

THE EFFECT OF UREA-AMMONIUM-NITRATE FERTILIZER AMENDED WITH  
UREASE AND NITRIFICATION INHIBITORS ON NITROGEN CYCLING IN HIGHLY  
ORGANIC AGRICULTURAL SOIL

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## **ABSTRACT**

JOELENE M. DIEHL: The effect of urea-ammonium-nitrate fertilizer amended with urease and nitrification inhibitors on nitrogen cycling in highly organic agricultural soil.

Starting in the mid 20<sup>th</sup> century the use of nitrogen fertilizers has steadily increased due to the rising demand in food production. Currently, urea-based nitrogen fertilizers are the most commonly used source of nitrogen in agriculture worldwide. Although there are benefits to urea fertilizers (high nitrogen content, high solubility, and low cost), its rapid decomposition to ammonia and carbon dioxide could lead to environmental and human health concerns. Laboratory experimentation was conducted to determine the rate of urea decomposition, ammonia volatilization, influence of soil moisture on urea decomposition, potential for fertilizer loss below the rooting zone, and the response of the microbial community to fertilization of highly organic agricultural soil with urea-ammonium-nitrate (UAN). In addition, urease and nitrification inhibitors were added to UAN fertilizer in order to determine if these amendments altered the rate of N transformations in soil.

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## ABBREVIATIONS

ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
CEC	Cation exchange capacity
DCD	Dicyandiamide
DEA	Denitrifying enzyme activity
GIS	Geographic information system
GPS	Global positioning system
IFA	International Fertilizer Industry
Mt	Million tones
NA	Nitrifying activity
NBPT	N-(n-butyl)thiophosphoric triamide
NK-N	Monoammonium phosphate
NPK-N	Diammonium phosphate
Nr	Reactive nitrogen
NUE	Nitrogen use efficiency
OM	Organic matter
PA	Precision agriculture
UAN	Urea, ammonium, and nitrate
UI	UAN and Agrotain
UNI	UAN and Agrotain Plus
WFPS	Water filled pore space
WHC	Water holding capacity

## CHAPTER 1

### INTRODUCTION

#### **Increased use of N fertilizer in agriculture**

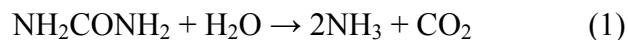
The use of nitrogen fertilizers for food production has continually increased globally since the mid 20<sup>th</sup> century. After World War II, the Haber-Bosch process was used to convert large amounts of atmospheric dinitrogen gas into reactive nitrogen (Nr). The International Fertilizer Industry Association (IFA; 2006) estimates that in 2004 there were approximately 90 Mt (million tonnes) of N fertilizer used worldwide. That was roughly 7.5 times more N fertilizer than was used in the 1960's. The use of N fertilizer has also increased in the United States by approximately three and half times over the last 40 years. The estimate of total N fertilizer use in the United States in 2004 was about 11 Mt (<http://www.fertilizer.org>). The ability to easily convert atmospheric N<sub>2</sub> into Nr increased human inputs of Nr from zero in the late 19<sup>th</sup> century to about 82 Mt in the year 2000 (Fixen and West 2002). Three-quarters of the N<sub>2</sub> fixed by humans is used for agricultural fertilizer production (Galloway et al. 2004). Currently, Asia, Europe, and N. America are the top three producers of Nr (Galloway et al. 2004). The commercialization of the Haber-Bosch process coupled with the industrial revolution and human population explosion has led to a worldwide increase in the application of synthetic N fertilizers to agroecosystems.

Nitrogen fertilizer is a broad term that includes a variety of different types of nitrogen- based soil additives. The IFA combines about seven different types of N fertilizers



in order to estimate total fertilizer-N application. Ammonium sulfate, urea, ammonium nitrate, ammonia, calcium ammonium nitrate, and also mixtures of various N forms like urea, ammonium, and nitrate (UAN) are common types of N fertilizers that are available to farm managers today (<http://www.fertilizer.org>). In addition, there are synthetic fertilizers that contain potassium and/or phosphorus in addition to nitrogen. Some of these fertilizer categories include monoammonium phosphate (NK-N) and diammonium phosphate (NPK-N) (Bouwman 1997). The N fertilizer best suited for a particular agricultural application is dependent on a variety of factors which include but are not limited to crop, soil type, and climate.

Despite the variety of synthetic N fertilizers available, urea-based fertilizers are the fastest growing and most commonly used source of N worldwide for agriculture (Bremner 1995). Urea can be applied as a synthetic fertilizer and is a major component in animal manure fertilizers. Currently, it is estimated that more than half of all fertilizer used globally is in the form of urea (Gilbert et al. 2006). The benefits of using urea as a fertilizer are due to its high nitrogen content (approximately 46% nitrogen), nonpolarity, high solubility, and low expense to manufacture, store, and transport (Prasad 1998). However, there are some problems associated with the use of urea fertilizers. Once urea is in soil, it rapidly decomposes (Eq. 1) to form ammonia (NH<sub>3</sub>) and carbon dioxide through hydrolysis (Bremner 1995):



In addition to increasing NH<sub>3</sub> volatilization, urea fertilizer can cause an increase in soil pH, and nitrite (Krogmeier et al. 1989; Bremner 1995). The increase in soil NH<sub>3</sub> (or NH<sub>4</sub><sup>+</sup>,

depending on soil pH) concentration from urea has been linked to a multitude of environmental and human health concerns (Bremner 1995; Townsend et al. 2003).

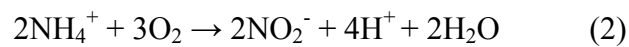
A major pathway by which N is lost from agricultural settings is through NH<sub>3</sub> volatilization. Mosier (2001) estimated that 65% of the global NH<sub>3</sub> flux to the atmosphere is emitted from agricultural systems, including animal production and the use of synthetic fertilizers. Bouwman et al. (2002) estimated that during the 1990s 11.2 of the 78.5 Mt of synthetic fertilizer-N used in agriculture was lost to NH<sub>3</sub> volatilization. Further, 7.3 of the 34.4 Mt of field-applied urea was lost to volatilization. The two categories of fertilizer that have the largest contribution of losses of N through volatilization are synthetic urea fertilizer and animal manure (Bouwman et al. 2002).

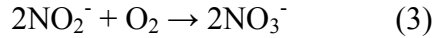
### **Plant uptake of nitrogen**

Plants can accrue N through the uptake of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in soil (Glass 2003; Miller and Cramer 2004). However, because NO<sub>3</sub><sup>-</sup> is typically more available, root uptake of N occurs mostly as NO<sub>3</sub><sup>-</sup> (Miller and Cramer 2004). Nitrate can be directly applied to soil or can become available for plant uptake as a result of the microbial process, nitrification. As such, a major factor in determining the amount of NO<sub>3</sub><sup>-</sup> that is available for plant uptake is partly determined by the activity of different microbial communities in soil.

### **Nitrification**

Nitrification is the term that describes the microbial conversion of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>, which follows a two step reaction (Schlesinger 1997):



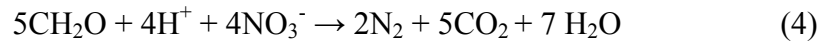


The total oxidation reaction is  $\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$ . The transformation of ammonium to nitrite (Eq. 2) is carried out by ammonium-oxidizing bacteria, and nitrate-oxidizing bacteria are responsible for the second reaction (Eq. 3) where nitrite is further oxidized to nitrate (Brady and Weil 2002).

Nitrification is influenced by soil physical and chemical properties. Soil structure and texture can influence the growth rate of nitrifying bacteria by providing substrate for growth and determining how tightly cations like  $\text{NH}_4^+$  are bound to soil particles through the cation-exchange capacity (CEC) of the soil. Soils with a high CEC can decrease the bioavailability of  $\text{NH}_4^+$  to nitrifying bacteria and thereby limit nitrification rates (Subbarao et al. 2006). The moisture, temperature and amount of  $\text{O}_2$  and  $\text{CO}_2$  in the soil can also influence nitrification rates (Gilmour 1984). Soil moisture and oxygen levels are inherently connected, as decreased soil moisture increases the diffusion of  $\text{O}_2$  in soils. Nitrification decreases in soil with high moisture content due to oxygen limitation. Nitrification is an aerobic process with optimal rates occurring when soil oxygen concentrations are similar to that found in the atmosphere (20%) and approximately 60% of the soil pore space is filled with water (Brady and Weil 2002; Subbarao et al. 2006). Nitrification is all but inhibited in saturated soils due to the absence or low diffusion of oxygen. The ideal temperature range for nitrification is between 25 and 35°C (Subbarao et al. 2006). Although nitrification can take place in highly acidic soils ( $\text{pH} \leq 4.0$ ), pH ranges from 6.7-8.5 are ideal (Subbarao et al. 2006). In general, low rates of nitrification are observed in soils with little oxygen, extremely high or low soil moisture, and low pH (Schlesinger 1997).

## **Denitrification**

Nitrate in soil can undergo three fates; denitrification, assimilation into plant and microbial biomass, or loss through leaching or runoff. Denitrification is the term used to describe the conversion of  $\text{NO}_3^-$  into nitrogen gas ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) and has the following general reaction (Schlesinger 1997):



Nitrogen gas formed through denitrification can be emitted to the atmosphere. Denitrifying bacteria are facultative anaerobes, meaning they can metabolize nutrients and remain active under oxic or anoxic soil conditions (Schlesinger 1997). Nevertheless, optimum denitrification rates are observed in anoxic and water saturated soil conditions (Brady and Weil 2002). However, depending on soil structure, denitrification can occur in anoxic microsites in soil that is for the most part well oxygenated (Schlesinger 1997). Temperatures that optimize denitrification range from 25-35°C. In addition, denitrifying bacteria are sensitive to pH. In acidic soil, denitrification occurs more slowly and  $\text{N}_2\text{O}$  is more likely to be the end product (Brady and Weil 2002).

## **Mineralization and immobilization**

Nitrogen mineralization describes the process by which organic forms of N are converted to inorganic-N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ). During N mineralization soil microbes decompose plant material or other organic matter as an energy source to break down amino compounds and release N (Brady and Weil 2002). Nitrogen immobilization is the opposite of mineralization, involving the incorporation of inorganic-N into organic material, either as microbial biomass or a component in soil humic matrices. As the term suggests, immobilized

N is biologically unavailable for plant uptake. Preference toward net nitrogen mineralization or immobilization in soil is dependent on the carbon to nitrogen ratio in soil (Brady and Weil 2002).

### **Volatilization**

Volatilization is another fate of soil nitrogen. Nitrogen as NO, N<sub>2</sub>O, and N<sub>2</sub> can be volatilized from the soil through microbial mediated processes such as nitrification and denitrification. In addition, NH<sub>3</sub> produced through the breakdown of organic residues can be volatilized from soil (Brady and Weil 2002). Much like nitrification and denitrification, rates of NH<sub>3</sub> volatilization are impacted by soil characteristics. Soils that are calcareous, high in pH, and/or low in CEC are all prone to increased NH<sub>3</sub> volatilization (Follett and Delgado 2002; Meisinger and Randall 1991). Also, soils that have manure or urea fertilizers surface-applied often show high rates of NH<sub>3</sub> volatilization (Follett and Delgado 2002). Brady and Weil (2002) report that by incorporating fertilizers a few centimeters below the soil surface ammonia volatilization can be reduced by up to 75%.

### **Nitrogen Fixation**

Previously described nitrogen transformations involve the cycling of the extant biologically available pool. New nitrogen can be made biologically available through biological nitrogen fixation (BNF), which involves the transformation of atmospheric N<sub>2</sub> into NH<sub>3</sub> in the pedosphere and hydrosphere. However due to the triple bond that forms between the two N atoms in dinitrogen gas, there is an extremely high energetic cost to this conversion (Schlesinger 1997). Some bacteria exist in soils that are capable of independent

BNF and other bacteria form symbiotic relationships with plants, specifically plant roots, in order to fix nitrogen. Irrespective of the organism, the enzyme responsible for nitrogen fixation is *nitrogenase* (Brady and Weil 2002).

### **Loss of nitrogen to surface waters and leaching**

Besides volatilization, nitrogen can also be lost from soil through leaching and surface runoff. Nitrogen in the form of  $\text{NO}_3^-$  is the most susceptible to leaching through the soil profile or lateral transport in surface water runoff because it is negatively charged and does not participate in cation exchange reactions (Follett and Delgado 2002). The amount of nitrate that is lost to surface water runoff is influenced by the infiltration potential of the soil, the severity of rain-fall events and the slope or topography of the land (Follett and Delgado 2002). Globally it is estimated that approximately 20 Tg of N is lost annually due to soil erosion and runoff (Smil 1999).

Factors such as soil and crop type, tillage practice and climate can also influence N leaching (Goulding 2004). Plant growth and uptake of N during the spring and summer months help to prevent the net downward movement of  $\text{NO}_3^-$ . However in the absence of growing plants, such as in the fall and winter months, there is a net downward movement of N through the soil profile (Kirchmann et al. 2002). In no till or conservation tillage agriculture, microbial communities are less disturbed and able to immobilize larger amounts of N than if conventional tillage agriculture is practiced (Kirchmann et al. 2002). Agricultural Best Management Practices (BMPs) involving nitrogen aim to reduce the amount of  $\text{NO}_3^-$  found in the soil rooting zone (0-15cm) beyond what is needed for plant growth and thereby

reduce the likelihood that N will be leached or incorporated into surface runoff (Keeney and Follett 1991).

### **Nitrogen use efficiency**

Despite advances in the understanding and forecasting when and how plants utilize nitrogen, there remain large inefficiencies in plant uptake of N per amount of fertilizer applied. Recent estimates indicate that only 30 to 40% of N applied as fertilizer is used by crops and converted to grain (Lea and Azevedo 2006). Nitrogen use efficiency (NUE) is the term used to describe the ratio of N utilized by the plant to the amount of N applied to the soil (Glass 2003; Lea and Azevedo 2006). NUE includes two main components; the ability of plants to remove N from the soil, and the efficiency in the transfer of N within the plant to the production of grain (Lea and Azevedo 2006). In a more encompassing definition, Cassman et al. (2002) describes NUE as the proportion of N inputs to the soil that are removed by a crop in the form of biomass harvested, remaining in the soil as a part of crop residue, or immobilized in inorganic pools.

### **Pollution of surface and ground waters with nitrogen**

As described previously, applied nitrogen that is not utilized by plants or soil microbes is subject to offsite loss through leaching to ground waters, lateral transport in surface runoff, or volatilization. Leaching of  $\text{NO}_3^-$  into groundwater can cause contamination of drinking water wells and other groundwater resources (Fletcher 1991; Townsend et al. 2003; Follett and Delgado 2002). In areas where agriculture is extensive, such as the Midwest region of the United States, agriculture is a significant contributor to  $\text{NO}_3^-$ -N

contamination of ground and surface waters (Udawatta et al. 2006; Fletcher 1991). High nitrate levels in drinking water can cause health problems especially for babies and young children. Methemoglobinemia, also known as ‘Blue-baby-syndrome’ can affect infants when high nitrate concentrations in drinking water reduce the ability of red blood cells to carry oxygen (Fletcher 1991; Kirchmann et al. 2002).

There are many environmental problems associated with the transport of  $\text{NO}_3^-$ -N in surface waters. For instance, high N levels can cause eutrophication of fresh and marine waters by promoting primary production (Follett and Delgado 2002; Townsend et al. 2003; Grant et al. 2006). Subsequent decomposition of phytoplankton blooms consume oxygen and can kill other aquatic life and otherwise disrupt the entire food web (Galloway 2003). Furthermore, Vitousek et al. (1997) noted that anoxic or hypoxic conditions resulting from increased N loading can lead to a loss of biodiversity in aquatic ecosystems.

### **Nitrogen loss due to volatilization**

In addition to losses of excess N fertilizer through leaching and surface water runoff, N can be lost through volatilization. Microbial activity in soil can lead to  $\text{NO}$ ,  $\text{N}_2\text{O}$ , and  $\text{N}_2$  emissions through nitrification and denitrification. Typically, nitrification is largely responsible for  $\text{NO}$  emissions, and denitrification for  $\text{N}_2\text{O}$  efflux (Liu et al. 2006). Ammonia can also be volatilized directly from the soil surface.

Once in the atmosphere, the various forms of nitrogen can have different fates. Nitric oxide in the atmosphere can become oxidized to form  $\text{HNO}_3$  in addition to interacting with hydrocarbons, light, and ozone to form nitric acid and atmospheric particulate nitrate (Mosier 2001). Small amounts of  $\text{NH}_3$  in the atmosphere can be oxidized to form  $\text{N}_2\text{O}$  (Ferm 1998).



The major sink for N<sub>2</sub>O in the atmosphere is stratospheric destruction (Schlesinger 1997). Tropospheric N<sub>2</sub>O is a greenhouse gas, while stratospheric N<sub>2</sub>O is involved in reactions that reduce ozone, allowing more ultraviolet to reach the Earth's surface. In contrast, NO and NH<sub>3</sub> can be returned to the earth's surface through wet and dry deposition. The deposition of these gases can cause eutrophication in aquatic ecosystems and soil acidification (Bouwman et al. 2002).

### **Future fertilizer use**

During the last 35 years of the 20<sup>th</sup> century global food production has doubled (Tilman et al. 2001). This doubling has resulted in 6.87-fold and 1.1-fold increases in N fertilization and area of land under cultivation, respectively. If this trend continues, it is estimated that N fertilizer use will increase 1.6 times by year 2020 (Tilman et al. 2001). In addition to increases in N fertilizer use, a doubling in food production will result in an 18% increase in land devoted to agriculture (Tilman 1999). An 18% increase represents the conversion of 268 million hectares of previously uncultivated land to agriculture. More recently, Zhang et al. (2006) estimated that by the year 2030 land devoted to agriculture will increase 6.6% from 2006 values. Strategies such as improved timing of fertilizer application, fertilizer placement, and use of chemical additives can be used to improve NUE in agroecosystems.

### **Timing of fertilization**

In addition to intrinsic plant properties such as the genetic makeup and C3 or C4 photosynthetic pathways, extrinsic factors such as timing and frequency of fertilization can

affect the NUE of plants (Dobermann and Cassman 2002). Fertilizer that is applied in one large application or during the fall has a greater chance of being lost from the system compared to fertilizer that is applied in correspondence with plant growth demands. In addition, timing fertilization application with plant demand for N can increase the amount of N utilized by plants (Dobermann and Cassman 2002). Inherent plant physiological limitations on NUE are unavoidable. However, matching N availability with plant N demand reduces the loss of N to the natural environment, minimizing the negative offsite environmental impacts that are commonly associated with agricultural activity.

### **Urease and nitrification inhibitors**

Chemical additives can also be used to increase NUE in crops and reduce N lost from soil to the surrounding environment. One such example of a chemical additive is a urease inhibitor. A urease inhibitor can be applied with urea fertilizers to reduce the rate that the urease enzyme catalyzes the hydrolysis of urea. By slowing the urease enzyme, N remains as urea-N longer, which increases the likelihood that urea-N will move deeper into the soil, thus reduce ammonia volatilization.

A urease inhibitor that has been identified as being particularly effective in upland soils is N-(n-butyl) thiophosphoric triamide (NBPT) (Bremner 1995). Researchers have found that it can be used to slow the transformation of urea-N to  $\text{NH}_3$ . In addition, NBPT has been useful in preventing ammonia volatilization from a broad range of agricultural settings such as cattle feedlots and swine lagoons to crops (Bremner 1995; Hendrickson and Douglass 1993; Watson et al. 1994; Varel 2002). The inhibitory action of NBPT is due to the formation

of its oxon analog (Creason et al. 1990) and its interaction with the two active Ni atoms in the urease enzyme (Manunza et al. 1999).

In addition to a urease inhibitor, a nitrification inhibitor can also be added to fertilizer alone or in concert with a urease inhibitor. Nitrification inhibitors slow the conversion of  $\text{NH}_4^+$ -N to  $\text{NO}_3^-$ -N and thereby reduce the likelihood that N will be lost from soil. Specifically, nitrification inhibitors can help to reduce N loss from leaching or denitrification (Fixen and West 2002). Research has shown that the incorporation of the nitrification inhibitor dicyandiamide (DCD) to urea fertilizer containing a urease inhibitor can increase NUE in crops and increase grain yield (Schwab and Murdock 2005).

### **Precision agriculture**

Precision agriculture (PA) is another method used to restrict N loss and improve fertilizer use efficiency and farm profitability. Precision agriculture is defined as a “management strategy that uses information technologies to bring data from multiple sources to bear on decisions associated with crop production” (National Research Council 1997). There are three main components to PA which include; collection of data at an appropriate scale, interpretation and analysis of gathered data, and implementation of management response at an appropriate spatial and temporal scale (National Research Council 1997). There are an array of technologies used in PA that include but are not limited to; microcomputers, geographic information systems (GIS), global positioning systems (GPS), yield mapping systems, ground based sensors and remote sensing (National Research Council 1997; Robert 2002). Through assembling a variety of data, farm managers can adjust

management practices so that timing and placement of field operations is site specific (Cassman 1999).

Although PA is more economically and environmentally beneficial than uniform management practices, there are obstacles preventing it from widespread adoption. Specifically, the cost associated with acquiring new technologies, the education or skill level of the farm manager, agronomic barriers, and development of technologies are all hurdles that must be overcome in order for the adoption of PA (Robert 2002). Daberkow and McBride (2003) conducted a study to identify farm and farm operator characteristics that are indicative of the likelihood that PA would be adopted. Factors such as farm size and locality, computer literacy, full time farming, and farm type were all identified as playing a role in the decision to adopt PA as a management strategy. As the knowledge gap narrows and technologies become more accessible, it is inevitable that PA practices will become more widespread.

## CHAPTER 2

### OBJECTIVES

The overall objective of this research was to evaluate rates of N transformation and transport from the rooted zone in an agricultural soil treated with UAN-type (urea-ammonium-nitrate) fertilizer. This study was conducted with bulk soils or intact soil cores from 0 to 20 cm in depth under controlled laboratory conditions. Plants were excluded to focus on the effects of physical and microbiological variables that influenced the transformation and transport of N applied at doses simulating field application practices, with an eye toward gaining a better understanding of the fate of N fertilizer applied to this soil. Specific goals were to compare for three fertilizer types (UAN, UAN amended with a urease inhibitor, UAN amended with a urease and nitrification inhibitor):

- 1) The post-application rate of urea decomposition and rate of  $\text{NH}_3$  volatilization;
- 2) The influence of soil moisture on rates of urea decomposition and subsequent transformations of fertilizer N;
- 3) The potential for fertilizer loss below the rooted zone during rainfall; and
- 4) The post-fertilization development and activity of the overall soil microbial population and microbes specifically involved in N cycling dynamics (nitrifying and denitrifying bacteria)

The study site, Open Grounds Farm, is located on a drained pocosin and is therefore characterized by highly organic soils. Relatively little information is available regarding the

impact of physical and microbiological variables on N cycling dynamics in fertilizer-amended, peaty agricultural soils. Information from this study is therefore of general interest to farm managers, but is also critical for developing informed fertilizer management strategies at the study site which is located adjacent to a sensitive estuarine complex that could serve as receiving waters for nutrients transported offsite.

## CHAPTER 3

### MATERIALS AND METHODS

#### 2.1 Site Description

The study site, Open Grounds Farm, is located at 34°54'N, 76°50'W in Carteret County, NC, USA (Fig 1). Open Grounds Farm was established in 1974 on undeveloped pine forest, swamp forest and pocosin and consists of 18,220 ha of row crops in a corn-wheat-soybean rotation. The farm is divided into a network of 260 ha blocks that are surrounded by field ditches that drain into canals. The canals have water control structures that can regulate water flow and can increase the water table during the growing season. During heavy rain events a portion of runoff drains into headwater creeks of the South River, a coastal plain estuary and tributary to the Neuse River Estuary which is part of the Albemarle-Pamlico Sound complex.

Management practices include no-till agriculture and precision application of liquid nitrogenous fertilizer by point or line injection with GPS-equipped planters that fertilize according to detailed soil fertility maps developed annually. The typical fertilizer is a 32% N solution by mass, consisting of a 16:8:8 mixture of urea, ammonium and nitrate (UAN). In some blocks, the standard UAN fertilizer is amended with Agrotain™ or Agrotain Plus™. The former is a urease inhibitor and the latter includes both a urease and a nitrification inhibitor. The N content by total mass and stoichiometry for these alternate fertilizers is consistent with the standard UAN fertilizer.

Soils are of the hydric series and are mapped as Deloss fine sandy loam (fine-loamy, mixed, semiactive, thermic Typic Umbraquult) overlying relic sand and shell. Soils are circumneutral (pH = 7.4) with a water holding capacity (WHC) of 67% and an organic content of 310 g kg<sup>-1</sup>. Bulk and particle densities are 860 and 1370 kg m<sup>-3</sup>. The average annual precipitation at the study site is 146 cm and the average daily winter and summer temperatures are 7.9 and 25.9°C, respectively.

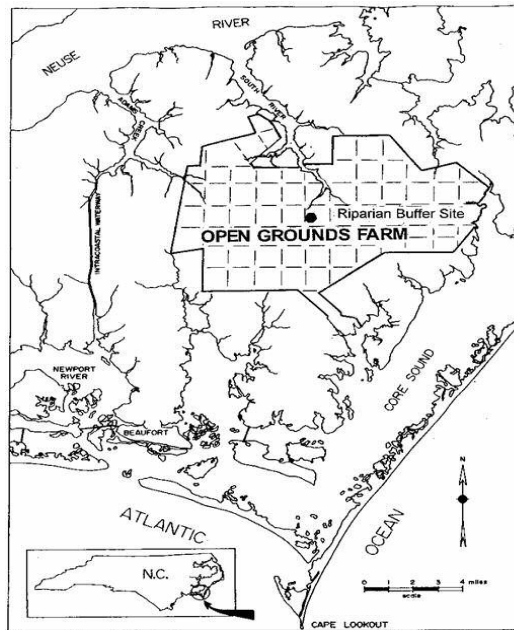


Figure 1. Location of the study site in eastern North Carolina.

## 2.2 Sample Collection and Preparation

Soils were obtained from field block 4 on five occasions beginning in the post-harvest period in October 2005 and ending in January 2007. Block 4 was planted with soybeans during 2006. Depending on the intended use, bulk soils from the surface 20 cm layer or intact soil cores to a similar depth were collected. Bulk soils were obtained with a hand trowel, while intact soil cores were collected with a hammer corer fitted with removable



stainless steel sleeves (4.8 cm i.d. x 30 cm length). Samples were stored at 4°C until experimentation. Bulk soils were homogenized and sieved (4-mm mesh) prior to distribution into experimental vessels, while soil cores were maintained in stainless steel collection sleeves during experimentation.

### **2.3 Experimentation**

Analysis of the fertilizer showed it was composed of 84 mg mL<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N and 168 mg mL<sup>-1</sup> of urea. Laboratory experimentation was conducted in an attempt to simulate field realism in fertilizer application. Based on farm data, fertilizer is line-applied at 14 mL 100 cm<sup>-1</sup>. Therefore, 0.71 mL of fertilizer was applied to a 5.1 cm diameter soil core. In order to ensure even distribution on the surface of soil cores, 0.71 mL of fertilizer was in all cases diluted in 5mL of deionized water prior to application. A similarly diluted volume of fertilizer was added per ~200g dry mass of homogenized soil in experiments involving bulk soils, as this approximates the dry mass of an intact soil core of the dimensions used here. Experiments were about 2 to 3 weeks in length (except for the simulated rainfall experiment) as this is the typical time frame between fertilizer applications in the field. Abbreviations used throughout for fertilizer treatments are as follows: UAN fertilizer (UAN), UAN with Agrotain™ (UI), and UAN with Agrotain Plus™ (UNI). All experiments were conducted at 20°C.

#### **2.3a Urea hydrolysis.**

Two experiments were conducted to assess the fate of urea (transformation to NH<sub>4</sub><sup>+</sup> and volatilization) as a function of fertilizer type. Both experiments consisted of two treatment groups. In the first experiment, 48 cores were collected; 24 were fertilized with UAN and the remaining with UI. All cores were sealed at the bottom with a polyethylene cap

and were fitted on the top with two horizontally stacked, acid-impregnated sorption pads (described below), allowing for a ~6 cm headspace between the soil surface and the lower sorption pad. The breathable sorption pads allowed air exchange between the atmosphere and soil surface. However, the lower pad absorbed  $\text{NH}_3$  volatilized from the soil surface, while the upper pad prevented  $\text{NH}_3$  contamination of the lower pad by ambient air.

Triplicate soil cores for each treatment were destructively sampled on days 0, 1, 2, 3, 5, 8, 14, and 21. The second experiment consisted of a total of 42 soil cores; half of the cores were fertilized with UAN and the other half with UNI. Triplicate cores for each treatment were destructively sampled in the second experiment on days 0, 1, 3, 5, 8, 14, and 21. The handling of soil cores for both experiments was identical. On days when cores were sacrificed, soil was divided into 10 cm sections (0-10cm, 10-20cm). Each section was then homogenized, subsampled (10g) and analyzed for urea-N,  $\text{NH}_4^+$ -N, and  $\text{NO}_3^-$ -N. Nitrogen lost to volatilization was determined in association with each soil nutrient analysis by analyzing the  $\text{NH}_4^+$ -N content of each lower sorption pad.

### **2.3b** *Moisture dependence*

The moisture-dependence of N cycling as a function of fertilizer type was assessed in homogenized soil samples. Percent water holding capacity (WHC) was adjusted to 22% higher or lower than the value for field moist soil (64% WHC) by misting or air drying soils with continuous mixing to bracket soil conditions that are typically observed in fields. The equivalent of 210g dry mass homogenized soil was added to 27 teflon-lined polycarbonate cylinders (9 cm i.d. x 35 cm long). Fertilizer treatments (UAN, UI, UNI) were triplicated at each of the three levels of %WHC (42%, 64%, and 86%) and cylinders were capped with duplicate horizontally stacked, acid-impregnated sorption pads as described above. On days

0, 1, 3, 6, 14, and 20 soil was subsampled (10g) from each cylinder and nutrient concentrations (urea-N,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N) were determined. Nitrogen lost to volatilization was measured in association with nutrient analysis by changing the sorption pads on days 1, 3, 6, 14, and 20 and analyzing the lower pad for  $\text{NH}_4^+$ -N content.

### **2.3c** *Simulated rainfall*

Intact soil cores were used to determine the influence of fertilizer type on the rate and form of N leached. Fertilizer treatments included UAN, UI, UNI, and a control that had the equivalent volume of deionized water added (S). Treatments were triplicated and subjected to simulated rainfall based on a historic record of volume and timing of rainfall at the time of planting near the site (<http://www.nc-climate.ncsu.edu/>). Rainfall of 2.5 cm over a 1 h period was added at weekly intervals for four weeks followed by a 5 cm rainfall over 2 h on the fifth week. A peristaltic pump was used to deliver rainfall as deionized water with the dripper positioned in the middle of the core to prevent channeling down the side of the core and to ensure constant delivery over an hourly period. Artificial rainwater was allowed to infiltrate the core and the leachate was collected. The volume of leachate was measured after each rainfall event and assessed for nutrient content. Cores were sealed at the bottom, but were left uncapped at the top between rainfalls to allow for natural gas exchange across the soil surface. At the termination of the experiment, cores were dismantled into 5 cm sections that were analyzed for residual N.

### **2.3d** *Microbial activity.*

Overall microbial community metabolism and the activity of the microbial groups specifically active in N cycling dynamics (nitrifiers and denitrifiers) were measured as a function of time and fertilizer treatment. Fifteen samples (1000g) of homogenized, field

moist soil were randomly assigned to five treatment groups which included field moist soil only (S), and soil amended with 35mL of deionized water (C) , or a similar volume of UAN, UI or UNI solutions. Treated soils were distributed into 2L Erlenmeyer flasks that were covered with perforated foil to allow gas exchange between the atmosphere and flask headspace. Flasks were subsampled (~100 g wet weight) on days 0, 1, 3, 5, 7, 10 and 14 to measure any or all of the following: gravimetric moisture content; community respiration (CO<sub>2</sub> production); short term nitrifier activity (NA); or denitrifying enzyme activity (DEA). On day 14, soil, subsamples were rendered anaerobic and assayed for DEA again after 4 days.

#### **2.4 Analytical**

Soil nutrients were extracted (10:1 volume/soil wet weight) with 2M KCl and filtered (Whatman no. 42 paper). Nutrient extracts were analyzed colorimetrically (Shimadzu Model UV-1201V spectrophotometer) for NO<sub>2</sub><sup>-</sup>-N + NO<sub>3</sub><sup>-</sup>-N (hereafter referred to as NO<sub>3</sub><sup>-</sup>-N) and NH<sub>4</sub><sup>+</sup>-N by the Cu-Cd reduction and indophenol blue methods, respectively (Bundy and Meisinger 1994). Urea-N was determined by the modified diacetyl monoxime method (Bremner 1982). Concentrations of N are expressed per gram of dry soil mass (g dw<sup>-1</sup>). Concentrations of NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N and urea-N in leachate were measured using similar colorometric methodologies adapted for aqueous samples (Parsons et al. 1984; Grasshoff 1999), while NO<sub>2</sub><sup>-</sup>-N (NA determination; below) was determined by diazotization (Parsons et al. 1984).

Ammonia volatilization was determined by the closed chamber, sorption pad technique (Marshall and Debell 1980). Duplicate polyfoam sorption pads impregnated with a 2:1 (v/v) solution of 2.2 N H<sub>3</sub>PO<sub>4</sub> and glycerine were emplaced in the headspace above

intact soil cores or homogenized soils in teflon-lined polycarbonate cylinders. The top pad prevented atmospheric contamination of the lower pad that intercepted  $\text{NH}_3$  diffusing upward from the soil surface. At prescribed intervals (above), the top pad was discarded and the bottom pad was rinsed with deionized water. Collected rinse water was analyzed for ammonium as described above.

Other soil physicochemical properties were determined following standard techniques (Klute et al. 1986). Soil pH was determined potentiometrically on 1:1 soil-deionized water slurries. Gravimetric soil moisture was determined by oven drying ( $105^\circ\text{C}$ ) for 24 hours. Particle density was measured pycnometrically. Organic matter content was determined by loss on ignition of oven-dried soil at  $550^\circ\text{C}$  for four hours. Water holding capacity (WHC) was determined by the soak and drain technique. Bulk density was determined as the ratio of oven-dried mass to volume of soil in core.

Carbon dioxide production was measured as an indicator of community microbial activity. Subsamples (20g) of field-moist soil equilibrated with laboratory air were sealed in air-tight 133-cc glass jars equipped with O-seal fittings and septa for syringe sampling of headspace gases. Using a syringe, 3mL of headspace gas was extracted at 0, 90, and 180 minutes from the time of sealing and  $\text{CO}_2$  was measured by thermal conductivity gas chromatography (Shimadzu GC-14A).

Short-term nitrifier activity (NA) was measured using the chlorate block technique (Schmidt & Belser 1994). Chlorate inhibits the oxidation of  $\text{NO}_2^-$ -N to  $\text{NO}_3^-$ -N. Therefore, in the presence of excess  $\text{NH}_4^+$ -N, the accumulation of  $\text{NO}_2^-$ -N provides an index of the population size of ammonium oxidizing bacteria. Subsamples (20g) of field moist soil in 250mL Erlenmeyer flasks were amended with 90mL of a phosphate buffer containing 0.2mL

of 0.25mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 1mL of 1 M KClO<sub>3</sub> solutions. Flasks were swirled and 2mL of slurry was immediately extracted and syringe-filtered (0.8µm polycarbonate filter). Flasks were placed on a rotary shaker at 150 rpm. At 24 and 48 h, additional samples were withdrawn and similarly filtered. All filtrate was analyzed for NO<sub>2</sub><sup>-</sup>-N.

Denitrifer enzyme activity (DEA) was measured as an indication of the potential for denitrification in soil samples following Tiedje (1994). The assumption is that the rate of N<sub>2</sub>O production is commensurate with the denitrifying enzyme concentrations when no other factors are limiting. Subsamples (20g) of field-moist soil placed in 133-cc glass jars (described previously) were amended with a deoxygenated medium consisting of 25mL of 1mM glucose, 1mM KNO<sub>3</sub><sup>-</sup>, and 1g L<sup>-1</sup> chloramphenicol. The latter inhibits *de novo* synthesis of denitrifying enzymes. Jars were then sealed with air tight screw top lids and the headspace was rendered anaerobic by repeated evacuation and filling with ultrahigh purity N<sub>2</sub>. Acetylene was added to 10kPa to inhibit reduction of N<sub>2</sub>O to N<sub>2</sub>. Jars were then hand shaken vigorously to ensure equilibration of C<sub>2</sub>H<sub>2</sub> between the gas and aqueous phases and placed on a rotary shaker (150 rpm). A 3mL sample of jar head space gas was withdrawn and analyzed for N<sub>2</sub>O by <sup>63</sup>Ni electron capture gas chromatography (Shimadzu GC 14A) at 30, 120, and 210 minutes after being placed on the shaker.

## 2.5 Calculations and Statistical Analysis

Rates of NA and DEA and total microbial activity were calculated by the time-linear rate of NO<sub>2</sub><sup>-</sup>-N, N<sub>2</sub>O-N and CO<sub>2</sub>-C accumulation, respectively. Total N<sub>2</sub>O-N at each time point in DEA experiments was calculated as the sum of the gas and liquid phase (determined from Bunsen solubility coefficients [Moraghan and Buresch 1977]) concentrations. Total NH<sub>3</sub> volatilization over the 2-3 week experiments was determined by adding the masses of

NH<sub>3</sub>-N captured on sorption pads while total CO<sub>2</sub> production was calculated by time-integrating data collected at discrete points throughout the observational period. Microbial activities are normalized per unit dry soil mass.

Data were analyzed by a one, two or three-way Analysis of Variance (ANOVA) using SAS software version 9.1.3 (SAS Institute, 2003). In the case of a three-way ANOVA, data were analyzed using a repeated measure approach. ANOVAs were performed using either the General Linear Model procedure (PROC GLM) or the Mixed procedure (PROC MIXED), and the MEANS procedure was used to calculate the mean and standard error of the various treatment replicates. Tukey's or Scheffe's methods were used for *post hoc* comparison of significant differences among means. All statistical analyses were performed at  $\alpha = 0.05$ .

## CHAPTER 4

### RESULTS

#### 3.1 Urea Hydrolysis

Urea-N declined rapidly from the time of fertilization for both UAN and UI treatments (Figure 2a). However, urea-N was retained in the soil significantly longer with UI relative to UAN. By day 8, less than 1% of urea-N remained in the soil in the UAN treatment, while 20% was still present for the UI treatment. By day 14, all of the initial urea-N was depleted from the UAN treatment and less than 1% remained in UI treated soil.

The disappearance of urea corresponded with a rapid increase in soil  $\text{NH}_4^+$ -N for several days for both fertilizers, indicating a high level of urease activity. In agreement with the rate of urea-N losses from soil, accumulation of  $\text{NH}_4^+$ -N in soil was more rapid for UAN than for UI (Figures 2a and 2b). Maximum soil  $\text{NH}_4^+$ -N concentrations occurred on day 3 for the UAN treatment and were 81% higher than the day 0 value. In contrast, maximum  $\text{NH}_4^+$ -N concentrations occurred on day 8 in UI treatment and were 101% higher than initial concentrations. On day 21, soil in the UAN treatment had an increase in  $\text{NH}_4^+$ -N from  $0.40 \pm 0.01 \text{ mg g dw}^{-1}$  ( $\bar{x} \pm 1 \text{ SEM}$ ) on day 0 to  $0.50 \pm 0.08 \text{ mg g dw}^{-1}$  soil. In contrast, the UI treatment declined from  $0.37 \pm 0.02 \text{ mg g dw}^{-1}$  on day 0 to  $0.35 \pm 0.16 \text{ mg g dw}^{-1}$  on day 21. These values represented changes of +27% and -6% with respect to initial concentrations.

Nitrate concentrations in soil cores amended with both fertilizer types were relatively constant up to day 8 followed by a slight increase until day 14 (Figure 2c). During the final 7 days of the experiment there was a small decrease in  $\text{NO}_3^-$ -N concentrations (Figure 2c). The



highest soil  $\text{NO}_3^-$ -N concentration of  $0.60 \pm 0.08$  and  $0.40 \pm 0.05$   $\text{mg g dw}^{-1}$  for the UAN and UI was observed on day 14 for both treatments. These concentrations represented increases of 87% and 52% over day 0 values for UAN and UI treatments, respectively. On day 21, soil  $\text{NO}_3^-$ -N concentrations increased from initial values of  $0.30 \pm 0.06$  to  $0.35 \pm 0.03$   $\text{mg g dw}^{-1}$  for the UAN treatment and decreased for the UI from  $0.29 \pm 0.04$  to  $0.20 \pm 0.04$   $\text{mg g dw}^{-1}$ . Most of the fertilizer (97%) remained localized in 0 to 10cm zone of the 20 cm cores throughout the experiment.

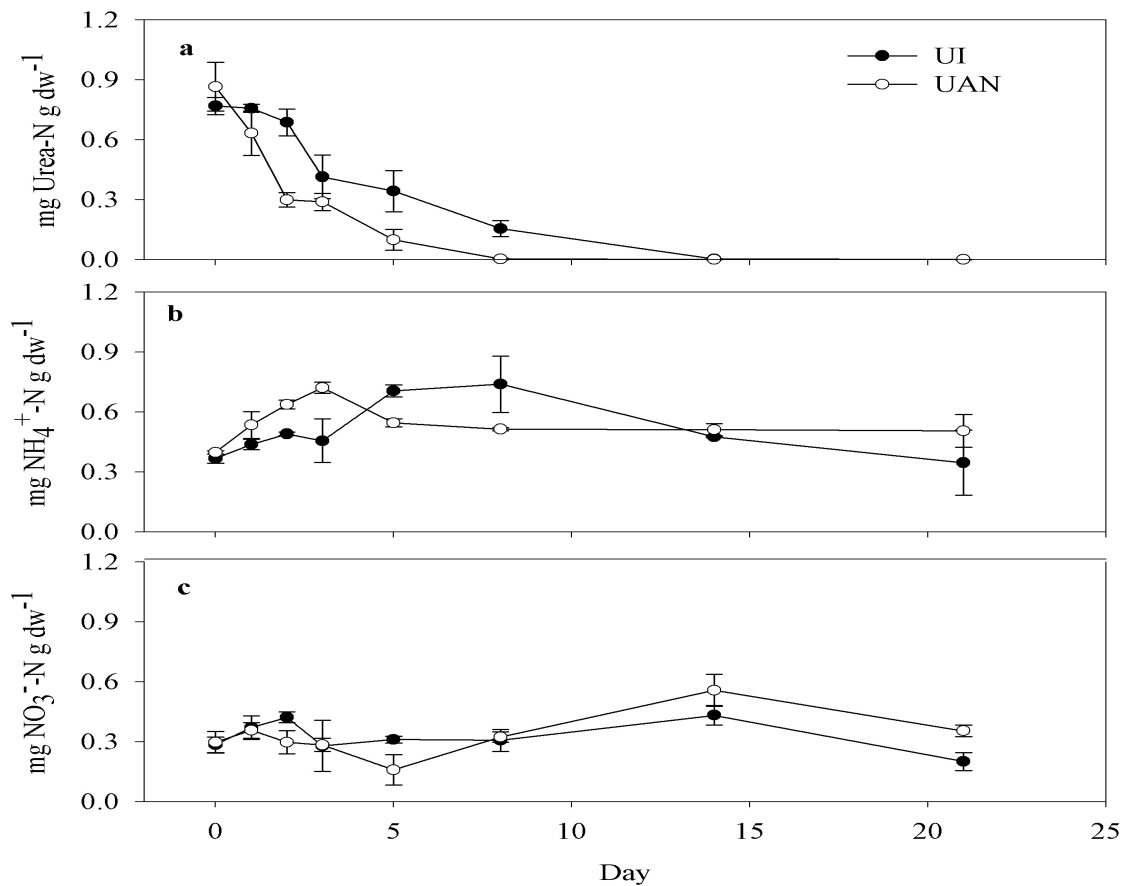


Figure 2. Time course for change in concentrations of nitrogenous nutrients in intact soil cores fertilized with a urea-ammonium-nitrate (UAN) fertilizer or a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor. Error bars represent  $\pm 1$  SEM for triplicate soil cores.

Ammonia volatilized from soil cores at least to day 14 for both fertilizer treatments (Figure 3a). With respect to time, the rate of volatilization from soil was often (days 5, 8 and 14) significantly more rapid for UAN than for UI. However, the total mass of N volatilized on day 21 from soils amended with UAN,  $20.2 \pm 1.7$  mg, was not significantly different than the total of  $16.7 \pm 2.1$  mg emitted from soils fertilized with UI. Loss to volatilization after 21 days represented 7% and 6% of the total N applied in the respective fertilizers.

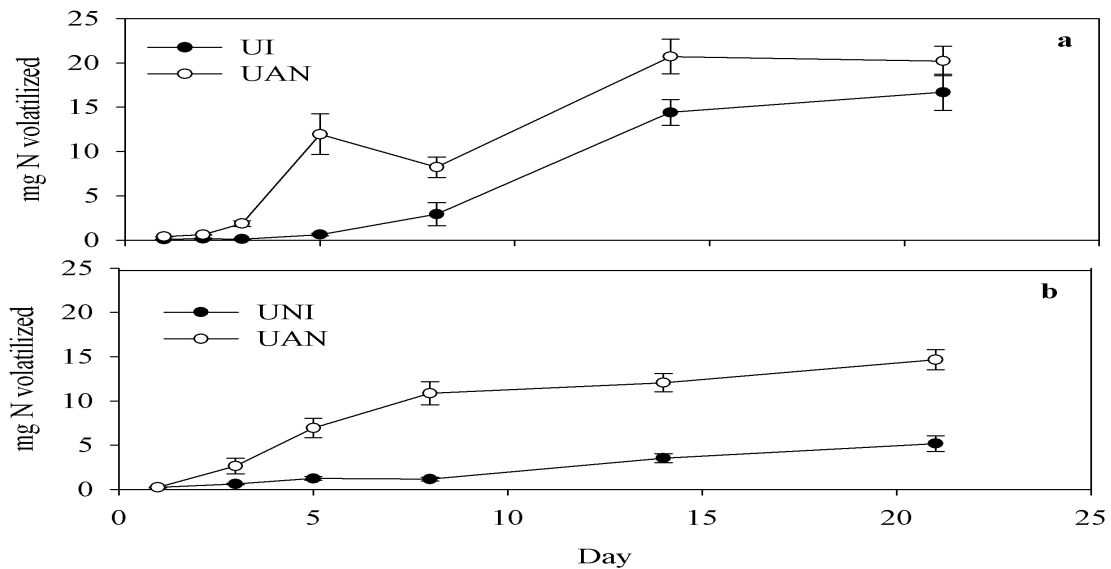


Figure 3. Time course for total NH<sub>3</sub> volatilized from soil cores fertilized with a urea-ammonium-nitrate (UAN) fertilizer or a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor, or Agrotain Plus™ (UNI), a urease and nitrification inhibitor. Error bars represent  $\pm 1$  SEM for triplicate soil cores.

The rate of urea-N decline in soil was more rapid for UAN than the UNI treatment (Figure 4a). By day 8, less than 2% urea-N remained in the UAN treatment, while 99% was still present for the UNI treatment. After 21 days, approximately 1% of the initial urea-N remained in soils treated with UAN. However, 50% of the urea-N that was present in the soil on day 0 remained on day 21 in the UNI treatment.

Differences in the soil concentrations of  $\text{NH}_4^+$ -N were also observed between the UAN and UNI treatments (Figure 4b). There was a rapid increase in soil  $\text{NH}_4^+$ -N concentrations for the UAN treatment and a slower rate of increase for UNI. Maximum soil  $\text{NH}_4^+$ -N concentrations occurred on day 8 for the UAN treatment and were 1.5 times higher than on day 0, at  $0.94 \pm 0.13 \text{ mg g dw}^{-1}$ . Unlike the UAN treatment, maximum soil  $\text{NH}_4^+$ -N concentrations for UNI treatment occurred on day 21 and were 1.3 times higher than initial concentrations. On day 21, soil in the UAN treatment had an overall increase in  $\text{NH}_4^+$ -N from  $0.38 \pm 0.08 \text{ mg g dw}^{-1}$  on day 0 to  $0.60 \pm 0.07 \text{ mg g dw}^{-1}$ . A more pronounced increase of  $0.29 \pm 0.03 \text{ mg g dw}^{-1}$  on day 0 to  $0.68 \pm 0.07 \text{ mg g dw}^{-1}$  on day 21 was observed in soil concentration of  $\text{NH}_4^+$ -N for the UNI treatment. These values represented changes of +28% and +133% with respect to initial concentrations.

Nitrate concentrations in soil cores amended with both UAN and UNI fertilizer solutions were relatively constant until day 8 and then increased slowly until day 21 (Figure 4c). The highest soil  $\text{NO}_3^-$ -N concentration of  $0.56 \pm 0.03 \text{ mg}$  and  $0.43 \pm 0.03 \text{ mg}$  for UAN and UNI was observed on day 21 for both treatments. These concentrations represented increases of 57% and 55% over day 0 values for UAN and UNI treatments, respectively. A majority of the fertilizer (97%) remained in the top 10cm of the 20cm cores throughout the experiment.

Ammonia volatilized from soil cores to day 21 for both fertilizer treatments UAN and UNI (Figure 3b). However, the rate of N volatilization from soil was significantly more rapid for UAN than for UNI on days 5, 8, 14, and 21. Accordingly, the total mass of N volatilized from soils amended with UAN ( $\pm 1 \text{ SEM}$ ) was significantly higher than for UNI,  $14.7 \pm 1.1$

mg versus  $5.2 \pm 0.9$  mg on day 21. Loss of N to volatilization represented approximately 5% and 2% of the total applied in the respective fertilizers.

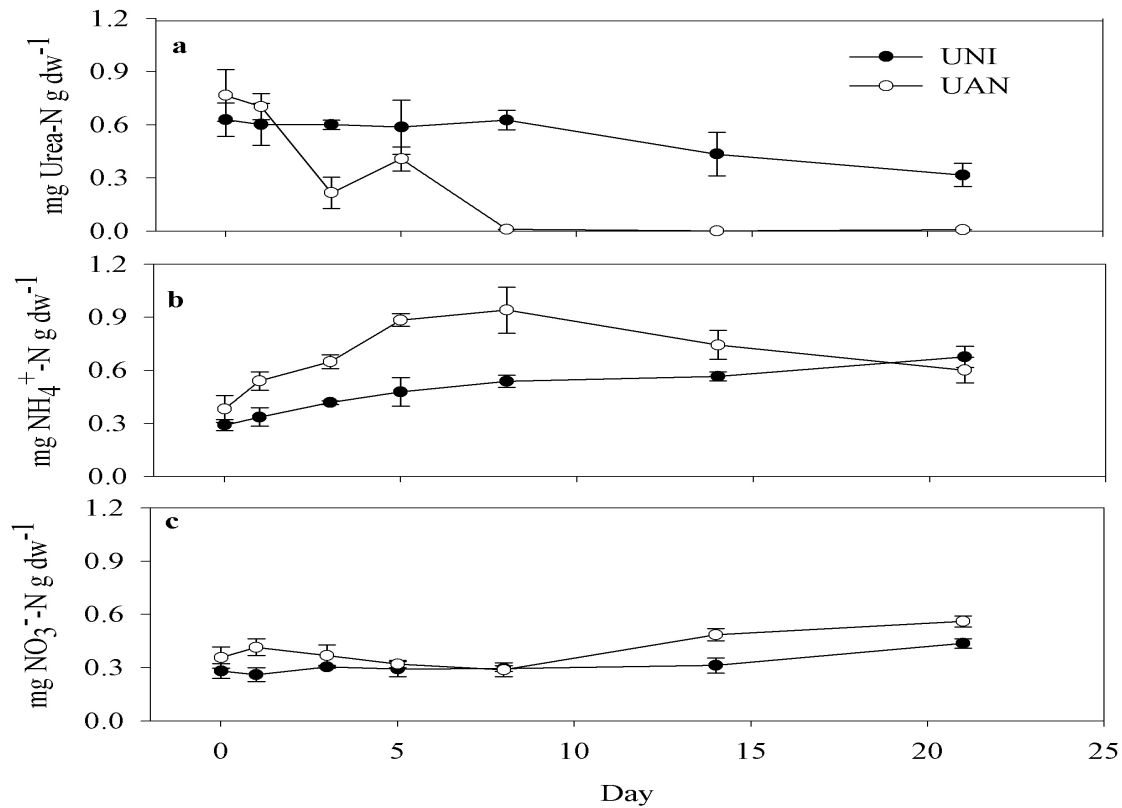


Figure 4. Time course for change in concentrations of nitrogenous nutrients in intact soil cores fertilized with a urea-ammonium-nitrate (UAN) fertilizer or a similar fertilizer amended with Agrotain Plus™ (UNI), a urease and nitrification inhibitor. Error bars represent  $\pm 1$  SEM for triplicate soil cores.

### 3.2 Moisture dependence

The rate of urea-N decline was faster in homogenized soil that was treated with UAN fertilizer compared to UI or UNI treatments at all levels of %WHC levels (42%, 64%, and 86%). On day 6, urea-N was not detectable in soil treated with UAN at all moisture levels. In contrast, there was a decrease in mean concentrations of urea-N by only  $15 \pm 14\%$  and  $37 \pm 13\%$  in soil treated with UI and UNI, respectively. At each of the three moisture levels there was no significant difference in the rate of urea-N transformation in soil treatments UI and

UNI. In addition, comparison of urea-N transformation rates among moisture levels within a fertilizer treatment showed that there were no differences in treatments UAN or UI.

However, UNI-treated soil at 86% WHC had a significantly faster rate of urea-N decline than at 64% WHC.

Ammonium concentrations in soil increased at the end of the experiment from initial day 0 concentrations for all fertilizer treatments at 86% WHC (Figure 5a). On day 20, soil  $\text{NH}_4^+$ -N increased from initial concentrations by  $65 \pm 19\%$ ,  $123 \pm 31\%$ , and  $156 \pm 24\%$  for the UAN, UI, and UNI treatments, respectively. There were significant differences in soil  $\text{NH}_4^+$ -N concentrations when the UAN treatment was compared to UI or UNI. However there was no significant difference in soil  $\text{NH}_4^+$ -N concentrations between the UI and UNI treatments. The UAN and UI treatments showed a net decrease in soil  $\text{NH}_4^+$ -N concentrations between days 0 and 20 at both 42% and 62% WHC. In contrast,  $\text{NH}_4^+$ -N concentrations in soil treated with UNI increased at 42% and 62% WHC by  $78 \pm 43\%$  and  $73 \pm 21\%$ , respectively. All fertilizer treatments showed significantly higher soil  $\text{NH}_4^+$ -N concentration at 86% WHC than 42%WHC. However, there was no significant difference between soil  $\text{NH}_4^+$ -N concentration between 42% and 64% WHC within a fertilizer treatment.

In contrast to increasing  $\text{NH}_4^+$ -N soil concentrations seen at 86% WHC between days 0 and 20 for all fertilizer treatments (Figure 5a), soil  $\text{NO}_3^-$ -N concentrations decreased by  $40 \pm 14\%$ ,  $32 \pm 9\%$ , and  $63 \pm 1\%$  at the highest moisture level for the UAN, UI, and UNI treatments, respectively (Figure 5b). There were no significant differences in soil  $\text{NO}_3^-$ -N concentrations at 86% WHC among fertilizer treatments (UAN, UI, UNI). Soil treated with UNI showed an overall decrease in  $\text{NO}_3^-$ -N concentrations at 42% and 64% WHC, whereas  $\text{NO}_3^-$ -N concentrations in soil treated with UAN and UI increased at the respective % WHCs.

At 64% and 42% WHC, soil  $\text{NO}_3^-$ -N concentrations in the UNI treatment were significantly different as concentrations declined at both moisture levels, but increased for other fertilizer treatments. Within the UAN treatment there were significant differences in soil  $\text{NO}_3^-$ -N concentrations among all moisture levels, with the observed value at 64% WHC representing a  $277 \pm 12\%$  increase on day 20 relative to the initial  $\text{NO}_3^-$ -N concentration. In contrast, for the UI and UNI treatments there were only significant differences within fertilizer treatment when 86% WHC was compared to 42% or 64% WHC.

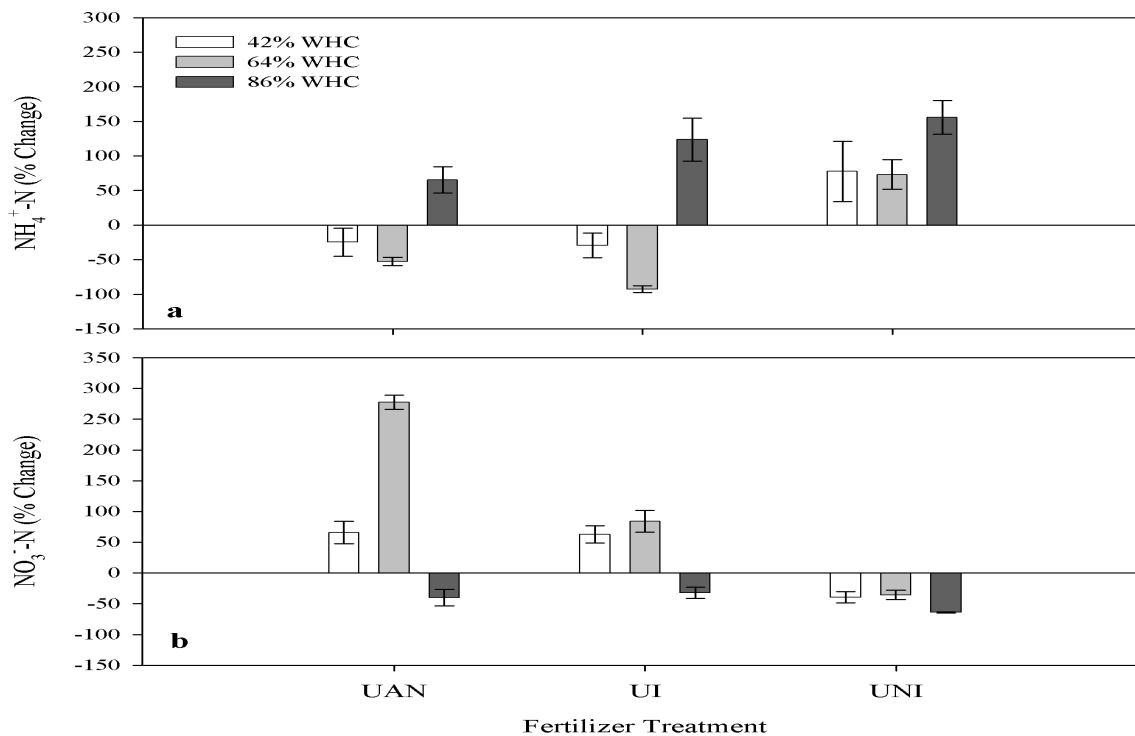


Figure 5. Percent change in concentrations of nitrogenous nutrients at 20 d relative to day 0 values in homogenized soil samples fertilized with urea-ammonium-nitrate (UAN) fertilizer; a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™ (UNI), a urease and nitrification inhibitor. Triplicate soil samples for each fertilizer were held at each of three values for percent of water holding capacity (WHC). Error bars represent  $\pm 1$  SEM.

The mass of ammonia volatilized from homogenized soil was significantly higher at 86% WHC for all fertilizer treatments than at 42% or 64% WHC (Figure 6). The respective amounts of  $\text{NH}_3\text{-N}$  volatilized after 20 days for the fertilizer treatments at 86%WHC were  $13.0 \pm 0.8$ ,  $17.1 \pm 0.3$  and  $18.0 \pm 1.1$  mg for the UI, UAN and UNI treatments. Among the fertilizer treatments at the three moisture levels, soil treated with UI had significantly less N volatilized than fertilizer treatments UAN or UNI. In addition, there was no statistical difference in mass of  $\text{NH}_3\text{-N}$  volatilized between the UAN and UNI treatment at any moisture level.

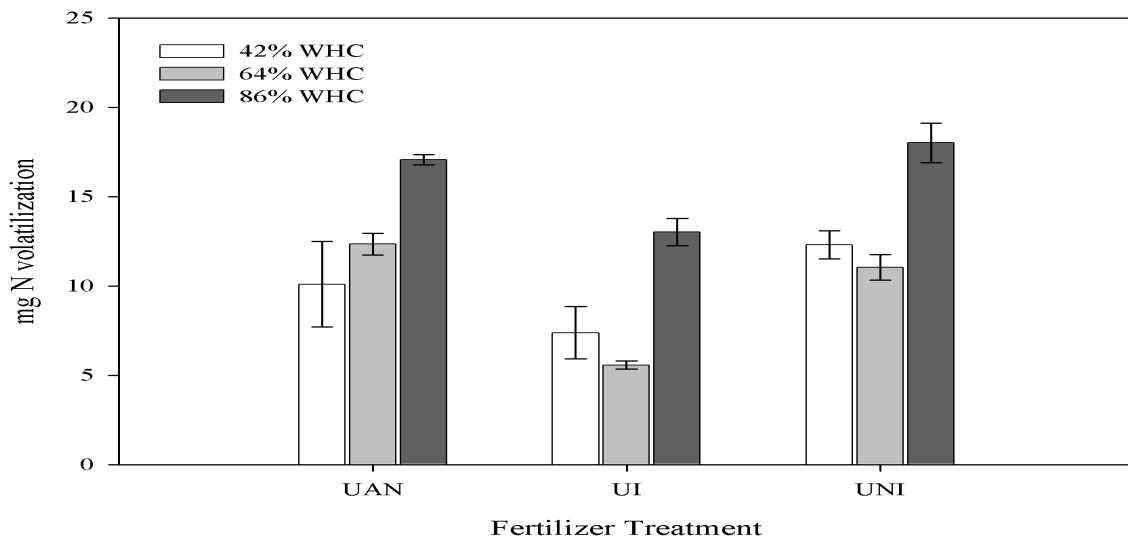


Figure 6. Total mass of  $\text{NH}_3$  volatilized after 20 days from homogenized soil at three values for percent of water holding capacity (WHC) for soils fertilized with urea-ammonium-nitrate (UAN) fertilizer; a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™ (UNI), a urease and nitrification inhibitor. Error bars represent  $\pm 1$  SEM ( $n=3$ ).

### 3.3 Simulated Rainfall

Leaching of nitrogenous nutrients occurred from all soils in response to simulated rainfall. Unsurprisingly, the mass of N leached from soil was related to the addition of fertilizer. Unfertilized soil (S treatment) had consistently lower masses of N leached (urea-N,

$\text{NH}_4^+$ -N, and  $\text{NO}_3^-$ -N) than fertilized (UAN, UI, and UNI) soil. Only trace amounts of urea-N mass leached from soil cores irrespective of treatment (data not shown). The highest mass of urea-N leached from soil occurred in the UI soil treatment during the first rain event ( $0.05 \pm 0.00$  mg), followed by  $0.003 \pm 0.000$  mg urea-N during the fourth rain event in the S treatment.

Ammonium was leached from fertilized soil cores during every simulated rainfall (Figure 7). Considering all fertilizer treatments, week 1  $\text{NH}_4^+$ -N leachate values varied from  $0.02 \pm 0.00$  (UNI) to  $1.6 \pm 1.6$  mg (UI). The mass of  $\text{NH}_4^+$ -N leached from fertilized cores showed a generalized increase with each successive rainfall, at least to the fourth event. At that time, the mass of  $\text{NH}_4^+$ -N leached varied from  $0.9 \pm 0.4$  to  $8.1 \pm 7.7$  mg in the UNI and UI treatment, respectively. However, due to high variability and low sample size, the only significant differences among weeks in the mass of  $\text{NH}_4^+$ -N leached were observed for the UI treatment. Week 4 had significantly more  $\text{NH}_4^+$ -N leached ( $8.1 \pm 7.7$  mg) than week 1, 2, or 3, where the mass of N leached varied from  $1.6 \pm 1.5$  to  $1.9 \pm 1.5$  mg. In contrast to the fertilized cores, the unfertilized cores (S treatment), showed little  $\text{NH}_4^+$ -N loss to leaching. The weekly mass of  $\text{NH}_4^+$ -N lost varied from  $0.005 \pm 0.000$  to  $0.016 \pm 0.004$  mg. These values represent 27% and less than 1% of the smallest and largest losses recorded for fertilized cores.

In accord with the data for  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N was leached from fertilized soil cores during every simulated rainfall (Figure 7). Considering all fertilizer treatments, the mass of  $\text{NO}_3^-$ -N leached through soil cores on week 1 varied from  $0.04 \pm 0.01$  (UNI) to  $0.80 \pm 0.70$ mg (UI). All fertilizer treatments showed increasing amounts of  $\text{NO}_3^-$ -N leached as the weeks progressed. For the UAN and UI treatments, there was significantly more  $\text{NO}_3^-$ -N



leached during week 5 than previous weeks. For the respective treatments,  $3.0 \pm 0.2$  and  $2.9 \pm 0.7$  mg of  $\text{NO}_3^-$ -N was leached. The largest mass of  $\text{NO}_3^-$ -N was also leached from the UNI treatment on week 5 ( $1.4 \pm 0.1$ mg). However, the mass of leachate was not significantly different compared to week 4 ( $1.1 \pm 0.2$ mg). In further agreement with the data for  $\text{NH}_4^+$ -N, the unfertilized cores showed comparatively little  $\text{NO}_3^-$ -N loss to leaching. The weekly mass of  $\text{NO}_3^-$ -N lost varied from  $0.003 \pm 0.001$  mg to  $0.040 \pm 0.014$  mg. These values represent 8% and 1% of the smallest and largest mass losses recorded for fertilized cores.

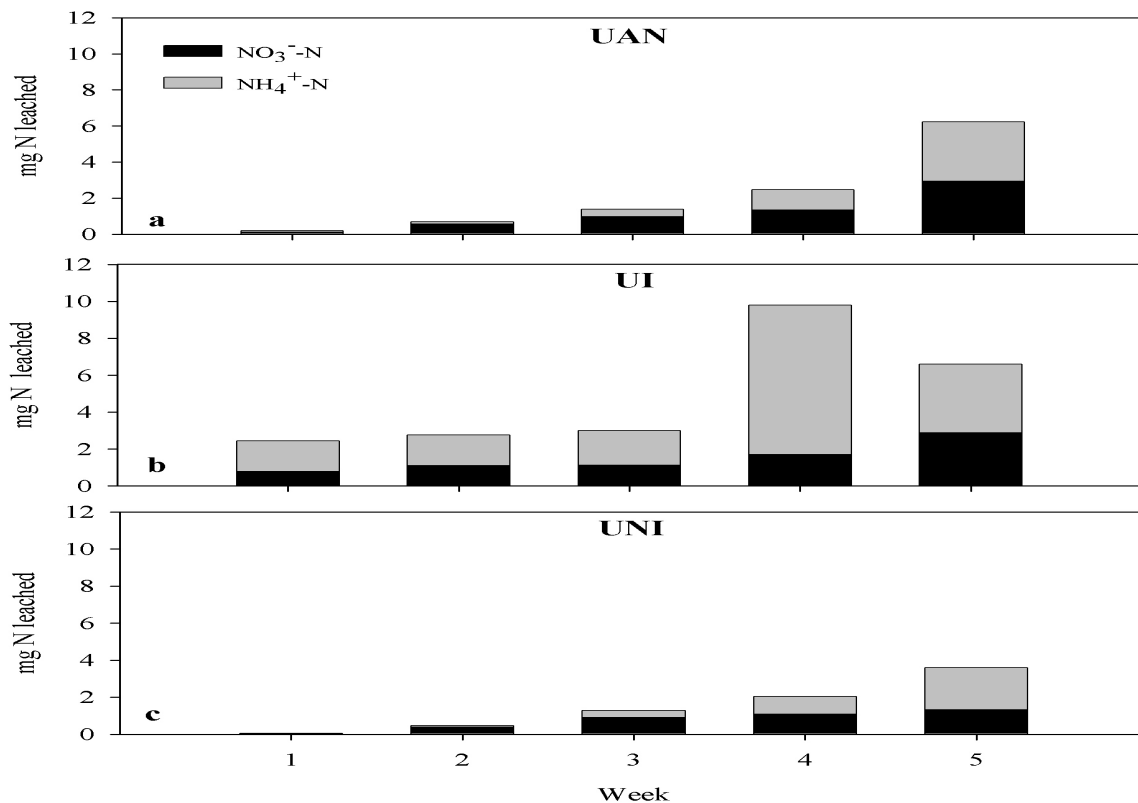


Figure 7. Time course for  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N leached from intact soil cores following simulated rainfall events of 2.5 cm (weeks 1 through 4) or 5 cm (week 5). Soil cores ( $n=3$ ; each treatment) were amended with one of three fertilizer treatments one week prior to the first simulated rainfall. Fertilizer treatments were a urea-ammonium-nitrate fertilizer (UAN) solution, or similar fertilizer solution amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™ (UNI) a urease and nitrification inhibitor. A fourth treatment included unfertilized, field moist cores (data not shown).

The S treatment showed the lowest total mass of  $\text{NH}_4^+$ -N leached,  $0.1 \pm 0.0\text{mg}$  (Figure 8a). In contrast, the total mass of  $\text{NH}_4^+$ -N leached for fertilized cores was  $16.9 \pm 3.2$ ,  $4.9 \pm 0.7$  and  $3.6 \pm 1.1 \text{ mg}$  for the UI, UAN and UNI treatments, respectively. Thus, the total mass of  $\text{NH}_4^+$ -N leached from soil in the S treatment ranged from 0.2% to 1.1% of that leached from fertilized soil treatments (UAN, UI, and UNI). Among fertilized treatments, there was significantly more  $\text{NH}_4^+$ -N leached from soil for the UI treatment than either the UAN or UNI treatments.

In agreement with the data for  $\text{NH}_4^+$ -N, the S treatment showed the lowest total mass of  $\text{NO}_3^-$ -N leached,  $0.1 \pm 0.0 \text{ mg}$  (Figure 8a and 8b). The total mass of  $\text{NO}_3^-$ -N leached through soil differed significantly among fertilizer treatments, ranging over a factor of approximately 2. The UNI treatment had the smallest value ( $3.8 \pm 0.3\text{mg}$ ) followed by the UAN ( $6.1 \pm 0.3\text{mg}$ ) and UI ( $7.7 \pm 0.3\text{mg}$ ) treatments. Nitrate captured in leachate from the S treatment represented from 0.9% to 1.8% of that leached from the treatments amended with fertilizer.

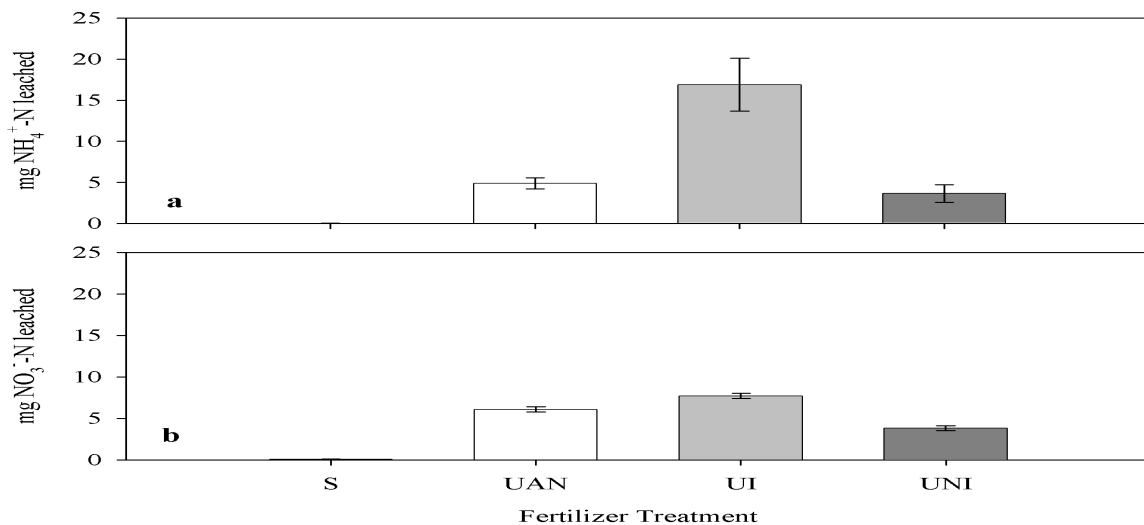


Figure 8. Total mass of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N leached from intact soil cores following simulated rainfall events of 2.5 cm (weeks 1 through 4) or 5 cm (week 5). Soil cores were amended with one of three fertilizer treatments one week prior to the first simulated rainfall.

Fertilizer treatments were a urea-ammonium-nitrate fertilizer (UAN) solution, or similar fertilizer solution amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™ (UNI) a urease and nitrification inhibitor. A fourth treatment included unfertilized, field moist cores (S). Error bars represent  $\pm 1$  SEM for triplicate soil cores, except for the UI treatment (n=2).

The total mass of N ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and urea-N) that was recovered as leachate from the UAN, UI, and UNI treatments was relatively small. For the respective fertilizer treatments, the percentage of fertilizer N that was collected in leachate was  $3.9 \pm 0.4\%$ ,  $8.8 \pm 1.0\%$ , and  $2.6 \pm 0.4\%$ . The differences among means were not significant. The percentage of total recovered N that was in the form of  $\text{NO}_3^-$ -N was  $55.8 \pm 2.2\%$ ,  $31.9 \pm 4.9\%$ , and  $53.4 \pm 7.5\%$  for the UAN, UI and UNI treatments, respectively. The remainder of N leached was largely in the form of  $\text{NH}_4^+$ -N, as urea comprised less than 1% of all N leached in the fertilizer treatments. In contrast, the relative distribution of N leachate from the S treatment was  $50.0 \pm 14.5\%$ ,  $40.6 \pm 11.6\%$ , and  $9.4 \pm 2.9\%$   $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and urea-N, respectively. The total mass of N leached from the S treatment was  $0.12 \pm 0.03$  mg, which represents less than 1% of the total mass of N leached from fertilized cores.

### 3.4 Microbial Activity

The rate of  $\text{CO}_2$  production was used as an index of community microbial activity. Carbon dioxide production was significantly higher for soil in fertilizer treatments (UAN, UI, and UNI) than soil without fertilizer (S and C) (Figure 9). The rates of soil  $\text{CO}_2$ -C production were similar in the UAN and UI treatments at approximately  $0.9 \text{ mg g dw}^{-1}$ . However, soil in the UNI treatment showed a significantly lower  $\text{CO}_2$ -C production rate ( $0.79 \pm 0.04 \text{ mg g dw}^{-1}$ ) than soil treated with UAN or UI. The rate of  $\text{CO}_2$ -C<sub>2</sub> production in soil for the S and C treatment were not significantly different from one another. The

respective rates of CO<sub>2</sub>-C production in soil for the S and C treatments were 0.29 ± 0.01 and 0.35 ± 0.002 mg g dw<sup>-1</sup>. The highest rate of soil CO<sub>2</sub>-C production among all treatments was observed in the UAN and UI treatments and was approximately 1.8 times the CO<sub>2</sub>-C production observed in the S and C treatments.

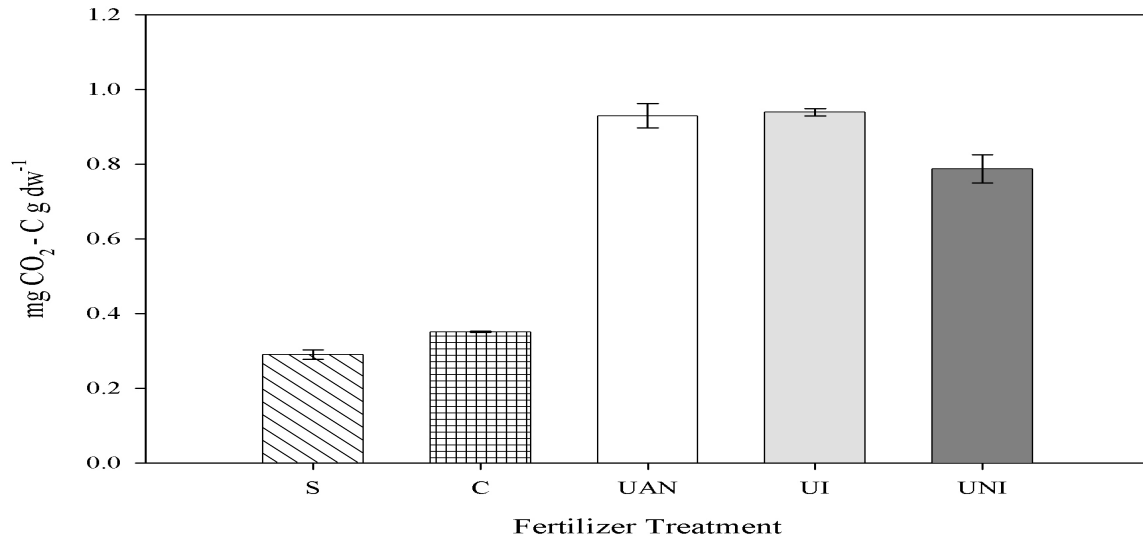


Figure 9. Total CO<sub>2</sub>-C production from homogenized soil for five treatments; field moist soil only (S), soil with the equivalent volume of deionized water added to soil as is added in fertilizer solutions (C), soil fertilized with urea-ammonium-nitrate (UAN) fertilizer solution, or a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor, or Agrotain Plus™, a urease and nitrification inhibitor (UNI). Error bars represent ± 1 SEM for triplicate soil samples.

Denitrifying enzyme activity (DEA) was used as a measure of the relative population size of denitrifying microbes in soil. Denitrifying enzyme activity increased for all soil treatments to day 10 followed by a decrease on day 14 (Figure 10). On day 10, soil in the UAN treatment had the largest increase in DEA, from an initial rate of 4.4 ± 1.5 ng N<sub>2</sub>O-N g dw<sup>-1</sup> h<sup>-1</sup> on day 0 to 121.1 ± 23.4 ng N<sub>2</sub>O-N g dw<sup>-1</sup> h<sup>-1</sup>. Thus, the rate increased by a factor of 27. Denitrifying enzyme activity on day 10 in the treatments UI and UNI increased to a lesser extent from initial levels, by factors of 2.3 and 5.5, respectively. Soil without fertilizer, the S

and C treatments, had low ( $< 10 \text{ ng N}_2\text{O-N g dw}^{-1} \text{ h}^{-1}$ ) DEA throughout the experiment. In addition, there was no significant difference in DEA between the S and C treatments. After soil was kept in anoxic conditions for four days there was an increase in DEA for all soils (data not shown) from the highest observed values (day 10). The soil in the UI treatment showed the greatest relative increase (factor of 73) from day 10 values. Soils in both the C and UAN treatments showed similar increases, by factors of 44 and 42, respectively. The UNI treatment showed an increase in DEA by a factor of 11 which was the lowest observed for all fertilizer treatments. Rates of DEA on day 18 were  $348 \pm 84$ ,  $304 \pm 141$ ,  $5230 \pm 705$ ,  $1712 \pm 582$ , and  $922 \pm 322 \text{ ng N}_2\text{O-N g dw}^{-1} \text{ h}^{-1}$  for the S, C, UAN, UI and UNI treatments, respectively.

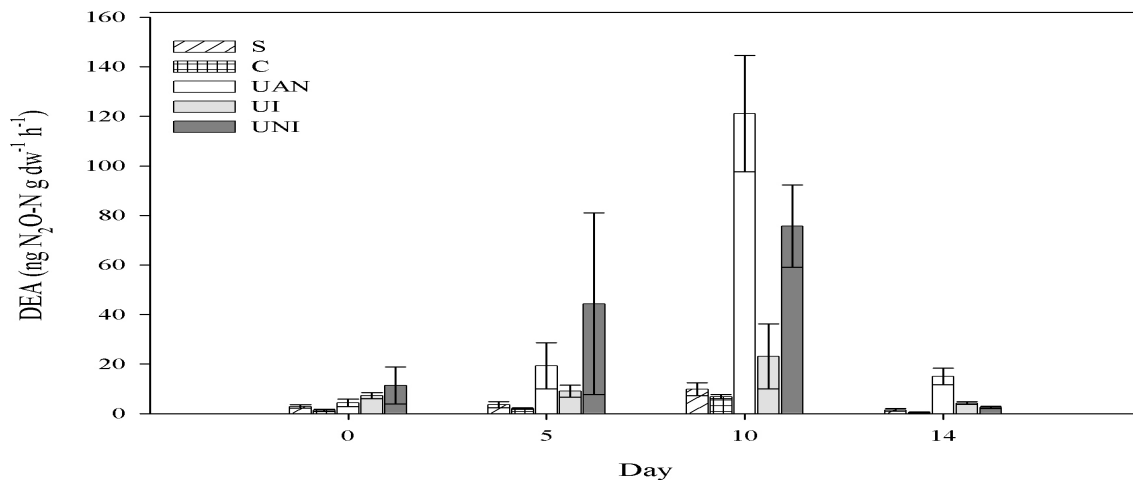


Figure 10. Time course for denitrifying enzyme activity (DEA) in homogenized soil for five treatments: field moist soil only (S); soil with the equivalent volume of deionized water added to soil as is added in fertilizer solutions (C); soil fertilized with urea-ammonium-nitrate (UAN) fertilizer solution; a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™, a urease and nitrification inhibitor (UNI). Error bars represent  $\pm 1$  SEM for triplicate soil samples.

Short-term nitrifier activity provided an index of the response of the ammonium-oxidizing microbial population to each treatment. Similar to DEA, NA increased in the UAN

and UI treatments to day 10 followed by a decline (Figure 11). On day 10, NA increased by 103% and 130% from day 0 values for the UAN and UI treatments, respectively. Maximum rates of NA for these treatments exceeded 500 ng NO<sub>2</sub><sup>-</sup>-N g dw<sup>-1</sup> h<sup>-1</sup>. In contrast to these two fertilizer types, rates of NA in soil treatments S and C remained low and fairly constant, varying from 161.4 ± 3.8 to 216.0 ± 0.8 ng NO<sub>2</sub><sup>-</sup>-N g dw<sup>-1</sup> h<sup>-1</sup>. Mean rates between these two treatments differed significantly only on day 5. Soil treated with a nitrification inhibitor (UNI) had the lowest rates of NA observed on all dates. Rates were only 24 to 57% of values recorded for the C treatment and 9 to 34% of the values observed for the UAN treatment. Short term nitrifier activity in soil was significantly lower in the UNI treatment than the C treatment throughout the experiment. In addition, NA rates declined significantly (57%) between day 0 to 14 for soil in the UNI treatment, from 91.7 ± 10.5 to 39.2 ± 3.5 ng NO<sub>2</sub><sup>-</sup>-N g dw<sup>-1</sup> h<sup>-1</sup>.

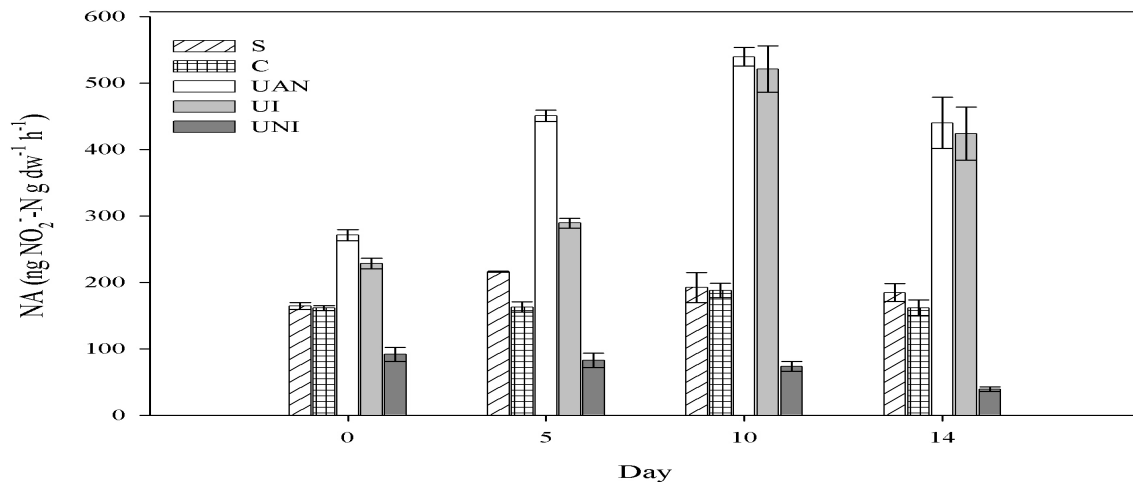


Figure 11. Time course for short-term nitrifier activity (NA) from homogenized soil for five treatments; field moist soil only (S); soil with the equivalent volume of deionized water added to soil as is added in fertilizer solutions (C); soil fertilized with urea-ammonium-nitrate (UAN) fertilizer solution; a similar fertilizer amended with Agrotain™ (UI), a urease inhibitor; or Agrotain Plus™, a urease and nitrification inhibitor (UNI). Error bars represent ± 1 SEM for triplicate soil samples.

## CHAPTER 5

### DISCUSSION

#### 4.1 Urea Hydrolysis

The extracellular urease enzyme is responsible for catalyzing urea hydrolysis, the transformation of urea-N to ammonia and carbon dioxide (Bremner 1995). Soil properties such as pH, texture, moisture, cation exchange capacity, and  $\text{CaCO}_3$  content can influence urease activity (Mulvaney and Bremner 1981). However, soil organic content is positively correlated with and perhaps exhibits the greatest effect on urease activity in soils (Myers and McGarity 1968; Zantua et al. 1977). Studies assessing the relationship between soil organic content and urease activity are limited to soils of 3.0 to 203.9  $\text{g kg}^{-1}$  organic carbon (Zantua and Bremner 1976; Zantua et al. 1977; Watson et al. 1994; Chakrabarti et al. 2004). Furthermore, it is difficult to compare rates of urea hydrolysis in soils with varying organic matter content because quality of organic material can influence urease activity (Perucci et al. 1982; Gill et al. 1999; Gioacchini et al. 2000). Accordingly, the experiments conducted in this study were to gain a better understanding of rates of urea hydrolysis and N transformations in soil with exceptionally high organic content.

Complete urea hydrolysis occurred between day 6 and 8 in all experiments when unamended UAN fertilizer was added to soil in this investigation. Previous laboratory experiments showed that in urea-fertilized soil with considerably lower organic content (2.8 to 24.6  $\text{g kg}^{-1}$ ) over 90% of applied urea-N was hydrolyzed within 10 d of application (Carmona et al. 1990; Zhengping et al. 1991b; Gill et al. 1999). Despite differences in

organic content and possibly other physiochemical properties, rates of urea hydrolysis from urea or UAN fertilized soil are comparable across studies. In a laboratory study involving a variety of soils with soil organic content ranging from 7.0 to 45.0 g kg<sup>-1</sup>, Kumar and Wagenet (1984) found no definitive relationship between urease activity and any one soil physiochemical property.

Ammonia volatilization ranged from 5.2 to 7.2% of N applied to soil cores treated with unamended UAN fertilizer during the 20 d incubation period in the current study. Similarly, in a laboratory experiment using homogenized Typic Ustochrept soil with a lower organic content (2.8 g kg<sup>-1</sup>), 7.12% of N applied as urea fertilizer volatilized after 16 days (Gill et al. 1999). However, in another study using 16 different homogenized soils from Northern and Southern Ireland with varying organic content (16.3 – 203.9 g kg<sup>-1</sup>), NH<sub>3</sub> volatilization from urea-fertilized soils ranged from 5.8 to 38.9% during a 9 day observational period (Watson et al. 1994). Analysis of the relationship between soil physicochemical properties and the rate of NH<sub>3</sub> loss from unamended urea- fertilized soil indicated that volatilization was most highly correlated with soil acidity.

Few studies have used (or cited the use of) Agrotain™ and Agrotain Plus™ products as enzyme or microbial activity inhibitors to study their effects on nitrogen transformations in soil. However, the chemical in Agrotain™ that acts as a urease inhibitor, N-(n-butyl) thiosphosphoric triamide (NBPT), and its effectiveness as an amendment to urea- based fertilizers has been extensively studied. The two major chemicals in Agrotain Plus™ that serve as urease and nitrification inhibitors are NBPT and dicyandiamide (DCD), respectively. While there have been studies conducted on the effectiveness of these inhibitors and their



ability to increase fertilizer and nitrogen use efficiency in agroecosystems, there is limited information on their effectiveness in highly organic soils.

Results of the first urea hydrolysis experiment showed that less than 1 and 20% of the initial urea-N remained in the soil on day 8 for the UAN and UI treatments, and hydrolysis was near complete by day 14 in both treatments (Figure 2a). These results compare favorably with previous laboratory studies. Hendrickson and Douglass (1993) found that 0 and 30% of urea-N remained in acidic soil fertilized with urea and urea plus NBPT, respectively, and hydrolysis was complete on day 14 for the NBPT-amended soil. Similarly, Vittori Antisari et al. (1996) reported that approximately 20-30% of applied urea-N remained in soil by day 8 when fertilized with urea plus NBPT with an application rate of NBPT equivalent to that used here (0.1% w/w<sub>urea</sub>). Both studies assessed rates of urea hydrolysis under varying physicochemical conditions (e.g. pH, texture, etc.) but soil organic contents ranged from 0.153 to 47 g kg<sup>-1</sup>. The time course for urea hydrolysis and its retardation by NBPT in this wide range of soil organic contents were similar possibly due to poor quality organic material or the interactive effects of physicochemical factors affecting hydrolysis and the effectiveness of NBPT.

Peak soil concentrations of NH<sub>4</sub><sup>+</sup>-N occurred between day 3 and 8 in the first urea hydrolysis experiment (Figure 2b), with the UAN treatment showing the most rapid NH<sub>4</sub><sup>+</sup>-N accumulation. A previous report (Vittori Antisari et al. 1996) also found that concentrations of NH<sub>4</sub><sup>+</sup>-N increased in general more rapidly in soil that was fertilized without a urease inhibitor versus soil that was fertilized with a urease inhibitor.

The addition of Agrotain™ to UAN fertilized soil in the first urea hydrolysis experiment resulted in a significant (45%) reduction in NH<sub>3</sub> volatilization over 21 days

(Figure 3a). Agrotain™ was more effective here at preventing NH<sub>3</sub> volatilization than in a laboratory study involving sandy soil cores (Singh et al. 2004), where a 27% reduction in volatilization was observed in urea-treated soils amended with Agrotain™. Differences in the percentage of N fertilizer lost to NH<sub>3</sub> volatilization could be due to differences in buffering capacity and pH of soil (Hargrove 1988).

The second urea hydrolysis experiment is the first study to directly assess the effectiveness of Agrotain Plus™ at delaying urea hydrolysis. A temporal disjunction between urea hydrolysis experiments precludes direct comparison of the efficacy of Agrotain™ and Agrotain Plus™ at delaying urea hydrolysis, as critical soil properties may have differed between sampling dates.

In agreement with results of the first urea hydrolysis experiment comparing UAN and UI, addition of NBPT and DCD resulted in a significant reduction (64%) in NH<sub>3</sub> loss to volatilization relative to UAN fertilizer. Although the percent reduction in NH<sub>3</sub> volatilization appears greater than the 45% observed for UI, simultaneous examination of the performance of all three fertilizers is necessary for confirmation. An 80 day field study on sandy loam soil (Gioacchini et al. 2002) showed that the addition of NBPT plus DCD to urea fertilizer reduced NH<sub>3</sub> volatilization by 58% relative to soil fertilized with unamended urea. This performance is comparable to results found here.

#### **4.2 Moisture dependence**

Moisture content appeared to have a fertilizer-specific impact on rates of urea hydrolysis as rates were not different among % WHCs for UAN and UI treatments, but soil at 86% WHC showed a significantly higher rate of urea hydrolysis than soil at lower

moisture levels for the UNI treatment. There are other references to inconsistencies in the relationship between urease activity and soil moisture in previous research (Mulvaney and Bremner 1981; Agehara and Warncke 2005). However, the results for the UNI treatment agree with most research, in that urea hydrolysis increases as moisture levels approach field capacity (Kumar and Wegnet 1984; Vlek and Carter 1983; Hongprayoon et al. 1991). Allowing for a direct comparison, Agehara and Warncke (2005) reported that urea-fertilized soil at 90% WHC had higher rates of urea-N decline than soil at 50 and 70% WHC. The reason for differences among fertilizers in the moisture sensitivity of urease activity in the current study is unknown.

In agreement with the results of the two urea hydrolysis experiments, urea hydrolysis in the moisture dependence experiment was delayed significantly in the UI and UNI treatments relative to the UAN treatment at all moisture levels. In addition, there was no significant difference in the rate of urea hydrolysis between the UI and UNI treatments within a moisture level. These results are consistent with previous research that the addition of DCD to NBPT in urea fertilizers does not further affect urea hydrolysis (Mulvaney and Bremner 1981). This further suggests that temporal differences in physicochemical properties influencing rates of urea hydrolysis account for the difference in performance of the UI and UNI treatments relative to the UAN treatment in the two urea hydrolysis experiments.

Results from the moisture experiment showed that soil at 86% WHC had an overall increase in soil  $\text{NH}_4^+$ -N concentrations for all fertilizer treatments (Figure 5). Soil conditions that are unfavorable for nitrification, such as high moisture and low oxygen levels, can cause

accumulation of  $\text{NH}_4^+$ -N in soil (Tisdale and Nelson 1975). It is likely that these factors contributed to the accumulation of soil  $\text{NH}_4^+$ -N at 86% WHC.

The simultaneous decline in soil  $\text{NH}_4^+$ -N and rise in  $\text{NO}_3^-$ -N for the UAN and UI treatments at 42 and 64% WHC in contrast to the increase in soil  $\text{NH}_4^+$ -N and decrease in  $\text{NO}_3^-$ -N in the UNI treatment, indicates that the DCD was effective at inhibiting nitrification throughout the 20 day experiment. In previous laboratory experiments using soils with varying physiochemical properties, the effectiveness of DCD ranged from 21 d to over 42 d (Bronson et al. 1989a; McCarty and Bremner 1989). The rate of DCD degradation in soil varies with soil temperature, texture, and moisture regime (Bronson et al. 1989).

There was an overall decrease in soil  $\text{NO}_3^-$ -N concentrations at 86% WHC in all fertilizer treatments. The decline in soil  $\text{NO}_3^-$ -N concentration suggests that soil moisture conditions were favorable for denitrification. As soil moisture increases, denitrification rates also increase (Tisdale and Nelson 1975) until optimum conditions are met when 100% of soil pore space is filled with water (Brady and Weil 2002). The decrease in soil  $\text{NO}_3^-$ -N concentrations in the UNI treatment at all moisture levels suggests that denitrification was occurring in all soil fertilizer treatments. Although denitrification is a facultative anaerobic process that has faster rates in anaerobic soils, denitrification can take place in well aerated soil due to anoxic zones in soil aggregates (Schlesinger 1997). Because nitrification is more rapid in aerobic soils, it was not until nitrification was inhibited that denitrification was inferred in soil at 42 and 64% WHC. In aerated soils, denitrification rates can be masked by high nitrification rates which allow a net accumulation of nitrate (Burns et al. 1996).

The highest net accumulation of soil  $\text{NO}_3^-$ -N occurred in the UAN treatment at 64% WHC, suggesting that this moisture level is ideal for nitrification in this soil. Brady and Weil

(2002) reported that the optimum moisture condition for nitrifying bacteria occurs when around 60% of total soil pore space is filled with water. Similarly, in a field study Abbasi and Adams (2000) measured the greatest accumulation of  $\text{NO}_3^-$ -N in soil at 63% water filled pore space (WFPS). In a laboratory study, soil  $\text{NO}_3^-$ -N concentrations increased with increasing water content in the range from 40 to 60% WFPS, leading researchers to conclude that nitrification rates increased with increasing moisture for moderately wet soils (Burns et al. 1996). This is consistent with previous reports that nitrification rates are lowest in excessively dry and wet soil conditions (Tisdale and Nelson 1975; Brady and Weil 2002).

Ammonia volatilization was significantly higher in all fertilizer treatments at 86% WHC than at 42 or 64% WHC. Previous research has shown that  $\text{NH}_3$  volatilization increases with soil moisture content (Fenn and Hossner 1985; Clay et al. 1990). Additionally, research has shown that maximum  $\text{NH}_3$  volatilization occurs when fertilized soil is at field capacity (Hargrove 1988). In a laboratory study conducted by Al-Kanani et al. (1991)  $\text{NH}_3$  volatilization increased with soil water content in UAN-fertilized soil. Moreover, field and laboratory experiments have shown that  $\text{NH}_3$  volatilization losses increase in urea-N fertilized soil following a rainfall event which leads to higher soil water content (Kissel et al. 2004).

Soil in the UI treatment had significantly less  $\text{NH}_3$  volatilized at all moisture levels compared to the other fertilizer treatments. Results from both laboratory and field studies conducted by Bronson et al. (1989b) showed that  $\text{NH}_3$  volatilization was significantly less in soil when urea fertilizer was amended with the urease inhibitor NBPT. Zhengping et al. (1991) showed similar results in a laboratory study, concluding that the addition of NBPT prevented the hydrolysis of urea and therefore reduced  $\text{NH}_3$  volatilization. In contrast to

NBPT, the addition of DCD increased  $\text{NH}_3$  volatilization from soil as shown in these results and in previous studies. In a laboratory experiment using sandy loam soil the application of DCD increased volatilization when applied with a urea fertilizer compared to unamended urea fertilizer (Praksa Roa and Puttanna 1987). In another experiment,  $\text{NH}_3$  volatilization losses were higher in both sandy loam and clay loam soil when DCD was added to soil with urea and NBPT in contrast to a treatment of urea-fertilizer amended with NBPT (Gioacchini et al. 2002) Since both types of soil showed a similar response to the addition of a nitrification and urease inhibitor, the authors concluded that DCD reduced the efficiency of NBPT. As an alternative explanation, Zhengping et al. (1991) speculated that the increased  $\text{NH}_3$  volatilization observed in aerated soils is due to the competition between nitrification and volatilization processes.

### **4.3 Simulated Rainfall**

Essentially no urea-N leached from soil cores regardless of fertilizer treatment. However, previous laboratory research using repacked soil columns found measurable concentrations of urea-N in collected leachate (Paramasivam and Alva 1997; Prakash et al. 1999). In a laboratory study using sandy soil, it was not until the second leaching event, approximately two weeks after fertilization, that urea-N was leached through the repacked soil column (Prakash et al. 1999). Additionally, there was no significant difference in the amount of urea-N leached between the urea and Agrotain™ fertilizer treatments. In another laboratory experiment using similar sandy soil Paramasivam and Alva (1997) also reported that the largest percentage of urea-N was leached from urea-fertilized soil approximately two

weeks after addition. In both experiments leaching of urea-N was complete by approximately four weeks.

Contrary to expectations, the proportion of N leached as  $\text{NH}_4^+$ -N was relatively large in this simulated rainfall experiment. There are conflicting reports of the percentage of N leached as  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N in previous field and laboratory studies. In a laboratory study using repacked soil columns 8 cm in height with homogenized sandy soils and fertilized with  $\text{NH}_4\text{NO}_3$ , approximately 85% and 37% of the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N applied was recovered in 1000 mL of leachate (Alva 1992). Further, it was determined that less  $\text{NO}_3^-$ -N leached because soil cores were nearly water-saturated, favoring denitrification. However in field experiments, researchers have found larger portions of N leached in the form of  $\text{NO}_3^-$ -N than  $\text{NH}_4^+$ -N regardless of N fertilizer type and irrigation practice (Timmons and Dylla 1981; Gioacchini et al. 2002; Portela et al. 2006). Despite six different treatments with varying combinations of granular or liquid nitrogen fertilizer and irrigation method,  $\text{NH}_4^+$ -N comprised a small percentage (0.02 to 0.12%) of the total N leached compared to  $\text{NO}_3^-$ -N in a field experiment conducted in a sandy Minnesota loam (Timmons and Dylla 1981). Similarly, Gioacchini et al. (2002) found that negligible amounts of  $\text{NH}_4^+$ -N leached from either sandy loam or clay loam soils fertilized with urea. Finally, a five year study involving silt loam soil fertilized with a variety of N fertilizers including urea and ammonium sulfate showed that  $\text{NH}_4^+$ -N concentrations in the leachate were below the detection limit (Portela et al. 2006).

Overall, less  $\text{NO}_3^-$ -N leached in the UNI treatment than either the UAN or UI treatments, although differences among treatments were not significant due to high within-treatment variability in the mass of  $\text{NO}_3^-$ -N leached. Qualitatively, however, reduced  $\text{NO}_3^-$ -N

in leachate from the UNI treatment is in agreement with results of a laboratory experiment (Alva 1992) that showed reduced leaching of  $\text{NO}_3^-$ -N from soil columns amended with controlled release versus traditional fertilizers. The nitrification inhibitor in the UNI treatment is functionally similar to the controlled release fertilizer by slowing the oxidation of  $\text{NH}_4^+$ -N to  $\text{NO}_3^-$ -N.

The total percentage of fertilizer lost to leaching ranged from 2.6% to 8.8% in the simulated rainfall experiment. These percentages are considerably lower than the values of 43% and 36% of applied N leached through soil columns containing moderately well drained sandy soil treated with urea and Agrotain™ fertilizer, respectively (Prakash et al. 1999). However, these data compare favorably with field studies assessing leaching loss of nitrogenous fertilizers. Portela et al. (2006) estimated that 0 to 3.5% of fertilizer derived N leached from silt and sandy loam soils in a field experiment, with the percentage loss linked to the crop and fertilizer type. Similarly, a field experiment conducted on a corn-soybean-wheat rotation showed that approximately 5% of urea fertilizer leached below the root zone (Riley et al. 2001). Direct comparison of the percentage of fertilizer-derived N lost to leaching is difficult due to an array of factors that influence N movement, including timing and volume of rainfall and soil texture.

#### **4.4 Microbial Activity**

The addition of water to soil did not stimulate the microbial community in the microbial activity experiment, as there was no difference  $\text{CO}_2$  production between soils in the S (no amendment) and C (water addition) treatments (Figure 9). Soil wetting has frequently been demonstrated in field and laboratory studies to enhance  $\text{CO}_2$  production as much as



500% compared with unwetted soils, with the CO<sub>2</sub> pulse persisting as long as 6 d following the wetting event (eg. Kieft et al. 1987; Franzluebbers et al. 2000; Borken et al. 2003). Enhanced CO<sub>2</sub> production following soil wetting has been attributed to improved C availability to active microbes from either previously killed microbial biomass or nonbiomass soil organic matter (Fierer and Schimel 2003). The lack of a respiratory response on wetting in the current study likely relates to soil moisture. Field moist soil (S treatment) was at 64% WHC, close to the optimum of 60% WHC for microbial respiration across diverse soils (Linn and Doran 1984), suggesting that the additional moisture would not stimulate microbial CO<sub>2</sub> production.

Soils treated with a volume of liquid UAN type fertilizer consistent with the volume of deionized water added in the C treatment show significantly elevated rates of CO<sub>2</sub> production relative to the C or S treatments (Figure 9), indicating that the enhanced CO<sub>2</sub> evolution was associated with fertilization rather than wetting. Previous studies assessing changes in CO<sub>2</sub> flux following application of urea fertilizers and inhibitors give mixed results. In a laboratory experiment conducted on sandy loam soils in Minnesota, Clay et al. (1990) found that CO<sub>2</sub> evolution was lower in soils treated with urea amended with NBPT and NBPT plus DCD relative to unamended controls. Although no explanation was given for the decline in CO<sub>2</sub> emission, soil pH increased from 6.5 to 9.0 in all fertilized samples, which could have influenced the distribution of CO<sub>2</sub> between the aqueous and gaseous phases. In contrast, a laboratory experiment conducted with 12 German soils showed either an increase and decrease in CO<sub>2</sub> production in soils amended with urea plus NBPT, depending on the soil (Guettes et al. 2002). Results likely reflect the sensitivity of urease activity to soil physicochemical properties as noted previously.

The reason for the CO<sub>2</sub> flush in the current study is unclear. If the time-integrated mass of CO<sub>2</sub>-C emission from the S and C treatments (0.3 mg C g dw<sup>-1</sup>) is considered to be the basal metabolic rate of unfertilized soil, the CO<sub>2</sub> efflux of 0.9 mg C g dw<sup>-1</sup> can be explained simply by urease activity, as total decomposition of the urea in the fertilizers used here will liberate 0.7 g CO<sub>2</sub>-C g dw<sup>-1</sup> soil, at the rate of fertilizer application in this experiment. An increase in denitrification in response to the increased availability of NO<sub>3</sub><sup>-</sup>-N following fertilization (Figure 10) may also be responsible for elevated CO<sub>2</sub> emission in fertilizer treatments. Simultaneous increases in denitrification and CO<sub>2</sub> production have been reported for natural soils (Robertson and Tiedje 1984; Groffman and Tiedje 1991) and DEA has been positively correlated with CO<sub>2</sub> evolution in a Kentucky soil (Parsons et al. 1991). Increases in the population of nitrifiers (Figure 11), should lead to net incorporation of CO<sub>2</sub> into microbial biomass. However, Miller and Johnson (1964) reported the simultaneous occurrence of maximum rates of nitrification and CO<sub>2</sub> production in Colorado soils. Carbon dioxide is an end product of the degradation of DCD, the nitrification inhibitor in the UNI fertilizer (Gioacchini et al. 2002), but will contribute insignificantly to the CO<sub>2</sub> mass balance at the application rate used here.

Proximal controls on denitrification (reviewed by Firestone and Davidson 1989; Robertson 2000) include O<sub>2</sub> and the availability of reductants (labile organic-C) and electron acceptors (nitrogen oxides). Soil moisture is often used as a proxy for soil O<sub>2</sub> status, as O<sub>2</sub> concentrations are inversely related to moisture (Davidson and Schimel 1995). Increasing rates of DEA in the fertilizer treatments from day 0 to 10 (Figure 10), and persistently low rates in the S and C treatments indicate that denitrification in these soils is at least in part constrained by NO<sub>3</sub><sup>-</sup>-N availability. Denitrification has been frequently documented in the

anoxic interior of soil aggregates in otherwise oxic bulk soils (Sextone et al. 1985). Moreover it is closely coupled with nitrification at low  $\text{NO}_3^-$ -N concentrations (Groffman 1994) due to diffusional constraints on substrate supply (Strong and Fillery 2002). The endogenous  $\text{NO}_3^-$ -N concentration of  $\sim 10 \mu\text{g g dw}^{-1}$  soil (= 0.8 mM) approaches the threshold of  $5 \mu\text{g g dw}^{-1}$  for denitrification (Ryden 1983) and is lower than  $K_m$  values (half-saturation constant) which range to 12 mM for denitrification in unstirred soil samples (Bowman and Focht 1974; Strong and Fillery 2002). Apparently, fertilization relaxed or eliminated diffusion limitation, as soil  $\text{NO}_3^-$ -N increased to about  $800 \mu\text{g g dw}^{-1}$ , allowing for denitrification uncoupled to nitrification (e.g. Nielsen and Revsbech 1998). It is unclear whether increased DEA in fertilized soils resulted from population growth of denitrifiers or *de novo* enzyme synthesis by existing microbes. Whalen and DeBerardinis (2007) demonstrated an increase in total microbial biomass in N-fertilized agricultural soils on a time horizon similar to that of the current experiment, but did not distinguish among functional groups.

The marked increase in DEA for all treatments following four days of anoxic incubation indicates that  $\text{O}_2$  was the dominant control over denitrification in this soil. The relative increase among fertilizer treatments (UAN > UI > UNI) was consistent with the rank order for  $\text{NO}_3^-$  availability. The DEA values of about 900 to 5200  $\text{ng N}_2\text{O-N g dw}^{-1}$  fertilized soils following anaerobic incubation rank toward the high end of previous reports for agroecosystems, which show DEA values ranging to 2000  $\text{ng N}_2\text{O-N g dw}^{-1}$  soil (Bergstrom and Beauchamp 1993; Sotomayor and Rice 1996; Loro et al. 1997; Mulvaney et al. 1997; Pelletier et al. 1999; D'Haene et al. 2003). Nitrogen loss to denitrification in agroecosystems has been estimated to account for 10 to 40% of fertilizer inputs worldwide (Galloway et al. 2004). High DEA following anaerobic incubation and the high organic content of these soils

point to a high potential for loss of fertilizer-N from denitrification during rainfall and argue for the use of the UNI fertilizer to restrict substrate availability for denitrifiers, and hence, gaseous N loss.

Active nitrification in all treatments in NA experiment (Figure 11) indicate that O<sub>2</sub> was present and corroborates the inference that denitrification was limited to anoxic microsites in these soils. In accord with data for DEA, at least some fertilizers (UAN and UI) elicited an increase in NA relative to S and C treatments. Initial soil concentrations of NH<sub>4</sub><sup>+</sup>-N were around 1 to 2 µg g dw<sup>-1</sup> in the S and C treatments, but values were increased to around 800 µg g dw<sup>-1</sup> in fertilized soils. Laboratory studies have shown an increase in nitrification with increasing NH<sub>4</sub><sup>+</sup>-N in the 50 to 200 µg g dw<sup>-1</sup> range (Malhi and McGill 1982; Nishio and Fujimoto 1990), while the reported K<sub>m</sub> for soil nitrification is 154 to 186 µg NH<sub>4</sub><sup>+</sup>-N g dw<sup>-1</sup>. Thus, the fertilizer-induced increase in NA is at least partly related to an increase in substrate supply. It is unclear whether population growth of ammonium oxidizers occurred, as Wheatly et al. (1997) demonstrated that NA can show wide temporal fluctuations without change in population size. Further, ammonium oxidizers have shown an uncommonly slow maximum specific growth rate (Hagopian and Riley 1998). The observed range of values for NA in this study when all treatments were considered (4 to 540 ng NO<sub>2</sub><sup>-</sup>-N g dw<sup>-1</sup> h<sup>-1</sup>) is similar to the 0 to 500 ng NO<sub>2</sub>-N g dw<sup>-1</sup> h<sup>-1</sup> given in previous reports for arable land and pastures (Berg and Roswall 1985, 1989; Hopkins et al. 1988; Bramley and White 1989).

The significant reduction in NA for soils in the UI treatment relative to the S and C treatments (Figure 11) is consistent with mode of action of DCD, namely suppression of activity of extant ammonium oxidizers (Subbarao et al. 2006). Previous research in mineral

soils (Amberger 1989) has demonstrated that DCD remains effective for 4 to 8 weeks. The current study clearly indicates that DCD inhibited  $\text{NH}_4^+$  oxidation to at least 2 weeks in this highly organic soil. This direct demonstration of the effectiveness of DCD as a nitrification inhibitor in this soil provides further evidence that its use will reduce offsite loss of fertilizer N from leaching and denitrification.

## CHAPTER 6

### SUMMARY

This study simulated field conditions to evaluate rates of N transformation and transport in highly organic agricultural soils treated with UAN type fertilizers. Rates of urea hydrolysis from UAN fertilizer were similar to previously reported rates for urea or UAN fertilizers in mineral soils, suggesting that the quality and/or quantity of organic material at the study site had no influence on urease activity. The urease inhibitor NBPT effectively slowed urea hydrolysis both alone and combined with nitrification inhibitor DCD. The influence of soil moisture on rates of urea hydrolysis appeared to be fertilizer specific, as only the UNI fertilizer showed sensitivity to % WHC. However, soil moisture did influence other nitrogen transformations that affect both the availability of fertilizer for plant assimilation and susceptibility for loss from the point of application. Specifically, soils fertilized with UAN and UI fertilizers at 42 and 64% WHC showed high rates of nitrate accumulation which can enhance offsite transport by subsequent denitrification or by leaching and surface runoff. Simulated rainfall experiments showed that soils subjected to UNI fertilizer experienced less leaching loss of both  $\text{NO}_3^-$ -N and total measured N (urea-N,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N) than soils fertilized with UAN or UI. However, differences among treatments in the latter were not significant due to high variability in the data. Although nitrification and denitrification were not specifically assessed in this study, soils fertilized with UAN and UI showed high potential for both microbial activities. In contrast, UNI soils

exhibited a reduction in NA relative to all other treatments over 14 d and severely limited DEA after a 4 d anaerobic incubation. Thus, UNI fertilizer can effectively limit the oxidation of  $\text{NH}_4^+$ -N supplied directly as fertilizer or from hydrolysis of fertilizer-urea, reducing the  $\text{NO}_3^-$ -N available for denitrification and offsite transport in these soils. Fractional N loss to volatilization was similar among fertilizer types, suggesting that this should have little influence in fertilizer selection at this site. Based on the effectiveness of the UNI fertilizer on suppressing nitrification and the pivotal role of  $\text{NO}_3^-$ -N in fertilizer loss from the site of application, use of this fertilizer is recommended for this agroecosystem, which abuts nutrient-sensitive marine waters.

## Appendix I:

### Urease Activity Experiment Treatments UAN and UI

Sample	Day	Urea-N	NH <sub>4</sub> <sup>+</sup> - N	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>3</sub> -N Volatilized
		mg g dw <sup>-1</sup>			mg
UAN1	0	0.7133	0.3864	0.3296	
UAN2	0	1.1072	0.3925	0.3700	
UAN3	0	0.7757	0.4148	0.1914	
UI1	0	0.8224	0.4112	0.3214	
UI2	0	0.6835	0.3364	0.2069	
UI3	0	0.8013	0.3546	0.3255	
UAN1	1	0.5051	0.4535	0.3147	0.4317
UAN2	1	0.5391	0.4849	0.3219	0.3602
UAN3	1	0.8542	0.6661	0.4337	0.4056
UI1	1	0.7587	0.4818	0.2991	0.0577
UI2	1	0.7919	0.3923	0.3268	0.1404
UI3	1	0.7228	0.4381	0.4871	0.1134
UAN1	2	0.3004	0.6526	0.3812	0.6432
UAN2	2	0.2372	0.5954	0.1838	
UAN3	2	0.3623	0.6669	0.3266	0.5906
UI1	2	0.6918	0.4725	0.4166	0.1171
UI2	2	0.7998	0.4996	0.4706	0.2238
UI3	2	0.5689	0.4994	0.3798	
UAN1	3	0.2905	0.7275	0.2310	1.5070
UAN2	3	0.3627	0.7678	0.3433	1.5956
UAN3	3	0.2143	0.6697	0.2779	2.5144
UI1	3	0.3626	0.4315	0.3881	0.1659
UI2	3	0.6246	0.6561	0.4268	0.1057
UI3	3	0.2562	0.2813	0.0247	0.0888
UAN1	5	0.0773	0.5652	0.0571	14.5074
UAN2	5	0.1985	0.5061	0.3092	7.3593
UAN3	5	0.0218	0.5672	0.1128	13.9630
UI1	5	0.5423	0.7086	0.3448	0.4150
UI2	5	0.1972	0.7564	0.2896	0.9164
UI3	5	0.2889	0.6534	0.2974	0.5945
UAN1	8	0.0102	0.5164	0.3477	5.9223
UAN2	8	0.0009	0.4994	0.3521	9.7219



UAN3	8	0.0000	0.5273	0.2704	9.0203
UI1	8	0.2324	0.8314	0.3107	0.6401
UI2	8	0.1012	0.4632	0.2105	2.9636
UI3	8	0.1326	0.9233	0.4010	5.1522
UAN1	14	0.0014	0.5476	0.6907	23.2635
UAN2	14	0.0000	0.5367	0.5651	16.8661
UAN3	14	0.0008	0.4523	0.4137	22.0114
UI1	14	0.0083	0.4808	0.5314	16.3867
UI2	14	0.0005	0.4695	0.3829	11.6144
UI3	14	0.0010	0.4754	0.3833	15.2163
UAN1	21	0.0000	0.3695	0.4111	23.2635
UAN2	21	0.0020	0.4966	0.3392	17.4754
UAN3	21	0.0003	0.6524	0.3122	19.9024
UI1	21	0.0004	0.5106	0.2428	18.7298
UI2	21	0.0021	0.5081	0.2473	14.6294
UI3	21	0.0000	0.0205	0.1122	

## Appendix II:

### Urease Activity Experiment Treatments UAN and UNI

Sample	Day	Urea-N	NH <sub>3</sub> -N		
			NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Volatilized
			mg g dw <sup>-1</sup>		
				mg	
UAN1	0	1.0585	0.5325	0.4755	
UAN2	0	0.6353	0.3252	0.3028	
UAN3	0	0.6053	0.2888	0.2917	
UNI1	0	0.6320	0.3047	0.2480	
UNI2	0	0.4654	0.2314	0.2308	
UNI3	0	0.7904	0.3352	0.3646	
UAN1	1	0.7487	0.5814	0.4520	0.2908
UAN2	1	0.8006	0.6022	0.4727	0.1417
UAN3	1	0.5608	0.4367	0.3221	0.2134
UNI1	1	0.7887	0.4244	0.3180	0.2944
UNI2	1	0.3820	0.2453	0.1870	0.2860
UNI3	1	0.6384	0.3401	0.2772	0.1689
UAN1	3	0.0910	0.7113	0.3066	4.3305
UAN2	3	0.3874	0.5778	0.4895	1.3442
UAN3	3	0.1710	0.6563	0.3115	2.2443
UNI1	3	0.5530	0.4111	0.2933	0.5194
UNI2	3	0.6439	0.4379	0.3200	0.5434
UNI3	3	0.6074	0.4036	0.3017	0.7873
UAN1	5	0.3136	0.9242	0.3478	7.9174
UAN2	5	0.3731	0.8146	0.3349	8.1416
UAN3	5	0.5396	0.9174	0.2783	4.7400
UNI1	5	0.6398	0.5323	0.3314	0.9468
UNI2	5	0.3005	0.3211	0.2074	1.6617
UNI3	5	0.8230	0.5854	0.3381	1.0838
UAN1	8	0.0092	0.6935	0.2172	10.7080
UAN2	8	0.0116	1.1355	0.3522	13.2082
UAN3	8	0.0128	0.9958	0.2976	8.6804
UNI1	8	0.5627	0.5374	0.2805	1.5609
UNI2	8	0.5811	0.4787	0.2771	0.9318
UNI3	8	0.7379	0.5999	0.3272	0.9829
UAN1	14	0.0038	0.7831	0.4880	13.5934
UAN2	14	0.0000	0.8624	0.4245	12.4466

UAN3	14	0.0000	0.5869	0.5462	10.1481
UNI1	14	0.3587	0.6133	0.3493	4.2477
UNI2	14	0.6739	0.5278	0.3599	2.5935
UNI3	14	0.2733	0.5590	0.2287	3.7480
UAN1	21	0.0095	0.5776	0.5638	16.8224
UAN2	21	0.0099	0.7386	0.6124	14.2139
UAN3	21	0.0092	0.4894	0.5072	12.9326
UNI1	21	0.2135	0.5613	0.3852	6.9074
UNI2	21	0.4372	0.7632	0.4751	4.4057
UNI3	21	0.3023	0.7040	0.4501	4.2101

### Appendix III:

#### Moisture Dependence Experiment

WHC	Sample	Day	Urea-N	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>3</sub> -N Volatilized
			mg g dw <sup>-1</sup>			mg
42%	UAN1	0	0.4500	0.2335	0.4472	
42%	UAN2	0	0.9790	0.4862	0.7000	
42%	UAN3	0	0.8489	0.3581	0.6095	
64%	UAN1	0	0.7746	0.3918	0.5933	
64%	UAN2	0	0.9467	0.4385	0.6146	
64%	UAN3	0	0.8545	0.3783	0.5580	
86%	UAN1	0	0.9917	0.3718	0.7032	
86%	UAN2	0	0.6853	0.3576	0.6041	
86%	UAN3	0	0.6149	0.2825	0.5765	
42%	UI1	0	0.4977	0.1920	0.4499	
42%	UI2	0	0.8221	0.2806	0.5795	
42%	UI3	0	0.8094	0.2807	0.4692	
64%	UI1	0	0.7351	0.2490	0.5792	
64%	UI2	0	1.0629	0.3839	0.6740	
64%	UI3	0	0.5767	0.2310	0.4862	
86%	UI1	0	0.5550	0.1977	0.4958	
86%	UI2	0	0.5973	0.2390	0.5466	
86%	UI3	0	0.7916	0.3297	0.6499	
42%	UNI1	0	0.8909	0.3430	0.5049	
42%	UNI2	0	0.5889	0.1861	0.4357	
42%	UNI3	0	0.7377	0.2454	0.4868	
64%	UNI1	0	0.8784	0.3038	0.5679	
64%	UNI2	0	0.9839	0.3179	0.5506	
64%	UNI3	0	0.6138	0.2263	0.4611	
86%	UNI1	0	0.9663	0.3216	0.5569	
86%	UNI2	0	0.7024	0.2291	0.5236	
86%	UNI3	0	0.5894	0.2263	0.4719	
42%	UAN1	1	0.2323	0.4216	0.2525	2.2453
42%	UAN2	1	0.4321	0.5024	0.3151	0.7077
42%	UAN3	1	0.4758	0.5016	0.1320	1.6386
64%	UAN1	1	0.3220	0.5155	0.2693	2.2361
64%	UAN2	1	0.3995	0.5265	0.2874	0.9121

64%	UAN3	1	0.5583	0.5491	0.3097	2.0777
86%	UAN1	1	0.3763	0.3802	0.2136	2.1818
86%	UAN2	1	0.4270	0.4399	0.1821	1.7370
86%	UAN3	1	0.4720	0.4415	0.2318	1.0695
42%	UI1	1	0.4842	0.2245	0.1850	1.2758
42%	UI2	1	0.8124	0.3133	0.3754	0.7712
42%	UI3	1	0.9023	0.3816	0.3726	1.2453
64%	UI1	1	0.4980	0.2317	0.1772	0.5806
64%	UI2	1	1.4117	0.6129	0.5798	1.0750
64%	UI3	1	0.6117	0.2265	0.2663	1.0631
86%	UI1	1	0.5547	0.2497	0.1746	1.3797
86%	UI2	1	0.7426	0.3198	0.1788	1.3310
86%	UI3	1	0.8899	0.4506	0.3177	1.7785
42%	UNI1	1	0.4454	0.1773	0.1307	1.1165
42%	UNI2	1	0.7185	0.3555	0.2790	1.6836
42%	UNI3	1	0.5719	0.2623	0.2068	1.2518
64%	UNI1	1	0.3885	0.1996	0.1166	0.4397
64%	UNI2	1	0.6500	0.3367	0.2212	0.9710
64%	UNI3	1	0.5173	0.2340	0.1755	1.1165
86%	UNI1	1	0.6397	0.3171	0.1869	1.6173
86%	UNI2	1	0.7321	0.3671	0.1975	2.3521
86%	UNI3	1	0.6289	0.3284	0.1889	2.1560
42%	UAN1	3	-0.0403	0.5309	0.2653	8.4562
42%	UAN2	3	0.0563	0.6985	0.3801	7.9552
42%	UAN3	3	0.0105	0.6310	0.2545	4.8469
64%	UAN1	3	-0.0070	0.7073	0.3451	6.9498
64%	UAN2	3	-0.0396	0.5750	0.3211	6.2399
64%	UAN3	3	0.0275	0.7769	0.4127	8.5030
86%	UAN1	3	0.0533	0.6018	0.0183	8.8612
86%	UAN2	3	0.1297	0.8201	0.2353	8.6089
86%	UAN3	3	0.1003	0.7003	0.1733	9.2470
42%	UI1	3	0.4371	0.2689	0.2271	3.5505
42%	UI2	3	0.6137	0.2971	0.3412	3.0597
42%	UI3	3	0.8363	0.3606	0.3297	4.1802
64%	UI1	3	0.5838	0.2465	0.2488	2.5654
64%	UI2	3	0.7987	0.4076	0.3639	3.6057
64%	UI3	3	0.5098	0.2566	0.2719	4.0919
86%	UI1	3	0.6163	0.3945	0.0512	4.2778
86%	UI2	3	0.6718	0.3432	0.2413	5.5430
86%	UI3	3	0.7265	0.4359	0.1372	6.4599

42%	UNI1	3	0.3699	0.2832	0.1449	4.3478
42%	UNI2	3	0.6331	0.3588	0.2661	4.1158
42%	UNI3	3	0.6526	0.3589	0.3020	4.5651
64%	UNI1	3	0.4538	0.2674	0.1398	2.2256
64%	UNI2	3	0.5496	0.3077	0.2004	2.9907
64%	UNI3	3	0.5294	0.3345	0.2047	3.7751
86%	UNI1	3	0.6869	0.3942	0.1979	4.2069
86%	UNI2	3	0.6157	0.3823	0.1960	6.2483
86%	UNI3	3	0.5143	0.3277	0.2010	6.8430
42%	UAN1	6	-0.0026	0.3632	0.4449	8.6301
42%	UAN2	6	-0.0035	0.6128	0.5409	13.1357
42%	UAN3	6	-0.0022	0.6256	0.3243	8.5450
64%	UAN1	6	-0.0029	0.6272	0.5873	10.7714
64%	UAN2	6	-0.0023	0.6639	0.5342	9.7879
64%	UAN3	6	-0.0038	0.7290	0.5303	11.9655
86%	UAN1	6	-0.0004	0.5529	0.2343	12.8743
86%	UAN2	6	0.0029	0.7320	0.2718	12.5704
86%	UAN3	6	-0.0004	0.8028	0.1625	14.0399
42%	UI1	6	0.2459	0.2361	0.2832	4.8429
42%	UI2	6	0.5851	0.3247	0.3069	4.7755
42%	UI3	6	0.7723	0.3583	0.3464	6.3389
64%	UI1	6	0.4739	0.2347	0.3363	4.0031
64%	UI2	6	0.7569	0.3355	0.3713	3.9312
64%	UI3	6	0.6002	0.3038	0.3129	5.6853
86%	UI1	6	0.4844	0.3784	0.2517	6.7800
86%	UI2	6	0.6560	0.4289	0.2679	8.5487
86%	UI3	6	0.5358	0.4277	0.1965	8.4668
42%	UNI1	6	0.2770	0.3258	0.1805	6.5819
42%	UNI2	6	0.4300	0.2748	0.2158	6.6252
42%	UNI3	6	0.7110	0.4154	0.3228	6.9476
64%	UNI1	6	0.5251	0.3723	0.2350	3.2122
64%	UNI2	6	0.5688	0.3678	0.0524	4.3557
64%	UNI3	6	0.5591	0.3786	0.2673	5.6254
86%	UNI1	6	0.4619	0.4613	0.1900	6.6732
86%	UNI2	6	0.5038	0.4452	0.2352	8.5893
86%	UNI3	6	0.4765	0.4143	0.2342	9.8985
42%	UAN1	14	-0.0018	0.0520	1.1863	10.6590
42%	UAN2	14	0.0006	0.4157	1.2811	16.2603
42%	UAN3	14	-0.0023	0.4109	1.3044	13.2745
64%	UAN1	14	0.0005	0.3182	1.9084	14.9529

64%	UAN2	14	-0.0008	0.3441	1.8385	13.2522
64%	UAN3	14	0.0005	0.4713	1.4684	15.7365
86%	UAN1	14	-0.0009	0.5489	0.3253	17.7022
86%	UAN2	14	-0.0023	0.6257	0.4003	18.4351
86%	UAN3	14	-0.0017	0.5108	0.3900	18.7859
42%	UI1	14	0.1508	0.0620	0.5715	6.1370
42%	UI2	14	0.0861	0.0241	0.6034	8.3955
42%	UI3	14	0.5731	0.2917	0.5113	9.6190
64%	UI1	14	0.4913	0.1698	0.5578	6.3478
64%	UI2	14	0.5244	0.1584	0.6262	6.8900
64%	UI3	14	0.4837	0.1707	0.5013	7.5881
86%	UI1	14	0.1924	0.4134	0.2501	10.8609
86%	UI2	14	0.3303	0.4945	0.2800	13.3435
86%	UI3	14	0.2521	0.6144	0.2191	11.9062
42%	UNI1	14	0.1866	0.2842	0.2155	10.1061
42%	UNI2	14	0.6049	0.3412	0.3458	10.4366
42%	UNI3	14	0.6162	0.4843	0.3394	11.7349
64%	UNI1	14	0.4359	0.4553	0.3197	7.0558
64%	UNI2	14	0.5972	0.4737	0.3923	8.7748
64%	UNI3	14	0.6003	0.4954	0.3719	9.6976
86%	UNI1	14	0.2429	0.5113	0.2558	12.7837
86%	UNI2	14	0.2725	0.5214	0.3043	16.1618
86%	UNI3	14	0.2068	0.5443	0.3022	17.1636
42%	UAN1	20	-0.0022	0.0030	0.8885	10.6807
42%	UAN2	20	-0.0010	0.2665	0.9456	18.6243
42%	UAN3	20	-0.0017	0.3425	0.9988	14.1845
64%	UAN1	20	0.0048	0.1440	2.3441	16.5785
64%	UAN2	20	0.0054	0.2146	2.3458	14.2683
64%	UAN3	20	0.0495	0.2156	1.9843	17.0260
86%	UAN1	20	0.0009	0.4781	0.3276	20.9630
86%	UAN2	20	0.0031	0.6269	0.1830	21.4906
86%	UAN3	20	0.0043	0.5424	0.4240	22.1167
42%	UI1	20	-0.0254	0.0000	0.8569	6.3586
42%	UI2	20	0.3025	0.1485	0.8940	9.5810
42%	UI3	20	0.4871	0.2494	0.6740	11.6121
64%	UI1	20	0.3237	0.0086	1.1684	6.5951
64%	UI2	20	0.3204	0.0652	1.0052	7.6308
64%	UI3	20	0.3263	0.0037	0.9806	8.1116
86%	UI1	20	-0.0022	0.5454	0.3826	14.3178
86%	UI2	20	0.1431	0.5427	0.4220	17.2948

86%	UI3	20	0.0984	0.5554	0.3225	15.5859
42%	UNI1	20	0.0201	0.3508	0.2185	13.8043
42%	UNI2	20	0.3172	0.4726	0.3281	16.4256
42%	UNI3	20	0.3699	0.4341	0.3074	15.2508
64%	UNI1	20	0.2319	0.4523	0.3990	10.7098
64%	UNI2	20	0.5927	0.4928	0.4055	13.3034
64%	UNI3	20	0.7124	0.4886	0.2272	13.5892
86%	UNI1	20	-0.0235	0.6668	0.2063	17.9099
86%	UNI2	20	0.0283	0.6529	0.1840	22.1074
86%	UNI3	20	0.0053	0.6219	0.0677	22.6737



### Appendix IV:

Simulated Rainfall Experiment – mass of Urea-N,  $\text{NH}_4^+$ -N, and  $\text{NO}_3^-$ -N leached

Sample	Week	Urea-N	$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N
		mg		
S1	1	0.0008	0.0084	0.0025
S2	1	0.0018	0.0171	0.0011
S3	1	0.0017	0.0223	0.0052
UAN1	1	0.0027	0.0200	0.1182
UAN2	1	0.0032	0.0357	0.1225
UAN3	1	0.0040	0.0997	0.1992
UI1	1	0.0621	3.2706	1.4995
UI2	1	0.0427	0.0176	0.1007
UNI1	1	0.0208	0.0172	0.0732
UNI2	1	0.0037	0.0229	0.0342
UNI3	1	0.0069	0.0151	0.0129
S1	2	0.0014	0.0024	0.0050
S2	2	0.0017	0.0079	0.0003
S3	2	0.0022	0.0072	0.0101
UAN1	2	0.0013	0.0512	0.6109
UAN2	2	0.0005	0.1140	0.6001
UAN3	2	0.0006	0.1362	0.5671
UI1	2	0.0047	3.1746	1.6549
UI2	2	0.0000	0.0676	0.6074
UNI1	2	0.0004	0.1646	0.4665
UNI2	2	0.0011	0.0268	0.6015
UNI3	2	0.0004	0.0064	0.1607
S1	3	0.0008	0.0069	0.0063
S2	3	0.0012	0.0033	0.0009
S3	3	0.0006	0.0040	0.0140
UAN1	3	0.0001	0.2148	1.0578
UAN2	3	0.0000	0.5401	1.0043
UAN3	3	0.0000	0.3568	0.9469
UI1	3	0.0019	3.3129	1.0864
UI2	3	0.0000	0.4066	1.2076
UNI1	3	0.0002	0.8379	0.9316
UNI2	3	0.0003	0.2357	1.1292
UNI3	3	0.0024	0.0169	0.7220

S1	4	0.0013	0.0031	0.0082
S2	4	0.0018	0.0054	0.0013
S3	4	0.0056	0.0064	0.0232
UAN1	4	0.0005	0.7089	1.2033
UAN2	4	0.0000	1.2641	1.4236
UAN3	4	0.0004	1.3332	1.4832
UI1	4	0.0011	0.3833	1.5945
UI2	4	0.0000	15.7631	1.8533
UNI1	4	0.0013	1.5817	0.9840
UNI2	4	0.0000	1.0524	1.4421
UNI3	4	0.0000	0.1867	0.8706
S1	5	0.0027	0.0114	0.0510
S2	5	0.0044	0.0122	0.0115
S3	5	0.0012	0.0103	0.0573
UAN1	5	0.0000	2.7851	2.5575
UAN2	5	0.0000	2.7845	2.9556
UAN3	5	0.0000	4.1908	3.3823
UI1	5	0.0016	3.5384	2.1669
UI2	5	0.0000	3.8480	3.6515
UNI1	5	0.0018	2.9287	1.1895
UNI2	5	0.0013	2.2508	1.2048
UNI3	5	0.0002	1.5576	1.6464

### Appendix V:

Microbial Activity Experiment: CO<sub>2</sub> production, denitrifying enzyme activity, and short term nitrification rates

Sample	Day	CO <sub>2</sub> -C	N <sub>2</sub> O-N	NO <sub>2</sub> -N
		ug	ng	
		g dw hr <sup>-1</sup>		
S1	0	2.3126	1.6005	174.6470
S2	0	2.3582	2.9369	157.3660
S3	0	2.5671	4.1023	161.9697
C1	0	1.6234	1.0729	155.3262
C2	0	1.6574	2.2167	168.4583
C3	0	1.8591	0.5577	160.2833
UAN1	0	5.9445	5.0448	257.2481
UAN2	0	5.8807	1.5772	286.1055
UAN3	0	6.7261	6.5738	269.8519
UI1	0	5.4156	9.2210	233.6718
UI2	0	5.2196	7.4205	238.9100
UI3	0	4.7592	5.0392	212.6671
UNI1	0	4.7187	3.7907	102.2965
UNI2	0	4.9044	26.2913	102.0282
UNI3	0	4.7661	4.0310	70.7870
S1	1	1.3883		
S2	1	1.5774		
S3	1	1.4905		
C1	1	1.3928		
C2	1	1.4418		
C3	1	1.4192		
UAN1	1	5.5058		
UAN2	1	5.7011		
UAN3	1	5.8475		
UI1	1	3.9574		
UI2	1	4.0235		
UI3	1	3.7561		
UNI1	1	2.9894		
UNI2	1	3.1049		
UNI3	1	3.0503		

S1	3	1.0995		
S2	3	1.1294		
S3	3	1.0960		
C1	3	1.1780		
C2	3	1.0751		
C3	3	1.2046		
UAN1	3	4.7743		
UAN2	3	4.8928		
UAN3	3	4.8527		
UI1	3	3.3441		
UI2	3	3.3966		
UI3	3	3.3787		
UNI1	3	2.4157		
UNI2	3	2.8077		
UNI3	3	2.9082		
S1	5	0.5434	5.9506	216.1641
S2	5	0.6594	1.8584	217.2899
S3	5	0.6863	2.9220	214.6794
C1	5	0.7743	1.9764	177.7733
C2	5	0.8409	1.6248	151.3140
C3	5	0.8085	2.5898	159.5064
UAN1	5	3.1107	37.8075	454.1125
UAN2	5	3.1217	12.1788	463.9477
UAN3	5	3.5148	7.8606	434.8904
UI1	5	2.2896	5.2907	284.7972
UI2	5	2.3164	8.1989	303.8930
UI3	5	2.4121	13.5471	279.0044
UNI1	5	1.9787	1.8815	63.7045
UNI2	5	2.1685	13.8842	83.4848
UNI3	5	2.1633	117.3073	101.3190
S1	7	0.6488		
S2	7	0.8419		
S3	7	0.7362		
C1	7	0.8257		
C2	7	1.1165		
C3	7	1.0498		
UAN1	7	2.9419		
UAN2	7	2.9289		
UAN3	7	3.1627		
UI1	7	2.6140		

UI2	7	2.8140		
UI3	7	2.7952		
UNI1	7	1.8588		
UNI2	7	2.3882		
UNI3	7	2.1947		
S1	10	0.5984	14.0439	226.2241
S2	10	0.7452	5.2346	201.0327
S3	10	0.8129	10.1684	149.6887
C1	10	1.0702	5.6736	208.9635
C2	10	1.0829	8.4417	180.4449
C3	10	1.1071	6.3933	173.5221
UAN1	10	0.4920	162.5983	567.4796
UAN2	10	0.6715	81.4538	522.4812
UAN3	10	0.9506	119.3807	528.8504
UI1	10	2.3572	165.6713	560.6481
UI2	10	2.5952	9.9341	451.7740
UI3	10	2.5681	36.1611	550.4691
UNI1	10	2.0202	103.1169	85.7573
UNI2	10	2.3711	45.7686	74.1492
UNI3	10	2.5022	78.3342	60.7514
S1	14	0.4053	2.2670	169.5048
S2	14	0.3606	0.4272	211.9589
S3	14	0.4506	1.9139	172.3822
C1	14	1.0009	0.9151	185.2188
C2	14	0.5567	0.3603	149.7355
C3	14	0.5587	0.4474	150.0218
UAN1	14	0.4753	13.1957	512.5598
UAN2	14	0.3803	10.2662	380.3388
UAN3	14	0.5415	21.4256	427.7384
UI1	14	1.8274	5.4195	503.1249
UI2	14	1.7018	3.8834	391.7127
UI3	14	1.9860	3.4652	376.8626
UNI1	14	1.1695	3.3386	43.5852
UNI2	14	1.4240	2.4150	41.7300
UNI3	14	1.6248	1.9276	32.3506
S2	21		432.8723	
S3	21		264.2799	
C1	21		540.2299	
C2	21		53.1076	
C3	21		320.1246	

UAN1	21	5930.1325
UAN2	21	5941.3191
UAN3	21	3818.6949
UI1	21	803.5925
UI2	21	1536.4995
UI3	21	2796.7726
UNI1	21	1458.7662
UNI2	21	313.2072
UNI3	21	994.0608

## Appendix VI:

### Microbial Activity Experiment: Soil nutrient concentrations

Sample	Day	Urea-N	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N
		mg g dw <sup>-1</sup>		
S1	0	0.0000	0.0019	0.0000
S2	0	0.0000	0.0011	0.0000
S3	0	0.0000	0.0015	0.0000
C1	0	0.0000	0.0025	0.0000
C2	0	0.0000	0.0025	0.0000
C3	0	0.0000	0.0018	0.0000
UAN1	0	2.2358	1.1232	0.0011
UAN2	0	2.0252	0.8573	0.0009
UAN3	0	2.4426	1.2899	0.0013
UI1	0	1.8266	0.8518	0.0009
UI2	0	2.2095	1.1561	0.0012
UI3	0	1.5653	0.7450	0.0007
UNI1	0	1.5576	0.7363	0.0007
UNI2	0	2.2227	0.4917	0.0005
UNI3	0	1.8446	0.9267	0.0009
S1	5	0.0000	0.0000	0.0000
C1	5	0.0000	0.0000	0.0000
C2	5	0.0000	0.0000	0.0000
C3	5	0.0000	0.0000	0.0000
UAN1	5	0.7307	2.1339	0.0021
UAN2	5	0.9019	2.4095	0.0024
UAN3	5	1.0900	2.4044	0.0024
UI1	5	2.0528	1.0806	0.0011
UI2	5	2.4917	1.2792	0.0013
UI3	5	1.9757	1.1149	0.0011
UNI1	5	1.6133	0.9826	0.0010
UNI2	5	1.8777	1.2320	0.0012
UNI3	5	2.0505	1.2115	0.0012
S1	10	0.0000	0.0000	0.0000
C1	10	0.0000	0.0000	0.0000
C2	10	0.0000	0.0000	0.0000
C3	10	0.0000	0.0000	0.0000
UAN1	10	0.0000	3.1424	0.0031

UAN2	10	0.0088	3.4545	0.0035
UAN3	10	0.0000	3.2457	0.0032
UI1	10	1.7563	1.5818	0.0016
UI2	10	1.7070	1.6328	0.0016
UI3	10	2.1197	1.6394	0.0016
UNI1	10	1.5339	1.4284	0.0014
UNI2	10	1.7614	1.6918	0.0017
UNI3	10	1.7617	1.5882	0.0016
S1	14	0.0000	0.0000	0.0000
C1	14	0.0000	0.0000	0.0000
C2	14	0.0000	0.0002	0.0000
C3	14	0.0000	0.0000	0.0000
UAN1	14	0.0000	2.8710	0.0029
UAN2	14	0.0000	3.2227	0.0032
UAN3	14	0.0000	2.9723	0.0030
UI1	14	1.0610	1.9552	0.0020
UI2	14	1.2757	2.1476	0.0021
UI3	14	1.1882	2.0825	0.0021
UNI1	14	0.8625	1.4645	0.0015
UNI2	14	1.0006	1.5725	0.0016
UNI3	14	1.2077	1.5270	0.0015



## REFERENCES

- Abbasi, M.K. and W.A. Adams. 2000. Gaseous N emissions during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil Biol. Biochem.* 32:1251-1259.
- Agehara, S. and D.D. Warncke. 2005. Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. *Soil Sci. Soc. Am. J.* 69:1844-1855.
- Al-Kanani, T., A.F. MacKenzie, and N.N. Barthakur. 1991. Soil water and ammonia volatilization relationships with surface-applied nitrogen fertilizer solutions. *Soil Sci. Soc. Am. J.* 55:1761-1766.
- Alva, A.K. 1992. Differential leaching of nutrients from soluble vs. controlled-release fertilizers. *Environ. Manage.* 16:769-776.
- Amberger, A. 1989. Research on dicyandiamide as a nitrification inhibitor and future outlook. *Comm. Soil Sci. Plant Anal.* 20:1933-1955.
- Berg, P. and T. Roswall. 1985. Ammonium oxidizer numbers, potential and actual oxidation rates in two Swedish arable soils. *Biol. Fertil. Soils* 1:131-140.
- Berg, P. and T. Roswall. 1989. 1989. Abiotic factors regulating nitrification in a Swedish arable soil. *Biol. Fertil. Soils* 8:247-254.
- Bergstrom, D.W. and E.G. Beauchamp. 1993. Relationships between denitrification rate and determinant soil properties under barley. *Can. J. Soil Sci.* 73:567-578.
- Borken, W., E.A. Davidson, K. Savage, J. Gaudinski and S.E. Trumbore. 2003. Drying and wetting effects on carbon dioxide release from organic horizons. *Soil Sci. Soc. Am. J.* 67:1888-1896.
- Bouwman, A.F. 1997. Long-term scenarios of livestock-crop-land use interactions in developing countries. *Land and Water Bulletin 5*. Food and Agriculture Organization of the United Nations, Rome, Italy.  
<http://www.fao.org/docrep/W5146E/W5146E00.htm>
- Bouwman, A.F., L.J. Boumans, and N.H. Batjes. 2002. Estimation of global NH<sub>3</sub> volatilization loss from synthetic fertilizers and animal manure applied to arable lands and grasslands. *Global Biogeochem. Cycles* 16: 8-1 – 8-14.
- Bowman, R.A. and D.D. Focht. 1974. The influence of glucose and nitrate concentration upon denitrification rates in sandy soil. *Soil Biol. Biochem.* 6:297-301.
- Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. 13<sup>th</sup> Ed. Prentice Hall.

Upper Saddle River, New Jersey.

- Bramley, R.G.V. and R.E. White. 1989. The effect of pH, liming, moisture and temperature on the activity of nitrifiers in a soil under pasture. *Aust. J. Soil Res.* 27:711-724.
- Bremner, J.M. 1982. Nitrogen - Urea. p. 702-705. In: A.L. Page (ed.) *Methods of Soil Analysis Part 2: Chemical and Microbiological Properties*, 2<sup>nd</sup> edition, Madison, WI.
- Bremner, J.M. 1995. Recent research on problems in the use of urea as a nitrogen fertilizer. *Fertilizer Research* 42:321-329.
- Bronson, K.F., J.T. Touchton, and R.D. Hauk. 1989a. Decomposition rate of dicyandiamide and nitrification inhibition. *Comm. Soil Sci. Plant Anal.* 20:2067-2078.
- Bronson, K.F., J.T. Touchton, A.E. Hiltbold, and L.L. Hendrickson. 1989b. Control of ammonia volatilization with N-(n-BUTYL) thiophosphoric triamide in loamy sands. *Comm. Soil Sci. Plant Anal.* 20:1439-1451.
- Bundy, L.G., and J.J. Meisinger. 1994. Nitrogen Availability Indices. p. 967-979. In: A. Klute (ed.) *Methods of Soil Analysis Part 2: Chemical and Microbiological Properties*, 2<sup>nd</sup> edition. Madison, WI, p. 967-979.
- Burns, L.C., R.J. Stevens, and R.J. Laughlin. 1996. Production of nitrite in soil by simultaneous nitrification and denitrification. *Soil Biol. Biochem.* 28:609-616.
- Carmona, G., C.B. Christianson, and B.H. Byrnes. 1990. Temperature and low concentration effects of the urease inhibitor N-(n-BUTYL) thiophosphoric triamide (nBTPT) on ammonia volatilization from urea. *Soil Biol. Biochem.* 22:933-937.
- Cassman, K.G. 1999. Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. *Proc. Natl. Acad. Sci. U S A* 96:5952-5959.
- Cassman, K.G., A. Dobermann, and D.T. Walters. 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio* 31:132-140.
- Chakrabarti, K., N. Sinha, A. Chakraborty, and P. Bhattacharyya. 2004. Influence of soil properties on urease activity under different agro-ecosystems. *Arch. Agron. Soil Sci.* 50:477-483.
- Clay, D.E., G.L. Malzer, and J.L. Anderson. 1990. Ammonia volatilization from urea as influenced by soil temperature, soil water content, and nitrification and hydrolysis inhibitors. *Soil Sci. Soc. Am. J.* 54:263-266.
- Creason, G.L., M.R. Schmitt, E.A. Douglass, and L.L. Hendrickson. 1990. Urease

- inhibitory activity associated with N-(n-BUTYL) thiophosphoric triamide is due to formation of its oxon analog. *Soil Biol. Biochem.* 22:209-211.
- Daberkow, S.G. and W.D. McBride. 2003. Farm and operator characteristics affecting the awareness and adoption of precision agriculture technologies in the US. *Precision Agric.* 4:163-177.
- Davidson, E.A. and J.P. Schimel. 1995. Microbial processes of production and consumption of nitric oxide, nitrous oxide and methane. p. 327-357. In: P.A. Matson and R.C. Harriss (ed.), *Biogenic trace gases: measuring emissions from soil and water.* Blackwell Science, Oxford
- D'Haene, K., E. Moreels, S. DeNeve, B.C. Daguilar, P. Boeckx, G. Hofman and O. Van Cleemput. 2003. Soil properties influencing the denitrification potential of Flemish agricultural soils. *Biol. Fertil. Soils* 38:358-366.
- Dobermann, A., and K.G. Cassman. 2002. Plant nutrient management for enhanced productivity in intensive grain production systems of the United States and Asia. *Plant and soil* 247:153-175.
- Fenn, L.B. and L.R. Hossner. 1985. Ammonia volatilization from ammonium or ammonium-forming nitrogen fertilizers. *Adv. Soil Sci.* 1:124-169.
- Ferm, M. 1998. Atmospheric ammonia and ammonium transport in Europe and critical loads: a review. *Nutr. Cycl. Agroecosys.* 51:5-17.
- Fierer, N. and J.P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Sci. Soc. Am. J.* 67:798-805.
- Firestone, M.K. and E.A. Davidson. 1989. Microbial basis of NO and N<sub>2</sub>O production and consumption in soil. p. 7-21. In: Andreae, M.O. and D.S. Schimel (ed.) *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere.* John Wiley and Sons, NY.
- Fixen, P.E., and F.B. West. 2002. Nitrogen fertilizers: Meeting contemporary challenges. *Ambio* 31:169-176.
- Fletcher, D.A. 1991. A national perspective. In: Follett, R.F. et al. (ed.) *Managing nitrogen for groundwater quality and farm profitability.* p. 9-17. ASA, CSSA, and SSSA, Madison, WI.
- Follett, R.F., and J.A. Delgado. 2002. Nitrogen fate and transport in agricultural systems. *J. Soil Water Conserv.* 57:402(7)
- Franzluebbers, A., R. Haney, C. Honeycutt, H. Schomberg and F. Hons. 2000. Flush of

- carbon dioxide following rewetting of dried soil relates to active organic pools. *Soil Sci. Soc. Am. J.* 64:613-623.
- Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Crosby. 2003. The nitrogen cascade. *BioScience* 53:341-356.
- Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, and C.J. Vorosmarty. 2004. Nitrogen cycles: past, present, and future. *Biogeochem.* 70:153-226.
- Gilbert, P.M., J.Harrison, C. Heil, and S. Seitzinger. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. 77:441-463.
- Gill, J.S., Bijay-Singh, C.S. Khind, and Yadvinder-Singh. 1999. Efficinecy of N-(n-butyl) thiophosphoric triamide in retarding hydrolysis of urea and ammonia volatilization losses in a flooded sandy loam soil amended with organic materials. *Nutr. Cycl. Agroecosys.* 53:203-207.
- Gilmour, J.T. 1984. The effects of soil properties on nitrification and nitrification inhibition. *Soil Sci. Soc. Am. J.* 48:1262-1266.
- Gioacchini, P., C. Giovannini, C. Marzadori, L. Vittori Antisari, A. Simoni, and C. Gessa. 2000. Effect of N-(n-butyl) thiophosphoric triamide added to peat and leather in urea-based fertilizers on urea hydrolysis and ammonia volatilization. *Comm. Soil Sci. Plant Anal.* 31:3177-3191.
- Gioacchini, P., A. Nastri, C. Marzadori, C. Giovannini, L. Vittori Antisari, and C. Gessa. 2002. Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. *Biol. Fertil. Soils* 36:129-135.
- Glass, A.D. 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorbtion. *Crit. Rev. Plant Sci.* 22:453-470.
- Goulding, K. 2004. Pathways and losses of fertilizer nitrogen at different scales. p. 209-211. In: Mosier, A.R., J.K. Syers, and J.R. Freney (ed.) *Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Prodcution and the Environment.* Island, Washington D.C.
- Grant, R., K. Nielsen, and J. Waagepetersen. 2006. Reducing nitrogen loading of inland and marine waters-evaluation of Danish policy measures to reduce nitrogen loss form farmland. *Ambio* 35:117-123.
- Grasshoff, K., K. Kremling, and M. Ehrhardt. 1999. Determination of Nutrients. p. 177-187. In: *Methods of Seawater Analysis*, 3<sup>rd</sup> edition. New York, NY

- Groffman, P.M. 1994. Denitrification in freshwater wetlands. *Curr. Topics Wetland Biogeochem.* 1:15-35.
- Groffman, P.M. and J.M. Tiedje. 1991. Relationships between denitrification, CO<sub>2</sub> production and air-filled porosity in soils of different texture and drainage. *Soil Biol. Biochem.* 23:299-302.
- Guette, R., W. Dott, and A. Eisentraeger. 2002. Determination of urease activity in soils by carbon dioxide release for ecotoxicological evaluation of contaminated soils. *Ecotoxicology* 11:357-364.
- Hagopian, D.S. and J.G. Riley. 1998. A closer look at the bacteriology of nitrification. *Aquacultural Eng.* 18:223-244.
- Hargrove, W.L. 1988. Evaluation of ammonia volatilization in the field. *J. Prod. Agric.* 1: 104-111.
- Hendrickson, L.L., and E.A. Douglass. 1993. Metabolism of the urease inhibitor N-(n-BUTYL) thiophosphoric triamide (NBPT) in soils. *Soil Biol. and Biochem.* 25:1613-1618.
- Hongprayoon, C., C.W. Lindau, W.H. Patrick, Jr., D.R. Bouldin, and K.R. Reddy. 1991. Urea transformations in flooded soil columns: I. Experimental Results. *Soil Sci. Soc. Am. J.* 55:1130-1134.
- Hopkins, D.W., A.G. O'Donnell and R.S. Shiel. 1988. The effect of fertilization on soil nitrifier activity in experimental grassland plots. *Biol. Fertil. Soils* 5:344-349.
- International Fertilizer Industry. 2006. Total fertilizer consumption statistics by region from 1970/71 to 2005/06. Retrieved April 16, 2007, from <http://www.fertilizer.org/ifa/statistics.asp>
- Keeney, D.R. and R.F. Follett. 1991. Overview and introduction. In: *Managing nitrogen for groundwater quality and farm profitability.* p. 9-17 Follett, R.F. et al. (ed.). ASA, CSSA, and SSSA, Madison, WI.
- Kieft, T., E. Soroker and M. Firestone. 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biol. Biochem.* 19:119-126.
- Kirchmann, H., A.E. Johnny Johnston, and L.R. Bergstrom. 2002. Possibilities for reducing nitrate leaching from agricultural land. *Ambio* 31:404-408.
- Kissel, D.E., M.L. Cabrera, N. Vaio, J.R. Craig, J.A. Rema, and L.A. Morris. 2004. Rainfall timing and ammonia loss from urea in a Loblolly Pine plantation. *Soil Sci. Soc. Am. J.* 68:1744-1750.

- Klute et al. Methods of soil analysis: Part I physical and mineralogical methods, 2<sup>nd</sup> edition, Klute A. ed. Madison, WI 1982,
- Krogmeier, M.J., G.W. McCarty, and J.M. Bremner. 1989. Potential phytotoxicity associated with the use of soil urease inhibitors. Proc. Natl. Acad. Sci. U S A 86:1110-1112.
- Kumar, V., and R.J. Wagenet. 1984. Urease activity and kinetics of urea transformation in soils. Soil Science 137:263-269.
- Lea, P.J., and R.A. Azevedo. 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from soil. Ann. of Appl. Biol. 149:243-247.
- Liu, X.J., A.R. Mosier, A.D. Halvorson, and F.S. Zhang. 2006. The impact of nitrogen placement and tillage on NO, N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes from clay loam soil. Plant and Soil 280:177-188.
- Linn, D.M. and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267-1272.
- Loro, P.J., D.W. Bergstrom and E.G. Beauchamp. 1997. Intensity and duration of denitrification following application of manure and fertilizer to soil. J. Environ. Qual. 26:706-713.
- Malhi, S.S. and W.B. McGill. 1982. Nitrification in three Alberta soils: effect of temperature, moisture and substrate concentration. Soil Biol. Biochem. 14: 393-399.
- Manunza, B., S. Deiana, M. Pintore, and C. Gessa. 1999. The binding mechanism of urea, hydroxamic acid and N-(N-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. Soil Biol. Biochem. 31:789-796.
- Marshall, V.G. and D.S. Debell. 1980. Comparison of four methods of measuring volatilization losses of nitrogen following urea fertilization of forest soils. Can. J. Soil Sci. 60:549-563.
- McCarty, G.W. and J.M. Bremner. 1989. Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. Comm. Soil Sci. Plant Anal. 20:2049-2065.
- Miller, A.J., and M.D. Cramer. 2004. Root nitrogen acquisition and assimilation. Plant and Soil 274:1-36.
- Miller, R.D. and D.D. Johnson. 1964. The effect of soil moisture tension on carbon dioxide evolution, nitrification and nitrogen mineralization. Soil Sci. Soc. Am. Proc. 28:644-647.

- Mosier, A.R. 2001. Exchange of gaseous nitrogen compound between agricultural systems and the atmosphere. *Plant and Soil* 228:17-27.
- Moraghan, J.T. and R. Buresh. 1977. Correction for dissolved nitrous oxide in nitrogen studies. *Soil Sci. Soc. Am. J.* 41:1201-1202.
- Mulvaney, R.L., and J.M. Bremner. 1981. Control of urea transformations in soils. In: *Soil Biochemistry Volume 5*. Paul, E.A. and J.N. Ladd (eds.) New York, NY, pp 153-196.
- Mulvaney, R.L., S.A. Kahn and C.S. Mulvaney. 1997. Nitrogen fertilizers promote denitrification. *Biol. Fertil. Soils* 24:211-220.
- Myers, M.G. and J.W. McGarity. 1968. The urease activity in profiles of five great soil groups from northern New South Wales. *Plant and Soil* 28:25-37.
- National Research Council. 1997. *Precision Agriculture in the 21<sup>st</sup> Century: Geospatial and Information Technologies in Crop Management*. National Academy Press, Washington D.C.
- Nielsen, T.H. and N.P. Revsbech. 1998. Nitrification, denitrification, and N-liberation associated with two types of organic hotspots in soil. *Soil Biol. Biochem.* 5:611-619.
- Nishio, T. and T. Fujimoto. 1990. Kinetics of nitrification of various amounts of ammonium added to soils. *Soil Biol. Biochem.* 22:51-55.
- Paramasivam, S. and A.K. Alva. 1997. Nitrogen recovery from controlled-release fertilizers under intermittent leaching and dry cycles. *Soil Science* 162:447-453.
- Parsons, L.L., R.E. Murray, and M.S. Smith. 1991. Soil denitrification dynamics: spatial and temporal variations of enzyme activity, populations, and nitrogen gas loss. *Soil Sci. Soc. Am. J.* 55:90-95.
- Parsons, T.R., Y. Maita, and C.M. Lalli. "Nutrients" in *A Manual of Chemical and Biological Methods for Seawater Analysis*. 1<sup>st</sup> edition, New York, NY 1984. p17-22.
- Pelletier, F. D. Prévost, G. Laliberté and E. van Bochove. 1999. Seasonal response of denitrifiers to temperature in a Quebec cropped soil. *Can. J. Soil Sci.* 79:551-556.
- Perucci, P., P.L. Giusquiani, and L. Scarponi. 1982. Nitrogen losses from added urea and urease activity of clay-loam soil amended with crop residues. *Plant and Soil* 69:457-463.
- Portela, S.I., A.E. Andriulo, M.C. Sasal, B. Mary, and E.G. Jobbagy. 2006. Fertilizer vs. organic matter contributions to nitrogen leaching in cropping systems of the Pampas:

- <sup>15</sup>N application in field lysimeters. *Plant Soil* 289:265-277.
- Prakasa Rao E.V.S. and K. Puttanna. 1987. Nitrification and ammonia volatilization losses from urea and dicyandiamide-treated urea in sandy loam soil. *Plant and Soil* 97:201-206.
- Prakash, O., A.K. Alva, and S. Paramasivam. 1999. Use of the urease inhibitor N-(n-BUTYL) thiophosphoric triamide decreased nitrogen leaching from urea in a fine sandy soil. *Water, Air, Soil Pollut.* 116:587-595.
- Prasad, R. 1998. Fertilizer urea, food security, health and the environment. *Current Science* 75:677-683.
- Riley, W.J., I. Oritz-Monasterio, and P.A. Matson. 2001. Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. *Nutr. Cycl. Agroecosys.* 61:223-236.
- Robert, P.C. 2002. Precision agriculture: a challenge for crop nutrition management. *Plant and Soil* 247:143-149.
- Robertson, G.P. 2000. Denitrification, pp. 181-190. In: Sumner, M.E. (ed.), *Handbook of Soil Science*. CRC Press, Boca Raton, FL.
- Robertson, G.P. and J.M. Tiedje. 1984. Denitrification and nitrous oxide production in successional and old-growth Michigan forests. *Soil Sci. Soc. Am. J.* 48:383-389.
- Ryden, J.C. 1983. Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. *J. Soil Sci.* 34:355-365.
- SAS. 2003. SAS/STAT user's guide, release 9.1.3. SAS Institute, Cary, North Carolina
- Schmidt, E.L., and L.W. Belser. "Nitrifying bacteria", in *Methods of Soil Analysis Part2: Chemical and Microbiological Properties*, 2<sup>nd</sup> edition, A. Klute, ed., Madison, WI 1994, p. 172-176.
- Schlesinger, W.H. 1997. *Biogeochemistry: An analysis of global change*, 2<sup>nd</sup> edition. Academic Press, San Diego, CA.
- Schwab, G.J. and L.W. Murdock. 2005. Nitrogen transformation inhibitors and controlled release urea. <http://www.ca.uky.edu/agc/pubs/agr/agr185/agr185.pdf>
- Sextone, A.J., N.P. Revsbech, T.P. Parkin and J.M. Tiedje. 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Sci. Soc. Am. J.* 49:645-651.
- Singh, J., S.Saggar, and N.S. Bolan. 2004. Mitigating gaseous losses of nitrogen from pasture



- soil with urease and nitrification inhibitors. SuperSoil 2004: 3<sup>rd</sup> Australian New Zealand Soils Conference.  
[http://www.regional.org.au/au/asssi/supersoil2004/s9/oral/1497\\_singhj.htm](http://www.regional.org.au/au/asssi/supersoil2004/s9/oral/1497_singhj.htm)
- Smil, V. 1999. Nitrogen in crop production: an account of global flows. *Global Biogeochem. Cycles* 13:647-662.
- Sotomayor, D. and C.W. Rice. 1996. Denitrification in soil profiles beneath grasslands and cultivated soils. *Soil Sci. Soc. Am. J.* 60:1822-1828.
- Stark, J.M. and M.K. Firestone. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl. Environ. Microbiol.* 61:218-221.
- Strong, D.T. and I.R. P. Fillery. 2002. Denitrification response to nitrate concentrations in sandy soils. *Soil. Biol. Biochem.* 34:945-954.
- Subbarao, G.V., O.Ito, K.L. Sahrawat, W.L. Berry, K. Nakahara, T. Ishikawa, T. Watanabe, K. Suenaga, M. Rondon, and I.M. Rao. 2006. Scope and strategies for regulation of nitrification in agricultural systems – challenges and opportunities. *Crit. Rev. Plant Sci.* 25: 303-335.
- Tiedje, J.M. 1994. Denitrifiers. p. 256-258. In: A. Klute (ed.) *Methods of Soil Analysis Part2: Chemical and Microbiological Properties*, 2<sup>nd</sup> edition. Madison, WI.
- Tilman, D., J. Farigone, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W.H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. *Science* 292:281-284.
- Tilman, D. 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proc. Natl. Acad. Sci. U S A* 96:5995-6000.
- Timmons, D.R. and A.S. Dylla. 1981. Nitrogen leaching as influenced by nitrogen management and supplemental irrigation level. *J. Environ. Qual.* 10:421-426.
- Tisdale, S.L. and W.L. Nelson. 1975. *Soil fertility and fertilizers*. 3<sup>rd</sup> Ed. Macmillan Publishing Co., Inc. New York, NY.
- Townsend, A.R., R.W. Howarth, F.A. Bazzaz, M.S. Booth, C.C. Cleveland, S.K. Collinge, A.P. Dobson, P.R. Epstein, E.A. Holland, D.R. Keeney, M.A. Mallin, C.A. Rogers, P. Wayne, and A.H. Wolfe. 2003. Human health effects of a changing global nitrogen cycle. *Front. Ecol. Environ.* 1:240-246.
- Udawatta, R.P., P.P. Motavalli, H.E. Garrett, and J.J. Krstansky. 2006. Nitrogen losses in runoff from three adjacent agricultural watersheds with claypan soils. *Agric., Ecosyst. Environ.* 117:39-48.

- Varel, V.H. 2002. Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review. *J. Animal Science* 80:E1-E7.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7:737-750.
- Vittori Antisari, L., C. Marzadori, P. Gioacchini, S. Ricci, and C. Gessa. 1996. Effects of the urease inhibitor N-(n-butyl) phosphorothioic triamide in low concentrations on ammonia volatilization and evolution of mineral nitrogen. *Biol. and Fertil. of Soils* 22:196-201.
- Vlek, P.L.G. and M.F. Carter. 1983. The effect of soil environment and fertilizer modifications on the rate of urea hydrolysis. *Soil Science* 136:56-63.
- Watson, C.J., H. Miller, P. Poland, D.J. Kilpatrick, M.D.B. Allen, M.K. Garrett, and C.B. Christianson. 1994. Soil properties and the ability of the urease inhibitor N-(n-BUTYL) thiophosphoric triamide (nBTPT) to reduce ammonia volatilization from surface applied urea. *Soil Biol. Biochem.* 26:1165-1171.
- Whalen, S.C. and J T. DeBerardinis. 2007. Nitrogen mass balance in fields irrigated with liquid swine waste. *Nutr. Cycl. Agroecosys.* 78:37-50.
- Wheatley, R.E., K. Ritz and B.S. Griffiths. 1997. Application of an augmented nitrification assay to elucidate the effects of a spring barley crop and manures on temporal variations in rates. *Biol. Fertil. Soils* 24:378-383.
- Zantua, M.I. and J.M. Bremner. 1976. Production and persistence of urease activity in soils. *Soil Biol. Biochem.* 8:369-374.
- Zantua, M.I., L.C. Dumenil, and J.M. Bremner. 1977. Relationships between soil urease activity and other soil properties. *Soil Sci. Soc. of Am. J.* 41:350-352.
- Zhang, W., Y Qi, and Z. Zhang. 2006. A long-term forecast analysis on worldwide land uses. *Environ. Monit. Assess.* 119:609-620.
- Zhengping, W., O. Van Cleemput, P. Demeyer, and L. Baert. 1991a. Effect of urease inhibitors on urea hydrolysis and ammonia volatilization. *Biol. Fertil. Soils* 11:43-47.
- Zhengping, W., O. Van Cleemput, L. Liantie, and L. Baert. 1991b. Effect of organic matter and urease inhibitors on urea hydrolysis and immobilization of urea nitrogen in an alkaline soil. *Biol. Fertil. Soils* 11:101-104.
- Zhengping, W., O. Van Cleemput, and L. Baert. 1996. Movement of urea and its hydrolysis products as influenced by moisture content and urease inhibitors. *Biol. Fertil. Soils* 22:101-108.