ABSTRACT

ALLEN, MARK BENJAMIN. Managing nitrogen from swine and poultry manure in North Carolina. (Under the direction of Robert L. Mikkelsen.)

With increasing pressure to regulate land application of animal manure, North Carolina faces a difficult dilemma, given the number of large-scale animal production facilities currently in operation. Poultry and swine industries in the state generate large volumes of animal manure that must be properly managed in order to avoid loss of N to ground water and surface water bodies. Using swine manure as an N source for soybean production is not commonly practiced due to soybean's ability to fix N, but recent research suggests that soybean may be a suitable receiver crop of anaerobic swine lagoon effluent. The objectives of this research were twofold: (1) determine the quantity of swine effluent-derived N taken up by soybean and estimate the degree of inhibition of symbiotic N-fixation and (2) determine how soil pH affects N mineralization, nitrification and immobilization when soil is amended with broiler litter. Swine effluent was spiked with $(^{15}NH_4)_2SO_4$ in order to attain a mean final ^{15}N enrichment of 5.765 atom % ^{15}N. The enriched effluent was applied 6 times at weekly intervals to nodulating and nonnodulating soybean growing in one-meter deep lysimeters at a rate of 185 kg PAN ha^{-1}. Additional lysimeters with nodulating and nonnodulating soybean received no applications of effluent. Leachate was collected on a weekly basis and analyzed for ^{15}N and total N. Soybean were harvested near maturity and analyzed for ^{15}N and total N. Biological N-fixation in soybean was not completely inhibited when swine effluent was added and accounted for 55% of the total N in the shoot. Nodulated and nonnodulated soybean shoots recovered similar

amounts of effluent N (36.6% and 33.4%, respectively). The addition of effluent and nodulation were both important sources of N for soybean growth, although the results suggest that nodulating and nonnodulating soybean behaved differently when they received effluent additions, as indicated by significant interactions. The experimental data showed that less than 1% of the added effluent N was accounted for in the leachate. An N budget of the plant-soil-water system showed that, of the effluent N added to nodulated soybean, 37% remained in the soil after the soybean were harvested, while 33% remained in the effluent-treated nonnodulated soybean. These results suggest that soybean can serve as an N receiver crop when swine effluent is the N source. To determine the effects of soil pH on N transformations in broiler litter amended soils, Wagram loamy sand with a pH of 4.4 was collected from a forested area near Clayton, NC, and sub-samples were limed to pH 4.8, 5.3, 5.8, 6.4, and 7.0. Broiler litter was added at a rate of 155 kg PAN ha^{-1} to the limed soils and incubated at 25^{o}C and 60% of field capacity for 112 d. Total inorganic N was measured at 0, 7, 14, 28, 56, 77, and 112 d. Cumulative net N mineralized was fitted to a first order model to determine potentially mineralizable N. Although nitrification rates increased as soil pH increased, there were significant inverse relationships between soil pH and net N mineralized, as well as soil pH and potentially mineralizable N. Isotope dilution measurements showed that gross and net mineralization rates were equivalent, refuting the notion that relatively more NH_4 immobilization had occurred in the high pH soils. The results indicate that N mineralization was enhanced at low soil pH, a phenomenon that presently is not fully understood.

MANAGING NITROGEN FROM SWINE AND POULTRY MANURE IN NORTH CAROLINA

by

MARK BENJAMIN ALLEN

A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

SOIL SCIENCE

Raleigh

2003

APPROVED BY:

Wei Shi	Michael G. Wagger
Philip W. Westerman	Robert L. Mikkelsen Chair of Advisory Committee

Personal Biography

Mark Benjamin Allen was born to Gary and Sheila Allen on March 15, 1977 in Twin Falls, Idaho. His parents moved into a small, three-bedroom house located in Filer, Idaho, where they lived for 22 years. Along with his three brothers and two sisters, he was raised on 700 acres of irrigated farmland, owned and operated by his family. It was here that Mark developed a love and interest of agriculture, although he vowed never to become a farmer himself. On the farm, he also developed a passion for off-road motorcycle riding and motocross racing, which continues with him today. In 1995, he graduated from Filer High School and moved to Provo, Utah to continue his academic studies at Brigham Young University. While at BYU, he met and fell in love with Jacqueline Toler, whom he married on August 13, 1999. After Mark changed majors several times, his older brother, Brett, convinced him to take some soil science classes. After taking the "Intro to Soil Science" class, Mark realized that a degree in agronomy would allow him to pursue both his interests in science and agriculture. Mark had many wonderful opportunities to work in the Department of Agronomy and Horticulture during his time at BYU. For two years, he worked as lab technician for Dr. Richard Terry, grinding hundreds of soil samples with a mortar and pestle. He also had the opportunity to be a lab instructor for the "Intro to Soil Science" class. In the summer of 2001, he completed his B.S. degree in Agronomy with an emphasis in Environmental Science. After graduation, Mark and his wife immediately set out for Raleigh, North Carolina so that he could begin studying for an M.S. degree in Soil Science.

ACKNOWLEDGEMENTS

I came to know many people in the NC State Soil Science Department who made my stay worthwhile and memorable. However, a few individuals made the completion of my thesis and graduate program possible. Dr. Mikkelsen's advice and assistance were invaluable throughout my research efforts and I have great respect for him as a scientist. Although for part of my stay, he was my advisor from afar, I could not have finished without his efforts. I owe a tremendous amount of thanks to Dr. Shi, who greatly stepped up her role as a member of my graduate student committee, even though she was not required to do so. I appreciate the patience and input of Dr. Wagger and Dr. Westerman as members of my graduate student committee. I could not have completed my thesis without the help of Nathan Nelson, who I foresee will become a great researcher. I admire his intelligence, attention to detail, and especially his craftiness. If I ever had a question or a problem, he knew how to solve it. I will greatly miss his friendship and advice. Thanks to all of the other graduate students who provided me with advice and diversionary activities. I especially enjoyed the weekly basketball games and summer softball games. I also want to thank my family and my wife's family for all of their support. Finally, the greatest recognition of all goes to my wife who suffered through two years of working as a lab technician at Duke University's Center for Human Genetics to put me through graduate school.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

CHAPTER I

CHAPTER II

CHAPTER III

APPENDIX A

APPENDIX B

LIST OF FIGURES

Page

CHAPTER II

CHAPTER III

CHAPTER I.

Swine and Poultry Manure Management in North Carolina

Introduction

Throughout history, societies have recognized the benefits of using animal manure as a fertilizer source in crop production. In pre-industrialized times, animal manure and other organic materials were viewed as the principle sources of plant nutrients. In the past century, crop production practices have changed immensely, as inexpensive, high-grade inorganic fertilizers have replaced organic materials as the chief source of plant nutrients. Synthetically produced fertilizers offer several key advantages over animal manures, making them a logical choice for modern crop production. For example, inorganic fertilizers are required by law to have a guaranteed chemical analysis and composition. Plant availability is usually a function of solubility rates and other well-known chemical properties. Animal manures, on the other hand, are far less predictable in terms of plant available nutrients and plant nutrient-use efficiency than commercial grade fertilizers. Indeed, the use of animal manure as a plant nutrient source is much more complex and uncertain compared with using inorganic fertilizers.

Recently, issues with manure disposal, especially from large, confined livestock operations have been a focal point of interest in North Carolina. Most of these issues involve concerns with ground or surface water contamination linked with the over

application of nutrients contained in manure. Water quality concerns associated with manures differ somewhat from industrial wastes, which may be highly concentrated and constitute a point source of pollution. Compared to inorganic fertilizer sources, animal wastes are typically far less concentrated with nutrients (Table 1). Most environmental problems with manure arise when it is viewed as a waste rather than a resource. From this perspective, there is little economic incentive to optimize nutrient use efficiency, and instead, management strategies are developed that maximize waste disposal.

Table 1. A comparison of selected macronutrients found in fresh animal manures (average values) and fertilizer sources expressed on a dry weight basis (*Adapted from* Barker and Zublena, 1996).

Material	N	P
	——————%——————	
Broiler manure	5.1	1.4
Beef manure	3.5	1.1
Swine manure	6.1	2.0
Ammonium nitrate	34	-
Urea	46	-
Anhydrous ammonia	82	-
Diammonium phosphate	18	46
Concentrated superphosphate	-	42-50

Some of the problems connected with modern manure management originate with the structure of the livestock industry. Just a few decades ago, livestock was principally produced on small, diverse, independently operated farms. In recent years, the face of livestock production has changed dramatically with small farms being replaced by larger, more specialized animal operation units, which often have lower costs associated with

production (National Agricultural Statistics Service, 2003). Modern swine and poultry producers in North Carolina generally operate under contracts with integrators or other middlemen. Westernized consumption patterns favor pre-processed foods and consumers typically do not want to handle raw farm products. Integrators operate as middlemen between producers and consumers, converting raw farm products into processed, packaged foods. Integrators usually provide growers (contractees) with feed and animals, while growers assume animal and waste management responsibilities from their individually owned production facilities. Under such circumstances, growers understandably seek the most inexpensive methods for manure disposal. Unfortunately, economic-based manure management may not correspond with the goal of minimizing environmental impacts.

To promote production efficiency, most large animal operations operate in small, confined areas that serve as feedlots for the animals. Over time, manure accumulates within the confined area and must be periodically removed to ensure healthy livestock growing conditions. In order to minimize transportation and handling costs, manure is commonly applied to pasture or field crops in close proximity to the production facility.

Although applying manure to meet crop nutrient requirements is certainly not an objectionable waste management practice, manure is usually applied at a rate that meets crop N requirements (Moore et al., 1995b). Repeated application of manure based on plant N needs can result in accumulation of P, which if lost through runoff, can lead to eutrophication of surface waters (Daniel et al., 1994; Sharpley et al., 1994). Other nutrients such as Cu and Zn have also been shown to accumulate in soil with repeated manure application (Mikkelsen, 1995; Shuman and McCracken, 1999). Nitrogen differs somewhat from P, Cu and Zn, because it is extremely transient. Even if N is applied in

excess of crop requirements, it will not accumulate in soils as does P, Cu and Zn. Once mineralized to an inorganic form, N is subject to several major loss pathways including volatilization, denitrification and leaching. In addition, N is the limiting plant nutrient in most agricultural soil systems, leading to significant crop uptake in non-leguminous crops. Therefore, if crops are harvested from a soil system, considerable N is also removed.

Concerns over animal manure management have reached the point that changes in legislation have been adopted. Recently, the U.S. EPA (2003) announced that manure applied to land from large, confined animal feeding operations (CAFOs) qualifies as a point source of pollution. Under these new regulations, CAFOs will be required to obtain a National Pollutant Discharge Elimination System (NPDES) permit. In addition, CAFOs will also be required to implement an approved nutrient management plan.

With increasing pressure to regulate land application of animal manure, North Carolina faces a difficult dilemma, given the number of large-scale animal production facilities currently in operation. Currently, North Carolina is the second largest swine producer in the nation and ranks fourth and second in broiler and turkey production, respectively (North Carolina Agricultural Statistics Division, 2003). Animal production has become so concentrated in certain regions of the state that the production of swine and poultry waste is greater than local crop nutrient demand (NCSU-SSSIRL, 2001). Clearly, appropriate animal manure management will be an essential if animal production and agronomic goals are to be met in an environmentally sound manner.

Swine Manure Issues

Modern swine production in North Carolina mandates high energy, high protein diets in the form of grain-based feeds (Mikkelsen, 1997). Proper swine nutrition is important because of the key role it plays in swine growth and maturity. However, like many other farm animals, swine excrete most of the nutrients they consume, typically incorporating less than 25% of ingested N and less than 20% of ingested P (Mikkelsen, 2000). Sources have reported that swine produce more manure per live weight than any other species of domestic livestock (Ensminger and Parker, 1984). Table 2 compares the manure production of several different animal species. Manure production also varies depending on the development stage of pigs, with the rate of excretion less for young pigs and substantially more for lactating sows, as illustrated in Table 3.

Table 2. Average animal manure production per 1000 kg live weight (*Adapted from* Barker and Zublena, 1996 and Mikkelsen, 1997).

Animal Type	Annual Manure Production	N (dry wt. basis)
	—Mg manure (1000 kg live weight)$^{-1}$ year^{-1}—	——%——
Broiler	9.0	5.1
Sheep	12.0	3.7
Beef	17.0	3.5
Dairy	24.0	3.7
Swine	32.0	6.1

Table 3. Manure production as excreted by various types of swine (*Adapted from* Barker, 1996b).

Type	Average Weight	Total Manure Production
	—kg—	—kg head^{-1} day^{-1}—
Feeder	14	1.5
Grower	41	3.8
Finisher	80	6.8
Gestating sow	182	7.3
Lactating sow	182	13.6
Boar	182	8.4

Since large quantities of waste are produced in large-scale swine production facilities, they must be stored and treated in an appropriate manner to avoid nutrient loss and environmental contamination. Sealed lagoons are commonly used for swine waste treatment and storage since they are relatively simple and economical to build and maintain. Lagoons can be classified according to the type of bacteria present that are responsible for organic matter decomposition. The three main types of lagoons are aerobic (bacteria require oxygen), anaerobic (bacteria exist solely in the absence of oxygen) or facultative anaerobic (combination of anaerobic and aerobic bacteria). Most lagoons in the United States are either anaerobic or facultative anaerobic units, since aerobic lagoons typically require mechanical pumping or very large surface areas in order to supply sufficient oxygen (Safley et al., 1993).

In most swine operations in North Carolina, urine and solid waste falls through slotted floors in the production houses where it is flushed with water several times a day to anaerobic lagoons for temporary storage. Liquid is pumped from the lagoon into the production houses and recycled as flush water. Since large amounts of water are used to

flush and transport swine manure, lagoon effluent is quite dilute compared to fresh manure (Tables 4 and 5). Within the lagoon, large solids settle to the bottom for slow decomposition while the liquid portion of the swine effluent is subject to rapid biological transformations. Anaerobic lagoons serve as treatment sites for swine effluent by providing a favorable environment for anaerobic decomposition. Anaerobic lagoons are advantageous because anaerobic bacteria can decompose more organic matter per unit lagoon volume than aerobic bacteria (Barker, 1996a). Since anaerobic metabolism is not dependent on dissolved oxygen concentration, lagoons can be deeper and occupy less surface area than aerobic lagoons (Barker, 1996a).

Anaerobic decomposition is brought about by a consortium of microorganisms that require a final electron acceptor other than oxygen for metabolism. There are three basic steps in the anaerobic digestion of the organic material present in swine waste (Marty, 1986). First, complex organic polymers are broken down to form volatile fatty acids, hydrogen, formate, lactate and alcohols in the processes of hydrolysis and acidogenesis. Microbes use these as substrates and convert them to acetate in the process of acetogenesis. Using acetate as an electron donor, methanogens convert the acetate to gaseous CH_4.

Table 4. Average N concentration in fresh swine manure expressed on a dry weight basis (*Adapted from* Barker, 1996b).

Swine Type	Total Kjeldahl N	NH_3-N
	——————g kg^{-1} ——————	
Feeder	41	34
Grower	34	29
Finisher	63	52
Gestating sow	95	58
Lactating sow	75	46
Boar	91	65

Table 5. Swine waste N characteristics as affected by storage method (*Adapted from* Barker, 1996b).

Manure type	Total Kjeldahl N	NH_3-N
	———— g kg^{-1} ————	
Paved surface scraped*	6.5	2.8
	———— g L^{-1} ————	
Liquid manure slurry	3.2	2.0
Anaerobic lagoon liquid	0.6	0.5
Anaerobic lagoon sludge	2.9	0.7

*Wet weight basis

Nitrogen is an important nutrient present in anaerobic swine lagoons. Generally, most of the N in anaerobic lagoon swine effluent is in the form of NH_4, sometimes accounting for more than 90% of the total effluent N (Mikkelsen, 1997). Urine and easily decomposable organic N compounds are rapidly converted to NH_4, while more complex organic N compounds may require many months to mineralize. Over time, a significant portion of N (70-80%) that enters a lagoon may be converted to NH_4 and subsequently volatilized as gaseous NH_3 (Safley et al., 1993). Ammonia volatilization increases as

lagoon pH and temperature increase, as well as when windy conditions persist at the lagoon surface (Safley et al., 1993). Volatilization typically reduces total N in anaerobic lagoon effluent to less than 600 mg N L^{-1} (Mikkelsen, 1997). Though NH_3 volatilization may account for considerable N losses, it is often viewed as a benefit to swine producers since the N concentration in the effluent is significantly reduced. Since North Carolina legislation mandates that loading rates be calculated by the N requirement of a growing crop, NH_3 volatilization reduces the N concentration in the effluent, allowing more waste to be land applied per volume of waste produced.

The most common receiver crops for swine effluent in the North Carolina Coastal Plain are corn (*Zea mays* L.) and Coastal bermudagrass (*Cynodon dactylon* L. Pers.). Bermudagrass is a popular receiver crop because of its ability to assimilate large amounts of N (Mueller et al., 1993). Although large amounts of N are removed in the harvested hay, it is not always marketable due to regional surpluses. In addition, the quality of the hay may suffer because of frequent summer rains. Such practices suggest that other manure management options should be investigated in an effort to prevent potential nutrient buildup and/or loss to sensitive water bodies.

Nonpoint source contamination of groundwater is a major concern in the Coastal Plain region of North Carolina owing to the proliferation of CAFO's, particularly swine production facilities. Since groundwater is the principal source of drinking water in the U.S. (Goodrich et al., 1991), and highly leachable sandy soils dominate the Coastal Plain, NO_3 contamination of groundwater is a valid concern. Elevated NO_3 concentrations have been linked with adverse human health conditions (U.S. EPA, 2003). For this reason, the U.S. EPA has set the maximum limit for NO_3-N in drinking water at 10 mg L^{-1} (U.S. EPA,

1992). However, researchers have suggested that under proper fertilization strategies, groundwater contamination can be avoided. Gilliam (1991) concluded that properly fertilized fields in the North Carolina Coastal Plain did not contribute to groundwater NO_3-N concentrations in excess of 10 mg L^{-1}. Spalding and Exner (1993) also reported acceptable concentrations of NO_3 in groundwater in areas of the southeastern U.S. under intensive agriculture. Clearly, adoption of BMPs combined with a good understanding of N dynamics will be imperative to properly managing swine manure in North Carolina.

Poultry Manure Issues

Recent consumer preference for low-fat, low cholesterol meat has fueled tremendous growth in the poultry industry. The number of broiler chickens produced annually in the U.S. more than doubled from 4 billion in 1983 to over 8 billion in 2001 (National Agricultural Statistics Service, 2003). North Carolina, as well as several other southeastern states, has been impacted tremendously by the growth trend. In 2001, 43 million turkeys and 653 million broiler chickens were produced in North Carolina, accounting for over $2.2 billion in cash receipts (North Carolina Agricultural Statistics Division, 2003).

In many regards, poultry and swine production share similar environmental concerns in North Carolina. Like swine production, poultry producers must contend with the tremendous amounts of manure generated by concentrated animal feeding operations. However, there are fundamental differences between the two manures, mostly in form and composition, which affect manure management strategies. The most important difference between the two manures is that swine manure is usually collected in a liquid form with

very little dry matter present, while poultry manure is stored and handled as a solid or slurry, depending on the type of bird grown. This important difference dramatically changes storage and handling characteristics and expands economically feasible transportation options. Most solid poultry waste is handled in the form of poultry litter, a mixture of manure and bedding materials such as wood chips, sawdust or peanut hulls. Compared to other animal manures, poultry litter is considered an excellent fertilizer source because of its low moisture content and relatively high nutrient status (Evers, 1998).

In the confining conditions of large poultry production houses, litter slowly accumulates and must be removed after a period of time. Typically, after five 10-week grow out periods, litter is removed from production houses and stored under barns or in piles until application to the field. The ability to temporarily store litter allows producers some flexibility in timing applications, an important aspect for matching nutrient release with plant nutrient needs (Moore et al., 1995b).

During handling and storage, the NH_3 fraction of manure may be quite unstable and be lost via volatilization. Recently, various types of acidifying chemical treatments have been shown to significantly reduce NH_3 volatilization, particularly the addition of alum. Research conducted by Moore et al. (1995a) showed that additions of alum ($Al_2(SO_4)_3$) to litter effectively reduced NH_3 volatilization by 99% and reduced P runoff. The authors suggest that such additions may be a cost-effective solution to conserving N and simultaneously reducing P loss potential.

Many management, environmental, and physiological factors influence poultry litter production and composition. According to Malone (1992), an average of 1.0 Mg litter (dry basis) is produced per 1000 birds in a 10-week growing cycle, although litter

production rates vary significantly depending on management factors such as the type of bedding materials used, the number of flocks grown in between bedding material replacement and the frequency of cleanout operations. Manure production rates can also vary greatly with breed and size of the bird. Simmons et al. (1987) recognized the variability in manure production rates for growing broilers and developed a model for predicting manure production based on bird growth stage. Their model is stated as:

$$M = 8.76 + 0.24D + 0.14D^2$$

where: M is daily manure production (g/bird) and D is the days of the brooding period.

Broiler and turkey litter, two of the most common poultry wastes in North Carolina, contain a variety of essential plant nutrients (Table 6). Edwards and Daniel (1992) estimated a N:P:K ratio of 2.3:1:1.6 for poultry litter, although nutrient concentrations vary greatly. Nutrient concentrations in litter vary because of feed ration, dietary supplements, litter type, number of batches of birds grown on the same litter, and handling and storage procedures (Sims and Wolf, 1994). Because of this variability, the only reliable method for determining litter characteristics is by analyzing the litter directly. Chemical analysis is especially important prior to land application in order to avoid over or under applying nutrients.

Table 6. Typical nutrient content of broiler and turkey litter as affected by storage method on a dry weight basis (*Adapted from* Barker, 1996b).

Litter Type	TKN	P	K	Ca	Mg	S
			g kg^{-1}			
Broiler house	46	19	25	27	6	8
Broiler stockpiled	27	27	22	52	7	8
Turkey house	34	17	21	24	5	5
Turkey stockpiled	26	25	21	37	6	6

One of the reasons poultry litter is an effective fertilizer is because of the relatively high N concentration. Estimates of total N in poultry litter range from 17-68 g kg^{-1} dry litter (Edwards and Daniel, 1992). Patterson et al. (1998) determined that approximately 31% of feed N is excreted in manure. Unlike swine effluent, where the majority of the N fraction is in the form of inorganic NH_4, poultry litter N mainly exists initially in the form of organic compounds. On average, poultry litter contains 1.7 to 6.8% total N by weight (Edwards and Daniel, 1992). Typically, 90% of N in poultry litter is organic, with about 50% of the organic N present in the form of easily decomposable uric acid (Overcash et al., 1983). Recalcitrant materials such as lignin make up the remaining portion of the organic N fraction (Gordillo and Cabrera, 1997). The inorganic N fraction of poultry litter is usually dominated by NH_4, which may exceed 90% of the inorganic fraction of the poultry litter (Chadwick et al., 2000). Westerman et al. found that 20% of total Kjeldahl N in broiler litter consisted of NH_4 (1988).

Because of favorable physical and chemical characteristics, broiler litter can serve as an effective plant nutrient source. Poultry litter has been shown to increase yields of many crops, including bermudagrass (*Cynodon dactylon*), fescue (*Festuca arundinacea*) (Huneycutt et al., 1988), and orchardgrass (*Dactylis glomerata L.*) (Hileman, 1973). In North Carolina, litter is also commonly used to supply nutrients to row crops. In many cases, poultry producers seek to maximize soil fertility and crop yields with the addition of poultry litter. With these goals in mind, the application rate is usually based on the N needs of the crop. Thus, the ability to predict plant-available N of poultry litter and the rate of nutrient release is vital in order to synchronize N release with crop N uptake.

Predicting N availability of land-applied broiler litter can be complex and difficult for many reasons. A fraction of the inorganic N portion can immediately become plant available once land applied, but in order for the organic portion of N in broiler litter to become plant available, it must first be mineralized. Although determining the inorganic N content of litter is relatively simple, measuring potentially mineralizable N is much more difficult and inconsistent. Mineralization of organic materials is generally controlled by the microbial population, soil temperature, soil moisture, and chemical and physical composition of the organic materials (Subbarao, 1999). In general, mineralization rates are optimized in well-aerated soils containing 50 to 70% water-filled pore space and at temperatures between 25 and 35º C, while they are suppressed by substrates containing lignin (Havlin et al., 1999). In a mineralization study with different types of animal manures, Chadwick et al. (2000) suggested that the C:N of the manure regulates the early phase of decomposition. Furthermore, the researchers suggested that as decomposition proceeds, the substrate changes and becomes more concentrated with lignin and other recalcitrant compounds. The recalcitrant material is then the primary mineralization rate-determining factor controlling decomposition over long-term periods.

An estimate of potentially mineralizable N of broiler litter is usually combined with an availability coefficient of inorganic N to form a comprehensive model that can be used to predict plant-available N in a growing season. In calculating plant-available N from broiler litter, Barker and Zublena (1993) suggested that the following model could be employed:

$$PAN = VR(NH_4\text{-}N) + 0.5(ON)$$

where PAN is N (g kg^{-1}) that is plant-available in the same year following application, VR is the availability coefficient, NH_4-N is the NH_4-N (g kg^{-1}) present in the applied litter and ON is the organic N (g kg^{-1}) applied in the waste. The availability coefficient (VR) for the above equation is equal to 0.75 if the litter is incorporated and 0.25 if the litter is surface applied.

The North Carolina Department of Agriculture takes a slightly different approach for calculating PAN. The model currently in use is based on two factors: total N of broiler litter and application method. The model can be represented as:

PAN = Total N * Availability Coefficient

For broiler litter, the availability coefficient is 0.45 if litter is surface applied and 0.57 if incorporated. The difference in these availability coefficients is mostly based on the assumption that the degree of volatilization will be lower when litter is incorporated.

Once mineralized, N is subject to several fates within the soil. For instance, soil microbes may immobilize N present in litter. Soil microbes maintain an average C:N ratio of 8:1 within their cells and thus this ratio in added organic matter largely controls whether or not inorganic N in the soil becomes immobilized or mineralized (Havlin, 1999). Chadwick et al. (2000) found that organic materials with C:N ratios of 15:1 or more will initially immobilize soil inorganic N while C:N ratios less than 15:1 result in mineralization. Gale and Gilmour (1986) have suggested that immobilization is responsible for reducing soil inorganic N for one to two weeks after application of poultry waste. The same researchers suggested that undigested feed and litter bedding materials with high C:N ratios were the likely cause of the immobilization patterns.

As previously discussed, one of the pathways by which N can be lost from poultry litter is through ammonia volatilization. Following land application of litter, pH of the soil-waste system usually rises and NH_4 is converted into volatile NH_3 (Edwards and Daniel, 1992). Estimates of N lost through NH_3 volatilization following land application of poultry litter vary widely in the literature. Ammonia losses from poultry litter have been found to range from 3.6 to 60% of total N applied (Cabrera et al., 1993; Cabrera and Chiang, 1994). In general, NH_3 losses increase with temperature up to 45º C as wind speed increases (Havlin, 1999). Experiments have shown that incorporation of litter at the time of application can virtually eliminate NH_3 volatilization losses (Wolf et al., 1988).

In addition to undergoing volatilization processes, NH_4 can also be nitrified. Crane et al. (1981) reported that little NO_3 accumulated in either clay or sandy soils in the first 5 days after surface application of poultry manure. Once nitrified, NO_3 is potentially subject to denitrification and leaching. Cabrera et al. (1993) found that denitrification losses accounted for less than 1% of total poultry manure N applied to sandy soils in the southeastern U.S. Marshall et al. (2001) also determined that denitrification was not a major pathway of N loss when litter was applied to fescue pastures in the southeastern U.S.

Several studies have shown that long-term applications of poultry litter in excess of crop needs promote NO_3 leaching (Liebhardt et al., 1979; Kingery et al., 1994). In Alabama, Adams et al. (1994) found that NO_3 concentrations in subsoils (120 cm depth) were correlated with litter application rates, although even at the maximum recommended litter application rate, NO_3 concentrations did not exceed 10 mg NO_3-N L^{-1}. Groundwater contamination via NO_3 leaching appears to be a site-specific phenomenon, highly dependent upon application rate, timing, and crop N requirements (Marshall et al., 2001).

As long as litter is applied to match crop N needs, there appears to be little risk of groundwater contamination by NO_3.

Ideally, poultry litter management decisions also consider site-specific environmental goals. However, management practices are usually dictated by two contrasting philosophies. In many cases, poultry producers seek to maximize soil fertility and crop yields with the addition of poultry litter. In contrast, some poultry producers may have entirely different economic goals, with incentives to minimize waste disposal costs. In this scenario, poultry litter may be applied in excess of crop nutrient requirements, leading to nutrient loss and potential surface and/or groundwater contamination. One important consideration for the future will be the economic feasibility in transporting poultry manure from concentrated areas of poultry production to areas where nutrients are deficient. Economic studies have already confirmed that the cost/benefit ratio is favorable for poultry litter transport in some areas (Bosch and Napit, 1992). In other areas, government incentives have been provided to facilitate poultry litter transport for beneficial purposes such as pelletization (Lichtenburg et al., 2002). Increased pressure to apply manure based on P instead of N in nutrient sensitive watersheds will also play a major role in the future of poultry manure management. Future research should focus on the dynamics of N and P when poultry litter is land applied. Using this knowledge, best management practices can be developed and implemented to satisfy both agronomic and environmental objectives.

References

Adams, P.L., T.C. Daniel, D.R. Edwards, D.J. Nichols, D.H. Pote, and H.D. Scott. 1994. Poultry litter and manure contributions to nitrate leaching through the vadose zone. Soil Sci. Soc. Am. J. 58:1206-1211.

Barker, J.C. 1996a. Lagoon design and management for livestock waste treatment and storage. [Online]. Available at http://www5.bae.ncsu.edu/programs/extension/pulblicat/wqwm/ebae103_83.html. (verified May 16, 2003).

Barker, J.C. 1996b. Swine and poultry manure production and characteristics. *In* M.B. Coelho and E.T. Kornegay (ed.) Phytase in animal nutrition and waste management: a BASF reference manual. BASF Corp, Mount Olive, NJ.

Barker, J.C., and J.P. Zublena. 1993. Livestock manure nutrient assessment in North Carolina. [Online]. Available at http://www.spatiallab.ncsu.edu/nutman/assessment.htm. (verified May 18, 2003).

Barker, J.C., and J.P. Zublena. 1996. Livestock manure nutrient assessment. *In* M.B. Coelho and E.T. Kornegay (ed.) Phytase in animal nutrition and waste management: a BASF reference manual. BASF Corp, Mount Olive, NJ.

Bosch, D.J., and K.B. Napit. 1992. Economics of transporting poultry litter to achieve more effective use as fertilizer. J. Soil Water Conserv. 47:342-346.

Cabrera, M.L. and S.C. Chiang. 1994. Water content effect on denitrification and ammonia volatilization in poultry litter. Soil Sci. Soc. Am. J. 58:811-816.

Cabrera, M.L., S.C. Chiang, W.C. Merka, S.A. Thompson, and O.C. Pancorbo. 1993. Nitrogen transformations in surface-applied poultry litter: effect of litter physical characteristics. Soil Sci. Soc. Am. J. 57:1519-1525.

Chadwick, D.R., F. John, B.F. Pain, B.J. Chambers, and J. Williams. 2000. Plant uptake of nitrogen from the organic nitrogen fraction of animal manures: A laboratory experiment. J. Agric. Sci. Camb. 134:159-168.

Crane, S.R., Westerman, P.W. and M.R. Overcash. 1981. Short-term transformations following land application of poultry manure. Trans. ASAE 24:382-390.

Daniel, T.C., A.N. Sharpley, D.R. Edwards, R. Wedepohl, and J.L. Lemunyon. 1994. Minimizing surface water eutrophication from agriculture by phosphorus management. J. Soil Water Conserv.

Edwards, D.R., and T.C. Daniel. 1992. Environmental impacts of on-farm poultry waste disposal—a review. Bioresour. Technol. 41:9-33.

Ensminger, M.E. and R.O. Parker. 1984. Swine science. Interstate Publ., Danville, IL.

Evers, G.W. 1998. Comparison of broiler poultry litter and commercial fertilizer for coastal bermudagrass production in the southeastern US. J. Sustainable Agric. 12:55-77.

Gale, P.M. and J.T. Gilmour. 1986. Carbon and nitrogen mineralization kinetics for poultry litter. J. Environ. Qual. 15:423-426.

Gilliam, R.W. 1991. Fertilizer nitrates not causing problems in North Carolina groundwater. Better Crops. Spring, p. 6-8.

Goodrich, J.A., B.W. Lykins Jr., and R.M. Clark. 1991. Drinking water from agriculturally contaminated groundwater. J. Environ. Qual. 20:707-717.

Gordillo, R.M., and M.L. Cabrera. 1997b. Mineralizable nitrogen in broiler litter: II. Effect of selected soil characteristics. J. Environ. Qual. 26:1679-1686.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers: An introduction to nutrient management. Prentice Hall, Upper Saddle River, New Jersey.

Hileman, L.H. 1973. Response of orchardgrass to broiler litter and commercial fertilizer. Rep. Ser. 207. Arkansas Agricultural Experiment Station. University of Arkansas. Fayetteville, AR.

Huneycutt, H.G., West, C.P. and J.M. Phillips. 1988. Responses of bermudagrass, tall fescue and tall fescue clover to broiler litter and commercial fertilizer. Bul. 913. Arkansas Agricultural Experiment Station. University of Arkansas. Fayetteville, AR.

Kingery, W.L., C.W. Wood, D.P. Delaney, J.C. Williams, and G.L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23:139-147.

Lichtenberg, E., D. Parker, and L. Lynch. 2002. Economic value of poultry litter supplies in alternative uses. Center for Agricultural and Natural Resource Policy. Policy analysis report No. 02-02. [Online] Available at: http://www.arec.umd.edu/policycenter/Policyreports/full-texts/02-02.pdf. (Verified July 7, 2003).

Liebhardt, W.C., C. Golt, and J. Tupin. 1979. Nitrate and ammonium concentrations of ground water resulting from poultry manure applications. J. Environ. Qual. 8:211-215.

Malone, G.W. 1992. Nutrient enrichment in integrated broiler production systems. Poult. Sci. 71:1117-1122.

Marshall, S.B., M.D. Mullen, M.L. Cabrera, C.W. Wood, L.C. Braun, and E.A. Guertal. 2001. Nitrogen budget for fescue pastures fertilized with broiler litter in major land resource areas of the southeastern US. Nutr. Cycl. Agroecosyst. 59:75-83.

Marty, B. 1986. Microbiology of anaerobic digestion. P. 72-87. In A. Kouzeli-Katsiri and P.J. Newman (ed.) Anaerobic digestion of sewage sludge and organic agricultural wastes. Elsevier Appl. Sci. Publ., London.

Mikkelsen, R.L. 1995. Swine waste disposal dilemma: A case study. J. Natural Res. Life Sci. Educ. 24:169-172.

Mikkelsen, R.L. 1997. Management issues of swine waste. p. 110-119. *In* J.E. Rechcigl and H.C. MacKinnon (ed.) Agricultural Uses of By-Products as Wastes. ACS Symposium Ser. 668. ACS. Washington, DC.

Mikkelsen, R.L. 2000. Beneficial Use of Swine By-Products: Opportunities for the Future. p. 451-480. *In* J.F. Power (ed.) Land application of Agricultural, Industrial, and Municipal by-products. SSSA Book Ser. 6. SSSA, Madison, WI.

Moore, P.A., Jr., T.C. Daniel, D.R. Edwards, and D.M. Miller. 1995a. Effect of chemical amendments on ammonia volatilization from poultry litter. J. Environ. Qual. 24:293-300.

Moore, P.A., Jr., T.C. Daniel, A.N. Sharpley and C.W. Wood. 1995b. Poultry manure management: environmentally sound options. J. Soil and Water Cons. 50:321-327.

Mueller, J.P., Green, J.T., Chamblee, D.S., Burns, J.C., Bailey, J.E., and Brandenburg, R.L. 1993. Bermudagrass management in North Carolina. North Carolina Cooperative Extension Service, AG-493, p.14.

National Agricultural Statistics Service. 2003. Agricultural charts and maps. [Online]. Available at http://www.usda.gov/nass/aggraphs/graphics.htm. (verified July 15, 2003).

North Carolina Agricultural Statistics Division. 2003. [Online]. Available at http://www.ncagr.com/stats/nc_rank/ncrallyr.htm. (verified June10, 2003).

North Carolina State University—Soil Science Spatial Information Research Lab. 2001. North Carolina nutrient management database project. [Online]. Available at http://www.spatiallab.ncsu.edu/nutman/main.htm (verified Aug. 25, 2003).

Overcash, M.R., Humenik, F.J. and R.J. Miner. 1983. Livestock Waste Management, vol. 1. CRC Press, Boca Raton, FL.

Patterson, P.H., E.S. Lorenz, and W.D. Weaver, Jr. 1998. Litter production and nutrients from commercial broiler chickens. J. Appl. Poultry Res. 7:247-252.

Safley, L.M., C.D. Fulhage, R.L. Huhnke and D.D. Jones. 1993. Lagoon management. Pork Industry Handbook. N.C. Cooperative Extension Service. PIH-62.

Sharpley, A.N., S.C. Chapra, R. Wedepohl, J.T. Sims, T.C. Daniel, and K.R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23:437-451.

Shuman, L.M. and D.V. McCracken. 1999. Tillage, lime, and poultry litter effects on soil zinc, manganese, and copper. Commun. Soil Sci. Plant Anal. 30:1267-1277.

Simmons, J.D., Huges, B.L., and W. H. Allen. 1987. Research note: growth and waste production of broilers during brooding. Poultry Sci. 66:762-764.

Sims, J.T., and D.C. Wolf. 1994. Poultry waste management: agricultural and environmental issues. Adv. Agron. 52:1-83.

Spalding, R.F. and M.E. Exner. 1993. Occurrence of nitrate in groundwater—A review. J. Environ. Qual. 22:392-402.

Subbarao, N.S. 1999. Soil Microbiology. Science Publishers, Inc. Enfield, New Hampshire.

U.S. Environmental Protection Agency. 1992. Drinking Water Regulations and Health Advisories. Washington, D.C. Office of Water, U.S. EPA.

U.S. Environmental Protection Agency. 2003. National pollutant discharge elimination system permit regulation guidelines and standards for concentrated animal feeding operations (CAFOs); final rule. Federal Register. Vol. 68 Num. 29. fr12fe03R [Online]. Available at: http://www.gpoaccess.gov/fr/index.html. (verified July 21, 2003).

Westerman, P.W., L.M. Safley Jr., and J.C. Barker. 1988. Available nitrogen in broiler and turkey litter. Trans. ASAE. 31: 1070-1075.

Wolf, D.C., Gilmour, J.T. and P.M. Gale. 1988. Estimating potential ground and surface water pollution from land application of poultry litter—II. Publication No. 137. Arkansas Water Resources Research Center. Fayetteville, AR.

Zublena, J.P., J.V. Baird, and J.P. Lilly. 1991. Soil facts: nutrient content of fertilizer and organic materials. North Carolina Cooperative Extension Service. AG-439-18. (Available online at http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-18). (Verified July 16, 2003).

CHAPTER II.

Determination of Nitrogen Use in Soybean with Swine Effluent as a Nitrogen Source

Introduction

Changing food consumption patterns have drastically affected agricultural enterprises in the southeastern U.S. (DiPietre, 2001). From the 1980's to the mid-1990's, North Carolina's swine industry underwent a rapid and significant expansion as primary production shifted from small independently owned farms to large vertically integrated production operations (Avery, 2003). In 1994, Rhodes and Grimes estimated that one-third of "super-producer operations" (>50,000 hogs) in the U.S. were located in North Carolina, owing to the inexpensive land and labor available in the area. Although the growth of swine operations in North Carolina has stopped in recent years due to a state mandated moratorium, pork production remains an important agricultural commodity, generating over 1.7 billion dollars in 2001 (North Carolina Agricultural Statistics Division, 2003). Currently, North Carolina houses 9.6 million hogs and pigs, making it the second largest swine producer in the nation (North Carolina Agricultural Statistics Division, 2003).

Swine production is one of the major contributors of animal waste-derived nutrients in North Carolina. Recent trends toward large enclosed feedlots have resulted in the

concentration of manure in smaller areas. Animal production has become so concentrated in certain regions that the production of swine and poultry waste meets local crop nutrient demand. In a recent statewide manure nutrient assessment, it was estimated that 3 counties in North Carolina had enough animal manure to meet 100% of non-legume and forage crop N requirements, while crop P requirements could be met in 20 counties (NCSU-SSSIRL, 2001). To make matters more complicated, several of these counties with large amounts of waste-derived nutrients are located in the Coastal Plain region, in close proximity to one another. Thus, the transport of swine waste to adjacent counties is not feasible and regions have become concentrated with swine waste-derived nutrients. On a statewide basis, it has been estimated that 28% of the N and 58% of the P crop requirements could be met with animal manure (NCSU-SSSIRL, 2001). Although swine waste is not the sole contributor to the statewide nutrient supply, it is a major source. Clearly, swine waste management is an essential component of a regional nutrient management plan if agronomic goals are to be met in an environmentally sound manner.

Lagoons are sealed, earthen basins commonly used to treat and store liquid swine waste. Anaerobic lagoons are attractive to producers because they are simple and economical to build and maintain (Andreadakis, 1992). Anaerobic lagoons serve as treatment sites for swine effluent by providing a favorable environment for anaerobic decomposition. When liquid in a hog lagoon reaches a regulatory maximum height, the effluent is usually pumped from the lagoon and land applied. In order to limit non-point source pollution, amendments of the Clean Water Act require that large confined animal operations adopt “best management practices” to improve manure handling (Geyer and Findley, 1994).

The state of North Carolina currently requires swine lagoon effluent to be applied based on the N needs of a growing crop. The most common receiver crops for swine effluent in the North Carolina Coastal Plain are corn (*Zea mays* L.) and Coastal bermudagrass (*Cynodon dactylon* L. Pers.). Bermudagrass is a popular receiver crop because of its ability to assimilate large amounts of N (Mueller et al., 1993). In an N rate study, Burton et al. (1963) found that inorganic fertilizer rates of 1008 kg N ha^{-1} increased dry matter production of Coastal bermudagrass by 12.8 Mg ha^{-1} compared with treatments receiving no N inputs. Burns et al. (1990) determined that bermudagrass dry matter yields were maximized when anaerobic swine effluent was applied at a rate of 1310 kg total N ha^{-1} yr^{-1}, although they also recognized that this high rate of application had unacceptable environmental consequences.

Some producers in North Carolina have taken advantage of high yielding bermudagrass in spray fields by converting them to pasture land and grazing cattle (Mikkelsen, 1995). However, there are concerns that bermudagrass may accumulate high concentrations of NO_3, which can be lethal to cattle when present in concentrations greater than 3400 mg NO_3-N kg^{-1} forage (Wright and Davidson, 1964). Using spray fields for grazing cattle has also raised concerns because like swine, cattle incorporate only a small percentage of N consumed in the forage. In a study of feed type and N digestibility in feedlot cattle, Bierman et al. (1999) estimated that between 65-80% of feed-N was excreted. Since most of the N consumed by cattle as forage is returned to the soil as manure, the potential remains for NO_3 leaching from the root zone into the groundwater.

Nonpoint source contamination of groundwater and surface waters is a major concern in the Coastal Plain region of North Carolina, owing to the proliferation of

concentrated animal feeding operations. Much of the Coastal Plain consists of highly weathered, sandy soils with low organic matter content. With a mean annual rainfall of 1000-1300 mm, NO_3 has the potential to leach into the shallow and deep groundwater. Lateral movement of shallow groundwater into surface streams has also been well documented in Coastal Plain soils (Hubbard and Sheridan, 1983; Hubbard et al., 1989). After percolating through the upper sandy layer of soil, water reaches the relatively impermeable subsoil and flows laterally down slope. This lateral movement can facilitate NO_3 transport, leading to nutrient enriched streams and lakes. Excess NO_3 in surface waters has been linked with eutrophication, where the decomposition of excessive plant and algae growth significantly lowers the dissolved oxygen content in water, resulting in fish kills and general degradation of water quality.

Many studies have confirmed that when animal waste products are land applied at rates higher than recommended, there is a high risk of NO_3 leaching into groundwater. At a research site in Duplin County, NC, Stone et al. (1998a) observed a significant increase of NO_3 in shallow groundwater at the edge of a spray field after application of anaerobic swine effluent was increased from a rate of 100 kg PAN ha^{-1} to 300 kg PAN ha^{-1}. Several of the wells sampled increased enough to exceed the drinking water standard of 10 mg NO_3-N L^{-1}. In a separate study, Stone et al. (1998b) measured NO_3 concentrations in groundwater where swine effluent had been applied at an excessively high rate of 2500 kg N ha^{-1} to a field with no permanent crop cover. Nitrate concentrations at the edge of the spray field were extremely high, averaging 87 mg NO_3-N L^{-1}. Evans et al. (1984) observed subsurface drainage water containing NO_3-N concentrations higher than 25 mg L^{-1} when swine waste was applied to Coastal Plain soils at four times the recommended rate. In

another study conducted by Stone et al. (1998c), 84 well sites on 21 farms in the Herrings Marsh Run, NC watershed were examined over two years. They found that 26% of the monitoring sites had NO_3-N concentrations higher than 10 mg L^{-1}. The researchers concluded that the farms with high measured NO_3 concentrations had likely received high rates of animal waste applications. Several investigators suggest that management plans should call for waste application rates to be governed by the estimated plant recoverable nutrients (Jackson et al., 1987; Zublena et al., 1990).

Soybean (*Glycine max* L. Merr.) has the potential to be a valuable receiver crop of anaerobic swine effluent. Although *Rhizobium* spp. are capable of producing N symbiotically in active, nodulated roots, high yielding soybean are in fact net consumers of N. In studies using ^{15}N, Varvel and Peterson (1992) found that soybean operate as an N-sink, removing large amounts of N when harvested. Heichel and Barnes (1984) reported that when harvested, soybean seed may remove significantly more soil-derived N than the symbiotic-derived N in residue returned to the soil. By exploiting the N-sink effect, soybean could be useful as an N accumulator in swine effluent sprayfields.

Soybean is commonly overlooked as a receiver crop for anaerobic swine effluent due to its ability to fix nitrogen. However, Varvel and Peterson (1992) reported that soybean is capable of using N from fertilizer or soil in addition to fixing N. Harper (1974) reported that 25 to 60% of the N in mature soybean comes from N-fixation, while the remaining N must be supplied by the soil. Though soybean may symbiotically fix a significant portion of N, the mechanism of N-fixation can at least be partially impeded. Previous research has shown that symbiotic N-fixation may be inhibited in soybean when a readily available source of N is present (Streeter, 1988). Angle et al. (1992) showed that

N-fixation was significantly reduced from 51% to 14% of total plant N harvested with the pre-plant addition of sewage sludge at a rate of 312 kg PAN ha^{-1}. Some investigations have linked delayed or reduced nodule formation in soils containing high concentrations of inorganic N (Weber, 1966; Hinson, 1975). Johnson et al. (1975) showed that reduced nodulation also caused a reduction in N-fixation, resulting in increased fertilizer N uptake.

Previous research has produced mixed results regarding soybean yield response when an additional source of N has been provided. Some researchers have concluded that inorganic fertilizer N addition can increase seed yield (Afza et al., 1987; Wesley et al., 1998; Seneviratne et al., 2000), while other studies have shown little or no yield benefit with fertilizer N addition (Johnson et al., 1975; Diebert et al., 1979; Schmitt et al., 2001). Some of the variability in results seems to be related to timing of fertilizer N addition, since soybean N requirement varies with growth stage. In the early growth stages of soybean, nodules are not developed and thus cannot contribute to plant N. During these early growth stages, the soybean's main source of N is the soil (Deibert et al., 1979). During the pod fill growth stages (R3 to R6), N is required in greater amounts. For example, a soybean crop yielding 2.7 Mg ha^{-1} (~40 bu A^{-1}) requires 155 kg N ha^{-1} to be translocated to the seed during the pod filling stage of growth (Wesley, 1998). Afza et al. (1987) found that N applied during the pod fill stage increased grain yield by 37% over controls. The authors hypothesized that fertilizer N was taken up and translocated directly into the pods for protein synthesis, as opposed to being used for vegetative growth.

Considering the unique characteristics of soybean and soybean production, it has the potential to become a valuable receiver crop of anaerobic swine effluent in North Carolina. Soybean seed contains a relatively high protein content, requiring large amounts

of N for synthesis. By harvesting soybean grain, large quantities of N could be removed from swine effluent spray fields. For example, harvesting 2.7 Mg ha^{-1} of soybean would remove approximately 200 kg N ha^{-1} (Table 1). In comparison, a typical corn yield of 6.3 Mg ha^{-1} (~100 bu A^{-1}) would remove approximately 85 kg N ha^{-1} (Table 1). Although bermudagrass certainly has the potential to remove more N from soil than soybean, markets for soybean production are not limited in the North Carolina Coastal Plain, unlike bermudagrass, which is cut and sometimes left to decompose at the edge of fields if no market is available. Soybean could also be advantageous as a receiver crop for swine effluent, because it could be used with other crops in currently practiced crop rotations.

Table 1. Typical annual N removal by the harvested portion of crops grown in North Carolina (*Adapted from* North Carolina Water Pollution Control System Operators Certification Commission, 2003)

Crop	Average Yield	Average N removed in harvested portion
	—Mg ha^{-1}—	—kg ha^{-1}—
bermudagrass	9.0	206
soybean	2.7	200
corn	6.3	85
cotton	0.8	53
wheat	3.4	65

With the aforementioned factors in mind, the objectives of this experiment were to determine the quantity of swine effluent derived N taken up by soybean and estimate the degree of inhibition of symbiotic N-fixation. To accomplish these objectives, we used swine effluent labeled with $^{15}NH_4$. In addition, we assessed the potential for NO_3 leaching

when swine effluent is applied to soybean. An N budget was constructed for the plant-soil-water system by measuring accumulated ^{15}N in leachate and soil.

Materials and Methods

This study was conducted in a greenhouse located in the North Carolina State University Method Road Research Station in Raleigh, NC. The upper 15 cm of a Goldsboro sandy loam was collected from the Ap horizon of a field in Johnston County, North Carolina that was in a corn, wheat, soybean rotation. The soil was passed through a 2-mm sieve and subsequently limed to a pH of 6.0. Sixteen soil lysimeters constructed of PVC were used in the experiment (diameter =15 cm, length = 1 m). The bottom of each lysimeter contained a fitted ceramic plate to prevent soil from escaping, while also allowing leachate to pass through when suction was applied via a vacuum pump. Each lysimeter was filled with approximately 16 kg of soil and secured to a wooden frame in an upright position.

Based on initial lagoon effluent total N, fifteen liters of anaerobic swine lagoon effluent were spiked with 1.57 g of 81 atom % ^{15}N-enriched $(NH_4)_2SO_4$ in an effort to attain an ^{15}N enrichment of approximately 5 atom%. Mean final ^{15}N enrichment was measured at 5.399 atom % excess of natural ^{15}N abundance (Table 2). The ^{15}N-enriched effluent was then frozen in six separate plastic containers and thawed one day prior to application. Effluent sub-samples were taken each week from the thawed effluent and frozen until analysis.

The experiment was organized in a completely randomized design with four replications. Ten mL of a bradyrhizobia-containing solution were injected 7 cm beneath

the soil surface of all the columns. Lysimeters were randomly selected and seeded with either a nodulating or nonnodulating Lee soybean cultivar. The nodulating and nonnodulating cultivars were near-isolines of the Lee cultivar, differing solely by the ability to form nodules through symbiotic relationships with bradyrhizobia. The recessive gene (r_{j1}) of the nonnodulating isoline inhibits the formation of nodules by either inoculation or soil strains of bradyrhizobia (Hartwig, 1994). Soybean response to the addition of anaerobic swine effluent was determined by measuring N accumulation in pods, stems, and leaves. Measurements of ^{15}N accumulation in nodulating and nonnodulating soybean isolines allowed us to evaluate the effectiveness of nodulated soybean to recovery of swine effluent N.

Four lysimeters containing nodulating soybean were randomly selected to receive swine effluent applications. Four lysimeters containing nonnodulating soybean were also randomly selected to receive swine effluent applications. The remaining nodulating and nonnodulating soybean lysimeters received only water and served as controls. Beginning 21 d after planting, 275 mL of ^{15}N-enriched effluent was applied each week to selected lysimeters for six wk, equivalent to 370 kg total N ha^{-1}. According to North Carolina State Extension Service, 50% of the N contained in anaerobic swine effluent becomes plant available in the first year when applied through irrigation (Zublena et al., 1990). Using this assumption and also assuming that there are 2.2×10^6 kg soil ha^{-1} in the surface 15 cm of soil with a bulk density of 1.5 g cm^{-3}, approximately 185 kg PAN ha^{-1} was added as swine effluent to the selected lysimeters. Nodulating and nonnodulating controls received a volume of distilled water equal to the volume of effluent applied to the treated experimental units in order to equalize soil water content. All treated and control soybean

were watered daily or as needed. Two days after each effluent addition, lysimeters were intentionally over-irrigated with 0.5 to 2.0 liters of distilled water. One day later, 100 to 700 mL leachate was collected into flasks by applying approximately -0.17 MPa pressure to the lysimeters with a vacuum pump. After recording leachate volumes, sub-samples were collected in 125-mL plastic containers and frozen until time of analysis. Leachate volumes and N concentrations are recorded in Tables 7A-9A.

Three weeks after initial effluent addition (42 days after planting), one plant was removed so that each lysimeter contained two soybean plants. Soybean shoots removed at this time were separated into pods, leaves and stems, oven dried at 60°C and weighed. At maturity (~R8 growth stage, 72 days after planting), the two remaining soybean shoots in each lysimeter were clipped at the soil surface and separated into pods, leaves and stems. After oven drying at 60°C and recording oven-dry weights, plant separates from the two harvest periods (three plants/lysimeter) were combined for grinding. Plant matter was ground with a Wiley mill and passed through a 1 mm sieve. Subsamples were further ground to a fine powder using a Crescent Wig-L-Bug ball mill (Reflex Analytical Corporation). Plant dry matter was later analyzed for ^{15}N and total N using a CE Elantech NC2500 elemental analyzer (EA) coupled to a ThermoFinnigan DELTA^{Plus} continuous flow isotope ratio mass spectrometer (CF-IRMS). Briefly, 2.000 mg of ground plant matter were weighed into tin capsules and loaded onto the elemental analyzer. Samples were flash combusted at over 1,500 ºC, thereby converting all forms of N in the sample to N_2 gas. The N_2 gas was separated on an analytical column and then passed on to the mass spectrometer via a Conflo II open split interface. The N_2 gas was ionized and the resulting signals for ^{14}N-^{14}N, ^{14}N-^{15}N and ^{15}N-^{15}N isotopes recorded.

Soil Analysis

At the conclusion of the experiment, soil was removed, mixed, and a composite sample was taken from each lysimeter. Prior to taking soil samples, large root masses were removed and inspected for active N-fixation by observing pink coloration within nodules. Roots were then oven dried and weighed. Soil samples were oven dried (60°C) and ground to a fine powder using a Crescent Wig-L-Bug ball mill (Reflex Analytical Corporation). Ten mg soil were then weighed into small tin capsules and analyzed for ^{15}N and total N using a CE Elantech NC2500 elemental analyzer (EA) coupled to a ThermoFinnigan DELTA^{Plus} continuous flow isotope ratio mass spectrometer (CF-IRMS) as previously described.

Leachate and Effluent Analysis

Leachate and effluent NO_3 and NH_4 concentrations were determined colormetrically with a Lachat QuikChem 8000 Automated Ion Analyzer (Lachat, 1995). Nitrate-N concentrations were analyzed using QuikChem Method 10-107-04-1-A, while NH_4-N concentrations were analyzed using QuikChem Method 10-107-06-2-A (Lachat, 1995).

The North Carolina Department of Agriculture Waste Analysis Laboratory determined total Kjeldahl N in the swine effluent using the method developed by Bremner (1960). Total N application as swine effluent was calculated by multiplying the volume of effluent added with the total Kjeldahl N concentration. Values for effluent total N and inorganic N are reported in Table 2. Additional effluent nutrient analysis and effluent pH is found in Table 3.

Table 2. Atom % excess ^{15}N, inorganic N, and total N values of anaerobic swine effluent added to lysimeters.

Week	Atom % ^{15}N excess of NH_4-N	NH_4-N	Inorganic N added to lysimeters	Total Kjeldahl N	Total N added to lysimeters
		—mg L^{-1}—	—mg—	—mg L^{-1}—	—mg—
1	5.302	205	56	360	99
2	5.612	289	79	438	120
3	5.496	242	67	362	100
4	5.437	203	56	348	96
5	5.256	272	75	491	135
6	5.289	228	63	363	100
mean	5.399	240	66	394	108
total	-	-	396	-	650

Table 3. Selected chemical characteristics of anaerobic swine lagoon effluent used in lysimeter study. Values are means of 6 batches of effluent applied to lysimeters at weekly intervals.

pH	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
	—mg L^{-1}—								
7.8	136	342	210	95	71	6.6	2.0	3.8	1.3

Leachate and effluent samples were prepared for ^{15}N isotope analysis using a modified diffusion procedure developed by Brooks et al. (1989) and Stark and Hart (1996). To prepare the acid trap, glass fiber filter discs (7 mm diameter) were acidified with 10 μL $KHSO_4$ and placed between a double-layer of Teflon® tape (2.5-cm width). The tape was pressed together using plastic grommets (Coghlan's Snap and Tap Grommets) to form a sealed bi-layer. Grommets were secured to lids of 125 mL plastic specimen containers with duct tape. Nitrate and NH_4 diffusion was initiated by adding 0.2 g MgO and 0.4 g

Devarda's alloy to samples of leachate containing approximately 50 μg (NH_4 + NO_3)-N. An acid-washed glass bead was also dropped into each specimen container to ensure adequate mixing. Specimen containers were shaken on a vertical rotary shaker at 100 rpm for 72 hr. After shaking, grommets were removed from specimen containers and acidified discs were placed into small plastic weighing dishes. Weighing dishes were placed into a dessicator containing Drierite® and allowed to dry for 24 hr. To avoid cross-contamination by volatile NH_3, a small beaker of 50 mL concentrated H_2SO_4 was placed into the dessicator. After drying, discs were removed with tweezers and inserted into tin capsules. Tin capsules were folded into small cubes and placed in a sample storage well until time of analysis. Samples were analyzed for ^{15}N and total N using a CE Elantech NC2500 elemental analyzer (EA) coupled to a ThermoFinnigan DELTA^{Plus} continuous flow isotope ratio mass spectrometer (CF-IRMS) as previously described.

Calculations

By using ^{15}N as a tracer in the system, the efficiency of effluent N recovery by nodulated and nonnodulated soybean could be calculated. The amount of pod, stem, leaf and total shoot N derived from the effluent was calculated using an equation adapted from Hauck and Bremner (1976):

$$\mathbf{W = \frac{T(A_T - A_C)}{A_E}}$$

where: W = weight of pod, stem, leaf or total shoot N of nodulated or nonnodulated soybean derived from effluent (mg)

T = total weight of N in pod, stem, leaf or total shoot of nodulated or nonnodulated soybean (mg)

A_T = atom % excess ^{15}N in pod, stem, leaf or total shoot of effluent-treated nodulated or nonnodulated soybean

A_C = mean (n = 4) atom % excess ^{15}N in pod, stem, leaf or total shoot of nodulated or nonnodulated control soybean

A_E = atom % excess ^{15}N of NH_4-N in effluent

Atom % excess is defined as the atom % ^{15}N in plant or effluent minus standard ^{15}N natural abundance in the atmosphere (0.3663 atom % ^{15}N). The % pod N, leaf N, stem N and total shoot N derived from effluent N was calculated as follows:

$$\mathbf{\% \text{ pod N derived from effluent N} = (W_P / T_P) \times 100}$$

where: W_P = weight of pod N derived from effluent (mg)

T_P = weight of total pod N (mg)

$$\mathbf{\% \text{ leaf N derived from effluent N} = (W_L / T_L) \times 100}$$

where: W_L = weight of leaf N derived from effluent (mg)

T_L = weight of total leaf N (mg)

$$\textbf{\% stem N derived from effluent N} = (W_S / T_S) \times 100$$

where: W_S = weight of stem N derived from effluent (mg)

T_S = weight of total stem N (mg)

Total constituent N (pod, leaf, stem) was calculated by multiplying the dry weight of each constituent by the %N. The % whole shoot N derived from effluent N was calculated as follows:

$$\textbf{\% whole shoot N derived from effluent N} = \frac{\sum (P_E + L_E + S_E)}{\sum (P_T + L_T + S_T)}$$

where: P_E = pod N derived from effluent (mg)

L_E = leaf N derived from effluent (mg)

S_E = stem N derived from effluent (mg)

P_T = total pod N (mg)

L_T = total leaf N (mg)

S_T = total stem N (mg)

The % of effluent N recovered in the soybean pods, leaves, stems and whole shoots was calculated as follows:

% of effluent N recovered by pods = (W_P / T_E) x 100

where: W_P = weight of pod N derived from effluent (mg)

T_E = weight of total effluent N (mg)

% of effluent N recovered by leaves = (W_L / T_E) x 100

where: W_L = weight of leaf N derived from effluent (mg)

T_E = weight of total effluent N (mg)

% of effluent N recovered by stems = (W_S / T_E) x 100

where: W_S = weight of stem N derived from effluent (mg)

T_E = weight of total effluent N (mg)

% of effluent N recovered by whole shoots = (W_{SH} / T_E) x 100

where: W_{SH} = weight of whole shoot N derived from effluent (mg)

T_E = weight of total effluent N (mg)

In addition to determining effluent N recovery, ^{15}N measurements in the constituents of nodulating and nonnodulating soybean allowed us to estimate the plant N contribution by N-fixation and soil N. The percent soybean shoot N derived from N-fixation was calculated for nodulated soybean that received effluent additions by the general isotope dilution method equation described by Warembourg (1993):

% shoot N derived from fixation = (1- (E / C)) x 100

where: E = ^{15}N atom % excess of whole shoot in effluent treated nodulated soybeans

C = mean ^{15}N atom % excess of whole shoot in effluent treated nonnodulated soybeans

In order to obtain E and C, weighted ^{15}N atom % excesses were calculated for nodulated and nonnodulated soybean using the ^{15}N atom % excesses of pods, stems and leaves as follows:

$$\textbf{Whole shoot weighted } ^{15}\textbf{N atom \% excess} = \frac{[(P_E \times P_T) + (L_E \times L_T) + (S_E \times S_T)]}{N_T}$$

where: P_E = pod ^{15}N atom % excess

L_E = leaf ^{15}N atom % excess

S_E = stem ^{15}N atom % excess

P_T = total pod N (mg)

L_T = total leaf N (mg)

S_T = total stem N (mg)

N_T = sum of pod, leaf and stem N (mg)

The percent soybean shoot N derived from soil N was calculated for nodulated soybean that received effluent additions using the following equation:

100 – % effluent-derived N recovered by soybean shoot – % shoot N derived from fixation

Cumulative leachate N was calculated by multiplying the volume of leachate collected at each sampling time with the measured inorganic N concentration and summing the N for all six collection periods. The amount of effluent-derived N measured in the leachate was calculated for experimental units that receive effluent additions using an equation adapted from Hauck and Bremner (1976):

$$\mathbf{W = \frac{T(A_T - A_C)}{A_E}}$$

where: W = weight of leachate N derived from effluent (mg)

T = total weight of N in leachate (mg)

A_T = atom % excess ^{15}N in leachate (effluent-treated nodulated or nonnodulated soybean)

A_C = mean (n = 4) atom % excess ^{15}N in leachate (control nodulated or nonnodulated soybean)

A_E = atom % excess ^{15}N in effluent

Atom % ^{15}N excesses were calculated by subtracting 0.3663 from leachate or effluent atom % ^{15}N values. The percent of leachate N derived from the effluent was calculated as follows:

$$\mathbf{L = (W/E)*100}$$

where: L = % of effluent-derived N measured in leachate

W = weight of leachate inorganic N derived from effluent (mg)

E = weight of total N added in effluent (mg)

The amount of effluent-derived N measured in the soil was also calculated for experimental units that received effluent additions using the equation adapted from Hauck and Bremner (1976):

$$\mathbf{W = \frac{T(A_T - A_C)}{A_E}}$$

where: W = weight of soil N derived from effluent (mg)

T = total weight of N in soil (mg)

A_T = atom % excess ^{15}N in soil of effluent-treated nodulated or nonnodulated soybean

A_C = mean (n = 4) atom % excess ^{15}N in soil of control nodulated or nonnodulated soybean

A_E = atom % excess ^{15}N in effluent

Atom % ^{15}N excess was calculated by subtracting 0.3663 from measured soil atom % ^{15}N values. The percent of soil N derived from the effluent was calculated as follows:

$$\mathbf{L = (W/E)*100}$$

where: L = % of effluent-derived N measured in soil

W = weight of soil N derived from effluent (mg)

E = weight of total N added in effluent (mg)

In experimental units that received effluent additions, gaseous N losses due to NH_3 volatilization and denitrification were estimated using the following equation:

Gaseous N losses = 100 – % effluent-derived N recovered by soybean shoot – % effluent-derived N remaining in soil – % effluent-derived N in leachate

The main effects of nodulation and effluent on shoot weight and N in pods, leaves, stems, shoots, leachate and soil, as well as nodulation x effluent interactions, were determined using the GLM Procedure of the Statistical Analysis System (SAS Institute, 1998). Regarding leachate N, the procedure used tested for effluent and nodulation main effects on leachate N that had accumulated by week 6, but not for a time effect. For all statistical analyses, the GLM Procedure assumes that the variance is homogenous for all treatments, thus a pooled estimate of the variance is computed from all the data and standard errors are the same for all treatment combinations. By using a pooled standard error, SAS calculated a better estimate compared with using individual standard deviations to compute standard error. Variances were confirmed to be homogeneous across treatments by plotting the residuals against predicted values. Proc GLM was also used to determine if there were statistical differences between pod-, leaf-, stem- and total shoot-^{15}N accumulation, % effluent-derived N in leachate, % effluent-derived N remaining in soil and % estimated effluent-derived N lost through gaseous emissions in effluent-treated nodulating and nonnodulating soybean.

Results and Discussion

Effluent addition had a significant ($p < 0.05$) effect on shoot (pods + stems + leaves) weight, but nodulation did not (Figure 1). There was no significant effluent x nodulation interaction on shoot weight. On average, the addition of effluent increased shoot weight by 30.2 g in the nodulated soybean and by 21.0 g in the nonnodulated soybean compared to control nodulated and nonnodulated soybean. The non-significant effect of nodulation on shoot weight is puzzling since there was a significant nodulation effect on shoot N ($p < 0.05$). Figure 1 illustrates that the weights of nodulated and nonnodulated controls were nearly identical, suggesting that the nodulated soybean were actively fixing N, but the N derived from fixation did not contribute to increased shoot mass. One possible explanation to this phenomenon is that at some point, N was no longer the limiting nutrient to plant growth. Other plant nutrients such as P, K or Ca may have inadvertently become the limiting factor after N was produced or taken up in sufficient amounts. The loss of foliage may have also played a part in the conflicting results since some leaves dropped off before harvest and were not collected. It may be possible that more leaves dropped off of the nodulating soybean relative to the nonnodulating soybean in non-random manner.

Statistical analysis revealed a significant ($p<0.05$) effluent by nodulation interaction on pod N (Figure 2), leaf N (Figure 3), total shoot N (Figure 4) and shoot N concentration (Figure 1A and Table 1A). No such interaction existed for stem N (Figure 5). Pod N, stem N, leaf N and whole shoot N also increased significantly ($p<0.05$) with nodulation and the addition of swine effluent. The effect of effluent addition on N accumulation in soybean supports findings in previous research where fertilizer N or easily degradable organic N increased shoot N concentration in soybean (Afza et al., 1987;

Wesley et al., 1998; Seneviratne et al., 2000). The significant interactions indicate that the addition of effluent and nodulation were both important N sources to growth, but when the two factors are combined, nodulated and nonnodulating soybean behave differently when they receive effluent additions. The differences in response are possibly due to physiological differences in the cultivars. Although the nodulating and nonnodulating Lee cultivars are theoretically very similar, there may have been differences in root structure that affected N uptake.

As observed in previous experiments, most of the shoot N at the final harvest was concentrated in the pods (Figure 6). The percentage of shoot N found in the pods was similar for all treatment groups, averaging 66% of the total shoot N. Leaves accounted for 25% of the total shoot N and stems the remaining 9%. Since soybean seed generally has a relatively high protein content, these results were not surprising.

The addition of effluent did not significantly ($p=0.33$) increase the amount of accumulated inorganic N removed from the system via leaching, nor did the presence of nodules ($p=0.58$) (Figure 7 and Table 2A). There was an initial large increase in inorganic leachate N in the first two leaching events, followed by a relatively small accumulation of N over the next four weeks. In 80% of the leachate samples, more than 95% of the inorganic N was in the form of NO_3. The NO_3-N concentrations decreased to near zero over the six-week sampling period (Figure 8). The NO_3-N concentration in the leachate collected one week following effluent application ranged from 199 mg L^{-1} in the effluent-treated nonnodulated soybean to 350 mg L^{-1} in the effluent-treated nodulated soybean. Nitrification of added effluent accounted for some of the collected NO_3 as evidenced by slightly elevated concentrations of ^{15}N in effluent treatments (Figure 10). However, as

previously stated, the addition of effluent did not account for significant differences in leachate N. It is probable that significant N mineralization and nitrification of soil organic matter had occurred before the columns were intentionally over-irrigated and this accounted for the high inorganic N concentrations collected from all experimental units during the first few weeks of leaching.

After the initial leaching front passed through the soil, there was only a small increase in leachate inorganic N for all treatments, suggesting that even with the addition of swine effluent, very little NO_3 was lost from the soil due to leaching. As the soybean grew larger over the period of the experiment, the plant N requirement probably increased, resulting in lower soil NO_3 concentrations and less NO_3 lost by leaching. Another possible explanation for the small amount of NO_3 collected in the leachate three weeks after the initial addition of swine effluent is that only 100 to 700 mL of leachate was collected each week. Since the soil in the lysimeters had an estimated pore volume of 6700 cm^3, only a fraction of the soil water would have been collected, leaving most of the effluent N in the soil. Even though some of the ^{15}N labeled effluent was removed from the lysimeters in the leachate (Figures 9 and 10), less than 1% of the added effluent N was accounted for in the leachate. Additionally, nodulation did not significantly increase the mg ^{15}N measured in leachate ($p=0.53$) (Table 4A). Since very little effluent N was lost by leaching, these results are encouraging given that some fields receiving swine effluent may have high concentrations of NO_3 early in the growing season after springtime applications of effluent. If soils in spray fields behave similarly to the soils in the present study, there is little risk of losing substantial NO_3 through leaching after applications of swine effluent at agronomic rates.

Nodulation did not significantly ($p=0.87$) increase total soil N present at the end of the experiment nor did the addition of effluent ($p=0.56$) (Table 2A). Total soil N averaged 772 kg/ha across all treatment groups and accounted for most of the measurable N in the lysimeter systems (Figure 11). The addition of effluent significantly ($p<0.05$) increased the ^{15}N atom % excess measured in the soil at the conclusion of the study, but nodulation had no significant effect on soil ^{15}N atom % excess ($p=0.96$) (Table 3A). Of the effluent N added to nodulated soybean, 37% remained in the soil after the soybean were harvested, while 33% remained in the soil in the effluent-treated nonnodulated soybean (Figure 12). With over one-third of the effluent N remaining in the soils after the soybean were harvested, this probably implies that N was applied in excess of soybean needs. However, since relatively small subsamples of soil were ground for ^{15}N analysis, (1.0 g soil), it is difficult to assume that soil samples accurately represented the soil N and ^{15}N for the entire volume of soil in the lysimeters. Additionally, since 40% of the effluent N was initially present in an organic form (Table 2), the measured soil ^{15}N in the effluent-treated soils only represents the portion of initial inorganic effluent N remaining in the soil. Effluent N present as organic compounds could not be traced with the experimental procedures employed, although we assume that some of the organic N probably mineralized during the experiment, adding to the pool of inorganic N derived from the addition of effluent.

A significant proportion of ^{15}N added in the swine effluent was never recovered (Figure 12). On average, 24% of the effluent ^{15}N was not recovered from the nodulated lysimeters while 33% of the ^{15}N was not recovered from the nonnodulated lysimeters. As previously mentioned, the North Carolina Department of Agriculture recommends a plant

availability coefficient of 0.5 for effluent that is applied through irrigation. The results from our experiment indicate that 67 to 76 % of the applied effluent was plant available (248 to 281 kg PAN ha^{-1}), significantly higher than NCDA's recommendation. Nitrogen losses were probably smaller because of limited NH_3 volatilization in the greenhouse setting. As previously discussed, most of the N in anaerobic swine lagoon effluent was present as NH_4, which is subject to volatilization in lagoons and when applied to plant or soil surfaces. The proportion of effluent N present as NH_4 and NH_3 is largely regulated by the solution pH, with NH_3 formation being favored above pH 9.3 (Havlin, 1999). Ammonia volatilization is often a mechanism of N loss under field conditions because of the inherent alkalinity of swine effluent. Even at a pH below 9.3, a significant proportion of NH_3 is usually present in swine effluent, which is subject to volatilization. Effluent is also usually applied with a sprinkler system, where small droplets with a relatively large surface area are emitted, increasing the potential for NH_3 volatilization. Hot, windy conditions also promote NH_3 volatilization, which are commonly encountered in field settings. However, in the present greenhouse experiment, effluent was applied by pouring it from a beaker, with little to no wind present at time of application. Therefore it is unlikely that a significant portion of the unrecovered ^{15}N was volatilized to NH_3.

Denitrification, the microbial mediated reduction of NO_3 to N_2, may have also accounted for a significant portion of lost N. Denitrifing bacteria thrive in anaerobic environments where easily decomposable organic matter is present. The effluent and soil certainly provided sources of soluble organic C, but it is not clear whether or not anaerobic conditions existed in the lysimeters. Since the soils in the lysimeters were irrigated enough to promote weekly collection of leachate, water may have accumulated in the bottom of the

lysimeters prior to removal with the vacuum pump. If the accumulated water had become stagnant enough to develop anaerobic conditions, denitrification may have occurred in the anaerobic region. Denitrification probably did not occur in the first two weeks following application of swine effluent since NO_3-N concentrations varied from 82 mg L^{-1} to 350 mg L^{-1}in the leachate (Figure 8). After the second week, it is possible that effluent N had nitrified and leached into the anaerobic region, where it was subsequently denitrified. However, redox measurements would be required to confirm the presence of reduced conditions.

In addition to volatilization and denitrification losses, some of the added effluent ^{15}N was probably present in the roots, which were not analyzed for total N or ^{15}N due to sample processing difficulties. Large root masses were removed prior to taking soil samples and subsequently oven dried and weighed, but excess soil adhering to roots preventing grinding for total N and ^{15}N analysis. Since total root N generally makes 7 to 10% of total plant N in mature soybean (D.W. Israel, personal communication, 2003), a proportion of the unrecovered ^{15}N could probably be accounted for in the roots.

Nodulation did not significantly increase the percentage of effluent N recovered by pods, stems or leaves (Table 5A-6A). Nodulated soybean that received effluent additions recovered an average of 36.6% of the total effluent ^{15}N added, compared to 33.4% for nonnodulated soybean that received effluent. The lack of significance suggests that the higher mass of shoot N measured in the nodulated soybean (Figure 4) was due to N-fixation. Although there were no statistical differences in effluent N recovered by nodulated and nonnodulated soybean, there were differences in the percent of whole shoot N derived from effluent. The effluent-treated nodulated soybean had three possible N

sources: soil N, effluent N and atmospheric N-fixation. On average, 17.5% of the whole shoot N in the nodulated soybean came from the effluent N, 27.2% originated from the soil and 55.2% was derived from atmospheric N-fixation. For the non-nodulated soybean treatments, 39.2% of the whole shoot N came from the effluent N and 60.8% originated from the soil.

Nitrogen fixation was not fully inhibited by the addition of swine effluent in this experiment, and other studies have also shown that N-fixation is often not completely inhibited when an external N source is available to soybean. Israel and Mikkelsen (2001) determined that in nodulated soybean supplied with 200 kg PAN ha^{-1} as anaerobic swine effluent, 19-36% of the harvested seed N was derived from N-fixation. Angle et al. (1992) showed that soybean shoot weight and N content increased with the pre-plant addition of sewage sludge up to 312 kg PAN ha^{-1}, while the contribution from N-fixation was significantly reduced from 51% to 14%. In the same study, there was no difference between the number and weight of soybean nodules in soybean receiving sludge applications and unfertilized control soybean. The authors hypothesized that nodules had probably developed after the readily available N from the sludge was no longer present. In our study, nodules were probably already developed by the time of effluent additions and therefore accounted for a greater proportion of shoot and seed N due to atmospheric fixation.

Conclusions

Biological N-fixation in soybean was not completely inhibited when swine effluent was added at a rate of 185 kg PAN ha^{-1} and accounted for 55% of the total N in the shoot.

Nodulated and nonnodulated soybean plants recovered similar amounts of effluent N in pods and in whole shoots. Effluent N recovered by nodulated and nonnodulated soybean shoots was 36.6 and 33.4%, respectively. The addition of effluent and nodulation were both important sources of N for growth, although our results suggest that nodulating and nonnodulating soybean behave differently when they receive effluent additions as indicated by significant interactions.

One of the main objectives of the experiment was to determine the potential for NO_3 to leach when swine effluent is applied to soybean at an agronomic rate. Using ^{15}N measurements, our data shows that less than 1% of the added effluent N was accounted for in the leachate. After most of the pre-existing native soil NO_3 had been removed in the first two weeks of leaching, NO_3-N concentrations in the leachate of effluent-treated nodulating and nonnodulating soybean were well below the EPA drinking water threshold value of 10 mg L^{-1}.

Future research should focus on analyzing the timing of effluent applications and how soybean responds to the added N during different growth stages. Our results suggest that soybean responds to applications of swine effluent prior to pod fill, however there may be a different response when effluent is applied at an earlier or later stage of growth. These results indicate that nodulated soybean could serve as an effective receiver crop of anaerobic swine effluent while maintaining environmental quality standards. These findings also support the notion that large amounts of N could be removed from spray fields by harvesting soybean seed. Since soybean is already a commonly grown crop in the North Carolina Coastal Plain, seed could be exported out of areas with concentrated animal production units, thus reducing regional nutrient surpluses.

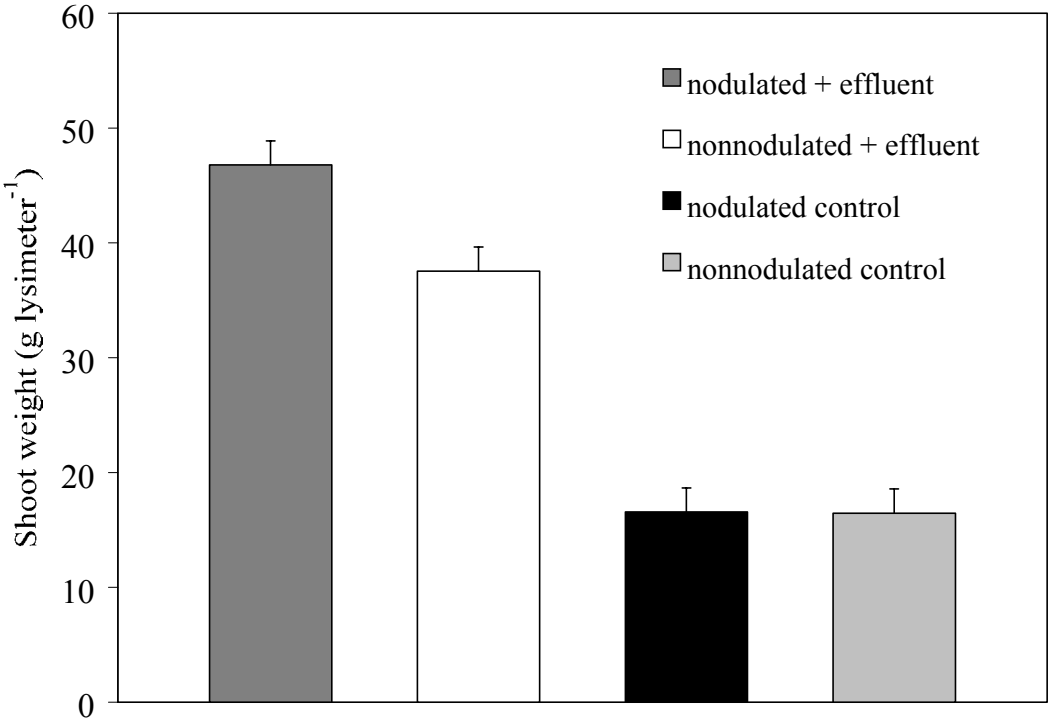


Figure 1. Mean dry weight of soybean shoots with thinned soybean and soybean harvested at conclusion of experiment combined. Bars indicate experimental standard error (n=4).

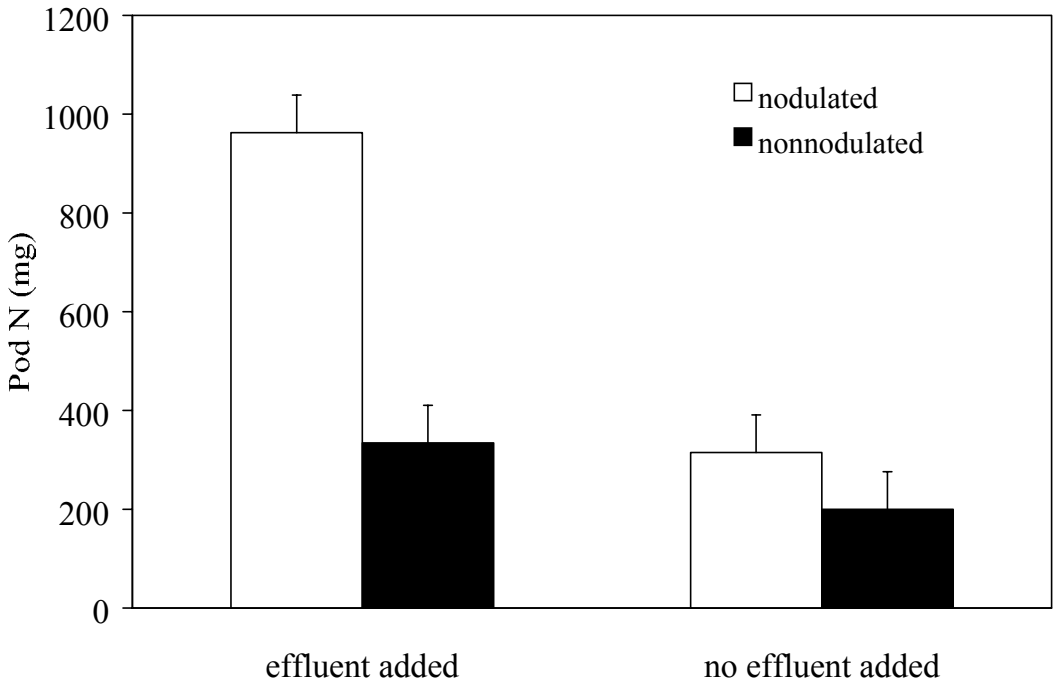


Figure 2. The effect of effluent addition on pod N of nodulated and nonnodulated soybean with thinned soybean and soybean harvested at conclusion of experiment combined. A significant ($p < 0.05$) interaction exists for effluent*nodulation. Bars indicate experimental standard error (n=4).

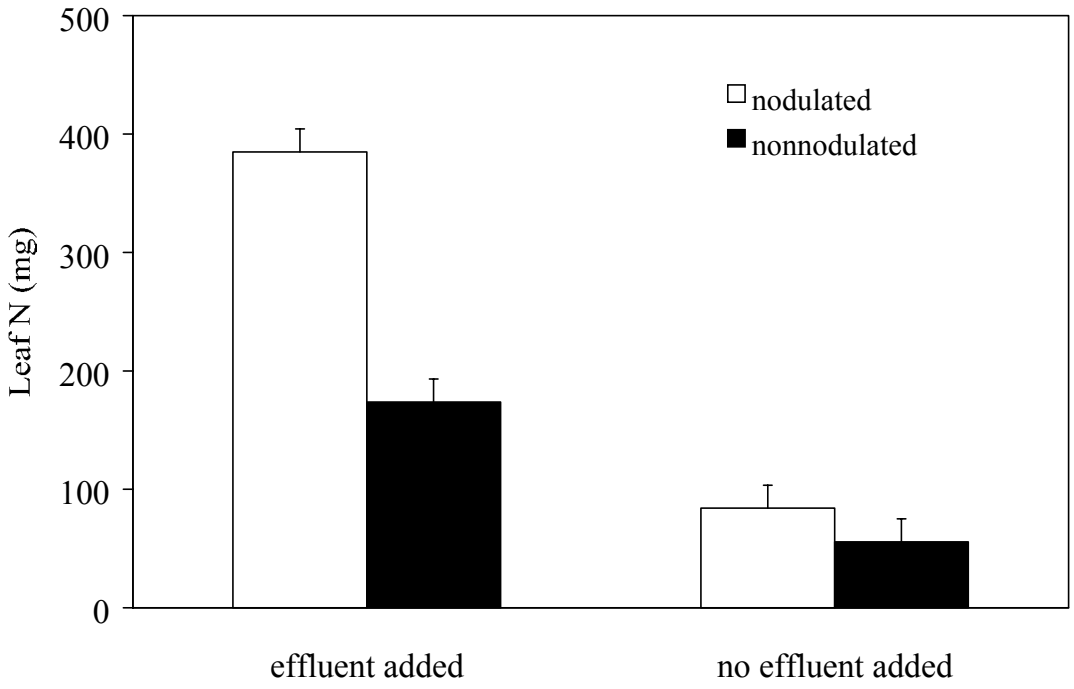


Figure 3. The effect of effluent addition on leaf N of nodulated and nonnodulated soybean with thinned soybean and soybean harvested at conclusion of experiment combined. A significant ($p < 0.05$) interaction exists for effluent*nodulation. Bars indicate experimental standard error (n=4).

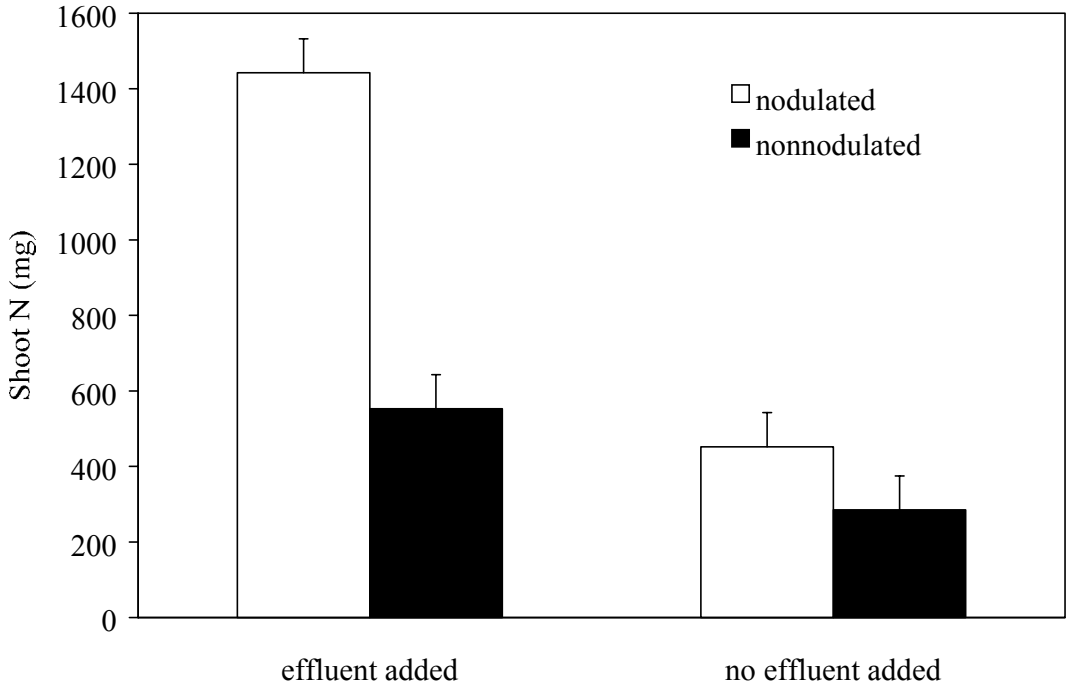


Figure 4. The effect of effluent addition on shoot N of nodulated and nonnodulated soybean with thinned soybean and soybean harvested at conclusion of experiment combined. A significant ($p < 0.05$) interaction exists for effluent*nodulation. Bars indicate experimental standard error (n=4).

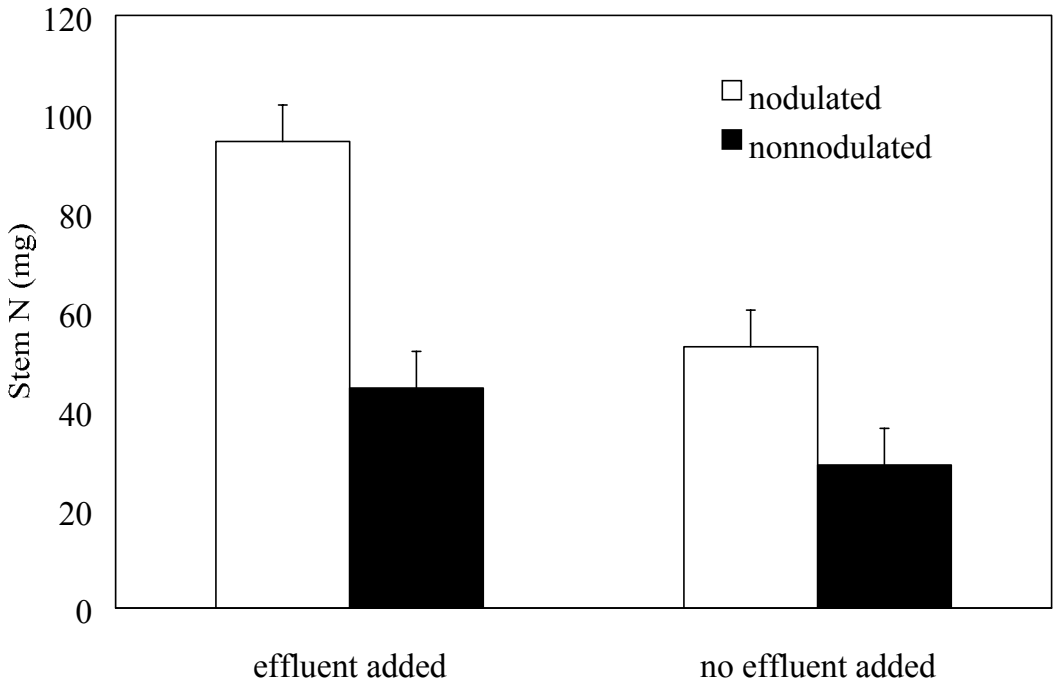


Figure 5. The effect of effluent addition on stem N of nodulated and nonnodulated soybean with thinned soybean and soybean harvested at conclusion of experiment combined. No interaction exists for effluent*nodulation. Bars indicate experimental standard error (n=4).

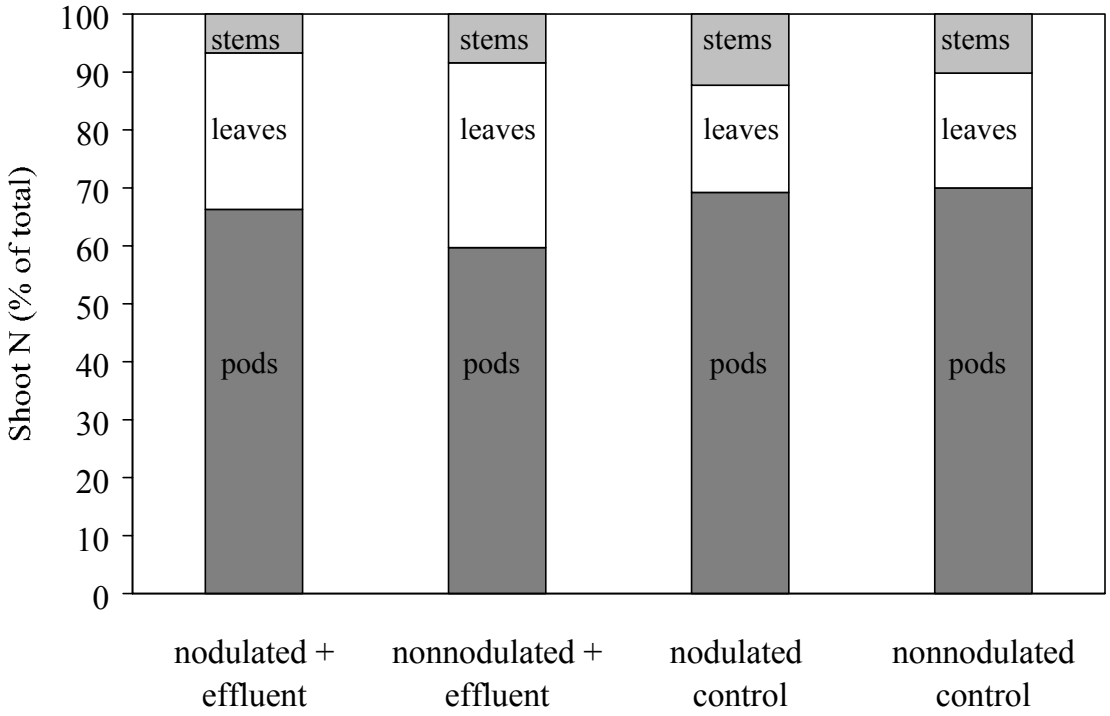


Figure 6. Nitrogen distribution in soybean pods, stems and leaves (thinned soybean and soybean harvested at conclusion of experiment combined).

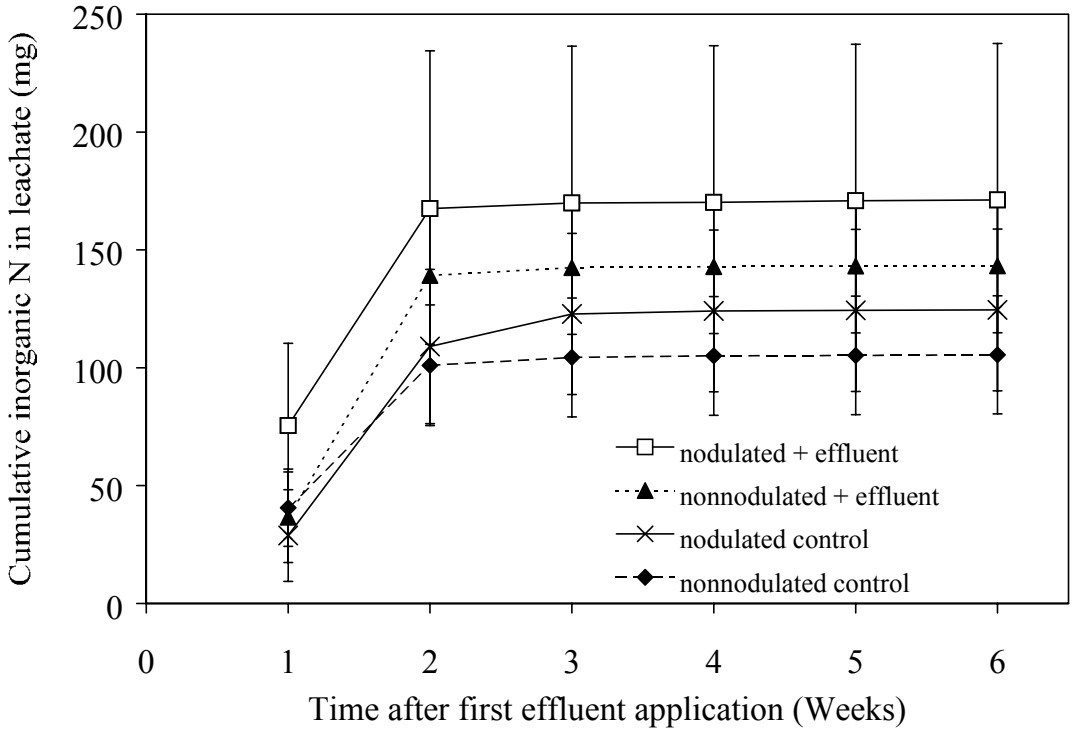


Figure 7. Cumulative inorganic N (NO_3-N + NH_4-N) over a six-week period. Bars indicate experimental standard error (n=4).

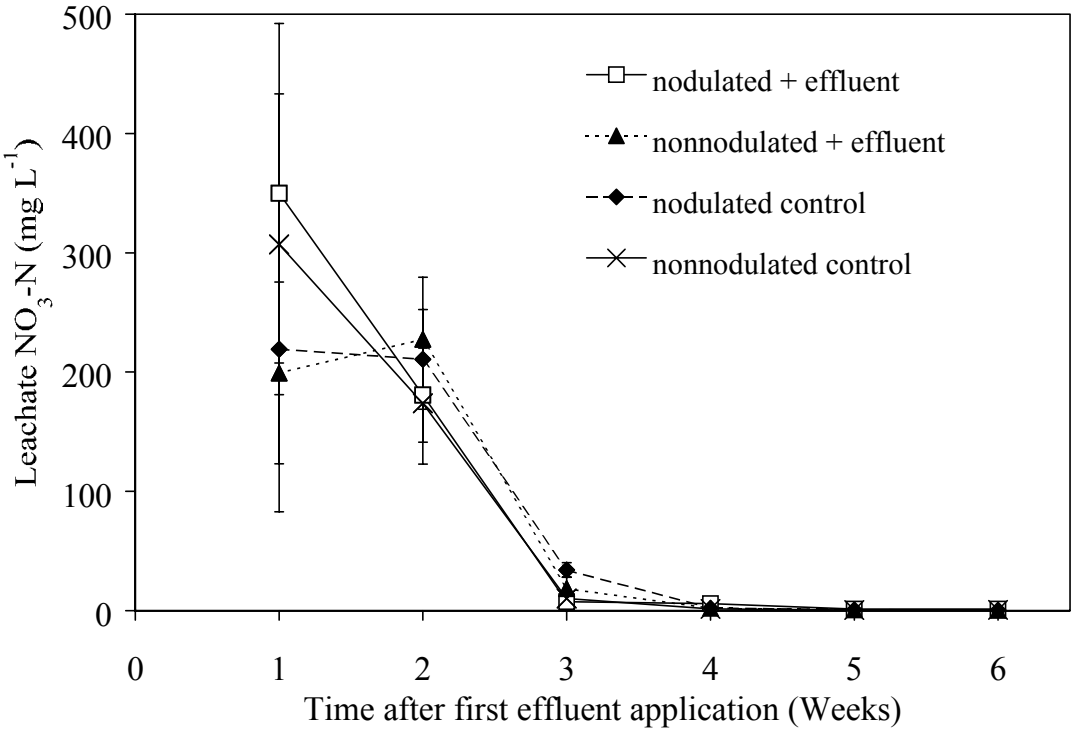


Figure 8. Average NO_3-N concentrations in leachate collected each week for six weeks after initial effluent application. Bars indicate experimental standard error (n=4).

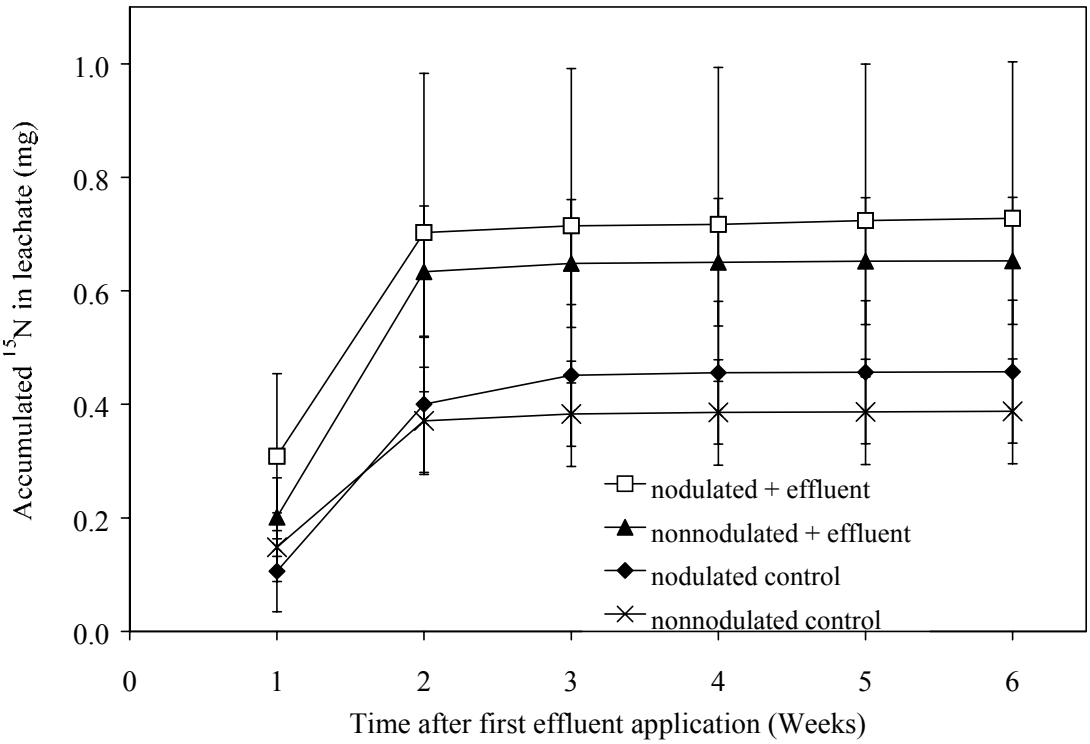


Figure 9. Mass of ^{15}N accumulation in leachate collected over a period of 6 weeks with or without swine lagoon effluent applied to nodulated or nonnodulated soybean. Bars indicate experimental standard error (n=4).

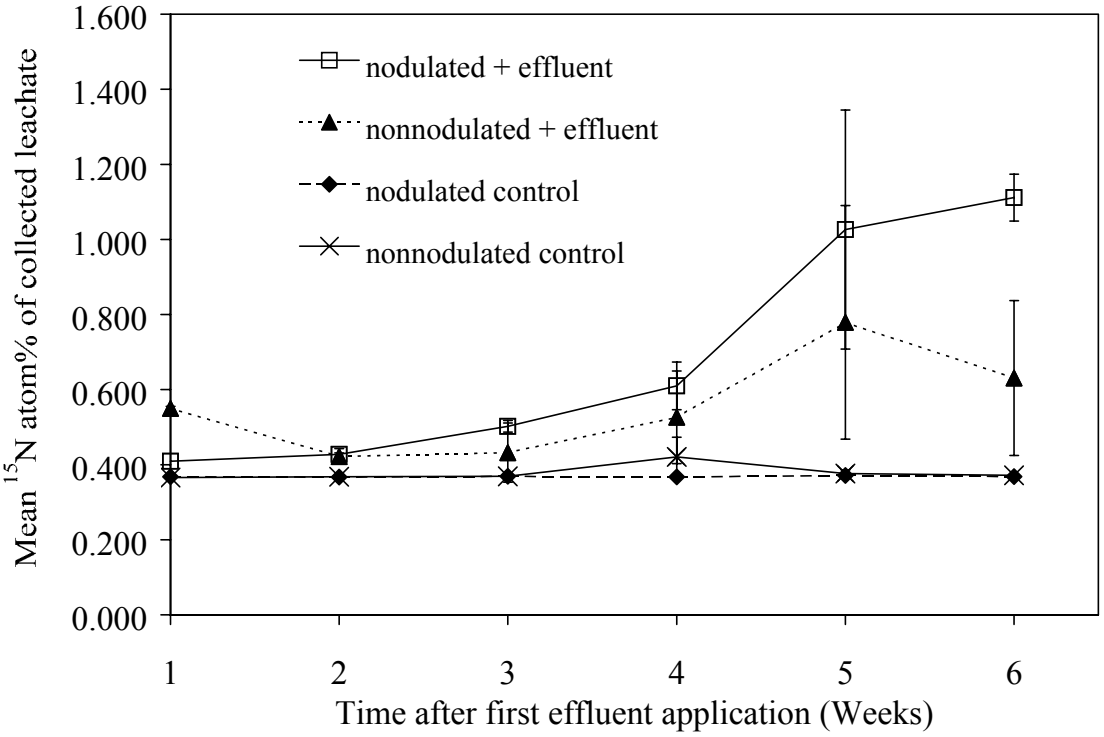


Figure 10. Mean ^{15}N atom % of leachate collected over a period of 6 weeks with or without swine lagoon effluent applied to nodulated or nonnodulated soybean. Bars indicate experimental standard error (n=4).

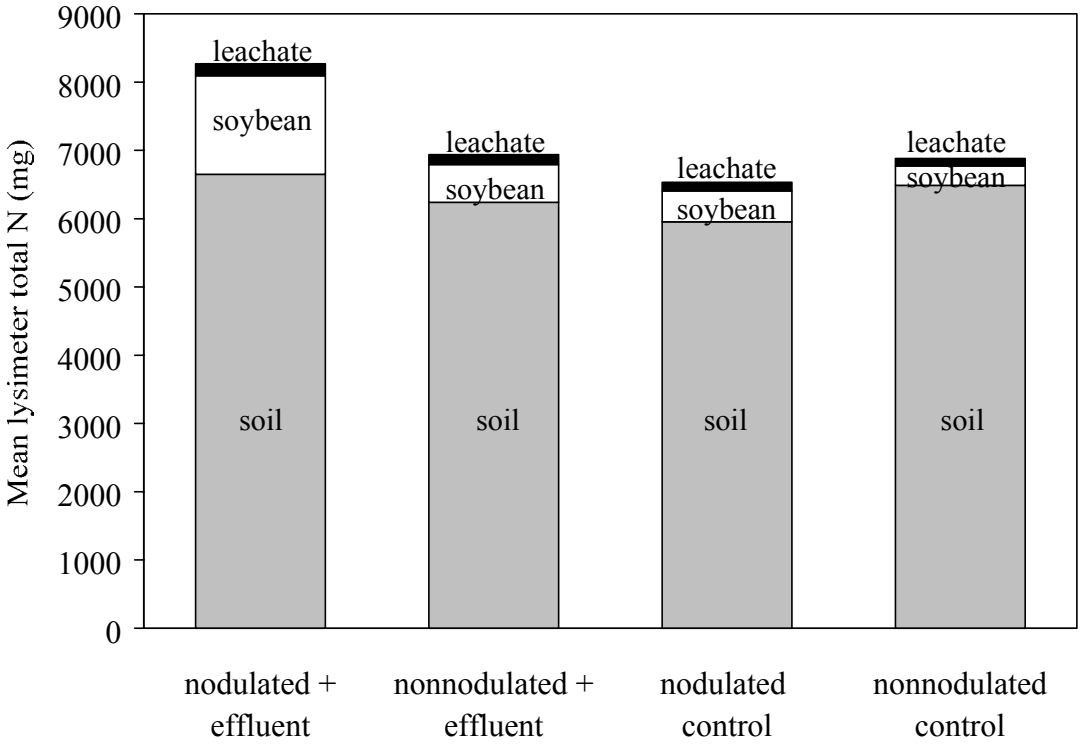


Figure 11. Nitrogen distribution in soybean, soil and leachate at the conclusion of the experiment as influenced by swine lagoon effluent and nodulated or nonnodulated soybean.

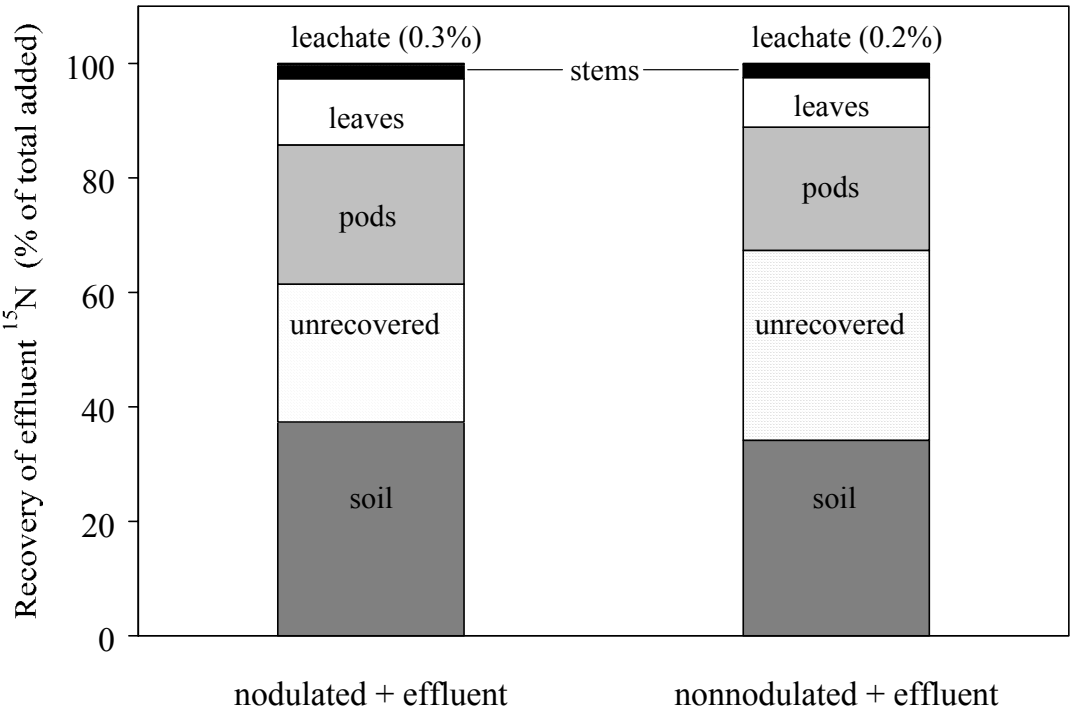


Figure 12. Recovery of ^{15}N as a percent of the total ^{15}N added in swine lagoon effluent to nodulated and nonnodulated soybean.

References

Afza, R., G. Hardarson, F. Zapata, and S.K.A. Danso. 1987. Effects of delayed soil and foliar N fertilization on yield and N2 fixation of soybean. Plant Soil. 97:361-368.

Andreadakis, A.D. 1992. Anaerobic digestion of piggery wastes. Water Sci. Technol. 25:9-16.

Angle, J.S., G.M. Madariaga, and E.A. Heger. 1992. Sewage sludge effects on growth and nitrogen fixation of soybean. Agric. Ecosyst. Environ. 41:231-239.

Avery, A. 2003. North Carolina Hog Farming and Water Quality: Time Series Analysis Fails to Reveal Significant Impacts. Hudson Institute, Center for Global Food Issues. [Online]. Available at http://www.cgfi.org/pdf/NCHogImpactReport.pdf. (verified May 19, 2003).

Bierman, S., G.E. Erickson, T.J. Klopfenstein, R.A. Stock and D.H. Shain. 1999. Evaluation of nitrogen and organic matter balance in the feedlot as affected by level and source of dietary fiber. J. Anim. Sci. 77:1645-1653.

Bremner, J.M. 1960. Determination of nitrogen in soil by the Kjeldahl method. J. Agric. Sci. 55:11-33.

Brooks, P.D, Stark, J.M., McInteer, B.B., and Preston, T. 1989. Diffusion method to prepare extracts for automated nitrogen-15 analysis. Soil Sci. Soc. Am. J. 53:1707-1711.

Burns, J.C., L.D. King, and P.W. Westerman. 1990. Long-term swine lagoon effluent applications on Coastal bermudagrass: I. Yield, quality, and element removal. J. Environ. Qual. 19:749-756.

Burton, G.W., J.E. Jackson, and R.H. Hart. 1963. Effects of cutting frequency and nitrogen on yield, in-vitro digestibility, and protein, fiber, and carotene content of Coastal bermudagrass. Agron. J. 55:500-502.

Deibert, E.J., M. Bijeriego, and R.A. Olson. 1979. Utilization of ^{15}N fertilizer by nodulating and non-nodulating soybean isolines. Agron. J. 71:717-723.

DiPietre, D. 2001. Proc. Annu. North Carolina Pork Conf., 45th, New Bern, North Carolina. 9-10 Jan. [Online]. Available at http://mark.asci.ncsu.edu/NCPorkConf/2001/dipietre.htm (verified May 19, 2003).

Evans, R.O., P.W. Westerman and M.R. Overcash. 1984. Subsurface drainage water quality from land application of swine lagoon effluent. Trans. ASAE. 27: 473-480.

Geyer, L.L. and M. Findley. 1994. Legal guidelines for swine manure management. Pork Industry Handbook. N.C. Cooperative Extension Service.

Harper, J.E. 1974. Soil and symbiotic nitrogen requirements for optimum soybean production. Crop Sci. 14:255-260.

Hartwig, E.E. 1994. Registration of near isogenic soybean germplasm lines D68-0099 and D68-0102, differing in ability to form nodules. Crop Sci. 34:822.

Hauck, R.D., and Bremner, J.M. 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 28:219-260.

Heichel, G.H., and D.K. Barnes. 1984. Opportunities for meeting crop nitrogen needs from symbiotic nitrogen fixation. P. 49-59. In D.F. Bezdicek et al. (ed.) Organic farming: Current technology and its role in a sustainable agriculture. ASA Spec. Publ. 46. ASA-CSSA-SSSA, Madison, WI.

Hinson, K. 1975. Nodulation response from nitrogen applied to soybean half-root systems. Agron. J. 67:799-804.

Hubbard, R.K. and J.M. Sheridan. 1983. Water and nitrate-nitrogen losses from a small, upland, Coastal Plain watershed. J. Environ. Qual. 12:291-295.

Hubbard, R.K., R.G. Williams and M.D. Erdman. 1989. Chemical transport from Coastal Plain soils under simulated rainfall: I. Surface runoff, percolation, nitrate, and phosphate movement. Trans ASAE. 32: 1239-1248.

Israel, D.W. and R.L. Mikkelsen. 2001. Soybean as a receiver crop for nitrogen in swine lagoon effluent. Soil Sci. 166: 453-464.

Jackson, G.D. Keeney, D. Curwen, and B. Webendorfer. 1987. Agricultural Management Practices to Minimize Groundwater Contamination. Environ. Resources Center, Univ. of Wis., Dept. Nat. Resources. Madison, Wis.

Johnson, J.W., L.F. welch, and L.T. Kurtz. 1975. Environmental implications of N fixation by soybeans. J. Environ. Qual. 4:303-306.

Lachat Instruments. 1995. QuikChem 8000 continuum series automated ion analyzer, Lachat, Inc., Milwaukee, WI.

Mikkelsen, R.L. 1995. Swine waste disposal dilemma- a case study. J. Natural Res. Life Sci. Educ. 24:169-172.

Mueller, J.P., J.T. Green, D.S. Chamblee, J.C. Burns, J.E. Bailey, and R.L. Brandenburg, 1993. Bermudagrass management in North Carolina. North Carolina Cooperative Extension Service, AG-493, p.14.

North Carolina Agricultural Statistics Division. 2003. [Online]. Available at http://www.ncagr.com/stats/livestoc/anihgi03.htm (verified May 19, 2003).

North Carolina State University—Soil Science Spatial Information Research Lab. 2001. North Carolina nutrient management database project. [Online]. Available at http://www.spatiallab.ncsu.edu/nutman/main.htm (verified Aug. 25, 2003).

North Carolina Water Pollution Control System Operators Certification Commission. 2003. Certification Training for Operators of Animal Waste Management Systems. [Online]. Available at http://www.soil.ncsu.edu/certification/Manual/a/intro_a.htm. (verified May 29, 2003).

Powlson, D.S. and D. Barraclough. 1993. Mineralization and assimilation in soil-plant systems. p. 209-242. *In* R. Knolwes and T.H. Blackburn (ed.) Nitrogen isotope techniques. Academic Press, San Diego.

Rhodes, V.J. and G. Grimes. 1994. The structure of the U.S. pork industry. Pork Industry Handbook. N.C. Cooperative Extension Service. PIH-85.

SAS Institute. 1998. The SAS System for Windows, version seven. Cary, NC 27513.

Schmitt, M.A., J.A. Lamb, G.W. Randall, J.H. Orf, and G.W. Rehm. 2001. In-season fertilizer nitrogen applications for soybean in Minnesota. Agron. J. 93:983-988.

Seneviratne, G., L.H.J. Van Holm, and E.M.H.G.S. Ekanayake. 2000. Agronomic benefits of rhizobial inoculant use over nitrogen fertilizer application in tropical soybean. Field Crops Res. 68:199-203.

Stark, J.M. and S.C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. Am. J. 60:1846-1855.

Stone, K.C., P.G. Hunt, F.J. Humenik, and M.H. Johnson. 1998a. Impact of swine waste application on ground and stream water quality in an Eastern Coastal Plain watershed. Trans ASAE. 41:1665-1670.

Stone, K.C., P.G. Hunt, M.H. Johnson, and S.W. Coffey. 1998b. GLEAMS simulation of groundwater nitrate-N from row crop and swine wastewater spray fields in the Eastern Coastal Plain. Trans. ASAE. 41:51-57.

Stone, K.C., P.G. Hunt, M.H. Johnson, and T.A. Matheny. 1998c. Nitrate-N distribution and trends in shallow groundwater on an Eastern Coastal Plain watershed. Trans. ASAE. 41:59-64.

Streeter, J. 1988. Inhibition of legume formation and N_2 fixation by nitrate. CRC Crit. Rev. Plant Sci. 7:1-23.

Varvel, G.E., and T.A. Peterson. 1992. Nitrogen fertilizer recovery by soybean in monoculture and rotation systems. Agron. J. 84:215-218.

Warembourg, F.R. 1993. N_2 fixation in soil and plant systems. p.127-156. *In* R. Knolwes and T.H. Blackburn (ed.) Nitrogen isotope techniques. Academic Press, San Diego.

Weber, C.R. 1966. Nodulating and nonnodulating soybean isolines: II. Response to applied nitrogen and modified soil conditions. Agron. J. 58:46-49.

Wesley, T.L., R.E. Lamond, V.L. Martin, and S.R. Duncan. 1998. Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. J. Prod. Agric. 11:331-336.

Wright, M.J., and D.L. Davidson. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. Adv. Agron. 16:197-217.

Zublena, J.P., J.C. Barker, and J.W. Parker. 1990. Swine manure as a fertilizer source. North Carolina Agricultural Extension Service Technical Bulletin No. 439-4.

Chapter III

Effect of Soil pH on Nitrogen Transformation Processes in Soils Amended with Broiler Litter

Introduction

In recent years, attitudes concerning human nutrition and health have swayed U.S. consumers to change their eating habits. From 1984 to 1994, annual U.S. per capita consumption of turkey and chicken increased from 28.1 to 40.9 kg (USDA, 1994). Due to the change in consumption habits, the poultry industry has been impacted tremendously. Broiler production in the U.S. increased 51% from 1986 to 1994 (USDA, 1994) and approximately 60% of broiler production now takes place in the southeastern U.S. (USDA, 1998). North Carolina currently ranks fourth and second in broiler and turkey production, respectively (North Carolina Agricultural Statistics Division, 2003). In 2001, 43 million turkeys and 653 million broiler chickens were produced, accounting for over $2.2 billion in cash receipts (North Carolina Agricultural Statistics Division, 2003).

Due to the rapid growth of the poultry industry, producers must contend with large quantities of manure that are generated. In North Carolina, poultry manure is usually

mixed with bedding materials such as peanut hulls or wood shavings to form a litter (Barker and Zublena, 1993). Since approximately 99% of broilers in the U.S. are grown under confined conditions, accumulated litter must be periodically collected and disposed (Overcash et al., 1983). According to a national survey conducted in 1992, 90% of poultry litter is land applied on the poultry grower's own acreage, most often on land with a crop or forage (Carpenter, 1992). Broiler litter differs from swine effluent and other types of liquid manures because it consists of relatively dry materials, an important characteristic that facilitates collection and handling. In addition, poultry litter has a greater nutrient content than most other animal manures (Evers, 1998).

Because of favorable physical and chemical characteristics, broiler litter can serve as an effective plant nutrient source. In many cases poultry producers wish to maximize crop yields with the addition of poultry litter as an N source. When the goal of poultry litter application is to supply nutrients to crops, the application rate is usually based on the composition of the litter and the N needs of the crop. However, predicting N availability of broiler litter can be complex and unreliable. On average, 10% of the total N in fresh poultry litter is the form of NH_4, which can immediately become plant-available once land applied (Overcash et al., 1983). In order for the organic portion of N in broiler litter to become plant-available, it must first be mineralized.

Nitrogen mineralization is the biological oxidation of organic N to NH_4 mediated by aerobic heterotrophic microorganisms and to a lesser extent, anaerobic heterotrophs (Havlin et al., 1999). The fraction of organic N that will be mineralized within a growing season is termed potentially mineralizable N. An estimate of potentially mineralizable N of broiler litter is usually combined with an availability coefficient of inorganic N to form

a comprehensive model that can be used to predict plant-available N in a growing season. In calculating plant-available N from broiler litter in North Carolina, Barker and Zublena (1993) suggested that the following model could be employed:

$$PAN = VR(NH_4\text{-}N) + 0.5(ON)$$

where PAN is N (tons/yr) that is plant-available in the same year following application, VR is the availability coefficient of the litter, NH_4-N is the NH_4-N present in the applied litter and ON is the organic N applied in the waste (Barker and Zublena, 1993). The availability coefficient (VR) for the above equation is equal to 0.75 if the litter is incorporated and 0.25 if the litter is surface applied.

The North Carolina Department of Agriculture takes a slightly different approach for calculating PAN. The model currently in use is based on two factors: total N of broiler litter and application method. The model can be represented as:

PAN = Total N * Availability Coefficient

For broiler litter, the availability coefficient is .45 if litter is surface applied and .57 if incorporated. The difference in these availability coefficients is based on the assumption that NH_3 volatilization will be lower when litter is incorporated.

Although determining the NH_4 content of litter is relatively simple, measuring mineralizable N is much more difficult and inconsistent, resulting in questionable estimates of plant-available N. Typically, 90% of N in poultry litter is organic, with about 50% of the organic-N present in the form of uric acid (Overcash et al., 1983). Since the vast majority of N in broiler litter is in the organic form, accurate estimates of potentially mineralizable N are essential if reliable models for predicting plant-available N are to be used.

In order to obtain a more reliable model for predicting potentially mineralizable N in broiler litter, Gordillo and Cabrera (1997a and 1997b) conducted a series of incubations and determined that organic N in broiler litter could be classified into two pools—rapidly mineralizable or slowly mineralizable. The rapidly mineralizable organic N in broiler litter consisted primarily of uric acid, and to a lesser extent, compounds such as allantoin, oxonic acid, and hypoxanthine produced during uric acid decomposition. From their studies, Gordillo and Cabrera formed a model to predict potentially mineralizable N (fast + slow pools) using soil and litter characteristics. The litter characteristics in the model consist of uric acid and C/N ratio as predictor variables, whereas the soil characteristics include soil pH and sand content: water content at field capacity ratio. Their model is stated as follows:

$$PMN = [26.68 + 1.04UAN - 1.22C/N]\,[1.6 + 0.068SAND{:}WC - 0.1624pH]$$

where: PMN = potentially mineralizable N (g N kg^{-1} dry litter)

SAND = sand content (kg sand kg^{-1} dry soil)

WC = water content at field capacity (kg water kg^{-1} dry soil)

UAN = uric acid nitrogen (g N kg^{-1} dry litter)

C/N = carbon: nitrogen ratio

pH = soil pH

Upon close examination, one can observe that the model predicts a negative linear relationship between PMN and soil pH. In other words, the model predicts that under the experimental conditions (soil pH between 5.1 and 7.0), PMN will decrease as initial soil pH increases. A comparison of the model proposed by Gordillo and Cabrera with that

currently recommended by the North Carolina Department of Agriculture is shown in Figure 1. The NCDA model predicts a constant PAN as soil pH changes, while Gordillo and Cabrera's model predicts that PAN decreases in a linear fashion as pH increases. In comparing the two models at soil pH 4.4, Gordillo and Cabrera's model predicts 36 g PAN kg^{-1} dry litter, 29% higher than that predicted by the NCDA model (28 g PAN kg^{-1} dry litter). At a soil pH of 7.0, the predicted difference between the two models is less, with Gordillo and Cabrera's model predicting 14% less PAN than the NCDA model. If the pH parameter in Gordillo and Cabrera's model is valid, potential agronomic and environmental incentives merit a full examination of the role that soil pH plays in N mineralization of broiler litter.

Many studies have confirmed that soil acidity inhibits nitrification in soils (Harmsen and van Schreven, 1955; Nyborg and Hoyt, 1978), however, the effect that soil acidity has on the process of ammonification is unclear. Many studies have suggested that N mineralization is not inhibited by low soil pH. Goovaerts and Chiang (1993) found that soil acidity did not influence the amount of mineralized N measured by anaerobic methods in fallowed soils. Olsen et al. (1970) applied dairy manure to soils at pH 4.5 and 7.3 and found similar amounts of net N mineralized after 21 weeks. Thompson et al. (1954) measured N mineralization in 25 pairs of virgin and cultivated soils and reported that soil pH had no significant effect on N mineralization. In order to avoid rapid artificial changes in soil pH, Dancer et al. (1973) measured N mineralization on soils that had been limed for 3 years and found similar amounts of mineralized N after 35 days of aerobic incubation. In a study by Williams (1994), un-limed soils accumulated more inorganic N than limed soils when different plant materials were added to the soils in a 16-week laboratory incubation.

Other studies have reported that when acid soils are limed, mineralizable N increases (Harmsen and van Schreven, 1955). Singh and Beauchamp (1986) limed two cropped acid soils in Ontario and found that more native soil organic N had mineralized to NH_4 after a 60-day incubation period compared with un-limed soils. Nyborg and Hoyt (1978) concluded that although liming increased the rate of N mineralization, the effect was temporary and did not affect the total mineralizable N. Given the conflicting results obtained in previous research, it is likely that the conclusions of these studies only apply to a small range of soil types or specific environmental circumstances.

In speculating on the nature of the pH parameter in their model, Gordillo and Cabrera (1997b) suggested that in their experiment, "the microbes performed better under slightly acidic conditions." However, their experimental design did not allow the direct effect of soil pH on PMN to be determined. Their results could also be explained by assuming that microorganisms simultaneously consumed (assimilated) relatively more inorganic N in the soils at higher pH, thus giving the appearance that the inorganic N pool was larger in the acid soils and more N mineralization had occurred. In this scenario, gross N mineralization (inorganic N production) may not have differed at all between the soils. If gross N ammonification rates were equivalent to net N ammonification rates and immobilization was not favored at high soil pH, enhanced N mineralization due to microbial adaptation of acid conditions is probably a correct assumption. Gross mineralization rate measurements require the use of isotopes and were not attempted in the study conducted by Gordillo and Cabrera, so such conclusions cannot be made with their data.

The purpose of our experiment was to determine how potentially mineralizable N of broiler litter is affected when applied to soils differing in pH. To accomplish this objective, we measured net N mineralization. Additionally, in order to separate inorganic N production and consumption processes, we measured gross mineralization, nitrification and immobilization using ^{15}N isotope pool dilution techniques.

Materials and Methods

Soil was collected from the top 20 cm of a forested area mapped as a Wagram loamy sand (loamy, siliceous, thermic Arenic Paleudults) located on the North Carolina State University Central Crops Research Station near Clayton, NC. The soil was subsequently air dried and passed through a 2 mm sieve. Subsamples were sent to the North Carolina State Soil Testing Laboratory for routine soil analysis. Soil chemical characteristics are summarized in Table 1. The soil was analyzed for texture using the hydrometer method (Gee and Bauder, 1986). Mass wetness at field capacity was determined by saturating 50 g of air-dry soil in a small funnel and allowing excess water to drain out for 24 h. Soil texture and water holding characteristics are summarized in Table 2. Since the soil pH was initially measured at 4.4, subsamples were limed using $Ca(OH)_2$ to attain additional pH's of 4.9, 5.3, 5.8, 6.4 and 7.0. Once lime was added and mixed with air-dry soils, deionized water was added in order to facilitate the reaction of the lime with the soil. Soils were incubated for one week and allowed to air dry before deionized water was added again to simulate another wetting-drying cycle. After incubating for another week, soil pH was measured in water with a glass electrode using a 1:1 soil to water ratio.

Table 1. Selected chemical properties of soil used in laboratory incubation†.

Humic matter	pH_{H2O}	NH_4-N	P	K	Ca	Mg	Na	Mn	Zn	CEC
%		——————————mg kg $soil^{-1}$——————————								$cmol_c$ kg^{-1}
0.5	4.4	13.9	9.1	20.0	43.9	11.5	16.8	2.6	2.0	2.3

†Soil nutrients extracted with Mehlich-3 solution (NCDA & CS, 2003).

Table 2. Soil particle size analysis and mass wetness at field capacity.

mass wetness	sand	silt	clay	USDA textural classification
—kg H_2O kg $soil^{-1}$—	————%————			
0.24	85.1	8.1	6.8	loamy sand

Broiler litter was obtained from a commercial broiler production facility located in Sampson County, North Carolina, which previously housed 14 flocks. Litter was collected directly from the poultry house and was air dry at the time of collection. Since the average particle size of the litter was relatively fine, it was passed through a 2 mm sieve as opposed to grinding it. Approximately 85% of the litter passed through the 2 mm sieve. Once sieved, litter was stored in plastic containers in a refrigerator at 4°C until the start of the incubation. Litter pH was determined in water with a glass electrode using a 1:1 litter to water ratio. Mass wetness at field capacity was determined by saturating 50 g of air-dry litter in a small funnel and allowing excess water to drain for 24 h. Litter subsamples were sent to the North Carolina Department of Agriculture Soil Testing Lab for routine waste analysis. Selected litter chemical and physical properties are summarized in Table 3. The Department of Soil Science Analytical Service Laboratory at North Carolina State University analyzed litter for total C and N via dry combustion. Litter inorganic N was determined by shaking 0.5 g litter with 40 mL of 1*M* KCl for 30 minutes. Extracts were

filtered with #2 Whatman filter paper, and frozen until analysis. Inorganic N (NH_4-N and NO_3-N) concentrations were determined colormetrically with a Lachat QuikChem 8000 Automated Ion Analyzer (Lachat, 1995). Nitrate-N concentrations were analyzed using QuikChem Method 10-107-04-1-A, while NH_4-N concentrations were analyzed using QuikChem Method 10-107-06-2-A (Lachat, 1995). Total C, N and inorganic N values are reported in Table 4.

According to analysis, approximately 5.4% of total N was present as NH_4-N, with a moisture content of 18%. Compared to other studies (Overcash et al., 1983; Westerman et al., 1988), % NH_4-N was slightly lower than average for broiler litter (10-20%) suggesting that significant NH_3 volatilization may have occurred prior to collection or during sieving and handling processes. Moisture content was also slightly lower than measured in previous studies (Westerman et al., 1988).

Table 3. Selected properties of broiler litter used in incubation.

Dry matter	pH_{H2O}	P	K	Ca	Mg	Zn	Cu	mass wetness
—g kg^{-1}—				—————g kg^{-1} litter—————				—kg H_2O kg^{-1} litter—
821	8.6	12.4	28.5	25.2	5.8	0.3	0.3	2.2

Table 4. Total C, N and inorganic N of broiler litter used in incubation.

Total C	Total N	NH_4-N	NO_3-N	PAN†	C:N
——————————g kg^{-1} litter——————————					
325.0	33.6	1.82	0.06	19.2	10:1

†PAN based on NCDA availability coefficient of 0.57.

Net N Mineralization Study

The net N mineralization experiment was based on a factorial design: 2 litter application rates (0, 155 kg PAN ha^{-1}) X 6 soil pH's (4.4, 4.9, 5.3, 5.8, 6.4, 7.0) replicated three times. To initiate the incubation study, 1.1 g of air-dry broiler litter was added to 300 g of air-dry soil at a rate of 270 kg total N ha^{-1} or 255 kg organic N ha^{-1} (assuming 2.2 x 10^6 kg soil ha^{-1} – 15 cm and bulk density = 1.5 g cm^{-3}) and mixed for 5 minutes in a rotating V-mixer. According to NCDA's model for predicting PAN, 57% of the litter N or 17 kg N ton^{-1} dry litter would become plant-available within the first year of application. Therefore the addition of litter in our study would be equivalent to 155 kg PAN ha^{-1}. The soil-litter mixtures were added to Ziploc® Sandwich bags (1.0 mil thickness) and water was added to bring the soil water content up to 60% of field capacity. Ziploc® bags were used to limit soil moisture loss throughout the incubation, while still permitting gas exchange. All bags were placed in a temperature-controlled incubator maintained at 25°C. To further prevent soil moisture loss, air was humidified by placing two pans of water inside the incubator. When required, water was added to the soils to bring the gravimetric water content to 60% of field capacity. Bags were opened at least once a week to ensure adequate aeration.

At 0, 7, 14, 28, 49, 77 and 111 days after litter addition, bags were removed from the incubator and 10 g subsamples were extracted with 25 mL of 1*M* KCl for 30 minutes. Extracts were filtered through #2 Whatman filter paper and frozen until analysis. Inorganic N (NO_3-N + NH_4-N) was determined colormetrically with a Lachat QuikChem 8000 Automated Ion Analyzer as previously discussed (Lachat, 1995). Soil water content

was measured by weighing 10 g of soil before and after drying at 105°C for 24 hr. Soil pH in water was also determined at each sampling time using a glass electrode and a 1:1 soil to water ratio.

Nitrification potential was determined at incubation day 7 and 28 for the 4.4, 4.9, 6.4 and 7.0 treated and control soils using the shaken soil slurry method (Hart et al., 1994). Briefly, 15 g of moist soil were weighed into 250-mL Erlenmeyer flasks and 100-mL phosphate buffer containing 1.5 mM NH_4-N was added into the flasks. The flasks were continuously shaken for 24 h at a speed of 200 rpm. After 2, 4, 22, and 24 h of shaking, 9-mL (approximate) of the slurry were removed and centrifuged at 8,000 rpm for 10 min. Clear aliquots were then poured into vials and frozen until analysis. Samples were analyzed colorimetrically for NO_3-N with a Lachat QuikChem 8000 Automated Ion Analyzer as previously discussed (Lachat, 1995). Nitrification potential (mg N kg^{-1} soil day^{-1}) was determined by plotting NO_3-N against sampling time and calculating the slope of the linear regression. Statistical analysis was performed using Proc GLM of the Statistical Analysis System (1998) to determine if there were differences in nitrification potentials due to length of litter incubation, soil pH, and litter addition.

Gross N Transformation Study

The gross N transformation study was conducted concurrently with the net mineralization study. Subsamples from two soil pH groups (4.4, 6.4) of the net N mineralization experiment were used for the gross N transformation study in an experimental design with 2 litter application rates (0, 155 kg PAN ha^{-1}), 2 soil pH's (4.4, 6.4), 2 incubation time periods (7, 28 d) and 3 replicates. At the beginning of the

incubation, 20 g of the soil-litter mixtures from the pH 4.4 and 6.4 treatments of the net N mineralization study were placed into 120 mL polyethylene specimen containers. Since the mixtures were dry, 3 mL of water were added to each specimen cup in order to bring the gravimetric water content to 14% (60% of field capacity). Soil water content was maintained by periodically weighing specimen containers and adding water when needed.

To measure gross N production and consumption rates simultaneously at day 7 and 28 of the incubation, an isotope pool dilution technique was employed (Hart et al., 1994). Gross N mineralization and microbial assimilation rates were measured by injecting 53 atom% enriched $^{15}NH_4Cl$ at a rate of 2.7μg $^{15}N\ g^{-1}$ soil (equivalent to oven dry weight) (Table 7B). The $^{15}NH_4Cl$ solution had a concentration of 100 mg N L^{-1} and a total of 1 mL was injected in 5 aliquots. For each soil pH and litter treatment combination, specimen cups were randomly paired, with a cup in each pair being designated with an extraction time (15 min or 2 days) following isotope injection. Each pair was replicated three times per treatment combination. To measure gross nitrification and microbial assimilation of NO_3, the same procedure was used as described above, except 56 atom% $K^{15}NO_3$ was injected at a rate of 2.8 μg $^{15}N\ g^{-1}$ soil (equivalent to oven dry weight) (Table 8B). At day 7, soils were extracted for NO_3-N and NH_4-N with 50 mL of 1*M* KCl at 0.25 hr and 41 hr after isotope injection. After shaking for 30 min, KCl extracts were filtered with #2 Whatman filter paper and frozen until analysis. At day 28, the same procedure was used except soils were extracted 0.25 hr and 46 hr after injection of $^{15}NH_4Cl$ and $K^{15}NO_3$. Some of the data from day 28 $^{15}NO_3$ measurements was not included in the analysis since negative values were calculated, indicating experimental error.

The KCl extracts were prepared for ^{15}N isotope analysis using a modified diffusion procedure developed by Brooks et al. (1989) and Stark and Hart (1996). To prepare the acid trap, glass fiber filter discs (7 mm diameter) were acidified with 10 μl $KHSO_4$ and placed between a double-layer of Teflon® tape (2.54 cm width). The tape was pressed together using plastic grommets (Coghlan's Snap and Tap Grommets.) to form a sealed bi-layer. Grommets were secured to lids of 125 mL plastic specimen containers with duct tape. Diffusion of NH_4 was initiated by adding 0.2 g MgO to extract samples containing approximately 20 μg NH_4-N. Before diffusing samples containing $^{15}NO_3$, NH_4 was first driven off by adding 0.2 g MgO and leaving specimen cups uncapped for 4 days while shaking on a vertical rotary shaker at 100 rpm. To initiate NO_3 diffusion, 0.4 g Devarda's alloy was then added to extract samples containing approximately 20 μg NO_3-N. An acid-washed glass bead was also dropped into each specimen container to ensure adequate mixing. Specimen containers were shaken on a vertical rotary shaker at 100 rpm for 72 hr. After shaking, grommets were removed from specimen containers and acidified discs were placed into small plastic weighing dishes. Weighing dishes were placed into a dessicator containing Drierite and allowed to dry for 24 hr. To avoid cross-contamination by gaseous NH_3, a small beaker with 50 mL concentrated H_2SO_4 was placed into the dessicator. After drying, discs were removed with tweezers and inserted into tin capsules. Tin capsules were folded into small cubes and placed in sample storage wells until time of analysis. Samples were analyzed for ^{15}N atom % and total N using a CE Elantech NC2500 elemental analyzer (EA) coupled to a ThermoFinnigan DELTA^{Plus} continuous flow isotope ratio mass spectrometer (CF-IRMS).

Net N Mineralization Calculations

The accumulation of NO_3, NH_4 and total inorganic N over time (t) due to mineralization of broiler litter was calculated for each replicate using the following equation:

Accumulated NO_3-N, NH_4-N or total inorganic N =

[(Inorganic $N_{treated}$) – (Inorganic $N_{control}$) – (Inorganic N_{litter})]

where: Inorganic $N_{treated}$ = NO_3-N, NH_4-N or total inorganic N of litter treated soils (mg Nx-N kg^{-1} soil); (x = NO_3, NH_4 or NO_3 + NH_4)

Inorganic $N_{control}$ = mean (n=3) NO_3-N, NH_4-N or total inorganic N of control soils (mg Nx-N kg^{-1} soil)

Inorganic N_{litter} = NO_3-N and NH_4-N present in soil due to inorganic N present in broiler litter at time 0 (mg Nx-N kg^{-1} soil)

The Proc GLM procedure by the Statistical Analysis System (SAS, 1998) was used to determine if there were differences in accumulated total inorganic N at the end of 111 days due to treatments. Where differences were detected, pair-wise comparisons of means were made using Fisher's LSD procedure. Accumulated inorganic N (mg NO_3-N + NH_4-N kg^{-1} soil) was converted to g accumulated inorganic N kg^{-1} organic N added and fit to a single-pool first-order model and using Proc NLIN (SAS, 1998), potentially mineralizable N and rate constants were calculated for individual replications using the following model:

$$\mathbf{N_m = N_o(1-e^{-kt})}$$

where: N_m = accumulated inorganic N (g NO_3-N+ NH_4-N kg^{-1} organic N added to soil) at time (t)

N_o = potentially mineralizable N (g N kg^{-1} organic N added to soil)

k = rate constant (day^{-1})

t = time (days)

The Proc GLM program by the Statistical Analysis System (SAS, 1998) was used to determine if there were differences in potentially mineralizable N (N_o) and rate constants (k). Where differences were detected, pair-wise comparisons of means were made using Fisher's LSD procedure.

Percent net litter N mineralized at time (t) was calculated for each replicate using the following equation:

$$\textbf{\% net litter N mineralized} = \frac{(N_t - S_t) - (N_i - S_i)}{N_{org}}$$

where: N_t = inorganic N of litter treated soils at time (t) (mg NO_3-N + NH_4-N kg^{-1} soil)

S_t = mean (n=3) inorganic N of control soils at time (t) (mg NO_3-N+ NH_4-N kg^{-1} soil)

N_i = mean (n=3) inorganic N of litter treated soils at t = 0 (mg NO_3-N+ NH_4-N kg^{-1} soil)

S_i = mean (n=3) control soil inorganic N at t = 0 (mg NO_3-N+ NH_4-N kg^{-1} soil)

N_{org} = initial organic N added in litter (mg litter organic N kg^{-1} soil)

N_{org} was calculated by subtracting the initial inorganic N in the litter ($N_i - S_i$) from the total N added (mg N kg^{-1} litter). Proc GLM by the Statistical Analysis System (SAS, 1998) was used to determine if there were differences in net litter N mineralized at 111 days due to treatments. Where differences were detected, pair-wise comparisons of means were made using Fisher's LSD procedure.

Gross N Transformation Calculations

Student t-tests were used to compare the differences of ^{15}N atom percent excesses measured 0.25 hr and 41 or 46 hr after injection in the paired samples. Where isotope excesses were significantly lower 41 or 46 hr after injection compared to initial excesses, gross N transformation rates were calculated using equations developed by Kirkham and Bartholomew (1954). Recovery of ^{15}N 0.25 hr and 41 or 46 hr after injection was also calculated for each sample pair. Ratios of recovery were calculated by dividing ^{15}N recovered 41 or 46 hr after injection with that recovered 0.25 hr after injection. Effects of soil pH, litter addition and incubation time on ratios of recovery were tested using Proc GLM of the Statistical Analysis System (SAS, 1998).

Results and Discussion

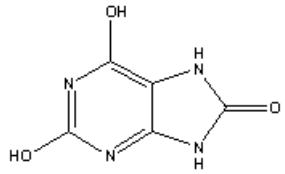
Ammonium accumulation patterns of all litter-treated soils are shown in Figure 2. Ammonium rapidly accumulated in all treatments in the first 14 days of the incubation. Hadas et al. (1983) also found an initial rapid accumulation of soil NH_4 when pelletized poultry manure was added to soils. By day 28, NH_4 accumulation peaked in the soils at pH

5.3, 5.8, 6.4 and 7.0 and NO_3 started to accumulate, particularly in the soil at pH 7.0. In general, NH_4 disappearance from all treatments at any given time was mirrored by the accumulation of NO_3 (Figure 3). Nitrate did not accumulate in any of the litter treated soils until day 14 of the incubation, indicating that nitrification was probably not a significant process in the first two weeks of the experiment (Figure 3). However, by day 49, NH_4 was no longer detected in the soil at pH 7.0, indicating that nitrification rates exceeded those of ammonification. Accumulated NH_4 also declined to zero in the soils at pH 6.4, 5.8 and 5.3, but not until day 77. The distribution of NH_4 and NO_3 by treatment group at the end of the study concur with results obtained by Macmillian et al. (1975), where NO_3 accumulated in soils at pH 7.1, while NH_4 production was favored in soils at pH 4.2 when poultry manure was added to soils.

In general, nitrification was delayed for longer periods of time as the soil pH decreased. For example, in the soils at pH 4.9, NH_4 concentration increased until day 49, where upon the concentration rapidly decreased. Ammonium-N concentration in the soils at pH 4.4 also increased until day 49 to nearly the same level (48 mg kg^{-1}) as the soil at pH 4.9 (47 mg kg^{-1}), but began to decrease at a much slower rate. After 111 days, NH_4-N concentration in the soil at pH 4.9 had declined to 9 mg kg^{-1}, whereas at pH 4.4, the NH_4-N concentration had only declined to 36 mg kg^{-1} in the same time period. Many previous studies have concluded that nitrifying bacteria are inhibited by acidic conditions (Dancer et al., 1973; Nyborg and Hoyt, 1978; Haynes and Swift, 1989). Morill and Dawson (1967) determined that there were different patterns of nitrification in soils that were largely dependent on soil pH. From short-term incubation studies, they found that few soils with a pH below 5.0 had the capability to nitrify available NH_4. In the same study, soils with a

pH between 5.0 and 5.4 accumulated NO_3, but at slower rate than soils with a higher pH. The results of our experiment tend to agree with these conclusions, although the soils at pH 4.9 and 4.4 both showed signs of nitrification after 49 days, suggesting that nitrification may have been performed to some degree by acid-tolerant nitrifiers as reported by other researchers (Persson and Wirén, 1995).

Soil pH increased an average of 1.2 pH units for all litter-treated soils within the first 14 days, followed by a subsequent decrease and stabilization period (Figure 4). Similar changes in soil pH were reported by Crane et al. (1981), where surface application of poultry manure caused an immediate rise in soil pH. The authors hypothesized that the immediate increase in soil pH was caused by the formation of NH_4 due to the rapid hydrolysis of uric acid and urea, acid consuming reactions illustrated as follows:



Uric acid

$$\longrightarrow (NH_2)_2CO + 2H_2O + H^+ \longrightarrow 2NH_4 + HCO_3^-$$

Urea

Although we did not measure uric acid concentration of the broiler litter, generally a large portion of the total N of broiler litter is in the form of uric acid (Nahm, 2003). In addition, the concentration of Ca was relatively high in the broiler litter (Table 3), indicating that $CaCO_3$ was probably added as a feed supplement. Thus, the initial increase in soil pH can probably be attributed to the rapid hydrolysis of uric acid and to a lesser extent, the presence of $CaCO_3$ in the litter.

For all litter-treated soils, soil pH leveled off between 14 and 56 days and declined by varying degrees, depending on initial soil pH. At incubation day 14, the soil pH of soils at pH 7.0 and 6.4 both started to decline. The decline in soil pH may have been related to

the nitrification rate since nitrification is an acid-producing reaction. Nitrate appeared 14 days after litter application in the soils at pH 7.0, but was undetected in pH 6.4 soils until day 28. The decline in pH could therefore be partially attributed to the natural buffering capacity of the soil. However, the decline in soil pH can generally be correlated with the appearance of NO_3 and is therefore probably linked to nitrification. For example, the soil pH of pH 4.4 soils did not start to decline until after incubation day 56. These soils also had the slowest rate of decline and were the only soils to have a net increase in soil pH at the end of 111 days. Even in the control soils, there was a decrease in soil pH over the incubation period for the soils at pH 6.4 and 7.0, but not for the other soil pH groups, indicating that nitrification was probably not inhibited above pH 6.4.

To assess net N mineralization (N_m), NO_3 and NH_4 accumulation was combined to form cumulative total inorganic N production (Figure 5). In general, the accumulation of total inorganic N was rapid in the first two weeks, followed by a slow accumulation over the next 14 weeks. The rate of net N mineralization was probably fast in the first two weeks because of the influx of easily decomposable constituents, such as uric acid N present in the broiler litter. After the easily degradable organic N was oxidized, the rate of N mineralization slowed considerably, probably because only recalcitrant organic N complexes remained from the original substrate.

The pattern of inorganic N accumulation was similar for all treatment groups, but there were significant differences ($p<0.05$) in the amount of net N mineralized after 111 days due to soil pH (Table 5). Values of net N mineralized ranged from 61.3 mg N kg^{-1} soil at pH 6.4 to 32.6 g N kg^{-1} soil at pH 4.4. Although soil at pH 6.4 had the lowest net N mineralization, there were no significant differences between it and soils at pH 7.0 and 5.3.

However, there was a significant difference between the soil at pH 6.4 and the soil at pH 5.8 in terms of net N mineralized, suggesting that the soil pH effect is somewhat confounding. This effect may have been due to the experimental design, where soil pH contrasted by 0.4 to 0.6 pH units between soils. Since these differences in soil pH were relatively small, treatment effects were also small between similar soil pH groups (i.e. soil pH 4.9 and 5.3) and sometimes confounding. In regressing soil pH against net N mineralized, there was a significant ($p<0.05$) linear relationship between the parameters ($r^2 = 0.77$) as shown in Figure 6. These results indicate that soil pH was negatively correlated with net N mineralization, suggesting that more N was mineralized in the soils at low pH. Although net N mineralization was highest in the pH 4.4 soil, it could be argued that relatively more N may have been immobilized by microorganisms in the soils at higher pH and thus gross N mineralization may not be different. Volatilization and denitrification losses also may have occurred, but relative differences between treatments are unlikely since litter was incorporated into all treatment groups and all soils were maintained at the same moisture content.

It appears that nitrogen accumulation in control soils heavily influenced the significant effect of soil pH on net N mineralization (Figures 1B-3B). Inorganic N accumulation was highest in the control soils at pH 6.4 and lowest in the pH 4.4 soils. In other words, more inorganic N accumulated in the soils at high pH compared to the soils at low pH. Significant soil pH effects may have been created when the accumulation of N in the controls was subtracted out from the accumulation of N in the litter treated soils, given that only 24 mg N kg^{-1} of inorganic N was subtracted out from the soil at pH 4.4 compared

52 mg N kg^{-1} from the soil at pH 6.4. Therefore, it appears that there may not have been significant differences in net N mineralized based on differences in soil pH.

Table 5. Mean values of actual net N mineralized (N_m), predicted potentially mineralizable N (N_o), predicted first order rate constants (k) of mineralization, predicted initial potential rates and actual % litter N mineralized for broiler litter incubated with soils of varying pH at 25°C for 111 days.

Soil pH	N_m†	N_o‡	k	Initial potential rate (N_ok)	Litter N mineralized
	—g N kg^{-1} organic N added to soil—		d^{-1}	g N kg^{-1} organic N d^{-1}	% of organic N added to soil
4.4	372a	388a	.027a	10.5a	56.9a
4.9	352ab	348a	.034a	11.8a	53.3a
5.3	296abc	284ab	.045a	12.8a	45.1ab
5.8	306ab	282ab	.043a	12.1a	45.6ab
6.4	198c	221b	.019a	4.2b	31.2b
7.0	249bc	222b	.042a	9.3ab	36.7b

† N_m means followed by the same letter are not significantly different according to Fisher's LSD at the 0.05 probability level.

‡N_o, k, N_o*k and litter N mineralized means followed by the same letter are not significantly different according to Fisher's LSD at the 0.1 probability level.

By fitting net mineralized N to a one-pool, first order model, parameters for potentially mineralizable N (N_o) and rate constants (k) were produced for each soil pH group receiving broiler litter (Table 5 and Table 1B). Values of N_o, the pool of N in broiler litter predicted to be mineralized over a period of 111 days, ranged from 221 g N kg^{-1} organic N in soil at pH 6.4 up to 388 g N kg^{-1} organic N in soil at pH 4.4. The ANOVA revealed marginal differences ($p< 0.07$) in potentially mineralizable N (N_o) between soil pH groups, but not for rate constants (k) ($p=0.29$). Regression analysis identified a

significant ($p<0.05$) inverse linear relationship ($r^2 = 0.92$) between soil pH and potentially mineralizable N in terms of organic N added (Figure 7) and dry broiler litter added (Figure 8). In a study where nine soils were incubated with broiler litter for 116 days, Gordillo and Cabrera (1997b) proposed that the size of N_o decreased as soil pH increased, although the range of soil pH used in their study was somewhat limited (5.5-7.1). The results from the present study augment those obtained by Gordillo and Cabrera, suggesting that in some cases, microbial communities in soils of low pH (4.4-5.5) are adapted to the acidic conditions and are able to mineralize broiler litter more quickly compared to soils at a higher pH. However, since net mineralized N was used to generate these results, there may not have been significant differences in N_o given the nitrogen accumulation patterns in control soils as previously noted.

Soil pH was also regressed against k (Figure 9), but there was no significant linear relationship ($p=0.28$), suggesting that the rate of N mineralization cannot be predicted based solely on soil pH. These results are somewhat puzzling since there was more potentially mineralizable N in the soils at low pH, yet there were no statistical differences in rates based on differences in soil pH. This apparent anomaly may be explained by the non-linear first-order reaction that was used to fit the data. Other researchers have also found that mineralization potentials and rates are sometimes conflicting factors, stemming from the assumption that there is only one pool of mineralizable N (Stanford and Smith, 1972; Shi et al., 1999). Although rate constants were not significantly different in our study, initial and final rates of N mineralization may have been different based on soil pH. It is possible that rates of N mineralization were greater in acid soils at the beginning of the incubation, but then leveled off sooner relative to soils at higher pH's. Soils at higher pH's

may have had slower, initial rates of mineralization but also more constant rates over the entire incubation period. In these scenarios, more N may have mineralized in the acidic soils over a period of 111 days, but the overall N mineralization rate would not necessarily be different from the higher pH soils.

To gain additional insight into the N supplying power of broiler litter, initial potential rates of N mineralization at time 0 ($N_o k$) were calculated for the soils amended with broiler litter (Table 5). The initial potential rate of N mineralization ($N_o k$) is defined as an index of short-term N supplying capacity and has been used to characterize N mineralization when estimates of potential mineralizable N and rate constants seemingly contrast (Campbell et al., 1991; Shi et al., 1999). In their analysis, Campbell et al. (1991) showed that the derivative of N_m with respect to time at $t = 0$ is equal to $N_o k$, equivalent to an instantaneous rate of N mineralization at time 0. Soil at pH 6.4 had significantly lower initial potential rates than soils at lower pH's. These results support the hypothesis that rates of N mineralization were greater in soils at low pH at the beginning of the experiment, but when averaged over the entire incubation period, mineralization rates were not different due to differences in soil pH.

Litter organic N that was mineralized during the incubation period, expressed as a percentage of added organic N, is shown in Figure 10. The percentage of litter organic N mineralized in 111 days varied from 31% for the soil at pH 6.4 up to 57% for the soil at pH 4.4. These results are comparable to a study conducted by Sims (1986), where 30 to 60% of broiler litter organic N was mineralized in a 150-day laboratory incubation. Statistical analysis revealed that there were marginal differences ($p < 0.06$) between soil pH groups in terms of the percentage of litter organic N that had mineralized by the end of 111 days.

According to Fisher's LSD mean comparisons, significant differences existed between the two most acidic soils (pH 4.4 and 4.8) and the other four soil groups (Table 5), suggesting that more litter organic N mineralized in soils at low pH. However, there were no significant differences in percentage of litter organic N mineralized between soils at pH 5.3 and 7.0, suggesting that the soil pH effect is somewhat confounding. As noted previously, this effect may have been due to the experimental design, where soil pH contrasted by 0.4 to 0.6 pH units between soils. Since these differences in soil pH were relatively small, treatment effects were also small between similar soil pH groups (i.e. soil pH 4.9 and 5.3) and sometimes confounding. Additionally, it appears that nitrogen accumulation in control soils heavily influenced the significant effect of soil pH on net N mineralization as previously stated. However, linear regression of the data revealed a significant ($p<0.05$) linear relationship ($r^2=0.84$) between soil pH and percent litter organic N mineralized (Figure 11).

These results suggest that microbial communities in this soil were probably adapted to low pH soils and were able to mineralize more broiler litter compared to soils limed to a higher pH, as commonly found in soils used for crop production. Microbial adaptation to acidic conditions by mechanisms such as proton exclusion and surface appendage alteration have been documented in the past (Paul and Clark, 1989). Although the acidic conditions may have played a direct role in N mineralization, the experimental conditions may have also indirectly affected microbial ability to mineralize organic N. For example, it is probable that once the soils were limed, the microbial community structure dramatically changed. Since the native soil was acidic (pH 4.4) and collected from a forested area, acidophilic fungi probably dominated the microbial community (Coyne,

1999). Since bacteria are usually more prone to dominate in neutral conditions, once the treatment groups were limed to pH 6.4 and 7.0, it is possible that community structure was in a transitory state and neutrophilic microorganisms were not well established at the time broiler litter was added. This phenomenon could be a partial explanation to why we observed more N mineralization in soils at lower pH.

Nitrification Potential

Nitrification potential was significantly impacted by the lime treatments (Figure 12). Statistical analysis revealed a significant ($p<0.05$) time x soil pH interaction (Table 2B). Previous studies have shown that nitrifying bacteria are normally quite sensitive to acidic environments (Haynes and Swift, 1989; Nyborg and Hoyt, 1978; Dancer et al., 1973), although they may also exist at alkali microsites in acidic soils (Persson and Wirén, 1995). The significant time by soil pH interaction suggests that soil pH affected the nitrification potential, but the nitrifying microorganisms from the various soil pH groups behaved differently at day 7 and day 28. The nitrification potential for the treated and the control soils decreased over time for all soil pH groups except for soils at pH 7.0, which slightly increased from day 7 to day 28 (Figures 13 and 14). These results were somewhat unexpected since the NO_3 pool was virtually undetected for all soil pH groups at day 7 and all treatment groups at day 28, with the exception of the pH 7.0 soil (Figure 3). It is possible that higher nitrification potentials at day 7 for the pH 4.4, 4.8 and 6.4 soils were due to the presence of heterotrophic nitrifiers, which have been previously observed in acid, forested soils (Sylvia et al., 1998). If heterotrophic nitrifiers were present in the native acid soils used in our experiment, they may have been responsible for most of the

nitrification observed at day 7, but not at day 28, due to changing organic substrates used as energy sources. In this scenario, the heterotrophic nitrification contribution may have decreased by day 28, but pH sensitive autotrophic bacteria populations could have increased in the pH 7.0 soil, due to the neutral conditions. This could also account for the higher NO_3 concentration that had accumulated in the soil at pH 7.0 by day 28.

There was no significant main effect of litter addition on nitrification potential (Figure 15). The non-significant effect is perplexing given that NH_4 is the main energy source for nitrifying bacteria and soil NH_4 concentration is one of the limiting factors of autotrophic nitrification (Sylvia et al., 1998). With more NH_4 and C present in litter-amended soils, it is logical to suppose that this addition induced more autotrophic and heterotrophic nitrifying organisms than in the control soils. However, the addition of litter played no significant role in nitrification potential, possibly because NH_4 and C were not limiting in treated or control soils prior to measurement.

Even if heterotrophic nitrifiers existed in this soil, nitrification potential results cannot fully explain NO_3 accumulation patterns in our experiment (Figure 3). These results suggest that nitrification potential must be complimented with isotopic measurements to fully understand the effect of soil pH on nitrification dynamics of the system.

Gross N Transformations

Gross N transformation measurements were useful for further elucidation of N dynamics in the experiment. Student's t-tests revealed that there were no significant differences in $^{15}NH_4$ atom % excesses between the long-term (41 or 46 hr) and short-term

(0.25 hr) incubations, suggesting that differences in gross N mineralization rates could not be detected using the isotope dilution technique. It appears that the level of ^{15}N atom% enrichment was too low to measure gross N mineralization in our study, since ^{15}N atom% enrichment was not diluted as hypothesized.

Although gross N mineralization rates could not be calculated by isotope dilution, percent ^{15}N recovery proved useful for further understanding into gross N mineralization. Percent $^{15}NH_4$ recovered 0.25 hr after ^{15}N injection also did not differ from % recovered 41 or 46 hr after ^{15}N injection for any of the treatments at day 7 and day 28 of the incubation (Figures 16 and 17; Tables 3B and 5B). In some instances, more ^{15}N was recovered after 0.25 hr than was added in the enriched solution, probably due to random and experimental error. However, in most cases, the high ratio of recovery (>0.9) for all treatments at both days (Figure 18) suggests that NH_4 loss through volatilization and immobilization processes were negligible, suggesting that gross production rates were equivalent to net rates, regardless of soil pH, litter addition, and incubation time. Thus according to the net mineralization results, N mineralization was probably favored at low pH for reasons previously discussed.

Student's t-tests revealed that there were no significant differences between $^{15}NO_3$ excesses 41 or 46 hr and 0.25 hr after ^{15}N injection. The lack of isotope dilution suggests that gross N nitrification rates were very small and could not be detected using the isotope dilution technique. As was the case with gross mineralization rates, it appears that gross nitrification rates were equivalent to net rates, regardless of soil pH, litter addition, and incubation time (Figures 19 and 20; Tables 4B and 6B). Although there were no treatment differences in the $^{15}NO_3$ recovered at day 7, the ratio of ^{15}N recovered was below 1.0 for

nearly all of the treatments, indicating that denitrification or immobilization occurred. Figure 19 shows that consumption processes occurred in all of the soils, regardless of treatment. Since the soils were maintained at 60% of field capacity, $^{15}NO_3$ losses via denitrification were probably negligible and NO_3 was likely immobilized by soil microorganisms. However, since there were no treatment differences in $\%^{15}NO_3$ recovered at day 7, immobilization was not preferentially favored at high or low soil pH. Data also suggests that NO_3 immobilization did not occur at day 28 in the pH 4.4 soil (Figure 20), but inference to other treatment groups cannot be made due to lost data.

Conclusions

Several conclusions can be drawn when considering the data of the current study. Soil pH increased during the first 14 days an average of 1.2 pH units with the addition of broiler litter, followed by a decrease in soil pH due to nitrification. Autotrophic nitrification was repressed by low soil pH, but heterotrophic nitrification was not. In general, nitrification was delayed for longer periods of time as the soil pH decreased. The accumulation of total inorganic N fit a one-pool, first order model, with a rapid accumulation occurring in the first two weeks followed by a slower accumulation over the next 14 weeks. There were significant ($p<0.05$) inverse relationships between soil pH and net N mineralized ($r^2 = 0.77$), soil pH and potentially mineralizable N ($r^2 =0.92$) and soil pH and percent litter N mineralized ($r^2=0.84$). However, there may not have been significant differences given that relatively more nitrogen accumulated in high pH control soils compared to low pH control soils. Using isotope dilution measurements, gross and net mineralization rates were equivalent, refuting the notion that relatively more NH_4

immobilization had occurred in the higher pH soils. All of these results indicate that N mineralization was probably enhanced at low soil pH, a phenomenon that presently is not fully understood. Microbial adaptation to acidic conditions offers one explanation, but a lack of microbial community structure data limits the inference we can make.

Future research will be required to fully elucidate the relationship between soil pH and N mineralization of poultry litter. One major question highlighted by this study is how broiler litter organic N mineralization might differ when applied to forested soils versus agricultural soils. Our study was conducted using forested soils where microbial populations and community structures may differ markedly from agricultural based soils. Future research is needed to determine how N mineralization dynamics differ between the non-limed forest soils and the limed agricultural soils and what specific role soil pH plays in the N transformation processes.

These results suggest that application rates of broiler litter may need to be adjusted for certain crops based on the pH of the soil where the litter is to be applied. As previously mentioned, potentially mineralizable N was 75% higher in the broiler litter when it was applied to the soils at pH 4.4 compared to when it was applied to soils at pH 6.4. Such large observed differences mandate that soil pH should be seriously considered when predicting PAN, especially where poultry litter is used as an N source for crops that thrive under low soil pH (i.e. blueberries, Christmas trees). By matching crop N requirements with soil pH and broiler litter N content, PAN predictions could be more reliable.

From a typical agronomic standpoint however, differences in potentially mineralizable N based on soil pH may not be significant enough to merit changes with PAN models currently in use. As previously discussed, broiler litter is often used as a

fertilizer source for row crops and pastures. To achieve optimum soil fertility for crop production, producers in the southeastern U.S. commonly lime agricultural soils to attain a soil pH of 5.5-6.0. Within this soil pH range, differences in PAN based on the Gordillo and Cabrera model and the NCDA model are small (Figure 1). At pH 5.5, the Gordillo and Cabrera model predicts 15% more potentially mineralizable N than the NCDA model, whereas at pH 6.0, the Gordillo and Cabrera model only predicts a 7% increase in potentially mineralizable N. Based on the data from the current study, differences in PAN due to soil pH in a typical agronomic setting (soil pH 5.5-6.0) would probably be negligible, especially considering the variability in PAN that would result from differences in litter quality, litter sample variability, soil environmental conditions at the time of field application, etc. In the present study, assuming 100% of initial litter NH_4 is available, 14 g PAN kg^{-1} dry litter (42% of total litter N) would be predicted if applied to a soil at pH 5.5 compared to 13 g PAN kg^{-1} dry litter (39% of total litter N) if applied to a soil at pH 6.0. Although these estimates are somewhat lower than the PAN predicted by NCDA's model (57% of total litter N or 19 g PAN kg^{-1} dry litter) and Westerman et al. (1988) (47 to 54% PAN of total litter N), the differences based on soil pH do not warrant that it be a major factor in calculating PAN where crops are produced under typical agronomic conditions (i.e. soil pH 5.5-6.0).

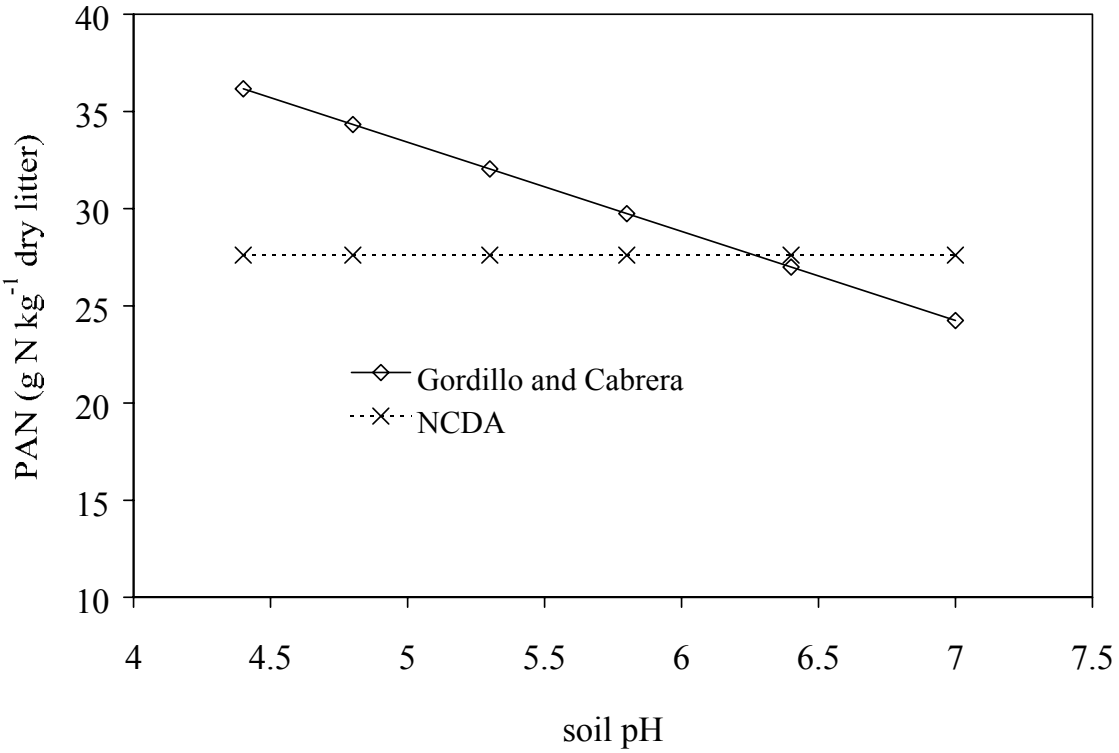


Figure 1. A comparison of two models used for predicting plant-available N as soil pH changes. In constructing the Gordillo and Cabrera model, all parameters were held constant except soil pH. Soil data used as input for the Gordillo and Cabrera model was measured on 40 soils collected in North Carolina and averaged for use in the model. Average uric acid, total N and C/N values were taken from Gordillo and Cabrera (1997a). In addition, we assumed that inorganic N was 90% available in the case of the Gordillo and Cabrera model. For both models, we assumed that litter was incorporated at time of application. The Gordillo and Cabrera model was extrapolated in this figure from a soil pH of 5.1 to 4.4 for comparison purposes.

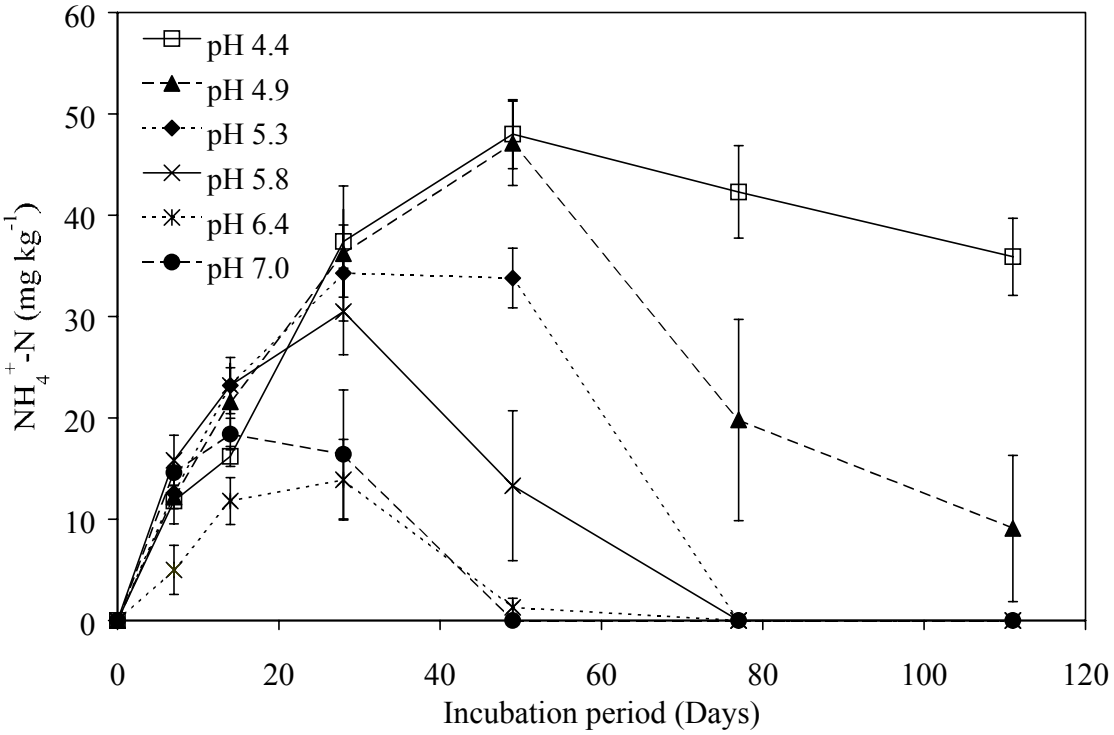


Figure 2. Soil NH_4-N concentrations for 6 soil pH groups incubated with broiler litter for 111 days at 25°C. Points represent mean values (n=3). Bars indicate experimental standard error.

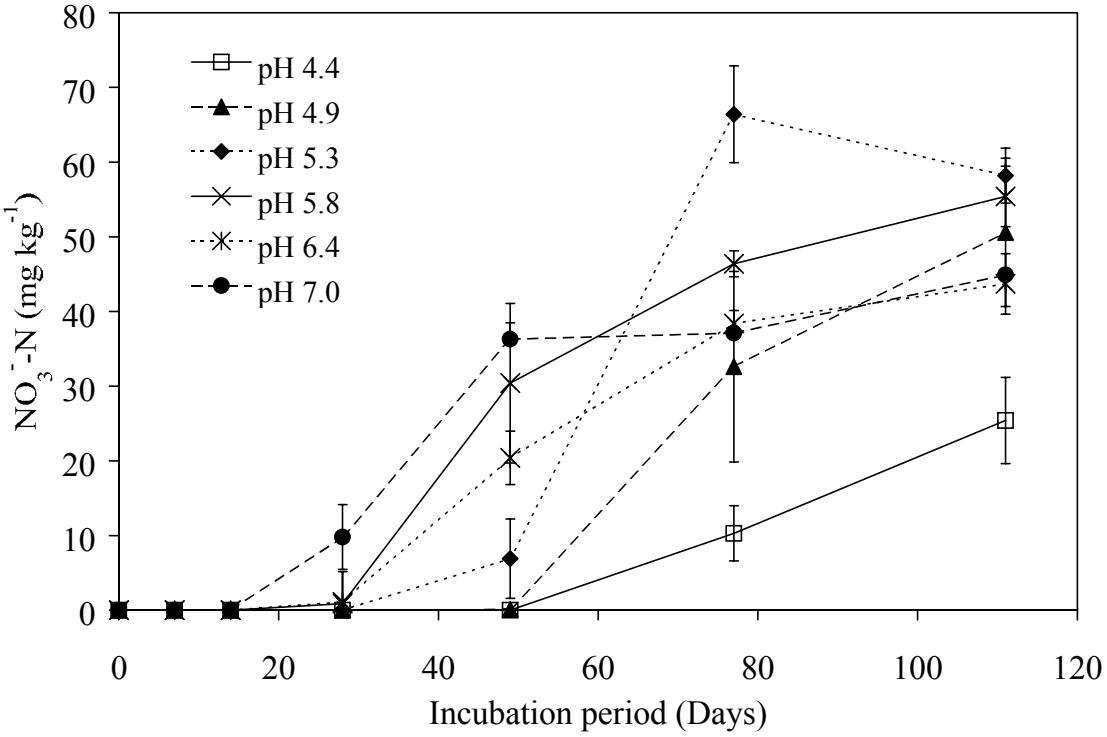


Figure 3. Soil NO_3-N concentrations for 6 soil pH groups incubated with broiler litter for 111 days at 25°C. Points represent mean values (n=3). Bars indicate experimental standard error.

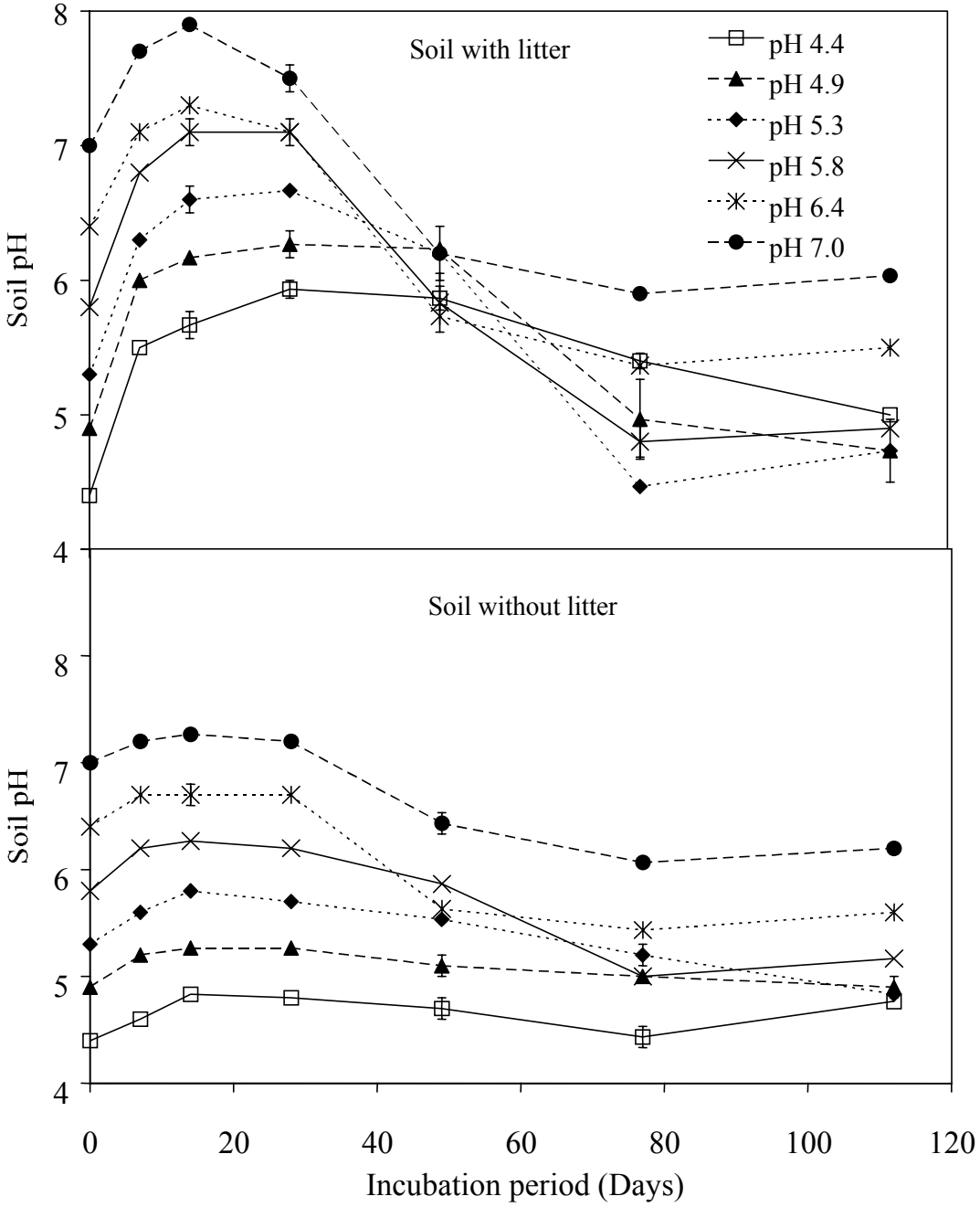


Figure 4. Soil pH of six soil pH groups incubated with broiler litter for 111 days at 25°C. Points represent mean values (n=3) of soil pH measured in a 1:1 soil:water ratio.

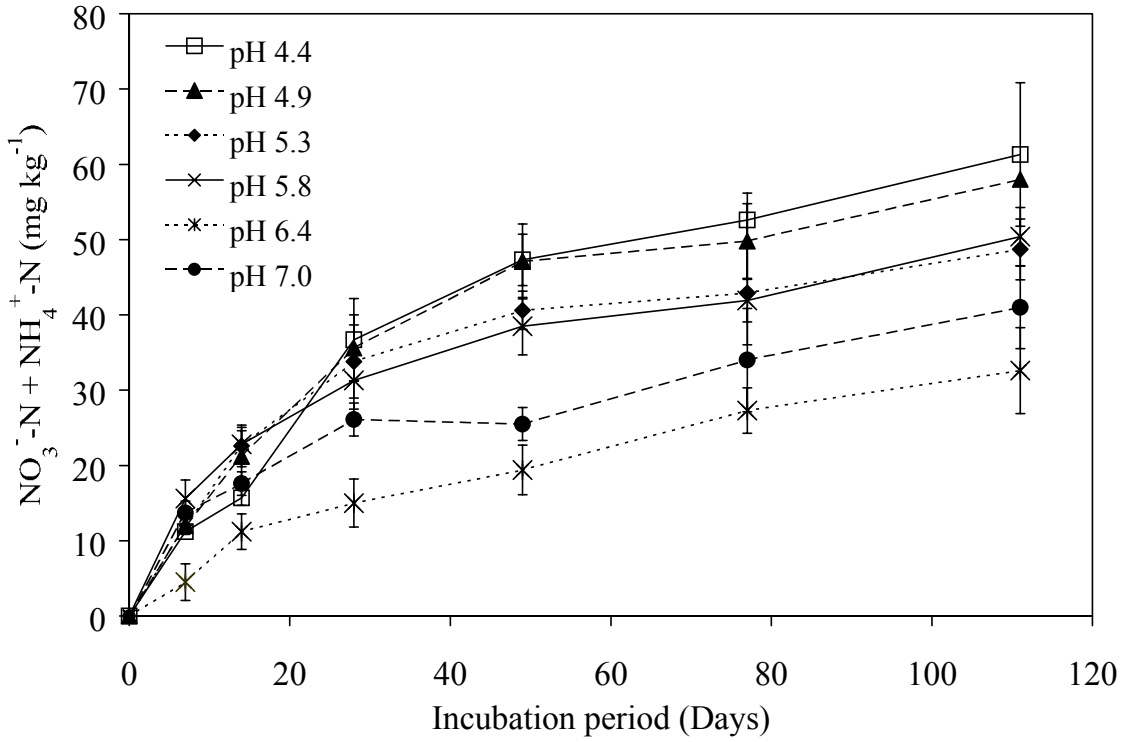


Figure 5. Soil inorganic N production (NO_3-N + NH_4-N) for 6 soil pH groups incubated with broiler litter for 111 days at 25°C. Bars indicate experimental standard error (n=3).

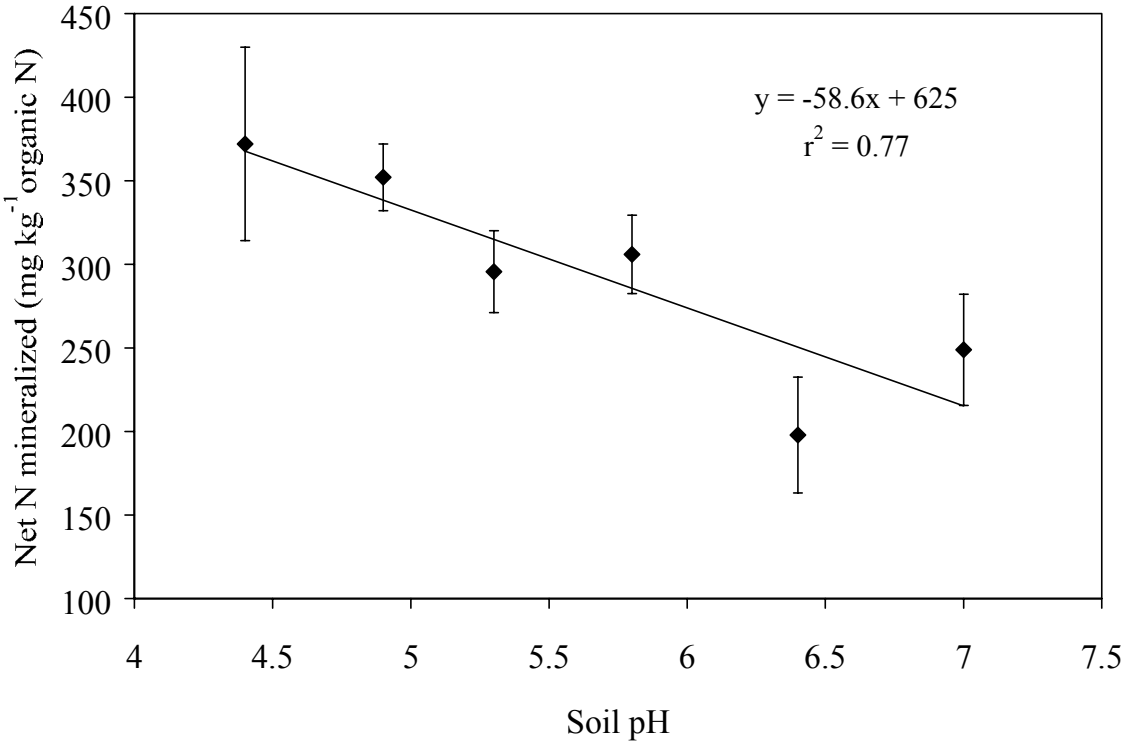


Figure 6. Relationship ($p<0.05$) between values of net N mineralized (N_m) after 111 days of incubation and soil pH. Bars indicate experimental standard error (n=3).

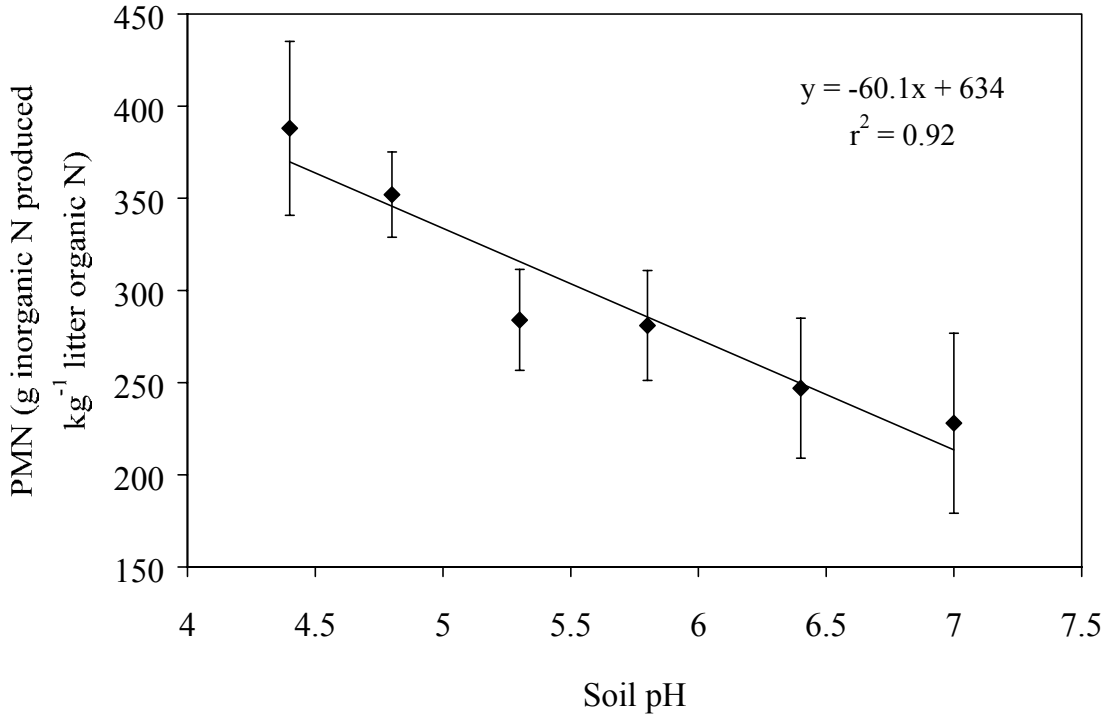


Figure 7. Relationship ($p<0.05$) between values of potential mineralizable N (PMN) based on organic N added and soil pH. Bars indicate experimental standard error (n=3).

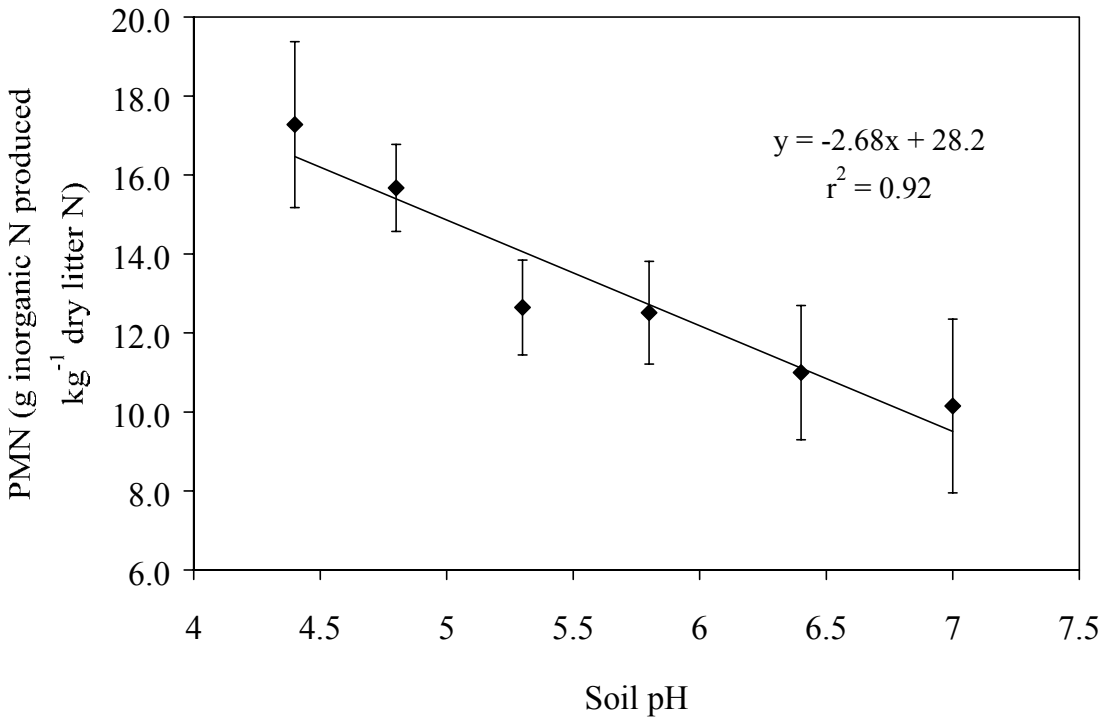


Figure 8. Relationship ($p<0.05$) between values of potential mineralizable N (PMN) based on litter added and soil pH. Bars indicate experimental standard error (n=3).

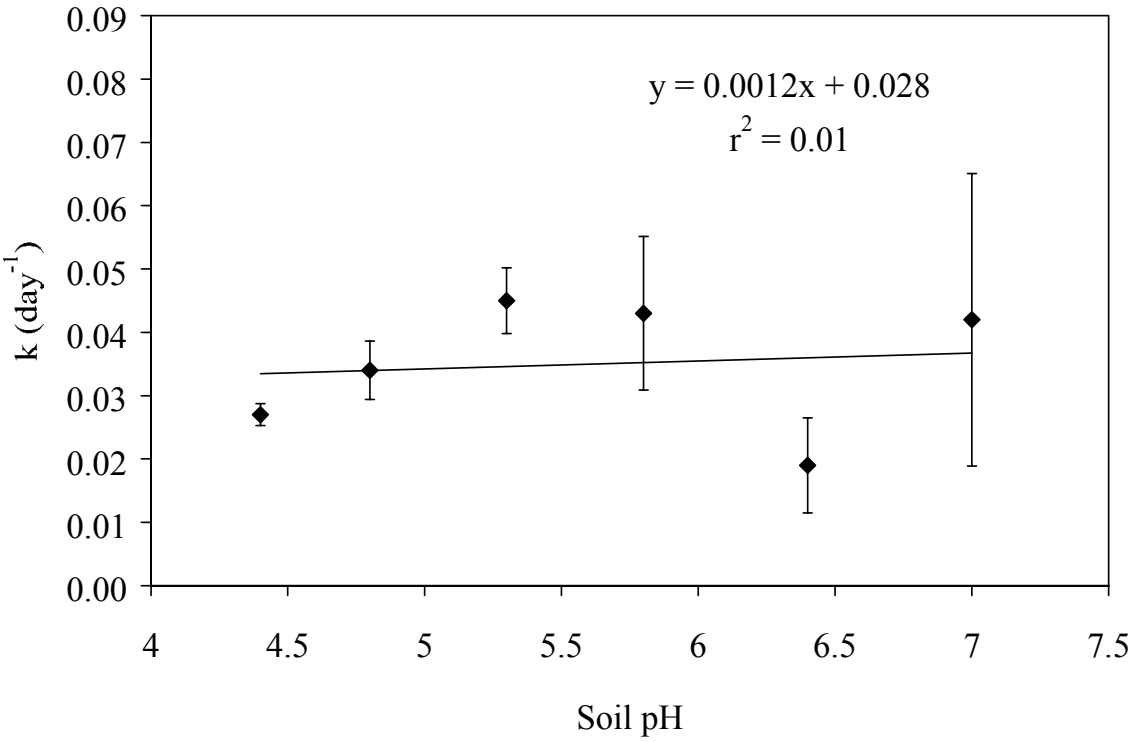


Figure 9. Relationship (p=0.28) between values of rate constant (k) and soil pH. Bars indicate experimental standard error (n=3).

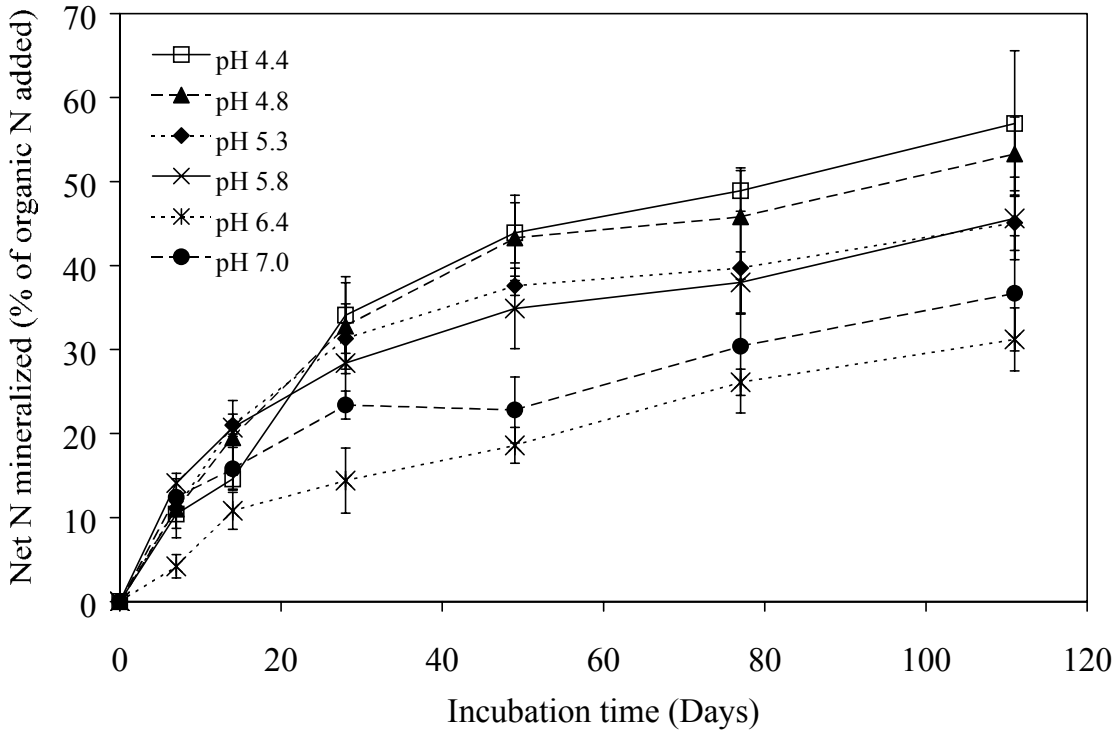


Figure 10. Net N mineralized as a percentage of litter organic N added for 6 soil pH groups incubated with broiler litter for 111 days at 25°C. Bars indicate experimental standard error (n=3).

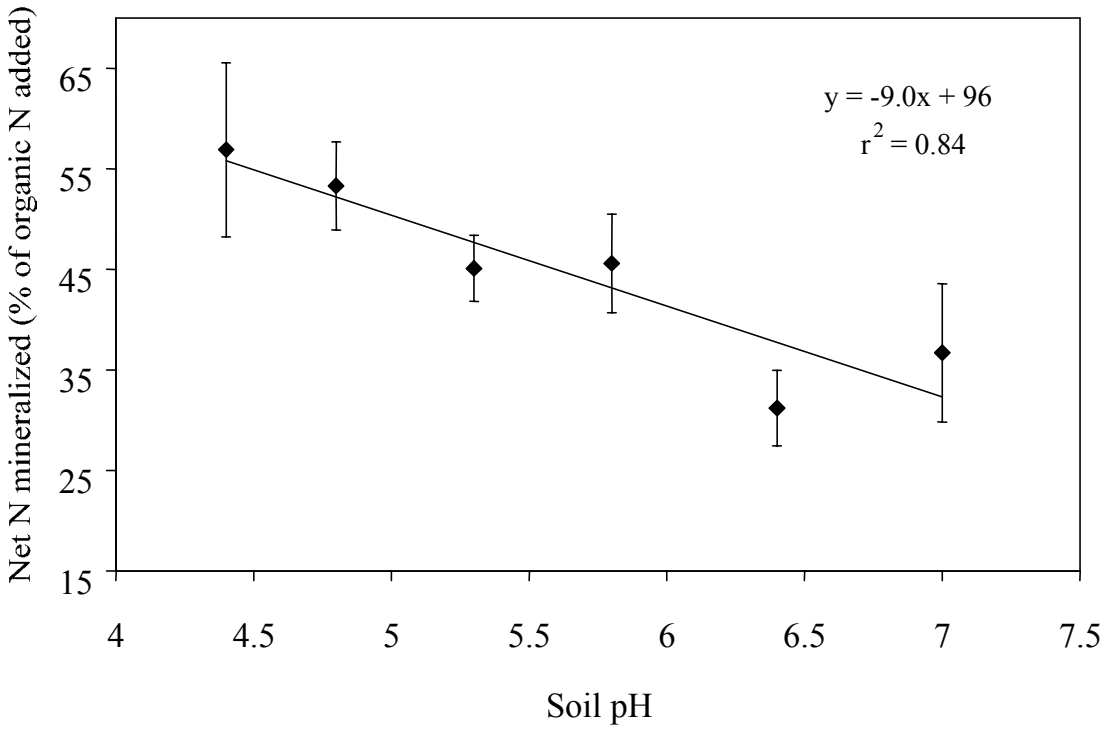


Figure 11. Relationship ($p<0.05$) between values of percent net litter organic N mineralized after 111 days and soil pH. Bars indicate experimental standard error (n=3).

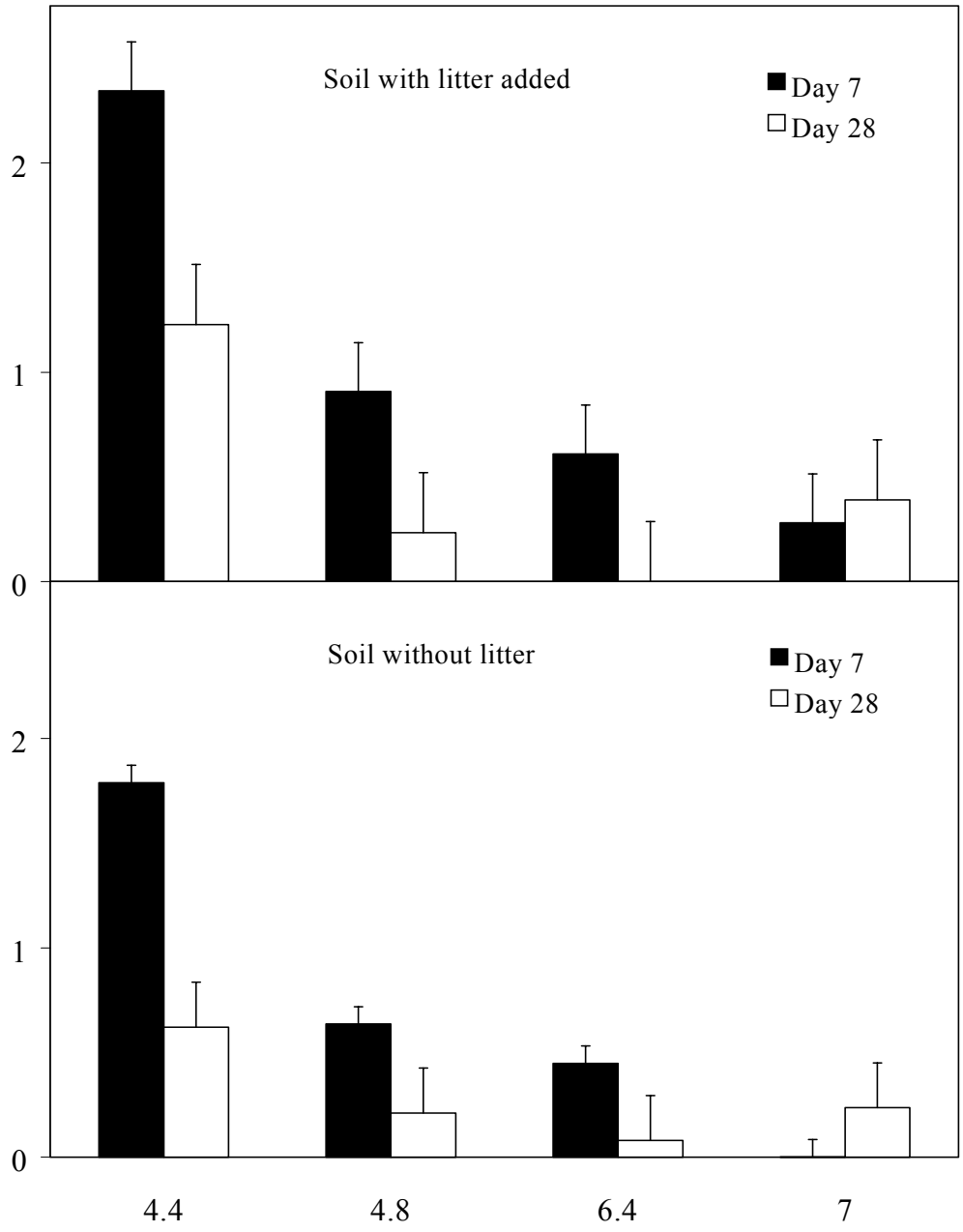


Figure 12. Nitrification potential of soils with and without addition of broiler litter. Bars represent measured nitrification potential at incubation days 7 and 28. Error bars represent standard error (n=3).

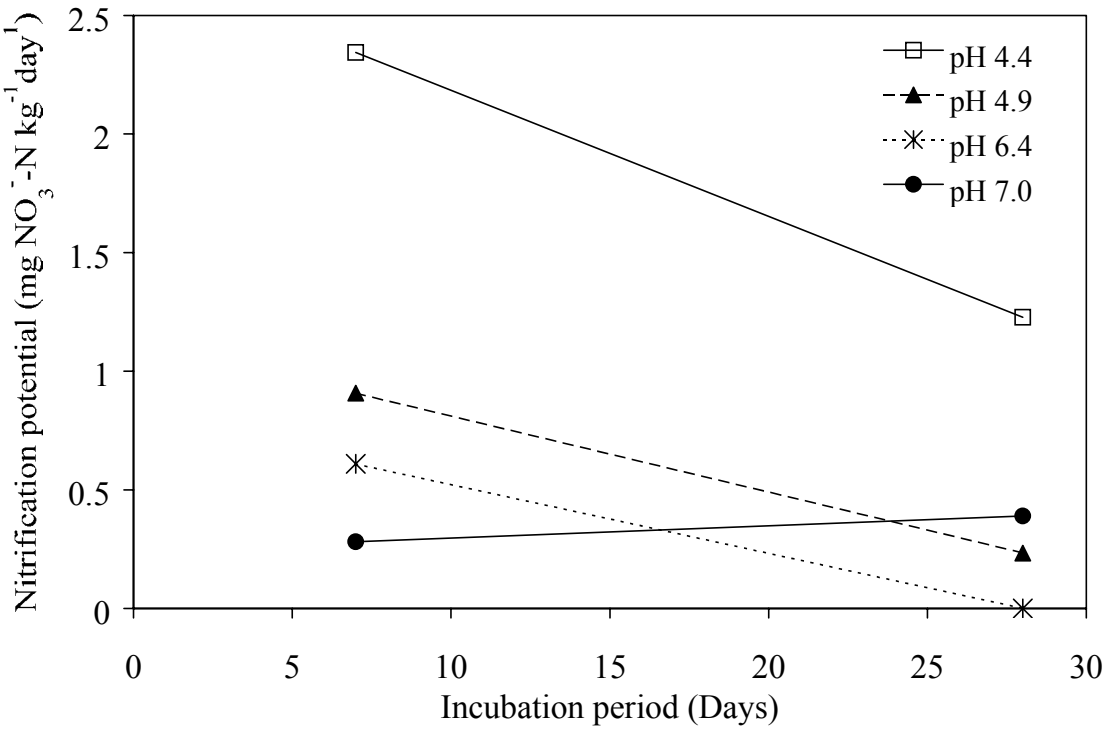


Figure 13. The effect of soil pH and incubation day on nitrification potential of soils amended with broiler litter, measured at 7 and 28 days in an aerobic laboratory incubation.

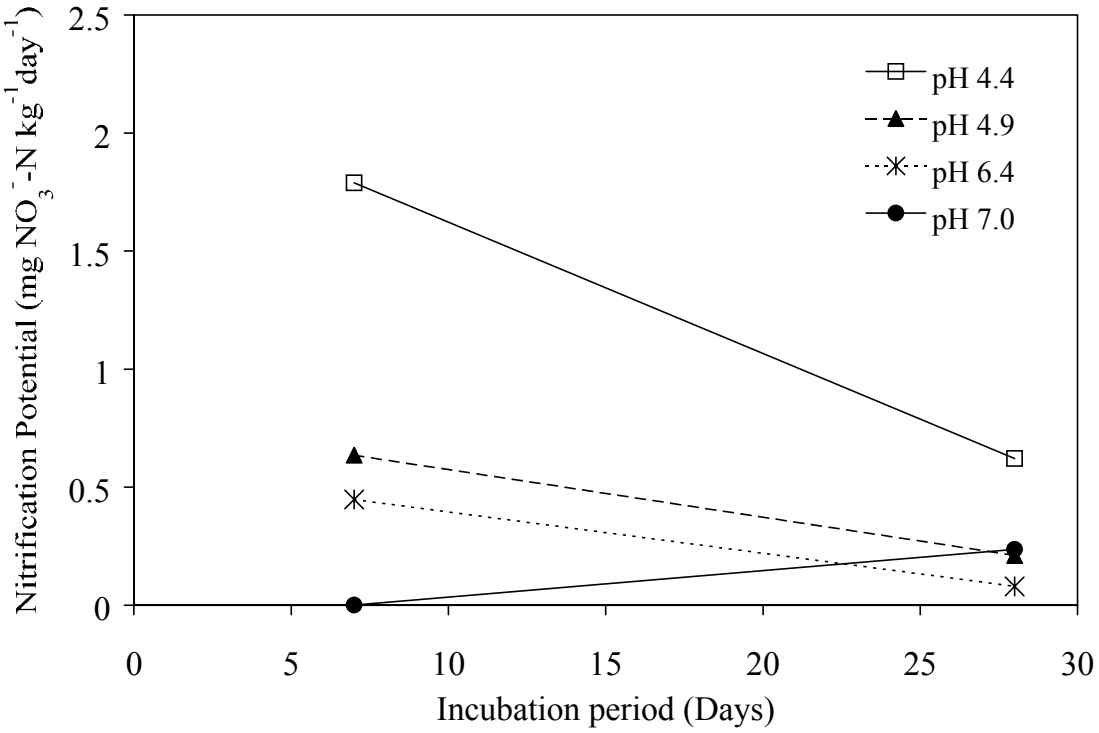


Figure 14. The effect of soil pH and incubation day on nitrification potential of control soils, measured at 7 and 28 days in an aerobic laboratory incubation.

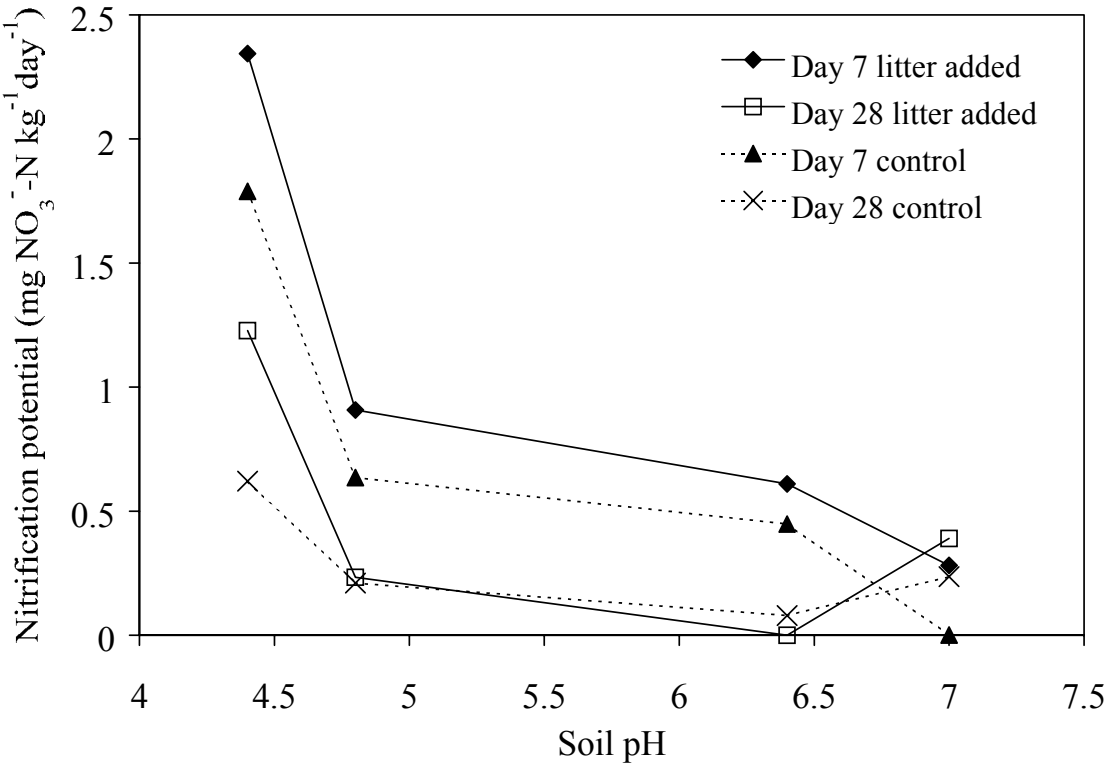


Figure 15. The effect of soil pH, incubation day and litter addition on nitrification potential of four soil pH groups with and without additions of broiler litter, measured at 7 and 28 days in an aerobic laboratory incubation.

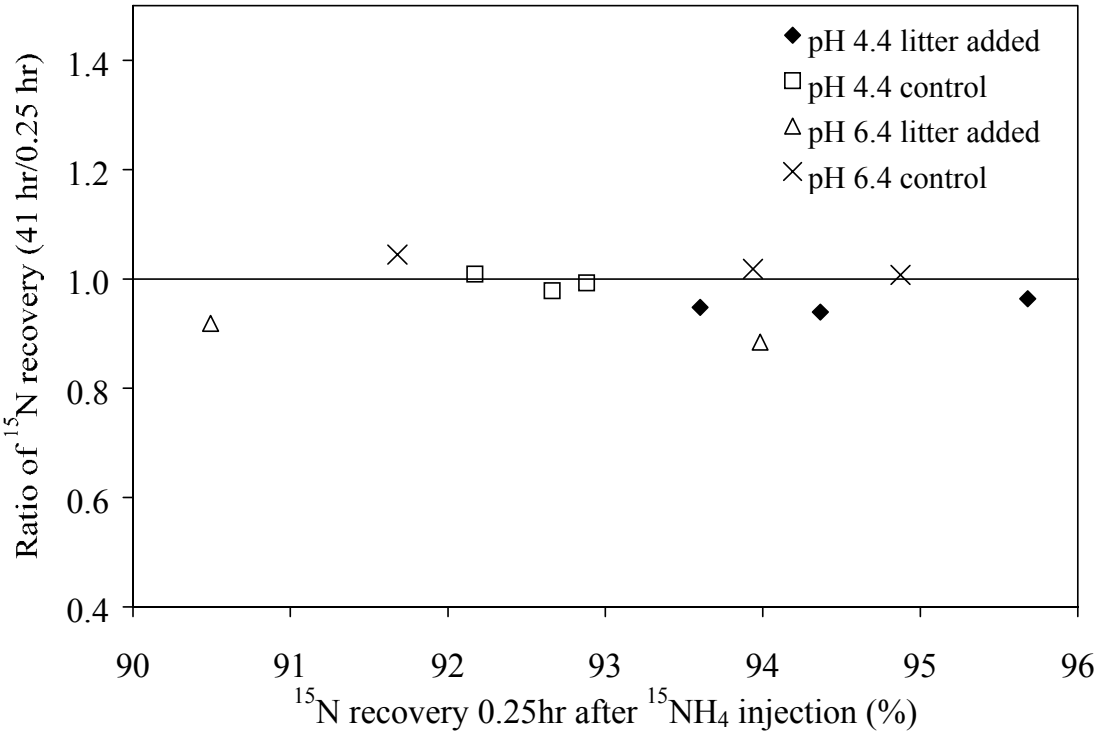


Figure 16. Percent ^{15}N recovery 0.25 hr after $^{15}NH_4$ injection and ratio of ^{15}N recovered 41 hr after ^{15}N injection to ^{15}N recovered 0.25 hr after ^{15}N injection (day 7).

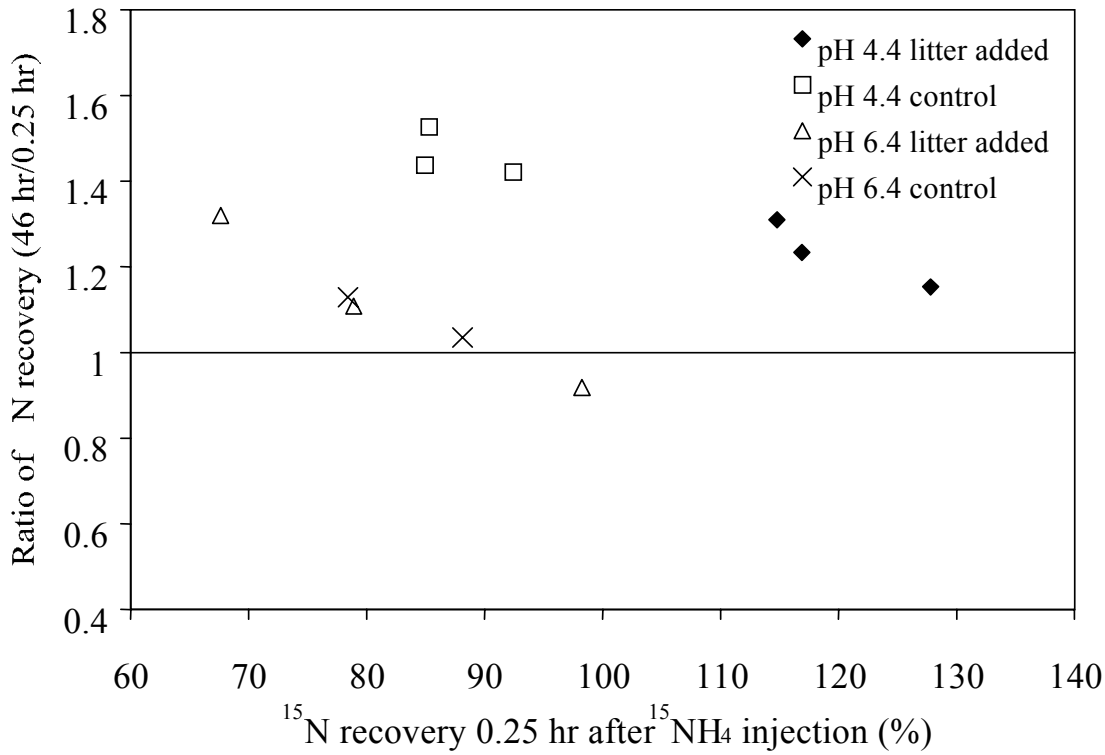


Figure 17. Percent ^{15}N recovery 0.25 hr after $^{15}NH_4$ injection and ratio of ^{15}N recovered 41 hr after ^{15}N injection to ^{15}N recovered 0.25 hr after ^{15}N injection (day 28).

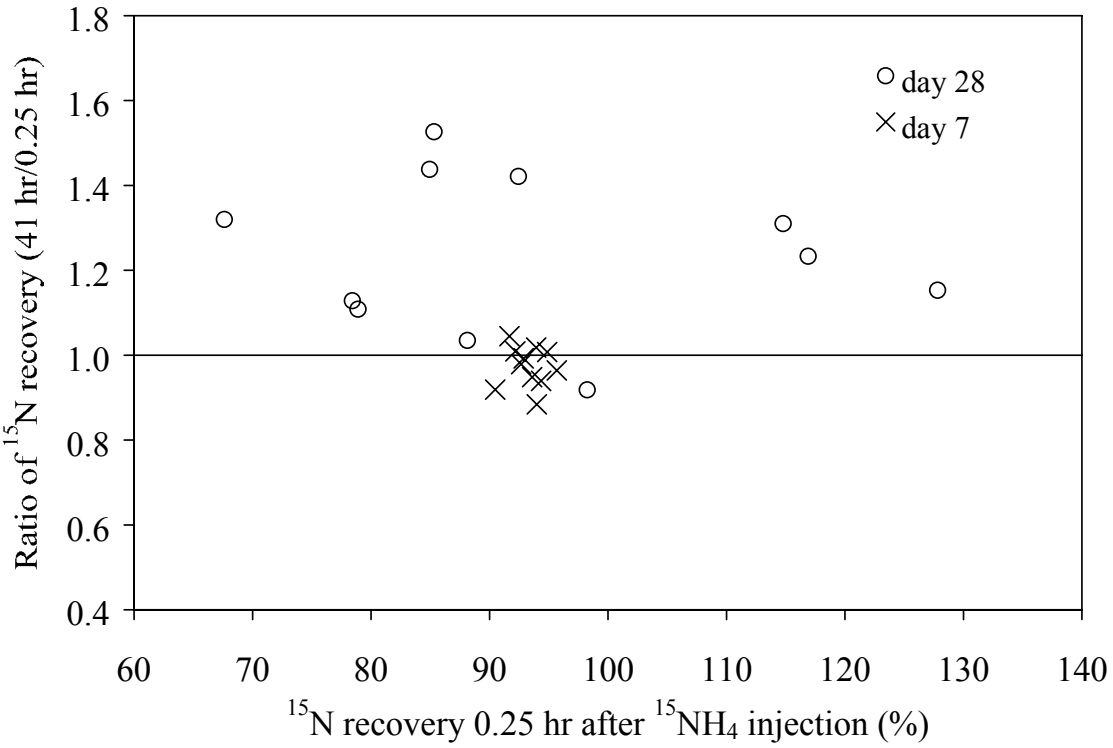


Figure 18. Percent ^{15}N recovery 0.25 hr after $^{15}NH_4$ injection and ratio of ^{15}N recovered 41 hr after ^{15}N injection to ^{15}N recovered 0.25 hr after ^{15}N injection (day 7 and 28).

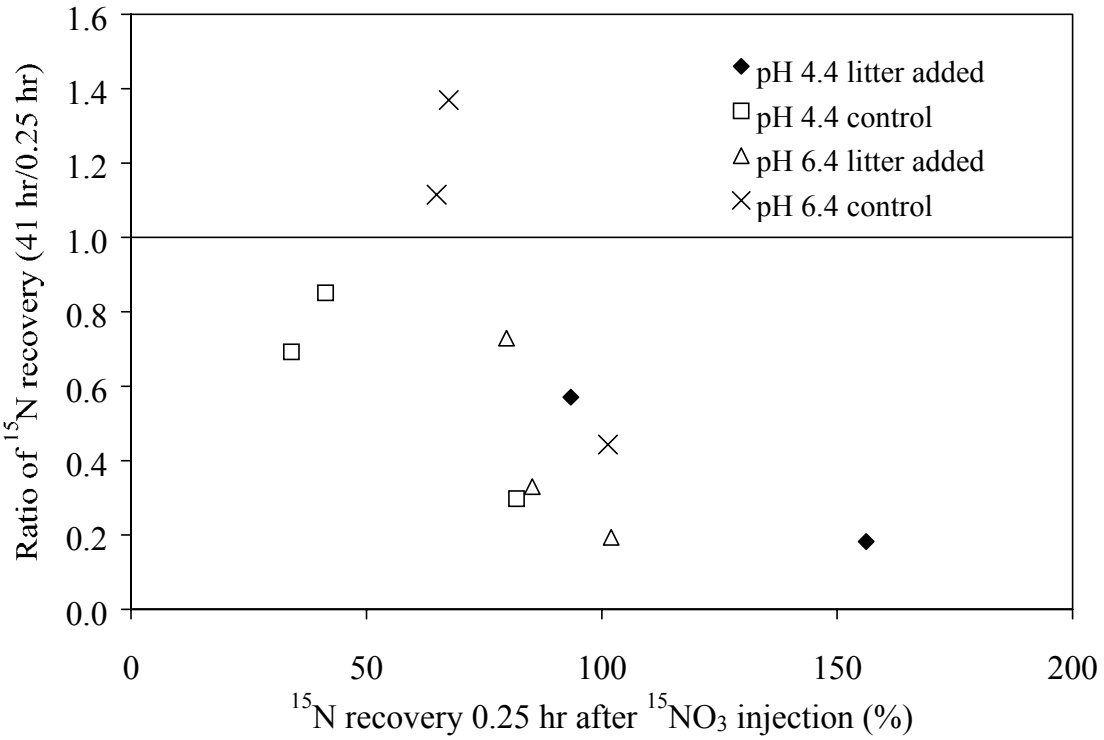


Figure 19. Percent ^{15}N recovery 0.25 h after $^{15}NO_3$ injection and ratio of ^{15}N recovered 41 h after ^{15}N injection to ^{15}N recovered 0.25 h after ^{15}N injection (day 7).

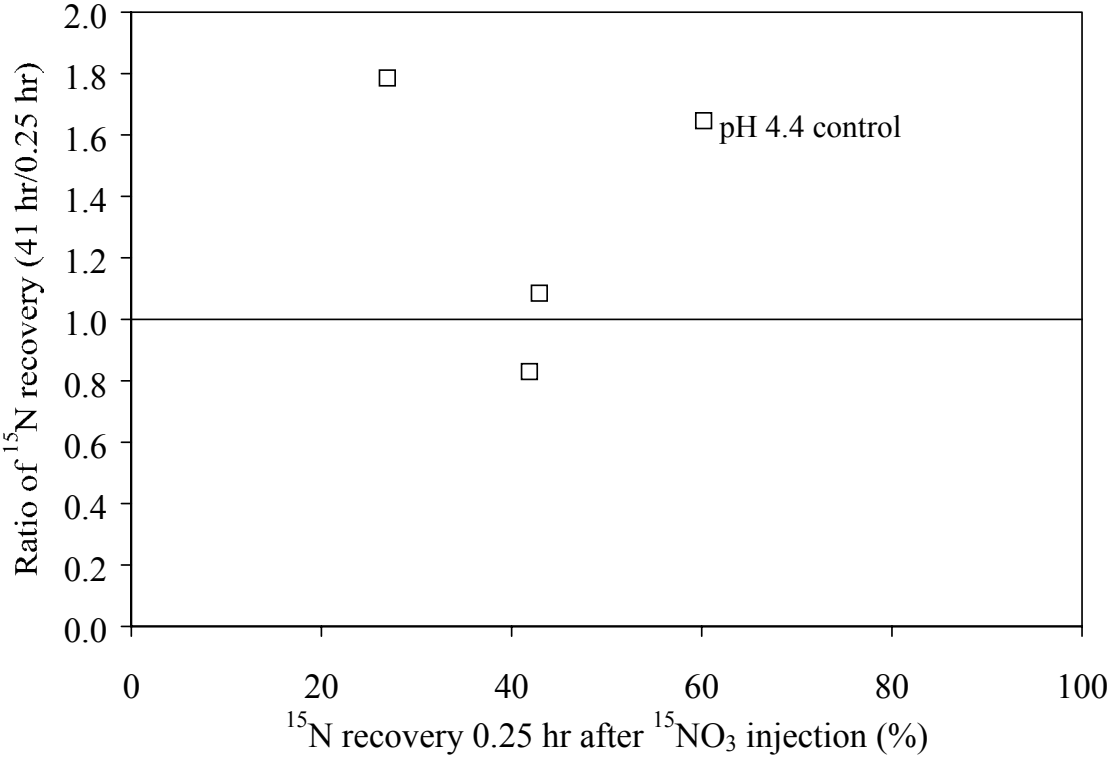


Figure 20. Percent ^{15}N recovery 0.25 hr after $^{15}NO_3$ injection and ratio of ^{15}N recovered 41 hr after ^{15}N injection to ^{15}N recovered 0.25 hr after ^{15}N injection (day 28).

References

Agricultural Statistics Board. 1994. Poultry: Production and value, annual summary. National Agricultural Statistics Service, USDA, Washington, DC.

Barker, J.C. and Zublena, J.P. 1993. Livestock Manure Nutrient Assessment in North Carolina- 1993. [Online]. Available at http://www.spatiallab.ncsu.edu/nutman/assessment.htm. (verified May 19, 2003).

Brooks, P.D., Stark, J.M., McInteer, B.B., and Preston, T. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci. Soc. Am. J. 53:1707-1711.

Campbell, C.A., G.P. Lafond, A.J. Leyshon, R.P. Zentner, and H.H. Janzen. Effect of cropping practices on the initial potential rate of N mineralization in a thin black chernozem. Can. J. Soil Sci. 71:43-53.

Carpenter, G.H. 1992. Current litter practices and future needs. *In* J.P. Blake, J.O. Donald and P.H. Paterson (ed.) Proc. 1992 National Poultry Waste Management Symposium. Auburn Univ. Printing Service, Auburn Univ., AL.

Coyne, M.S. 1999. Soil microbiology: an explanatory approach. Delmar Publishers, Albany, New York.

Crane, S.R., P.W. Westerman, and M.R. Overcash. 1981. Short-term chemical transformations following land application of poultry manure. Trans. ASAE 24:382-390.

Dancer, W.S. L.A. Peterson, and G. Chesters. 1973. Ammonification and nitrification of N as influenced by soil pH and previous N treatments. Soil Sci. Soc. Amer. Proc. 37:67-69.

Evers, G.W. 1998. Comparison of broiler poultry litter and commercial fertilizer for coastal bermudagrass production in the southeastern US. J. Sustainable Agric. 12:55-77.

Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. Agron. J. 9:383-411.

Goovaerts, P., and C.N. Chiang. 1993. Temporal persistence of spatial patterns for mineralizable nitrogen and selected soil properties. Soil Sci. Soc. Am. J. 57:372-381.

Gordillo, R.M., and M.L. Cabrera. 1997a. Mineralizable nitrogen in broiler litter: I. Effect of selected litter chemical characteristics. J. Environ. Qual. 26:1672-1679.

Gordillo, R.M., and M.L. Cabrera. 1997b. Mineralizable nitrogen in broiler litter: II. Effect of selected soil characteristics. J. Environ. Qual. 26:1679-1686.

Hadas, A., B. Bar-Yosef, S. Davidov, and M. Sofer. 1983. Effect of pelleting, temperature, and soil type on mineral nitrogen release from broiler litter and dairy manures. Soil Sci. Soc. Am. J. 47:1129-1133.

Harmsen, G.W. and van Schreven, D.A. 1955. Mineralization of organic nitrogen in soil. Adv. Agron. 7:299-398.

Hart, S.C., J.M. Stark, E.A. Davidson and M.K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. p. 985-1018. *In* R.W. Weaver et al. (ed.) Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. SSSA Book Ser. 5. SSSA. Madison, WI.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers: An introduction to nutrient management. Prentice Hall, Upper Saddle River, New Jersey.

Haynes, R.J. and R.S. Swift. 1989. Effect of rewetting air-dried soils on pH and accumulation of mineral nitrogen. J. Soil Sci. 40:341-347.

Kirkham, D., and Bartholomew, W.V. 1954. Equations for following nutrient transformation in soil utilizing tracer data. Soil Sci. Soc. Am. Proc. 19:189-192.

Lachat Instruments. 1995. QuikChem 8000 continuum series automated ion analyzer, Lachat, Inc., Milwaukee, WI.

Macmillan, K.A., T.W. Scott, and T.W. Bateman. 1975. Corn response and soil nitrogen transformations following varied application of poultry manure treated to minimize odor. Can. J. Soil Sci. 55: 29-34.

Morrill, L.G., and J.E. Dawson. 1967. Patterns observed for the oxidation of ammonium to nitrate by soil organisms. Soil Sci. Soc. Am. Proc. 31:757-760.

Nahm, K.H. 2003. Evaluation of the nitrogen content in poultry manure. World Poultry Sci. J. 59:77-88.

North Carolina Agricultural Statistics Division. 2003. [Online]. Available at http://www.ncagr.com/stats/nc_rank/ncrallyr.htm. (verified June 10, 2003).

North Carolina Department of Agriculture and Consumer Services. 2003. Soil test methodologies. [Online]. Available at http://www.ncagr.com/agronomi/stmethod.htm. (verified August 15, 2003).

Nyborg, M. and Hoyt, P.B. 1978. Effects of soil acidity and liming on mineralization of soil nitrogen. Can. J. Soil Sci. 58:331-338.

Olsen, R.J., R.F. Hensler, and O.J. Attoe. 1970. Effect of manure application, aeration, and soil pH on soil nitrogen transformations and on certain soil test values. Soil Sci. Soc. Am. Proc. 34:222-225.

Overcash, M.R., Humenik, F.J. and R.J. Miner. 1983. Livestock Waste Management, vol. 1. CRC Press, Boca Raton, FL.

Paul, E.A. and F.E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, Inc., San Diego, CA.

Persson, T. and A. Wirén. 1995. Nitrogen mineralization and potential nitrification at different depths in acid forest soils. p. 55-66. *In* L.O. Nilsson, R.F. Huttl and U.T. Johansson (ed.) Nutrient uptake and cycling in forest ecosystems. Kluwer Academic Publishers. Dordrecht, The Netherlands.

SAS Institute. 1998. The SAS System for Windows, version seven. Cary, NC 27513.

Shi, W., J.M. Norton, B.E. Miller, and M.G. Pace. 1999. Effects of aeration and moisture during windrow composting on the nitrogen fertilizer values of dairy waste composts. Appl. Soil Ecol. 11:17-28.

Sims, J.T. 1986. Nitrogen transformations in a poultry manure amended soil: temperature and moisture effects. J. Environ. Qual. 15:59-63.

Singh, Y. and E.G. Beauchamp. 1986. Nitrogen mineralization and nitrifier activity in limed and urea-treated soils. Commun. Soil Sci. Plant Anal. 17:1369-1381.

Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Amer. Proc. 38:99-102.

Stark, J.M., and S.C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. Am. J. 60:1849-1855.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer. 1998. Principles and applications of soil microbiology. Prentice-Hall, Inc. Upper Saddle River, New Jersey.

Thompson, L.M., C.A. Black, and J.A. Zoellner. 1954. Occurrence and mineralization of organic phosphorus in soils, with particular reference to associations with nitrogen, carbon, and pH. Soil Sci. 77: 185-196.

USDA. 1994. Livestock, dairy, and poultry statistics and outlook. Econ. Res. Serv. USDA. Washington, DC.

USDA. 1998. Poultry: production and value—supplement (PBH-BB) 1997 summary. NASS Washington, DC.

Westerman, P.W., L.M. Safley Jr., and J.C. Barker. 1988. Available nitrogen in broiler and turkey litter. Trans. ASAE. 31: 1070-1075.

Williams, M.L. 1994. Regulation of nitrogen mineralization in acid soils by polyphenols. PhD. diss. North Carolina State Univ., Raleigh.

APPENDICES

APPENDIX A

Additional Data and Statistical Analysis from Soybean Nitrogen Use Study (Chapter II)

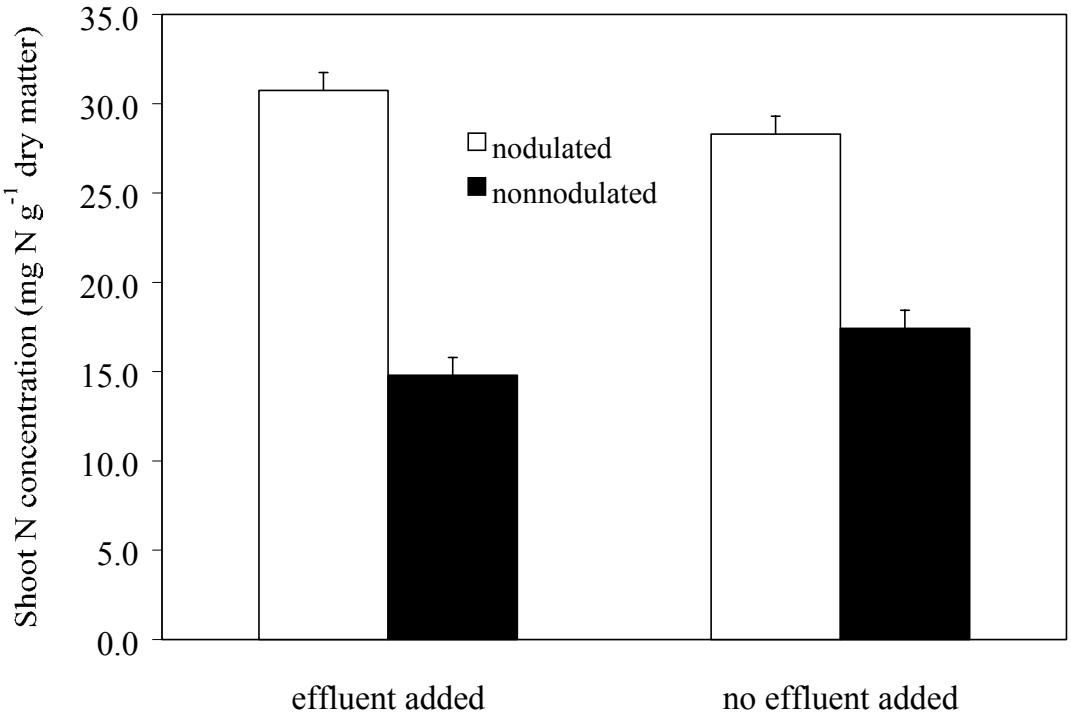


Figure 1A. The effect of effluent addition on shoot N concentration of nodulated and nonnodulated soybean with thinned soybean and soybean harvested at conclusion of experiment combined. A significant ($p < 0.05$) interaction exists for effluent*nodulation. Bars indicate experimental standard error (n=4).

Table 1A. Analysis of variance for pod N, leaf N, stem N, shoot N and shoot dry weight with thinned soybean and soybean harvested at conclusion of experiment combined.

Variable	C.V.	Source	d.f.	F value	*p* value
Pod N	33.6	nodulation	1	23.92	<0.001
		effluent	1	26.48	<0.001
		nodulation*effluent	1	11.40	0.006
Leaf N	22.2	nodulation	1	38.19	<0.001
		effluent	1	116.71	<0.001
		nodulation*effluent	1	22.23	<0.001
Stem N	26.8	nodulation	1	24.97	<0.001
		effluent	1	14.95	0.002
		nodulation*effluent	1	3.08	0.105
Shoot N	26.4	nodulation	1	34.34	<0.001
		effluent	1	48.67	<0.001
		nodulation*effluent	1	16.03	0.002
Shoot dry weight	20.3	nodulation	1	2.48	0.141
		effluent	1	74.63	<0.001
		nodulation*effluent	1	2.38	0.149

Table 2A. Analysis of variance for soil N (mg) at the end of the experiment and leachate N (mg) accumulated over a period of six weeks.

Variable	C.V.	Source	d.f.	F value	*p* value
Soil N	11.9	nodulation	1	0.03	0.873
		effluent	1	0.35	0.564
		nodulation*effluent	1	1.56	0.235
Leachate N	60.4	nodulation	1	0.32	0.584
		effluent	1	1.02	0.333
		nodulation*effluent	1	3.08	0.105

Table 3A. Analysis of variance for soil ^{15}N atom % excess (APE) and soil ^{15}N (mg) at the end of the experiment.

Variable	C.V.	Source	d.f.	F value	*p* value
Soil ^{15}N APE	45.7	nodulation	1	0.00	0.960
		effluent	1	70.83	<0.001
		nodulation*effluent	1	0.00	0.987
Soil ^{15}N (mg)	21.0	nodulation	1	0.01	0.905
		effluent	1	17.79	0.001
		nodulation*effluent	1	0.55	0.472

Table 4A. Analysis of variance for leachate ^{15}N (mg) accumulated over a period of six weeks.

Variable	C.V.	Source	d.f.	F value	*p* value
Leachate ^{15}N	61.9	nodulation	1	0.27	0.612
		effluent	1	1.99	0.183
		nodulation*effluent	1	0.05	0.827

Table 5A. Mean mass N, ^{15}N atom % excess (APE), mass effluent N recovered, total mass N added as effluent and % effluent N recovered by pods, leaves, stems and shoots with thinned soybean and soybean harvested at conclusion of experiment combined.

Treatment	Mass N	^{15}N	Effluent N recovered	Total N added as effluent	Effluent N recovered of total N added
nodulated + effluent	—mg—	—APE—	—mg—	—mg—	—%—
pods	957	0.907	153	650	23.5
leaves	386	1.077	70	650	10.8
stems	96	0.886	15	650	2.3
shoots	1439	0.949	238	650	36.6
nonnodulated + effluent					
pods	336	2.358	146	650	22.5
leaves	173	1.750	56	650	8.6
stems	45	1.777	15	650	2.3
shoots	554	2.120	217	650	33.4
nodulated control					
pods	324	0.003	-	-	-
leaves	83	0.010	-	-	-
stems	60	0.006	-	-	-
shoots	468	0.005	-	-	-
nonnodulated control					
pods	203	0.006	-	-	-
leaves	55	0.011	-	-	-
stems	29	0.007	-	-	-
shoots	287	0.006	-	-	-

Table 6A. Analysis of variance for % of effluent N recovered in pods, leaves, stems, shoots (thinned soybean and soybean removed at final harvest combined), soil, leachate and unrecovered effluent N.

Variable	C.V.	Source	d.f.	F value	*p* value
Pods	17.7	nodulation	1	0.94	0.369
Leaves	22.9	nodulation	1	3.23	0.122
Stems	16.1	nodulation	1	0.21	0.662
Shoots	12.2	nodulation	1	3.70	0.103
Soil	44.8	nodulation	1	0.08	0.788
Leachate	62.5	nodulation	1	0.24	0.643
Unrecovered	46.4	nodulation	1	0.94	0.370

Table 7A. Leachate (NO_3 + NH_4)-N concentration, volume and ^{15}N atom % (wk 1-2).

	Week 1			Week 2		
Treatment/rep	Concentration	Volume	^{15}N atom %	Concentration	Volume	^{15}N atom %
Nodulated + effluent	—mg L^{-1}—	—mL—		—mg L^{-1}—	—mL—	
1	769.5	226	0.415	219.2	828	0.420
2	280.2	125	0.407	272.9	461	0.424
3	156.6	118	0.420	124.1	226	0.411
4	193.5	381	0.395	106.5	311	0.455
Mean	349.9	213	0.409	180.7	456	0.428
S.E.	142.2	61	0.010	39.49	133	0.010
Nonnodulated + effluent						
1	41.5	114	0.906	349.7	500	0.427
2	262.2	17	0.477	115.3	536	0.462
3	381.5	241	0.384	272.1	392	0.401
4	111.9	169	0.433	173.1	386	0.397
Mean	199.3	160	0.550	227.5	453	0.422
S.E.	76.2	30	0.120	52.01	38	0.020
Nodulated control						
1	69.2	115	0.368	231.1	111	0.367
2	115.7	161	0.368	219.6	263	0.367
3	65.2	118	0.368	296.0	574	0.366
4	627.0	138	0.368	96.0	708	0.367
Mean	219.3	133	0.368	210.7	414	0.367
S.E.	136.4	11	0.000	41.74	137	0.000
Nonnodulated control						
1	279.8	110	0.366	260.6	162	0.367
2	176.7	159	0.364	82.5	190	0.368
3	669.4	133	0.367	88.8	521	0.369
4	102.7	144	0.366	262.9	524	0.368
Mean	307.1	137	0.366	173.7	349	0.368
S.E.	126.1	10	0.000	50.86	100	0.000

Table 8A. Leachate (NO_3 + NH_4)-N concentration, volume and ^{15}N atom % (wk 3-4).

	Week 3			Week 4		
Treatment/rep	Concentration	Volume	^{15}N atom %	Concentration	Volume	^{15}N atom %
Nodulated + effluent	—mg L^{-1}—	—mL—		—mg L^{-1}—	—mL—	
1	2.3	307	0.433	0.0	0	0.000
2	4.1	324	0.429	2.0	159	0.413
3	4.3	270	0.409	4.3	46	0.887
4	19.2	330	0.738	11.7	55	0.530
Mean	7.5	308	0.502	4.5	65	0.457
S.E.	3.93	13	0.08	2.56	34	0.180
Nonnodulated + effluent						
1	10.8	295	0.461	1.3	237	0.466
2	15.0	32	0.438	2.4	163	0.704
3	2.6	397	0.441	1.3	289	0.411
4	45.8	191	0.389	2.7	152	0.523
Mean	18.5	229	0.432	2.1	210	0.526
S.E.	9.46	78	0.020	0.30	32	0.060
Nodulated control						
1	37.8	368	0.369	1.1	447	0.367
2	23.3	367	0.371	4.5	289	0.368
3	49.7	423	0.366	1.0	830	0.367
4	25.4	469	0.370	2.2	1131	0.368
Mean	34.0	407	0.369	2.2	674	0.368
S.E.	6.12	25	0.000	0.81	190	0.000
Nonnodulated control						
1	5.8	207	0.368	0.7	456	0.369
2	15.5	429	0.371	3.0	354	0.370
3	1.2	396	0.366	0.5	982	0.577
4	18.2	277	0.370	1.6	388	0.368
Mean	10.2	327	0.369	1.5	545	0.421
S.E.	3.99	52	0.000	0.57	147	0.050

Table 9A. Leachate (NO_3 + NH_4)-N concentration, volume and ^{15}N atom % (wk 5-6).

	Week 5			Week 6		
Treatment/rep	Concentration	Volume	^{15}N atom %	Concentration	Volume	^{15}N atom %
Nodulated + effluent	—mg L^{-1}—	—mL—		—mg L^{-1}—	—mL—	
1	1.0	316	0.467	0.8	393	0.847
2	2.4	600	1.696	1.9	281	1.580
3	1.2	539	0.917	1.9	139	1.326
4	0.8	442	no data	0.7	474	0.693
Mean	1.3	474	1.026	1.3	322	1.111
S.E.	0.35	62	0.31	0.34	73	0.210
Nonnodulated + effluent						
1	0.7	470	0.436	0.4	363	0.547
2	1.1	327	1.733	0.3	192	0.639
3	0.4	214	0.430	0.1	665	0.805
4	0.3	574	0.517	0.2	341	0.533
Mean	0.6	396	0.779	0.3	390	0.631
S.E.	0.16	79	0.32	0.05	99	0.060
Nodulated control						
1	0.3	272	0.369	0.4	420	0.369
2	1.9	108	0.369	0.7	473	0.370
3	0.5	445	0.381	0.3	420	0.363
4	0.5	720	0.367	0.3	655	0.372
Mean	0.8	386	0.371	0.4	492	0.369
S.E.	0.38	131	0.00	0.10	56	0.000
Nonnodulated control						
1	0.5	608	0.406	0.4	421	0.367
2	0.6	191	0.367	2.1	304	0.378
3	0.3	823	0.365	0.2	905	0.369
4	0.3	338	0.367	0.2	736	0.372
Mean	0.4	490	0.376	0.7	592	0.372
S.E.	0.08	141	0.01	0.46	139	0.000

APPENDIX B

Additional Data and Statistical Analysis from

Soil pH Effects on N Transformations (Chapter III)

Table 1B. Analysis of variance for net N mineralization (N_m) (mg N kg^{-1} soil), potentially mineralizable N (N_o) (mg N kg^{-1} organic N), rate constant (k) (day^{-1}), initial potential rate (N_ok) (g N kg^{-1} organic N d^{-1}), and % N mineralized (of organic N added) by 111 days.

Variable	C.V.	Source	d.f.	F value	*p* value
N_m	20.3	soil pH	5	3.46	0.036
N_o	21.6	soil pH	5	2.75	0.070
k	55.5	soil pH	5	1.23	0.283
N_ok	30.1	soil pH	5	3.23	0.045
% N mineralized	21.8	soil pH	5	2.96	0.057

Table 2B. Analysis of variance for nitrification potential (mg NO_3-N kg^{-1} soil day^{-1}) at two incubation days (7, 28) for six soil pH groups with and without broiler litter addition.

Variable	C.V.	Source	d.f.	F value	*p* value
Nitrification potential	81.47	day	1	12.12	0.002
		soil pH	3	16.77	<0.001
		litter	1	2.74	0.108
		day*soil pH	3	3.48	0.027
		soil pH*litter	3	0.74	0.536
		day*litter	1	0.16	0.692
		day*soil pH*litter	3	0.05	0.985

Table 3B. Analysis of variance for $^{15}NH_4$ ratio of recovery at two incubation days (7, 28) for six pH groups with and without broiler litter addition.

Variable	C.V.	Source	d.f.	F value	*p* value
Ratio of recovery	122.5	day	1	6.91	0.020
		soil pH	1	2.79	0.117
		litter	1	2.11	0.168
		day*soil pH	1	0.92	0.353
		soil pH*litter	1	0.34	0.571
		day*litter	1	0.44	0.518
		day*soil pH*litter	1	1.66	0.218

Table 4B. Analysis of variance for $^{15}NO_3$ ratio of recovery at incubation day 7 for six pH groups with and without broiler litter addition.

Variable	C.V.	Source	d.f.	F value	*p* value
Ratio of recovery	27.1	soil pH	1	0.37	0.560
		litter	1	13.98	0.007
		soil pH*litter	1	0.17	0.691

Table 5B. Mean (n=3) % ^{15}N recovery of added $^{15}NH_4$ at two incubation days (7, 28) for 2 soil pH groups with and without broiler litter addition.

Soil pH	Incubation day	Litter addition	^{15}N injection time	^{15}N Recovered	^{15}N injection time	^{15}N Recovered
			hours	%	hours	%
4.4	7	yes	0.25	94.6	41	88.6
4.4	7	no	0.25	92.6	41	92.0
6.4	7	yes	0.25	92.2	41	83.9
6.4	7	no	0.25	93.5	41	95.7
4.4	28	yes	0.25	119.8	41	147.3
4.4	28	no	0.25	87.6	41	127.9
6.4	28	yes	0.25	81.6	41	89.0
6.4	28	no	0.25	83.3	41	90.4

Table 6B. Mean (n=3) % ^{15}N recovery of added $^{15}NO_3$ at two incubation days (7, 28) for 2 soil pH groups with and without broiler litter addition.

Soil pH	Incubation day	Litter addition	^{15}N injection time	^{15}N Recovered	^{15}N injection time	^{15}N Recovered
			hours	%	hours	%
4.4	7	yes	0.25	45.4	41	16.1
4.4	7	no	0.25	46.0	41	33.3
6.4	7	yes	0.25	29.7	41	18.4
6.4	7	no	0.25	38.4	41	40.8
4.4	28	no	0.25	50.21	41	65.52

Table 7B. Mean (n=3) soil ^{15}N atom % for soils injected with $^{15}NH_4$ at two incubation days (7, 28) for 2 soil pH groups with and without broiler litter addition.

Soil pH	Incubation day	Litter addition	^{15}N injection time	^{15}N atom %	^{15}N injection time	^{15}N atom %
			hours		hours	
4.4	7	yes	0.25	7.472	41	7.676
4.4	7	no	0.25	17.000	41	16.170
6.4	7	yes	0.25	5.012	41	4.562
6.4	7	no	0.25	0.181	41	0.142
4.4	28	yes	0.25	11.868	41	8.851
4.4	28	no	0.25	25.500	41	24.860
6.4	28	yes	0.25	5.270	41	4.263
6.4	28	no	0.25	9.370	41	6.260

Table 8B. Mean (n=3) soil ^{15}N atom % for soils injected with $^{15}NO_3$ at two incubation days (7, 28) for 2 soil pH groups with and without broiler litter addition.

Soil pH	Incubation day	Litter addition	^{15}N injection time	^{15}N atom %	^{15}N injection time	^{15}N atom %
			hours		hours	
4.4	7	yes	0.25	22.394	41	13.588
4.4	7	no	0.25	25.270	41	23.490
6.4	7	yes	0.25	19.894	41	22.750
6.4	7	no	0.25	22.740	41	26.460
4.4	28	yes	0.25	28.271	41	16.857
4.4	28	no	0.25	32.430	41	37.280
6.4	28	yes	0.25	0.120	41	0.144
6.4	28	no	0.25	0.051	41	0.260

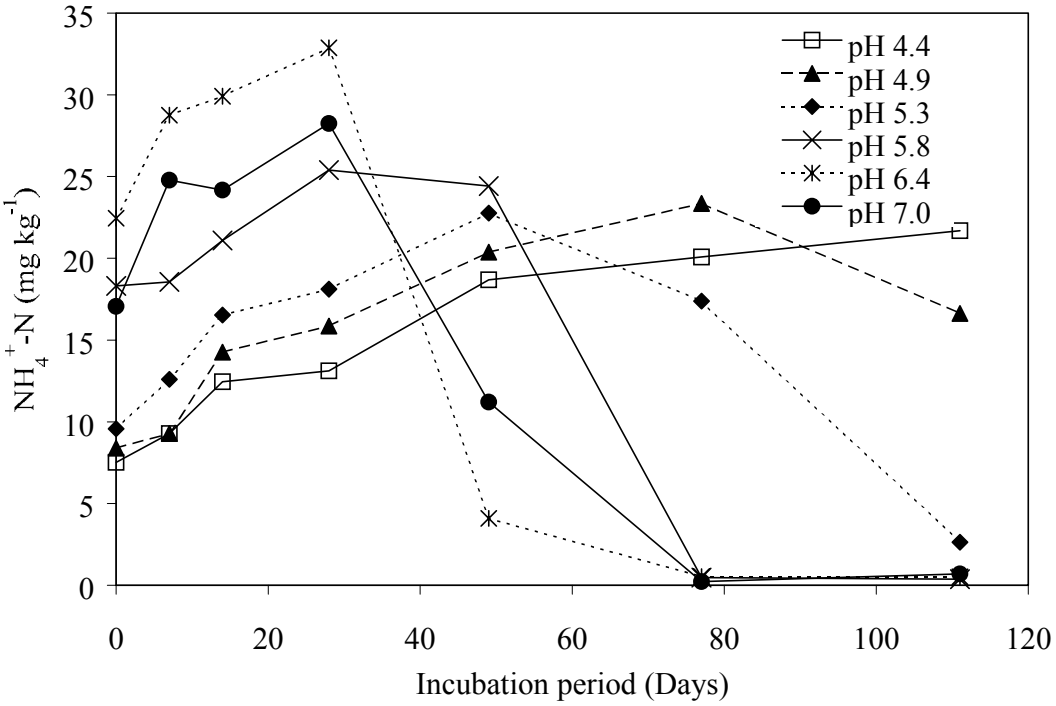


Figure 1B. Soil NH_4-N concentration for 6 control soil pH groups incubated for 111 days at 25°C. Symbols represent mean values (n=3).

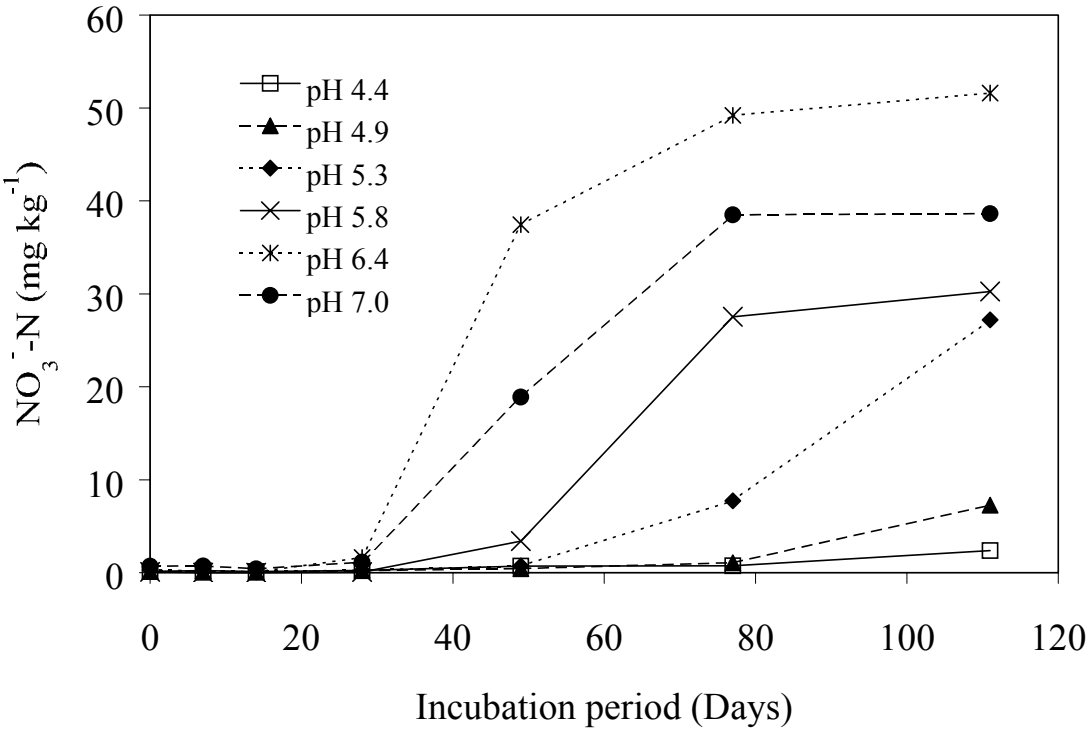


Figure 2B. Soil NO_3-N concentration for 6 control soil pH groups incubated for 111 days at 25°C. Symbols represent mean values (n=3).

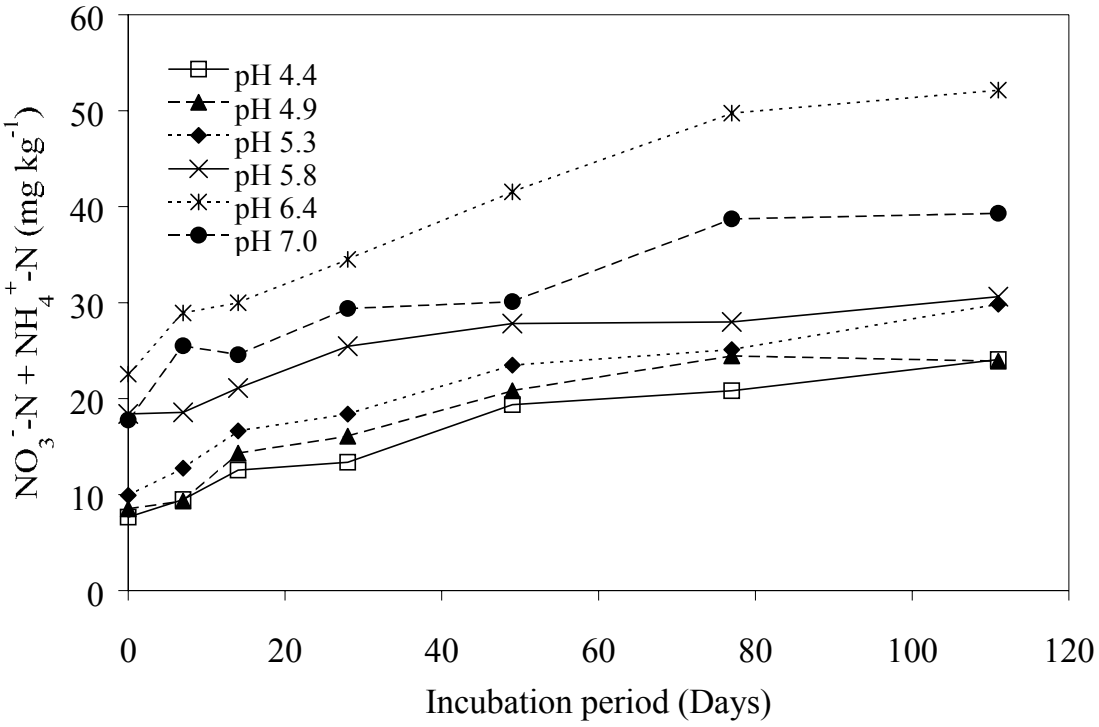


Figure 3B. Soil inorganic N concentration for 6 control soil pH groups incubated for 111 days at 25°C. Symbols represent mean values (n=3).