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NITROGEN AVAILABILITY AND USE EFFICIENCY IN CORN TREATED WITH
CONTRASTING NITROGEN SOURCES

by

Avneet Kakkar

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

Jeanette M. Norton, Ph.D.
Major Professor

Jennifer R. Reeve, Ph.D.
Committee Member

David Hole, Ph.D.
Committee Member

Mark R. McLellan, Ph.D.
Vice President for Research and
Dean of the School for Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2017

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ABSTRACT

Nitrogen Availability and Use Efficiency in Corn Treated with Contrasting
Nitrogen Sources

by

Avneet Kakkar, Master of Science

Utah State University, 2017

Major Professor: Dr. Jeanette M. Norton
Department: Plants, Soils, and Climate

Nitrogen (N) is one of the essential macronutrients needed for growth and reproduction of all plants. N is required in relatively large quantities for corn production and is often considered as the limiting nutrient for crop production and yield. Most croplands require additional N to that existing in the soil to achieve maximum yields. For this reason, agricultural systems often receive additional N in the form of fertilizers. The proper management of different N sources can improve N use efficiency (NUE) which is considered key to agroecosystem sustainability. Improved understanding of the interacting processes determining NUE is essential to increase sustainability in crop systems and thereby promote food security.

We examined NUE in an irrigated silage corn field in Northern Utah (Site 1 N cycle plots) under different N source treatments over 5 years. This small plot study compared a control (no N amendment) with ammonium sulfate at two levels of N

application (112 kg N per hectare (AS100) and 224 kg N per hectare (AS200)) and steer compost at the rate of 224 kg total N per hectare as sources of N. We determined the treatment effects on yield, plant N uptake, soil N availability, and NUE. We found that the inorganic N treatments of AS100 and AS200 had higher yields as compared to the organic N treatment, compost. N uptake was the highest in AS200 (186 kg N/ha) followed by AS100 (115 kg N/ha) and compost (80 kg N/ha). N uptake increased over time in the compost treatment for the first three years but then declined in the last two years. The AS200 treatment had the highest NUE (62%) overall followed by AS100 (60%) and compost (14%). The NUE rates in compost treatment were the lowest and witnessed a gradual decline over the years. The corn was likely nitrogen deficient in all the treatments as indicated by in season plant tissue analysis.

The study of nitrogen mineralization and nitrification is essential for an understanding of processes occurring in the soil that affect availability of N to plants. Mineralization and nitrification were examined in soils from Site 1 and from a nearby long-term organically managed experiment studying N source, cover crops and crop rotations (Site 2 Organic rotational plots). The N source experiment at site 2 compared a control (no amendment) with steer manure and steer compost both applied at the rate of 224 kg total N ha⁻¹. We conducted 84-day laboratory incubations with soils sampled late season in 2015 to determine carbon mineralization, net nitrogen mineralization and net nitrification rates. Carbon mineralization rates were found to be higher in compost under Site 1 and compost and manure under Site 2 as compared to control. There was a significant increase in net N mineralization and net nitrification with compost and AS200

at site 1 and with manure and compost treatments at site 2. There was a similar increase in net nitrification rates with N amendment for both the sites.

Estimates of gross mineralization rates and gross nitrification rates in agricultural soils are crucial where production and consumption processes occur simultaneously. Laboratory ^{15}N isotope dilution experiments were conducted with the Site 1 soils sampled in August 2015 and June 2016 for determination of gross mineralization and nitrification rates. We found that gross mineralization and gross nitrification rates were not significantly affected by treatment in 2015 (average $3.5 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ and $1.2 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ respectively). Similar to the results in 2015, gross mineralization rates were not statistically different by treatment in 2016 (average $1.7 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$). Gross nitrification rates were found to be higher in the AS200 treatment versus control or compost. Approximately 30 % of the nitrification in the AS200 soils from 2016 was attributed to ammonia oxidizing bacteria. There was high variability in the gross nitrogen transformation rates in compost treatments of both years indicating presence of hot spots of labile organic matter or N immobilization.

The results obtained from our study focus on the need for optimization of fertilizer rates and their timing for corn production to increase crop yields sustainably. To improve NUE, it is necessary to follow integrated management strategies that take into consideration improved fertilizer and soil and crop management practices. Also, results from our second study indicate that the compost treatment significantly increased the C and N mineralized under conventional management while the impacts were less obvious under organic management that included crop rotations and cover crop inputs. The high

variability in N transformation rates in compost treatment (2016) might be due to presence of hot spots of mineralization, immobilization and nitrification.

(134 pages)

PUBLIC ABSTRACT

Nitrogen Availability and Use Efficiency in Corn Treated with Contrasting
Nitrogen Sources

Avneet Kakkar

The plant-soil nitrogen cycle plays a significant role in allocation of available N to plants, and improved understanding of N cycling helps sustainably increase fertilizer use efficiency. There are various processes (nitrogen mineralization and nitrification) involved in the availability and mobility of nitrogen in the soil. The primary objective of this study was to determine the NUE under contrasting nitrogen treatments over a period of five years. Additionally, we examined the effect of different N treatments on N mineralization and nitrification in conventional and organic farming systems.

This project was funded by Agriculture and Food Research Initiative Competitive Grants Program Grant no. 2011-67019-30178 from the USDA National Institute of Food and Agriculture and by the Utah Agricultural Experiment Station. We established silage corn field plots in northern Utah, and silage corn was grown using ammonium fertilizers or manure composts over five years. Nitrogen use efficiency was found to be higher in ammonium sulfate fertilizer treatments as compared to compost treated soils. Nitrogen mineralization and nitrification rates were examined for soils from the silage corn field plots and also for additional soils from certified organic field plots receiving steer compost, steer manure and crop rotations. There was a significant overall nitrogen

treatment effect for both conventional and organic rotational plots. Carbon mineralization rates were found to be higher in compost under conventional plots and manure under organic rotational plots as compared to control. There was no significant treatment effect found in gross mineralization and nitrification rates in 2015 and 2016. Gross nitrification rates were found to be the higher in AS200 treatment versus compost and control in 2016.

Improved knowledge of the timing and rates of nitrogen supply is vital for improving NUE and for reducing excessive use of fertilizers while maintaining an acceptable yield. The optimization of fertilizer rates according to crop demand at different stages of growth will be helpful in the efficient management of available N especially for composts and manures.

DEDICATION

This thesis is dedicated to my grandfather, Sh. K.L. Kakkar. Thanks for all your love and guidance throughout my life, without which I would not be the person I am today. I know your blessings are always with me. I will try my best to be the most hardworking person that you wanted me to be. I will always miss you.

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This project was funded by Agriculture and Food Research Initiative Competitive Grants Program Grant no. 2011-67019-30178 from the USDA National Institute of Food and Agriculture and Utah Agricultural Experiment Station.

My loving family has always been very supportive. I am profoundly thankful to my grandmother Smt. Chand Rani, my father, Sh. Udesch Kakkar and my mother Darshana Devi Kakkar for their selfless love and support throughout my life. I would also like to thank my sisters, Dr. Navneet Kakkar and Dr. Kirandeep Kaur for their valuable feedback and constructive criticism on various aspects of my study. I am highly indebted to my

uncle, Sh. Ramji Das for believing in me and motivating me to opt for a Bachelors' degree in Agriculture Sciences. I am grateful to my elder brother Mohit Dhawan for all the financial support and helping me get settled in the United States. Thanks for always being there for me. Special thanks to my friend Dr. Daljit Singh for his valuable feedback and encouragement during my entire Master's program.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Corn is one of the most important crops in the United States and worldwide. Globally, 40.8 billion bushels of corn were produced in 2016-17. In the United States (US) in 2016, corn was planted on 94 million acres with 86.7 million acres planted for grain while 6.1 million acres of land was used for silage corn. The harvested land under corn has increased from 67 million to 87 million acres in the US in the past 80 years from 1936 to 2016. In 2016, the average yield was around 175 bushels/acre, and total US production was 15.1 billion bushels. In Utah, 90,000 acres of land was used for corn production out of which 29,000 acres of corn was used for grain production. The total production in Utah (2016) was around 5 million bushels, and average yield was 175 bushels/acre (National Corn Growers Association, World of Corn 2017).

Nitrogen (N) is one of the essential macronutrients needed for plant growth and reproduction. N is required in relatively large quantities for corn production and is often considered as the limiting nutrient for growth and high yield. Deficiency of N results in stunted growth, low chlorophyll contents, yellowing of older leaves and low crude protein in plants. The immediately available N in agricultural soils is primarily in the inorganic forms of ammonium and nitrate. Soil organic N is a reserve that is made available to the plants through mineralization over time. Nitrogen is abundant as N₂ gas in the atmosphere, but this form is not readily available to plants. Nitrogen gas may be made available to plants by biological N fixation which is performed by certain bacteria

and archaea such as *Azotobacter*, *Rhizobium*, and many others. Most croplands require additional N to that existing in the soil to achieve maximum yields. For this reason, agricultural systems often receive additional N in the form of fertilizers.

Sources of N fertility for crops include inorganic N fertilizers such as ammonia, urea, and nitrates, organic N fertilizers such as manure and compost, N₂ fixing cover crops and soil reserves. Worldwide nearly 56 percent of the inorganic N fertilizer is used for the production of cereal crops including rice, maize and wheat (IFA 2002). N fertilizers have resulted in an approximately 40% increase in per capita food production in the past 50 years (Brown 1999; Smil 2002). These cereals and other crops use about 50 percent of the N applied for producing biomass while the remaining N is stored in the soil or dissipated to the environment causing many environmental and ecological side effects (Galloway and Cowling 2002). NUE may be defined as the ratio of the crop N uptake to the total input of N fertilizer. In this study, we examined the NUE in silage corn receiving contrasting N sources over the period of 5 years (Chapter 2).

The goal of our study was to evaluate NUE in silage corn treated with contrasting N treatments and understand the factors affecting NUE. Additionally, we aimed to investigate the N mineralization rates under different N treatments by conducting long-term laboratory incubation experiments with soils from two experimental sites. Improved knowledge of the N mineralization rates will increase our understanding of crucial processes of making N available to plants. Eventually, understanding of the N cycling processes affecting N availability will help in formulating management techniques and policies to improve NUE in cropping systems.

LITERATURE REVIEW

Nitrogen Use Efficiency

Nitrogen use efficiency represents the ratio between the amount of fertilizer N removed from the field by the crop and the amount of fertilizer N applied. As the global human population is expected to reach 9.6 billion by 2050 and with little new land suitable for cultivation of crops, it is essential to increase the yield per unit area of major cereals (Gerland et al., 2014). Plants often take up only 20 to 50% of the N fertilizer applied to soil for cereal crop production. NUE may vary from 30% or lower in the case of rice production up to 70% in case of intensive maize production (Cassman et al., 2002). About 85 million Mt of nitrogenous fertilizers were applied globally in 2002 (FAO, 2004). The demand for N fertilizers was 116 million Mt in 2016 and is expected to increase in the future (FAO 2015). It is possible to increase the yields in most of the areas around the world by applying additional N fertilizer or by increasing NUE in plants. For decades, improving NUE has been a primary goal for crop and soil scientists (Raun and Johnson, 1999; Raun et al., 2002; Khosla et al., 2002). Increased knowledge of different plant mechanisms is vital for improving NUE and for reducing excessive use of fertilizers while maintaining an acceptable yield.

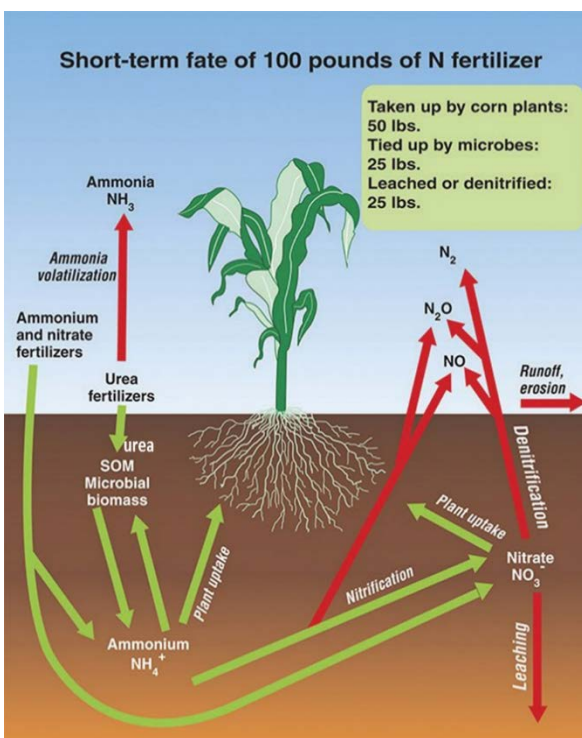


Fig. 1. A simplified Nitrogen cycle showing the typical fate of 100 pounds of N fertilizer applied to a corn field. (Millar et al., 2014).

A simplified N cycle depicting the typical fate of 100 pounds of ammonium or urea fertilizer applied to a corn field is shown in Fig. 1. Fertilizer enters the soil as ammonium, some of which is taken up by the plants while some part enters soil organic matter (SOM) assimilated by heterotrophic microbes. The ammonium may be converted into nitrate by the process of nitrification. Nitrate produced by this process is also available for plant uptake although this nitrate is also prone to loss via leaching and denitrification. Typically, about 50% of the fertilizer applied is taken by plants while 25% is added to the SOM (mostly through the microbial biomass) and the remaining 25% is lost via leaching and denitrification.

Factors Affecting NUE

Factors that influence the N-assimilation into crop plants and N loss from soil systems alter NUE. Environmental factors such as temperature, rainfall, soil texture, etc. affect plant growth and development, soil N availability and soil N losses such as leaching and denitrification. Crop management plays a vital role in determining N loss (Neeteson et al., 1998) which includes factors like cropping density and spatial arrangement of plants (Shapiro and Wortmann, 2006), placement and timing of N fertilization rate and application methods (Li, 2003; Ma and Kalb, 2006; Osborne, 2006), and water management (Battilani et al., 2003; Remie et al., 2003; Singandhupe et al., 2003). The simultaneous management of fertilizer and water by fertigation is of special interest for NUE in intensively managed systems.

NUE is affected by plant factors like N uptake efficiency and absorbed efficiency. The former is the ability of crops to take up N from the soil (Burns, 2006; Greenwood et al., 1989), and latter is defined as the efficiency with which absorbed N is used by crops to grow and provide yield (Janssen, 1998; Schenk, 2006). These efficiencies may differ within the same crop as they are dependent on various organs and processes and environmental factors as well. Additionally, different species and cultivars are believed to play a key role. N uptake and use of absorbed N are affected by the genotype because each genotype has its own morphological and functional traits for roots, leaves, etc. (Schenk, 2006; Thorup-Kristensen and Sørensen, 1999; Thorup-Kristensen and Van der Boogard, 1999). While more N is typically recovered by crops under low fertilizer N rates; NUEs may vary at different levels of available N even within the same genotype (Burns, 2006).

Large losses of N from the soil through leaching, ammonia volatilization or denitrification results in low NUE (Raun and Johnson 1999). The N losses mainly occur through the processes of: (1) ammonia volatilization: release of ammonia gas into the atmosphere that is more prevalent in high pH soils, (2) nitrate leaching: mass flow of nitrate out of root zone and (3) denitrification: natural soil microbial process where nitrate is transformed to N gases which are lost to the atmosphere. The use of urea or ammonia fertilizers as the main fertilizers leads to N losses via ammonia volatilization whereas losses through leaching and denitrification occur from soils where nitrate forms are dominant (Hubbell 1995). Denitrification occurs at a much faster rate in warm, wet soils with higher nitrate levels. The use of high rates of inorganic N fertilizers may result in inorganic N accumulation in the soil and promote N loss. These N losses from soil systems reduce soil fertility, crop yield and adversely affect the environment.

Previous studies have investigated the optimization of fertilizer rates and how to reduce N losses (Agostini et al., 2010; Burns, 2006; Neeteson and Carton, 2001; Rahn, 2002). Rahn (2002) studied factors including plant-soil interactions, plant N demand and root structure of plants which need to be understood for reducing N losses in crops and making site-specific recommendations. Losses via ammonia volatilization from fertilizers or manures can be controlled by soil incorporation along with tillage or by subsurface injection. Losses can be reduced by 50 to over 90% compared to the surface application (Thompson and Meisinger, 2002; Powell et al., 2011). N losses and availability are also affected by the timing of manure applications. Laboski et al., (2013) conducted a four-year experiment to evaluate the effect of dairy manure application methods on corn yield, fertilizer N credits, and N losses. They found ammonia volatilization decreased with

immediate incorporation or injection of manure, but higher losses by N₂O emissions occurred. Under the use of injection methods, more N was available to corn even when less fertilizer was applied which led to economic and environmental benefits.

Methods for Calculating NUE

Several methods are used for calculating NUE in cropping systems around the world. Some relevant methods include the difference method and the isotopic method (using depleted or enriched fertilizer material) (Raun et al., 2002). Difference method uses the formula described below:

$$\text{NUE} = (\text{NF}) - (\text{NC}) / \text{R} \quad (1)$$

NF = total N uptake in corn from N fertilized plots

NC = total N uptake in corn from unfertilized plots

R = rate of fertilizer N applied

Isotopic method (Depleted material) involves the following equation to calculate NUE:

$$\text{NUE} = ((\text{NF}) \times (\text{C} - \text{B}) / \text{D}) / \text{R} \quad (2)$$

B = atom % 15N of plant tissue from N fertilized plots

C = atom % 15N of plant tissue from unfertilized plots (0.366% natural abundance)

D = depleted atom % 15N in applied N fertilizer

Lastly, NUE can also be calculated using the isotopic method (enriched material)

which is described below:

$$F = \frac{A_s - A_r}{A_f - A_r} \quad (3)$$

F = fraction of total N uptake derived from ^{15}N enriched fertilizer

A_s = atom % ^{15}N measured in the harvested plant sample

A_f = atom % ^{15}N in the enriched fertilizer

A_r = atom % ^{15}N of the reference harvested plant material from non ^{15}N enriched fertilizer treatments

$E_f = F \times \text{total N uptake}$

E_f = uptake of ^{15}N enriched fertilizer

We have used the difference method in our study to calculate NUE rates for all five years (2012-2016). Despite the type of method used, most results in estimated mean NUEs ranging between 30 and 35% for cereal production. However, mid-season topdress or side-dress N fertilizer applications can increase the NUE to more than 50% in crops (Vetsch and Randall, 2004).

Improvements in NUE

The goal of improvement of NUE of crops is to achieve high yields with reduced N fertilization rates. Some approaches that are used to increase the fertilizer NUE include: optimal utilization of fertilizer through adjusting rate and method of application,

improved matching of crop demand with the supply of N, providing the irrigation water with the fertilizer, split application schemes, using the fertilizer according to the conditions in the field, use of slow release fertilizers and minimizing applications in the wet season to reduce leaching (Ladha et al., 2007). NUE can be further increased through the use of cultivars with improved nitrogen acquisition and internal-use efficiency traits. In USA, NUE in corn increased from 42 to 57 kg grain kg N⁻¹ between 1980 and 2000 (Cassman and Dobermann et al., 2002) and several factors were involved in this were (1) modern maize hybrids with greater stress tolerance; (2) effective and improved management techniques such as higher plant densities, conservation tillage, better quality of seeds, pest and weed control; and (3) improved matching of the applied N and crop demand (Cassman and Dobermann et al., 2002). The goal of increasing NUE and reducing N losses should be accomplished at the farm level using improved technologies and farmer friendly policies (Dobermann, 2005).

Nitrogen Fertility Sources for Crops

Many N sources are available for supplying N to crops. These sources include inorganic N fertilizers such as ammonia, urea, and nitrates, organic N fertilizer amendments such as manure and compost, SOM reserves and biological N₂ fixation. Most inorganic N fertilizers are readily available for plant uptake from the soil solution. In contrast, most N from organic sources is less readily available because it needs to be released by mineralization to the soil solution before plant uptake. Biological N₂ fixation by legumes and residues from cover crops are a major source of N for crop production.

Long-term application of one type of fertilizer may be harmful to crop sustainability. For example, over-reliance on inorganic fertilizers in farming systems can decrease SOM and deteriorate the soil fertility and quality. While modest applications of organic fertilizers generally reduce the potential for nitrate leaching, over-application of organic fertilizers over the long term can also have adverse effects on the crop sustainability. According to a review by Edmeades (2003), several long-term experiments showed that soils treated with cattle manure had increased levels of OM, P, K, Ca and Mg in the surface soil, and increased levels of nitrate-nitrogen (NO_3^- -N), Ca and Mg in the subsoil, in comparison with soils treated with inorganic fertilizers. In the Broadbalk experiment, Goulding et al., (2000) witnessed greater N leaching loss from the plots treated with farmyard manure (FYM). Fall applications of manures may contribute to this problem because N mineralized in fall and early winter may not be used by the crop, and thus is subject to leaching. The increased porosity in manured soils may also facilitate nitrate leaching.

The efficient use of organic and inorganic fertilizers may lead to more sustainable nutrient management systems. After reviewing 14 long-term experiments conducted in North America and Europe and having yield data for over 20 years, Edmeades (2003) reported that under well managed systems, manure application can give similar crop yields to inorganic N fertilizers. In another 28-year long-term experiment conducted in India, Hati et al. (2007) found that combined application of NPK and FYM resulted in significantly higher soybean and wheat yields as compared to NPK treatment alone but also increased soil organic carbon (SOC) by 56.3% in comparison to the initial SOC value. In a long-term maize-wheat-cowpea experiment started in 1971 in India,

Kanchikerimath and Singh (2001) witnessed increases in the crop yield, SOC, total N, mineralizable C and N, microbial biomass C and N, and dehydrogenase, urease, and alkaline phosphatase activities under the inorganic fertilizer (NPK) treatment, however, manure applied together with inorganic fertilizer increased these parameters more strongly. There is considerable evidence indicating that the combined application of NPK fertilizers and organic fertilizers (NPK+OF) generally results in higher crop yields than either of these applied alone (Poulton, 1995; Greenland, 1997; Manna et al., 2007; Hati et al., 2007; Bhattacharyya et al., 2008; Xu et al., 2008; Pan et al., 2009; Zhang et al., 2009a, b, c; Diacono and Montemurro, 2010; Zhao et al., 2010).

Previous Studies of NUE in Corn

Several studies have examined the NUE in corn over the last several decades. In a study in North Dakota, USA, Wienhold, et al., (1995) studied effects of irrigation and N fertility levels on growth and NUE by corn using a nitrogen-15 isotope approach. Three different levels of irrigation were used in this study: precipitation plus irrigation equal to one, two, and three times the calculated evapotranspiration (ET) rate. The ^{15}N -enriched fertilizer was applied at rates equivalent to 100 and 200 kg N ha⁻¹ and NUE was determined by recovery. They found that grain and dry matter yields, N content, and utilization of fertilizer N all showed annual variations, which might be the result of yearly weather patterns, especially temperature. On average, 35% and 15% of applied fertilizer at the rate of 100 kg N ha⁻¹ and 200 kg N ha⁻¹ was used by grain and stover, while 30% remained in the upper 0.6 m of the soil profile at the end of the growing season. Approximately, 20% of the applied fertilizer was lost via leaching or denitrification. They

found that under higher mean temperatures, corn responded to increasing N fertility with 60% greater yields, 75% greater N content, and 60% higher percentage N derived from fertilizer with the higher N fertility treatment. One study (Liang and MacKenzie 1994) was conducted in southwestern Quebec from 1988 to 1990 where they studied the fertilizer NUE in corn. Two sites with contrasting soil textures were chosen to get an estimate of optimum N fertilizer rate for corn production. They found that maximum grain yields of corn at 300 to 350 kg N ha⁻¹ fertilizer application, with optimum economic rates at 179-273 kg N ha⁻¹. Large amounts of N fertilizer at both the 285 kg ha⁻¹ and 400 kg ha⁻¹ were not recovered by the crop. Total fertilizer nitrogen recovery (FNR) varied from 30-58% at the 170 kg N ha⁻¹ rate while from 23-40 % at the 400 kg N ha⁻¹. Habbib et al., (2016) conducted a study in northern France to evaluate the combined impact of N fertilization and tillage on various NUE related traits like N harvest index, N remobilization and N remobilization efficiency and grain yield in maize cultivated in the presence of a cover crop. Four years after conversion to no-tillage, they found a significant increase in NUE and N harvest index under both no and high N fertilizer conditions. Furthermore, they found that grain yield and grain N content were higher under no-tillage conditions only when N fertilizers were applied. Hence, continuous no-tillage practices appear to be promising for increasing NUE in maize.

There is a common belief among the growers that high rate of N fertilizer is an "insurance" for crop yields, but it has been found that reduced application of N fertilizer along with crop rotation in maize has resulted in good yields. A 3-year experiment was conducted by Montemurro et al., (2006) in Italy under Mediterranean conditions on two maize-barley crop rotations to study N indicators, uptake and use efficiency under five

contrasting N treatments. The treatments included mineral N (200 kg N ha^{-1}); organic-mineral N (100 kg N ha^{-1} as olive pomace compost plus 100 kg ha^{-1} of mineral N); mineral N (100 kg N ha^{-1}); organic N (100 kg N ha^{-1} as olive pomace compost); and an unfertilized control. Winter barley was cultivated without fertilization. They found that by using organic fertilizer, as partial substitution of mineral N, similar yields were achieved as by the highest mineral fertilizer treatment in both the crops. Additionally, NUE indices did not change under highest N treatments in both the crops. The N uptake was strongly affected by the high amounts of available soil N during the growing seasons (57.4% in first and 45.2% in the second season) and this N uptake further affected the yield and NUE. The N indicator in case of maize was pre-sowing soil mineral N whereas in the case of barley, mean stem nitrate content was the best N indicator showing a linear relationship with the yield. Therefore, under high fertility conditions, crop rotation could be an important agronomical tool to increase NUE and minimize possible N losses. While most previous NUE measurements have focused on inorganic fertilizers, the role of organic nitrogen sources and mineralization of reserves of SOM are increasingly realized to impact N uptake by crops and therefore NUE.

Nitrogen Mineralization

Nitrogen mineralization is the process by which organic N from various sources like organic matter, crop residues and manures are converted to plant-available inorganic forms by microbial decomposition. Soil N is present in four major forms: (1) SOM such as plant and animal residues and stabilized humus; (2) living organisms and microorganisms; (3) ammonium ions held in clay interlayers and (4) mineral N forms in

soil solution, including ammonium, nitrate and low concentrations of nitrite. N mineralization transforms large and complex organic N compounds to simple N monomers or ammonium. Mineralization is one of the key processes that enables plant growth by releasing nutrients to plants in available form.

N mineralization in cropping systems exhibits high spatial and temporal diversity (Knoepp & Swank, 1998), which is controlled by factors including temperature (Guntinas et al., 2012), moisture (Paul et al., 2003), and land use type (Templer et al., 2005). Growers need estimates of N available from mineralization to effectively manage soil N. The study of nitrogen mineralization rates in different soils will help to develop simple tools and models for estimation of field-specific N mineralization rates.

Different Models in N Mineralization

Several models have been used for estimating N mineralization rates under different conditions (Benbi et al., 2002). We studied N mineralization by conducting long-term incubations under controlled conditions. The mineralizable N pool is defined as the amount of N present in soil which is released over a period and is often expressed in mg N /kg soil. Potential mineralizable N is defined as the amount of N that mineralizes under optimum and constant environmental conditions and is often estimated by fitting a first-order kinetic model to inorganic N concentrations over time during a laboratory incubation (Stanford & Smith, 1972). The equation used in the first order model is described below:

$$N_t = N_o(1 - e^{-kt}) \quad (4)$$

N_t = mineralizable N present at time t

N_o = mineralizable N initially present

k = first order rate constant

t = time

Multi-fraction first order models have also been used for estimation using the following double exponential equation:

$$N_t = N_o S(1 - e^{-ht}) + N_o(1 - S)(1 - e^{-kt}) \quad (5)$$

where S and $(1 - S)$ represent the labile and recalcitrant organic N fractions getting decomposed at specific rates h and k , respectively.

The other models used for describing N mineralization kinetics in soils are non-compartment models, multi-compartment models, and food web models. The two compartment models like First Order Double Compartment (FODC) are useful for modeling N dynamics in soils and comparing data in tabular forms. Multi-compartment models are based on three or more organic N pools and are difficult to validate as most of the functional pools cannot be evaluated by physical, chemical or biological techniques. Under food web models, organisms are classified as functional groups, and their consumption rates are used to determine N mineralization rates (Benbi et al., 2002).

Methods for Estimating N Mineralization Rates in Soils

The process of mineralization may be further described by its net, potential and gross mineralization rates (Norton and Schimel, 2011). The net mineralization rate is the outcome of two opposite processes: gross mineralization (N release) and immobilization (N assimilation) by the micro-organisms. Net mineralization is positive when the gross rates of mineralization are higher than rates of combined consumptive processes (i.e., immobilization plus losses). The change in soil inorganic N pool size over a specified period estimates the net rates of mineralization (NMR) (Hart et al., 1994a). The net rate of mineralization which occurs during the absence of plant uptake and leaching is called potential mineralization rate. The laboratory procedures used for estimating net and potential mineralization rates are built on a primary assertion that quantity of accumulated inorganic N is estimated over a particular period under a defined temperature and moisture conditions (Norton and Schimel, 2011).

$$\text{Net Mineralization} = (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_{t+1} - (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_t$$

Net rates can also be defined as:

Net N mineralization =

Gross N mineralization – microbial immobilization of inorganic N (in the absence of plant uptake and denitrification)

Due to its simple procedure, net mineralization is extensively used as an indicator of N availability in soils (Schimel and Bennett, 2004). Nonetheless, net mineralization

ignores the prospective role of inorganic N assimilation by microorganisms and denitrification, and the yield and uptake of simple organic compounds by both plants and microorganisms (Norton and Schimel, 2011; Schimel and Bennett, 2004). Comparing net and gross rates of mineralization and nitrification may allow for better insights into the effects of nitrogen sources on availability.

Nitrification

Nitrification is the biological oxidation of ammonium to nitrite or nitrate. Nitrate is more mobile than ammonium because it is negatively charged and is repelled by cation exchange sites. Therefore, nitrate is more easily lost via leaching and denitrification than ammonium (Norton, 2008; Prosser, 1990). Nitrification occurs at a faster rate in moist, warm and well-aerated soils. There are mainly three types of bacterial groups involved in this process: (1) autotrophic ammonia oxidizers, (2) autotrophic nitrite oxidizers and (3) heterotrophic nitrifiers. The first two groups oxidize ammonia to nitrate under aerobic conditions whereas heterotrophic nitrifiers oxidize organic compounds to produce nitrate (NO_3^-) or nitrite (NO_2^-). The accessibility and activity of N in soils are considerably controlled by the N transformations of mineralization and nitrification.

Methods for Estimating Nitrification Rates in Soils

There are several methods to measure nitrification rates in soils. Some of them involve field and laboratory measurements. In our study, we determined the net nitrification rates (NNR) by conducting aerobic incubation experiments similar to those used to study mineralization described earlier. Net nitrification rates are calculated by

subtracting initial nitrate concentrations from the final values. Estimates of NMR and NNR are helpful in the evaluation of potential N losses from the ecosystems and the availability of N for plant uptake (Norton 2000).

RESEARCH RATIONALE AND HYPOTHESES

Nitrogen is required by all the crops for their growth and development, thus affecting the crop yield. Cereal crops also need N for storing protein in grains. In modern agricultural systems, most supplemental N is applied in the form of inorganic fertilizers, while some is applied in organic forms like urea, compost, farm yard manure, etc. The use of chemical N fertilizers first started in the 19th century and increased greatly with the development of Haber-Bosch process. Currently, more than 50% of the chemically fixed N is used by agriculture. The efficient use of N is important for sustainable agriculture production. At present, typically less than 50% of the applied N is taken up by plants leading to low N use efficiencies (NUE). The lower NUEs can be due to over application of chemical N fertilizer causing N losses, and poor synchrony between crop N demand and N supply. Improved understanding of N cycling is required to understand the different processes involved in the release of N to plants, plant N uptake and the use of N by plants for growth. Predictions of N mineralization help in understanding the rates at which N is being made available to the plants for uptake.

Our study investigates the NUE in silage corn receiving contrasting N sources over the period of five years (Chapter 2). We examined the N mineralization rates in two sites under different N treatments by conducting long-term laboratory incubation

experiments at different time intervals in the soil and pool-dilution measurements of N mineralization and nitrification (Chapter 3). We believe that the knowledge gained from this study will improve our understanding of N uptake and cycling under contrasting N fertility sources. The long-term goals of this work are to understand approaches useful for improving NUE. The information from this study may be helpful in improving N management strategies so that we can maximize plant yields while keeping our environment safe. The study of N mineralization processes will help in improved understanding of N cycle which is beneficial for sustainable agricultural production. The sites selected for conducting these studies allowed for replicated experimental design and site maintenance over the period of five years (2012-2016).

Research Objectives

Objective 1: To evaluate the NUE in silage corn over several years in the same system (Chapter 2).

Hypothesis 1: Higher level ammonium additions will have lower NUE.

Objective 2: To evaluate N uptake by silage corn in plots under contrasting N management (Chapter 2).

Hypothesis 2 (a): The annual N uptake will differ by treatment.

Hypothesis 2 (b): The N uptake from compost treated soils will increase over time (years).

Objective 3: Determine the effects of contrasting N management treatments on the amount and rate of N mineralization in laboratory incubations and using N-15 pool dilution rate determinations (Chapter 3).

Hypothesis 3 (a): After multiple years of compost amendment, mineralization rates will be higher in these soils. Nitrification rates will be higher in soils receiving ammonium sulfate fertilizers.

Hypothesis 3 (b): Higher mineralization rates will result in increased NUE over time in compost-treated plots.

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CHAPTER II

NITROGEN USE EFFICIENCY IN CORN TREATED WITH CONTRASTING NITROGEN TREATMENTS¹

ABSTRACT

Improved understanding of mechanisms and interacting processes determining nitrogen use efficiency (NUE) is essential to increase sustainability in crop systems. A silage corn field was treated with ammonium sulfate at two levels of nitrogen (N) application of 112 kg per hectare (AS100) and 224 kg per hectare (AS200) and compost at the rate of 224 kg per hectare as sources of nitrogen over five years. The N pool sizes were observed to study the effects on yield, N availability, and NUE. We found that the inorganic N treatments of AS100 and AS200 had higher yields as compared to the organic N treatment of compost. N uptake was the highest in AS200 (186 kg N/ha) followed by AS100 (115 kg N/ha) and compost (80 kg N/ha). N uptake increased over time in compost for the first three years but then declined in the last two years. The AS200 treatment had the highest NUE (62%) overall followed by AS100 (60%) and compost (14%). The NUE rates in compost treatment were the lowest and witnessed a gradual decline over the years. All the treatments were likely N deficient as indicated by in season plant tissue analysis. The results obtained from this study focus on the need for optimization of fertilizer rates and their timing for corn production to increase crop yields sustainably. To improve NUE, it will be important to follow integrated management

¹ Avneet Kakkar, Jeanette Norton

strategies that take into consideration improved fertilizer, soil and crop management practices.

INTRODUCTION

Corn is one of the most important crops in the United States and the world. Nitrogen is required in relatively large quantities for corn production and is often considered as the limiting nutrient for growth and high yield. Deficiency of N results in stunted growth, low chlorophyll contents, yellowing of older leaves and low crude protein in plants. The immediately available N in agricultural soils is primarily in the inorganic forms of ammonium and nitrate. Soil organic N is a reserve that is made available to the plants through mineralization over time. Nitrogen is abundant as N₂ gas in the atmosphere, but this form is not readily available to the plants. Nitrogen gas may be made available to plants by biological N fixation which is performed by certain bacteria and archaea such as *Azotobacter*, *Rhizobium*, and many others. Most crops require additional N to that existing in the soil to achieve high yields. For this reason, agricultural systems often receive additional N in the form of fertilizers.

Sources of N fertility for crops include inorganic N fertilizers such as ammonia, urea, and nitrates, organic N fertilizers such as manure and compost, N₂ fixing cover crops and soil reserves. Worldwide nearly 56 percent of the inorganic N fertilizer is used for the production of cereal crops including rice, maize and wheat (IFA 2002). Unfortunately, the recovery of fertilizer nitrogen (N) in corn cropping systems is generally low (e.g., mean N recovery efficiency = 37 [Cassman et al., 2002] and 41% [Kaizzi et al., 2012]). This low nitrogen recovery is generally caused by the loss of

fertilizer N through leaching below the root zone, ammonia volatilization and denitrification.

Nitrogen use efficiency (NUE) is defined as the ratio of the crop N uptake to the total input of N fertilizer. As the global human population is expected to reach 9.6 billion by 2050 and with little new land suitable for cultivation of crops, it is essential to increase the yield per unit area of major cereals including corn (Gerland et al., 2014). Plants often take up only 20 to 50% of the N fertilizer applied to soil for cereal crop production. NUE may vary from 30% or lower in the case of rice production up to 70% in case of intensive maize production (Cassman et al., 2002). It is possible to increase crop yields in most of the areas around the world by applying additional N fertilizer or by increasing NUE in plants. The main goal of soil scientists and agronomists for decades has been the improvement of NUE (Raun and Johnson, 1999; Raun et al., 2002; Khosla et al., 2002). Increased knowledge of different plant mechanisms is vital for improving NUE and for reducing excessive use of fertilizers while maintaining an acceptable yield.

Factors that influence the N-assimilation into crop plants and N loss from soil systems affect NUE. Environmental factors such as temperature, rainfall, soil texture, etc. affect plant growth and development, soil N availability and soil N losses such as leaching and denitrification. Crop management plays a vital role in determining N loss (Neeteson et al., 1998) which includes factors like cropping density and spatial arrangement of plants (Shapiro and Wortmann, 2006), placement and timing of N fertilization and its application method (Li, 2003; Ma and Kalb, 2006; Osborne, 2006), and water management (Battilani et al., 2003; Remie et al., 2003; Singandhupe et al., 2003).

Many studies have examined the NUE in corn over the last several decades. In a study in North Dakota, USA, Wienhold, et al., (1995) studied effects of irrigation and N fertility levels on corn growth and NUE using a nitrogen-15 isotope approach. Three different levels of irrigation were used in this study: precipitation plus irrigation equal to one, two, and three times the calculated evapotranspiration (ET) rate. The ^{15}N -enriched fertilizer was applied at rates equivalent to 100 and 200 kg N ha⁻¹ and NUE was determined by recovery. They found that grain and dry matter yields, N content, and utilization of fertilizer N all showed annual variations, which might be the result of yearly weather patterns, especially temperature. On average, 35% and 15% of applied fertilizer at the rate of 100 kg N ha⁻¹ and 200 kg N ha⁻¹ was used by grain and stover, while 30% remained in the upper 0.6 m of the soil profile at the end of the growing season. Approximately, 20% of the applied fertilizer was lost via leaching or denitrification. They found that under higher mean temperatures, corn responded to increasing N fertility with 60% greater yields, 75% greater N content, and 60% higher percentage N derived from fertilizer with the higher N fertility treatment.

There is a common belief among the growers that high rate of N fertilizer is an "insurance" for crop yields, but it has been found that reduced application of N fertilizer along with crop rotation in maize resulted in good yields. A 3-year experiment was conducted by Montemurro et al., (2006) in Italy under Mediterranean conditions on two maize-barley crop rotations to study N indicators, uptake and use efficiency under five contrasting N treatments. The treatments included mineral N (200 kg N ha⁻¹); organic-mineral N (100 kg N ha⁻¹ as olive pomace compost plus 100 kg ha⁻¹ of mineral N); mineral N (100 kg N ha⁻¹); organic N (100 kg N ha⁻¹ as olive pomace compost); and an

unfertilized control. Winter barley was cultivated without fertilization. They found that by using organic fertilizer, as partial substitution of mineral N, similar yields were achieved as by the highest mineral fertilizer treatment in both the crops. Additionally, NUE indices did not change under highest N treatments in both the crops. The N uptake was strongly affected by the high amounts of available soil N during the growing seasons (57.4 % in first and 45.2 % in the second season) and this N uptake further affected the yield and NUE. The N indicator in case of maize was pre-sowing soil mineral N whereas in the case of barley, mean stem nitrate content was the best N indicator showing a linear relationship with the yield. Therefore, under high fertility conditions, crop rotation could be an important agronomical tool to increase NUE and minimize possible N losses. While most previous NUE measurements have focused on inorganic fertilizers, the role of organic nitrogen sources and mineralization of reserves of SOM are increasingly realized to impact N uptake by crops and therefore NUE.

The goal of our study was to evaluate NUE in silage corn treated with contrasting nitrogen source treatments and understand the factors affecting NUE. Eventually, understanding of the N cycling processes affecting N availability will help in formulating techniques and policies to improve NUE in crops.

MATERIALS AND METHODS

Experimental Site

The site is located at the Greenville farm (41°45' N, 111°48'52 W), located in North Logan, Utah. The soil is an irrigated, very strongly calcareous Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll) with pH of 8.2. The plots were established in 2011 to study N cycling and the various N transformations under contrasting N management (Ouyang et al. 2016). The experiment is a completely randomized block design experiment consisting of four N treatments with four replications for a total of 16 plots (Fig. A-1). The treatments are a control (no N fertilization), ammonium sulfate (AS, 112 & 224 kg N per hectare), and compost made from steer manure, slaughter by-products and woodchips (applied 224 kg total N per hectare). Each plot is 9.1 m long and 3.8 m wide. There is a 1.2 m alley between each block and a 4.6 m alley between each plot in a block. Silage corn is planted each year in May since 2012.

Field Operations

Pre-planting soil was sampled from each plot at 0-30 and 30-60 cm depth early spring of each year. Soils were analyzed for phosphorous, potassium (P&K) and for ammonium and nitrate N as previously described (Ouyang et al., 2016). The plots were fertilized for P&K according to the recommendation for silage corn in the Utah Fertilizer Guide (James and Topper, 2010). The fertilizer and compost treatments were applied in early May every year to individual plots using a small spreader and incorporated by tillage within one day. The site was tilled after amendments were added and the corn seed DKC35-18 RR (glyphosate tolerant) was planted on 30" row spacing. Approximately five rows of silage corn were planted in each block at the rate of 50,000 plants per hectare

using a John Deere planter. We used a sprinkler irrigation system and applied water weekly as needed and as available. We applied herbicide (Killzall with 41% glyphosate, 18.7 g L⁻¹ of water) for killing weeds once per season. The corn ear leaf samples for tissue N analysis were taken at approximately 80 days after planting each year. After corn reached maturity in late September the aboveground plant material was harvested using machetes from 3 m (10 ft.) of the inner two rows of each plot. Plant counts and fresh wet weight was determined for each plot. Bundled corn was then dried at 60° C for about one week, and dry weight was determined. The dried corn was then chopped and a subsample was finely ground using Willey Mill.

Plant and Soil Analysis

A subsample of plant tissue was more finely ground to pass a 0.5 mm sieve and then analyzed for total N by dry combustion (Primacs^{SLC} for organic carbon, Primacs^{SN} for total N, Skalar, Inc, GA, USA). About 200 mg of a sub-sample was run with high range N standards. The soil was sampled at depths of 0-15, 0-30 and 30-60 cm in May, June, August and November from 2012-2015 as previously described (Ouyang et al., 2016). The soil sampling for 2016 was done in May and December. The samples were analyzed for ammonium and nitrate using flow injection analyzer (QuikChem 8500, methods 12-107-06-1-A, 12-107-04-1-J Lachat Instrument, Loveland, CO).

Corn Yield and NUE

The corn weights were adjusted to equivalent of 17.4 feet of row and yield was estimated (Rankin 2017; Blonde 2017). Dry matter yield was used to calculate NUE. The

difference between the N uptake in fertilized plots and the control plots was used to determine NUE for five years (2012-2016) by the equation given by Vetsch and Randall, (2004).

$$NUE = \frac{NF - NC}{R}$$

NF = total N uptake in corn from N fertilized plots

NC = total N uptake in corn from unfertilized plots

R = rate of fertilizer N applied

Statistical Analysis

The data from Yield, N uptake, NUE, corn ear leaves N and total soil N were analyzed as repeated measures analysis of variance (ANOVA) with the Mixed model in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Residuals were evaluated for normality using the UNIVARIATE procedure of SAS and for common variance using scatterplots of residuals vs. predicted values (Weisberg, 2005). The treatment and year were used as fixed effects and block as a random effect. All statistical analyses were carried out at 95% confidence level ($P \leq 0.05$). When a significant treatment and year interaction was present, the data is presented by year.

RESULTS

Yield, N uptake, and NUE

There were significant effects of treatment, time and treatment-time interactions overall for yield (dry weight basis). The yield was found to be the higher in inorganic N treatments of AS100 and AS200 as compared to compost treatment. The control was found to be the overall lowest in terms of yield as expected. There was a significant treatment and time interaction for yield, there was no treatment effect within year 2016. The yield was found to be higher in 2014, 2012 as compared to 2013 and 2015. However, the yield in 2016 was not significantly different from all the other years.

N uptake was significantly different with treatment, time and treatment-time interaction (Table 2-2). The mean aboveground plant N uptakes (2012-2016) from the AS200, AS100, and compost treated soils were 186, 115 and 80 kg ha⁻¹, respectively. We witnessed increased N uptake in compost for the first three years (2012, 2013 and 2014) but declined in the last two years (2015 and 2016) as shown in Fig. 2-2. The N uptake was found to be the highest in AS200 followed by AS100, compost and control.

The difference method (Vetsch and Randall, 2004) used to calculate NUE showed that NUE was highest in AS200 (62%) and AS100 (60%) treatments versus compost (14%) treatments. NUE in compost treatment was found to be the lowest because this organic source of N is less readily available (Fig. 2-3). There was a significant treatment and time interactions for NUE in all the years except 2012 and 2016 (Table 2-3). NUE was the highest in 2014 for AS100 and AS200 treatments exceeding 100% which shows that there was residual N left from the previous years which later became available for uptake, resulting in higher yields and higher NUE eventually.

We found a significant effect of treatment, time and treatment-time interaction overall in corn ear leaf N for all the years (Table 2-4). The mean values for the N in corn

ear leaves for AS200, AS100 and compost were found to be 2.21, 1.52 and 1.24%, respectively (Fig. 2-4). The interpretation of these values indicates N deficiency in all the treatments because the N levels at the third leaf stage (70-90 days after planting) should be in the range of 3.5 to 4.5 %, respectively (Schulte and Kelling, 1999). The results showing the total soil N are shown in the Fig. 2-5.

DISCUSSION

The yield, N uptake and NUE showed significant effects of treatment, time and treatment-time interaction and were similar to the previous studies. A similar study conducted by Habteselassie et al., (2006) studied the repeated application of different N treatments in silage corn and found higher yields in soils treated with N fertilizers as compared to control. Another study by Taghizadeh and Sharifi (2011) investigated the effects of different N levels (0, 80, 160 and 240 kg ha⁻¹) in corn on NUE. They found that there was a significant effect of N fertilizer on the yield and NUE. The highest yield was given by the higher levels of fertilizer N (240 and 160 kg ha⁻¹). However, NUE decreased around 28% with the increased application of N.

The N uptake values indicate that NUEs in AS200 were higher than AS100 and compost treatments because inorganic N provides nitrate and ammonium that is readily taken up by the soil as compared to the organic treatments like compost. After looking at the soil N pool sizes (Ouyang et al., 2017), we found that there was an average of 30 mg N/kg soil in the form of nitrate (0-30 cm) present in August 2013 which corresponds to about 60 kg N/ha in the top 0-15 cm of the soil. This nitrate was available in the

following year of 2014 resulting in higher yields and increasing NUE. The nitrate levels were found to be high in May 2014 as well which could have possibly contributed to higher yields and NUE. As far as compost is concerned, NUE was negative in year 2012 which could have been due to N loss via leaching or immobilization. There was less than 2 mg N/kg soil in the form of nitrate present in compost treated soils in August 2012 which was the reason for really low NUE. AS100 had the highest NUE in year 2013 and 2014 but declined in the next two years as described in Fig. 2-3. We witnessed a gradual decline in NUE in compost over the years.

After five years of annual application of N fertilizers ($112\text{-}224\text{ kg N ha}^{-1}$) in corn grown under conventional tillage, we were able to recover only 45% on an average (all treatments). These kinds of results are common and congruous with worldwide NUE data of major cereal crops. Another study by Olson and Swallow (1984) in winter wheat that received an annual application of N fertilizer ($57\text{-}112\text{ kg N ha}^{-1}$) for five years demonstrated only 27-33% NUE. There is a need to initiate a collaborative global approach to improve NUE.

The improvement in NUE depends on many factors like water, light, temperature, carbon status and soil type and these factors further affect N uptake, assimilation and remobilization efficiency (Kant et al. 2008). Previous studies have focused on the optimization of N fertilizer rates to increase the NUE. It has been reported that the N losses via nitrate leaching were over 27% of the total fertilizer N applied which averaged to about 52 kg/ha in continuous corn in Nebraska (Klocke et al., 1996). Andraski et al. (2000) also investigated nitrate leaching losses in corn production in Wisconsin and

found that losses occurred in the range of 3 to 88 kg/ha. These losses were dependent on fertilizer N rate applied and other crop and manure management practices.

CONCLUSIONS

The present study investigated the effects of ammonium sulfate versus steer compost treatments on the silage corn yield, N uptake, and NUE. We witnessed differences in yield, N uptake, and NUE under different sources of N. The overall effect of treatment, time and treatment-time interaction was significant in yield, N uptake, NUE, corn ear leaves total N, and total soil N. The performance of AS100 and AS200 was not consistent over the five years. There were lower recoveries in silage treated with organic fertilizer (compost) as compared to inorganic fertilizers. The year 2014 was found to be the best year in terms of yield, N uptake, and NUE. The synchronization of N supply with plant demand is important for ensuring adequate quantity of N uptake and utilization and optimum yield. The use of integrated management practices can help in increasing N uptake and minimize N losses. The increase in N recoveries by crops will help growers to produce the same crop at low N fertilizer rates. This will not only help in reducing N losses but also cut the fertilizer costs and raise growers' profits.

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TABLES AND FIGURES

Table 2-1. Yield (dry weight basis) for the years 2012-2016

Treatments	Yield (kg ha ⁻¹)				
	2012	2013	2014	2015	2016
Control	7352 ab	10235 b	15456 b	10927 b	10544 a
AS100	10058 a	13378 a	21757 a	11392 a	11097 a
AS200	10676 a	11319 a	24194 a	19943 a	10090 a
Compost	9683 b	5828 a	10037 b	5613 ab	8100 a

Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Table 2-2. N uptake (kg N ha⁻¹) in harvested corn silage for the years 2012-2016

Treatments	N uptake (kg N ha ⁻¹)				
	2012	2013	2014	2015	2016
Control	48 b	43 b	62 c	39 b	45 bc
AS100	59 b	146 a	180 b	77 b	114 b
AS200	95 a	145 a	304 a	208 a	179 a
Compost	43 b	93 b	109 c	77 b	77 c

Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Table 2-3. Nitrogen use efficiency (NUE) (%) for the years 2012-2016

Treatments	NUE (%)				
	2012	2013	2014	2015	2016
AS100	9.9 ab	92.2 a	105.3 a	33.5 ab	61.3 a
AS200	21.0 a	45.8 b	108.1 a	75.1 a	59.8 a
Compost	-2.2 b	22.6 b	20.9 b	16.7 b	14.1 a

Abbreviation: NUE- nitrogen use efficiency. Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Table 2-4. Corn ear leaves total N for the years 2012-2016

Treatments	Total N (% dry weight)				
	2012	2013	2014	2015	2016
Control	0.96 c	1.11 b	1.13 c	1.60 ab	1.22 b
AS100	1.69 b	1.42 b	1.61 b	1.37 b	1.17 b
AS200	2.47 a	2.20 a	2.42 a	1.77 a	1.96 a
Compost	1.11 c	1.15 b	1.18 c	1.55 ab	1.15 b

Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Table 2-5. Total Soil N for samples from May pre-fertilizer application (2012-2016).

Treatments	Total N (% dry weight)				
	2012	2013	2014	2015	2016
Control	0.08 ab	0.11 a	0.10 a	0.11 a	0.10 a
AS100	0.09 a	0.11 a	0.10 a	0.14 a	0.10 a
AS200	0.06 b	0.12 a	0.09 a	0.16 a	0.09 a
Compost	0.09 a	0.10 a	0.10 a	0.14 a	0.14 a

Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$

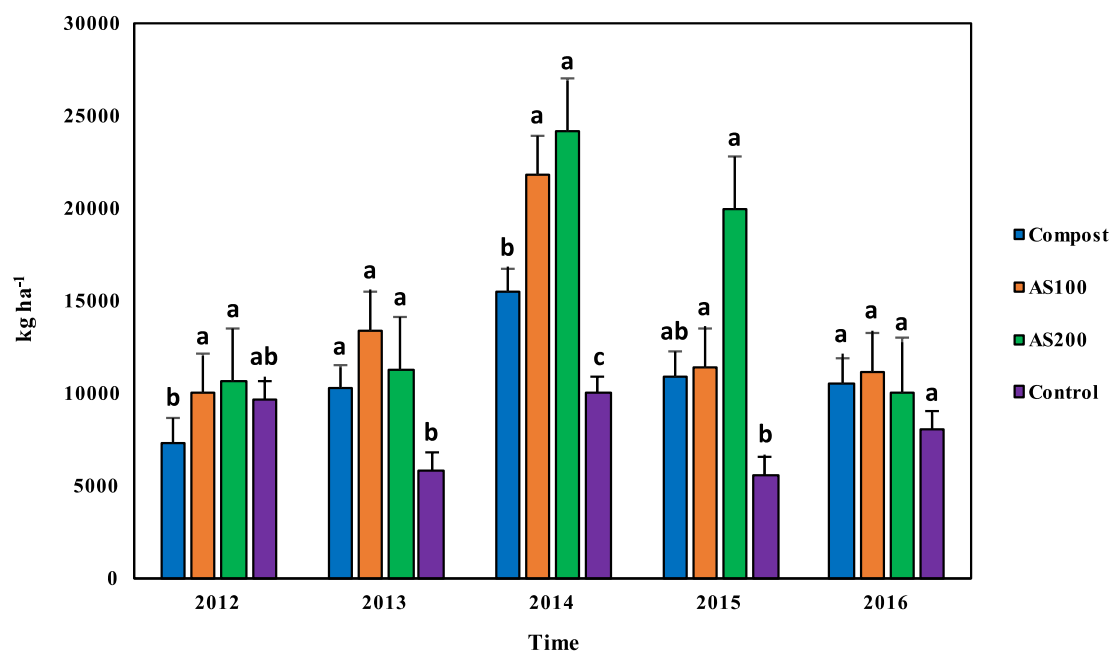


Fig. 2-1. Silage corn yield on a dry weight basis over five years for four treatments (compost (224 kg N ha⁻¹), and ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and (control (no N fertilization)). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures Proc Mixed.

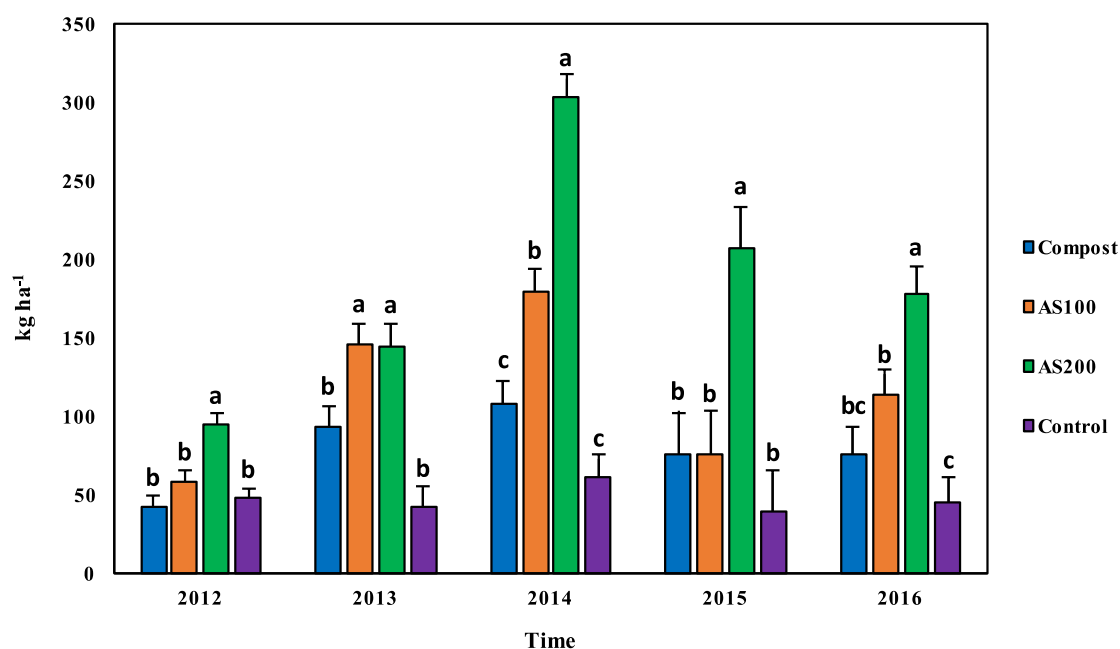


Fig. 2-2. N uptake in silage corn calculated over five years for four treatments (compost (224 kg N ha⁻¹), and ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and (control (no N fertilization))). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures Proc Mixed.

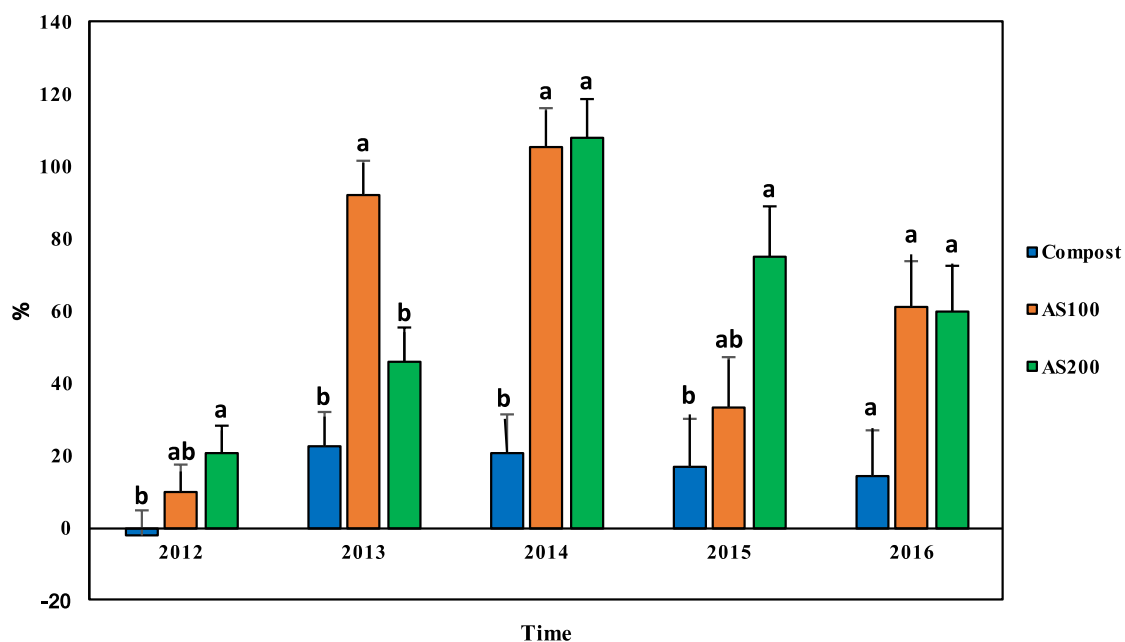


Fig. 2-3. NUE in silage corn calculated over five years for three treatments (compost (224 kg N ha⁻¹), and ammonium sulfate (AS 112 & 224 kg N ha⁻¹)). Error bars represent standard errors (n = 3). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures Proc Mixed.

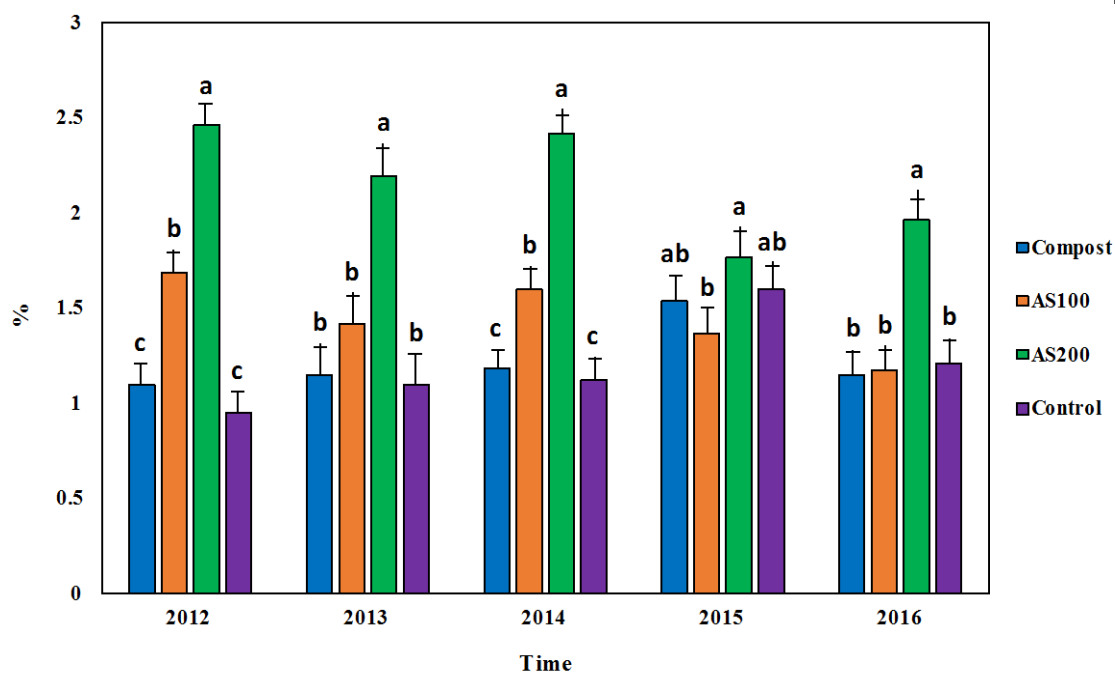


Fig. 2-4. Total N in corn ear leaves at 80 days over five years for four treatments (compost (224 kg N ha⁻¹), and ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and (control (no N fertilization)). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures Proc Mixed.

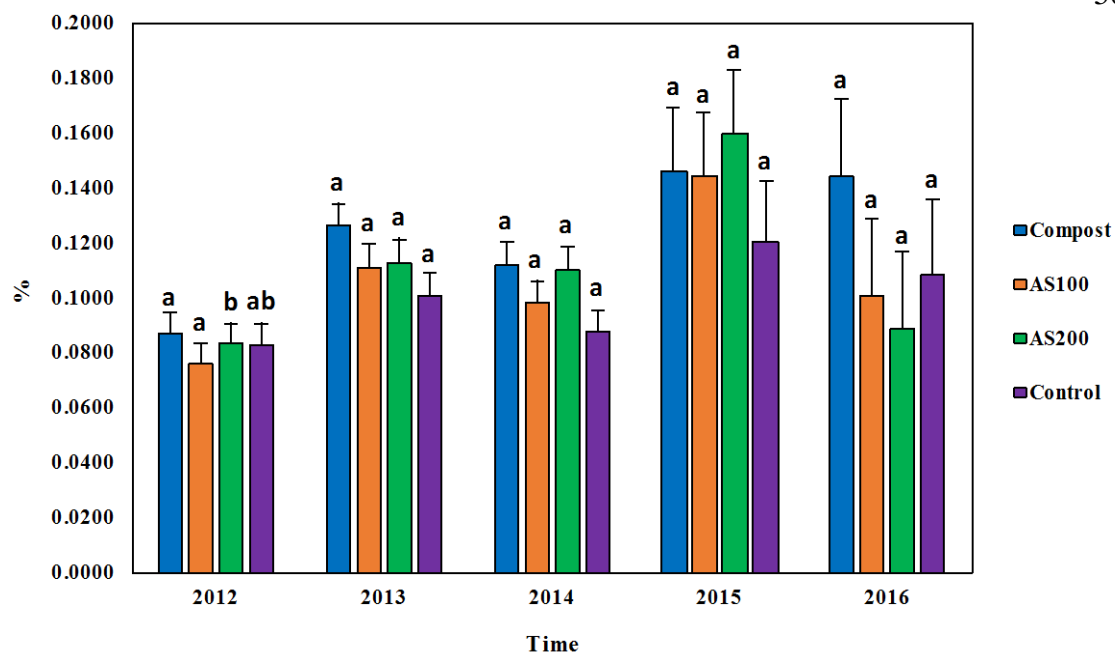


Fig. 2-5. Total soil N at 0-15 cm depth over five years for four treatments (compost (224 kg N ha⁻¹), and ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and (control (no N fertilization))). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures Proc Mixed.

CHAPTER III

NITROGEN MINERALIZATION AND NITRIFICATION UNDER CONTRASTING NITROGEN TREATMENTS²

ABSTRACT

The study of nitrogen (N) mineralization and nitrification is essential for an understanding of the effects of these soil processes on the availability of N to plants. Estimates of gross mineralization rates and gross nitrification rates in agricultural soils are crucial where the production and consumption processes occur simultaneously. Soils were sampled from silage corn field plots (Site 1) that had been treated for five years with contrasting N sources: control (no additional N), ammonium sulfate at 112 and 224 kg total N ha⁻¹ (AS100 & AS200) and steer manure compost at 224 kg total N ha⁻¹ (compost). The second experiment was conducted with soils from an organic farming system that had received steer manure or steer compost at the rate of 224 kg total N ha⁻¹ or control (Site 2). We conducted laboratory incubation experiments to determine carbon mineralization, net nitrogen mineralization and net nitrification rates. Carbon mineralization rates were found to be the highest in soils treated with compost under Site 1 and with manure under Site 2 as expected. There was a significant treatment effect for net N mineralization rates under site 2 but none under site 1. There were significant treatment effects for net nitrification rates in both sites. Laboratory ¹⁵N isotope dilution

² Avneet Kakkar, Jeanette Norton, Jennifer Reeve, Yang Ouyang

experiments were conducted with the Site 1 soils sampled in August 2015 and June 2016 for determination of gross mineralization and nitrification rates. We found that gross mineralization and gross nitrification rates were not significantly affected by treatment in 2015 (average 3.5 mg N kg⁻¹ soil d⁻¹ and 1.2 mg N kg⁻¹ soil d⁻¹ respectively). Similar to the results in 2015, gross mineralization rates were not statistically different by treatment in 2016 (average 1.7 mg N kg⁻¹ soil d⁻¹). Gross nitrification rates were found to be higher in the AS200 treatment versus control or compost. Approximately 30 % of the nitrification in the AS200 soils from 2016 was attributed to ammonia oxidizing bacteria. There was higher variability in the gross nitrogen transformation rates in compost treatment indicating presence of hot spots of labile organic matter or N immobilization.

INTRODUCTON

Link between Nitrogen Mineralization and NUE

Nitrogen (N) is one of the most important nutrients required for plant growth and development. However, in many systems more than 50% of applied N fertilizer is lost via leaching, ammonia volatilization, surface run off and denitrification. The ultimate goal of the producers is to increase crop yields while minimizing losses to the environment. This can be achieved by synchronizing crop N demand with the supply of N also known as N synchrony (Crews and Peoples, 2005). N synchrony is divided into three parts: (1) soil net N mineralization, (2) soil inorganic N available for crop uptake, and (3) crop N uptake. The mineralization of soil organic N sources like soil organic matter (SOM), plant residues and organic amendments helps in providing plant available inorganic N, of

which 50-100% is ultimately taken up by crops (Robertson 1997). Increase in the knowledge of different ways of soil management and application of organic amendments would be helpful in improved understanding of N mineralization. This would ultimately help in formulating techniques to increase the crop yields by synchronizing the plant N demand with N supply. Achieving higher yields while minimizing losses would eventually lead to higher N use efficiency and increased sustainability.

Nitrogen Mineralization and Nitrification Rates

Nitrogen mineralization is the process by which organic N from various sources like organic matter, crop residues and manures is converted to plant-available inorganic forms by microbial decomposition. Soil N is present in four major forms: (1) soil organic matter (SOM) such as plant and animal residues and stabilized humus; (2) living organisms and microorganisms; (3) ammonium ions held in clay interlayers and (4) mineral N forms in soil solution, including ammonium, nitrate and low concentrations of nitrite. N mineralization transforms large and complex organic N compounds to simple N monomers or ammonium. Mineralization is one of the key processes that enables plant growth by releasing nutrients to plants in available form.

N mineralization in cropping systems exhibits high spatial and temporal diversity (Knoepp & Swank, 1998), which is controlled by factors including temperature (Guntinas et al., 2012), moisture (Paul et al., 2003), and land use type (Templer et al., 2005). The growers need estimates of N available from mineralization to effectively manage soil N. The study of nitrogen mineralization rates in different soils is used to develop simple tools and models for estimation of field-specific N mineralization rates.

Estimating N Mineralization Rates in Soils

The process of mineralization may be further described by its net, potential and gross mineralization rates (Norton and Schimel, 2011). The net mineralization rate is the outcome of two opposite processes: gross mineralization (N release) and immobilization (N assimilation) by the micro-organisms. Net mineralization is positive when the gross rates of mineralization are higher than rates of combined consumptive processes (i.e., immobilization plus losses). The change in soil inorganic N pool size over a specified period estimates the net rates of mineralization (NMR) (Hart et al., 1994a). The net rate of mineralization which occurs during the absence of plant uptake and leaching is called potential mineralization rate. The laboratory procedures used for estimating net and potential mineralization rates are built on a primary assertion that the quantity of accumulated inorganic N is estimated over a particular period under a defined temperature and moisture conditions (Norton and Schimel, 2011).

$$\text{Net Mineralization} = (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_{t+1} - (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_t$$

Net rates can also be defined as:

Net N mineralization =

Gross N mineralization – microbial immobilization of inorganic N

Due to its simple procedure, net mineralization is extensively used as an indicator of N availability in soils (Schimel and Bennett, 2004). Nonetheless, net mineralization ignores the prospective role of inorganic N assimilation by microorganisms and

denitrification, and the yield and uptake of simple organic compounds by both plants and microorganisms (Norton and Schimel, 2011; Schimel and Bennett, 2004). Comparing net and gross rates of mineralization and nitrification may allow for better insights into the effects of nitrogen sources on availability.

Models of N Mineralization

Several models have been used for estimating N mineralization rates under different conditions (Benbi et al., 2002). We studied the N mineralization by conducting long-term incubations under controlled conditions. The mineralizable N pool is defined as the amount of N present in soil which is released over a period and is often expressed in mg N /kg soil. Potential mineralizable N is defined as the amount of N that mineralizes under optimum and constant environmental conditions and is often estimated by fitting a first-order kinetic model to inorganic N concentrations over time (Stanford & Smith, 1972). The equation used in the first order model is described below:

$$N_t = N_o(1 - e^{-kt}) \quad (1)$$

N_t = mineralizable N present at time t

N_o = mineralizable N initially present

k = first order rate constant

t = time

The multi-fraction first order models have also been used for estimation using the following double exponential equation:

$$N_t = N_o S(1 - e^{-ht}) + N_o(1 - S)(1 - e^{-kt}) \quad (2)$$

where S and $(1 - S)$ represent the labile and recalcitrant organic N fractions getting decomposed at specific rates h and k , respectively.

The other models used for describing N mineralization kinetics in soils are non-compartment models, multi-compartment models, and food web models. The two compartment models like First Order Double Compartment (FODC) are useful for modeling N dynamics in soils and comparing data in tabular forms. Multi-compartment models are based on three or more organic N pools and are difficult to validate as most of the functional pools cannot be evaluated by physical, chemical or biological techniques. Under food web models, organisms are classified as functional groups, and their consumption rates are used to determine N mineralization rates (Benbi et al., 2002).

Nitrification

Nitrification results in the rapid oxidation of ammonium to nitrite or nitrate. Nitrate is more mobile than ammonium because it is negatively charged and is repelled by cation exchange sites. Therefore, nitrate is easily lost via leaching and denitrification (Norton, 2008; Prosser, 1990). Nitrification occurs at a faster rate in moist, warm and well-aerated soils. There are mainly three types of microbial groups involved in this process: (1) autotrophic ammonia oxidizers, (2) autotrophic nitrite oxidizers and (3) heterotrophic nitrifiers. The first two groups oxidize ammonia to nitrate under aerobic conditions whereas heterotrophic nitrifiers oxidize organic compounds to produce nitrate

(NO₃⁻) or nitrite (NO₂⁻). The accessibility and activity of N in soils are considerably controlled by the N transformations of mineralization and nitrification.

Estimating Nitrification Rates in Soils

There are several methods to measure nitrification rates in soils. Some of them involve field and laboratory measurements. In our study, we determined the net nitrification rates (NNR) by conducting aerobic incubation experiments similar to those used to study mineralization described earlier. The soil samples are extracted with 2M KCl solution before the start of incubation to calculate the initial nitrate concentration and another extraction is done at the end of incubation with a salt solution using 2M KCl. The net nitrification rates were calculated by subtracting initial concentrations from the final values. Estimates of NMR and NNR are helpful in the evaluation of potential N losses from the ecosystems (Norton 2011).

Nitrogen Sources Affect N Mineralization and Nitrification

Different sources of N affect the rates of N mineralization and nitrification. Organic amendments having high N and low C: N ratios mineralize enough N for plant growth (Cordovil et al., 2005; Seneviratne, 2000) whereas immobilization occurs in organic amendments with low N and high C: N ratios (Manojlović et al., 2010). The inorganic N treatments like ammonium sulfate and ammonium nitrate are readily available for plant uptake as compared to organic N treatments and are more likely to get nitrified. In general, nitrification rates are higher in inorganic N treatments as compared to organic treatments.

The goal of our study was to study N mineralization rates under different N treatments by conducting long-term laboratory incubation experiments with soils from two experimental sites. We studied gross mineralization and nitrification rates to predict release, availability, and mobility of the different forms of N. Improved knowledge of the N mineralization and nitrification rates will increase our understanding of crucial processes of N cycling.

MATERIALS AND METHODS

Experiment Sites and Soil Sampling

Site 1. The details of the agricultural site (North Logan, Utah, USA), experimental design, treatments, soil sampling, and soil characteristics have been previously described in Chapter 2. Briefly, the experimental design is a randomized complete block with four blocks and four nitrogen treatments: control (no N fertilization), ammonium sulfate (AS 112 and 224 kg N ha⁻¹), and steer-waste compost (224 kg total N ha⁻¹). Treatments were surface applied in May of each year and incorporated by tilling immediately after application. The soil is an irrigated, very strongly calcareous Millville silt loam (Coarse-silty, carbonatic, mesic Typic Haploxeroll).

Site 2. This site was established in fall 2007 for studying the transition to organic farming following different crop rotations to improve soil quality and increase economic return (Fig. B-1). This site is also known as the “organic rotation plots”. This is located at the Greenville Research Farm in North Logan, Utah (41°46' N, 111°49'W). The soil is a Millville Silt Loam with a pH of 7.8-8.2. This is a completely randomized split-split plot

design consisting of three different crop rotations involving three different cover crops with or without manure or compost. A cover crop is the main plot (buckwheat, millet, legume), crop rotation the sub-sub plot and fertility treatment the sub-plot (compost, manure, nothing) each with three replicates. Each main plot is 27m long and 11m wide. Each rotation differs in the level of farming intensity (no. of cash crops grown, and input intensity, fertilizers applied one or twice in a four-year rotation). For this proposed study, we propose using the composts in the most intensive rotation (potatoes, cover crops, beans, sweet corn) receiving an application of 224 kg/ha total N in the form of a high C: N ratio compost, lower C: N ratio cattle manure or nothing applied to the potato and corn crop in each rotation. The plots are irrigated with an overhead sprinkler irrigation system.

Long-term Incubations

A long-term incubation experiment was conducted with soils sampled from both the sites to study effects of different N source treatments on the amount and rate of nitrogen mineralization. The soil from site 1 was sampled during August 2015. Two big soil cores were taken from each plot (0-15 cm depth), one in the middle of corn plants and other in between rows. The soils were composited and thoroughly mixed, sieved to 2mm, adjusted to 18% moisture content and eight subsamples weighing 15 g (o.d. equivalent) were placed into plastic containers for incubation. One of these subsamples was extracted with 75 ml 2M KCl to determine the amount of inorganic N present at the start of incubation (Day 0). The additional cups were placed inside quart mason jars, and one ml of deionized water was added to the bottom to avoid any loss of moisture from the

soil. These jars were then sealed using lids provided with septa and time was noted for the start of incubation. All the jars were incubated in the dark at 25⁰C.

The soil samples were extracted after 7, 14, 21, 35, 42 and 84 days using 2M KCl extraction. Each sample of 15g (o.d. equivalent) was extracted with 75 ml of 2M KCl. The filtrate was collected and the container was sealed and frozen at -40⁰C for inorganic N analysis. Ammonium and nitrate+nitrite N was determined using a flow injection analyzer (QuikChem 8500, methods 12-107-06-1-A, 12-107-04-1-J Lachat Instrument, Loveland, CO).

A similar procedure was followed for the long-term incubation experiment for the soil sampled from Site #2. The soil was sampled in October 2015 using 12 cores in each plot at 0-15 cm depth. The soils were composited and thoroughly mixed, sieved to 2mm and adjusted to 20% gravimetric moisture content. The incubation was performed as for site 1 soils.

Carbon Mineralization

The carbon mineralization was determined by measuring carbon dioxide (CO₂) released during the soil incubation. Briefly, a 10ml syringe with a hypodermic needle was used to sample about six ml of the headspace atmosphere at day 2, day7, day14, day21, day35, day42 and day84 of the incubations. The gas sample was then injected into clean and well labelled sealed evacuated vials. These evacuated vials contained calcium sulfate desiccant inside to absorb H₂O present. After sampling the sealed jars were opened and flushed with fresh moisturized air for about five seconds to replenish atmosphere at each sampling and then the jars were resealed. A record of time and date of sampling was kept.

These samples were then run for CO₂ analysis using an HP6890 Series gas chromatograph with a TCD detector (Agilent) with appropriate calibration standards.

Net Mineralization and Nitrification Rates

The N pool sizes obtained from Lachat analysis were used to determine the net mineralization (NMR) and net nitrification rates (NNR). The difference of inorganic N present at the start and end of incubation was used to calculate the NMR. This difference was then divided by the number of days of incubation period to get the net mineralization rate per day. Similarly, the difference of the nitrate present at the start and end of incubation was used to calculate the NNR. This difference was divided by the number of days of incubation period to get the net nitrification rate per day.

Gross N mineralization and Nitrification Rates

Pool Dilution Experiments

Experiments were conducted to calculate gross mineralization (GMR) and gross nitrification rates (GMR) using ¹⁵N isotope pool dilution technique as described previously (Ouyang et al., 2016, Habteselassie et al., 2006; Hart et al., 1994b; Stark, 2000). The site 1 soil was sampled in August 2015 (twelve weeks after fertilization). The soil was sampled at 0-15 cm depth and eight cores were taken from each plot and composited. Soil was sieved to 2 mm, well-mixed, moisture content determined and adjusted with an allowance for moisture addition with the labeled N. In 2015, four subsamples (40 g o.d.) were weighed into plastic specimen cups from each plot. Then, 1.6 ml of ¹⁵(NH₂SO₄) (6 ppm at 98 atom % ¹⁵N) solution or K¹⁵NO₃ solution (2 ppm at 98

atom % ^{15}N) was added and mixed carefully bringing the final moisture content in the soil to 18%. One of the subsamples for each label was harvested immediately after mixing and extracted using freshly prepared cold 2M KCl (200 ml) to determine the ^{15}N concentrations at time-0. The remaining two subsamples were placed inside 1-L mason jars with one ml of water in the bottom to minimize any loss of moisture from the soil, and jars were sealed with lids with septa. The jars were incubated in the dark for 24 hours at 25°C. After 24 hours, the headspace in the mason jars was sampled for CO_2 release as described above. The soil samples were extracted with 2M KCl at time 0 and after 24 hours of incubation to determine pools of NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$ using flow injection analyzer (QuikChem 8500, methods 12-107-06-1-A, 12-107-04-1-J Lachat Instrument, Loveland, CO).

Pool Dilution with Selective Inhibition of Nitrification Using Octyne

A similar procedure was adopted in 2016 with soils sampled less than two weeks after fertilization in June. The soil was sampled at 0-15 cm depth and eight cores were taken from each plot, composited, sieved to 2 mm, well mixed, and then moisture content determined and adjusted. Five subsamples of 40 g o.d. equivalent were measured into specimen containers, two samples were for determination of mineralization, three samples were used for determination of nitrification with and without the selective inhibitor octyne (Ouyang et al 2017). The octyne inhibits nitrification by the ammonia-oxidizing bacteria (AOB) and was used to distinguish the contributions of ammonia oxidizing archaea (AOA) and the AOB to nitrification (Ouyang et al 2017, Taylor et al., 2015; Taylor et al., 2013). We labeled with 1 ml of $^{15}(\text{NH}_4\text{Cl})$ solution (6 ppm at 99 atom % ^{15}N) solution or 1 ml K^{15}NO_3 solution (6 ppm at 98 atom % ^{15}N) per 40 g soil sample.

We added 24 ml gas containing 1-octyne to the headspace of one sample labeled with nitrate ($4 \mu\text{M } C_{\text{aq}}$ final concentration) to inhibit nitrification by the AOB. Incubation and extraction procedures were as for 2015 pool dilution experiment.

Diffusion Procedure and N-15 Isotope Determination

The extracts from the ^{15}N pool dilution experiments done in 2015 and 2016 were processed for isotope analysis by diffusion using the procedure described previously (Habteselassie et al., 2006; Hart et al., 1994b; Stark, 2000; Stark and Hart 1996). Briefly, acid traps for ammonium were made using a 7 cm x 12.5 mm PTFE (Teflon) tape strips. Two filter paper disks (7mm diameter, pre-rinsed in KCl and deionized water then dried) were placed 3.5 cm apart on the PTFE strip and five μl of 2.5 M KHSO_4 was pipetted onto each disk. The other half of the PTFE strip was carefully folded over to cover both the disks and sealed using an 11-mm diameter glass culture tube. Extra care was taken during all these steps to avoid contamination. We measured a known amount of the extracts into a Mason jars (425 ml) to pour the solutions to be diffused and one acid trap was placed into each jar immediately after adding 0.2 g of magnesium oxide (MgO) which makes the solution basic. The jars were closed immediately and incubated at 22°C for seven days. The released ammonia is captured on the acidified filter paper disks. Jars were inverted two to three times every second day to make sure all the reagents mix well.

For the recovery and determination of ^{15}N enrichment of nitrate, the solutions were weighed into the mason jars with their lids open and 0.2 g of MgO was added to each jar. Jars were allowed to sit in the open for a week to get rid of the ammonia present in the extracts. After seven days, 0.4 g of Devarda's Alloy was added to each jar along with the acid trap, sealed and incubated for seven days at 22°C . The role of Devarda's

alloy is to reduce the NO_3^- into NH_4^+ which then is captured as ammonia onto the acidified filter paper disks. The same procedure was followed as described above. The ^{15}N standards were made using ammonium sulfate at 5.521 atom % ^{15}N 50 $\mu\text{g}/\mu\text{l}$ and potassium nitrate at 5 atom % ^{15}N 50 $\mu\text{g}/\mu\text{l}$. Five standards were diffused along with the samples and two standards that were not diffused.

After the incubation was completed, the jars were opened and acid traps were removed and placed on a clean surface. Deionized water was used to clean the traps by dipping them briefly in water using clean forceps. These traps were transferred to 24 wells plate, placed and dried inside the desiccator containing concentrated sulfuric acid for at least three days. The dried disks were then placed inside 5x8 mm tin capsules and placed into 96 wells micro-titer plate. The plate was then analyzed for total N and ^{15}N enrichments of NH_4^+ and NO_3^- using continuous-flow direct dry combustion and mass spectrometry with an ANCA 2020 system (Europa Scientific, Cincinnati, OH) at the USU Stable Isotope Laboratory. The gross N transformation rates were calculated using the equation of Norton and Stark (2011).

The enrichments were calculated following the guidelines given by (Stark and Hart 1996). The mass of N in the blank was used to estimate the blank-corrected enrichment of the sample. The mean value of all the blanks run along with other samples was taken. Although we made diffused and non-diffused standards for blank estimation we did not complete sufficient diffused standards at the same time as the non-diffused standards. Therefore, we concluded that the actual blanks were our best estimate for the mass of N in the blanks. The following equation was used to calculate the enrichment in samples:

$$E_s = E_m + \frac{M_b (E_m - E_b)}{M_s}$$

E_s = Corrected ^{15}N enrichment of the sample

E_b = Enrichment of the blank (assumed to 0.366%)

E_m = ^{15}N enrichment measured in the diffused standards

M_s = Mass of N in the sample

M_b = Mass of N in the blank

Statistical Analysis

The data from NMR, NNR, GMR, and GNR were analyzed as Proc Generalized Linear Model (GLM) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Residuals were evaluated for normality using the UNIVARIATE procedure of SAS and for common variance using scatterplots of residuals vs. predicted values (Weisberg, 2005). The treatment and year were used as fixed effects and block as a random effect. The data from carbon mineralization was analyzed as repeated measures analysis of variance (ANOVA) with the MIXED model in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). All statistical analyses were carried out at 95% confidence level ($P \leq 0.05$).

RESULTS

Carbon and Nitrogen Mineralization and Nitrification Rates during Long-term Incubations

The effects of treatment, time and treatment-time interaction on carbon mineralization were significant for site 1. Significant differences were found between the N source treatments between the compost and the ammonium sulfate fertilizer treated soils. The first week of incubation witnessed the highest carbon mineralization in all the treatments with compost treatment being the highest initially (Table 3-1). There was no significant difference in carbon mineralization between AS100 and AS200 treatment initially. The initial high rates are linked with the flush of carbon mineralization that follows disturbance and addition of moisture to the soil as described by Birch (1958).

There was a difference between the different treatments at first that became less with time (Table 3-1, Fig. 3-1). Compost treatment was found to have the higher C mineralization rates overall versus control which was expected because carbon flux increases with organic amendments. There was no significant difference between the two levels of AS treatments.

For the site 2 plots, we found that there was a significant effect of treatment, time and treatment-time interaction. The respiration rates were found to be higher in manure as compared to the control treatment over the entire period of incubation (Table 3-3, Fig. 3-2). We calculated that C mineralization rates for compost, manure and control treatments at Day2 of incubation were 12.8, 17.2 and 9.9 mg C kg⁻¹ soil day⁻¹ respectively whereas

the rates at the end of incubation were 2.5, 3.5 and 1.8 mg C kg⁻¹ soil day⁻¹, respectively (Table 3-2).

There was a significant overall treatment effect on NMR and NNR for both the sites. There was no significant difference between the different N treatments for NMR and NNR under both the sites. Pearson analysis showed that there was significant correlation between NMR and NNR for both sites indicating that mineralized N is quickly nitrified.

Gross Mineralization and Nitrification Rates

The overall treatment effect was not significant on gross mineralization rates (GMR) in 2015. The level of N treatment was also not significant. The gross rates were averaged to be 3.48 mg N kg⁻¹ soil d⁻¹ for compost, AS200, AS100 and control treatments (Table 3-8). There was no significant difference between the average GMR of all treatments as compared to control. There was no significant treatment effect overall on gross ammonium consumption rates (GACR) in the year 2015. GACR for compost, AS100, AS200 and control were found to be averaged 7.64 mg N kg⁻¹ soil d⁻¹ (Table 3-8). There was no significant difference within the treatments for GACR. Contrary to the GMR, we did find a significant difference between the average GACR of all treatments as compared to control. Pearson analysis showed that there was a significant correlation between the GMR and GACR.

Gross nitrification rates (GNR) were found to be averaged 1.12 mg N kg⁻¹ soil d⁻¹ for compost, AS200, AS100 and control treatments (Table 3-9). There was no significant treatment effect overall for GNR in 2015 which is similar to results reported by Ouyang

et al. (2017). As far as nitrate consumption rates are concerned, we found that there was no overall significant treatment effect. GNCR for compost, AS200, AS100 and control treatments were found to be averaged $0.52 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ (Table 3-9). Pearson analysis showed that there was a significant correlation between the GNR and GNCR.

In the year 2016, we found that there was no significant treatment effect on GMR. GMR were found to be averaged $1.73 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ for compost, AS100, AS200 and control treatments (Table 3-10). There was no overall significant treatment effect on GACR. The ammonium consumption rates for compost, AS100, AS200 and control were averaged to be $8.05 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ (Table 3-10). There was no significant difference within the treatments for GMR and GACR. Pearson analysis showed that there was no significant correlation between the GMR and GACR.

We found no significant treatment effect overall in nitrification and nitrate consumption rates for samples with and without octyne (Fig. 3-10). Under no-octyne conditions, GNR and GNCR were found to be the higher in AS200 as compared to compost and control. GNR for compost, AS100, AS200 and control were found to be 1.19, 2.74, 6.84 and $0.97 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ (Table 3-11). We found that AS200 was significantly different from compost treatment in terms of GNR under the absence of octyne. However, this was not the case in the presence of octyne where there was no significant difference within the treatments in terms of GNR. Under octyne conditions, GNR for compost, AS100, AS200 and control were found to be averaged $2.57 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$ (Table 3-11). Additionally, there was no significant treatment effect in GNCR under octyne and no-octyne conditions.

DISCUSSION

Treatment effects on carbon mineralization

Organic amendments of manure and compost increased C mineralization during the initial stages of incubation suggesting a small increase in the labile C pools at both sites. Previous studies have shown that C mineralization rates are higher in organic amendments as compared to control. In a similar study by Habteselassie et al., (2006), increased C mineralization rates were found at the start of incubations but stabilized with time. Other studies have shown substantial increases of C mineralization rates by 42 to 400% during the incubation after the addition of dairy manure in comparison with control during an incubation (Calderon et al., 2004). Another study by Sanchez et al. (2004) demonstrated larger C mineralization rates in composted dairy manure treated soils as compared to soils treated with synthetic N fertilizer. The steer compost used at both sites although relatively high in total nitrogen had fairly high carbon to nitrogen ratios suggesting that the organic matter was fairly stable.

Treatment effects on nitrogen mineralization and nitrification rates

Net mineralization and nitrification rates were increased by organic amendments and by high ammonium sulfate fertilization in both soils. The first order model that assumes one pool of labile N was not a good fit at site 1 because there was no plateau of mineralized N and within the 84-day incubation. This may be due to the presence of multiple organic N pools with different lability mineralizing simultaneously at different rates. We also tried to use a polynomial model but there was not much difference

between the results obtained through the linear and polynomial model. Hence, a linear model was used to calculate the NMR and NNR for the incubation data for site 1.

However, the first order model was a good fit for the site 2 (Fig. 3-7). The potentially mineralizable N (N_0) estimates for the plots receiving the compost and manure treatments were significantly different from control treatment. There was no significant effect of the cover crops. The model k is the decomposition rate constant for the labile organic N pool. The k values associated with the manure and compost treatments were significantly lower than control treatment (Table 3-7). These results are in agreement with the previous studies where under organically managed systems, the N supplying potential was double that under conventionally managed system (Burger and Jackson, 2003).

There are different models used for calculating mineralization rates which mainly involve the estimation of an active fraction of potentially mineralizable N and a rate constant to estimate mineralization rate (Benbi et al., 2002). Two approaches are generally used to calculate mineralization rates: (1) simple functional approaches to predict NMR and this includes first order model, multi-fraction approaches, and empirical models and (2) mechanistic approaches like compartment and non-compartment models, food web models, etc.

The first order model has been used under varying climate and cropping conditions (Stanford & Smith, 1972). There are some shortcomings reported with this model as it underestimated mineralization during the initial stages of incubation and overestimated in the intermediate stages leading to deviations in predicted and measured data values (Bonde and Rosswall 1987; Seyfried and Rao 1988). Multi-fraction

approaches are based on the fact that different organic N fractions mineralize at different rates in the soil. One of the best examples of this approach is the double exponential model (Benbi et al., 2002) which is considered better than the simple first-order model by many authors (Lindemann and Cardenas 1984; Deans et al. 1986; Cabrera and Kissel 1988; Diaz-Fierros et al. 1988). Similarly, some empirical approaches involve the use of polynomial and parabolic functions to calculate the net N mineralization in soils.

The other models used for describing N mineralization kinetics in soils are non-compartment models, multi-compartment models, and food web models. The two compartment models like First Order Double Compartment (FODC) are useful for modeling N dynamics in soils and comparing data in tabular forms. Multi-compartment models are based on three or more organic N pools and are difficult to validate as most of the functional pools cannot be evaluated by physical, chemical or biological techniques. Under food web models, organisms are classified as functional groups and their consumption rates are used to determine N mineralization rates (Benbi et al., 2002). The results found are congruous with the previous studies conducted at the same site. Ouyang et al. (2017) reported that there was no significant difference found between manure and compost treatments under organic rotation plots in October 2014 and 2015. Also, NMR and NNR were found be higher in manure treatment as compared to compost treatment in July 2015. The estimates of NMR and NNR are crucial for predicting the plant available N and the likelihood of nitrate to leach out of the system.

Treatment effects on gross mineralization and nitrification rates

Gross mineralization and nitrification rates are crucial to anticipate release, availability, and mobility of the various forms of N. The results found are congruous with

the previous studies conducted at the same site. (Ouyang 2016) reported that the GMR were not significantly different at the August 2014 sampling date. An infield study by Habteselassie et al. (2006) found that GMR and GNR were higher in the treatments receiving higher levels of nitrogen regardless of source. Zaman et al. (1999) also reported GMR of 6.1 and 3.4 mg N kg⁻¹ soil d⁻¹ in soils treated with dairy shed affluent and ammonium chloride as compared to 1.5 mg N kg⁻¹ soil d⁻¹ for control treatment. There was a higher variability in N transformation rates in soils treated with compost in June 2016. This might be due to the presence of hot spots of mineralization, immobilization and nitrification as a result of non-uniform distribution of the waste (Korsaeth et al., 2001).

We expected that gross rates of nitrification would be strongly affected by the time of sampling after fertilization. We found the AS200 treatment nitrification was significantly higher in June sampled soils. For soils sampled in August 2011 and 2014 at site 1 Ouyang et al (2016) found that there was no significant difference between treatments for NNR and GNR in 2011 but found significantly higher GNR for AS200 and compost treated soils in 2014 (Ouyang et al 2016). Shi and Norton (2000) found higher nitrification rates in soils treated with ammonium sulfate as compared to dairy waste compost after 1 year of treatment. Mendum et al. (1999) also noted higher GNR in soils treated with ammonium nitrate as compared to farmyard manure sampled three days of fertilizer application. They reported that the GNR for farmyard manure, ammonium nitrate and control were found to be 2.3, 7.8 and 0.2 mg N kg⁻¹ soil d⁻¹. While gross rates of nitrification reflect actual rates their dependence on short-term measurements means that they are dependent on the availability of the substrate ammonium during the rate

determination, hence temporal aspects of fertilization are critical to interpretation. We observed that the increased GNR for AS200 treatment was significant in the absence of octyne suggesting that the ammonium fertilizers affected the AOB activity in these soils.

Carbon and nitrogen interactions under different N sources

After looking at the long-term incubation results from both the sites, we can see that the compost treatment behaved differently. The amount of N mineralized under site 2 was almost double ($0.44 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$) as compared to site 1 ($0.23 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$). This was similar to results reported by (Burger and Jackson, 2003) where they found N supplying potential was twice under organically managed systems as compared to conventional managed systems. This might be due to presence of cover cropping and crop rotation at site 2. Although, the original soils at the two sites were similar and the compost treatment applied was the same, we observed higher carbon mineralized under site 1 ($6.15 \text{ mg C kg}^{-1} \text{ d}^{-1}$) as compared to site 2 ($2.48 \text{ mg C kg}^{-1} \text{ d}^{-1}$). The compost might be immobilizing N and mineralizing more carbon. Further investigation needs to be done to study the carbon and nitrogen interactions under conventional versus organic management including crop rotation at both the sites.

Implications for Agricultural Management

The present study helps to understand the N mineralization rates under contrasting nitrogen sources. This will improve our understanding of the different factors affecting the observed effects of N sources. The results show that we need to take into consideration the effects of other management practices like crop rotation on N

mineralization instead of focusing on N sources only. A collaborative approach should be taken to better understand the complex processes occurring inside the soil profile.

Improved knowledge of the role of different management practices like crop rotation will definitely help in development of effective crop management. This would further enhance the crop yields and productivity while maintaining a healthy soil profile. The results obtained from this study indicate that the compost treatment significantly increased the C and N mineralized under conventional management while the impacts were less obvious under organic management including crop rotations and cover crop inputs. The higher variability in N transformation rates in the compost treatment (2016) might be due to presence of hot spots of mineralization, immobilization and nitrification.

ACKNOWLEDGMENTS

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TABLES AND FIGURES

Table 3-1

Carbon mineralization rates during laboratory incubation for site 1 soils sampled in August 2015.

Sampling day	Treatments			
	Control	AS200	AS100	Compost
2	5.52 b	7.88 ab	7.40 ab	13.92 a
7	5.66 b	7.54 b	10.46 ab	15.01 a
14	4.97 b	8.99 ab	7.90 ab	13.11 a
21	3.15 c	7.32 ab	6.42 bc	10.07 a
35	4.18 b	5.10 b	5.17 b	9.15 a
42	3.50 a	2.64 a	2.98 a	4.30 a
63	3.23 a	4.64 a	6.56 a	7.55 a
84	1.97 b	3.85 ab	3.98 ab	6.15 a

Different letters within a row indicate significantly different treatment means within the sampling day, $p < 0.05$.

Unit: $\text{mg CO}_2\text{-C kg}^{-1}\text{d}^{-1}$ for the time period preceding each sampling day.

Table 3-2

Cumulative carbon mineralization during 84-day laboratory incubation for site 1 soils sampled in August 2015.

Treatment	Cumulative C
Control	32.46 b
Compost	80.12 a
AS100	51.43 b
AS200	48.42 b

Different letters within a column indicate significantly different treatment means $p < 0.05$.

Unit: $\text{mg CO}_2\text{-C kg}^{-1}$

Table 3-3

Carbon mineralization during laboratory incubation for site 2 soils sampled in October 2015.

Sampling day	Treatments		
	Control	Manure	Compost
2	9.92 a	17.16 a	12.81a
7	12.08 a	16.30 a	13.51 a
14	5.95 b	8.88 a	8.45 a
21	7.60 a	8.34 a	9.05 a
35	4.50 b	6.50 a	5.54 ab
49	8.32 a	12.07 a	12.60 a
64	2.25 a	4.71 a	2.91 a
84	1.81 b	3.47 a	2.48 b

Different letters within a column indicate significantly different treatment means within the sampling day, $p < 0.05$.

Unit: $\text{mg CO}_2\text{-C kg}^{-1}\text{d}^{-1}$ for the time period preceding each sampling day.

Table 3-4

Cumulative carbon mineralization during 84-day laboratory incubation for site 2 soils sampled in October 2015.

Treatment	Cumulative C
Control	52.44 b
Manure	77.43 a
Compost	67.35 ab

Different letters within a column indicate significantly different treatment means $p < 0.05$.

Unit: $\text{mg CO}_2\text{-C kg}^{-1}\text{soil}$

Table 3-5

NMR and NNR for laboratory incubations for site 1 soils sampled in August 2015.

Treatments	NMR	NNR
Control	0.23 b	0.23 b
AS100	0.27 ab	0.27 ab
AS200	0.33 a	0.34 a
Compost	0.36 a	0.36 a

Abbreviation: NMR-Net mineralization rate, NNR-Net nitrification rate. Different letters within a column indicate significantly different treatment means, $p < 0.05$.

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

Table 3-6

Net mineralization (NM) and net nitrification (NN) for over 84-day laboratory incubations for site 2 soils sampled in October 2015.

Treatments	NM	NN
Control	22.68 b	22.87 b
Manure	37.27 a	37.46 a
Compost	36.77 a	36.86 a

-, -. Different letters within a column indicate significantly different treatment means, $p < 0.05$.

Unit: mg N kg^{-1}

Table 3-7

Potentially mineralizable N (N_0) and decomposition rate constant (k) based on the first order kinetics model fit for laboratory incubations for site 2 sampled in October 2015.

Treatments	N_0	k
Control	28.14 b	0.03 a
Manure	56.01 a	0.01 b
Compost	53.46 a	0.02 b

Abbreviation: N_0 -Potentially mineralizable N (mg N kg^{-1}), k-decomposition rate constant (d^{-1}). Different letters within a column indicate significantly different treatment means, $p < 0.05$.

Table 3-8

Gross mineralization and ammonium consumption rates for site 1 soils sampled in August 2015.

Treatments	GMR	GACR
Control	2.53 a	6.42 a
AS100	3.88 a	7.48 a
AS200	3.52 a	8.33 a
Compost	3.98 a	8.32 a

Abbreviation: GMR-Gross mineralization rate, GACR-Gross ammonium consumption rate. Different letters within a column indicate significantly different treatment means $p < 0.05$.

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

Table 3-9

Gross nitrification and nitrate consumption rates for site 1 soils in August 2015.

Treatments	GNR	GNCR
Control	0.54 a	0.43 a
AS100	0.76 a	0.14 a
AS200	1.81 a	1.07 a
Compost	1.39 a	0.46 a

Abbreviation: GNR-Gross nitrification rate, GNCR-Gross nitrate consumption rate. Different letters within a column indicate significantly different treatment means within the column, $p < 0.05$.

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

Table 3-10

Gross mineralization and ammonium consumption rates for site 1 soils in June 2016.

Treatments	GMR	GACR
Control	1.56 a	5.87 a
AS100	2.43 a	7.84 a
AS200	1.80 a	13.96 a
Compost	1.13 a	4.55 a

Abbreviation: GMR-Gross mineralization rate, GACR-Gross ammonium consumption rate. Different letters within a column indicate significantly different treatment means within the column, $p < 0.05$.

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

Table 3-11

Gross nitrification and nitrate consumption rates for site 1 soils in June 2016.

Treatments	GNR	GNCR	GNR*	GNCR*
Control	0.97 b	-0.20 a	2.71 a	4.17 a
AS100	2.74 ab	4.95 a	1.87 a	1.73 a
AS200	6.84 a	17.86 a	4.76 a	17.29 a
Compost	1.19 b	3.78 a	0.94 a	3.44 a

Abbreviation: GNR-Gross nitrification rate, GNCR-Gross nitrate consumption rate.

Different letters within a column indicate significantly different treatment means within the column, $p < 0.05$.

Asterisks: * highlight rates with octyne added, ** highlight rates by AOB

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$ **Table 3-12**

Gross nitrification rates by AOB for site 1 soils in June 2016.

Treatments	GNR (AOB)	SE
Control	-0.48 a	1.0
AS100	0.88 a	0.48
AS200	2.07 a	2.9
Compost	0.25 a	1.1

Abbreviation: GNR-Gross nitrification rate, SE-Standard error. Different letters within a column indicate significantly different treatment means within the column, $p < 0.05$. Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

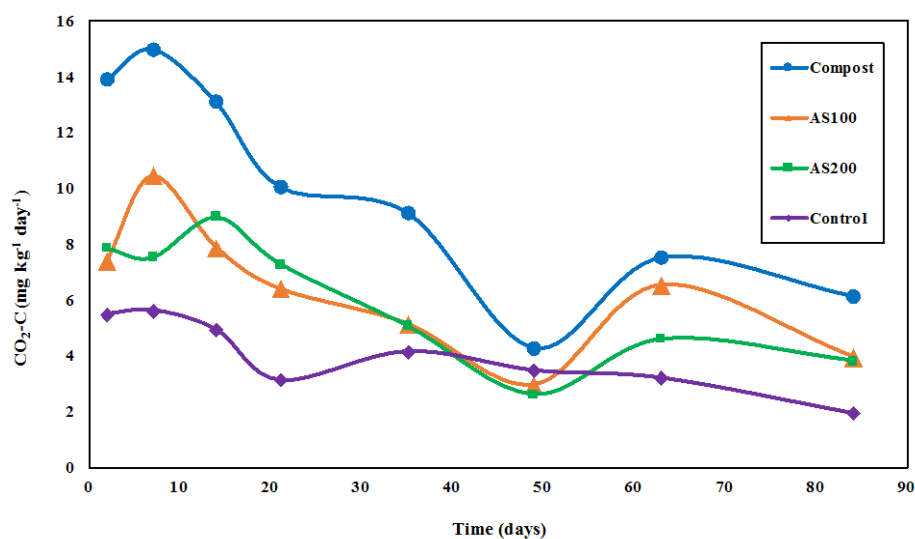


Fig. 3-1. Carbon mineralization rates during laboratory incubation of site 1 soils under four treatments (compost, ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and control (no N fertilization)).

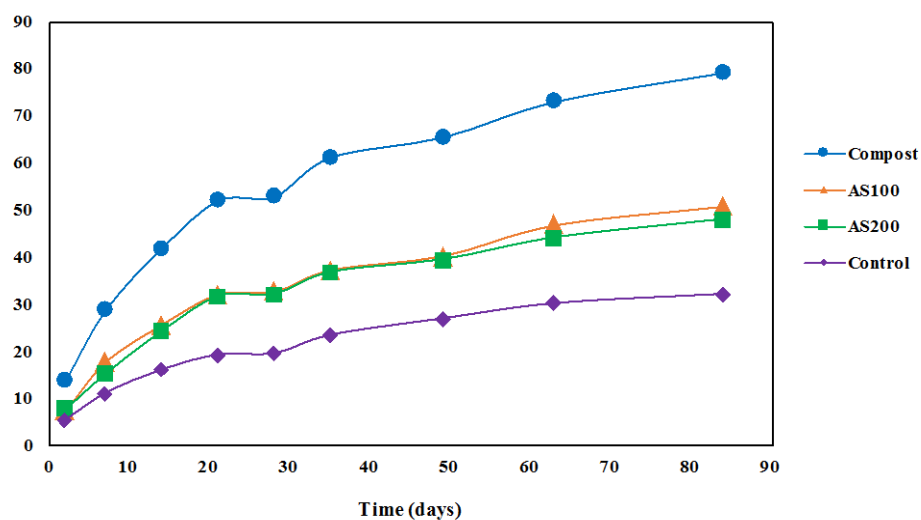


Fig. 3-2. Cumulative CO₂-C (mg C kg⁻¹soil) mineralized during laboratory incubation of site 1 soils under four treatments (compost, ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and control (no N fertilization)).

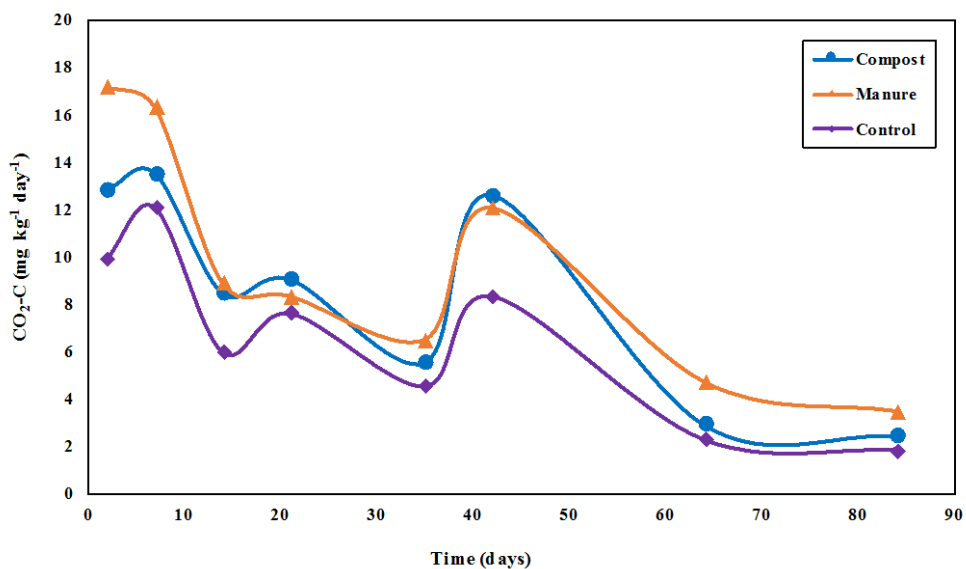


Fig. 3-3. Carbon mineralization rates during laboratory incubation of site 2 soils under three treatments (compost, steer manure, and control (no N fertilization)).

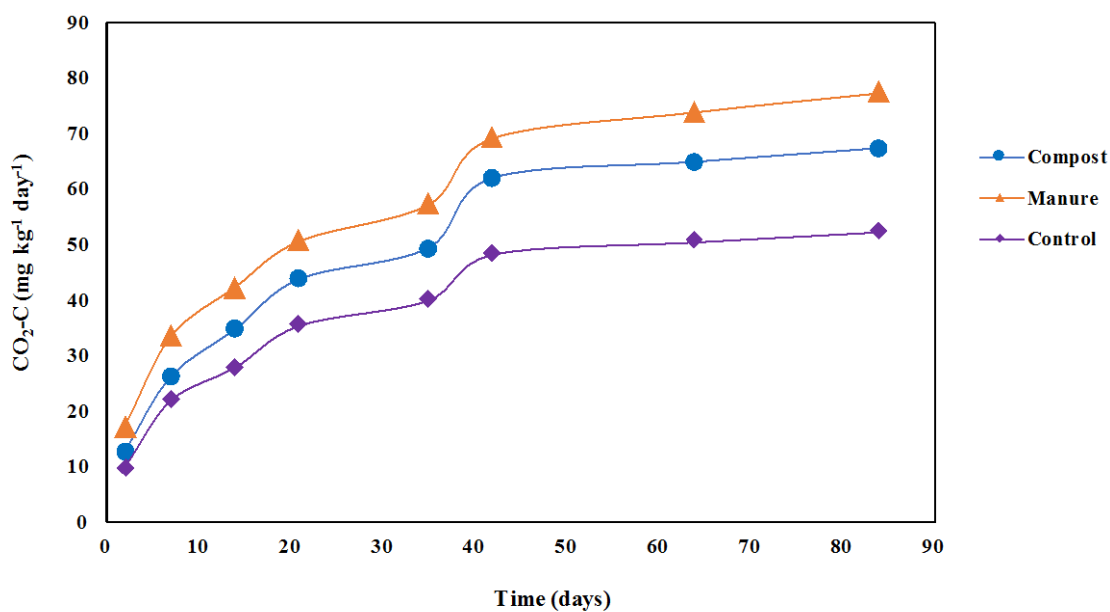


Fig. 3-4. Cumulative C mineralization during laboratory incubation of site 2 soils under three treatments (compost, steer manure, and control (no N fertilization)).

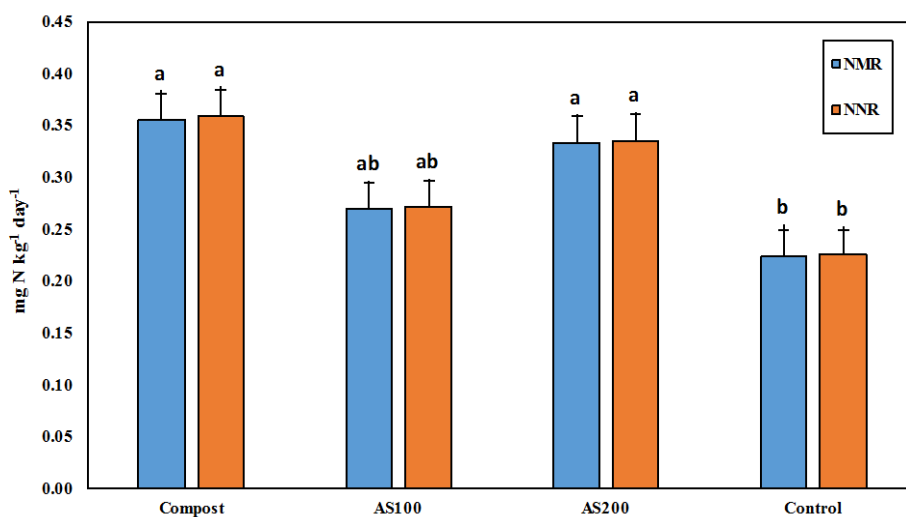


Fig. 3-5. NMR and NNR of soils from site 1 under four treatments (compost, ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and control (no N fertilization)). Error bars represent standard errors (n = 4).

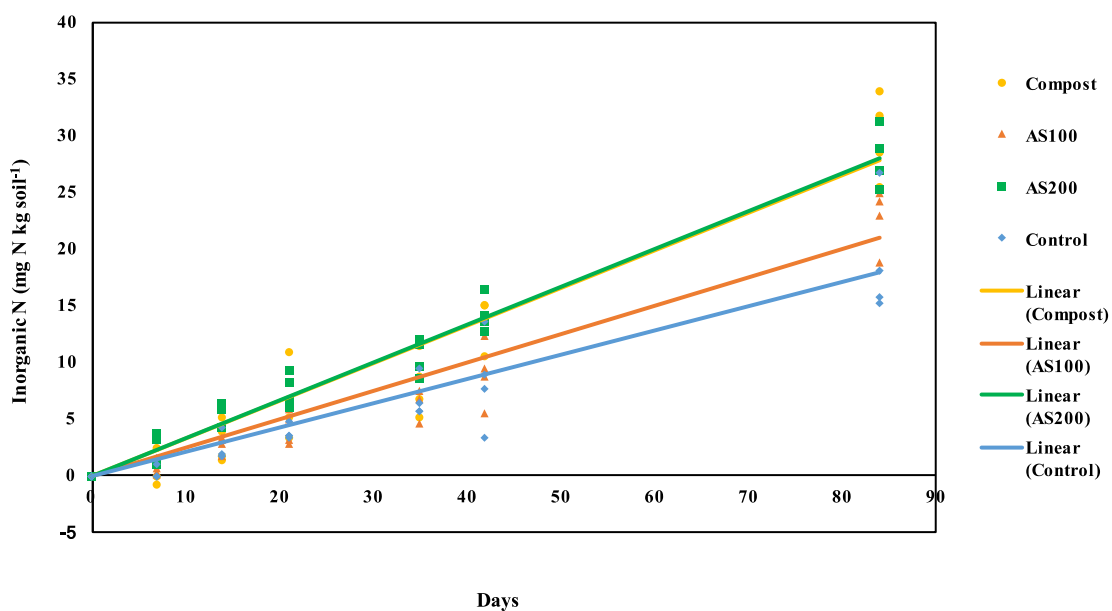


Fig. 3-6. Cumulative inorganic N during laboratory incubation of site 1 soils under four treatments (compost, ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and control (no N fertilization))

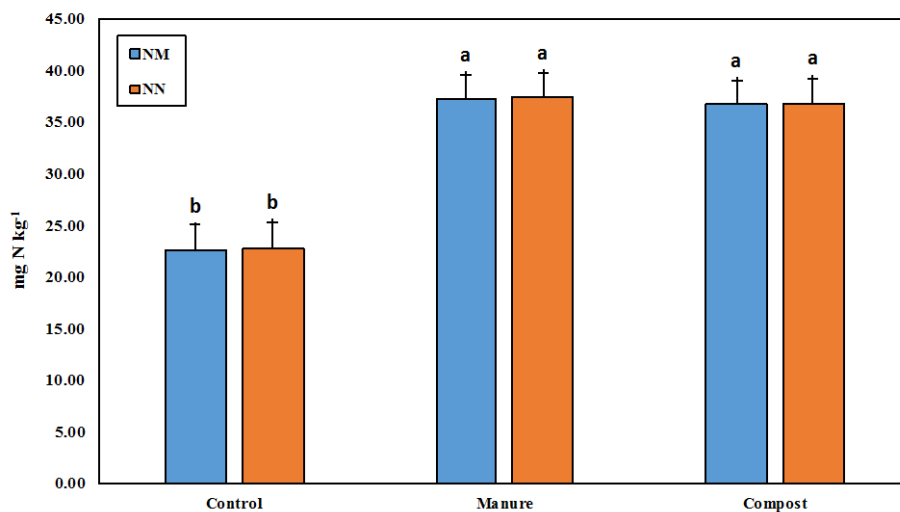


Fig. 3-7. Net mineralization (NM) and net nitrification (NN) of soils from site 2 under three treatments (compost, steer manure, and control (no N fertilization)). Error bars represent standard errors (n = 3).

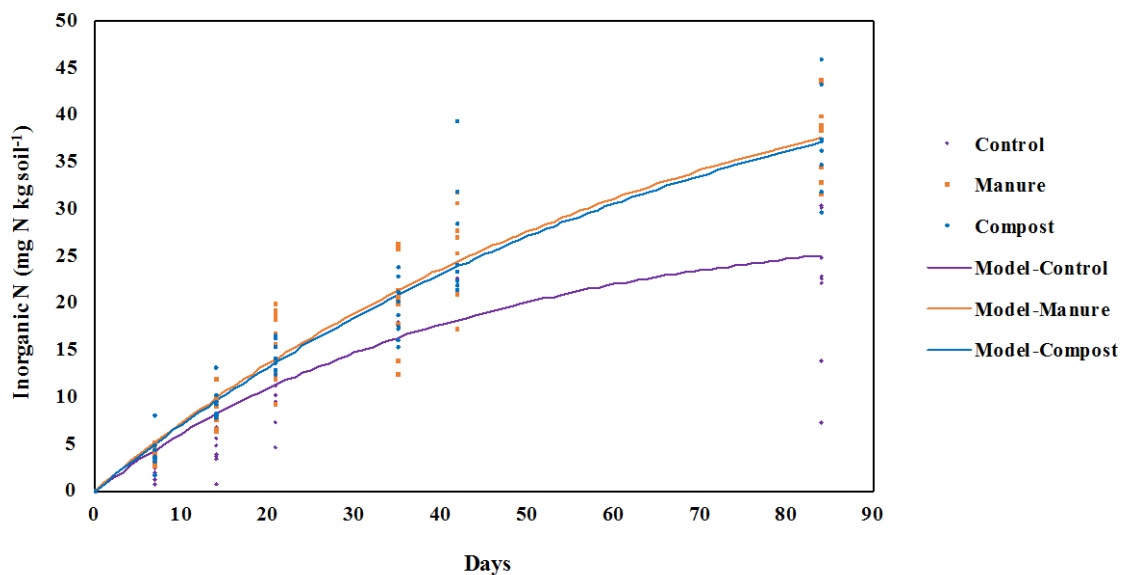


Fig. 3-8. Cumulative inorganic N during laboratory incubation of site 2 soils under three treatments (compost, steer manure, and control (no N fertilization)).

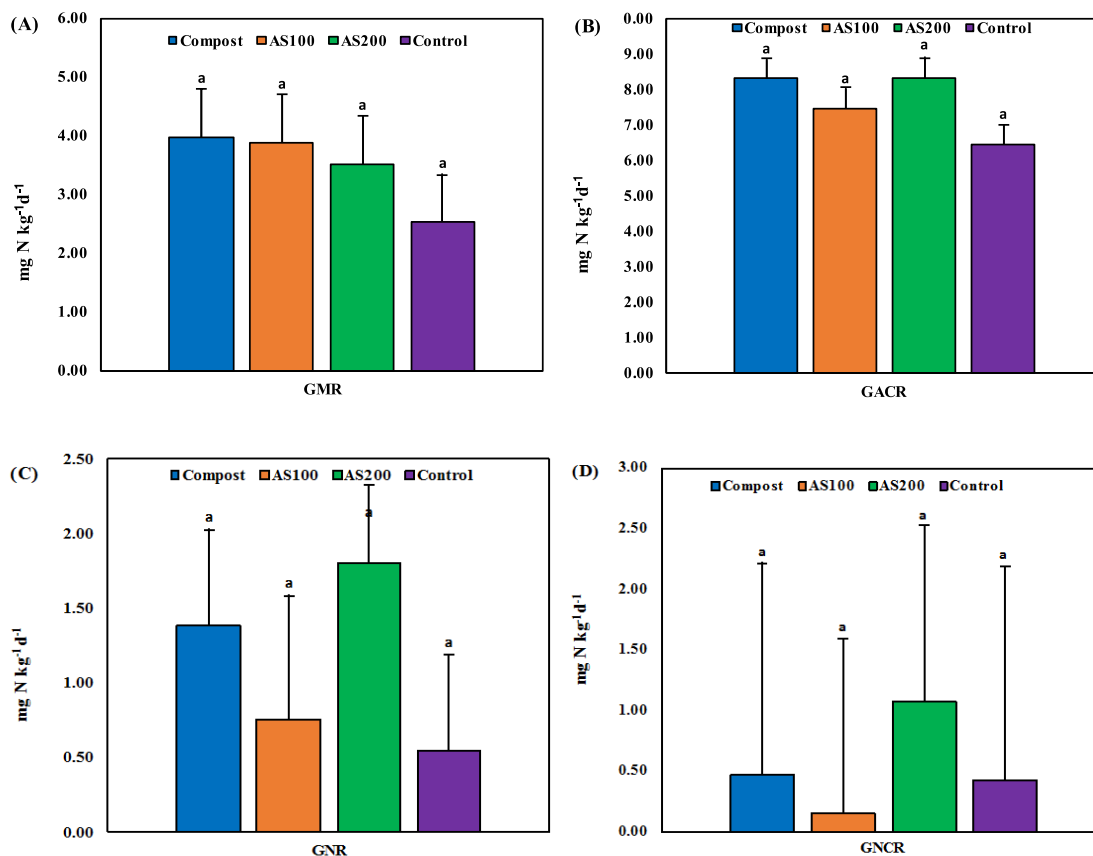


Fig. 3-9. Gross mineralization (A), ammonium consumption (B), nitrification rate (C), and nitrate consumption rates (D) for soils from site 1 sampled in August 2015. Different lowercases above the bars indicate a significant difference among treatments based on Proc GLM. ($p < 0.05$),

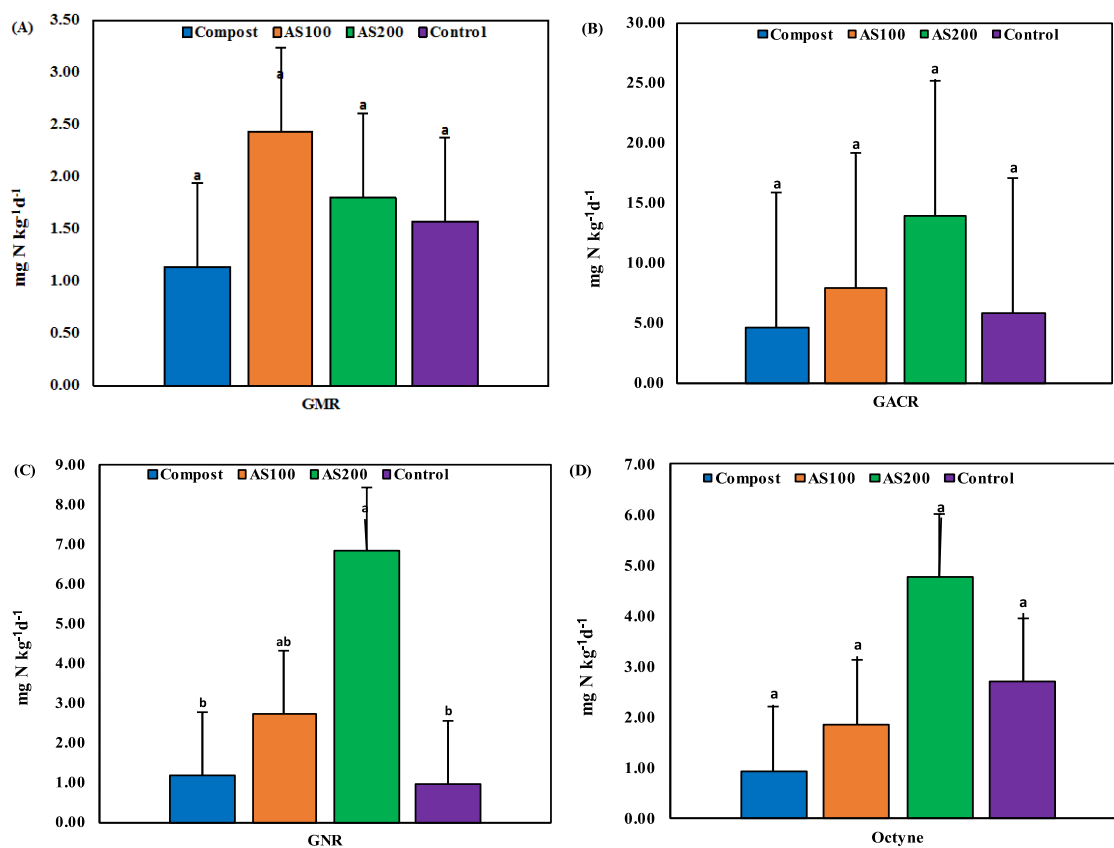


Fig. 3-10. Gross mineralization (A), ammonium consumption (B), nitrification rate (C), and nitrification rates in the presence of octyne (D) for soils from site 1 sampled in June 2016. Different lowercases above the bars indicate a significant difference among treatments based on Proc GLM ($p < 0.05$).

CHAPTER IV

SUMMARY AND CONCLUSIONS

Nitrogen is required by all the crops for their growth and development and thus N availability affects crop yields. While in most agricultural systems the majority of the needed N is applied in the form of inorganic fertilizers; some N is also applied in organic amendments like compost, farm yard manure, etc. The use of chemical N fertilizers first started in the 19th century and increased greatly with the development of Haber-Bosch process. Currently, more than 50% of the chemically fixed N is used by agriculture for fertilization. The efficient use of these N fertilizers is important for sustainable agriculture production. At present, typically less than 50% of the applied N is taken up by plants leading to low N use efficiencies (NUE). The low NUEs can be due to over application of chemical N fertilizer causing N losses, and poor synchrony between crop N demand and N supply. N mineralization helps in understanding the rates at which organic N is being made available to plants for uptake. Deeper understanding of N cycling is required to manage the soil processes involved in the release of N to plants for their growth.

The present study investigated the effects of ammonium sulfate versus steer compost treatments on silage corn yield, N uptake, and NUE. We witnessed differences in yield, N uptake, and NUE under different sources of N. The overall effect of treatment, time and treatment-time interaction was significant in yield, N uptake, NUE, corn ear leaves total N, and total soil N. The performance of AS100 and AS200 was not consistent over the five years. The irrigation water and precipitation played an important role in

determining the differences between different treatments in terms of yield, N uptake, and NUE. There was lower plant N uptake from the organic fertilizer as compared to inorganic fertilizers. The year 2014 was found to be the best managed year in terms of yield, N uptake, and NUE. The synchronization of N supply with plant demand is important for ensuring adequate quantity of N uptake and utilization and optimum yield. The use of integrated management practices can help in increasing N uptake and minimize N losses. The increase in N recoveries by crops will help growers to produce the same crop at low N fertilizer rates. This will not only help in reducing N losses but also cut the fertilizer costs and raise growers' profits.

Furthermore, this study helps in understanding the N mineralization rates under contrasting nitrogen sources. This will improve our understanding of the different factors affecting the observed effects of N sources. The results show that we need to take into consideration the effects of other management practices like crop rotation on N mineralization instead of focusing on N sources only. A collaborative approach should be taken to better understand the complex processes occurring inside soil profile. Improved knowledge of the role of different management practices like crop rotation will definitely help in development of effective crop management. This would further enhance the crop yields and productivity while keeping a healthy soil. The results obtained from this study indicate that the compost treatment significantly increased the C and N mineralized under conventional management while their impacts were less obvious under organic management that received significant organic C and N inputs from crop rotations and cover crop inputs. The high variability in N transformation rates in compost treated soils

in 2016 might be due to presence of hot spots of mineralization, immobilization and nitrification.

Our study provides useful assessment of silage corn yield, N uptake, NUE and N transformation rates that are crucial in enhancing our understanding of NUE and N transformations in agricultural soils treated with ammonium sulfate and steer compost. This should help in the development of sound management practices to maximize crop yields while minimizing negative environmental impacts. The data collected during the course of this study will be helpful in the future formulation of optimized models that could be used in estimation and management of soil N transformations. The improved estimates of soil N dynamics will not only help in improving yields but may also decrease costs. Improved management to prevent over application of fertilizers may be instituted for local farms and for other areas with similar edaphic and climatic conditions.

APPENDICES

APPENDIX A

SUPPLEMENTARY MATERIAL AND STATISTICAL ANALYSIS FOR
CHAPTER II

Results of statistical analysis for Chapter II

Yield calculated over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	13.51	<.0001
Time	4	57	69.95	<.0001
Treatment*Time	12	57	6.55	<.0001

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	4.41	0.0074
Treatment*Time	2013	3	57	8.04	0.0001
Treatment*Time	2014	3	57	162.86	<.0001
Treatment*Time	2015	3	57	8.48	<.0001
Treatment*Time	2016	3	57	0.39	0.7630

Yield sliced by treatment over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	13.51	<.0001
Time	4	57	69.95	<.0001
Treatment*Time	12	57	6.55	<.0001

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	4.41	0.0074
Treatment*Time	2013	3	57	8.04	0.0001
Treatment*Time	2014	3	57	162.86	<.0001
Treatment*Time	2015	3	57	8.48	<.0001
Treatment*Time	2016	3	57	0.39	0.7630

N uptake calculated over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	47.41	<.0001
Time	4	57	44.98	<.0001
Treatment*Time	12	57	8.65	<.0001

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	10.96	<.0001
Treatment*Time	2013	3	57	13.24	<.0001
Treatment*Time	2014	3	57	53.19	<.0001
Treatment*Time	2015	3	57	7.83	0.0002
Treatment*Time	2016	3	57	12.20	<.0001

N uptake sliced by treatment over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	47.41	<.0001
Time	4	57	44.98	<.0001
Treatment*Time	12	57	8.65	<.0001

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	10.96	<.0001
Treatment*Time	2013	3	57	13.24	<.0001
Treatment*Time	2014	3	57	53.19	<.0001
Treatment*Time	2015	3	57	7.83	0.0002
Treatment*Time	2016	3	57	12.20	<.0001

NUE calculated over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	42	30.98	<.0001
Time	4	42	29.18	<.0001
Treatment*Time	8	42	5.10	0.0002

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	2	42	3.07	0.0569
Treatment*Time	2013	2	42	15.09	<.0001
Treatment*Time	2014	2	42	24.08	<.0001
Treatment*Time	2015	2	42	5.17	0.0098
Treatment*Time	2016	2	42	2.31	0.1115

NUE sliced by treatment over five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	42	30.98	<.0001
Time	4	42	29.18	<.0001
Treatment*Time	8	42	5.10	0.0002

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	2	42	3.07	0.0569
Treatment*Time	2013	2	42	15.09	<.0001
Treatment*Time	2014	2	42	24.08	<.0001
Treatment*Time	2015	2	42	5.17	0.0098
Treatment*Time	2016	2	42	2.31	0.1115

Corn ear leaves N for five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	107.55	<.0001
Time	4	57	5.20	0.0012
Treatment*Time	12	57	9.08	<.0001

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	85.37	<.0001
Treatment*Time	2013	3	57	23.51	<.0001
Treatment*Time	2014	3	57	65.20	<.0001
Treatment*Time	2015	3	57	3.23	0.0291
Treatment*Time	2016	3	57	23.88	<.0001

Total soil N (0-15 cm depth) for five years (2012-2016).

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	57	0.87	0.4620
Time	4	57	35.43	<.0001
Treatment*Time	12	57	3.69	0.0004

Tests of Effect Slices					
Effect	Time	Num DF	Den DF	F Value	Pr > F
Treatment*Time	2012	3	57	6.53	0.0007
Treatment*Time	2013	3	57	1.23	0.3069
Treatment*Time	2014	3	57	0.45	0.7195
Treatment*Time	2015	3	57	1.17	0.3285
Treatment*Time	2016	3	57	0.88	0.4573

Table A-1

Compost nutrient composition for the years 2012-2016.

Compost	2012	2013	2014	2015	2016
N	1.48	ND	1.56	1.56	1.68
P	0.60	ND	ND	0.47	0.58
K	1.04	ND	ND	1.07	1.07

Abbreviation: N- Nitrogen, P- Phosphorus, K- Potassium

Unit: %

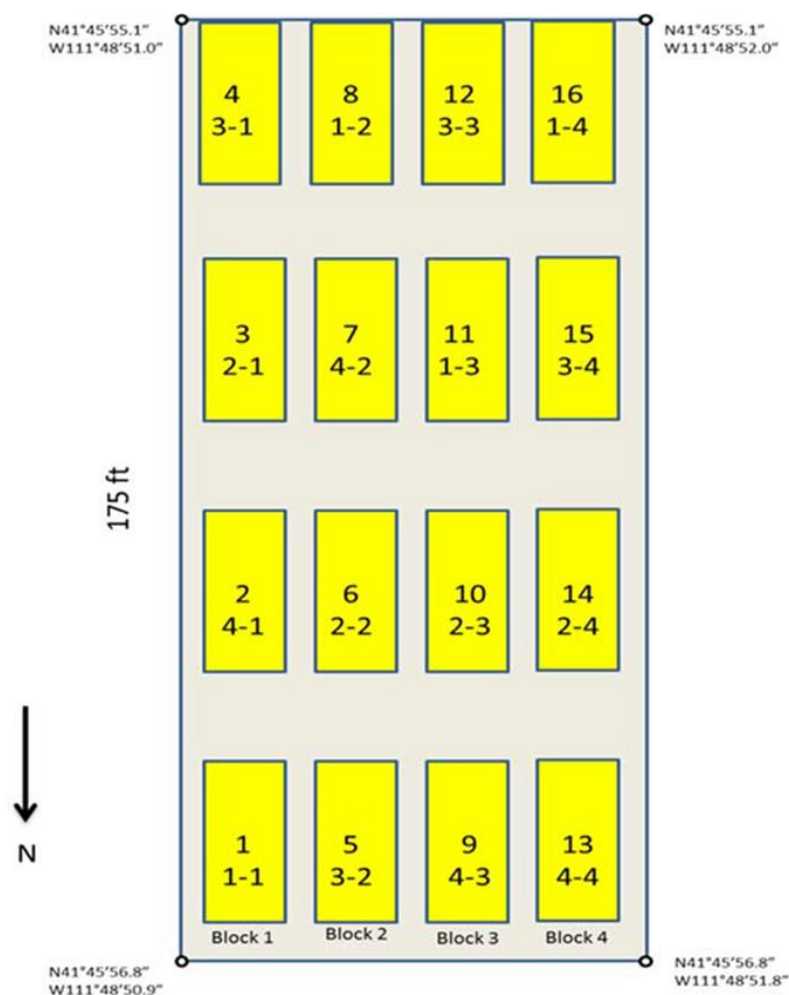


Fig. A-1: Plot layout for N fertilization studies on nutrient levels in corn tissue at the Greenville Farm in North Logan, UT. Top number refers to plot number and the bottom number refers to treatment and block. The four treatments: (1) control, (2) ammonium sulfate 100 (AS100, 112 kg/ha), (3) ammonium sulfate 200 (AS200, 224 kg/ha), (4) compost. Each plot is 3.8 x 9.1 m (4.6 m between rows and 1.2 m between blocks).

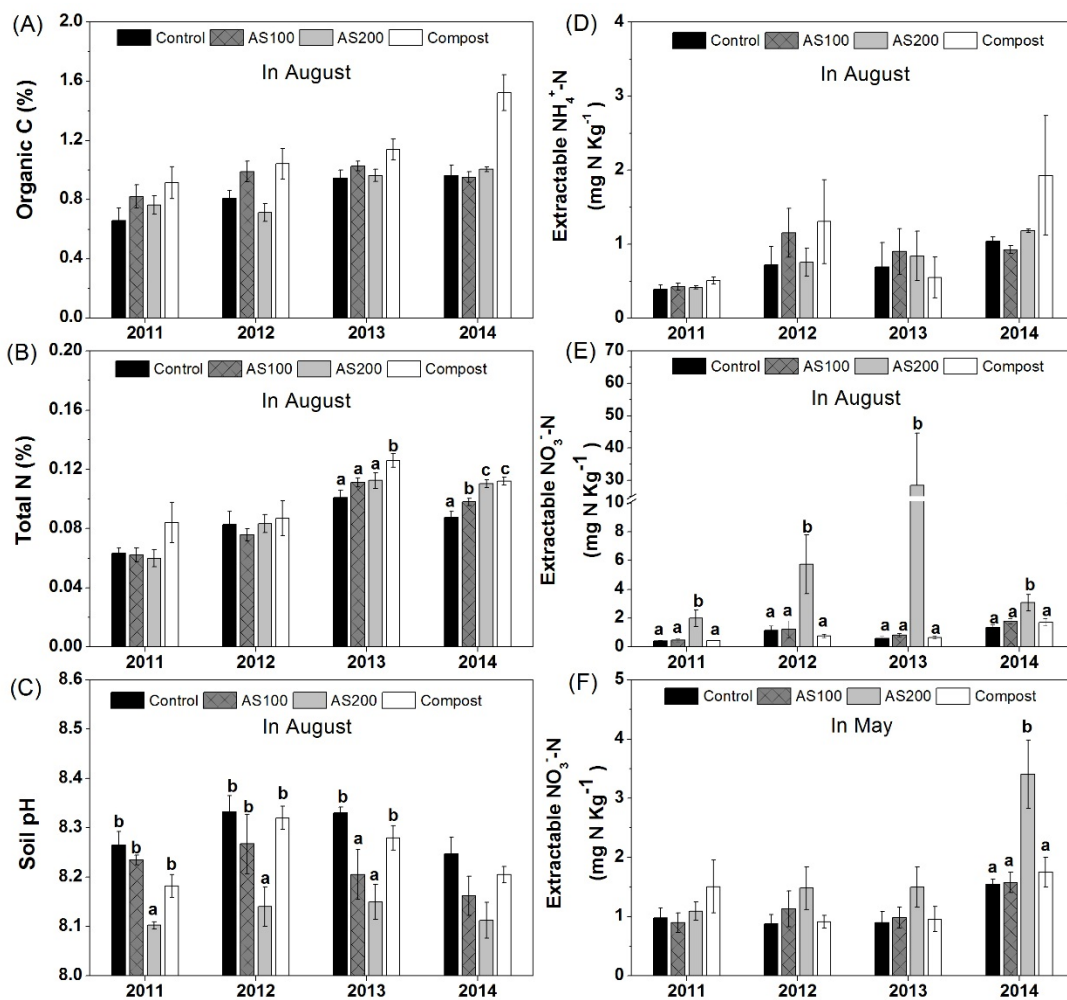


Fig. A-2. Adapted from Ouyang et al., 2017. Soil organic C (A), total N (B), soil pH (C), extractable ammonium (D), and extractable nitrate (E) in August, and extractable nitrate (F) in May for four N treatments (control (no N fertilization), ammonium sulfate (AS 100 & 200 kg N ha⁻¹), and compost (200 kg N ha⁻¹)). Error bars represent standard errors (n = 4). Different lowercases above the bars indicate a significant difference among treatments in a specific year (p < 0.05), based on repeated measures ANOVA. mg N kg⁻¹

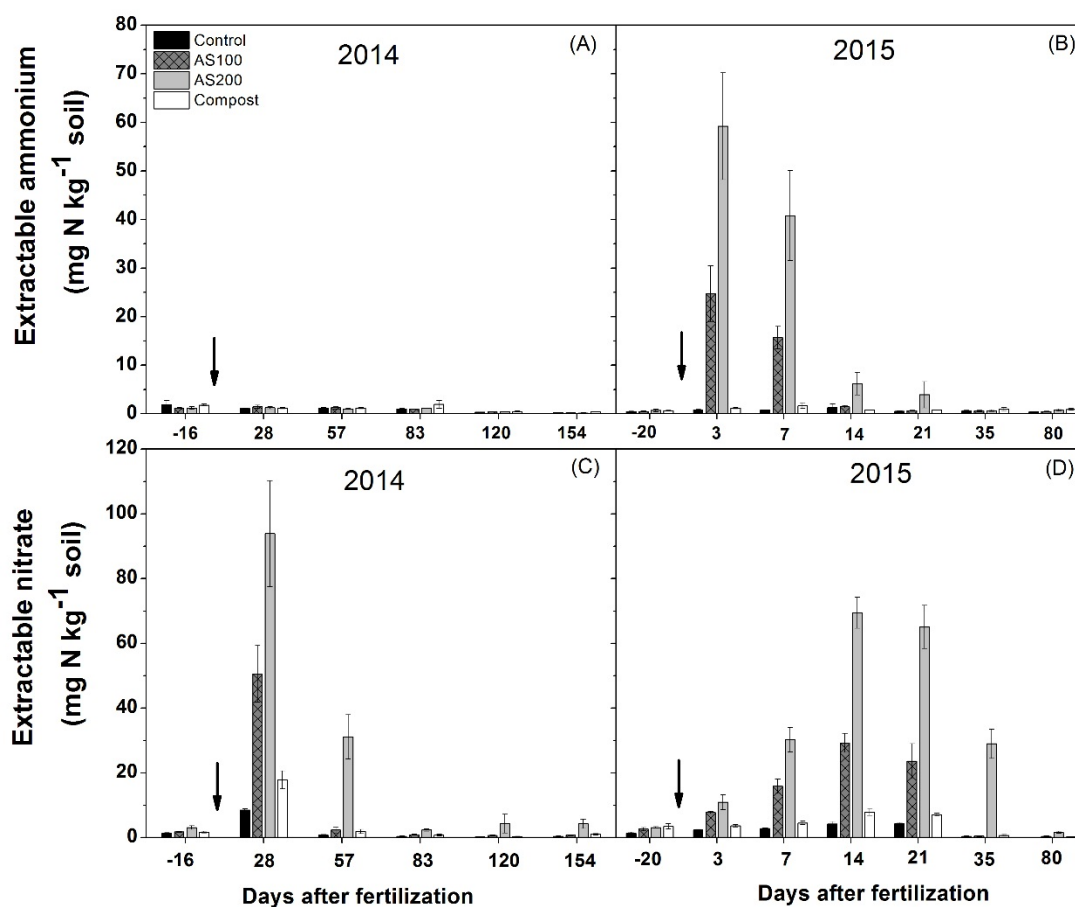


Figure A-3 Adapted from Ouyang et al., 2017. Soil KCl-extractable ammonium in 2014 (A) and 2015 (B), and KCl-extractable nitrate in 2014 (C) and 2015 (D) across four N treatments (control (no N fertilization), ammonium sulfate (AS, 100 & 200 kg N ha⁻¹), and compost (200 kg N ha⁻¹)). Error bars represent standard errors (n = 4).

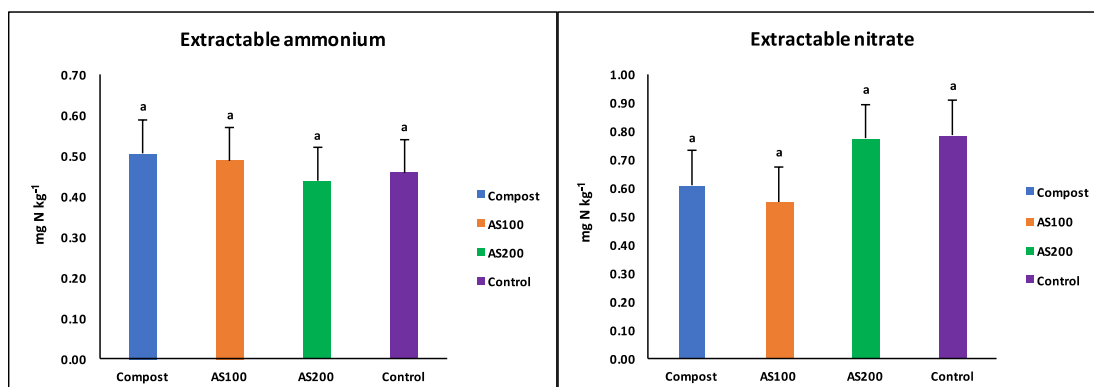


Fig. A-4. Extractable nitrate in May 2016 (0-30cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

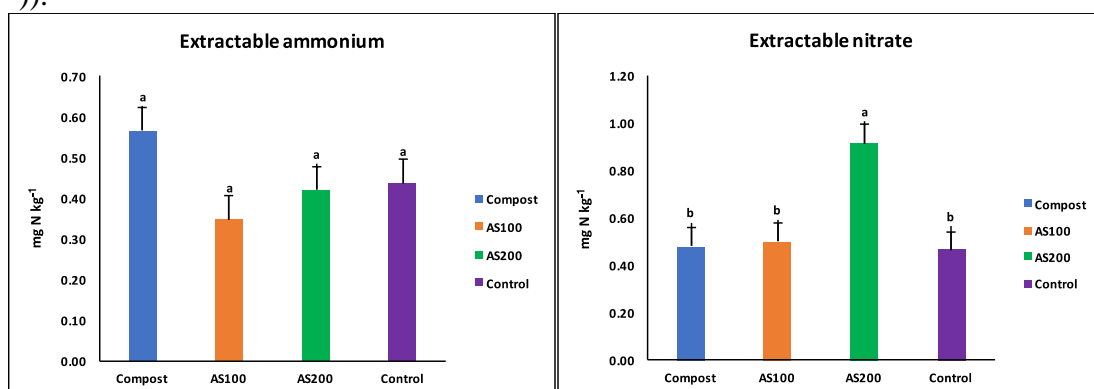


Fig A-5. Extractable nitrate in May 2016 (30-60cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

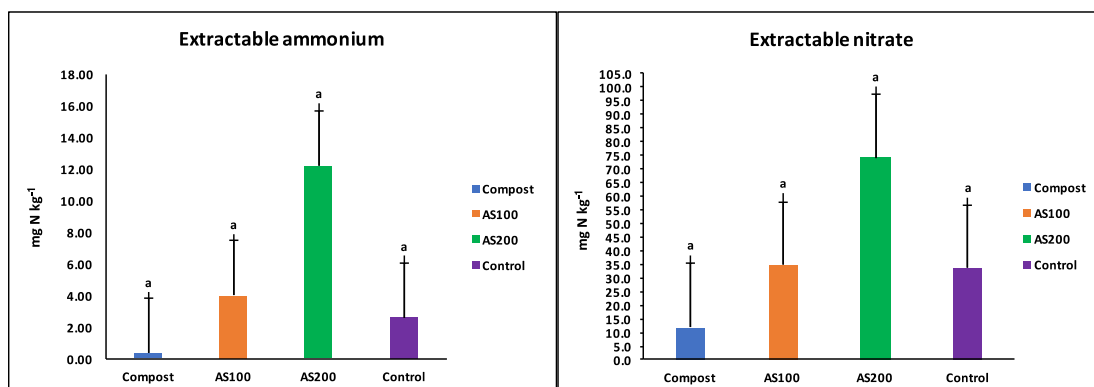


Fig. A-6. Extractable ammonium in June 2016 (0-15cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

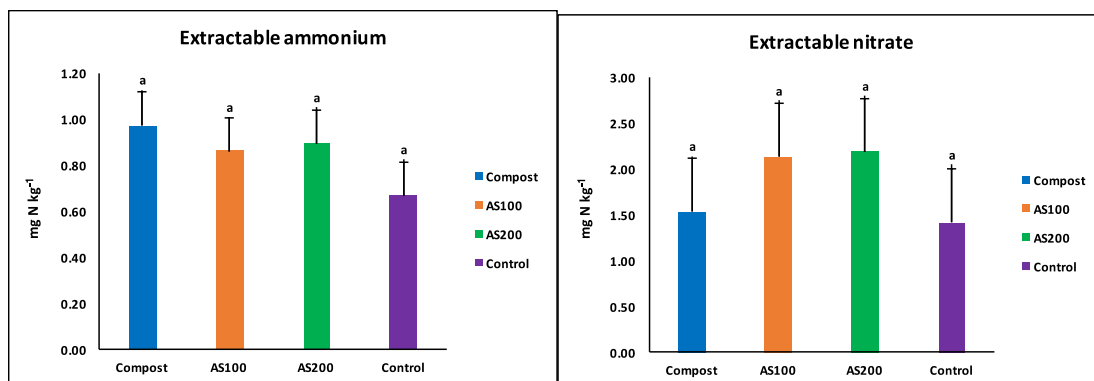


Fig. A-7. Extractable ammonium in December 2016 (0-15cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

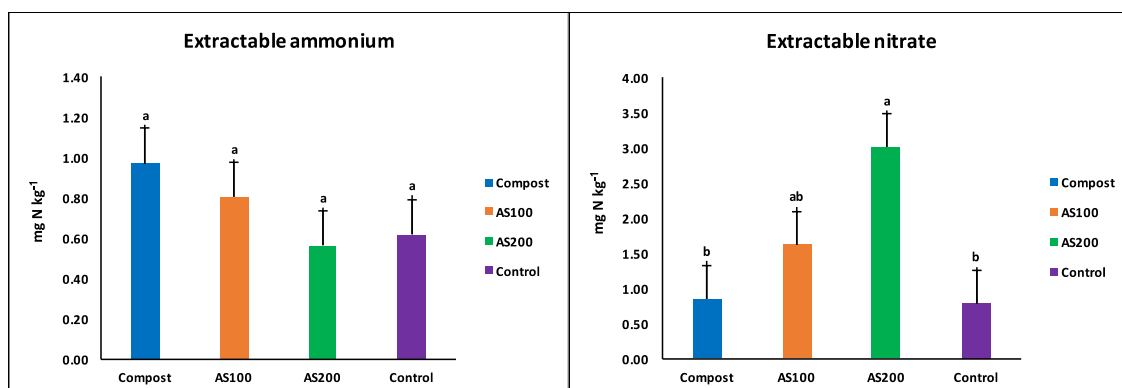


Fig. A-8. Extractable ammonium in December 2016 (0-30cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

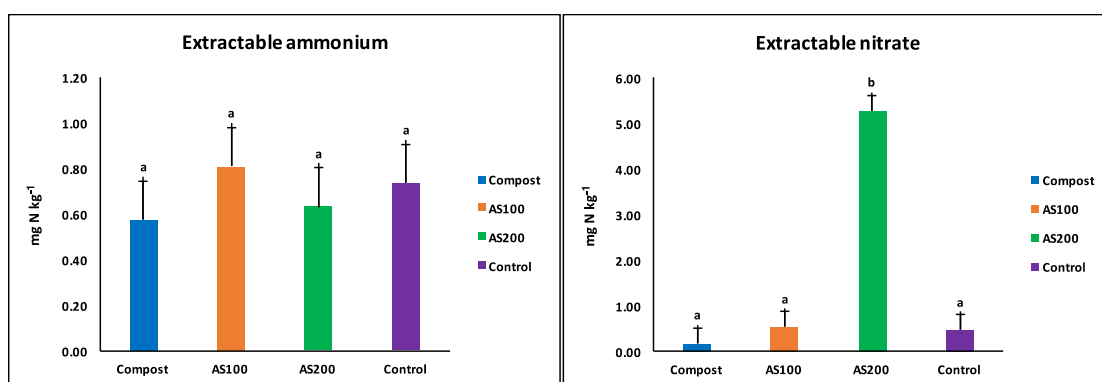


Fig. A-9. Extractable ammonium in December 2016 (30-60cm) for four N treatments (control (no N fertilization), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and compost (224 kg N ha⁻¹)).

APPENDIX B

SUPPLEMENTARY MATERIAL AND STATISTICAL ANALYSIS FOR CHAPTER

III

Results of statistical analysis for chapter III

Net mineralization rates for laboratory incubations under conventional plots site 1.

R-Square	Coeff Var	Root MSE	NMR Mean
0.695401	16.10717	0.047617	0.295625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	0.00446875	0.00148958	0.66	0.5987
Treatment	3	0.04211875	0.01403958	6.19	0.0143

Net nitrification rates for laboratory incubations under conventional plots site 1.

R-Square	Coeff Var	Root MSE	NNR Mean
0.717211	15.51982	0.046368	0.298766

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	0.00486240	0.00162080	0.75	0.5473
Treatment	3	0.04421295	0.01473765	6.85	0.0106

Net mineralization rates for laboratory incubations under organic rotation plots (site 2).

R-Square	Coeff Var	Root MSE	NMR Mean
0.604590	19.06824	0.073194	0.383853

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.00519579	0.00259789	0.48	0.6222
Treatment	2	0.17501793	0.08750896	16.33	<.0001

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CoverCrop	2	0.00500000	0.00250000	0.47	0.6301
Treatment	2	0.17360000	0.08680000	16.38	<.0001

First order model fit using SAS for organic rotation plots (site 2).

Iterative Phase							
Iter	Nc	Nm	Nn	Kc	Km	Kn	Sum of Squares
0	50.0000	50.0000	50.0000	0.0200	0.0200	0.0200	6826.5
1	50.6785	50.9476	28.7446	0.0147	0.0151	0.0221	1381.0
2	53.1504	53.0011	29.0417	0.0142	0.0147	0.0235	1323.3
3	53.1799	53.0207	29.0812	0.0142	0.0147	0.0234	1323.2

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Model	6	62310.5	10385.1	1436.26	<.0001
Error	183	1323.2	7.2306		
Uncorrected Total	189	63633.7			

Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
Nc	53.1799	4.4308	44.4378	61.9219
Nm	53.0207	4.1862	44.7614	61.2801
Nn	29.0812	2.0334	25.0692	33.0932
Kc	0.0142	0.00180	0.0107	0.0178
Km	0.0147	0.00178	0.0112	0.0182
Kn	0.0234	0.00300	0.0175	0.0293

Net nitrification rates for laboratory incubations under organic rotation plots (site 2).

R-Square	Coeff Var	Root MSE	NNR Mean
0.601489	19.04669	0.073464	0.385706

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.00528012	0.00264006	0.49	0.6196
Treatment	2	0.17392942	0.08696471	16.11	<.0001

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CoverCrop	2	0.00587407	0.00293704	0.55	0.5866
Treatment	2	0.17254074	0.08627037	16.06	<.0001

Potentially mineralizable N (N_0) and decomposition rate constant (k) based on the first order kinetics model fit for laboratory incubations for site 2 sampled in October 2015.

R-Square	Coeff Var	Root MSE	sqrt_N0 Mean
0.540478	15.73967	1.080203	6.862935

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CoverCrop	2	0.89050741	0.44525371	0.38	0.6872
Treatment	2	29.30249269	14.65124634	12.56	0.0002

R-Square	Coeff Var	Root MSE	sqrt Mean
0.358762	21.02631	0.027668	0.131588

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CoverCrop	2	0.00019205	0.00009603	0.13	0.8827
Treatment	2	0.00923048	0.00461524	6.03	0.0082

GMR for pool dilution experiment in 2015.

R-Square	Coeff Var	Root MSE	GMR Mean
0.604827	46.52495	1.617033	3.475625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	30.78106875	10.26035625	3.92	0.0482
Treatment	3	5.23736875	1.74578958	0.67	0.5928

GACR for diffusion experiment in 2015.

R-Square	Coeff Var	Root MSE	GACR Mean
0.723252	14.78316	1.129249	7.638750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	20.23227500	6.74409167	5.29	0.0224
Treatment	3	9.76127500	3.25375833	2.55	0.1208

GNR for diffusion experiment in 2015.

R-Square	Coeff Var	Root MSE	GNR Mean
0.573499	86.56676	1.049622	1.212500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	4.13520143	1.37840048	1.25	0.3846
Treatment	3	3.37485976	1.12495325	1.02	0.4573

Parameter	Estimate	Standard Error	t Value	Pr > t
Trts vs Control	3.06381579	2.19379064	1.40	0.2214

GNCR for diffusion experiment in 2015.

R-Square	Coeff Var	Root MSE	GNCR Mean
0.319027	278.9868	2.907441	1.042143

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	22.60981958	7.53660653	0.89	0.4911
Treatment	3	7.36044458	2.45348153	0.29	0.8313

Parameter	Estimate	Standard Error	t Value	Pr > t
Trts vs Control	2.45650000	5.95848078	0.41	0.6925

GMR for pool dilution experiment in 2016.

R-Square	Coeff Var	Root MSE	GMR Mean
0.291375	93.19817	1.611163	1.728750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	6.09512500	2.03170833	0.78	0.5329
Treatment	3	3.51122500	1.17040833	0.45	0.7229

GACR for pool dilution experiment in 2016.

R-Square	Coeff Var	Root MSE	GACR Mean
0.409800	80.51782	6.485207	8.054375

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	54.5705187	18.1901729	0.43	0.7349
Treatment	3	208.2524688	69.4174896	1.65	0.2460

GNR for pool dilution experiment in 2016.

R-Square	Coeff Var	Root MSE	GNR Mean
0.589412	107.8179	3.166476	2.936875

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	40.81061875	13.60353958	1.36	0.3168
Treatment	3	88.73056875	29.57685625	2.95	0.0908

GNCR for pool dilution experiment in 2016.

R-Square	Coeff Var	Root MSE	GNCR Mean
0.519630	167.0906	11.02693	6.599375

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	448.8088688	149.6029563	1.23	0.3543
Treatment	3	734.9693187	244.9897729	2.01	0.1824

GNR with octyne for diffusion experiment in 2016.

R-Square	Coeff Var	Root MSE	GNROctyne Mean
0.379090	98.38362	2.528459	2.570000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	3.16305000	1.05435000	0.16	0.9173
Treatment	3	31.96620000	10.65540000	1.67	0.2427

GNCR with octyne for diffusion experiment in 2016.

R-Square	Coeff Var	Root MSE	GNCROctyne Mean
0.448752	177.9852	11.84603	6.655625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	3	412.7297188	137.5765729	0.98	0.4442
Treatment	3	615.3972188	205.1324063	1.46	0.2891

Carbon mineralization during laboratory incubation in conventional plots.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	93	28.16	<.0001
Days	7	93	14.27	<.0001
Treatment*Days	21	93	1.29	0.2035

Tests of Effect Slices					
Effect	Days	Num DF	Den DF	F Value	Pr > F
Treatment*Days	2	3	93	3.97	0.0104
Treatment*Days	7	3	93	6.19	0.0007
Treatment*Days	14	3	93	5.73	0.0012
Treatment*Days	21	3	93	8.84	<.0001
Treatment*Days	35	3	93	17.95	<.0001
Treatment*Days	49	3	93	0.50	0.6860
Treatment*Days	63	3	93	2.12	0.1028
Treatment*Days	84	3	93	6.41	0.0005

Carbon mineralization during laboratory incubation under organic rotation plots.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	46	6.00	0.0048
Days	7	46	53.24	<.0001
Treatment*Days	14	46	0.68	0.7846

Tests of Effect Slices					
Effect	Days	Num DF	Den DF	F Value	Pr > F
Treatment*Days	2	2	46	1.12	0.3362
Treatment*Days	7	2	46	2.18	0.1245
Treatment*Days	14	2	46	6.67	0.0029
Treatment*Days	21	2	46	0.37	0.6935
Treatment*Days	35	2	46	5.44	0.0076
Treatment*Days	42	2	46	1.19	0.3143
Treatment*Days	64	2	46	2.93	0.0636
Treatment*Days	84	2	46	10.92	0.0001

Table B-1

Carbon mineralization after 24 hours for the ^{15}N pool dilution experiment 2016.

Treatments	NH_4	NO_3	NO_3^*
Control	0.1347 a	0.3160 a	0.5470 a
AS100	0.1347 a	0.6934 a	0.6021 a
AS200	0.7212 a	0.3918 a	0.9152 a
Compost	0.4896 a	0.4762 a	0.7731 a

Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Asterisks highlight rates with octyne added.

Unit: $\text{mg kg}^{-1}\text{d}^{-1}$

Table B-2

NMR and NNR for laboratory incubations for site 2 sampled in October 2015.

Treatments	NMR	NNR
Control	0.27 b	0.27 b
Manure	0.44 a	0.45 a
Compost	0.44 a	0.44 a

Abbreviation: NMR-Net mineralization rate, NNR-Net nitrification rate. Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

Table B-3

Gross nitrification rates for site 1 soils in June 2016.

Plots	Treatment	GNR	GNR*	GNR**
1	Control	0.99	1.26	-0.27
2	Compost	0.55	1.44	-0.89
3	AS100	0.09	0.15	-0.06
4	AS200	4.00	8.12	-4.12
5	AS200	8.61	5.36	3.25
6	AS100	5.88	3.80	2.08
7	Compost	3.67	0.07	3.60
8	Control	0.25	-0.55	0.80
9	Compost	0.14	0.95	-0.81
10	AS100	3.18	2.89	0.29
11	Control	0.34	4.47	-4.13
12	AS200	14.11	4.57	9.54
13	Compost	0.41	1.30	-0.89
14	AS100	1.83	0.62	1.21
15	AS200	0.64	1.01	-0.37
16	Control	7.36	5.66	1.70

Abbreviation: GNR-Gross nitrification rate. Different letters within a column indicate significantly different treatment means within the year, $p < 0.05$.

Asterisks: * highlight rates with octyne added, ** highlight rates by AOB

Unit: $\text{mg N kg}^{-1}\text{d}^{-1}$

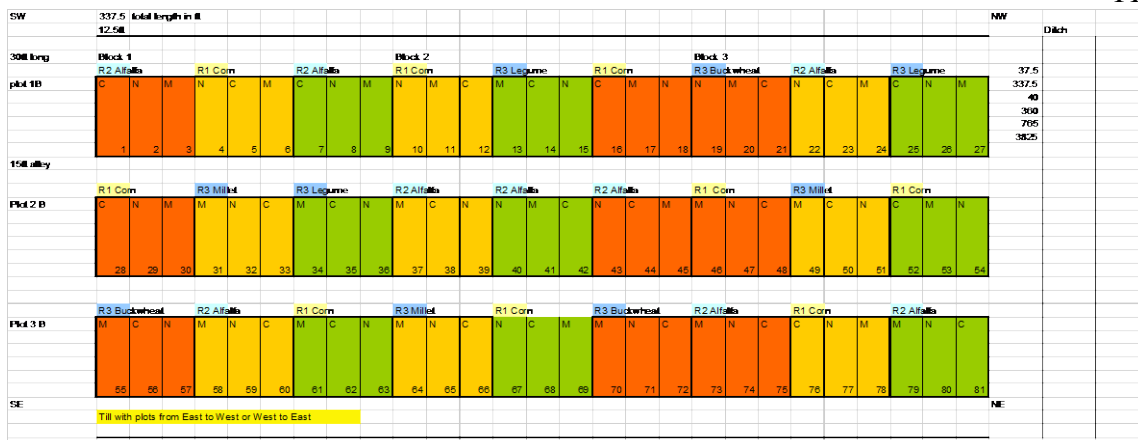


Fig. B-1. Plot layout for N fertilization studies on nutrient levels in corn tissue at the Greenville Farm in North Logan, UT. Also, known as the “Organic Rotation Plots” or “GRO-6 Field”.

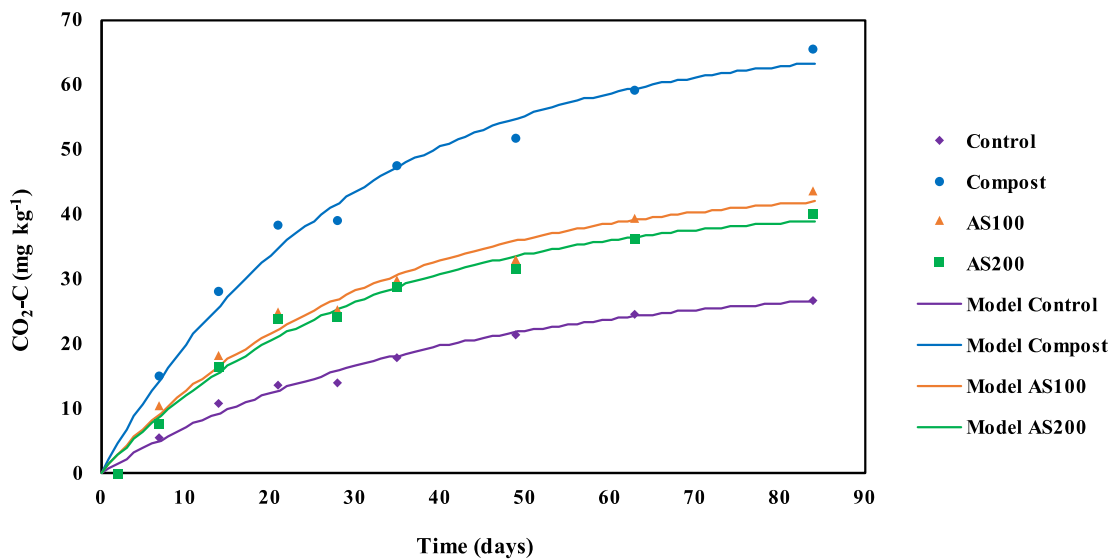


Fig. B-2. Cumulative CO₂-C mineralization using a first order model during laboratory incubation of site 1 soils under four treatments (compost (224 kg N ha⁻¹), ammonium sulfate (AS 112 & 224 kg N ha⁻¹), and control (no N fertilization)).

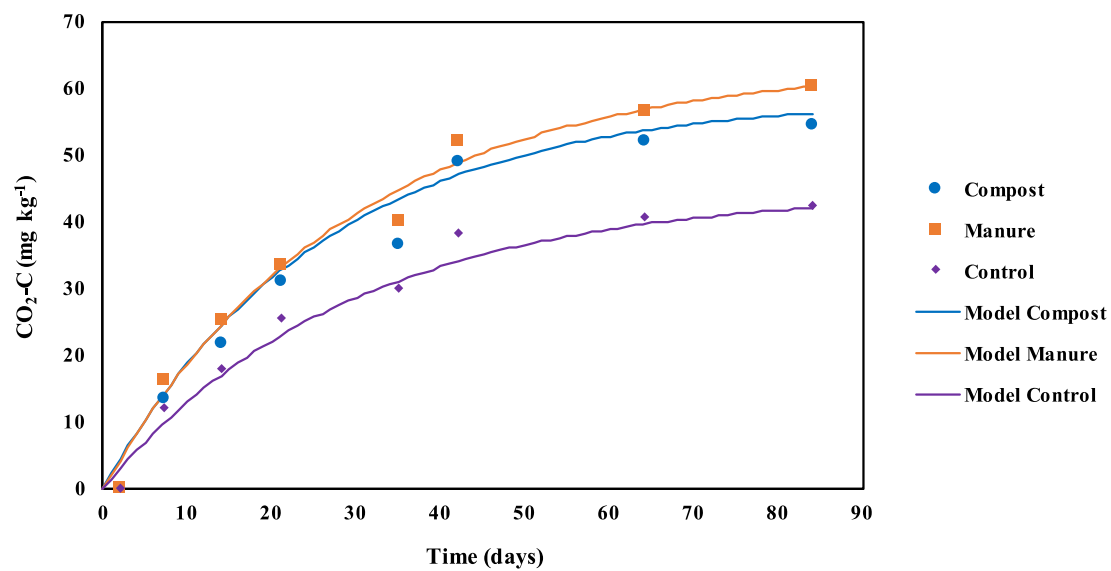


Fig. B-3. Cumulative CO₂-C mineralization using a first order model during laboratory incubation of site 2 soils under three treatments (compost (224 kg N ha⁻¹), steer manure, and control (no N fertilization)).

CURRICULUM VITAE

Avneet Kakkar

Department of Plants, Soils and Climate,
Utah State University, Logan, UT 84322-4820
Cell Phone: (435)-294-7893
E-mail: avneetkakkkar007@aggiemail.usu.edu

PERSONAL DATA

Date of Birth: March 15, 1991
Place of Birth: Kotkapura, Punjab, India
Gender: Male
Nationality: Indian

EDUCATION

2015-present Masters' candidate (Plant Science).
Utah State University, Logan, Utah.

2009-2013 B.Sc. Agriculture Sciences, *graduated with First Class*.
Punjabi University, Punjab, India.

EXPERIENCE

2015-present Research Assistant, Utah State University, Logan, Utah.
Thesis: Nitrogen availability and use efficiency in corn treated with contrasting nitrogen sources.

SKILLS

Lab Skills:

Soil analysis techniques: soil nutrients (TC, TN, NO₃⁻, NH₄⁺); Stable Isotope N¹⁵ pool dilution, Gas chromatography measurement for CO₂.
Molecular biology techniques: genomic DNA extraction, quantitative real-time PCR.

Field work:

Plant tissue and soil sampling.
Installed sprinkler irrigation system and applied organic and inorganic fertilizers using broadcasting method.
Led different farming operations from sowing to harvesting.
Worked and interacted with growers at various extension programs and farm exhibitions at Punjab Agricultural University.

Computer Skills:

Data analysis: SAS.
Office Tool: Microsoft Excel, Word, PowerPoint.
Web Designing and Development (PHP).

Language:

Punjabi (Fluent).
Hindi (Fluent).
English (Fluent.)

TEACHING EXPERIENCE

Guest Lecturer:

2017: Nitrogen inputs and losses. In PSC 5530 Soils and Plant Nutrient Bioavailability. Utah State University.

Teaching Assistant:

2017: Analytical techniques for the soil environment (PSC 5560). Utah State University.

PRESENTATIONS

Poster Presentations

Avneet Kakkar, Yang Ouyang and Jeanette Norton. Nitrogen availability and use efficiency in corn treated with contrasting nitrogen sources, PSC Graduate Seminar, Utah State University, Logan, UT, USA, December 2016.

Avneet Kakkar, Yang Ouyang and Jeanette Norton. Nitrogen use efficiency and nitrogen mineralization in corn treated with contrasting nitrogen sources, USU Spring Forum, Utah State University, Logan, UT, USA, April 2017.

Avneet Kakkar, Yang Ouyang and Jeanette Norton. Nitrogen availability and use efficiency in corn treated with contrasting nitrogen sources, Western Nutrient Management Conference, Reno, NV, USA, March 2017.

Oral Presentations

Avneet Kakkar and Jeanette Norton. Nitrogen availability and use efficiency in corn treated with contrasting nitrogen sources, PSC Graduate Seminar, Utah State University, Logan, UT, USA, December 2015.

Avneet Kakkar, Yang Ouyang and Jeanette Norton. Nitrogen availability and use efficiency in corn treated with contrasting nitrogen sources, PSC Graduate Seminar, Utah State University, Logan, UT, USA, December 2016.

REFERENCES

Dr. Jeanette M. Norton: Principal Investigator; jeanette.norton@usu.edu; Phone: 435-797-2166.

Dr. David Hole: Professor (Plant Breeding/Genetics); david.hole@usu.edu; Phone: 435-797-3455.

Dr. Jennifer Reeve: Professor (Organic/Sustainable Agriculture); jennifer.reeve@usu.edu; Phone: 435-797-3192.