NITROGEN DYNAMICS UNDER CONTRASTING MANAGEMENT SYSTEMS

by

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(Under the Direction of Mussie Habteselassie)

ABSTRACT

The abundance of poultry litter (PL) in states such as Georgia makes it an ideal choice as a source of N and other nutrients to grow organic produce that have high demand. However, research is needed to exploit such benefits in a way that minimizes the undesirable impacts. We evaluated the use of PL in an organic N management system to determine if it could be used to provide comparable yield to conventional fertilizers without significant accumulation of potential pollutants. Sweet corn was grown in plots receiving four treatments: Control (no N), ammonium sulfate at 112 (AS1) or 224 (AS2) kg N $ha^{-1}$ and PL (in combination with a cover crop) at 112 kg available N $ha^{-1}$. Average 3-year yield was greater in the AS and PL treatments than Control, with no significant difference among AS and PL. Post-season $NO_3^-$-N in the 15-30 cm for the 3-year average was the greatest for AS2 (16.0 kg N $ha^{-1}$), followed by AS1 (7.8 kg N $ha^{-1}$), with no significant difference between PL and Control. Thus downward movement of N in PL was limited, possibly due to the use of cover crop. We also calibrated and validated a computer simulation submodel, CERES-N, which was used to predict plant-available N from the cover crops. Crimson clover was incorporated in the soil and samples were collected over 120 d for three years. Root mean squared error (18.9 to 63.1 kg $ha^{-1}$), $F_{LOFIT}$ ($p > 0.05$), and 95% confidence intervals indicated an adequate fit of modeled and measured values. The use of such models to predict N availability

from cover crops that are used in combination with PL avoids excess application of PL and hence N loss and accumulation of pollutants. Finally, we examined impacts of the two N management systems on abundance and function of ammonia-oxidizing bacteria and archaea (AOB and AOA), which mediate the rate-limiting first step in production of nitrate that is highly mobile in soils. AOA estimates of abundance based on *amoA* gene copy numbers were higher than (or no different from) AOB abundance for all treatments, with the highest AOA to AOB ratios in control and organically managed systems. However, the abundance of AOB showed stronger correlation with nitrification potential than AOA, indicating their functional dominance in the AS treatments. The differential response of AOB and AOA suggests the need for targeted approaches to maximize N-use efficiency in the two systems. The study addressed interrelated soil, plant and microbial factors that are important in achieving N efficiency in an organic system that uses PL as an amendment.

INDEX WORDS: Ammonia Oxidizers, Nitrogen, Poultry Litter, Cover Crops, Nitrification, Nitrogen Mineralization

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# DEDICATION

This dissertation is dedicated to my mother, Beverly Vinson, who is kind, strong, funny, and wise, and who showed me that it is both possible and worth it to try to "have it all" and pursue education, career, family, etc. and that laughter is important.

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# CHAPTER 1

# INTRODUCTION

Nitrogen (N) is often the most limiting nutrient for crop production and can account for a large portion of fertilizer budgets. N-fertilizer use increased 800% from 1960 to 2000 (Bao, 2000). By the mid-1990's roughly 40% of the world's dietary protein was made available through the Haber-Bosch synthesis of ammonia (Smil, 2001). N-fertilizer costs account for nearly half of total fertilizer costs per year in the USA 2010-2015 (NASS, 2015). Considering the great demand for and cost of N fertilizers, many growers include alternative sources such as manure (Sims and Wolf, 1994; Burger and Venterea, 2008; Woli et al, 2013) or cover crops (Schomberg and Cabrera, 2001; Tribouillois et al., 2015). Improved understanding of nitrogen (N) cycling in agroecosystems, especially with alternative N sources, is essential for increasing nitrogen use efficiency and enhancing the sustainability of food, fiber, and fuel production (Cassman et al., 1998).

Poultry litter is a common source of N and other nutrients in agronomic systems. Nutrient ratios and other characteristics of litter and manures are subject to variability and it can be difficult to predict the timing and amount of plant-available N (Whitmore, 2007; Endale et al., 2010). Georgia is the largest producer of poultry in the country and, hence, of poultry litter, which is often land-applied. In 2010, the state of Georgia produced over $3 billion worth of broilers leaving around 2 million Mg of litter (USDA, 2014). Poultry litter is unique as compared to other animal wastes because it contains metals and antibiotics (Li et al., 2011) and the

potential for excessive P accumulation in the soil (Reddy et al., 2009). These could have unique effects on soil microbial populations as compared to other types of animal waste. There is limited research examining long-term impacts of poultry litter application on soil microorganisms that mediate these transformations and on nutrient pools in the soil. As demand for organic produce increases, there is a potential for increased use of poultry litter as an organic amendment in farms. More study is needed to develop best management practices for different soil types in different regions.

The decomposition and N mineralization of cover crop residues is another common alternative (or supplemental) source of N for crops. However, the timing and amount of available N is difficult to predict. Previous work in Georgia indicated that the N subroutine of the DSSAT (Decision Support System for Agrotechnology Transfer) family of models, CERES-N, can be calibrated to provide reasonable estimates of N released from cover crop residues when decomposing on the surface of a Cecil soil. Additional research is needed to validate the model for surface-applied and incorporated cover crop residues. Cover crops have been shown to mitigate P accumulation in cotton systems with repeated poultry litter applications (Nyakatawa et al., 2001). There is potential for integrating these alternative sources of N (and other nutrients) in other cropping systems as well but more field studies are needed with different crops on different soil types. Best management practices could be developed to maximize N-use efficiency while mitigating excessive buildup of other nutrients such as P and metals.

Several biotic and abiotic factors contribute to the complexity of N transformations including substrate characteristics (Whitmore, 1996; Cabrera et al., 2005), timing of application (Woli et al., 2013), soil water content (Kruse et al., 2004) and soil temperature (Vigil and Kissel, 1995; Quemada and Cabrera, 1996). There is a great need to better understand the

transformations which make N available to plants and subject to loss, especially at the field scale (Vitousek and Howarth, 1991; Habteselassie et al., 2006; Koper et al., 2010). Because of the variability in these and other factors, it is important to gather data for different soil types under different climatic conditions in regards to ammonification and nitrification, two important processes discussed in detail below which result in plant-available forms of nitrogen. These processes are complex, especially in the field, making it difficult to estimate the amount of N that will be plant available over a growing season. Risk-averse growers seek to avoid both under-application, which could reduce plant growth, and over-application which can have negative economic, agronomic, and environmental (Davidson et al, 1998; Cameron et al., 2013) effects.

Processes of N transformation including mineralization, immobilization, volatilization, nitrification, and denitrification have been widely studied; however, characterizing the microbial contributions to these cycles is an ongoing task. The transformation of organic N in litter or other fertilizers into ammonium and nitrate, forms that can be used by plants and microorganisms, is achieved through a sequence of microbially-mediated processes. N mineralization, also called ammonification, is the first process that converts the organic N forms into ammonium. Ammonium is then converted to nitrate via nitrite through a process called nitrification. The first step of nitrification is mediated by an enzyme called ammonia monooxygenase (AMO) which is produced by archaea and bacteria collectively referred to as ammonia oxidizers (AO). Nitrification is crucial from both environmental and agronomic perspectives because its product, nitrate, can easily be lost from soils through leaching or denitrification after conversion to other gaseous forms (Davidson et al., 1998).

We designed a field study to compare alternative (organic) N management systems, using poultry litter and cover crops as N sources, with conventional N management systems, using

ammonium sulfate as a source of N. We assessed the corn yield response along with other soil and plant parameters to the different N management systems. We also conducted a companion field study to calibrate and validate the CERES-N submodel to predict plant-available N from cover crops. Finally, we used novel molecular tools in conjunction with measurements of N transformation rates to examine the function and abundance of microorganisms responsible for selected N-cycle processes in soils under organic or conventional N management systems. We took a holistic approach where the role of microorganisms in cycling nitrogen was examined in plots where sweet corn (*Zea mays* convar. *saccharata* var. *rugosa*) was grown under contrasting systems of N management.

The following review (Chapter 2) will examine previous work on N management impacts on soil nutrients, plant parameters, and ammonia oxidizers. We will also review what is known about the nitrogen cycle, N-fertilizer demand, poultry litter as a nutrient source, cover crops as a nutrient source, factors affecting N mineralization, nitrification and ammonia oxidizers, management impacts on N-cycling microorganisms, techniques for studying soil N mineralization and techniques for studying ammonia-oxidizing bacteria and archaea in agricultural systems.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Nitrogen Cycle

Agricultural production of food, fiber, and fuels depends on plant-available N and other nutrients (Cassman et al., 1998). Therefore, a better understanding of the transformations, which make this nutrient available to plants, is essential (Vitousek and Howarth, 1991). Plant-available N from organic sources results from the enzymatic processes of N mineralization and N immobilization. A significant contribution to plant-available N can come from mineralization of soil organic matter (Cabrera et al., 1994). N mineralization, or ammonification, is the oxidation of organic N to inorganic N in the form of ammonium ($NH_4^+$). Nitrification is the oxidation of ammonium ($NH_4^+$) to nitrate ($NO_3^-$) via hydroxylamine ($NH_2OH$). The $NO_3^-$ can be easily lost from soils through leaching or denitrification, resulting in N losses which can have detrimental economic and environmental impacts.

The transformation of organic N in fertilizers into inorganic forms which can be used by plants and microorganisms is achieved through a sequence of microbially-mediated processes. Inorganic N becomes available to plants through mineralization, the conversion of organic N to inorganic N ($NH_4^+$ and $NO_3^-$). While these processes have been widely studied, the broad range of microorganisms mediating these processes has not been as well characterized. Nitrification is crucial from both environmental and agronomic perspectives because its product, $NO_3^-$, can easily be lost from soils through leaching or denitrification. Over-application of N fertilizers has led to financial losses, increases in greenhouse gas emissions such as nitrous oxide ($N_2O$)

(Davidson et al., 1998), and eutrophication and hypoxic zones in bodies of water (Smith and Schindler, 2009; Stoate et al., 2009; Cameron et al., 2013). Great strides have been made in the last several decades in terms of N-use efficiency but there is still room for improvement.

**2.2. N-Fertilizer Demand and Alternative Sources**

The demand for certified organic produce has steadily increased in recent years. The demand for composts and organic fertilizers (mostly derived from animal waste) is increasing along with the growth of the organic farming sector where use of synthetic fertilizers is excluded (Chalk et al., 2013). In the United States in 1995 there were less than 500,000 hectares of certified organic farmland whereas in 2008 there were almost 2,000,000 hectares (USDA, 2013). These organic farms are required to use alternative sources of N and other nutrients in order to provide the products demanded by the marketplace. The current global usage rate of synthetic N fertilizers is high in terms of the energy and cost to manufacture, house, and transport (Galloway et al., 2008). Without fossil-fuel-derived N fertilizers, it is estimated that about half of the world's human population would not be alive today. By the mid-1990's roughly 40% of the world's dietary protein was made available through the Haber-Bosch synthesis of ammonia (Smil, 2001). While use of synthetic fertilizers is necessary and, arguably, one of the greatest accomplishments of mankind, non-synthetic, or organic, fertilizers are used in many farming systems.

Synthetic fertilizers are not an option for the majority of farmers in the world who either lack physical access to these inputs because of road and climate conditions and/or who lack access to the money or credit needed to purchase these inputs. It is estimated that 2 billion people live and work on small farms in developing countries and most of the world's smallholder farmers earn less than $2 a day on average (IFAD, 2010). Many of these smallholder farmers go

without fertilizer inputs altogether and/or rely on traditional techniques such as slash-and-burn which can become unsustainable. These types of farming operations could benefit from studies of alternative N inputs such as locally sourced animal waste and cover crops, which are widely used in organic farming systems and could be incorporated in low-input, low-capital farms. There is a need to study organic N fertilizers, especially in organic farming systems, which could be extrapolated to smallholder farming systems in the developing world.

### 2.3. Poultry Litter as a Nutrient Source

Animal waste is a common source of N, P, K and other nutrients in agronomic systems (Stephenson et al., 1990; Endale et al., 2010). Animal waste is an ideal soil amendment for organic and smallholder cropping systems due to nutrient content and availability. In the USA, over 8 billion broilers (*Gallus gallus domesticus*) are produced annually. The state of Georgia often ranks number one in poultry production in the USA with broilers accounting for the highest percentage of the state's farm gate value (32%) in 2014 worth over $4 .5 billion (USDA-NASS, 2015). Over 1.3 billion birds produced in 2014 resulted in vast quantities of litter, a mixture of bedding (such as wood shavings or peanut hulls), feathers, feed waste, and excreta (Cabrera et al., 2005). At an estimated 1.5 kg litter produced per bird, approximately 2 million Mg dry litter were produced in Georgia, out of around 12 million Mg dry litter in the entire USA. This volume of poultry litter could provide vast quantities of N and other nutrients required by crops with approximately 40-60% of the N available in the first year following application (Bitzer and Sims, 1988; Whitmore, 2007). Litter is commonly land-applied in pastures or cropping systems both as a nutrient source and as a means of waste disposal (Stephenson et al., 1990; Pote et al., 2011). Nutrient ratios and other characteristics of these manures are subject to variability and it can be

difficult to predict the timing and amount of plant-available N (Whitmore, 2007; Endale et al., 2010).

Poultry litter is unique compared to other animal wastes because some of its constituents are metals and antibiotics (Li et al., 2011). These could have unique effects on soil microbial populations as compared to other types of animal waste. Management decisions such as surface-application or incorporation of poultry litter can change the timing and extent of N release. Some studies have found that poultry litter can be used efficiently in no-till (Nyakatawa et al., 2001; Reddy et al., 2009) and conservation tillage (Endale et al., 2010) cropping systems. However, poultry litter incorporated into the soil can release greater amounts of plant-available N at a faster rate than poultry litter surface-applied, and decrease environmental contamination from $NH_3$ volatilization (Giddens and Rao, 1975; Egdell et al., 2015).

Several field studies have shown increased soil productivity and soil organic matter from poultry litter surface-applied or subsurface applied in no-till soils (Pote et al., 2011). Soil organic matter (SOM) has been shown to stabilize soil pH (Campbell et al., 1996), increase pH-buffering capacity (pHBC) (Magdoff et al., 1987; Weaver et al., 2004), and sequester environmental toxins (Alexander, 2000) including those introduced with land-applied poultry litter (Durant et al., 2012). There is limited research examining long-term impacts of poultry litter application on soil microorganisms that mediate these transformations. As demand for organic produce increases, there is a potential for increased use of poultry litter as an organic amendment in farms.

Due to the difficulty of predicting the amount and timing of plant-available N, some common practices used by farmers include applying fertilizer two to four weeks before planting and/or applying up to 50% more poultry litter than recommended (Boyhan et al., 2010). Land

application of poultry litter can lead to detrimental environmental effects such as $NO_3^-$ contamination of groundwater (Bitzer and Sims, 1988; Liebhardt et al., 1979), production and loss of ammonia ($NH_3$) gas (Rothrock et al., 2010; Cassity-Duffey et al., 2014;), buildup of heavy metals (Sheppard and Sanipelli, 2012), accumulation of P (Schomberg et al., 2009), and subsequent P loss (Kuykendall et al., 1999).

Some studies have shown potential for mitigating environmental contamination from N and P released from poultry litter with amendments such as biochar (Doydora et al., 2011), dry acid amendments (Rothrock et al., 2010) or with cover crops planted subsequently to take up N and P (Nyakatawa et al., 2001). Additional field studies with multi-year poultry litter applications are needed to develop best management practices for agricultural soils in proximity to the massive stores of poultry litter which will be land-applied, as is the case in Georgia.

### 2.4. Factors Affecting N Mineralization and Nitrification

Nitrogen mineralization supplies the substrate for nitrification when N is applied in organic form. Thus, the two processes are closely linked, especially in waste-treated soils. Some factors that affect these processes include soil pH (Kissel et al., 1988), soil and atmospheric temperature (Cameron et al., 2013), soil cation exchange capacity (Cameron et al., 2013), soil type (Gordillo and Cabrera, 1997), C:N ratio of amendments (Nahm, 2005), and soil moisture (Cabrera, 1993).

Changes in soil moisture, such as the drying and rewetting cycles commonly experienced in agricultural soils, can greatly influence N mineralization. There is usually a flush of N mineralization following the rewetting of a dry soil which leads to the release of significant amounts of N (Cabrera, 1993). This is subject to the influence of several factors, including the availability of microbial biomass from microorganisms which did not survive the drying and

serve as a source of carbon upon soil rewetting (Marumoto et al., 1982); the increase in the availability of organic substrates through the increase in exposure of organic surfaces (Birch, 1958); and through desorption from the cation exchange sites of soil surfaces (Seneviratne, 2008) during drying and rewetting. These and other factors combine to contribute to the N flush in a way that follows first-order kinetics, where the rate of decomposition of the substrate is proportional to the amount of substrate available.

Several other kinetic models are commonly used to describe decomposition of a substrate including zero-order kinetics, where the rate of change is constant and second order kinetics, where the rate of change is proportional to both the substrate and microbial biomass. The Monod equation includes growth of a microbial population in the decomposition of a substrate. The Michaelis-Menten model [Eq. 1] relates the reaction rate to the concentration of the substrate while Haldane [Eq. 2] accounts for reaction-rate inhibition at high substrate concentrations.

$$V = V_{max}S/ (K_m + S) \quad \text{[Eq. 1]}$$

Where V is the $NH_4^+$ oxidation rate, $V_{max}$ is the maximum reaction rate under non-limiting conditions, $K_m$ is the half-saturation constant, and S is the $NH_4^+$ (substrate) concentration. When the reaction rate is inhibited at high substrate concentrations the Haldane model can be used:

$$V = V_{max}S/ (K_m + S + S^2/K_i) \quad \text{[Eq. 2]}$$

Where $K_i$ is an inhibition parameter equal to the maximum substrate concentration that produces a rate of ½ $V_{max}$, and $K_m$ is equal to the minimum substrate concentration that produces a rate of ½ $V_{max}$ (Haldane, 1965; Koper et al., 2010).

Due to the finite amount of N present in the soil, zero-order kinetics for N mineralization cannot be sustained indefinitely (Cabrera, 1993), although it can occur over long periods of measurement (Bonde et al., 1988). The appearance of zero-order N mineralization could be due

to a variety of factors including extension of fungal hyphae through soil aggregates as they continually colonize new substrate; the upper limit for microbial biomass due to protozoan grazing; or, in some cases, the fixation of mineralized $NH_4^+$ by 2:1 clays (Bonde et al., 1988). In a study done by Cabrera in 1993 it was found that a two-pool system more accurately described N mineralized in soils that were dried and rewetted. While zero-order background mineralization was confirmed through un-dried soil samples that were incubated and sampled periodically, the cumulative net N mineralized in dried and rewetted samples was more adequately described by a model with two N pools. One pool followed first-order kinetics. This N mineralization was occurring simultaneously over the background of N mineralization following zero-order kinetics. This background, zero-order rate was significantly greater in the dried and rewetted soils, indicating that N actually transferred from a passive pool to the zero-order pool. The two-pool model was determined to be superior based on the root mean square error and the extra sum of squares. Simulation models are more accurate when allowing for user input of pool sizes based on crop residue chemical composition, especially for rye and crimson clover (Quemada and Cabrera, 1995; Quemada et al., 1997; and Schomberg et al., 2001). Simulation models can be useful in estimating plant-available N from cover crops, giving credit for the N from both the cover crops and soil organic matter. These can be calibrated and made available to growers to increase N use efficiency and decrease the amount of N fertilizer that needs to be applied for subsequent crops in conventional or organic systems.

**2.5. Nitrification and Ammonia Oxidizers**

The first (and rate-limiting) step of nitrification is mediated by the enzyme ammonia monooxygenase which is produced by archaea and bacteria, collectively called ammonia oxidizers (AO). Ammonia monooxygenase catalyzes the oxidation of ammonia to

hydroxylamine (Leininger et al., 2006). This important and potentially detrimental process (because of potential loss of N) is mediated by the microbial populations whose functions and diversities are still not fully understood. Until recently it was believed that the microorganisms responsible for this process in soils were bacteria, specifically chemolithoautotrophic β- or ϒ-proteobacteria. Now it has been shown that archaea, *Thaumarchaeota* (Brochier-Armanet et al., 2008; Prosser and Nicol, 2012) and, possibly mesophilic *crenarchaeota* (Treusch et al., 2005), perform this function in soils as well. Homologs of ammonia monooxygenase genes have been discovered in archaea, and some archaeal ammonia oxidizers have been cultivated (Schleper 2010). The presence of archaea has been documented in almost all natural environments, and it is clear that archaea play a large role in the global nitrogen cycle in both terrestrial and marine ecosystems (Leininger et al., 2006; Nicol et al., 2008; Tourna et al., 2008; Reeve and Schleper 2011).

Recently more work has been done to reevaluate what is known about the soil AO community and its role in nitrification. The AMO enzyme catalyzes the first step in ammonia oxidation. This enzyme is mediated by both AOA and AOB. The *amoA* gene encodes the subunit A of the AMO enzyme. This gene is ideal for molecular analysis of ammonia-oxidizing communities because it is a functional trait, it is highly specific, and it has fine-scale resolution in closely-related populations (Rotthauwe et al., 1997). Quantitative PCR (qPCR) targeting the *amoA* gene suggests that AOA are abundant in soils and, therefore, may have a larger role than previously thought in soil nitrification. AOA and AOB have been evaluated in terms of ammonia affinity, mixotrophy, and pH growth optimum through physiological and genomic analyses of environmental samples (metagenomics), soil microcosms, and cultivated organisms (Prosser and

Nicol, 2012). Research is needed to better understand the relative contributions of these two groups of organisms to nitrification in soils receiving repeated applications of poultry litter.

Both the form and the amount of N available in soils can affect microorganism populations. In fertilized soils receiving nitrogen input, AOB tend to perform the bulk of the ammonia-oxidizing activity even when archaeal *amoA* gene copies outnumber bacterial *amoA* gene copies. In low-nutrient soils, specifically those with low N, AOA tend to be responsible for ammonia oxidation and, therefore, nitrification (Leininger et al., 2006; Jia and Conrad, 2009; Gubry-Rangin et al., 2010; Di et al., 2010; Zeng et al., 2011). In some cases, nitrification was linked to AOB abundance but not to AOA abundance indicating that AOA may grow mixotrophically, not using ammonia oxidation as their primary energy source (Di et al., 2010) while in other studies autotrophic ammonia oxidation by archaea was confirmed using DNA Stable Isotope Probing (Jia and Conrad, 2009). The nitrification rate was significantly higher in incubations of soil with organic N amendments while it was unaffected by the addition of inorganic N in the form of ammonium (Levicnik-Hofferle et al., 2012). The increase in nitrification rate was accompanied by increases in the abundance of the *amoA* gene but no changes in community structure. It should be noted that bacterial *amoA* genes were not detected in these soils. Koper et al. (2010) found agricultural management practices affected the size of AOA and AOB communities seasonally, while parameters such as substrate affinity and sensitivity to substrate inhibition remained relatively resistant to change over multiple seasons of fertilization. Significant nitrification inhibition appeared to occur in soils with high ammonium-N content from treatments receiving ammonium sulfate or dairy waste compost. The kinetics describing measured concentrations of product and substrate fit better with Haldane models than with Michaelis-Menten, indicating nitrification inhibition. These results suggest that both N

sources increased the size of the nitrifier community but did not shift community structure in ways that would influence enzyme affinity or sensitivity to ammonium (Koper et al., 2010). Nitrification rates driven by AOB can decrease at low pH (especially below 7) (de Boer and Kowalchuck, 2001; Nicol et al., 2008).

**2.6. Techniques for Studying N Mineralization**

Many techniques are available for studying the nitrogen (N) cycle and the microorganisms which mediate this cycle. As previously mentioned, some of the important processes in the conversion of nitrogen include ammonification and nitrification. These are the two main processes that result in plant-available N. The net result of N mineralization, immobilization, and nitrification can affect primary productivity (Hart et al., 1994). Often, estimates of N ammonification and nitrification are obtained by measuring these net rates from two or more processes occurring simultaneously. Net rates of N mineralization are determined by calculating the change in the soil inorganic-N ($NH_4$-N + $NO_3$-N) pool size over time. These pool sizes are measured by KCl extraction (Hart et al., 1994). Immobilization, as opposed to mineralization, would be indicated by a negative net value for the change in pool size from d 0 to the sampling time. These techniques can build mineralization curves over a sampling time period. Net nitrification is calculated similarly as the net change in the soil $NO_3^-$-N pool size. Ammonium and nitrate/nitrite can be measured colorimetrically with an automatic flow injector as described in Methods of Soil Analysis (Mulvaney, 1996).

Net rates of N mineralization and nitrification can also be calculated as the differences in gross process rates. Measuring gross rates can be more difficult, time consuming, and expensive. While measuring net changes in N concentration is a more common method of assessing N transformations, it does not provide information about productive and consumptive processes

that may be taking place simultaneously (Hart et al., 1994). It is important to consider both decreasing production and/or increasing consumption of inorganic N to maximize N use efficiency (Habteselassie et al., 2006). In order to resolve these contrasting processes that occur simultaneously, gross rates of N transformations can be determined using isotope techniques (Hart et al., 1994). Gross rates can be estimated by techniques using two N isotopes, $^{14}N$ and $^{15}N$. These include natural abundance, tracer, and isotope-dilution techniques. Net N transformation rates can be measured in the field or in the lab.

Potentially mineralizable N (PMN) can be estimated using hot KCl extraction of $NH_4^+$. Ammonium released by the hot 2*M* KCl method was strongly correlated with potentially mineralizable N in 30 different Iowa soils ($r^2 = 0.96$; Gianello and Bremner, 1986) and with net N mineralized over 24 d in 60 Cecil sandy loam soil samples from Georgia ($r^2 = 0.76$; Picone et al., 2002). In the hot KCl method, soil is mixed with 2*M* KCl and heated to 100˚C for 4 hours. The $NH_4^+$ released is measured, and $NH_4^+$ extracted in room-temperature 2*M* KCL is subtracted to calculate the $NH_4^+$ released by heating.

Net N transformation rates can be estimated by measuring pool sizes of $NH_4$-N and $NO_3$-N at different times throughout the season. Lab incubation studies can be used as indices of available N in soil. Nitrification potential can be measured by incubation of a shaken-soil slurry. In this case samples are incubated under optimized conditions in terms of water content, $NH_4^+$availability, aeration, and P availability. This index can be used to assess the size of the ammonia oxidizer community in the soil sample (Hart et al., 1994). These techniques can be used to gain a better understanding of process kinetics. Techniques for studying ammonia-oxidizing bacteria and archaea will be discussed in more detail in the section below.

Mineralization of organic N is essentially a sequence of enzymatic reactions. Most of the organic N is contained in large polymers which enzymes break down. The substrates for this depolymerization include a range of proteins, nucleic acids, and microbial cell wall constituents (amino sugars and their polymers, chitin and peptidoglycan). Enzymes in soils come primarily from the microbial biomass, though they can originate from plant and animal residues as well. Enzyme activities in soils can come from exoenzymes, which are free enzymes from living cells, endoenzymes, those released from decomposing cells, and enzymes bound to nonproliferating cells or cell constituents. The depolymerization of the organic-N-containing, large polymers is carried out by extracellular enzymes, primarily microbial in origin, including the proteinases, chitinases, kinases, amidases and amidohydrolases (Tabatabai, 1994). Exoenzymes play the most significant role in decreasing the size and complexity of organic N molecules, facilitating their further decomposition or assimilation. This depolymerization, including the cleavage of proteins to amino acids, is often the rate-limiting step in the mineralization process (Burns et al., 2013; Jan et al., 2009). Some of the enzymes that are involved in mineralization and their reactions include amidohydrolases such as urease, which facilitates the hydrolysis of urea to CO2 and NH3, and L-glutaminase, which facilitates the hydrolysis of L-glutamine, proteases such as subtilisin which hydrolyzes alkaline proteinase, amidohydrolases such as arginase which facilitates the hydrolysis of arginine to ornithine and urea, and glycosidases such as chitinase which hydrolyzes chitin linkages.

Simulation models are useful tools for estimating N mineralized from cover crops. Models that account for all N transformations in a crop-soil system are ideal but development and validation of such models is intensive in terms of human resources and cost (Quemada and Cabrera, 1995). Some of the most widely used models for simulating an entire crop-soil system

are CERES models (Godwin and Jones, 1991; Quemada and Cabrera, 1995). Previous work in Georgia showed that the N subroutine of the DSSAT family of models, CERES-N, could be calibrated to provide accurate estimates of N released from rye (*Secale cereal* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and crimson clover (*Trifolium incarnatum* L.) residues when decomposing on the surface of a Cecil soil (Quemada et al., 1997; Schomberg and Cabrera, 2001).

The CERES-N model originally assigned fixed percentages of plant residues to three pools, carbohydrates, cellulose, and lignin, with different first-order rate constants of decomposition derived from the PAPRAN model (Seligman and Van Keulen, 1981; Godwin and Jones, 1991). The original rate constants assigned to these fixed pools were found to overestimate decomposition of plant residues (Vigil et al., 1991; Bowen et al., 1993), which led to suggested reductions in rate constants. Vigil et al. (1991) also modified the minimum C/N ratio, below which no decrease in the rate constant occurs, and decreased the microbial N demand. Schaaf et al. (1995) adjusted the model to allow for user input of C/N ratios, and Quemada and Cabrera (1997) modified the model to allow user input of actual pool sizes for carbohydrates, cellulose, and lignin. Schomberg and Cabrera (2001) found that using residue pool sizes as well as rate constants calibrated by Quemada et al. (1997) provided better estimates of N released from crimson clover and rye cover crops under field conditions. Additional research is needed to validate the model for surface-applied and incorporated cover crop residues. Field studies are needed to validate the model for surface applied and incorporated cover crops under different climatic conditions in different soil types.

## 2.7. Techniques for Studying Ammonia-oxidizing Bacteria and Archaea

A variety of techniques have been used to study ammonia oxidizing bacteria and archaea in agricultural systems. Recently, knowledge of archaeal molecular biology, metabolism, and phylogenetics has been gathered without the need for laboratory cultivation (Brochier-Armanet et al., 2011). Genetics and genomics have been the tools of choice because the conserved protein markers used to identify certain types of archaea require complete genome sequences which are now gathered in environmental genomic libraries (Spang et al., 2010). Archaea with the potential to oxidize ammonia, AOA, are identified by targeting the *amoA* and 16SrRNA genes. AOA actually outnumber AOB, often by orders of magnitude, in most environments based on gene counts using quantitative polymerase chain reaction or qPCR (Schleper, 2010; Schauss et al., 2009). It is not known whether these bacteria and archaea are functionally redundant, competing for the same resources, or if they occupy different niches (Schleper, 2010). AOA of moderate terrestrial and marine environments initially assigned to *Crenarchaeota* now belong to a novel phylum *Thaumarchaeota* (Spang et al., 2010). This sets the stage for continued research and new discoveries about the role and function of this novel phylum of archaea which are abundant in agricultural soils and play an important role in nitrogen cycling in agricultural soils and on a global scale.

## 2.8. Current and Future Research

In this comparative study we evaluated conventional systems of N management (with ammonium sulfate) and organic systems of N management (with cover crops, poultry litter, and blood meal) in terms of crop yield, soil and plant N, and the function and abundance of the microorganisms that mediate nitrification. Our study of N management impacts on the plant-soil-microbe nexus focused on three main objectives. The first objective was to examine how the

primary productivity of sweet corn responds to the contrasting N management systems, which can affect the behavior of the microorganisms ultimately responsible for the availability of plant-usable forms of N. We measured primary productivity parameters such as corn crop yield, plant height, and corn leaf and cornstalk N content to better understand the impact of the different systems of N management on a cropping system.

The second objective was to validate and calibrate a web-based submodel used to predict plant-available N from cover crops over a growing season. We used the CERES-N simulation submodel to evaluate plant-available N from cover crops. N mineralization from crimson clover and rye was assessed in a preliminary field study. Model predictions were evaluated for crimson clover in the sweet corn plots once they were established.

The third objective was to compare the abundance and function of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in organically or conventionally managed soil. We compared the functional response of these microorganisms in the contrastingly managed soil by measuring nitrification potential, quantifying functional genes of microorganisms, and assessing correlations. Under this objective, we also examined changes in mineralization patterns of organic N forms, which are the sources of substrate for nitrification. Recent work has shown greater gene copy numbers, biological activity, and diversity in organically managed soils compared to soils managed conventionally (Reeve and Schleper, 2011). More work is needed to examine these and other parameters in different soil types under contrasting systems of N management.

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# CHAPTER 3

# COMPARISON OF SOIL AND PLANT NUTRIENT DYNAMICS IN CONVENTIONAL AND ORGANIC SWEET CORN PRODUCTION SYSTEMS [1]

[1]Woodruff, L.K., M.Y. Habteselassie, J.M. Norton, G.E. Boyhan, M.L. Cabrera. To be submitted to *Soil Sci. Soc. Am. J.*

## Abstract

Poultry litter is abundant in Georgia and is an inexpensive source of N in an organic system. However, poultry litter characteristics are variable and nutrient availability for crops, along with potential accumulation of nutrients or metals in soils, depends on a number of litter and soil factors, necessitating site-specific studies. Our objective was to determine if organic N management with poultry litter and cover crops as amendments could provide comparable yield to synthetic fertilizers without accumulation of nutrients or metals. Sweet corn was grown in plots receiving four treatments: Control (no N), ammonium sulfate at 112 (AS1) or 224 (AS2) kg N $ha^{-1}$ and PL (in combination with a cover crop) at 112 kg available N $ha^{-1}$. The average 3-year yield was greater in the AS and PL treatments than in Control, with no significant difference among AS and PL. The greatest difference in cornstalk N was observed between AS2 and Control, with means of 1.75 and 0.79 g $kg^{-1}$ $NO_3^-$-N, respectively. Post-season $NO_3^-$-N in the 15-30 cm soil depth for the 3-year average was the greatest for AS2 (16.0 kg N $ha^{-1}$), followed by AS1 (7.8 kg N $ha^{-1}$), with no significant difference between PL and Control. This suggests the downward movement of N in PL was limited as compared to the AS1 treatment. This could be due to the use of PL with cover crop that kept the N in the top layer after the corn was harvested. There was significant buildup of Zn in PL-treated plots over time but did not affect yield parameters as compared to AS1 that provided comparable rate of N. Overall, the use of PL resulted in comparable levels of yield and plant N parameters as AS treatments. The combination of PL and cover crop seemed to have limited the downward N movement as compared to the AS treatments.

Abbreviations: N, Nitrogen; AS, ammonium sulfate; PL, poultry litter

## 3.1. Introduction

Animal waste is available in abundance near many agricultural areas and is an inexpensive source of N, P, K, and other nutrients required for crop growth (Stephenson et al., 1990; Endale et al., 2010). In the United States, over 8 billion broilers (*Gallus gallus domesticus*) are produced annually necessitating disposal of approximately 12 million Mg dry litter, a mixture of bedding (such as wood shavings or peanut hulls), feathers, feed waste, and excreta. (USDA-NASS, 2015). This litter is commonly land-applied in pastures or cropping systems both as a nutrient source for pasture or crops and as a means of waste disposal (Stephenson et al. 1990; Pote et al., 2011). Animal waste is of particular importance in organic systems. The demand for certified organic produce and, therefore, the area of land under organic management has increased in recent years (Cavigelli et al., 2008; O'Connell et al., 2015). Land-application of organic fertilizers such as animal waste has increased in conjunction with the growth of the organic farming sector where use of synthetic fertilizers is excluded (Chalk et al., 2013; Egdell et al., 2015). Many factors contribute to the biotic and abiotic processes that make N from poultry litter plant-available. The interaction of these factors is difficult to replicate in the laboratory or extrapolate across regions, necessitating field-based studies which are essential to determining best management practices for poultry litter land application.

Nutrient ratios and other characteristics of manures are variable and it can be difficult to predict the timing and amount of plant-available N (Whitmore, 2007; Endale et al., 2010). Nitrification, the conversion of $NH_4^+$ to $NO_3^-$, is crucial from both environmental and agronomic perspectives because its product, $NO_3^-$, is easily lost from soils through leaching or through denitrification after conversion to other gaseous forms. Inefficient use of N fertilizers has led to

financial losses, increases in greenhouse gas emissions such as nitrous oxide (Davidson et al., 2000), and eutrophication and hypoxic zones in bodies of water (Cameron et al., 2013; Smith and Schindler, 2009; Stoate et al., 2009). When poultry litter is applied at a rate based on N requirement, other nutrients including metals (Mg, Zn, and Mn) are often applied in excess of crop demand (Endale et al., 2010). Metals and other nutrients contained in poultry litter may be toxic for crops and pose environmental concerns (Reddy et al, 2009; Endale et al., 2010). These and other constituents of poultry litter should be monitored when land-applied, especially for repeated applications where accumulation to toxic levels can occur.

Land application of poultry litter can lead to detrimental environmental effects such as $NO_3^-$ contamination of groundwater (Bitzer and Sims, 1988; Liebhardt et al., 1979), production and loss of ammonia ($NH_3$) gas (Rothrock et al., 2010; Cassity-Duffey et al., 2014;), buildup of heavy metals (Sheppard and Sanipelli, 2012), accumulation of P (Schomberg et al., 2009a), and subsequent P loss (Kuykendall et al., 1999). Due to the difficulty of predicting the amount and timing of plant-available N, some common practices used by farmers include applying fertilizer 2 to 4 weeks before planting and/or applying up to 50% more poultry litter than the recommendation (Boyhan et al., 2010). Some studies have shown potential for mitigating environmental contamination from N and P released from poultry litter with amendments such as biochar (Doydora et al., 2011), dry acid amendments (Rothrock et al., 2010) or with cover crops planted subsequently to take up N and P (Nyakatawa et al., 2001). Additional field studies with multi-year poultry litter incorporation are needed to develop best management practices for agricultural soils in proximity to the massive stores of poultry litter which will be land-applied, as is the case in Georgia.

We used a holistic approach in a multi-year study comparing conventional N management (ammonium sulfate) and organic N management (a combination of poultry litter, as a nutrient source, and cover crops, both as a nutrient source and a means to mitigate potential environmental losses of N and P). We compared how repeated incorporation of poultry litter or ammonium sulfate affects plant and soil parameters including sweet corn yield, N content in plant tissue, and soil-nutrient pools in an Ultisol over multiple growing seasons. Sweet corn (*Zea mays* L. var. *saccharata)* was chosen for this comparative study because it has a relatively high N requirement for high yield and is a common cash crop grown on many different soil types. Our objective was to determine if poultry litter and cover crops could be used efficiently in a typical cropping system under conventional tillage, giving similar results to conventional N management (ammonium sulfate) in terms of plant parameters such as yield with multi-year application without a significant build-up in nutrients or metals that are potential pollutants.

## 3.2. Materials and Methods

### 3.2.1 Experimental Field Site and Amendments

Field plots were established at the Durham Horticulture Farm in Watkinsville, GA (33˚ 55´ N lat.; 83˚ 25´ W long.) on an irrigated Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapludults) in March 2012. The area where the plots were established had been fallowed for the previous six years. Precipitation and temperature were measured by the Georgia Automated Weather Monitoring Network with a weather station located at the farm (http://www.georgiaweather.net/). Total precipitation for the sweet corn growing seasons for 2012, 2013, and 2014 was 27, 66, and 18 cm, respectively. Mean temperatures for the growing seasons of the three years ranged from 22 to 24˚C (Fig. 3.1). Initial pH was $5.3_{1:1}$ and pH

buffering capacity (pHBC) was 257$_{(ppm\ CaCO3/pH)}$. Selected soil properties are summarized in Table 3.1. Initially the entire area (1,650 $m^2$) was chisel-plowed, followed by disking. In subsequent years the 16 individual plots were chisel-plowed, once pre-season, and disked after amendments were added.

A randomized complete block design was used with four treatments, Control (no N added), AS1 (ammonium sulfate at 112 kg N $ha^{-1}$), AS2 (ammonium sulfate at 224 kg N $ha^{-1}$), and PL (poultry litter, blood meal, and crimson clover (*Trifolium incarnatum* L.)) at 112 kg N $ha^{-1}$ total). Each treatment had four replications and each plot was 3.8 by 9.1 m with 2 m between blocks and 6 m between rows. Fresh litter was retrieved from local poultry houses each year and composite samples were land-applied within one week. Selected properties of litter are indicated in Table 3.2.

For the PL plots, the N-requirement from poultry litter was calculated by subtracting predicted plant-available N from cover crops using a computer simulation model that implements the N subroutine of CERES models (Godwin and Jones, 1991) from the total available N amendment rate of 112 kg N $ha^{-1}$. CERES-N is a subroutine that considers three pools of cover crop biomass (cellulose, carbohydrates, and lignin) (Schomberg and Cabrera, 2001; Quemada et al., 1997; Goodwin and Jones, 1991). Each pool decomposes according to first-order kinetics with a maximum potential rate constant (based on recalcitrance) which is modified daily by temperature and moisture factors (Schomberg and Cabrera, 2001). Crimson clover was planted each fall in October, flail-mowed in April, and incorporated into the soil before planting.

All amendments used on the PL plots were Organic Materials Review Institute (OMRI) certified; animal waste is allowed in certified organic systems if it is applied at least 90 d before

harvest for crops with the harvested portion not in contact with the soil. Potash was applied in one organic plot in Year 2 and blood meal was applied as an N side-dressing each year. In 2012, 7.0 Mg $ha^{-1}$ of poultry litter provided all of the N requirement (112 kg N $ha^{-1}$) and other nutrient requirements based on soil test recommendations. In 2013 and 2014, N contribution from cover crop was considered and PL application rates were reduced to 2.9 and 2.3 Mg $ha^{-1}$, respectively. Lime, N, and P amendments are listed in detail for all treatments in Table 3.3. In 2014, the poultry litter added in the PL treatment based on N rate did not supply sufficient P and K based on soil test recommendations. Rock phosphate (0-3-0) was applied at variable rates of 29 to 49 kg P $ha^{-1}$ to each of the PL plots to fulfill the recommendations of each plot. Sulfate of potash (0-0-51) was added at 43 kg K $ha^{-1}$ to each plot. Nutrient availability from poultry litter was estimated to be 58% of total N measured by combustion (Evers, 1999; Meisinger and Jokela, 2000; Whitmore, 2007). Amendments were weighed, distributed to plots, and spread by hand before being tilled in to a depth of 15 cm. Side-dressings (1/3 of total N amendments) were withheld from pre-season applications and applied several weeks after planting when corn plants reached stage V6 to V7 (Ritchie et al., 1997; Kitchen et al., 2010). Side-dressings were ammonium sulfate for AS1 and AS2, and blood meal (13.25 % N) for PL. Blood meal was the side dressing for the PL plots because according to OMRI regulations, poultry litter cannot be applied less than 90 d before harvest.

Sweet corn variety “Silver Queen,” purchased from Johnny’s Selected Seeds (Winslow, ME), was planted following amendment application and tillage each year in April. Corn was planted at a rate of two seeds (thinned to one plant after germination) every 23 cm with rows 90 cm apart per seed package recommendation. The plots were irrigated with a central-pivot

sprinkler irrigation system as needed. Amendment application rates for all nutrients except N were based on soil test recommendations from the AES Laboratories in Athens, GA (aesl.ces.uga.edu). Conventional amendments for other nutrients included potassium sulfate (0-0-60), triple superphosphate (0-45-0), and borax. The poultry litter provided all other nutrients needed for the PL plots except for potassium needed for one plot in year two of the study.

Soil samples from 0-15 and 15-30 cm were collected at least three times per season each year (pre-season, mid-season, and post-season). Pre-season samples were collected before amendment application and planting. Mid-season samples were collected at day 45-49 after planting. Post-harvest samples were taken a few days after the final harvest in June or July.

### 3.2.2 Soil and Poultry Litter Analysis

Soil and poultry litter analyses were performed by the Agricultural and Environmental Services (AES) Laboratories unless otherwise noted. Pre-season each year, composite soil samples (0-15 cm) were analyzed for P, K, Ca, Mg, Zn, and Mn using Mehlich I extractant (Mehlich, 1953). Soil pH was measured using 0.01 M $CaCl_2$ (1:1 ratio) because using a dilute salt solution to measure pH gives more stable values for soils because of seasonal variations in soil salinity due to rainfall and fertilization. A dilute salt solution masks these seasonal variations. Poultry litter was analyzed each year for N, P, K, Mg, S, Mn, Fe, B, C, Zn, and Na (ICP AES EPA method 6020b; USEPA, 2014).

Soil parameters such as soil organic matter (pre-season each year; 0-15 cm depth), inorganic N pools (pre-, mid-, and post-season $NH_4^+$-N and $NO_3^-$-N; 0-15 and 15-30 cm depths), and soil water content were measured. Soil organic matter was determined using dry combustion (Matejovic, 1997) with a LECO CHN-2000 (LECO Corporation, St. Joseph, MI). The inorganic

N pool sizes were determined by mixing 15 g of soil at field moisture with 75 mL of 2 *M* KCl, followed by shaking for 1 h, and centrifuging at 8000 x *g* for 15 min. Supernatant was frozen and analyzed later for $NH_4^+$-N and $NO_2^-$- and $NO_3^-$-N using an Alpkem Autoanalyzer (Mulvaney, 1996).

### 3.2.3 Corn Plant Nitrogen and Yield

The end of season cornstalk nitrate test (CSNT) was used to assess N uptake (Binford et al., 1992; Blackmer 1996; Balkcom et al., 2003). Cornstalk samples were processed following procedures outlined by Beegle and Rotz (2009). Briefly, a 20-cm section of cornstalk, starting 12 cm above the ground, was cut from ten randomly selected plants from each plot. Samples were oven-dried at 65ºC for 72 hours. The dried samples were then ground in a Thomas-Wiley Laboratory Mill Model 4 (Arthur H Thomas Company, Philadelphia, PA) and passed through a 1 mm sieve. Ground-sieved samples were packed into circular cells and scanned to collect near infrared spectra for every 2 nm from 400 to 2498 nm on a FOSS NIRSystems model 6500 scanning monochrometer (FOSSNIRSystems, Silver Spring, MD) in reflectance mode. Each sample was scanned 13 times and the results were averaged to produce a single spectrum. The average spectral properties were used to predict crude protein (CP) using the NIRS calibration equation for grass species (13gh50b2.eqa) developed by NIRS Forage and Feed Testing Consortium (http://nirsconsortium.org). The total N concentration of residue was estimated as "CP/ 6.25". The $NO_3^-$-N content was estimated as 10% of total N. Corn yield was measured each year by harvesting corn ears by hand from the middle two rows (10 m per row) of each plot when silks had begun to turn brown and ears were deemed mature (83 to 85 days after planting; Cerrato and Blackmer, 1990).

### 3.2.4 Agricultural Cropping Efficiency Coefficient

An agricultural cropping efficiency (ACE) coefficient was adapted to compare N management systems in terms of potential pollutants (N and P) and yield (Egdell et al., 2015). The ACE coefficient is the proportion of total sweet corn yield per unit of potential pollutant (N or P). The higher the coefficient, the greater the yield in relation to potential pollutants; the lower the coefficient, the lower the yield and/or greater the potential pollutant. A higher coefficient indicates a more efficient system in terms of the parameters examined; a lower coefficient indicates lower yield per unit of available N or P post-season, therefore, a less efficient system. The coefficient was calculated by dividing total sweet corn yield by potential pollutant load (dimensionless because both factors were in kg $ha^{-1}$), and normalized by setting the highest (most efficient) value each year to 100 and proportioning the other values to it.

### 3.2.5 Statistical Analysis

Data were analyzed statistically with repeated measures analysis of variance (PROC MIXED) with year as a repeated measures factor. All statistical analyses were performed using least significant difference at 0.05 probability level ($LSD_{0.05}$) for multiple comparisons among means with SAS Software Version 9.3 (SAS Institute, Inc., 2013. SAS. Release 9.3. Cary, NC).

## 3.3. Results and Discussion

### 3.3.1 Soil Organic Matter and pH

Soil organic matter showed no change over the course of the study (no significant treatment, year, or year x treatment interaction effects), with means ranging from 2.6 to 2.8% throughout the study (Table 3.1). The experimental plot area was left fallow for the six years before the study began; initial soil organic matter was 2.7%, relatively high for agricultural soils

in the area typically ranging from 0.9 to 1.7% (Schomberg et al., 2009b). Throughout the study tillage was the same for all treatments. Weed pressure was considerable in the off-season as no herbicides were applied. Each spring the biomass of the weeds was chisel plowed and disked into the soil. This likely served to replenish SOM lost to tillage each year and could account for the lack of difference in SOM across treatments and years. Off-season weed pressure was mitigated in the PL plots with the planting of crimson clover. However, organic matter input was 1,680 to 2,240 kg dry matter $ha^{-1}$ when the crimson clover was tilled in each Spring 2013 and 2014, respectively (Table 3.3).

Soil pH showed a significant treatment effect ($p = 0.0439$) and year effect ($p < 0.0001$) but no treatment x year interaction. The overall treatment average pH in PL and Control was greater than AS2. By year, the greatest overall pH was observed in 2014 (both pre and post-season means of 6.3) followed by 2013 (6.0) and 2012 (5.3). Soil pH was the most acidic before treatments were applied, 5.3 in 2012 (Table 3.1), increasing for most treatments each study year as plots were limed based on soil test recommendations. In 2013, pH was significantly lower for the AS2 treatment (5.5) than AS1, PL, and Control, with no difference among the three. Lime application rates among treatments varied each year and decreased (for AS1 and AS2) or were eliminated (for Control and PL) by 2014 (Table 3.3). The highest soil-nitrate pools were observed in AS2 (section 3.3), indicating the greatest nitrification activity, a process which results in net release of protons ($H^+$). The lowest soil nitrate concentrations were observed in the Control plots where no N was added, so the pH was raised with the initial liming and did not suffer the subsequent drop observed among the N-added treatments (AS1 and AS2). After year 2, no lime was required for the Control or the PL treatments.

### 3.3.2 Available Phosphorous, Potassium, and Metals

Available phosphorous (P) showed treatment (0.0113) and year ($p < 0.0001$) effects but no treatment x year interaction ($p = 0.1871$). Potassium pools in the soil differed with treatment ($p = 0.0028$), year ($p < 0.0001$), and treatment x year interaction effects ($p = 0.0355$). Overall, PL had the greatest P with no differences among the AS treatments and Control. Phosphorous accumulation was highest for post-season 2014 (41 mg $kg^{-1}$). Soil phosphorous level was 5 mg $kg^{-1}$ soil before amendment applications (Table 3.1). Phosphorous was significantly higher in both 2013 and 2014 than 2012, with no difference between the two. In pre- and post-season 2014, the Control had significantly greater potassium than AS or PL but none of the treatments were significantly different from each other before 2014. Overall, potassium showed a decreasing pattern over time in all the treatment except for control.

When manure is land-applied, phosphorous and potassium can build up to high levels (Habteselassie et al., 2006; Smith et al., 1998). Poultry litter application significantly increased soil P after 5 years of repeated application at rates of 5 and 20 Mg $ha^{-1}$ (Liechty et al., 2009). In our study, P and K buildup was mitigated by the incorporation of cover crops in subsequent years. Phosphorous and potassium amendments were applied based on soil test recommendations for all treatments as needed except for PL treatments where poultry litter was added based on N rate (Table 3.3). The amount of poultry litter applied in the second and third years of treatment were reduced to 2,945 and 2,340 kg $ha^{-1}$ respectively because of the N credit from the cover crop. This was, on average, 62% reduction in amount of poultry litter applied in comparison to the first year. This type of management system, coupling the use of poultry litter with a cover

crop can potentially provide a means to minimize the build-up of potential pollutants while providing adequate amount of N.

Soil Ca and Mg increased significantly over time ($p < 0.0001$), but there was no main effect from treatment or treatment x year interaction for Ca. Magnesium in the soil had treatment ($p = 0.0118$) and treatment x year interaction ($p = 0.0117$) main effects. Calcium in the soil was significantly higher in 2013 and 2014 than in 2012 with no difference between the two. In 2013, Calcium was higher in PL than in AS2. Magnesium was greater in PL than both AS treatments. Magnesium was greatest overall post-season 2014, followed by pre-season 2013 and 2014 (with no difference between the two), and 2012. Plots were amended with dolomitic lime so Magnesium was added each year in the AS treatments and the first 2 years in PL causing Magnesium concentrations to increase over time. Treatment effects were similar to Calcium because the two were added in conjunction.

Zinc and manganese concentrations in the soil are shown in Table 3.1. Zinc had treatment ($p < 0.0001$), year ($p < 0.0001$), and treatment x year effects ($p = 0.0073$). Zinc was greatest in PL, with no difference among initial soil concentrations, AS1, AS2, and Control treatments over the course of the study. Zinc accumulation decreased over time in the PL treatment because the amount of PL applied was reduced after the first year. Manganese had main effects from treatment ($p = 0.0002$) and year ($p < 0.0001$) but no treatment x year interaction. Overall, Mn was greatest in AS2 and PL with no difference between the two. In 2013, extractable Mn was greater in AS2 (31 mg $kg^{-1}$) than the Control (22 mg $kg^{-1}$) (Table 3.1). Final sampling, post-season 2014, showed AS2 Mn concentrations greater than AS1 and Control. Unlike Zn, there was not a consistent increase of Mn due to PL.

Because poultry litter was applied at a rate based on N, other nutrients including metals (Mg, Zn, and Mn) were often applied in excess of crop demand. Metals and other nutrients contained in poultry litter may be toxic for crops and pose environmental concerns (Reddy et al, 2009; Endale et al., 2010). Zinc concentration in the soil increased with poultry litter amendments accumulating at rates of up to 6.2 kg $ha^{-1}$ $y^{-1}$ with long-term repeated applications of poultry litter (Endale et al., 2010). The highest rate of Zn increase observed over the course of this study was 2.4 kg $ha^{-1}$ $y^{-1}$ in PL the first year with decreases in concentrations in subsequent years. While accumulation of these metals sometimes occurs after repeated applications of poultry litter (Endale et al., 2010), this was only the case for Zn in the current study. As with soil P accumulation, integration of cover crops may have mitigated the buildup of metals following repeated applications of poultry litter.

### 3.3.3 Inorganic N Pools

*Pre-season*

Inorganic N ($NH_4^+$-N and $NO_3^-$-N) pools were measured pre-season each year to evaluate plant-available N status in the soil before planting. Main effects for year ($p = 0.0025$) and treatment x year interaction ($p = 0.0012$) were significant for pre-season $NO_3^-$-N pools (0-15 cm), with no treatment differences for 2012 and 2013. For the three-year study, there was no difference in the average among treatments (Fig. 3.2). A treatment effect ($p < 0.0001$) was observed in 2014. For the PL treatment in 2014, $NO_3^-$-N (0-15 cm) was greatest, 18.8 kg N $ha^{-1}$, followed by the AS1 and AS2, with no difference between the two. Pre-season $NO_3^-$-N was lowest in the Control, 7.7 kg N $ha^{-1}$. The PL treatment had the highest concentration of $NO_3^-$-N by the third year of the study possibly because of the cumulative effect of yearly applications of

PL and the relatively slow decay of diverse organic N forms in the litter. This residual fertility from PL can increase yield (Reddy et al., 2009).

Main effects for year, treatment, and treatment x year interaction were significant for pre-season $NO_3^-$-N pools 15-30 cm ($p < 0.0001$). For the average of the three years, AS2 had the highest $NO_3^-$-N concentration, 13.4 kg N $ha^{-1}$, followed by AS1 and PL (with no difference between the two), and the lowest concentration was in the Control, 5.2 kg N $ha^{-1}$ (Fig. 3.2). The $NO_3^-$- N concentrations in 15-30 cm were greatest in 2013, and the treatment effect was significant ($p < 0.0003$) with AS2, 29.7 kg N $ha^{-1}$, greater than the other three treatments. In 2014, PL and AS1 were slightly higher (by 2-5 kg N $ha^{-1}$) than the Control and AS2.

The main effect of time (year) was significant for pre-season $NH_4^+$-N, 0-15 cm ($p < 0.0001$) with the highest concentrations in 2012, averaging 9.0 kg N $ha^{-1}$across treatments. The $NH_4^+$-N concentrations pre-season tend to be lower than the $NO_3^-$-N concentrations in agricultural soils. Once the study was established, plant uptake of N and nitrification throughout each growing season likely contributed to the depletion of $NH_4^+$-N pools in the soil accounting for the lower concentrations pre-season in subsequent years. There were no treatment, time, or treatment x time interaction effects of pre-season $NH_4^+$-N at the 15-30 cm depth.

*Mid-season*

Mid-season soil $NO_3^-$- N and $NH_4^+$-N were measured each year to assess plant-available N at a time of high demand for the crop and, presumably, relatively high rates of nitrification due to temperature and substrate availability in the N-amended treatments. Main effects of treatment, year, and treatment x year interaction were significant ($p < 0.0001$) for mid-season $NO_3^-$-N concentrations (0-15 cm), with AS2 the greatest for 2012 and 2013 (131.2 and 26.1 kg N $ha^{-1}$)

with no differences among AS1, PL, and Control. The AS2 and PL treatments (129.8 and 116.4 kg N $ha^{-1}$) were greatest in 2014 with no difference between AS1 and Control (Fig. 3.3).

Main effects of treatment ($p < 0.001$), year ($p < 0.001$) and treatment x year interaction ($p = 0.0138$) were significant for mid-season $NO_3^-$- N (15-30 cm). AS2 was greatest in 2012 (60 kg N $ha^{-1}$) with no difference among the other three treatments (Fig. 3.3). AS2 and PL were greater than the other treatments in 2013 and 2014, respectively. In 2012, total rainfall from amendment applications to mid-season sampling was 9 cm. In 2013, total rainfall over the same period was 33 cm with 17 cm of rainfall over the 7 d period before mid-season sampling (Fig 1). Greater precipitation in 2013 combined with warmer average temperatures (i.e., no daily averages below 20˚C in the 20 days before mid-season sampling, unlike in 2012 and 2014) likely led to increased nitrification earlier in the season and subsequent leaching in 2013 than in the other two study years when average conditions were cooler and drier the first half of the season.

The $NH_4^+$-N in the 0-15 cm depth was significantly affected by treatment ($p = 0.0158$), year ($p = 0.0326$), and treatment x year interaction ($p = 0.0346$). Soil $NH_4^+$-N in the 0-15 cm depth was greatest in 2012 in AS1 and AS2 treatments with no significant difference between PL and Control. Soil $NH_4^+$-N in 0-15 cm was relatively low in 2013 and 2014 (both lower than 2012) with no difference among treatments. N pools mid-season for 2012 and 2014 experienced the greatest differences in speciation at 0-15 cm, with greater $NO_3^-$-N than $NH_4^+$-N concentrations. The $NO_3^-$-N form was the dominant form of the inorganic N pool in all the years across the treatments, suggesting that $NH_4^+$-N was being quickly nitrified to $NO_3^-$-N. The greatest concentrations of inorganic N were associated with the AS2 treatment, which stands to reason as it had the highest rate application.

Similar trends were observed for mid-season $NO_3^-$-N and $NH_4^+$-N at 15-30 cm. There were significant treatment ($p < 0.0001$) and year ($p = 0.0240$) effects, but no treatment x year interaction. AS2 $NH_4^+$-N (15-30 cm) was greatest in 2012 with no differences among the other three treatments. In 2013 there was no difference among treatments. In 2014, concentrations were greater than in the other two years, with AS2 greater than Control. Mid-season $NO_3^-$-N was significantly greater than $NH_4^+$-N for all treatments for all years. Since the amendments were mainly added in the top 0-15 cm, this result might suggest some movement of $NO_3^-$-N down the profile. Although the considerable levels of $NH_4^+$-N at same depth might also suggest some came from mineralization of soil organic matter.

*Post-season*

Post-season N pools were measured to assess the potential for leaching after harvest. Significant differences were observed for treatment, year, and treatment x year interaction effects ($p < 0.0001$ for all) for $NO_3^-$-N, 15-30 cm. Similar trends were observed each year so the average of the three is presented in Fig. 3.2. Inorganic N pools for the average of the three years showed the greatest amount of $NO_3^-$-N at the 15-30 cm depth for the AS2 treatment with a mean of 16.0 kg N $ha^{-1}$, followed by AS1 (7.8 kg N $ha^{-1}$), with no difference between PL and Control (Fig. 3.2). The PL $NO_3^-$-N leaching potential in terms of $NO_3^-$-N pool size was the same as that of the Control indicating that the PL treatment did not significantly increase the risk of post-season $NO_3^-$-N leaching, unlike AS1 and AS2, most likely due to the use of cover crop after the corn harvest. The AS2 treatment had the greatest potential for $NO_3^-$-N leaching, suggesting excessive N application in this treatment.

### 3.3.4 Corn Yield and Cornstalk Nitrate Content

The main effects on yield by treatment ($p = 0.0014$) and by treatment x year interaction ($p = 0.0112$) were significant. Main effects on yield by year were not significant (Table 3.4). For the average of the three years, yield for all three N-added treatments (AS1, AS2, and PL) was significantly greater than the Control with no difference among the three. Results were similar for 2012 and 2014. Year 2 of the study (2013) showed significant differences among all treatments with the greatest yield in AS2, followed by AS1 and PL. Yield was lower for PL in 2013 than in the other 2 study years. Timing of N release from organic inputs can be difficult to predict and may not always coincide with plant demand. Nitrate N pools (0-15 cm) were uncharacteristically low mid-season in PL in 2013 as well (Fig. 3.3) and could be due to climatic factors that might have caused nitrate leaching as described above. Overall, the PL treatment supplied sufficient N for comparable yield to the AS treatments without leaving a significantly greater pool of $NO_3^-$-N in the soil post-season.

Sweet corn under similar N management yields 13.14 to 20.59 Mg $ha^{-1}$ for conventional treatments and 4.72 to 8.58 Mg $ha^{-1}$ for organic treatments, with yield up to 3 times higher in conventional treatments than organic for the same growing season (Egdell et al., 2015). In 2012 the yield for AS2 (14.45) was almost twice that of the organic PL treatment (7.67 Mg $ha^{-1}$). Yield reductions of 25% to 68% have been attributed to weed competition in organic systems (Cavigelli et al., 2008; Teasdale et al., 2012). However, several studies have shown organic treatments produce corn yields no different from conventional treatments (Walker et al., 2009; Johnson et al., 2010). In some systems, corn yield can be similar in organic and conventional treatments despite greater weed biomass in the organic treatments (Poffenbarger et al., 2015).

This could be due to shifts in weed community composition for organically managed systems (Davis et al., 2005) or N-resource partitioning (Poffenbarger et al., 2015).

The end-of-season cornstalk nitrate test (CSNT) concentrations showed main effects from treatment, year, and treatment x year interaction ($p < 0.0001$ for all). The CSNT concentration was greatest in AS2 in 2012 and 2014 (1.7 and 1.6 g $kg^{-1}$) (Fig. 3.4). In 2013, no difference was observed among treatments, and AS1, AS2, and PL were all lower than in the other two study years. In 2014, the CSNT concentration was greatest in AS2 followed by AS1 and PL, with no difference between the two. The CSNT identifies four categories for N sufficiency or excess in corn plants interpreted as follows: low (0-0.25 g $kg^{-1}$), marginal (0.25-0.70 g $kg^{-1}$), optimal (0.70-2.0 g $kg^{-1}$), and excessive stalk N (2.0 g $kg^{-1}$) (Blackmer and Mallarino, 1996). For the low and marginal categories, yield is likely decreased due to N deficiency in the plant. For the excessive category, N inputs could be decreased with no corresponding decrease in yield. For the optimal category, N uptake by the plant was adequate to meet the nutrient requirements of the plant during the growing season. The CSNT concentrations were well within the optimal range (0.70-2.0 g $kg^{-1}$) for AS2 and PL for all study years and for AS1 in 2012 and 2014. Control CSNT concentrations were not significantly different from the lower optimum limit (0.70) in 2012 and 2013 and were low (0.19 g $kg^{-1}$) in 2014.

In 2012, N uptake by the corn plants appears to have been less efficient, with N remaining in the leaves instead of being used to produce ears. A similar volume of ears was produced by all three N-added treatments, but if N present in the cornstalks at harvest had been used in ear production, the differences may have been the inverse of the relationship observed, with no difference among treatments for cornstalk N and significant differences for yield

volume. This inverse relationship between CSNT and yield volume was observed the following year. In 2013, conditions throughout the season included greater volume of precipitation and greater soil water content (Fig. 3.1b).

Poultry litter can be an alternative fertilizer source for crops without the negative environmental effects caused by N and P losses and accumulation of metals in the soil if used efficiently. Previous studies have shown positive yield responses from other animal waste such as liquid swine manure (Woli et al., 2013) and dairy compost and liquid dairy waste (Habteselassie et al., 2006b). One field study showed positive correlation with onion yield, comparable with response from commercial fertilizers, over a range of approximately 8.9 to 13.5 Mg poultry litter $ha^{-1}$ (Boyhan and Hill, 2008). Several field studies have shown increased soil productivity and soil organic matter from poultry litter surface-applied or subsurface applied in no-till soils (Pote *et al*., 2011). Some studies have found that poultry litter can be used efficiently in conservation tillage (Endale et al., 2010), no-till cotton-cover crop-corn rotations (Reddy et al., 2009) and no-till and mulch-till cotton-winter rye rotations (Nyakatawa et al., 2001).

### 3.3.5 Agricultural Cropping Efficiency Coefficient (ACE)

The ACE coefficient for inorganic N ($NO_3^-$-N + $NH_4^+$-N) 15-30 cm was greatest (indicating greatest efficiency) for PL in 2012, AS2 in 2013, and AS1 in 2014. The least efficient N-management systems were AS2 for two study years (19 in 2012 and 60 in 2014) and PL (38) in 2013 (Table 3.5). In a study by Egdell et al. (2015) ACE coefficients for yield:$NO_3^-$-N (from surface runoff) ranged from 9 to 19 for conventionally managed treatments and 20 to 99 for organic treatments (with N inputs from cover crops and pelletized poultry litter). For both study years no-till, conventional treatments had the highest ACE coefficients (100) (Egdell et al.,

2015). These results differ from previous work by Egdell et al. (2009), which found that organically managed plots were consistently more efficient than conventional plots under conventional-tillage. However, in the same study, conventional plots were consistently more efficient than organic under no-tillage (Egdell et al., 2009). The difference between the previous study and our study could be due to a number of factors, including differences in type of amendments (fresh vs pelletized PL), soil and climactic conditions. Overall, the AS1 and PL treatments were most efficient over the 3 study years in regards to N and the AS2 treatment was 70% as efficient (Table 3.5).

Inorganic N pools at the 15-30 cm depth were used for calculating ACE because $NO_3^-$-N is subject to leaching. Total inorganic N, $NO_3^-$-N + $NH_4^+$-N, was considered because $NH_4^+$-N is the substrate for nitrification and is readily converted to $NO_3^-$-N. The ACE coefficient, expressed as total sweet corn yield per unit of potential pollutant, indicates a more efficient system with a greater value (when yield volume is higher and/or potential pollutant concentration is lower) and a less efficient system with lower values (when yield volume is lower and/or potential pollutant concentration is higher).

### 3.4. Summary and Conclusions

Overall, the PL treatment resulted in comparable levels of yield and plant N parameters as the AS treatments. The PL treatment resulted in Zn accumulation over time but this did not affect yield as compared to the AS1 treatment that supplied comparable level of available N. The potential for Zn and other metal accumulation was reduced through the use of cover crop during winter, resulting in N credit and hence reduction in the amount of PL applied in the subsequent years. Cornstalk nitrate levels indicated that the PL and AS treatments provided a sufficient

amount of N to the corn, but PL was consistently more reliable than the AS1 treatment over the three-year time. Overall, the PL treatment had comparable soil $NO_3^-$–N concentration in the 15-30 cm depth as that of the Control, suggesting that the PL treatment did not significantly increase the risk of post-season $NO_3^-$-N loss (via leaching), unlike AS1 and AS2. As opposed to the AS treatments, the nitrogen from PL is mineralized slowly over time and could be susceptible to leaching after corn harvest. It is most likely that the use of cover crop along with PL prevented this from happening. The normalized ACE coefficient provided further support that AS1 and PL were the most efficient management systems in terms of inorganic N post-season, and AS2 was the most likely to have conditions favorable to nitrate leaching losses over the three-year study

Studies comparing conventional N management to organic management with poultry litter incorporated in the soil are lacking. Our results show that an organically managed cropping system (with a combination of poultry litter and cover crops as N amendments) was competitive with conventionally managed systems in terms of yield and other plant parameters, while mitigating N losses in comparison to the conventional systems. Further study is needed to develop best management practices for incorporation of poultry litter as a fertilizer and as a means of waste disposal.

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Table 3.1. Selected soil properties for 2012 (Initial), 2013, and 2014 for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments.

| | | **P** | **K** | **Ca** | **Mg** | **Zn** | **Mn** | **pH** | **SOM** |
|---|---|---|---|---|---|---|---|---|---|
| | | ——Mehlich I Extractant—— | | | | | | | % |
| | | ——*mg kg$^{-1}$*—— | | | | | | | |
| ***2012*** | Initial | 5 | 58 | 310 | 41 | 1.5 | 13 | 5.3 | 2.7 |
| ***2013*** | Control | 16ab† | 90a | 750ab | 73ab | 1.7b | 22b | 6.3a | 2.7a |
| ***Pre-season*** | AS1 | 16ab | 81a | 626ab | 57b | 1.5b | 24ab | 5.9a | 2.7a |
| | AS2 | 11b | 71a | 480b | 50b | 1.5b | 31a | 5.5b | 2.8a |
| | PL | 26a | 95a | 823a | 87a | 2.5a | 28ab | 6.3a | 2.8a |
| ***2014*** | Control | 19a | 102a | 784a | 80ab | 1.6b | 17b | 6.4a | 2.5a |
| ***Pre-season*** | AS1 | 11b | 80b | 700a | 63b | 1.5b | 19ab | 6.2a | 2.6a |
| | AS2 | 15ab | 88ab | 882a | 73ab | 1.5b | 22ab | 6.3a | 2.7a |
| | PL | 18a | 73b | 912a | 86a | 2.2a | 23a | 6.5a | 2.5a |
| ***2014*** | Control | 20b | 97a | 616a | 76b | 0.9b | 15b | 6.3a | 2.7a |
| ***Post-season*** | AS1 | 22ab | 71bc | 780a | 78ab | 0.6b | 16b | 6.5a | 2.4a |
| | AS2 | 27ab | 57c | 904a | 100a | 0.7b | 22a | 6.1a | 2.6a |
| | PL | 41a | 83ab | 852a | 86ab | 1.8a | 19ab | 6.6a | 2.7a |

† Different letters indicate a significant difference within sampling time according to Fisher's $LSD_{0.05}$; n = 4 for each treatment for each sampling time.

Table 3.2. Selected properties of poultry litter composite samples (analyzed by ICP-MS) used as an amendment in 2012, 2013, and 2014.

| Year | Total N | P | K | Ca | Mg | S | Mn | Zn |
|---|---|---|---|---|---|---|---|---|
| | | | *g kg$^{-1}$* | | | | | |
| 2012 | 27.6 | 18.3 | 29.7 | 27.7 | 7.2 | 7.5 | 0.5 | 0.4 |
| 2013 | 32.2 | 10.3 | 19.8 | 21.4 | 6.2 | 10.6 | 0.5 | 0.4 |
| 2014 | 31.7 | 8.1 | 13.6 | 17.2 | 4.2 | 5.6 | 0.4 | 0.3 |

Table 3.3. Amendments for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter, blood meal, and cover crops).

| Treatment | N Source | Applied N and Total Amendment Applied | | | Applied P (from 0-46-0 except for PL) | | | Applied Lime | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 | 2012 | 2013 | 2014 |
| | | *kg $ha^{-1}$* | | | | | | | | |
| **Control** | -- | -- | -- | -- | 58 | 41 | 40 | 3,923 | -- | -- |
| **AS1** | Ammonium sulfate N | 112 | 112 | 112 | 58 | 43 | 49 | 3,923 | 1,821 | 1,261 |
| **AS2** | Ammonium sulfate N | 224 | 224 | 224 | 58 | 49 | 44 | 3,923 | 4,904 | 701 |
| **PL** | Poultry litter N and P | 112 | 47 | 32 | 58 | 50 | 39 | 3,923 | 1,821 | -- |
| | Cover crop N | -- | 46 | 47 | -- | -- | -- | -- | -- | -- |
| | Blood meal N | -- | 19 | 33 | -- | -- | -- | -- | -- | -- |
| **PL (Total Amendment Applied)** | Poultry litter | 7,025 | 2,945 | 2,340 | -- | -- | -- | -- | -- | -- |
| | Cover crop | -- | 2,240 | 1,680 | -- | -- | -- | -- | -- | -- |
| | Blood meal | -- | 145 | 250 | -- | -- | -- | -- | -- | -- |

Table 3.4. Corn yield for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treated soil.

| | **2012** | **2013** | **2014** | **Mean** |
|---|---|---|---|---|
| | | *Mg Corn $ha^{-1}$* | | |
| Control | 6.44b† | 4.28d | 3.78b | 4.57b |
| AS1 | 11.26a | 11.27b | 11.07a | 11.17a |
| AS2 | 9.39a | 14.45a | 9.51a | 10.72a |
| PL | 12.47a | 7.67c | 11.31a | 10.69a |

† Different letters indicate a significant difference according to Fisher's $LSD_{0.05}$; n = 4 for each treatment for each sampling time.

Table 3.5. Normalized agricultural cropping efficiency coefficients by inorganic N pools ($NO_3^-$ - N + $NH_4^+$ - N, 15-30 cm) post-season for AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments.

| Treatment | Inorganic N | | | |
|---|---|---|---|---|
| | 2012 | 2013 | 2014 | Mean Yield: Mean Inorganic N |
| AS1 | 48b† | 42b | 100a | 100a |
| AS2 | 19c | 100a | 60b | 70b |
| PL | 100a | 38b | 74b | 95a |

† Different letters indicate a significant difference according to Fisher's $LSD_{0.05}$; n = 4 for each treatment for each sampling time.

Figure 3.1. Daily precipitation (cm), daily average air temperature (˚C), and daily average soil water contents,0-15 cm, (g $g^{-1}$) for the 2012 (a), 2013 (b) and 2014 (c) growing seasons with reference line denoting drained upper limit (field capacity) of the soil.

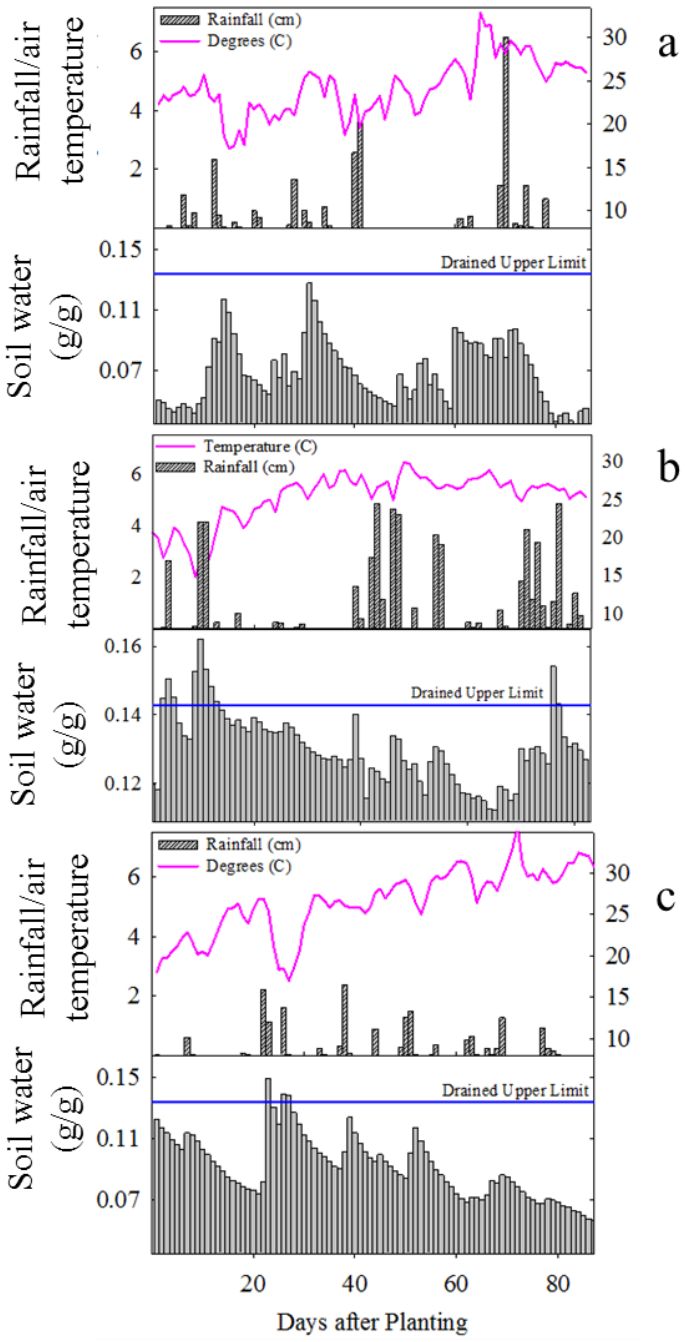


Figure 3.2. Three-year averages of inorganic N pre-season and post-season from plots with the following treatments: Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter, blood meal, and cover crops) treatments.

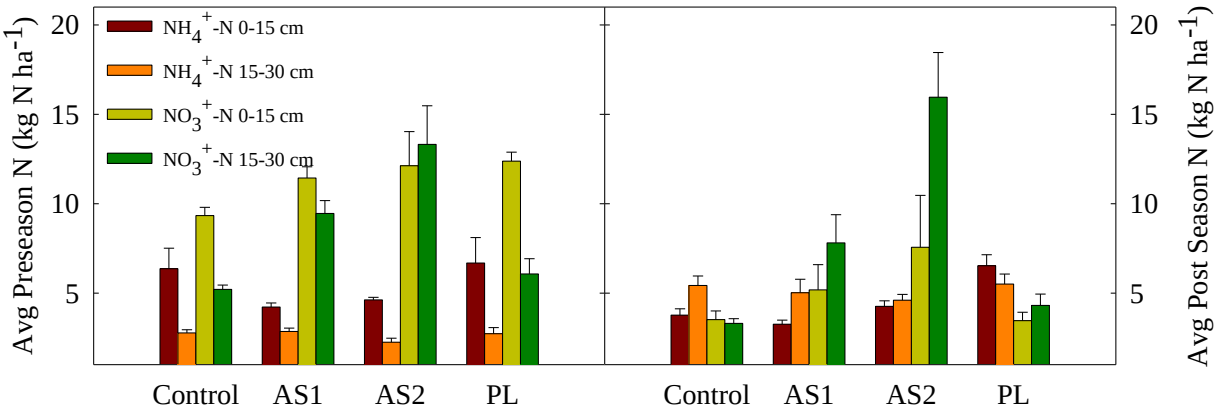


Figure 3.3. Mid-season inorganic N pools for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments in 2012, 2013, and 2014.

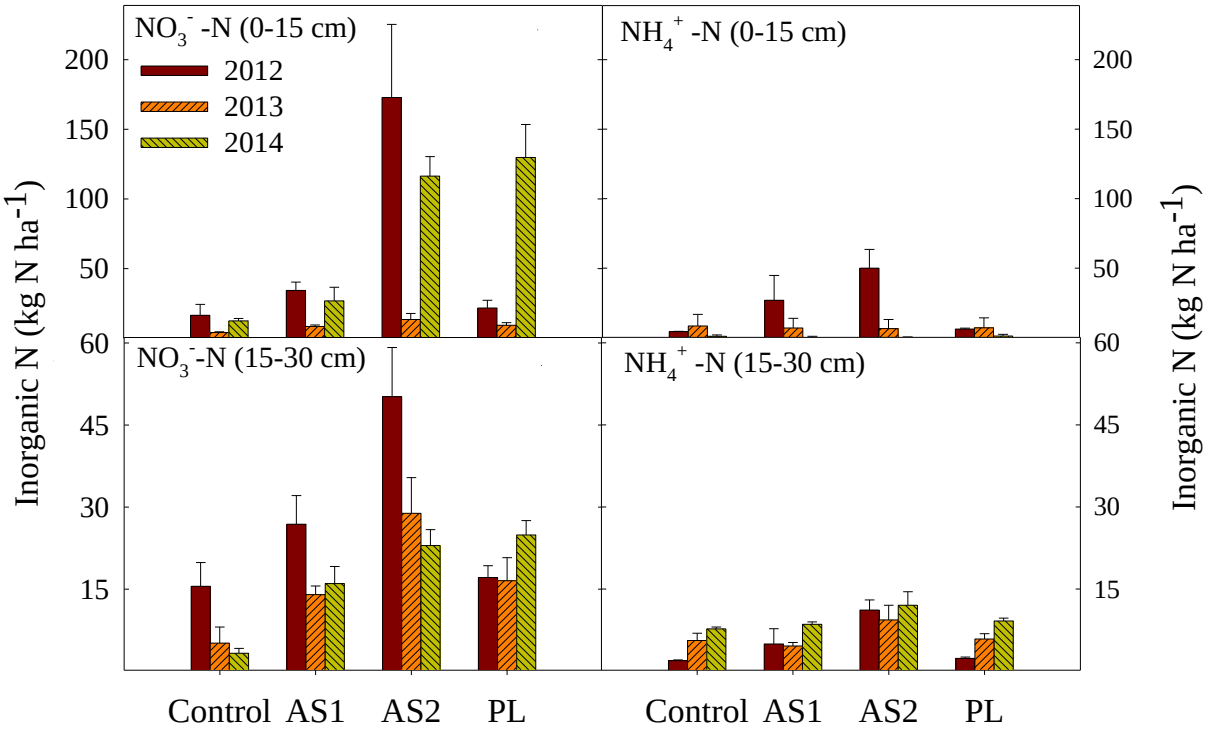
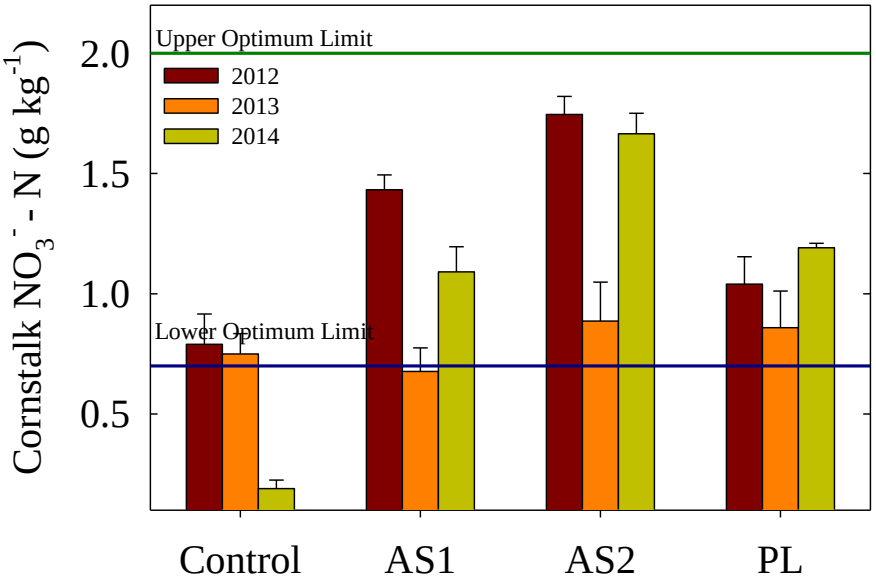


Figure 3.4. Cornstalk $NO_3^-$ - N (g $kg^{-1}$) in plants that received the Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), or PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments.



# CHAPTER 4

# A WEB-BASED MODEL OF N MINERALIZATION FROM CROP RESIDUE DECOMPOSITION[1]

[1]Woodruff, L.K., D.E. Kissel, M.L. Cabrera, J. Gaskin, L. Sonon, U. Saha, R. Hitchcock, M.F. Vigil, M.Y. Habteselassie, and J. Rema. To be submitted to *Soil Sci. Soc. Am. J.*

**Abstract**

Cover crops can provide substantial quantities of N for subsequent crops, but estimating the amount of N that will be mineralized from residues is challenging because of the complexity of the processes and the variety of factors involved, including residue quality, temperature, water content, and soil characteristics. Simulation models of N mineralization have been shown to provide reasonable estimates of N mineralized as crop residues decompose. To improve the reliability of simulations further, model inputs could be improved by linking models to more specific soil water and soil temperature data from weather stations, and by residue testing for better input for model simulations. Our objectives were to describe a web-based N mineralization model and its operation, calibrate the model with results from published laboratory studies, and validate it with field studies conducted with surface-applied or incorporated crimson clover (*Trifolium incarnatum* L.) or rye (*Secale cereale* L.) during three years. Modifications to rate constants and some parameters led to good fit between estimated and measured values in the calibration studies. In the validation studies, the model performed well for incorporated residues, but additional work is needed to improve its performance for surface-applied residues.

Abbreviations: N, Nitrogen; CC, crimson clover; R, rye; PL, poultry litter.

## 4.1. Introduction

Reports of routine soil tests for soil fertility evaluation typically include nitrogen (N) fertilizer recommendations for the crops to be grown. However, it is well known that the previous crop affects a soil's N fertility. Therefore, statements on the reports typically suggest N credits (reduction in the N fertilizer recommendation) if the crop to be grown follows a legume crop such as alfalfa, peanuts, soybeans, or a winter cover crop. These statements are very general and do not take into account the field-to-field variability and complex processes that affect N availability from decomposition of the previous crop's plant residues. Improved understanding of plant residue decomposition and N mineralization can improve quantitatively the recommendations of N credits for different kinds and amounts of crop residues and for different field environments.

Cover crops can provide all or part of the (N) required for subsequent crops (Tribouillois et al., 2015) in addition to decreasing erosion, suppressing weeds and reducing nitrate ($NO_3^-$-N) leaching (Nyakatawa et al., 2001; Justes et al., 2009). Therefore, the ability to accurately estimate plant-available N from cover crops and subtract this N credit from the fertilizer N to be applied could reduce environmental contamination (from runoff and leaching) from over-application of mineral fertilizers (Quemada et al., 1997). This would allow growers to maximize the critical services that cover crops provide to agricultural productivity (Palm et al., 2000). Estimating the amount of N that will be mineralized from cover crop residues is challenging, however, because of the complexity of the process and the variety of factors involved, including residue quality, temperature, water content, and soil characteristics (Vigil and Kissel, 1995; Quemada et al., 1997; Kruse et al., 2004; Cabrera et al., 2005). Such complexity suggests that computer simulation models may be useful tools for that purpose.

Because of the complexity of the processes involved, numerous efforts have been made to develop computer simulation models to understand quantitatively the residue decomposition processes and their effects on N mineralization. A review of these models is not intended here, but briefly, some of the models are those described by Seligman and Van Keulen (1981); Molina et al. (1983); Parnas (1975); Knapp et al. (1983); Godwin and Jones (1991). A description of the processes involved in residue decomposition and N mineralization, as well as research needed to improve these models have been provided by Cabrera et al. (2005). Some key factors to consider in estimating the rate of N mineralization from crop residues are how decomposition rates change quantitatively with changes in soil temperature and soil water content, and how decomposition rates change with changes in soil properties important in N cycling, such as soil texture, soil inorganic N content, soil organic carbon content, and finally a better understanding of the rate of N mineralization from soil humus itself. Also important in residue decomposition are the chemical properties of the residues, including the residue's concentrations of N (Frankenberger and Abdelmajid, 1985; Vigil and Kissel, 1991) as well as their concentrations of lignin (Muller et al., 1988), cellulose and hemicellulose, and nonstructural carbohydrates, each of which has a different rate of decomposition (Seligman and van Keulen, 1981).

To improve the reliability of simulations further due to uncertainties in the field, however, better data on the environmental conditions and specific information about the chemical composition of crop residues are needed. Model inputs could be improved by linking models to more specific soil water and soil temperature data from weather stations, and by residue testing for better input for model simulations. Our objectives were to describe a web-based N mineralization model and its operation, including its links to and the selection of weather related data and crop residue properties. In addition, we compared model simulations

with multiple sets of published N mineralization data to adjust the model coefficients to obtain the best fit to the data. Finally, we collected field data to validate the model for surface applied and incorporated crimson clover and rye cover crops.

## 4.2. Materials and Methods

### 4.2.1 Description of the Model

The model described here is a modified version of the N mineralization/immobilization subroutine from the CERES-N submodel described in more detail by Godwin and Jones (1991). The CERES-N submodel, which was adopted and modified from the model originally published by Seligman and Van Keulen (1981), simulates N mineralization/immobilization of crop residues or green manure (called fresh organic matter, FOM) as well as from soil humus (HUM) in a rational but simplified way. It is the decay of FOM that is the primary focus of this version of the model in that the model's calculations of crop residue decomposition and the corresponding N accounting result in an amount of inorganic N mineralized (or immobilized) and therefore a value of N credit (or debit) that may be used to modify N fertilizer recommendations.

The FOM is divided into three components which differ in their decomposition rates. These are nonstructural carbohydrates, cellulose and hemicellulose (hereafter referred to as cellulose), and lignin. The CERES-N model originally used the first-order decay constants for these three respective components of 0.80, 0.05, and 0.0095 $day^{-1}$ when decay was not limited by soil water, soil temperature, or available nitrogen needed for decomposition. In the field, the decomposition rate is most often slowed by one or more of these factors. The CERES-N model also assumed a set proportion of non-structural carbohydrates, cellulose, and lignin respectively to be 20%, 70%, and 10%, but these vary widely by the kind of FOM. Because the concentrations of these three components are now easily measured by NIR spectroscopy for

samples submitted by the farmer, they are provided as input to our model implementation, along with the N concentration of the residue.

Some model parameters and decay rates for the three crop residue components in the model have been changed based on several published studies. First, Vigil, et al (1991) found that the C/N ratio above which the decomposition rate is reduced gave a better fit to experimental data when it was reset to 13 from the original value of 25 used in CERES-N. The decay rates for carbohydrate, cellulose and lignin have been changed by various investigators so that the model predictions gave the best agreement to their experimental data but these were all somewhat different perhaps due to the plant materials or environmental conditions employed in the studies (Seligman and van Keulen, 1981; Vigil et al., 1991; Bowen, et al., 1993; and Quemada and Cabrera, 1995). The decay rate for lignin at optimum soil water content and 30$^{o}$C, as used in the original CERES-N, was 0.0095 day$^{-1}$. This was changed to 0.00095 day$^{-1}$ based on studies by Vigil, et al (1991), who found a better fit to their data with this slower decay rate.

The daily gross N mineralized from the three components is the product of the modified first-order decay rates and the size of each pool (first-order kinetics). The N contained in the residue is considered to be uniformly distributed within the three fresh organic matter components. Separately, any immobilization is calculated as described by Godwin and Jones (1991). Net N mineralized is calculated as Gross N mineralized minus N immobilized.

The decay rates are modified by a C/N ratio factor, as well as soil water content and soil temperature factors (all three that vary from 0 to 1). The soil water and temperature factors are calculated from data taken from weather stations located within the state of Georgia (http://www.griffin.uga.edu/aemn/) as described below. A description of how the rates are

adjusted for less than optimum conditions is described by Godwin and Jones (1991). Briefly, the daily decay rates are reduced by the product of the TF, MF, and the CNRF.

*Model input for soil water content and soil temperature*

The soil temperature data are taken at a soil depth of 10 cm at each of the weather stations of the Georgia Automated Environmental Monitoring Network. The temperature factor (TF) used varies linearly from zero at a soil temperature of 5$^{o}$C to one at a soil temperature of 35$^{o}$C, which, as cited by Godwin and Jones (1991), has described ammonification response to temperature in laboratory studies (Stanford, et al., 1973; Myers, 1975). This function is

$$TF = (ST-5)/30 \qquad \text{Eq. [1]}$$

where ST is soil temperature and TF is the temperature factor that varies from zero to one.

The soil water factor (MF) is also a unitless factor that varies from zero at air dry (AD) to a value of one at the soil's water content at the drained upper limit (DUL). This function is

$$MF = (SW-AD)/DUL-AD) \qquad \text{Eq. [2]}$$

for which SW is the soil water content. At values above the DUL, the value of MF varies linearly from 1 at the DUL to 0.5 at saturation (SAT). Two unitless functions set this daily value

$$XL = (SW-DUL)/SAT-DUL) \qquad \text{Eq. [3]}$$

Then,

$$MF = 1-0.5 *XL \qquad \text{Eq. [4]}$$

The values of MF calculated depend on the establishment of location specific soil water contents for AD, DUL, and SAT. These were determined as follows:

$$SAT = (1-(BD/2.65)) *1/BD \qquad \text{Eq. [5]}$$

The DUL for soil at each weather station was determined from mean daily soil water content ($cm^3$ $H_2O/cm^3$ soil) for the 0-10 cm depth by selecting from the most recent 5-year record the

median soil water content on day 3 following a day of heavy precipitation (>51 mm) in which no precipitation occurred on day 2 of the wet period. The lower limit for evapotranspiration (LL) was determined as the minimum soil water content determined for the 0-10 cm depth for the 5-year record. The soil water content for AD was calculated as one half of the LL as done in the CERES models.

*Determination of Model inputs for Crop Residue*

The cover crop biomass samples received from farmers (sent overnight in zip lock bags) are weighed and then dried in an oven at 65$^{o}$C for 24 h. After drying, dry weights of the samples are taken and their moisture contents (M1) are calculated. The dried samples are then ground in a Thomas-Wiley Laboratory Mill Model 4 (Arthur H Thomas Company, Philadelphia, PA) and passed through a 1 mm sieve. Ground-sieved samples are packed into circular cells and scanned to collect near infrared spectra for every 2 nm from 400 to 2498 nm on a FOSS NIRSystems model 6500 scanning monochrometer (FOSSNIRSystems, Silver Spring, MD) in reflectance mode. Each sample is scanned 13 times and the results are averaged to produce a single spectrum. The average spectral properties are used to predict moisture (M2), dry-matter, crude protein (CP), fat, non-fibrous carbohydrates (NFC), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, and ash contents using the NIRS calibration equation for mixed hay (12mh50-2.eqa) or grass hay (13gh50b2.eqa) developed by NIRS Forage and Feed Testing Consortium (http://nirsconsortium.org). The NIRS calibration equation "12mh50-2.eqa" is used when the cover crop is a legume or grass+legume mixture with >40% legume, whereas the calibration equation "13gh50b2.eqa" is used when the cover crop is a grass species or grass+legume mixture with <40% legume.

Based on the principles of detergent fiber analyses (Van Soest, 1963a, 1963b; Van Soest and Wine, 1967), the contents of hemicellulose and cellulose are estimated using NIR predicted ADF, NDF, Lignin, and Ash contents as follows:

$$\% \text{ Hemicellulose} = \%\text{NDF} - \%\text{ADF} \qquad \text{Eq. [6]}$$

$$\%\text{Cellulose} = \%\text{ADF} - (\%\text{Lignin} + \%\text{Ash}) \qquad \text{Eq. [7]}$$

The nitrogen concentration of residue is estimated as "CP/ 6.25". The sum of CP, NFC, and fat contents are taken as the "carbohydrate" content; the sum of hemicellulose and cellulose contents are the "cellulose" content; and the lignin content is predicted directly by the calibration equation. For final model inputs, the carbohydrate content, the lignin content, and the adjusted cellulose and hemicellulose contents are normalized to 100%. The dry cover crop biomass yield, needed as input to the model is estimated as follows:

Dry cover crop biomass yield (kg $ha^{-1}$) =

$$\text{Wet cover crop biomass yield (kg ha}^{-1}) \times \frac{100 - M1}{100} \times \frac{100 - M2}{100} \qquad \text{Eq. [8]}$$

The wet cover crop biomass yield is determined from the weight of biomass obtained from harvesting 30 cm × 45 cm or 60 x 75 cm rectangular areas from multiple locations within a field.

*Model User Interface and Inputs*

The model can be accessed on the website of the Agricultural and Environmental Services Laboratories under online calculators at http://www.aesl.ces.uga.edu/mineralization. The first input screen shows an outline of the state of Georgia showing locations of the weather stations. The user may select the weather station nearest the client's farm.

Average daily temperature, moisture, and other parameters from the weather stations are stored in a database on the Web server. When a weather station is selected, the program queries this database to determine DUL, AD, and the 5-year average of the weather station's temperature and moisture data to be used in the calculations for each day. After selecting the nearest weather station, the model input screen appears for soil's input data (upper left), weather data (upper right), and cover crop data (lower left). Another user option is to run the model at a set soil temperature for testing laboratory incubation data. If this option is selected, soil water content is considered optimum and MF=1.

Much of the soils input data, such as soil organic C, inorganic N, and hot 2 M KCl (for predicting the rate of N mineralization from soil humus) is pre-selected based on previous data for area of the state (North and South Georgia) and whether the farm is conventional or organic. The prediction of mineralization rates from soil humus is based on the relationship established by Egelkraut, et al. (2003) between the zero-order rate constant of mineralization and the amount of ammonium released by the hot 2 M KCl method (Gianello and Bremner, 1986). Other required inputs on this screen are the date of cover crop killing/incorporation, and the planting date of the upcoming crop. Both of these dates are provided by the client on the sample submission form with the cover crop sample.

The cover crop input screen consists of cover crop biomass provided on the submission form by the client, and the four chemical properties of the cover crop provided by the NIR Spectroscopy analysis of the cover crop sample submitted by the client. Those inputs are the cover crop % N, % non-structural carbohydrate, % cellulose, and % lignin.

*Model Output Screen*

For use by clients, only the output of N mineralized from the cover crop is provided for the first 90 days after killing/incorporation of the cover crop. The data are provided in both tabular and graphic form as a cumulative amount of N mineralized in kg N $ha^{-1}$. An explanation of how the data may be used to adjust N fertilizer rates is also provided. Advanced options allow the user to increase the length of time of the simulation or select a constant temperature for modeling mineralization.

### 4.2.2 Model Calibration

A set of simulations were carried out to calibrate the model with data from six laboratory studies and one field study (Table 4.1). All studies except for the study by Van Schreven provided values for lignin concentration. Two of the studies (both of Vigil's studies) provided laboratory values for concentrations of carbohydrates, and cellulose but the others did not. Because the composition of these two components affects the rate of mineralization, we estimated the values for % non-structural carbohydrates using a relationship obtained from analysis of data from the UGA Agricultural and Environmental Services Laboratories (AESL) database. These data were for % non-structural carbohydrates as a function of % nitrogen in 108 cover crop samples that were submitted to AESL from 2013 to 2015 (Fig. 4.1). All data values were obtained by use of Near Infrared Reflectance Spectroscopy (NIRS). These data were primarily from cowpea (*Vigna unguiculata* L.), sunn hemp (*Crotalaria juncea* L.), black oats (*Avena strigosa* L.), and rye (*Secale cereale* L.), with lesser numbers of samples of sudex (*Sorghum bicolor* × *S. bicolor* var. *sudanense* L.) and millet (*Pennisetum glaucum* L.). Use of this relationship allowed an estimation of % non-structural carbohydrates for five of the seven

studies for which % non-structural carbohydrates were not available. Then, values for % cellulose were obtained from

$$\% \text{ cellulose} = 100 - (\% \text{ non-structural carbohydrate} + \% \text{ lignin}) \quad \text{Eq. [9]}$$

Application rates for the laboratory studies varied and were based on the concentrations applied in each study (mg residue $kg^{-1}$ soil) and assuming that the residues were mixed in the top 10 cm of soil that had a bulk density of 1.5 g $cm^{-3}$. Actual loading rates in kg $ha^{-1}$ were used for the one field study. In all of the seven N incubation studies from the literature, the crop residues were incorporated into the soil (Table 4.1). The incubation temperatures varied from 25 to 35ºC, and the time of incubation varied from 77 to 168 days. Both temperature and time were adjusted accordingly for the simulations to match the conditions of the experiments. The range of residue components covered a wide range. Lignin proportions ranged from 3.8 to 30.6% and cellulose ranged from 21.2 to 53.5% (estimated based on the data from Fig. 4.1). The N concentrations of residues varied from 5 to 54.5 g $kg^{-1}$ across the seven studies.

### 4.2.3 Experimental Field Plots for Model Validation

Field plots were established in 2011, 2012, and 2013 at the Durham Horticulture Farm of the University of Georgia (lat. 33˚ 55′ N, long. 83˚ 25′ W) on a Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapludults). Rye and/or Crimson clover were drill planted in October of the previous year and aboveground plant parts were collected in April or May of each year, air dried, and cut in small sections (2-5 cm long) to be used in the N mineralization studies. PVC cylinders (7.5 cm ID x 20 cm long) were used to collect intact soil cores by driving them into the soil followed by retrieval. These cores received completely randomized assignments of the following treatments applied at a rates of 2,800 to 3,700 kg dry matter $ha^{-1}$: crimson clover incorporated (CC), rye incorporated (R), or control (no residue; C) in 2011; crimson clover

incorporated (CC), crimson clover surface-applied (CC (S)), or control (C) in 2012 and 2013. Analytical-grade Rexyn-300 (Fisher Scientific, Pittsburg, PA), a cation/anion exchange resin, was mixed with the bottom 2.5 cm of the soil in the core at a rate of 30 g in 150 g of soil, and was repacked into the core to maintain the continuity of the soil pore space and trap any $NO_3^-$ - N or $NH_4^+$ -N that otherwise could potentially be leached from the core (Hanselman et al., 2004; DiStefano and Gholz, 1986). Cores were repacked based on field-measured bulk densities each year, 1.16, 1.35, and 1.40 g $cm^{-3}$ for 2011, 2012, and 2013 respectively. Bulk density was measured according to procedures outlined by Blake (1965). Cores were then replaced in the field and four cores of each treatment were removed at 30-d increments up to 120 d to measure inorganic N in the form of KCl extracted $NH_4^+$-N and $NO_3^-$ N (Mulvaney, 1996). At each sampling time (30 d, 60 d, 90 d, and 120 d), the total amount of soil in the core was divided in half and each half was mixed with 3.5 of 1 *M* KCl, shaken for 1 h, and centrifuged at 8000 x *g* for 15 min. The supernatant was frozen and analyzed later for $NH_4^+$ and $NO_3^-$ using an Alpkem Autoanalyzer.

### 4.2.4 Cover Crop Characteristics

Cover crop aboveground biomass was collected and total biomass was determined after drying at 65˚C for 48 h. The % C and N, non-structural carbohydrates (CARB) and lignin (LIGN) were measured using near-infrared reflectance (NIR) spectrometry (McLellan et al., 1991) as described above.

### 4.2.5 Soil Chemical Analysis

Initial soil samples were analyzed each year of the study for inorganic N ($NH_4^+$-N and $NO3^-$-N) by KCl extraction. Potentially mineralizable N (PMN) was determined using the hot

KCl method (Picone et al., 2002). Briefly, Pyrex tubes were filled with 3 g of field moist soil and 20 mL of 2 M KCl. Tubes were submerged in a water bath for 4 h at 100˚C. Supernatant was frozen and analyzed later for $NH_4^+$ using an Alpkem Autoanalyzer. PMN was calculated as $NH_4^+$-N measured by KCl extraction subtracted from $NH_4^+$-N measured by hot KCl.

### 4.2.6 Simulation Methods

Decomposition rates from previous calibration studies were used for model simulations for the current study. Soil temperature and soil volumetric water content ($\theta_v$) in the upper 15 cm were measured and recorded every 15 min with Decagon EM50 dataloggers and sensors (Decagon Devices, Inc., City, State). Volumetric water content was converted to gravimetric water content ($\theta_g$) using bulk density of the soil. Daily averages of temperature (˚C) and $\theta_v$ were used to drive the model. Separate model simulations were conducted using the 5-year average of soil water content and temperature because this is the data growers would most readily be able to access. Net nitrogen mineralized from the cover crop residue was estimated by subtracting inorganic N in the control cores. The measured values of net N mineralized were compared with estimated values by the CERES-N model.

### 4.2.7 Statistical Analysis

Analyses used to evaluate model performance included the mean difference between measured and simulated data (*M*) as well as its standard deviation, root mean squared error (RMSE), lack of fit (LOFIT), and confidence intervals (95%) (Addiscott and Whitmore, 1987; Whitmore, 1991). These and other statistical methodologies used are described in detail by Quemada and Cabrera (1995) and Shomberg and Cabrera (2001). Sum of squares of the LOFIT was calculated by subtracting the error (SSE) from the residual sum of squares (RSS):

$$RSS = \sum_{i=1}^{N} \sum_{j=1}^{n} \left(measured_{ij} - simulated_i\right)^2 \quad [10]$$

$$\text{SSE} = \sum_{i=1}^{N} \sum_{j=1}^{n} \left( measured_{ij} - \langle measured_i \rangle \right) \quad [11]$$

$$\text{LOFIT} = \text{RSS} - \text{SSE} \quad [12]$$

Where $N$ is the number of sampling dates, $n_i$ is the number of replications each sampling time, $measured_{ij}$ is the measurement for each $j$th replicate at the $i$th sample time, and $simulated_i$ is the simulated value for the $i$th sample time, and $<measured_i>$ is the mean of the measurements at the $i$th sampling time. $F_{\text{LOFIT}}$ is determined by dividing mean square lack of fit (MSLOFIT) by mean square error (MSE):

$$F_{\text{LOFIT}} = \frac{\text{MSLOFIT}}{\text{MSE}} = \frac{\text{LOFIT}}{\text{df}_{\text{LOFIT}}} \div \frac{\text{SSE}}{\text{df}_{\text{SSE}}} \quad [13]$$

The RMSE evaluates the difference between simulated and measured data

$$\text{RMSE} = [\Sigma (\text{simulated} - \text{measured})^2 / \text{N}]^{0.5} \quad [14]$$

Generally, low M and RMSE, and a non-significant MSLOFIT indicate a statistically satisfactory model simulation. Data were analyzed using the Statistical Analysis System (SAS, 2014).

### 4.3. Results and Discussion

#### 4.3.1 Crop Residue Structural and Non-Structural Components and Rates of Decomposition

The % non-structural carbohydrates in crop residue can significantly affect the amount of N mineralized that is available to the following crop. To illustrate, we did two simulations in which we compared the amount of N mineralized from a cover crop of 5600 kg ha$^{-1}$ with 2 % N, incorporated April 1. We used weather and soil data from a weather station approximately 15 km from Athens, GA. The only factors that differed were the relative amounts of non-structural carbohydrates and cellulose. In one simulation, we used 25% carbohydrate and 70% cellulose,

and in the other 50% carbohydrate and 45% cellulose (we used 5% lignin for both). The model predicted 20 kg N $ha^{-1}$ mineralized by 90 days after soil incorporation with 25% non-structural carbohydrates, and 38 kg N $ha^{-1}$ mineralized with 50% non-structural carbohydrate. It is therefore important to have reliable data for concentrations in residue of lignin, cellulose and non-structural carbohydrates for input to the model. For this reason, we applied the relationship from Fig. 4.1 to estimate unknown information of % non-structural carbohydrate and cellulose for some of the seven calibration studies listed in Table 4.1 that lacked this information. In a routine testing environment, this information can be most easily obtained for cover crop samples using NIRS analysis.

### 4.3.2 Model Prediction Compared to Measured N for Calibration Studies

*Incorporated Crop Residue*

The model was calibrated by adjusting the rates of decomposition for nonstructural carbohydrates, cellulose, and lignin. The rates that gave the best fit for incorporated residues were 0.119 $d^{-1}$ for carbohydrates and 0.000808 $d^{-1}$ for lignin. A further change to the original version of the model was to make the cellulose decay rate an exponential function of the lignin content of the residue, similar in principle to the function used by Parton et al (1987). In the model described here, the equation for cellulose decomposition in incorporated residues is:

$$\text{Cellulose rate } (d^{-1}) = 0.00217 \exp(-12 * \text{lignin}) \qquad \text{Eq. [15]}$$

where lignin is expressed as a proportion (g $g^{-1}$) instead of percentage. This equation reduces the cellulose decomposition rate from 0.001922 $day^{-1}$ for a residue with 0.01 g $g^{-1}$ lignin, to 0.00065 $day^{-1}$for a residue with 0.1g $g^{-1}$ lignin, to 0.00020 $day^{-1}$ for a residue with 0.20 g $g^{-1}$ lignin. This change allowed the model to predict more accurately the N mineralization from incorporated

residues high in lignin, such as soybean stems. An additional modification made during calibration was to change the percentage of N mineralized that is recycled back into soil OM from 20% to 25% when the crop residue is incorporated.

The overall performance of the calibrated model can be shown with the following comparison of simulated and measured N mineralized from the seven studies from the literature (Fig. 4.2). The statistical relationship between modeled and measured N mineralized for all 85 treatments indicated a slope of 1.00 and an $R^2 = 0.93$. Considering the range of values mineralized and an intercept of -5.3 kg N $ha^{-1}$, it appears that the model simulates values of N mineralized reasonably well when N is immobilized and also for cases in which greater amounts of N are mineralized.

*Surface-Applied Crop Residue*

In contrast to the previous simulations (Fig. 4.2), the crop residues in the study of Quemada and Cabrera (1995) were applied on the soil surface and not incorporated. In this study, the N concentration of crop residues ranged from 0.5 to 4.4%, and the lignin concentration varied from 2.6 to 14.2% (Table 4.2). There was also a more than twofold range in cellulose and non-structural carbohydrate proportions. Soil moisture was kept near optimum throughout their study. The rates of decomposition for surface-applied residues were reduced to 82% of the rates for incorporated residues. In the initial simulations, the model under-predicted the N mineralized in 162 days by about 10 to 15%. To improve the agreement with measured values, we changed the recycle of mineralized N to organic matter to 12.5%, half of the value used for incorporated residue. It can be argued that surface applied residue interacts with less soil than incorporated residue because it has no soil above it. The comparison between measured and modeled N mineralized in kg N $ha^{-1}$ for the 12 treatments in Quemada and Cabrera (1995) study is shown in

Fig. 4.3. The statistical relationship between modeled and measured N mineralized for all treatments indicated a slope of 1.00 and an $R^2 = 0.97$.

The output of cumulative N mineralized can be selected to be daily or for longer time intervals if desired. For purposes of testing the dynamics of N mineralization, the cumulative N mineralized for three different plant materials from the study of Quemada and Cabrera (1995) were compared with values simulated by the model (Fig. 4.4). Throughout the 160-day incubation, the model predicted reasonably well the measured cumulative values of N mineralized. There was in general a small over prediction of cumulative N mineralized from 30 to 60 days but from 95 to 160 days after incorporation, the predicted values were within the small standard error of the laboratory measurements. By 160 days, predicted values were within 5% of measured values for these three treatments.

### 4.3.3 Crop Residue Structural and Non-Structural Components for Validation Field Studies

In our validation field studies, cover crop N content ranged from 23.5 to 42.5 g $kg^{-1}$ over the three years of the study with the lowest value for rye in 2011 and the highest value for crimson clover in 2012 (Table 4.3). Nitrogen (N) mineralization has been observed to proceed consistently over N concentrations ranging from 15 to 20 g $kg^{-1}$ (Harmsen and Van Schreven, 1955) and immobilization observed to proceed at N concentrations lower than 10 g $kg^{-1}$ (Vigil and Kissel, 1991). Concentrations in each study year exceeded these concentrations. Lignin concentration can also be a good predictor of net N mineralized (Muller et al., 1988). Lignin concentration was highest in crimson clover in 2013, 8.0%, and lowest in crimson clover in 2011, 4.5%. Rye had the smallest total amount of carbohydrate with a pool size of 32.3% (Table 4.3).

### 4.3.4 Soil Properties for Validation Field Study

Soil properties considered by the model included potentially mineralizable nitrogen (PMN), inorganic N pools, and organic C. PMN by hot KCl ranged from 2.4 to 11.5 mg N $kg^{-1}$ over the three years of the study in 2013 and 2012, respectively (Table 4.4). Soil N in 2012 had the greatest mineralization potential which may have contributed to cover crop-N mineralization as well. Inorganic N pools were similar for 2011 and 2012 and slightly greater in 2013. Organic C ranged from 12.7 to 20.0 g $kg^{-1}$ in 2011 and 2013, respectively. The study year (2013) with the greatest organic C also had the greatest inorganic N pools so soil C/N ratios were similar for all three years.

### 4.3.5 Model Prediction Compared to Measured N for Validation Field Study

*2011 Field Study*

In 2011 daily average temperatures during the study ranged from 18 to 33˚C. Daily average gravimetric water content ranged from 0.003 to 0.045 g $cm^{-3}$, a relatively dry season compared to the other two study years (Fig. 4.5,4. 6, and 4.7). Net N mineralized for crimson clover incorporated was relatively low for the first three sampling times, day 30, 60, and 90, finally peaking at 42 kg N $ha^{-1}$ (± 8) on day 120 (Fig. 4.5). Ranges of net N mineralized after 35 days of 25-30 kg N $ha^{-1}$ have been reported (Schomberg and Cabrera, 2001) but that was under conditions of favorable early season soil water availability. This was a relatively dry season with low gravimetric soil water content throughout the season (Fig. 4.5) so N mineralization rates were slower.

Characteristic moisture curves for leaves and stems of clover residues were measured by Quemada and Cabrera (2002) with water contents below 0.05 g $g^{-1}$ corresponding to water potentials (Ψ) of -2 to -6 MPa. Apparent net N mineralized from crimson clover residue was

shown to increase with Ψ of the residue up to a maximum point between -0.5 and -0.003 MPa (Quemada and Cabrera, 1997), corresponding to gravimetric water contents of 3.5-4.3 g $g^{-1}$. With soil water content below 0.5 g $g^{-1}$ for most of the season (Fig. 4.5) residue water content and Ψ could not have spent much time in the ranges where maximum N mineralization was observed by Quemada and Cabrera (2002). This likely accounts for the low rates of N mineralization, especially early in the season, compared to previous studies.

For the rye-amended soils, net immobilization was observed for the first three sampling times with final net N mineralized at day 120 of 43 kg N $ha^{-1}$ (± 23) (Fig. 4.5). Lower N content and a relatively small pool size of carbohydrate in rye in 2011 corresponded with the lower predicted net N mineralized, 9.4 kg N $ha^{-1}$. Mineralization of N from rye can occur at about half the rate of N mineralization from crimson clover, 0.32 and 0.65 kg N $ha^{-1}$ $d^{-1}$, respectively, averaged over a two-year study (Schomberg and Cabrera, 2001). Characteristic moisture curves for leaves and stems of rye residue were also reported by Quemada and Cabrera (2002). Even at the higher end of the range of water content recorded (1.9-2.8 g $g^{-1}$), rye water potential (approximately -1 MPa) was relatively lower than crimson clover at the same water content. This is likely due to physical and chemical differences in the residues including their proportions of carbohydrates, cellulose, lignin, and N. Because of this, residue decomposition and N mineralization rates were not as efficient from rye residues as from crimson clover residues. With net cumulative N immobilization from rye at days 60 and 90 (Fig. 4.5), rye alone would not have provided adequate N for most cropping systems, despite the similar (to crimson clover) cumulative net N mineralized by d 120.

The RMSE was lower for crimson clover (25.5 kg $ha^{-2}$) than rye (38.3 kg $ha^{-2}$), and the F value of MSLOFIT was not significant for either at $\alpha = 0.05$ indicating a better fit of predicted

values to observed for crimson clover (Table 4.5). Predicted values fell within the 95% confidence intervals for measured values with both measured climate data and 5-year averages of climate data for both crimson clover and rye, though predictions were slightly higher using the 5-year average (Fig. 4.5). Greater net N mineralization and better fit with model predictions was observed for crimson clover than for rye which agrees with the results of Schomberg and Cabrera (2001). Because of this and the lower net N mineralization from rye early in the season, we chose to focus on crimson clover in subsequent study years 2012 and 2013. Field studies with both surface-applied and incorporated crimson clover treatments are lacking so we included both treatments in subsequent study years.

*2012 Field Study*

In 2012, average daily temperatures ranged from 21 to 35˚C, slightly warmer than the previous year, and average daily gravimetric water content ranged from 0.034 to 0.134 g $cm^{-3}$, never dropping below the lower limit (LL) of plant uptake, 0.034 g $cm^{-3}$(Fig. 4.6), derived from a soil moisture release curve (data not shown). The PVC cores were installed in the fallow space between sweet corn plots which were irrigated with central pivot irrigation on an as-needed basis. This was by far the most time spent in a study season under optimal conditions of temperature and moisture for N mineralization to occur. Crimson clover 2012 had predicted net N at 120 d of 81.2 and 91.3 kg N $ha^{-1}$ for incorporated and surface-applied, respectively, and the highest measured net N of 83.0 kg N $ha^{-1}$ (± 33.0 SE) from crimson clover incorporated at 120 d after application. The greatest net N mineralization observed over the three years of the study was from crimson clover in 2012 when lignin was lowest 6.56 %. The carbohydrate pool represented the largest proportion of the three pools for crimson clover in 2012 with 67.5 % g. This is the least recalcitrant pool decomposing at the highest rate of 0.17 $d^{-1}$. Consistently higher

soil water content was also a factor in 2012 (Fig. 4.6). Net N mineralization from crimson clover incorporated was much greater than surface-applied for all four sampling times. Values predicted with measured climate data overlapped with 95% confidence intervals for measured values for all four sampling times for both incorporated and surface-applied clover (Fig. 4.6). Model predictions using the 5-year average for climate data were slightly higher, still falling within confidence intervals for crimson clover incorporated but with over predictions for day 30 and 60 for crimson clover surface-applied.

*2013 Field Study*

In 2013, average daily temperatures ranged from 19 to 31˚C, slightly cooler than the previous year, and average daily gravimetric water content varied from 0.115 to 0.134 g $cm^{-3}$, exceeding the LL of 0.029 g $cm^{-3}$ for the entire season (Fig. 4.7). The mean difference between measured and simulated values for crimson clover incorporated was lower than that of crimson clover surface-applied, -3.1 and -26.9 kg N $ha^{-1}$, respectively (Table 4.5). However, confidence interval assessments indicated that the model performed better for incorporated treatments than surface-applied with 4/4 sample means within the 95% CI for incorporated and 3/4 for surface-applied using both measured climate data and the 5-year average. Thus the model predictions for crimson clover incorporated were more satisfactory. The model appears to generate reasonable data in most cases but more field data are needed to calibrate the model for different soil types and validate the model to the system from which the observed data are derived, especially for surface-applied cover crops.

**4.4. Summary and Conclusions**

The N mineralization subroutine modified from CERES-N has been tested and modified previously (Vigil et al, 1995; Quemada and Cabrera, 1995), but these authors used smaller sets of

N mineralization data from their laboratories. In the present study, a more robust set of N mineralization data from eight different studies were used to test the model and to modify the rate constants for decomposition of the three plant components, non-structural carbohydrates, cellulose, and lignin to give the best possible estimation of the available data of N mineralized. The data from the seven studies in which crop residues were incorporated had a wide range of properties, most significantly the % N which ranged from 0.5 to 5.45%, and the % lignin, which ranged from 3.8 to 30.6%. The samples with high lignin and cellulose led to using an equation for the cellulose component that slowed its decomposition considerably at higher levels of lignin, which allowed better agreement for those samples.

In the validation field study, simulated values of *in situ* net N mineralization from cover crops fell within the 95% CI of measured values for all but one sample time. N mineralization from incorporated sources was generally more accurate over a wider range of statistical parameters than from surface-applied. Overall, prediction was most accurate for crimson clover incorporated in 2013 and least accurate for crimson clover surface-applied in 2013. According to our results CERES-N can be a valuable tool for managing N inputs in a Georgia Ultisol. Further study is needed to assess the prediction capacity of the model for different soil types, under ranges of climactic conditions, and with different cover crops. Future work should also address the potential incorporation of other driving factors for the model for surface-applied crimson clover including water potential and/or relative humidity

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Table 4.1. Seven studies from the literature showing the residue type, their ranges in chemical composition, and the temperature and time of incubation.

| Treatments | Study[†] | Residue Type | Nitrogen | Carbohydrates | Cellulose | Lignin | Temperature | Time |
|---|---|---|---|---|---|---|---|---|
| | | | -g kg$^{-1}$- | ----------% of Total C---------- | | | ˚C | d |
| 1-10 | M.F. Vigil (field) | sorghum plants parts | 9.2-21.0 | 39.1-47.1 | 47.4-53.8 | 5.1-7.4 | Station data | 110 |
| 11-38 | M.F. Vigil (lab) | sorghum and soybean plant parts | 10.8-43.5 | 34.4-72.8 | 22.4-47.8 | 4.3-18.9 | 35 | 98 |
| 39-50 | W.T. Frankenberger | legume foliage, stems, roots | 13.4-54.5 | 33.5-71.1[‡] | 21.2-53.5[§] | 3.8-30.6 | 28 | 140 |
| 51-57 | J.L. Jensen | legume and grass plant parts | 5.4-44.5 | 23.8-61.5[‡] | 33.5-71.2[§] | 5.0-8.0 | 25 | 168 |
| 58-69 | H.C. Millar | legume and grasses | 5.0-31.4 | 23.4-48.9[‡] | 34.2-61.1[§] | 11.8-17.6 | 30 | 77 |
| 70-81 | M.H. Fu | corn, soybeans, sorghum and alfalfa plant parts | 7.0-28.3 | 25.3-45.9[‡] | 46.1-68.5[§] | 6.2-15.0 | 30 | 140 |
| 82-85 | D.A. Van Schreven | wheat, weeds, alfalfa | 8.6-26.7 | 26.9-44.3[‡] | 50.7-68.1[§] | 50[¶] | 25 | 112 |

[†]Each study is referenced by the first author.
[‡] % Carbohydrates estimated from database values, see Fig. 4.1.
[§]Estimated from %cellulose = 100 - (%carbohydrate + %lignin)
[¶] Lignin not measured, estimated based on typical values for these plant materials

Table 4.2. The 12 crop residue types, their chemical composition, and the measured net N mineralized after surface application of these residues to soil at 35 C for 162 days from Quemada and Cabrera (1995).

| Cover Crop Residue | Nitrogen | Soluble Carbohydrates | Cellulose Hemicellulose | Lignin |
|---|---|---|---|---|
| | -g $kg^{-1}$- | ----------% of Total C ---------- | | |
| Clover (50% L, 50% S) | 29 | 49.7 | 40.4 | 9.9 |
| Clover Stems | 14 | 33.2 | 52.6 | 14.2 |
| Clover Leaves | 44 | 66.2 | 28.2 | 5.6 |
| Rye (50% L, 50% S) | 10 | 32.6 | 63.0 | 4.4 |
| Rye Stems | 5 | 26.7 | 67.0 | 6.3 |
| Rye Leaves | 16 | 38.6 | 58.8 | 2.6 |
| Wheat (50% L, 50% S) | 18 | 44.4 | 49.3 | 6.2 |
| Wheat Stems | 5 | 49.0 | 42.7 | 8.3 |
| Wheat Leaves | 31 | 39.8 | 56.0 | 4.2 |
| Oat (50% L, 50% S) | 21 | 38.1 | 55.6 | 6.3 |
| Oat Stems | 6 | 31.4 | 59.6 | 9.0 |
| Oat Leaves | 37 | 44.8 | 51.6 | 3.6 |

Table 4.3. Properties of cover crop residues, C/N ratio, carbohydrate (CARB), cellulose (CELL), and lignin (LIGN) pools of cover crop residues from validation field study.

| Year | Cover Crop Residue | C/N | N | CELL | CARB | LIGN |
|---|---|---|---|---|---|---|
| | | | -g $kg^{-1}$- | ----------*% of Total C*---------- | | |
| *2011* | Rye | 28 | 23.5 | 61.3 | 32.3 | 6.4 |
| | Crimson clover | 25 | 30.3 | 40.4 | 55.1 | 4.5 |
| *2012* | Crimson clover | 23 | 42.5 | 26.0 | 67.5 | 6.6 |
| *2013* | Crimson clover | 25 | 31.7 | 35.4 | 56.7 | 8.0 |
| Ł Rate Constants ($d^{-1}$): | | | | | | |
| | Incorporated | | | 0.0119 | 0.1445 | 0.0008 |
| | Surface-applied | | | 0.0098 | 0.1190 | 0.0007 |

Ł Rate constants determined by Schomberg and Cabrera, 2001.

Table 4.4. Soil parameters from validation field study used to initiate the CERES-N computer model to predict plant-available N.

| Year | PMN Ł | $NH_4^+$ – N | $NO_3^-$ – N | Organic C |
|---|---|---|---|---|
| | ————kg N $ha^{-1}$———— | | | g $kg^{-1}$ |
| *2011* | 9.26 | 16.54 | 5.32 | 12.7 |
| *2012* | 11.52 | 17.89 | 5.15 | 15.4 |
| *2013* | 2.39 | 2.87 | 25.69 | 20.0 |

Ł Potentially mineralizable N.

Table 4.5. Statistical comparison from validation field study for measured and simulated values of *in situ* net N mineralization from cover crops, incorporated unless otherwise noted with (S) for surface-applied. Simulated values obtained from CERES-N using rate constants (Table 4.3) and pool sizes of residues (Table 4.4).

| **Amendment** | **M† (kg ha$^{-1}$)** | **±SD of M (kg ha$^{-1}$)** | **RMSE (kg ha$^{-1}$)** | **$F_{LOFIT}$** | **Pr > F** | **Predictions within 95% CI** |
|---|---|---|---|---|---|---|
| *2011* | | | | | | |
| Rye | 8.4 | 36.1 | 38.3 | 3.85 | 0.0511 | 4/4 |
| Crimson Clover | 8.2 | 25.0 | 25.4 | 1.85 | 0.1995 | 4/4 |
| *2012* | | | | | | |
| Crimson Clover | -48.1 | 63.1 | 63.1 | 2.49 | 0.1246 | 4/4 |
| Crimson Clover (S) | -21.2 | 49.1 | 47.0 | 0.00 | 0.9954 | 4/4 |
| *2013* | | | | | | |
| Crimson Clover | -3.1 | 20.3 | 20.8 | 0.67 | 0.5295 | 4/4 |
| Crimson Clover (S) | -26.9 | 19.2 | 18.9 | 0.08 | 0.9257 | 3/4 |

† M is the mean difference between measured and simulated; RMSE is the root mean square error for the difference between measured and simulated; $F_{LOFIT}$ is MSLOFIT divided by MSE; CI is the 95% confidence interval.

Figure 4.1. Data of % non-structural carbohydrates as a function of % N for a wide range of cover crop samples submitted to the University of Georgia Agricultural and Environmental Services Laboratories.

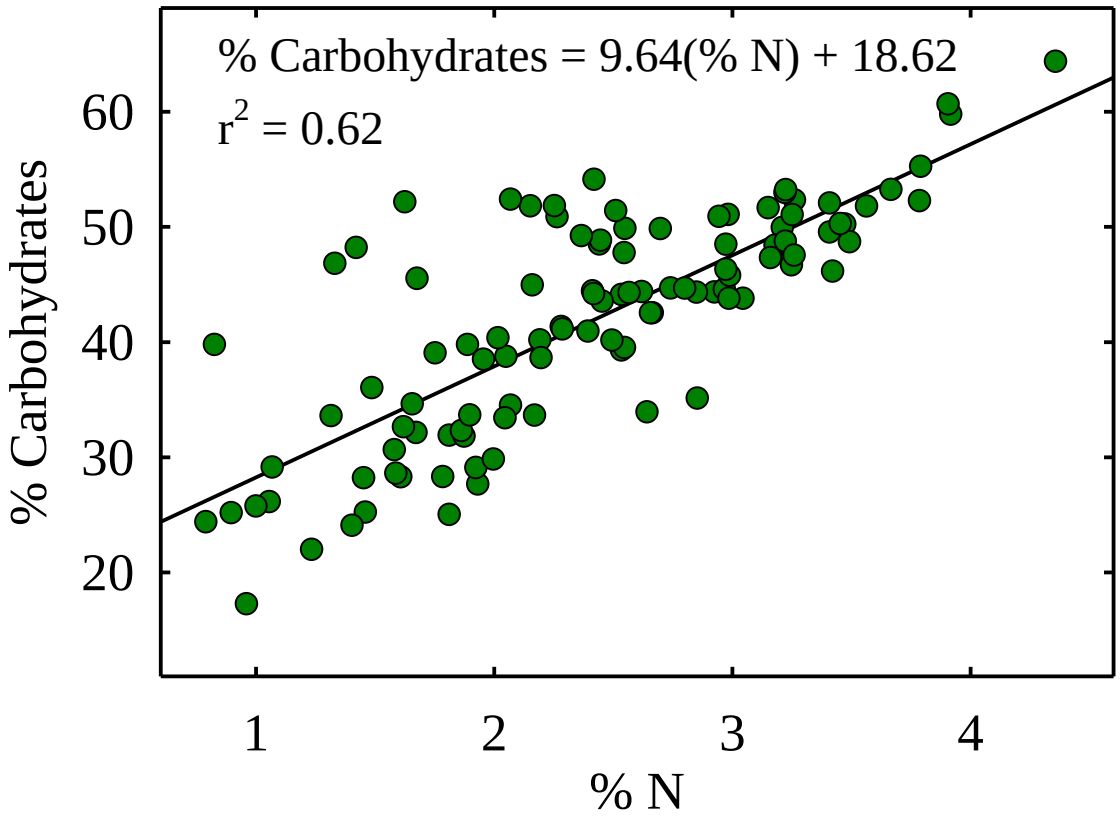


Figure 4.2. A comparison of measured N mineralized from seven studies (listed in Table 4.1) vs simulation of N mineralized by the model.

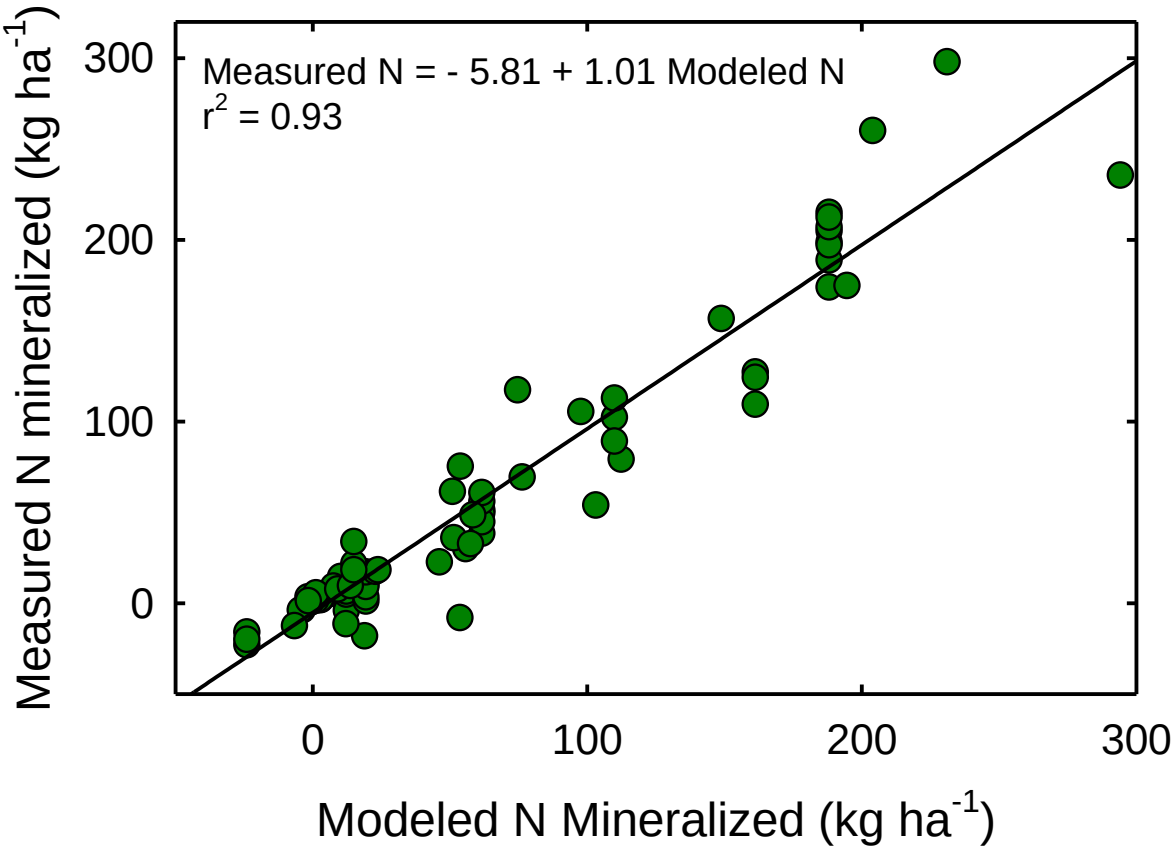


Figure 4.3. Modeled vs measured N mineralized after 162 days for the study of Quemada and Cabrera (1995) in which residue was surface applied under optimal soil water and 35˚ C.

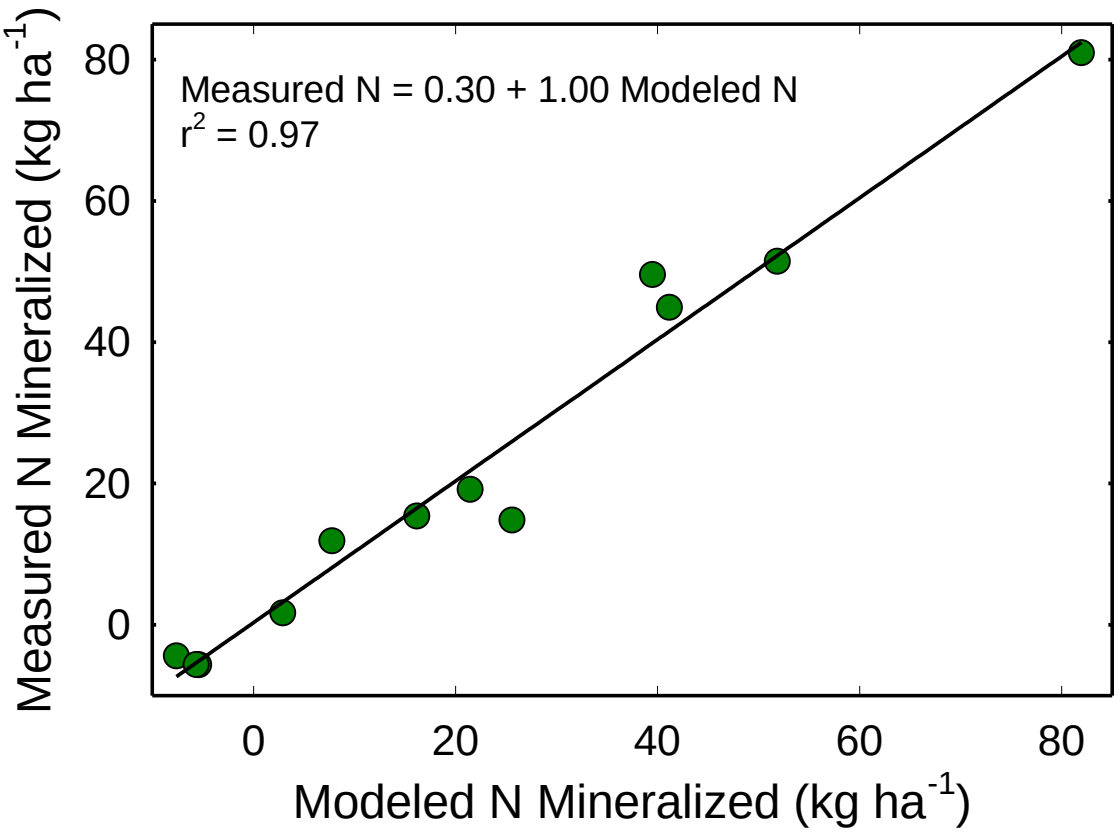


Figure 4.4. Cumulative net N mineralized (mean with standard error) for measured values from Quemada and Cabrera (1995). The lines are cumulative N mineralized simulated with the model.

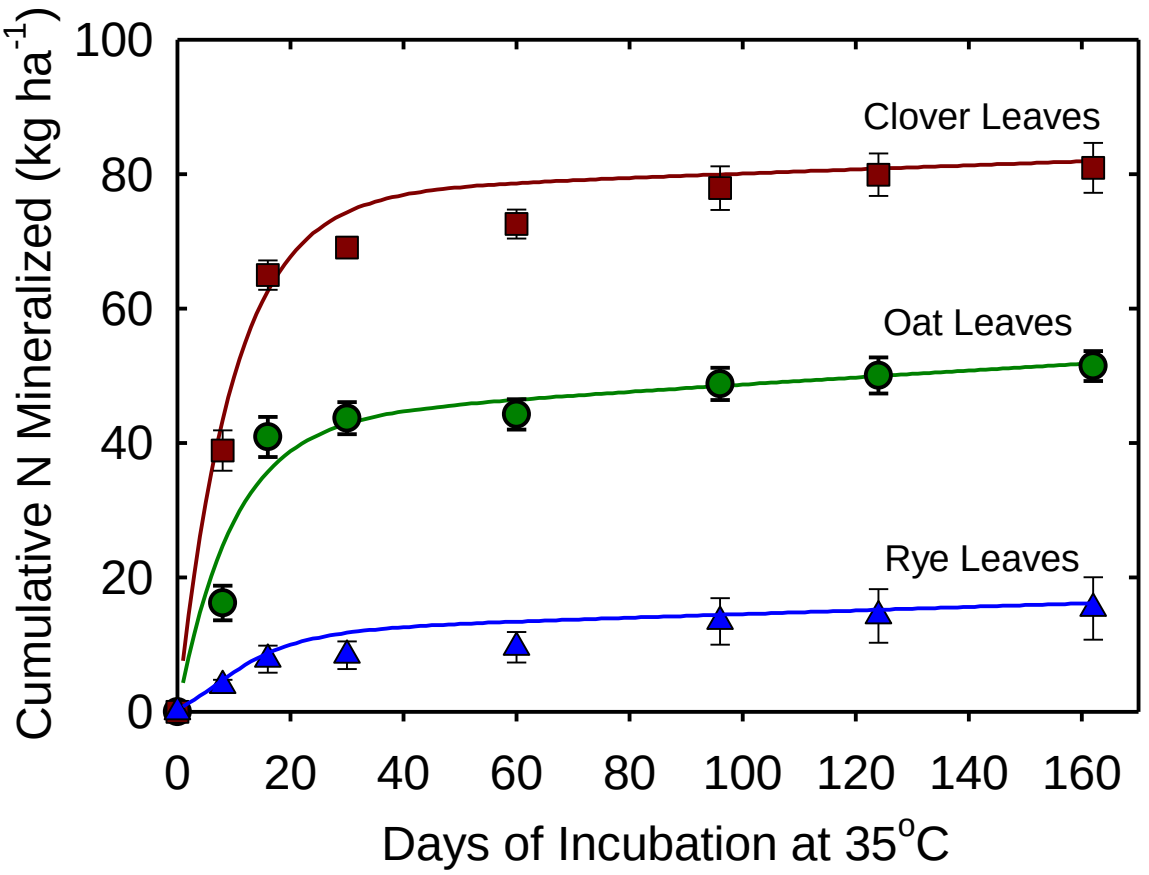


Figure 4.5. Net Inorganic N mineralized/immobilized from rye and crimson clover (error bars show 95% CI), soil temperature (˚C) and soil water content (g $g^{-1}$) over 120 d in 2011.

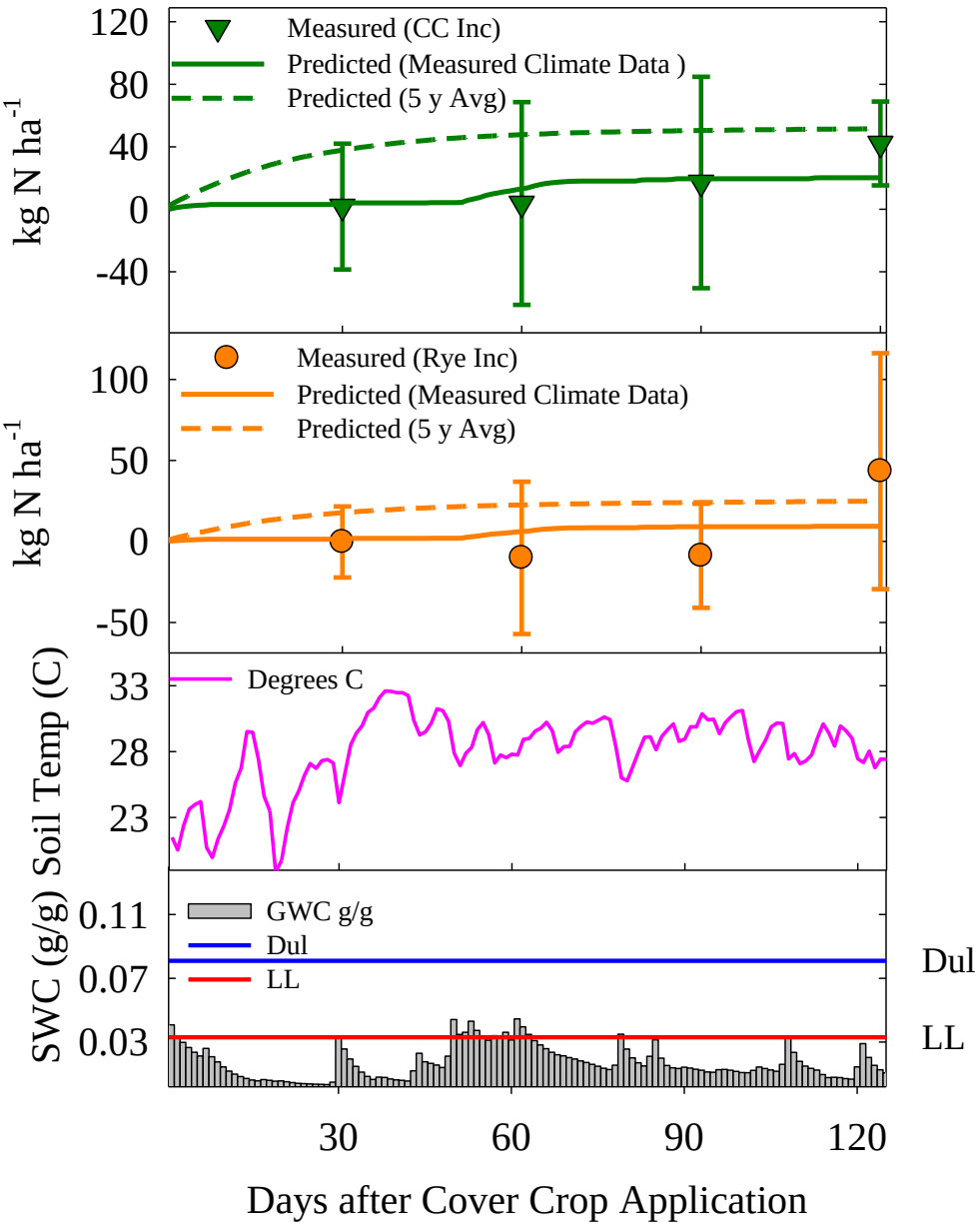


Figure 4.6. Net Inorganic N mineralized/immobilized from crimson clover (surface-applied or incorporated with error bars showing 95% CI), soil temperature (˚C) and soil water content (g g$^{-1}$) over 120 d in 2012.

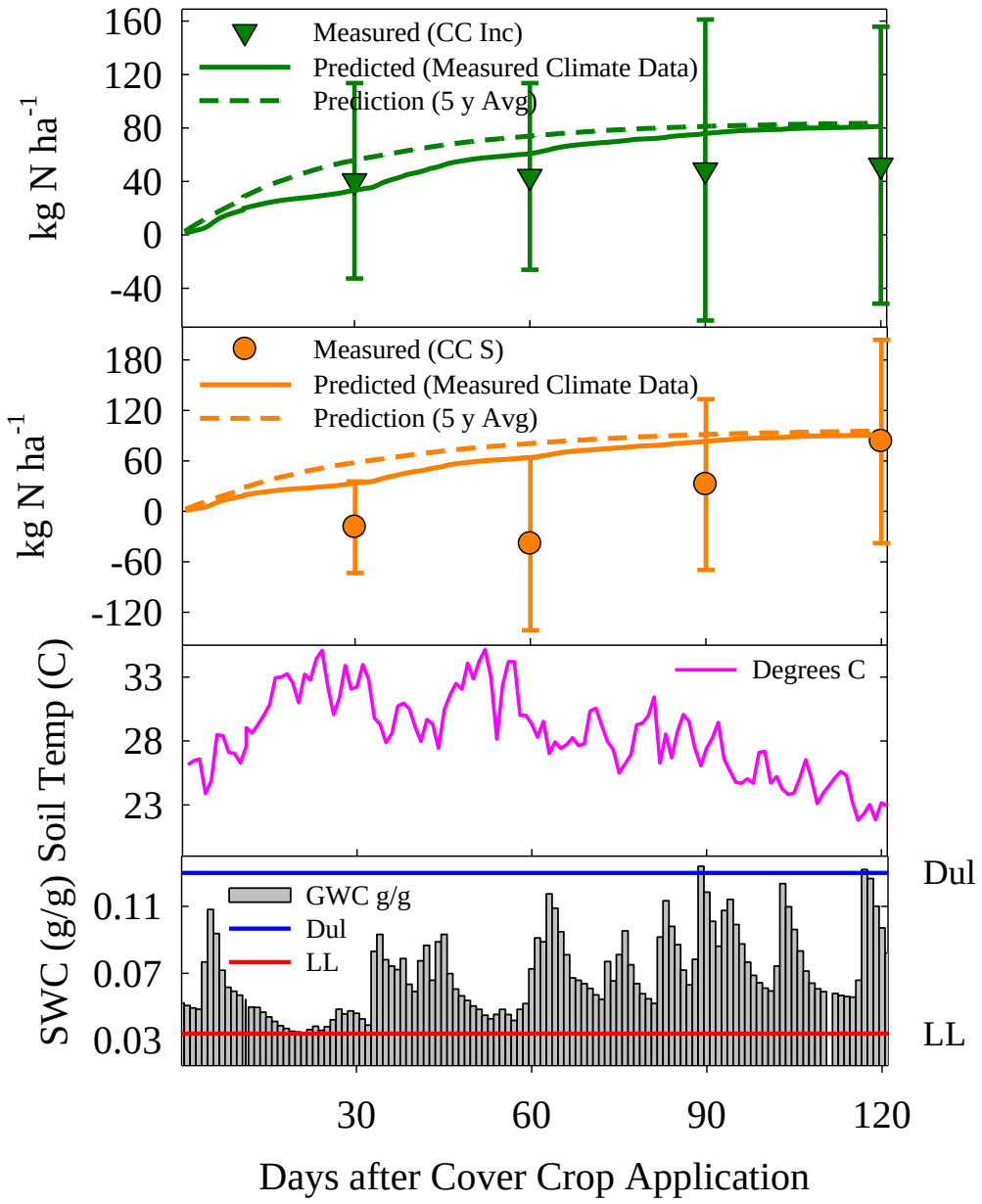
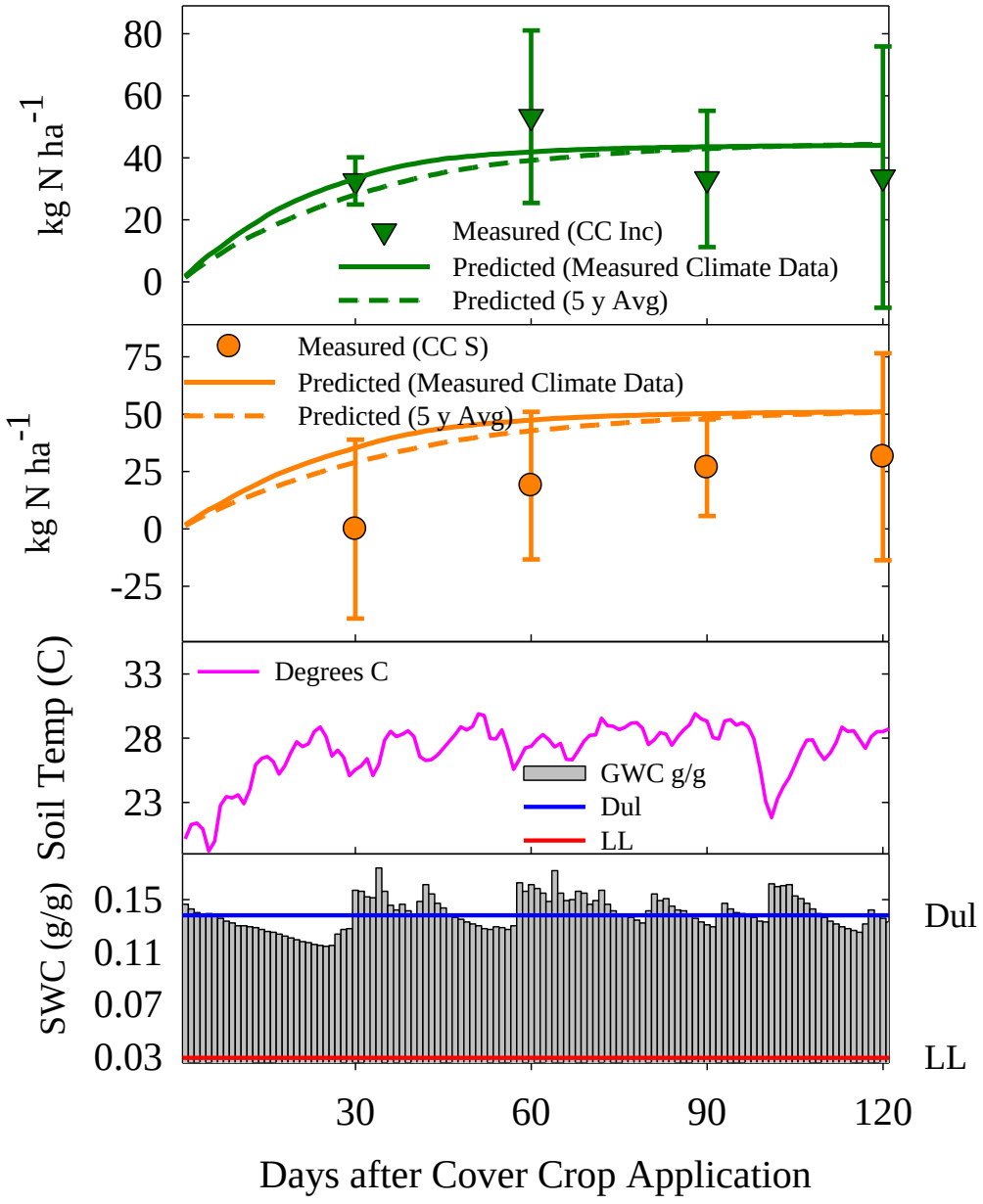


Figure 4.7. Net inorganic N mineralized/immobilized from crimson clover (surface-applied or incorporated with error bars showing 95% CI), soil temperature (˚C) and soil water content (g g$^{-1}$) over 120 d in 2013.



# CHAPTER 5

# AMMONIA OXIDIZER ABUNDANCE AND FUNCTION UNDER CONVENTIONAL AND ORGANIC SYSTEMS OF NITROGEN MANAGEMENT[1]

[1]Woodruff, L.K., A. Mundepi, J.M. Norton, and M.Y. Habteselassie. To be submitted to *Environ. Microbiol*.

## Abstract

Different systems of nitrogen (N) management can affect ammonia oxidation, which results in production of nitrate that can easily be lost from soil. In a multiple-year field study, we examined the impacts of organic and conventional N management systems on the abundance and function of ammonia-oxidizing bacteria (AOB) and archaea (AOA), which mediate the first and rate-limiting step in nitrate production. Sweet corn was grown in plots receiving four treatments: Control (no N), ammonium sulfate at 112 (AS1) or 224 (AS2) kg N $ha^{-1}$ and poultry litter, cover crops and blood meal (PL) at 112 kg $ha^{-1}$. Abundance was measured by targeting the *amoA* gene in a quantitative polymerase chain reaction and was significantly affected by the type and application rate of N source. Bacterial *amoA* abundance was highest in y 3 and was significantly higher than the Control in all N-added treatments ($P = 0.021$). Archaeal *amoA* abundance was highest in y 3 and was the lowest in the treatment with the greatest amount of N input, AS2. Numerically, AOA were most dominant over AOB in y 3 in Control (14X). AOB correlated significantly with $NO_3^-$-N pools ($r^2 = 0.34$; $P = 0.020$) for mid-season 2014, while AOA had no significant correlation with soil $NO_3^-$-N. AOB had the most significant correlation with nitrification potential, which was used to measure potential activity, on d 86 after planting in y 3 of the study ($r^2 = 0.95$; $P < 0.027$). These results indicate AOB were the most responsive to N additions in terms of function. The differential response of AOB and AOA suggests the need for targeted approaches to maximize N use efficiency in the two systems.

Abbreviations: N, Nitrogen; AS, ammonium sulfate; PL, poultry litter.



Keywords: ammonia-oxidizing archaea; ammonia-oxidizing bacteria, nitrification.

## 5.1. Introduction

Ammonia oxidation is the first and often rate-limiting step in nitrification, a microbially-mediated key process in both cropping systems and the global N cycle (Kowalchuk and Stephen, 2001; Levicnik-Hofferle et al., 2012). Ammonia oxidizers play a critical role in nitrogen cycling. They facilitate the enzymatic process of nitrification, the oxidation of ammonium ($NH_4^+$) to nitrate ($NO_3^-$) via nitrite ($NO_2^-$), which provides N to plants in agronomic systems; however, nitrate is the form of nitrogen most prone to loss via soil leaching and runoff and/or atmospheric deposition in the form of $N_2O$, a potential greenhouse gas with global warming potential of up to 300 times that of $CO_2$ (Lashof and Ahuja, 1990; Ravishnakara et al., 2009). The enzymatic process of aerobic ammonia oxidation with ammonia monooxygenase (AMO) was previously thought to be mediated exclusively by bacteria but it has now been shown that archaea mediate the reaction as well and are often more abundant in soils than bacteria (Treusch et al., 2005; Leininger et al., 2006).

Molecular techniques provide a means to quantify functional genes, such as *amoA*, the active subunit of AMO, and compare correlations to substrate availability, nitrate pools, and other parameters that may influence nitrification. Over the last decade, it has been well documented that archaea, in addition to bacteria, function as ammonia oxidizers in soils as well as in every other ecosystem on earth (Leininger et al., 2006; Tourna et al., 2008; Jia and Conrad, 2009; Norton, 2011). In a number of agricultural soils archaea, rather than bacteria have been found be more abundant (Schleper, 2010) and contribute more to nitrification than bacteria (Gubry-Rangin et al., 2010). Since this relatively recent discovery, a series of experiments have determined influencing factors for the relative contributions of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) such as substrate concentration (Levicnik-

Hofferle et al., 2012, Schauss et al., 2009), pH (Yao et al., 2011; Zhang et al. 2012), spatial distribution (Wessen et al., 2011, Sher et al., 2013), and multi-factorial drivers such as land use, soil type, and climate (Yao et al., 2013). Multi-year soil management practices can also be an influencing factor for the abundance and diversity of AOA and AOB (Wessen et al., 2010).

Ammonia-oxidizers have been widely studied in agricultural soils and many other ecosystems (Treusch et al., 2005; Jia and Conrad, 2009; Schleper, 2010; Wessen et al., 2010; Zhang et al.; 2012; Habteselassie et al., 2013; Monteiro et al., 2014; Ouyang et al., 2016). However, their abundance and function has not been well-characterized in agricultural soils after repeated incorporation of poultry litter in organically managed systems. Nitrogen losses from poultry litter can be substantial, through processes such as $NH_3$ volatilization (Rothrock et al., 2010) and nitrification followed by $NO_3^-$ leaching, and $N_2O$ emissions (Xie et al., 2012). Incorporation of poultry litter into the soil has been shown to decrease $NH_3$ volatilization by up to 90% compared to surface application (Pote et al., 2011). Therefore, in our study, nitrification and related parameters were the main focus. A better understanding of the abundance and function of the microorganisms, which mediate nitrification when poultry litter is incorporated into soil, will help determine management practices to mitigate N losses and maximize N availability at times when plant demand is high. This is important in Georgia where poultry litter is abundant and can be used as a cheap source of N in organic farming. As such, the main objective of this study was to evaluate the response of ammonia oxidizers in abundance and function to organic and conventional N management systems in which sweet corn was grown over multiple years in an Ultisol, the most common type of soil in GA.

## 5.2. Materials and Methods

### 5.2.1 Experimental Field Site and Amendments

Detailed descriptions of the field site and amendments are given in Chapter 3. Brief descriptions are provided here for convenience. Field plots were established at the Durham Horticulture Farm (lat. 33˚ 55′ N, long. 83˚ 25′ W) on an irrigated Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapludults) from 2012 to 2014. Precipitation and temperature were measured by the Georgia Automated Weather Monitoring Network with a weather station located at the farm. Annual total precipitation for 2012, 2013, and 2014 was 95, 164, and 106 cm, respectively. Mean temperatures for the three growing seasons was 21˚C. Rainfall and temperature were measured by the Georgia Automated Weather Monitoring Network with a weather station located at the farm (http://www.georgiaweather.net/). Initial pH was $5.3_{1:1}$ and soil organic matter content was 2.7%. The study area had been fallow for the previous six years leading up to 2012. Initially the entire area (1,650 $m^2$) was chisel-plowed, followed by tillage. In subsequent years only the 16 individual plots were chisel plowed, once before planting, and tilled, once before amendments were added and once just before planting.

A randomized complete block design was used with 4 treatments, Control (no N added), AS1 and AS2 (ammonium sulfate at 112 and 224 kg N $ha^{-1}$), and PL (poultry litter, cover crops, and blood meal at 112 kg N $ha^{-1}$). Each treatment had 4 replications for a total of 4 blocks with a total of 16 plots. Each plot was 3.8 by 9.1 m with 2 m between blocks. For the organic plots the nitrogen needed from poultry litter was calculated by subtracting predicted plant-available N from cover crops using the simulation submodel CERES-N from the total N amendment rate of 112 kg N $ha^{-1}$. Fresh broiler litter was retrieved from a local farm each year and applied within one week of collection.

Sweet corn was planted within a week following amendment application and tillage. The plots were irrigated with a sprinkler irrigation system as needed. Amendment application rates for all nutrients besides N were based on soil test recommendations from the Agricultural and Environmental Services (AES) Laboratories in Athens, GA (aesl.ces.uga.edu). Conventional amendments for other nutrients have included potassium sulfate (0-0-60), triple superphosphate (0-45-0), and borax. The poultry litter provided all the other nutrients needed for the PL plots except for sulfate of potash (0-0-51) which was needed for one plot in year 2 of the study. All amendments used on the PL plots were Organic Materials Review Institute (OMRI) certified. Poultry litter was analyzed at the Soil Testing Lab for % N and other nutrients (Table 3.2). Nutrient availability from poultry litter was estimated to be 58% of total N measured by combustion based on the suggestion of the aforementioned AESL.

Soil samples were collected 3 times per season each year (pre-season, mid-season, and post-season), and 5 times in the final year from 0-15 cm depth. Pre-season samples were collected before amendment application and planting. Mid-season samples were collected at day 45-49 after planting. Post-harvest samples were taken after the final harvest.

**5.2.2 Soil Analysis for Inorganic N and Nitrification Potential**

Soil samples were analyzed for inorganic N pools ($NH_4^+$, $NO_2^-$- N, and $NO_3^-$-N) colorimetrically with an automatic flow injector as described in Methods of Soil Analysis (Mulvaney 1996). Each sampling time, 15 g of soil at field moisture was mixed with 75 mL of 2 *M* KCl, shaken for 1 h, and centrifuged at 8000 x *g* for 15 min. Supernatant was frozen and analyzed later for $NH_4^+$ and $NO_3^-/NO_2^-$ using an Alpkem Technicon Autoanalyzer (Saskatoon, Saskatchewan, Canada). Analysis of other basic soil properties (e.g., pH, organic matter, metals) is found in Chapter 3.

Nitrification potential was determined for 0-15 cm by the shaken-soil-slurry method under optimized conditions in terms of water content, $NH_4^+$ concentration, and aeration (Hart et al., 1994). Erlenmeyer flasks (250 mL) were filled with 15 g of field moist soil and 100 mL phosphate buffer with 1 m*M* $NH_4^+$-N. Flasks were shaken continuously at 200 rpm for 24 h with 20 mL of aliquot removed at 2, 4, 22, and 24 h. Aliquots were centrifuged at 8000 x *g* for 15 min and analyzed for $NO_2^-$-N and $NO_3^-$-N using an Alpkem Autoanalyzer. Nitrification potential was determined by the slope of linear regression of time versus concentration of $NO_2^-$-N and $NO_3^-$-N.

### 5.2.3 Abundance of Ammonia Oxidizers

The abundance of ammonia oxidizers was determined using the quantitative polymerase chain reaction method (qPCR) by targeting the *amoA* gene. Soil DNA was extracted from 0.25 g fresh soil from each sample using a MoBio PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions and quantified with a TBS-380 Fluorometer (Turner BioSystems, Sunnyvale, CA). Archaeal *amo*A genes were quantified using ArchamoAF (5' TTATGGTCTGGCTTAGACG 3') and ArchamoAR (5' GCGGCCATCCATCTGTATGT 3') primer pair (Francis et al., 2005). Standard curve was prepared using serial dilution of the archaeal plasmid DNA with copies ranging from $3 \times 10^7$ to $3 \times 10^3$. Conventional PCR amplicon of ArchamoAF/AR from a soil sample was used as a template for ligation in the cloning. The PCR product was cloned with pGEM®-T and pGEM®-T Easy Vector System (Promega, Madison, WI, U.S.A) and plasmid DNA was extracted with PureYield™ Plasmid Miniprep System (Promega, Madison, WI, U.S.A). Plasmid concentration (nanograms per microliter) was measured with a TBS-380 Fluorometer (Turner BioSystems, Sunnyvale, CA). Total volume for each qPCR reaction was 20 µl with 2 µl of soil DNA, 0.6 µM

*o*f each primer, and 10 µl of SYBR® Select Master Mix (Applied Biosystems, Austin, TX). The standards were run in triplicate and samples in duplicate. Cycling conditions, according to (Francis et al., 2005; Sher et al., 2013) were modified with denaturation at 95°C for 10 min, 40 cycles of denaturation 95°C for 35 sec, 56°C annealing for 45 sec and 72°C elongation for 3 min. Product specificity was confirmed by melting curve analysis with an efficiency of 80.0% with an $r^2$ of 0.95 and a slope of -3.9.

Bacterial *amo*A genes were quantified with SYBR® GreenI fluorescent dye using primers *amo*A-189F (5´-GGHGACTGGGAYTTCTGG-3´) and *amo*A-2R (5´-CCCCTCKGSAAAGCCTTCTTC-3´) (Okano et al., 2004; Habteselassie et al., 2013). Quantifications were standardized using a dilution series of a known amount of a linearized plasmid containing the *amo*A gene of *Nitrosomonas europaea ATCC 19718*. The standard curve containing $3 \times 10^7$ to $3 \times 10^3$ copy numbers was prepared in the same way as archaea; using PCR amplicon of *amoA* gene from *Nitrosomonas europaea ATCC 19718* with amoA189F and amoA-2R primer pair. Each q-PCR reaction was quantified based on the fluorescence intensity of SYBR® Green dye in a total volume of 20 µl consisting of 2 µl of soil DNA, 0.5 µM of each primer, and 10 µl of SYBR® Select Master Mix (Applied Biosystems, Austin, TX). Cycling conditions according to Okano et al. (2004) and Habteselassie et al. (2013) were modified to 10 min at 95 ˚C, 40 cycles of 45 sec at 95 ˚C, 1 min at 60 ˚C, 2 min at 72 ˚C followed by a melt curve 15 sec at 95°C, 1 min at 60°C, and 15 sec 95°C. Product specificity was confirmed by melting curve analysis with an efficiency of 96.4% with an $r^2$ of 0.99 and a slope of -3.4. All real-time PCR reactions were performed in Step One Plus™ Real Time PCR system (Applied Biosystems, Grand Island, NY, USA) and analyzed with StepOne Software version 2.3.

### 5.2.4 Statistical Analysis

Statistical analysis was performed with repeated measures analysis of variance (Proc Mixed) with year as a repeated measures factor. Regression analysis was performed to assess correlation of archaeal and bacterial ammonia-oxidizer abundances with nitrification-related parameters. Log transformations of data were performed as needed to meet normality assumptions. All statistical analyses were performed at $P < 0.05$ with SAS Software (SAS Institute Inc., Cary, NC, 2014).

## 5.3. Results and Discussion

### 5.3.1 Abundance of Archaeal and Bacterial *amoA*

Bacterial *amoA* abundance was significantly impacted by treatment ($P = 0.0403$) and year ($P = 0.0020$), with no treatment x year interaction. Bacterial *amoA* abundance was significantly greater in PL than Control, with AS1 and AS2 showing no difference from PL or Control. Bacterial *amoA* abundance was greater overall in 2014 and 2012 than in 2013. Archaeal *amoA* abundance was also significantly impacted by treatment ($P = 0.0196$) and year ($P = 0.0014$) with no treatment x year interaction. Archaeal *amoA* abundance was greater in Control than in the N-added treatments with no difference among AS1, AS2, or PL. AOA abundance was greatest in 2014 and 2012, with no difference between the two.

Archaeal *amoA* abundance was greater than (or not significantly different from) bacterial abundance in all treatments in mid-season 2013 and 2014 samples. Archaeal abundance ranged from 5.2 to 6.8 log copies $g^{-1}$ soil in 2013 and 2014, with 2014 abundance bein$^{g\ g}$reater than 2013 for all treatments (Fig. 5.1). The abundance numbers from this study are generally lower than previously reported (Zhang et al., 2012; Leininger et al., 2006; He et al., 2007). A study by Liu et al. (2014) found abundance of AOA and AOB decreased in metal-polluted rice paddies. PL

treatments in our study had significant accumulations of Zn, 1.8 to 2.5 g $kg^{-1}$, over the 3 years of the study and could potentially be the reason for the lower abundance in PL treatment. However, Zn accumulation in our study was much lower than the Zn concentrations reported by Liu et al. (2014) ranging from 9.5 to 24.8 g $kg^{-1}$.

The ratio of bacterial to archaeal *amoA* abundance (AOA/AOB) is generally taken as an indicator of the relative importance of AOA and AOB in nitrification. The ratio ranged from 0.5 to 14.1 throughout the study (Fig. 5.1). The highest ratios were in Control and PL and the lowest in the AS treatments. Other studies have found similar ranges such as Wessen et al. (2010) who reported AOA/AOB ranging from 0.7 to 7.8 for organic and conventional treatments with no significant differences between the two. He et al. (2007) reported AOA/AOB in agricultural soils ranging from 1.02 to 12.36, with archaeal *amoA* abundance exceeding those of AOB in both summer and winter. In a study by Di et al. (2010) AOA/AOB ranged from 5.7 to 21.9 in intensively grazed pastures treated with animal urine.

Other studies have found higher ranges of AOA/AOB. In a study comparing ammonium sulfate and composted dairy manure amendments AOA/AOB ranged from 2.9 to 158.8 with the highest ratios in compost and control treatments and lowest in the AS treatments (Ouyang et al., 2016). Leininger et al. (2006) found that AOA/AOB ranged from 1.2 to 230 in 12 pristine and agricultural soils. Agricultural soils ranged from 7 to 73 in a study by Schauss et al. (2009). While AOA:AOB may vary widely across soil types and treatments, the trends observed in our study were similar to previous studies such as Ouyang et al. (2016) with AOA/AOB higher in control plots (and sometimes organic (PL)) and lower in plots with conventional N amendments (AS1 and AS2) (Fig. 5.1). Habteselassie et al. (2013) also found AOA/AOB higher in control and compost-treated soils.

AOA/AOB tend to be higher in low substrate environments such as control treatments with no N input or organic treatments with low $NH_4^+$ concentrations. AOA/AOB tend to be lower under conventional N-management because AOB abundance is more responsive to increases in $NH_4^+$. This indicates that there can be niche specialization among bacteria and archaea. In treatments where AOB are the main drivers of nitrification, management efforts could be targeted towards bacteria to minimize N losses and maximize N-use efficiency. High concentrations of ammonium in soils can lead to shifts in AOB communities from a more diverse population to predominance of *Nitrosospira* clusters 1 and 3 (Stein et al., 2005; Dharni et al., 2010).

### 5.3.2 Inorganic N pools and Ammonia Oxidizers

Main effects of treatment, year, and treatment x year interaction were significant ($p < 0.0001$) for mid-season $NO_3^-$-N concentrations (0-15 cm), with AS2 the greatest for 2012 and 2013 (131.2 and 26.1 kg N $ha^{-1}$), with no differences among AS1, PL, and Control (Fig. 5.2). The AS2 and PL treatments (129.8 and 116.4 kg N $ha^{-1}$) were greatest in 2014 with no difference between AS1 and Control. The $NH_4^+$-N in the 0-15 cm depth was significantly affected by treatment ($P = 0.0158$), year ($P = 0.0326$), and treatment x year interaction ($P = 0.0346$). Soil $NH_4^+$-N in the 0-15 cm depth was greatest in 2012 in AS1 and AS2 treatments with no significant difference between PL and Control. Soil $NH_4^+$-N in 0-15 cm was relatively low in 2013 and 2014 (both lower than 2012), with no difference among treatments. N pools mid-season for 2012 and 2014 experienced the greatest differences in speciation at 0-15 cm, with greater $NO_3^-$-N than $NH_4^+$-N concentrations. The $NO_3^-$-N was the dominant form of the inorganic N pool in all the years across treatments, suggesting that $NH_4^+$-N was being quickly

nitrified to $NO_3^-$-N. The greatest concentrations of inorganic N were associated with the AS2 treatment as it had the highest rate application.

While ammonium concentration is indicative of substrate availability to ammonia oxidizers, nitrate concentration is indicative of their activity. As indicated above, most of the ammonium was quickly nitrified to nitrate. Nitrate concentration was correlated significantly with bacterial *amoA* abundance rather than archaeal *amoA* abundance, with $r^2 = 0.42$, 0.86, and 0.34 for AOB for 2012, 2013, and 2014, respectively, and $r^2 < 0.13$ for AOA for all three years (Fig. 5.3). This indicates that AOB had a more significant quantitative relationship with $NO_3^-$-N production than AOA, as has been documented in previous studies of agricultural soils (Leininger et al., 2006; Jia and Conrad, 2009). The Regression analysis between *amoA* abundance and $NO_3^-$-N concentration for 2014 mid-season samples is shown below as a sample (Fig. 5.3):

$y = 5.77 + 0.004x$

$r^2 = 0.34$; $P = 0.02$ [Eq. 1]

where *y* is the bacterial *amoA* abundance (log copies $g^{-1}$ soil) and *x* is the $NO_3^-$-N concentration (kg N $ha^{-1}$).

$y = 6.50 + 0.0009x$

$r^2 = 0.08$; $P = 0.718$ [Eq. 2]

where *y* is the archaeal *amoA* abundance (log copies $g^{-1}$ soil) and *x* is the $NO_3^-$-N concentration (kg N $ha^{-1}$).

The correlations between ammonium concentrations and archaeal and bacterial amoA were also examined. The only significant correlation was between ammonium concentrations and bacterial *amoA* abundance for midseason 2014.

$y = 6.31 - 0.12x$

$r^2 = 0.23$; $P = 0.04$ [Eq. 3]

where *y* is the bacterial *amoA* abundance (log copies $g^{-1}$ soil) and *x* is the $NH_4^+$-N concentration (kg N $ha^{-1}$).

Ammonium is the substrate for nitrification so correlation with AOB can indicate which population is more active in mediating ammonia oxidation and production of $NO_3^-$-N. AOA have a higher substrate affinity than AOB and have been observed to decrease in abundance in response to high $NH_4^+$ concentrations (Valentine, 2007). The first cultivated isolate of AOA, *Nitrosopumilus maritimus,* was shown to have an extremely low substrate threshold and half-saturation constant, apparently adapted for the oligotrophic conditions of the open ocean (Martens-Habbena et al., 2009). AOB abundance tends to increase with $NH_4^+$ concentrations in soil within the normal range of fertilizer application rates. Nitrate concentrations were higher in mid-season in all three N-treated soils ($P < 0.05$) (Fig. 5.3). This indicates that nitrification activity was significantly lower in untreated (Control) soils where AOA outnumbered AOB by the widest margins (Fig. 5.1). The nitrification response (in terms of $NO_3^-$-N pools) to N

treatments showed a similar pattern to the bacterial *amoA* abundance response, indicating that AOB are most likely the main drivers of nitrification in this study soil.

Nitrate production has shown positive correlations with bacterial *amoA* abundance in oceans (Wutcher et al., 2006), and it is widely accepted that AOA are the primary drivers of ammonia oxidation in ocean plankton (Schleper 2010). However, studies of agricultural soils have yielded mixed results. Zhang et al. (2012) found that for 5 soils, $NO_3^-$-N had significant positive correlations with archaeal *amoA* abundance while correlation with bacterial *amoA* abundance was negative (in 2 soils) or not significant (3 soils). Positive correlations ranged from $r^2$ of 0.73 to 0.92 ($P < 0.05$). AOB had a more significant correlation with $NO_3^-$-N production than AOA in several other studies of agricultural soils (Leininger et al., 2006; Jia and Conrad, 2009).

### 5.3.3 Nitrification Potential and Ammonia Oxidizers

Nitrification potential (NP) was measured mid-season 2013 and 5 sampling times in 2014 to assess temporal trends between years and within year (over a growing season). Mid-season 2013 NP was measured for d 45 after planting. Sampling times in relation to planting date for 2014 were d -8, d 49, d 63, d 77, and d 86. NP is an indicator of the capacity of a soil to produce $NO_3^-$-N under non-limiting conditions of substrate, pH, and temperature. Correlations of ammonia-oxidizer abundance with NP can provide evidence for which group of organisms (bacteria or archaea) are the main drivers of nitrification. Overall, the treatment difference for the 2014 sampling times was significant ($P = 0.006$) with AS2 and PL greater than the Control with no difference between the two. The AS1 treatment was not significantly different from AS2, PL, or Control. Nitrification potential was significantly greater for PL preseason (d -8) with no difference among AS1, AS2, and Control. Midseason, AS2 had greater NP than Control, with no

difference between AS1 and PL. At d 63 after planting, AS2 had the greatest NP in AS2 and PL with no difference among Control, AS1, and PL.

NP rates in 2013 ranged from 1.9 to 3.6 mg $NO_3^-$-N $kg^{-1}$ soil $d^{-1}$, with AS2 and PL significantly greater than Control and AS1. NP rates in 2014 ranged from 1.9 to 12.8 mg $NO_3^-$-N $kg^{-1}$ soil $d^{-1}$ (Fig. 5.4). This is similar to previous studies such as Yao et al. (2013) where NP rates varied greatly ranging from 0.1 to 12.1 mg $NO_3^-$-N $kg^{-1}$ soil $d^{-1}$. On average, agricultural soils had rates of 7.0 mg $NO_3^-$-N $kg^{-1}$ soil $d^{-1}$, 3 to 6 times higher than soils without N amendments. This study included 713 soils under a wide range of management including agricultural, grassland, bog, etc. In a study of agricultural soils with fresh manure amendments by He et al. (2007), NP rates ranged from 0.3 to 4.4 mg $NO_3^-$-N $kg^{-1}$ soil $d^{-1}$ and increased in the manure-amended soils. In the current study, NP rates were highest at d 63 after planting for the 3 N-added treatments (AS1, AS2, and PL), with no difference between AS2 and PL. NP was approximately two times higher in AS2 and PL than in AS1, which was about twice as high as Control. PL had the highest NP rate in the pre-season sampling time (d -8). Otherwise, all 3 N-added treatments generally had higher NP rates than the Control for all other sampling times. These results are consistent with previous studies (He et al., 2007; Wessen et al., 2010; Rudsill et al. 2016) where soils receiving fresh manure, crop residues, or a mixture of composted poultry litter and crop residues showed increases in NP.

The relationships between NP and bacterial and archaeal *amoA* copy numbers were assessed. In 2013, NP correlated significantly with AOB ($r^2 = 0.58$; $P = 0.03$) but correlation with AOA was not significant. Significant correlation was found between NP and AOB on d 86 in 2014 ($r^2 = 0.95$; $P = 0.027$; Fig. 5.5). This provides evidence that AOB were the main drivers of nitrification in the soil after harvest. No significant correlation was found between NP and AOA.

This is similar to the results of Ouyang et al. (2016) who also found that archaeal *amoA* abundance did not correlate significantly with NP rates while bacterial *amoA* abundance did ($P = < 0.0001$). Yao et al. (2013) similarly reported NP correlated significantly with bacterial amoA abundance ($r^2 = 0.26$; $P < 0.001$).

**5.4. Conclusions**

Overall, the different systems of nitrogen (N) management affected ammonia oxidizer abundance and function. AOB increased in response to N additions. AOA estimates of abundance based on *amoA* gene copy numbers were higher than (or no different from) AOB abundance for all treatments, with the highest AOA to AOB ratios in Control, 14.1 and 3.5 for 2013 and 2014, respectively, and in PL, 2.7 in 2013. Abundance was influenced by the type and application rate of the N source. Even though archaeal *amoA* abundance was higher than bacterial in most instances, bacterial *amoA* abundance had the most significant correlation with nitrate concentration and nitrification potential. This is indicative of AOB being more responsive to N additions in terms of abundance and two indicators of activity. The ratio of AOA to AOB was highest each year in the Control where the substrate for nitrification was primarily mineralization from soil organic matter. Ratios were lower in the AS treatments in y 2 and in all three N-added treatments in the first and last years of the study. Lower AOA to AOB ratios appear to occur in soils with readily-available ammonium such as inorganic N fertilizers. AOB abundance increased in response to increased substrate availability while AOA generally showed no difference among treatments. The differential response of AOB and AOA suggests the need for targeted approaches to maximize N use efficiency in the two systems, likely focusing on AOB for short-term conventional N management using inorganic N fertilizers and AOA for organic N management using fresh manure.

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Figure 5.1. Abundance of *amoA* gene in soil (0-15 cm) for archaea (AOA) and bacteria (AOB) (0-15 cm) for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments mid-season in 2012, 2013, and 2014 with AOA:AOB ratios shown above the standard error bars for each treatment; n = 4 per treatment per sampling time.

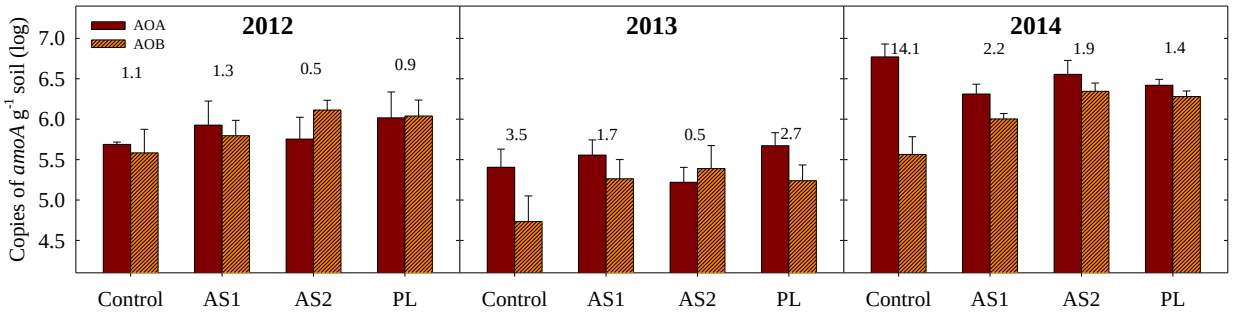


Figure 5.2. Mid-season inorganic N pools ($NO_3^-$-N and $NH_4^-$-N) 0-15 cm for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments in 2012, 2013, and 2014; standard deviation bars shown for each mean; n = 4 per treatment per sampling time.

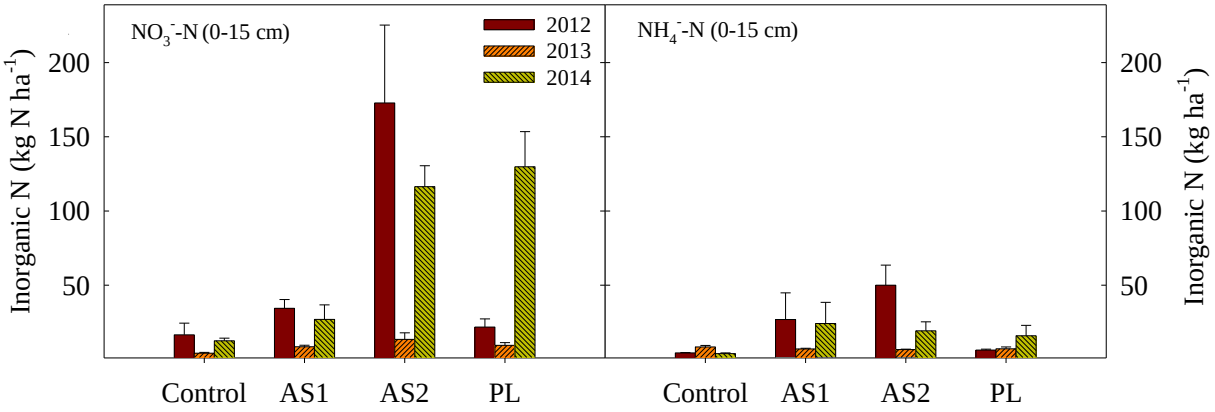


Figure 5.3. Relationship between bacterial and archaeal *amoA* gene abundance and nitrate mid-season 2012 (left), 2013 (middle), and 2014 (right) for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments with error bars showing standard deviations; n = 4 per treatment per sampling time.

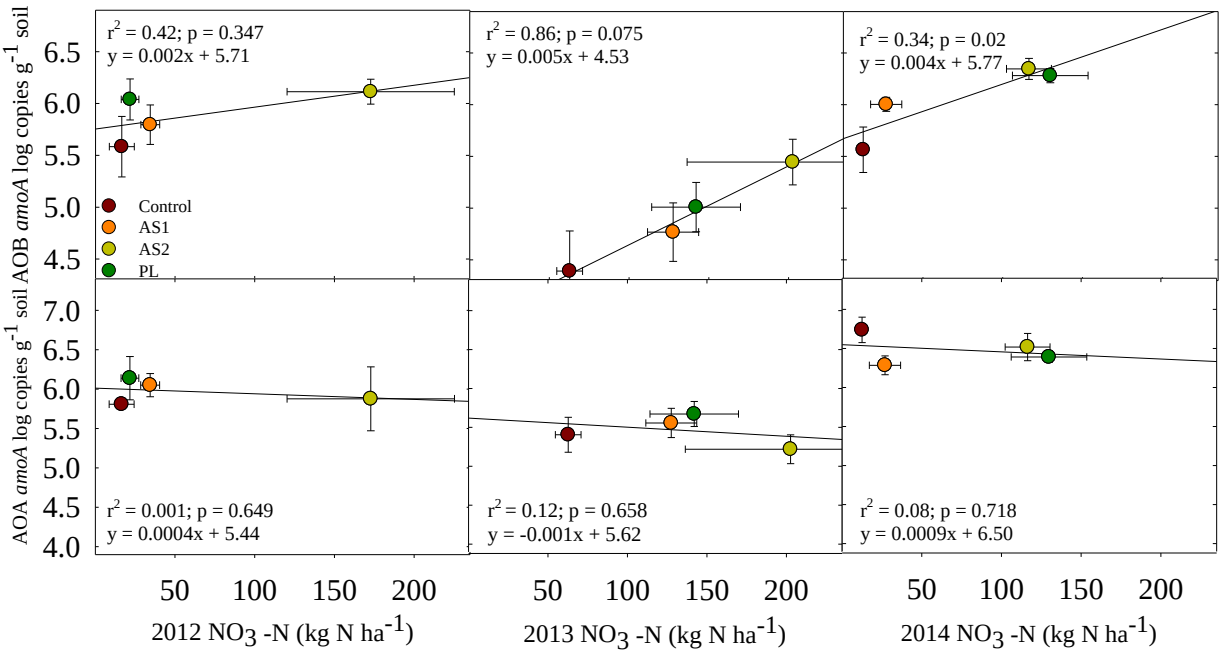


Figure 5.4. Nitrification potential over a range of 5 sampling times in y 3 (2014) for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments with error bars showing standard deviations; n = 4 per treatment per sampling time.

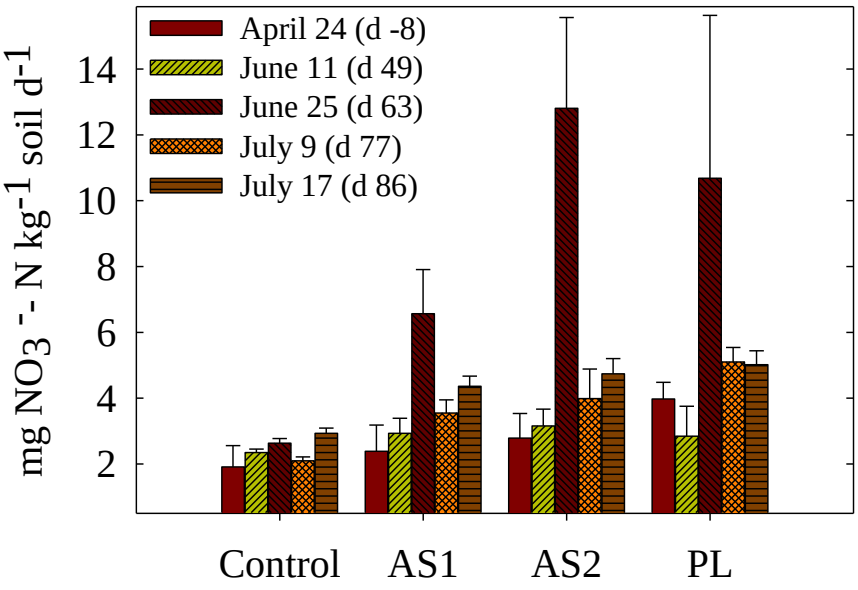
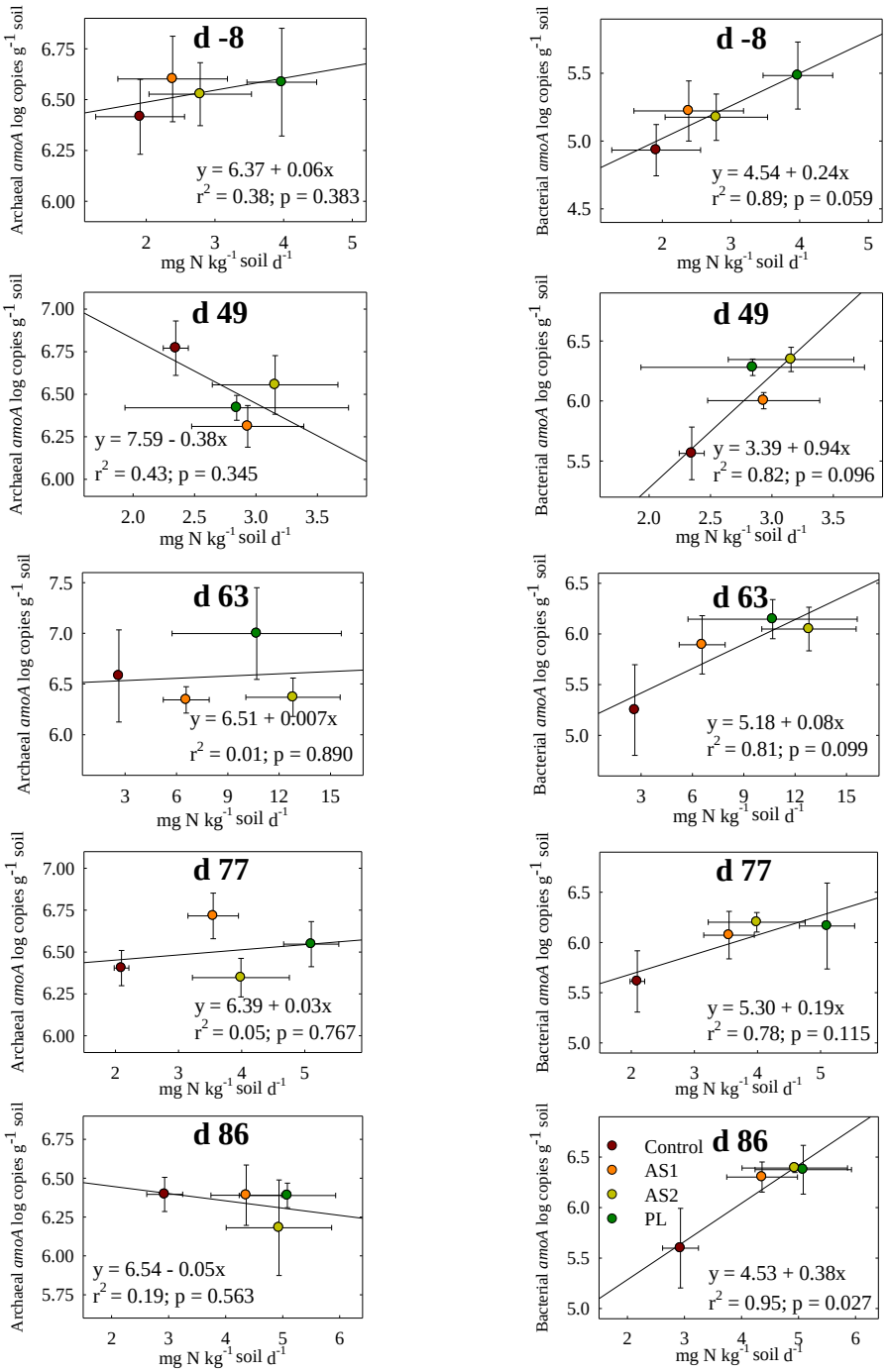


Figure 5.5. Relationship between bacterial and archaeal *amoA* abundance and nitrification potential over 5 sampling dates (d -8, d 49, d 63, d 77, and d 86 in relation to planting date) in 2014 for Control (no N added), AS1 (112 kg N $ha^{-1}$ from ammonium sulfate), AS2 (224 kg N $ha^{-1}$ from ammonium sulfate), and PL (112 kg N $ha^{-1}$ from poultry litter and cover crops) treatments with error bars showing standard deviations; n = 4 per treatment per sampling time.



# CHAPTER 6

# SUMMARY AND CONCLUSIONS

In this comparative study we evaluated conventional systems of N management (with ammonium sulfate) and organic systems of N management (with cover crops, poultry litter, and blood meal) in terms of crop yield, soil and plant N, and the function and abundance of the microorganisms that mediate nitrification. Our study of N management impacts on the plant-soil-microbe nexus focused on three main objectives. The first objective was to examine how the primary productivity of sweet corn responds to the contrasting N management systems, which can affect the behavior of the microorganisms ultimately responsible for the availability of plant-usable forms of N. We measured primary productivity parameters such as corn crop yield and cornstalk N content to better understand the impact of the different systems of N management on a cropping system.

The second objective was to validate and calibrate a web-based submodel used to predict plant-available N from cover crops over a growing season. We used the CERES-N simulation submodel to evaluate plant-available N from cover crops. N mineralization from crimson clover and rye was assessed in a preliminary field study. Model predictions were evaluated for crimson clover in the sweet corn plots once they were established.

The third objective was to compare the abundance and function of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in organically or conventionally managed soil. We compared the functional response of these microorganisms in the contrastingly managed soil by measuring nitrification potential, quantifying functional genes of microorganisms, and assessing

correlations. Recent work has shown greater gene copy numbers, biological activity, and diversity in organically managed soils compared to soils managed conventionally (Reeve and Schleper, 2011). More work is needed to examine these and other parameters in different soil types under contrasting systems of N management.

Overall, the PL treatment resulted in comparable levels of yield and plant N parameters as the AS treatments. The PL treatment resulted in Zn accumulation over time but this did not affect yield as compared to the AS1 treatment that supplied comparable level of available N. The potential for Zn and other metal accumulation was reduced through the use of cover crop during winter, resulting in N credit and hence reduction in the amount of PL applied in the subsequent years. Cornstalk nitrate levels indicated that the PL and AS treatments provided a sufficient amount of N to the corn, but PL was consistently more reliable than the AS1 treatment over the three-year time. Overall, the PL treatment had comparable soil $NO_3^-$–N concentration in the 15-30 cm depth as that of the Control, suggesting that the PL treatment did not significantly increase the risk of post-season $NO_3^-$-N loss (via leaching), unlike AS1 and AS2. As opposed to the AS treatments, the nitrogen from PL is mineralized slowly over time and could be susceptible to leaching after harvest. It is most likely that the use of cover crop along with PL prevented this from happening. The normalized ACE coefficient provided further support that AS1 and PL were the most efficient management systems in terms of inorganic N post-season, and AS2 was the most likely to have conditions favorable to nitrate leaching losses over the three-year study

Studies comparing conventional N management to organic management with poultry litter incorporated in the soil are lacking. Our results show that an organically managed cropping system (with a combination of poultry litter and cover crops as N amendments) was competitive with conventionally managed systems in terms of yield and other plant parameters. Further study

is needed to develop best management practices for incorporation of poultry litter as a fertilizer and as a means of waste disposal.

The N mineralization subroutine modified from CERES-N has been tested and modified previously (Vigil and Kissel, 1995; Quemada and Cabrera, 1995), but these authors used smaller sets of N mineralization data from their laboratories. In the present study, a more robust set of N mineralization data from eight different studies were used to test the model and to modify the rate constants for decomposition of the three plant components, non-structural carbohydrates, cellulose, and lignin to give the best possible estimation of the available data of N mineralized. The data from the seven studies in which crop residues were incorporated had a wide range of properties, most significantly the % N which ranged from 0.5 to 5.45%, and the % lignin, which ranged from 3.8 to 30.6%. The samples with high lignin and cellulose led to using an equation for the cellulose component that slowed its decomposition considerably at higher levels of lignin, which allowed better agreement for those samples.

In the validation field study, simulated values of *in situ* net N mineralization from cover crops fell within the 95% CI of measured values for all but one sample time. N mineralization from incorporated sources was generally more accurate over a wider range of statistical parameters than from surface-applied. Overall, prediction was most accurate for crimson clover incorporated in 2013 and least accurate for crimson clover surface-applied in 2013. According to our results CERES-N can be a valuable tool for managing N inputs in a Georgia Ultisol. Further study is needed to assess the prediction capacity of the model for different soil types, under ranges of climactic conditions, and with different cover crops. Future work should also address the potential incorporation of other driving factors for the model for surface-applied crimson clover including water potential and/or relative humidity.

Overall, the different systems of nitrogen (N) management affected ammonia oxidizer abundance and function. AOB increased in response to N additions. AOA estimates of abundance based on *amoA* gene copy numbers were higher than (or no different from) AOB abundance for all treatments, with the highest AOA to AOB ratios in Control, 14.1 and 3.5 for 2013 and 2014, respectively, and in PL, 2.7 in 2013. Abundance was influenced by the type and application rate of the N source. Even though archaeal *amoA* abundance was higher than bacterial in most instances, bacterial *amoA* abundance had the most significant correlation with nitrate concentration and nitrification potential. This is indicative of AOB being more responsive to N additions in terms of abundance and two indicators of activity. The ratio of AOA to AOB was highest each year in the Control where the substrate for nitrification was primarily mineralization from soil organic matter. Ratios were lower in the AS treatments in y 2 and in all three N-added treatments in the first and last years of the study. Lower AOA to AOB ratios appear to occur in soils with readily-available ammonium such as inorganic N fertilizers. AOB abundance increased in response to increased substrate availability while AOA generally showed no difference among treatments. The differential response of AOB and AOA suggests the need for targeted approaches to maximize N use efficiency in the two systems, likely focusing on AOB for short-term conventional N management using inorganic N fertilizers and AOA for organic N management using fresh manure.

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