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MANAGEMENT OF MICROBIAL NITRATE PRODUCTION
IN AGRICULTURAL SOILS

by

Wei Shi

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

of

DOCTOR OF PHILOSOPHY

in

Soil Science
(Soil Microbiology)

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Logan, Utah

1998

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ABSTRACT

Management of Microbial Nitrate Production in Agricultural Soils

by

Wei Shi, Doctor of Philosophy

Utah State University, 1998

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

Nitrate (NO_3^-) is of central importance in the internal soil nitrogen (N) cycle. While animal wastes and nitrification inhibitors have been used in modern agriculture for decades, their effects on soil NO_3^- concentrations in relation to microbial NO_3^- production have not been well characterized. The objective of this research was to determine microbial NO_3^- production in relation to ammonium (NH_4^+) availability and nitrifier population activity in agricultural soils receiving animal wastes or nitrification inhibitors.

Several laboratory and field studies assessed the impacts of variously treated dairy wastes and the effects of repeated long-term use of a nitrification inhibitor, nitrapyrin, on microbial NO_3^- production and soil NO_3^- concentrations in Utah agricultural soils. The various process rates of N mineralization, nitrification, and microbial N immobilization were determined in laboratory and field systems using ^{15}N

isotope dilution techniques. Nitrification potentials were used simultaneously to measure the nitrifier population size and activity.

Microbial NO_3^- immobilization was not observed in the laboratory and field experiments. The lack of microbial NO_3^- consumption indicates that nitrification was the primary process controlling soil NO_3^- concentrations. Nitrifiers were not weaker competitors than heterotrophs for utilizing soil NH_4^+ ; about 50% of the NH_4^+ mineralized was used by nitrifiers. Low carbon availability may have limited heterotrophic microbial growth, thereby minimizing the heterotrophic microbial consumption of NH_4^+ and NO_3^- .

Effects of dairy wastes on soil NH_4^+ availability depend on the treatment systems of dairy wastes and their application rates. The N mineralization potentials were approximately 5% of the organic N in dairy-waste compost versus 90% of the organic N in dairy waste digested anaerobically. Dairy-waste compost at appropriate application rates did not increase nitrification rates, nitrification potentials, or soil NO_3^- concentrations for several months following application. However, even stabilized dairy-waste compost led to high nitrification rates and potentials, and elevated soil NO_3^- concentrations when it was applied at an excessive rate (i.e., 100 Mg dry wt. ha^{-1}).

In a dryland wheat agroecosystem, repeated use of nitrapyrin for 8 years had a 2-year residual effect observed as lower nitrification potentials in soils with a history of nitrapyrin use compared to soils without that history.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Soil inorganic nitrogen (N) consists primarily in the ammonium (NH_4^+) and nitrate (NO_3^-) ionic forms and is the direct source of plant available N. The microbial conversion of organic N or NH_4^+ to the oxidized nitrite (NO_2^-) and NO_3^- forms is the process of nitrification. In agricultural soils, inorganic N in excess of plant demand generally accumulates as NO_3^- , which can leach to ground water or be lost as N gases to the atmosphere by denitrification. The losses of NO_3^- from agricultural soils may lead to high NO_3^- levels in ground and surface waters and to the production of atmospherically active trace gases such as N_2O and NO_x . Highly concentrated NO_3^- in drinking water may also have deleterious effects on humans, especially infants where high NO_3^- in blood causes methemoglobinemia (Paul and Clark, 1989). The trace gases N_2O and NO_x , which may be produced both by nitrification and denitrification, contribute to global warming and stratospheric ozone depletion. Nitrate losses also decrease N fertilizer use efficiency, which is an economic consideration for producers. Controlling NO_3^- losses from agricultural soils therefore has become an active research area. Most previous studies have focused on the factors and management practice influencing leaching and denitrification for controlling NO_3^- losses (Owens, 1990; Peterson and Russelle, 1991; Weier et al., 1993a, 1993b; Bergstrom et al., 1994; Maag and Vinther, 1997). The potential for the management of NO_3^- production for preventing the adverse effects of

surplus soil NO_3^- has not been thoroughly examined. This dissertation focuses on the dynamics of NO_3^- production in agricultural soils receiving animal wastes and N fertilizers.

Nitrate in soil is produced by microbe-mediated processes of autotrophic and heterotrophic nitrification. As heterotrophic nitrification is generally not significant in agricultural soils (Belser, 1979), autotrophic nitrification is considered the dominant process for producing soil NO_3^- . Autotrophic nitrification is a two-step, consecutive process of NH_4^+ oxidation by two groups of gram-negative chemolithotrophic bacteria known as nitrifiers or nitrifying bacteria. Ammonium oxidizing bacteria transform NH_4^+ to NO_2^- , then nitrite oxidizing bacteria transform NO_2^- to NO_3^- . The extent and rate of nitrification generally depends on NH_4^+ availability and nitrifier population activity. Many N management practices may affect microbial NO_3^- production through their effects on NH_4^+ availability or nitrifier population activity. Two common N practices in agricultural soils are the application of animal wastes to replace mineral N fertilizers, and the application of nitrification inhibitors with mineral N fertilizers to limit the short-term microbial NO_3^- production. It is not clear how these N management practices affect NH_4^+ availability and nitrifier population activity, and the subsequent microbial NO_3^- production. The overall goal of this dissertation was to determine microbial NO_3^- production in relation to NH_4^+ availability and to nitrifier population activity in agricultural soils after the application of animal wastes or nitrification inhibitors.

The management of soils amended with animal wastes contrasts with those receiving mineral fertilizers. Organic N in animal wastes is slowly released as NH_4^+ by the

process of ammonification. The slow release of NH_4^+ and uptake of NH_4^+ by plants and microbes are assumed to limit NH_4^+ availability to nitrifiers. The slow release of NH_4^+ may also coincide with crop NH_4^+ uptake. The synchrony between crop uptake and supply of soil NH_4^+ may further decrease NH_4^+ available for nitrifiers.

Nitrification inhibitors, such as nitrapyrin and acetylene (C_2H_2), inactivate an essential enzyme involved in microbial NH_4^+ oxidation. The inactivity of the essential enzyme limits the nitrifier population activity typically for a few months following application. However, the long-term repeated use of these inhibitors may have residual effects on the ammonia oxidizer community. Repressed populations and selection for ammonium oxidizing bacteria less sensitive to the inhibitor may occur after long-term repeated applications of inhibitors.

Increased understanding of soil microbial NO_3^- production in relation to the status of available NH_4^+ and of nitrifier population activity may help identify appropriate agricultural N management practices. Suitable management of animal wastes and nitrification inhibitors should benefit crop production and minimize the environmental problems caused by surplus NO_3^- . This dissertation includes five chapters summarizing research related to the management of NO_3^- production in agricultural soils, each focusing on one specific area not previously addressed in the literature. Chapter 2 addresses the N fertilizer values of dairy-waste composts as affected by turning and watering during windrow composting; Chapter 3 compares the N mineralization dynamics of dairy wastes treated by aerobic composting or anaerobic lagoon digestion; Chapter 4 evaluates microbial NO_3^- production and consumption in an agricultural soil treated with dairy-

waste compost or ammonium fertilizer; Chapter 5 determines nitrification rates and potentials in a corn field treated with liquid or composted dairy waste; and Chapter 6 evaluates the effects of long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification. The overall goal is to increase our understanding of the dynamics and controls of NO_3^- production and accumulation in agricultural soils.

Literature Review

Nitrogen Mineralization of Animal Wastes

In contrast to a one-time application of a large amount of mineral N fertilizers, inorganic N is gradually released from animal wastes through mineralization or ammonification. The slow release of inorganic N may limit NH_4^+ availability to nitrifiers, thereby decreasing microbial NO_3^- production. However, agricultural soils fertilized with animal wastes may still cause serious NO_3^- environmental pollution because animal wastes are often applied at high rates or at unsuitable times due to poor management or uncertainties about the amount and rate of N mineralized. The adverse effects of excess animal waste on crop, soil, and water quality have been widely reported (Shortall and Liebhardt, 1975; Liebhardt, 1976; Liebhardt et al., 1979; Burns et al., 1990; Roth and Fox, 1990; Kandeler et al., 1994). The investigation of N mineralization from animal wastes is key for environmentally sound N management.

Decomposition of organic N is a biochemical process mediated by microorganisms. Using chemical indices such as total N, initial inorganic N, and C:N ratio for predicting the amount and rate of decomposition is inadequate (Castellanos and

Pratt, 1981; Beauchamp, 1986; O'Keefe et al., 1986; Bitzer and Sims, 1988; Hadas and Portnoy, 1994). Biological incubation, although time consuming and labor intensive, has been considered to be a good method for monitoring the decomposition of organic N with time. The amount and rate of N mineralization are determined from the first-order model: $N_m = N_0(1 - e^{-Kt})$, where N_m is the accumulated N mineralized at time t , N_0 is the N mineralization potential, and K is the mineralization rate constant (Stanford and Smith, 1972). High N_0 means a large pool size of mineralizable organic N, and high K means fast decomposition of organic N (Fig. 1.1). Many studies have evaluated the N mineralized from various soils or organic wastes (Stanford and Smith, 1972; Castellanos and Pratt, 1981; Bitzer and Sims, 1988; Sierra, 1990; Aoyama and Nozawa, 1993; Chèneby et al., 1994), while little information is available for the amount and rate of N mineralized from animal waste treated by different systems (Kirchmann, 1991; Bernal and Kirchmann, 1992). Moreover, it is not clear how mineralization controls subsequent nitrification after the application of treated animal wastes.

Microbial N Transformations

Plant and microbial N uptake may decrease N remaining in soil. The role of plants in reducing NO_3^- concentrations in soil profiles has been studied using deep-rooted alfalfa (Schertz and Miller, 1972; Mathers et al., 1975). Theoretically, NH_4^+ uptake by plants may reduce NH_4^+ available for nitrifiers, thereby reducing microbial NO_3^- production. Nitrification rates have been found to be lower with plant growth versus without plant growth (Zak et al., 1990; Verhagen et al., 1994). Nitrifier population sizes have also

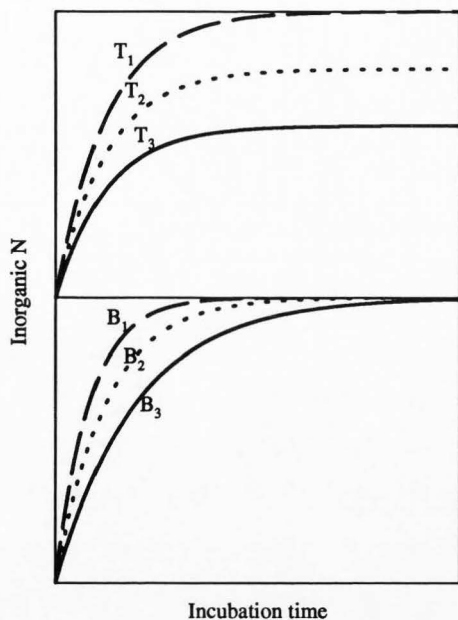


Fig. 1.1. The meanings of N mineralization potential (N_0) and mineralization rate constant (K). Three organic materials have the same K , but different N_0 (top of the figure). T_1 has the largest mineralizable organic N pool size, while T_3 has the smallest mineralizable organic N pool size. At the bottom, three organic materials have the same N_0 , but different K . B_1 decomposes faster than B_2 and B_3 .

been observed to be lower in the presence of plants (Verhagen et al., 1994). The majority of the recent work on the interaction of nitrifiers and plants has been done in the natural ecosystems of forests and grasslands (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990; Norton and Firestone, 1996). Although these studies have examined the partitioning of inorganic N between plants and microbes with qualitative and quantitative analyses by isotope ^{15}N dilution and tracer techniques, the results may not directly be applicable to agricultural soils. Therefore, nitrification rates should be determined in fertilized and cropped agricultural soils.

It is generally accepted that many agricultural plants prefer NO_3^- , while soil microbes prefer NH_4^+ for their growth. The partitioning of NH_4^+ and NO_3^- between plants and microbes is controlled by NH_4^+ and NO_3^- availability and mobility in the soil (Jackson et al., 1989; Schimel et al., 1989; Norton and Firestone, 1996). Plants may utilize more NH_4^+ if the proportion of NH_4^+ to NO_3^- is high (Crawford and Chalk, 1993). Because nitrifiers are considered weaker competitors for NH_4^+ than plants (Rosswall, 1982), NH_4^+ uptake by plants may decrease NH_4^+ availability to nitrifiers, in which case the nitrification rate may be reduced. The limited data on the effect of plant NH_4^+ uptake on soil nitrifiers and nitrification are available for natural ecosystems that are not receiving N fertilizers (Schimel et al., 1989; Zak et al., 1990; Verhagen et al., 1994). Investigations of nitrification in soils with crop growth and the application of animal wastes are rare (Laanbroek and Gerards, 1991; Kandeler et al., 1994). With increasing concern over NO_3^- environmental pollution from agricultural soils, especially from soils with the application of animal wastes, quantitative analysis of microbial NO_3^- production

in relation to soil available NH_4^+ and NO_3^- will be necessary for the appropriate N management.

Net N process rates, which are determined by the changes of inorganic N pool sizes over time, usually confound two or more individual processes of N production and consumption. In contrast, gross N process rates may provide more detailed information for controlling N transformations and may be uniquely determined by isotope ^{15}N dilution or tracer techniques. Figure 1.2 shows the concepts for determining individual process rates by ^{15}N pool dilution techniques. Briefly, nitrification rate is measured by adding $^{15}\text{NO}_3^-$ and observing the rate at which $^{15}\text{NO}_3^-$ is diluted due to the oxidation of $^{14}\text{NH}_4^+$ to $^{14}\text{NO}_3^-$. Similarly, a gross N mineralization rate is measured by adding $^{15}\text{NH}_4^+$ and observing the rate at which $^{15}\text{NH}_4^+$ is diluted due to the production of $^{14}\text{NH}_4^+$ from the mineralization of native organic ^{14}N . Consumption of NO_3^- or NH_4^+ does not affect the ^{15}N enrichment. Thus, gross nitrification and N mineralization rates can be calculated from the rates of dilution of pool enrichments. Gross rates of NO_3^- and NH_4^+ consumption can be calculated from disappearance of the ^{15}N label.

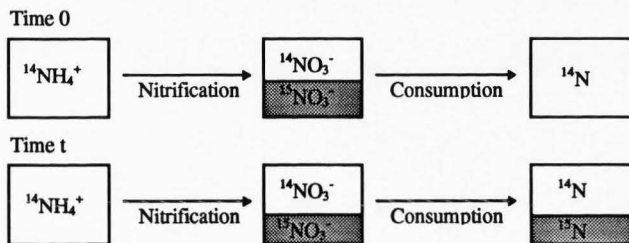


Fig. 1.2. The ^{15}N pool dilution approach to estimate rates of gross nitrification and NO_3^- consumption. At time 0, NO_3^- pool is labeled with $^{15}\text{NO}_3^-$. From time 0 to time t, the ^{15}N label is diluted.

Even in well-designed laboratory experiments in which plant N uptake, NO_3^- leaching, denitrification, or ammonia (NH_3) volatilization do not occur, net N process rates still confound microbial N production with microbial N immobilization. As a result, using net rates to evaluate an ecosystem may lead to false conclusions. For instance, net nitrification rates in young coniferous forest soil significantly differed from those in old coniferous forest soil, while the gross nitrification rates in both soils were similar (Davidson et al., 1992). In that study, gross rate measurements demonstrated that microbial NO_3^- immobilization in young and old coniferous forest soils was significantly different, causing the difference in net NO_3^- accumulation.

It is generally considered that microbial NO_3^- immobilization is negligible and that even relatively low levels of soil NH_4^+ may inhibit microbial utilization of NO_3^- (Jansson, 1958; Jones and Richards, 1977). Such traditional concepts have been contradicted by recent observations in forest and grassland soils based on gross rate measurements of N processes (Davidson et al., 1990; Stark and Hart, 1997). Recent studies that simultaneously determined net and gross rates of N processes (Davidson et al., 1992; Zou et al., 1992; Hart et al., 1994) have shown that net and gross N transformation rates were not well correlated. The work of these authors has indicated that environmental factors may have different effects on N consumption and production processes. Measurement of gross rates is thus potentially very valuable to provide detailed information for managing N fertilizers.

Role of Nitrification Inhibitors

Nitrification inhibitors are chemical compounds that can inactivate essential

enzymes involved in the oxidation of NH_4^+ and thus decrease nitrification rates (Hynes and Knowles, 1982; Hyman and Wood, 1985; Powell and Prosser, 1985). Once nitrification inhibitors are decomposed, the nitrification rate is presumed to recover. Therefore, nitrification inhibitors are used to delay nitrification and to retain inorganic N in the soil root zone for plant uptake. The extent of nitrification inhibitor has been related to the type of nitrification inhibitor, soil properties, and the amount of nitrification inhibitor used (Gomes and Loynachan, 1984; Keeney, 1986; Powell and Prosser, 1986; Chancy and Kamprath, 1987; McCarty and Bremner, 1990; Powell and Prosser, 1991). These observations, based on experiments that measured nitrification rates immediately after one-time use of nitrification inhibitors, have led to the practice of annual application of nitrification inhibitors with NH_4^+ fertilizers in agricultural soils. However, one study has indicated that nitrification potential did not recover in the next year after the application of C_2H_2 at 1 Pa pressure (Klemedtsson and Mosier, 1994). Thus far, little information related to a long-term, repeated application of nitrification inhibitors is available. It is not clear if a long-term application of nitrification inhibitors has an irreversible effect on the nitrification process. From the management standpoint, it is important to investigate the effect of a long-term, repeated application of nitrification inhibitors on soil nitrification.

Studies have revealed that nitrification inhibitors generally function for a short time and that the effects of these inhibitors on nitrification are related to their persistence in soils (Touchton et al., 1978; McCarty and Bremner, 1990). Yet, it has been hypothesized that soil nitrification rates may never recover to the prior rates after the

application of a nitrification inhibitor (Keeney, 1986), which suggests that nitrification inhibitors may have an irreversible effect on soil nitrification or soil nitrifier population activity. To our knowledge, there are no published studies that examine the effect of nitrification inhibitors after long-term, repeated applications on soil nitrifier population activity.

Since nitrification inhibitors were developed and authorized for application in agricultural soils, studies have focused on the effectiveness of nitrification inhibitors on the basis of crop yields and soil NH_4^+ or NO_3^- pool sizes (Gomes and Loynachan, 1984; Chancy and Kamprath, 1987; McCarty and Bremner, 1990). Crop yields do not always respond to the application of a nitrification inhibitor because other factors including application rates and timing of N fertilizers, and soil and climate conditions may also significantly affect crop yields. A response of crop yields to a nitrification inhibitor will not be expected if an excessive rate of N fertilizers is applied, or if an appropriate amount of N fertilizers is applied while little or no N loss is likely to occur (Peterson and Frye, 1989). Consequently, it is unsuitable to use only crop yields for evaluating the role of nitrification inhibitors. In addition, if we do not know the inputs and outputs of NH_4^+ and NO_3^- , the role of nitrification inhibitors may be equivocal when based on soil NH_4^+ and NO_3^- pool sizes alone. In this dissertation, we directly determine soil nitrifier population activities to evaluate the role of the repeated application of nitrapyrin (N-Serve) in a dryland wheat system.

Determination of application rates and timing for the application of animal wastes to replace mineral fertilizers will always be a potential problem. The effects of

nitrification inhibitors on crop yields and soil NO_3^- concentrations will also depend on other factors, such as soil and climate conditions. The biological and physical-chemical environment of the wide variety agricultural soil systems is difficult to assess adequately. However, the goal of the following studies is to answer some mechanistic questions and thereby help to promote environmentally sound management of microbial NO_3^- production in agricultural soils.

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CHAPTER 2

EFFECTS OF AERATION AND MOISTURE DURING WINDROW
COMPOSTING ON THE NITROGEN FERTILIZER
VALUES OF DAIRY WASTE COMPOSTS¹

Abstract

The objective of this work was to evaluate the effects of turning and moisture addition during windrow composting on the N fertilizer values of dairy-waste composts. Composted-dairy wastes were sampled from windrow piles, which received four treatments in a 2×2 factorial of turning (turning vs. no turning) and moisture addition (watering vs. no watering), at two stages of maturity (mature vs. immature). Composts were characterized for their chemical properties. An 84-day laboratory incubation of soils with addition of the composts at two levels was conducted to evaluate the inorganic N accumulation patterns from the variously treated composts.

Chemical analyses of variously treated composts did not differ between compost treatments or maturity. In contrast, the inorganic N accumulation patterns differed between soils that received immature versus mature turned composted-dairy wastes. The results suggested that turning was more important than moisture addition in the composting process. There was no significant difference in inorganic N accumulation patterns among soils that received different immature composts, while the N accumulation patterns observed for soils that received different mature composts

¹ Coauthored by W. Shi, J.M. Norton, B.E. Miller, and M.G. Pace.

depended on compost treatments. Soils amended with mature composts treated by frequent turning had higher N mineralization potentials (N_0), mineralization rate constants (K), and initial potential rates (N_0K) in comparison to soils with composts that had not been turned. Soils with mature composts treated by watering had a higher N_0 , lower K, and, therefore, similar N_0K when compared to soils with composts that had not been watered. Soils that received mature composts treated by watering and frequent turning had higher N mineralization potentials and N_0 to total organic N ratios than soil alone, which suggested that intensive management of composting would ensure positive N fertilizer values of dairy waste composts, if the appropriate composting duration is completed.

Introduction

Composting has been defined as a controlled-microbial aerobic decomposition process with the formation of stabilized organic materials that may be used as soil conditioners and/or organic fertilizers (Golueke, 1973; Wilson and Dalmat, 1986; Buchanan and Gliessman, 1991; García et al., 1992; Schlegel, 1992). The stabilization of organic materials, however, is relative because the agricultural utility of composts as sources of plant nutrients depends on their further decomposition in soils. Mature compost can be of high value for crop nutrition, in contrast to immature compost, which may result in net immobilization of soil N into the microbial biomass and may induce N deficiency in crops (Golueke, 1973; Inbar et al., 1993). Although many physical, chemical, and biological indices have been linked to the maturity of composts (Golueke,

1973; Forster et al., 1993; Mathur et al., 1993), it is unlikely that any single index will be valid for all types of composts (Inbar et al., 1993). Farmers who compost to manage agricultural wastes often judge the maturity of composts by their own methods, due to the lack of criteria of maturity or to the inconvenience of some indices. Consequently, the application of composted materials may sometimes decrease available soil N to crops, therefore decreasing crop yields.

Windrow composting is a commonly used processing method. The microbial decomposition of organic wastes is controlled by environmental factors affecting microbial activity within the windrow piles. Aeration and moisture are two very important factors influencing microbial activity; therefore, intensive management of the composting process by turning and moisture addition is likely to affect the N fertilizer value of the finished compost. However, it has been suggested that intensive management is not necessary if time is not a constraint (Golueke, 1973). Longer-duration composting with little disturbance may be used to manage organic wastes. It is currently unknown if intensive management of composting will ensure positive N fertilizer values compared to less intensive management. Since composts mainly contain organic N, the rate and amount of N mineralization are important for predicting N availability in soil receiving compost.

Incubation experiments are reliable for assessing soil N availability (Stanford and Smith, 1972; Stanford et al., 1974), and have been used extensively to compare the N supplying capacity of organic wastes and to monitor the short-term behavior of organic wastes added to soils (King, 1984; Bitzer and Sims, 1988; Kirchmann, 1991; Bernal and

Kirchmann, 1992; Nugroho and Kuwatsuka, 1992; Aoyama and Nozawa, 1993; Chèneby et al., 1994). The first-order mathematical model for simulating the inorganic N accumulation patterns is a useful tool for estimating the amount of mineralizable organic N and the rate at which it is mineralized. The quantity of organic N that is susceptible to mineralization, according to first-order kinetics, has been defined as the N mineralization potential (N_0) (Stanford and Smith, 1972). Both the N_0 and rate constants derived by first-order models indicate the quality of organic wastes and, to some extent, can predict the productivity of soil systems affected by addition of these wastes (Campbell et al., 1991). Although a long-term incubation experiment of 16-30 weeks (Stanford and Smith, 1972; King, 1984; O'Keefe et al., 1986) can ensure that most of potentially mineralized N is released and improve the accuracy of estimated N_0 , the data from short-term incubation experiments are also useful for assessing the relative N availability of different organic N sources (Castellanos and Pratt, 1981; Kirchmann, 1991; Bernal and Kirchmann, 1992; Beloso et al., 1993).

The aim of this study was to examine the effects of turning and moisture addition during windrow composting on the simple chemical properties and the N fertilizer values of composts at two stages of maturity. We compared the inorganic N accumulation patterns and evaluated the N mineralization kinetics of the variously treated composts added to an agricultural soil in laboratory incubation experiments.

Materials and Methods

Compost Sampling

The starting compost material was feces and urine of dairy cows with bedding material and additional straw collected from the Animal Science Farm of Utah State University. The C:N ratio of the starting compost material was 38:1. The wastes were arranged in 12 windrow piles (2.4-2.7 m wide, 1.2-1.5 m high, and 9-10 m long) in a complete randomized-block design with three blocks and four treatments. Aeration and moisture of windrow piles were controlled by turning and watering designed as a complete 2×2 factorial (turning (T) vs. no turning (NT), and watering (W) vs. no watering (NW)) to form four treatments: 1) no turning/no watering (NTNW); 2) no turning /watering (NTW); 3) turning /no watering (TNW); and 4) turning /watering (TW). The T treatments were turned weekly, while the W treatments were watered only when the moisture of windrow piles dropped to or below 40%. Composting began on September 20, 1993, and finished on November 22, 1993. During the composting period, 416 and 832 L of water were added to the water-treated windrow piles on September 29, 1993, and October 6, 1993. For details of the composting process, see Pace (1995). Three compost samples (about 6 kg each) were taken by coring to include different zones (upper vs. lower, and inner vs. outer) of each windrow pile. These samples were composited and about 500 g subsamples retained for further chemical analysis and incubation experiments. Windrow compost was sampled at two dates: 1 month and 2 months after the initiation of composting. Temperature of the windrow piles was monitored every other day and used to judge the maturity of composting (Pace,

1995). Because the temperature in 1-month windrow piles was significantly above ambient air temperature, the 1-month composts are defined as immature composts. In contrast, the temperature in 2-month windrow piles was approximately equal to the air temperature, and did not increase in the days following turning. The 2-month composts are therefore defined as mature composts.

The composts were analyzed for total C and total N by dry combustion methods (Leco-CHN 1000, St. Joseph, MI), for inorganic N by extracting with 2M KCl (1:10 compost:KCl) followed by colorimetric analysis using a Lachat Flow Autoanalyzer (QuikChem Systems, 1992; 1993), and for optical density (OD) of the water extract by ultraviolet absorption method (Mathur et al., 1993). The chemical properties of composted-dairy wastes are given in Tables 2.1 and 2.2.

Incubation Experiment

The Millville silt loam soil (coarse-silty, carbonatic, mesic Typic Haploxeroll) from 0-15 cm depth (30% sand, 53% silt, and 17% clay) was collected in bulk (approximately 30 kg) (Greenville Farm, Utah State University) for the incubation experiment. The soil chemical characteristics were: 1.17% organic C (Walkley-Black method), 0.10% total N (direct combustion method), C:N ratio 11.7, 43.7% CaCO₃ (acid-neutralization method), and pH 8.2. Moist soil was sieved through 2-mm screen before use.

For evaluating the effects of turning and moisture addition during windrow composting on the N fertilizer values of composted-dairy wastes, the soil and composts were mixed at levels of 1.1 g (low) or 3.3 g (high) compost (dry wt. basis) per 100 g soil (corresponding approximately to 22 or 66 Mg (dry wt. basis) compost ha⁻¹). The soil-

Table 2.1. The chemical properties of immature composted-dairy wastes.

Chemical properties	Treatments [†]				Treatment effects
	NTNW	NTW	TNW	TW	
Total C (%)	25.4 (3.3) [‡]	20.1 (2.5)	25.6 (1.3)	23.4 (3.0)	NS [§]
Total N (%)	2.0 (0.2)	1.9 (0.1)	2.0 (0.1)	1.8 (0.2)	NS
NH ₄ ⁺ -N (µg/g)	105 (83)	16 (11)	82 (77)	55 (47)	NS
NO ₃ ⁻ -N (µg/g)	900 (398)	1312 (518)	885 (144)	921 (178)	NS
C:N ratio [¶]	3.5 (1.7)	11.4 (1.1)	13.6 (1.0)	13.5 (1.0)	NS
pH (1:5 H ₂ O)	8.5 (0.3)	8.3 (0.2)	8.6 (0.1)	8.6 (0.2)	NS
OD of 1:400 H ₂ O extract (260 nm)	0.7 (0.1)	0.6 (0.1)	0.9 (0.0)	0.8 (0.1)	NS

[†] Compost treatments: NTNW, no turning/no watering; NTW, no turning/watering; TNW, turning/no watering; and TW, turning/watering. See materials and methods for details.

[‡] Values are means and (standard errors) for n = 3.

[§] Not significant (p > 0.05).

[¶] C:N ratio was calculated after subtracting the inorganic N from the total N.

Table 2.2. The chemical properties of mature composted-dairy wastes.

Chemical properties	Treatments [†]				Treatment effects
	NTNW	NTW	TNW	TW	
Total C (%)	21.9 (2.3) [‡]	18.3 (3.0)	23.4 (0.1)	23.0 (2.2)	NS [§]
Total N (%)	1.7 (0.1)	1.7 (0.2)	1.8 (0.1)	1.9 (0.1)	NS
NH ₄ ⁺ -N (µg/g)	231 (154)	406 (34)	83 (72)	61 (28)	NS
NO ₃ ⁻ -N (µg/g)	379 (202)	823 (258)	292 (175)	661 (91)	NS
C:N ratio [¶]	13.7 (1.4)	11.6 (3.0)	13.2 (0.4)	12.5 (0.6)	NS
pH (1:5 H ₂ O)	8.6 (0.2)	8.3 (0.3)	8.7 (0.2)	8.7 (0.2)	NS
OD of 1:400 H ₂ O extract (260 nm)	0.7 (0.1)	0.4 (0.1)	1.0 (0.2)	0.8 (0.2)	NS

[†] See Table 2.1 for compost treatments.

[‡] Values are means and (standard errors) for n = 3.

[§] Not significant (p > 0.05).

[¶] C:N ratio was calculated after subtracting the inorganic N from the total N.

compost mixtures (equivalent to 10 g dry wt.) were put into 120-ml specimen cups with a 2-mm dia. hole in the cover for gas exchange, and placed in an incubator at 20 ± 2 °C. The soil gravimetric water content was adjusted to 21% (about 60% of field capacity) every 3 days. Eight cups were prepared for each composting treatment replication. Ten-gram soil samples without compost were also incubated as controls. After 0, 14, 42, and 84 days, two randomly selected samples were withdrawn from each treatment replication and extracted with 2 M KCl (1:5 soil wt.:KCl vol.) by shaking for 1 h. Extracts were filtered through pre-rinsed Whatman #1 filter papers and filtrates were frozen until analyzed for inorganic NH_4^+ - and $(\text{NO}_3^- + \text{NO}_2^-)$ -N as described above.

Statistical Analysis

The effects of treatments NTNW, NTW, TNW, and TW on the chemical properties of composted-dairy wastes were statistically analyzed using a randomized-block design. The means of inorganic N of the two lab incubation replications were used to analyze the effects of composting treatments. The inorganic N produced by soil alone was not subtracted from that of the soil treated with compost before data analysis. The effects of composting treatments (NTNW, NTW, TNW, and TW) and factors (T and W) on the accumulated soil inorganic N dynamic patterns were statistically analyzed by a split-plot method (SuperANOVA, 1989, Abacas Concepts, Berkeley, CA). To compare the inorganic N accumulation patterns, a nonlinear regression (SigmaPlot 3.0, 1995, Jandel Scientific, San Rafael, CA) was used to derive the best fit to the first-order model given by $N_m = N_0(1 - e^{-Kt})$, where N_m is the accumulated N mineralized at time t , N_0 is the mineralization potential, and K is the mineralization rate constant (Stanford and Smith,

1972). The standard errors of the N_0 and K were calculated using SigmaPlot 3.0 (Jandel Scientific, 1995, San Rafael, CA). See Appendix A for the details of statistical analysis.

Results

Soil N Mineralization as Affected by the Additions of Mature Composts

Soil inorganic N accumulation patterns were significantly different following the additions of the differently treated mature composts (NTNW, NTW, TNW, and TW) at both low and high levels (Fig. 2.1). Throughout the 84-day incubation period, NO_3^- -N was the major form of inorganic N. The model parameters, N mineralization potentials, and rate constants are given in Table 2.3.

Soil N mineralization potentials and rate constants increased with the higher rate of compost added. The soil with composted-dairy wastes treated by TW had the highest N_0 at both low and high levels. Although the soil with addition of TNW composts had a lower N_0 in comparison to that with addition of TW composts, the release of available N was similar for a short period of time (about 40 days) due to the higher rate constant (Fig. 2.1, Table 2.3).

The treatment effects on soil N mineralization kinetics can be subdivided into the effects of turning (T vs. NT), moisture addition (W vs. NW), and their interaction. Significantly different N mineralization kinetics occurred in those soils with addition of composts treated by T versus NT, and W versus NW. The turning and moisture addition factor effects during composting on the soil N mineralization parameters are given in Table 2.4.

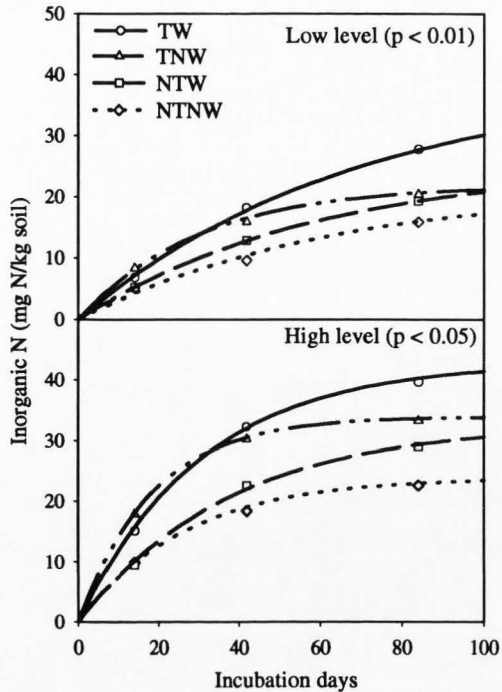


Fig. 2.1. Experimental (symbols, $n = 3$) and simulated (lines) inorganic N accumulation in the soils receiving mature composts treated by NTNW (no turning/no watering), NTW (no turning/watering), TNW (turning/no watering), and TW (turning/watering) during composting process. Low level, 1.1 g compost per 100 g soil; high level, 3.3 g compost per 100 g soil. See Materials and Methods for details.

Table 2.3. The N mineralization potentials (N_0) and rate constants (K) of soils receiving mature composted-dairy wastes treated by NTNW, NTW, TNW, and TW during composting process.

Levels [†]	Treatments [‡]	N_0 ($\mu\text{g/g}$)	SE_{N_0} [§]	K ($\times 10^3, \text{d}^{-1}$)	SE_K [§] ($\times 10^3$)
Low	NTNW	22	4.3	15	1.6
	NTW	26	0.3	16	2.7
	TNW	22	0.6	33	0.1
	TW	40	2.4	14	1.7
High	NTNW	24	0.4	38	5.2
	NTW	33	1.5	26	0.3
	TNW	34	0.2	55	2.2
	TW	43	0.9	33	1.3

[†] Low level, 1.1 g compost per 100 g soil; high level, 3.3 g compost per 100 g soil.

[‡] See Table 2.1 for compost treatments.

[§] Standard error of N_0 . [¶] Standard error of K.

Table 2.4. The N mineralization potentials (N_0) and rate constants (K) of soils receiving mature composted-dairy wastes treated by T vs. NT and W vs. NW during composting process.

Levels [†]	Factors [‡]	N_0 ($\mu\text{g/g}$)	SE_{N_0} [§]	K ($\times 10^3, \text{d}^{-1}$)	SE_K [§] ($\times 10^3$)
Low	NT	24	1.9	16	2.1
	T	29	0.2	21	0.3
	NW	20	1.5	25	0.4
	W	33	1.2	15	1.0
High	NT	28	0.3	31	1.0
	T	38	0.2	42	0.6
	NW	28	0.2	48	1.0
	W	38	1.1	30	2.1

[†] See footnote for Table 2.3.

[‡] Factor: NT, no turning; T, turning, NW, no watering; W, watering.

[§] Standard error of N_0 . [¶] Standard error of K.

Both the N mineralization potentials and rate constants of soils that received T composts were higher than those with the addition of NT composts. When comparing the effects of composts treated with W to NW, the soil N mineralization potentials and rate constants had opposing effects. Therefore, for a short time (< 40 days), composts treated with W or NW have similar N supplying capacity (Fig. 2.2). The N mineralization potential and rate constant of the control soil was $27 \mu\text{g g}^{-1}$ and 0.016 day^{-1} , respectively.

Soil N Mineralization as Affected by the Additions of Immature Composts

Effect of variously treated immature composts on soil N mineralization was evaluated from soil amended with the low-level composts. There was no significant difference in inorganic N accumulation patterns among differently treated immature composts (Fig. 2.3). Also, no significant difference existed for the effects of T versus NT and W versus NW (Fig. 2.4). Results for soils treated with high-level additions of immature composts were similar.

Comparison of Soil N Mineralization with the Additions of Mature vs. Immature Composts

The N supplying capacity of composted-dairy wastes was related to the composting duration (Fig. 2.1 vs. Fig. 2.3), and to the aeration and moisture conditions during the composting process (Table 2.3, and Table 2.4). Figure 2.5 shows the effects of turning and moisture addition on N accumulation patterns of soils mixed with immature and mature composts. The soil inorganic N accumulation patterns were not

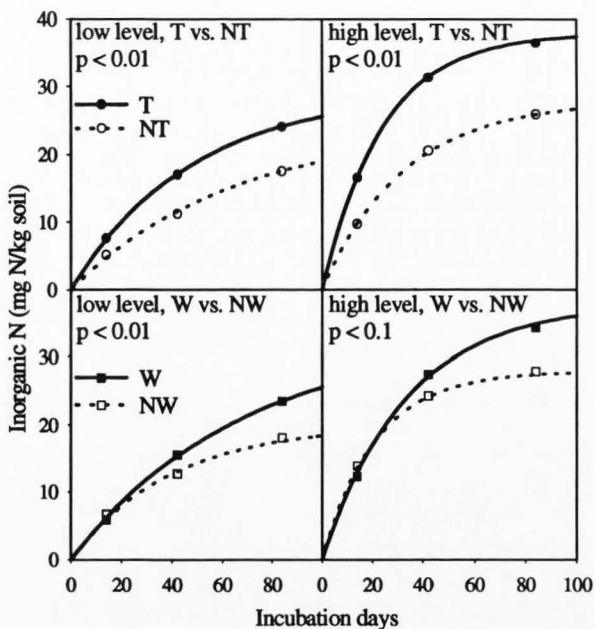


Fig. 2.2. Experimental (symbols, $n = 6$) and simulated (lines) inorganic N accumulation in the soils receiving mature composts treated by T (turning) vs. NT (no turning) and W (watering) vs. NW (no watering) during composting process. See Fig. 2.1 for level definitions.

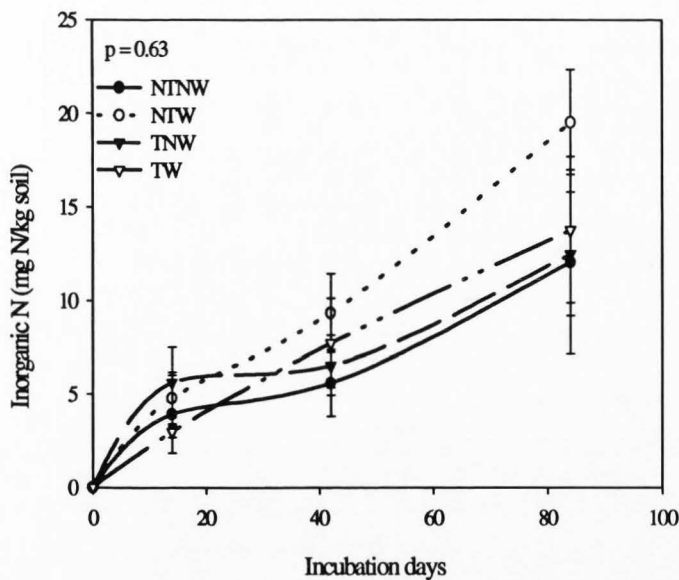


Fig. 2.3. The inorganic N accumulation patterns in the soils receiving low-level immature composts treated by NTNW, NTW, TNW, and TW during composting process. See Fig. 2.1 for treatment definitions.

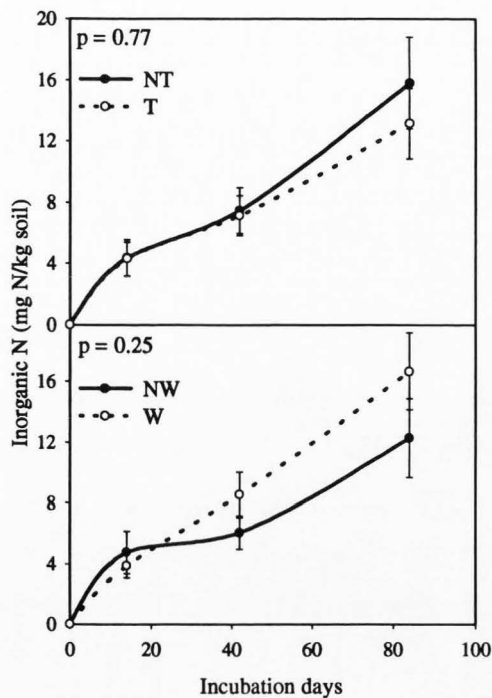


Fig. 2.4. The inorganic N accumulation patterns in the soils receiving low-level immature composts treated by T vs. NT and W vs. NW during composting process. See Fig. 2.2 for factor definitions.

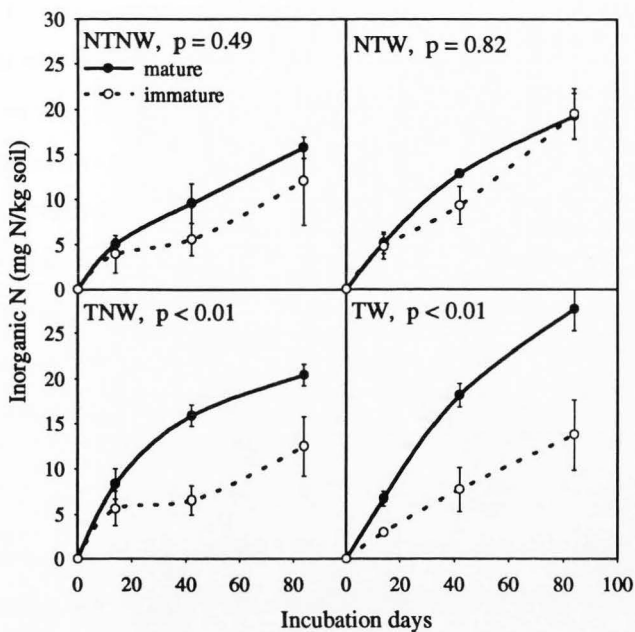


Fig. 2.5. The inorganic N accumulation patterns in the soils receiving low-level immature or mature composts treated by NTNW, NTW, TNW, and TW during composting process. See Fig. 2.1 for treatment definitions.

significantly different between immature and mature composts for the NTNW and NTW treatments, while there was a significant difference in inorganic N accumulation patterns between immature and mature composts for the TNW or TW treatments.

The turning and moisture addition effects on the N supplying capacity of composts can easily be observed in Fig. 2.6. There was a significant difference in inorganic N accumulation patterns following addition of turned compost (immature vs. mature), while no significant difference existed between unturned immature and mature composts. There were significant differences in inorganic N accumulation patterns between immature and mature composts treated by both W and NW.

Discussion

One way to evaluate the N fertilizer value of organic wastes from incubation experiments is to subtract the contribution of mineralized organic N from the soil alone. If the N produced by the soil alone is subtracted, our data show that only soils treated with mature compost that had been turned and watered can supply substantial available N, which is about 3 and 6% of the organic N in the composts for the high and low levels, respectively. Our results are consistent with those obtained by Castellanos and Pratt (1981), where the net N mineralization of composted-dairy wastes was about 5% of the organic N in a 10-week incubation with soil and at least 4% of the organic N was available to plants in a 10-month greenhouse experiment.

On the assumption that N availability is related to soil organic N content, the N fertilizer value of composts can also be assessed by the index of N_0 /soil organic N. It has

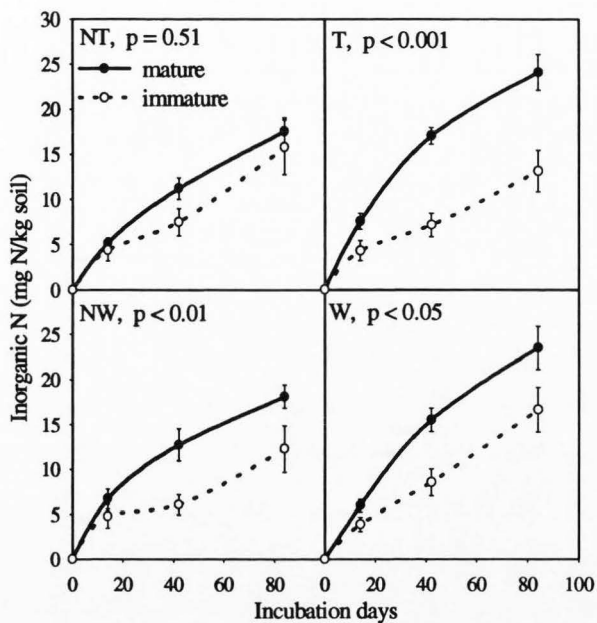


Fig. 2.6. The inorganic N accumulation patterns in the soils receiving low-level immature or mature composts treated by T vs. NT and W vs. NW during composting process. See Fig. 2.2 for factor definitions.

been suggested that if the ratio of soil N_0 /soil organic N is increased, then the compost is having a beneficial effect on the soil N availability (Campbell et al., 1991). The results calculated with our data are given in Fig 2.7. After normalized to soil organic N, it is shown that the soil with mature composted-dairy wastes treated by T and W at both low and high levels can improve or at least maintain the soil N supplying capacity.

We found that mineralization potentials and rate constants were often opposing factors. With increasing time, estimated N mineralization potential increased, whereas rate constant decreased, as observed by Stanford and Smith (1972). Also, the rate constant varies with the calculation methods (Lindemann and Cardenas, 1984). Such drawbacks are believed to come from the simplified assumption in the first-order model that there is only one pool of mineralizable organic N. Some efforts to overcome this problem have concentrated on using relatively complicated mathematical models (Lindemann and Cardenas, 1984; Sierra, 1990; Hadas and Portnoy, 1994). However, models of mineralization that represent multiple pools of mineralizable N, each with their individual rate constants, are often over-parameterized for the available data (Richter and Benbi, 1996).

An alternative for evaluating N supplying capacity of organic wastes is to use the product of N mineralization potential and rate constant defined as the initial potential rate of N mineralization (Campbell et al., 1991) as an index of mineralization. It has been demonstrated that initial potential rate of C mineralization (comparable to N_0K) is a more suitable index for linking decomposition process with chemical composition than C_0 and K used separately, and it is thought that C_0K can be a more precise index than the

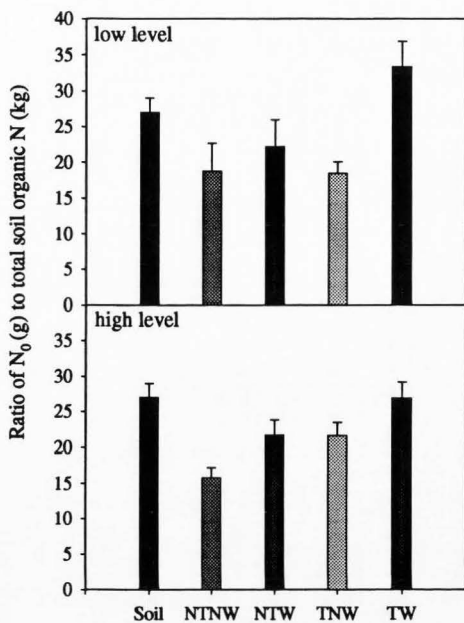


Fig. 2.7. The ratios of N₀ (g) to organic N (kg) in the control soil, and the soils receiving mature composts treated by NTNW, NTW, TNW, and TW during composting process. See Fig. 2.1 for treatment definitions.

individual parameters C_0 or K (Saviozzi and Riffaldi, 1993). The product N_0K has been effectively applied as an index of short-term N supplying capacity (Campbell et al., 1991) for distinguishing the change in soil organic N due to various cultural and management practices. The initial potential rates of N mineralization (N_0K) for soils freshly amended with composted-dairy wastes are given in Table 2.5. The trend is that T composts have higher values of initial potential N rates than NT composts, whereas there is no difference in the N_0K between the W and NW.

Composting is a microbial decomposition process; therefore, any environmental factors beneficial to microbial activities will increase the decomposition rate and potentially improve the physical and biochemical nature of composts. The amounts and composition of amendments such as straw will also impact the compost characteristics. In windrow composting, the aeration is performed by turning the windrow piles periodically. Significant effects of turning on microorganisms within the windrow piles have been reported (Insam et al., 1996). Although a functional change in the microbial

Table 2.5. Initial potential rates (N_0K) of N mineralization in the soils receiving mature composts treated by NTNW, NTW, TNW, and TW, or treated by T vs. NT, and W vs. NW during composting process.

Treatments [†]	Low level	High level	Factors	Low level	High level
	(µg N g ⁻¹ soil day ⁻¹)			(µg N g ⁻¹ soil day ⁻¹)	
NTNW	0.34	0.90	NT	0.38	0.86
NTW	0.42	0.88	T	0.62	1.58
TNW	0.73	1.85	NW	0.50	1.33
TW	0.56	1.44	W	0.49	1.14

[†] See Tables 2.1, 2.3, and 2.4 for treatment, level and factor definitions, respectively.

community with the composting process is a basic characteristic, the change is more rapid when the compost windrows are turned (Insam et al., 1996). In our experiments, the turning accelerated the decomposition process, resulting in mature composts with relatively higher N_0 , K, and N_0K . The T treatment resulted in a significant difference in N accumulation patterns between immature and mature composts. The effect of turning on the decomposition rate of composting process may also be demonstrated by the temperatures of windrow piles monitored near the end of the composting process (Pace, 1995). The T windrow piles cooled down faster than the NT piles, indicating a more complete decomposition process.

The influence of watering on the finished composts can be shown by the higher N_0 , lower K, and similar N_0K when compared to the NW treatments. The similar N_0K of W- and NW-treated composts reflects that these composts have similar short-term N supplying capacity (< 40 days). Subsequently, those that were watered will supply more available N than those not watered. During the composting period, there were only two times when the windrow moisture was found at or below 40% and water was added to windrow piles of the W treatments. Even with these relatively minor additions, the temperature of windrows that received water was generally higher through the composting period (Pace, 1995), which reflects higher microbial activities. The results suggest that if composting is performed in a dry environment when evaporation is high and precipitation is insufficient to maintain the windrow moisture above 40%, watering windrow piles might increase the N fertilizer values of composts.

The N supplying capacity of composts following compost application depends on

the degree of stabilization of organic wastes, which is usually identified by indices of compost maturity. However, it is not easy to assess the biological maturity of composts, partly because defined indices are not completely valid for all composts from different sources of organic wastes under different management. The maturity of composts has been reflected in a number of physical, chemical, and biological indices of color, odor, temperature, pH, cation exchange capacity, C:N ratio, $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ ratio, patterns of organic C to N ratio, soluble organic matter, and dehydrogenase activity (Golueke, 1973; Forster et al., 1993; Inbar et al., 1993; Mathur et al., 1993). The composted-dairy wastes sampled at 1 month and 2 months after initiation of composting are definitely at different stages of maturity, as shown by the different soil inorganic N accumulation patterns (Fig. 2.1, Fig. 2.2, Fig. 2.5, and Fig. 2.6). However, the chemical properties of organic C to organic N ratio, pH, and soluble organic matter were not significantly different between immature and mature composts and among differently treated mature composts (Table 2.1 and Table 2.2). Similarly, in a study of the composting process with cattle manure, the investigators observed that a change occurred in the chemical properties of C:N ratio, soluble organic matter, cation exchange capacity, and humus component during the first month (Inbar et al., 1989; Inbar et al., 1993), followed by a period with little change of the chemical indices. However, the changes in chemical structure and functional characteristics were easily identified by C-13 nuclear magnetic resonance and infrared spectroscopy (Inbar et al., 1989). Our observations show that changes in the N supplying capacity of composts treated by extended composting are not indicated by their simple chemical characteristics.

Conclusions

The N supplying capacity of composted-dairy wastes is controlled by the quality and quantity of formed stabilized organic matter, which is determined by the composting duration, and the aeration and moisture of windrow piles. Immature and mature composted-dairy wastes may not be distinguished from one another by pH, C:N ratio, soluble organic matter, or other simple chemical properties, but may have different inorganic N accumulation patterns, especially when turned frequently. While the mature composted-dairy wastes with different turning and watering treatments could not be differentiated by simple chemical properties or temperatures, they could be distinguished by their inorganic N accumulation patterns.

The chemical indices of C:N ratio, pH, and soluble organic matter were not suitable for predicting the positive or negative N fertilizer value of composted-dairy wastes. Watering and frequent turning accelerate the decomposition rate of dairy wastes during the composting process, and the composted-dairy wastes treated by frequent turning and watering have higher N fertilizer values than those not turned or not watered. Consequently, intensive aeration and moisture management (turning and watering) during composting of dairy wastes will ensure positive N fertilizer values in soils following the application of composts.

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CHAPTER 3

COMPARISON OF NITROGEN MINERALIZATION DYNAMICS OF
DAIRY WASTES TREATED BY AEROBIC COMPOSTING
OR ANAEROBIC LAGOON DIGESTION

Abstract

Aerobic composting and anaerobic lagoon digestion are used to treat and stabilize dairy wastes prior to land application. The different conditions during treatments of dairy wastes in these two techniques produce end products that may differ in their chemical, physical, and biological properties. Consequently, soils receiving compost may have different N-release characteristics from those receiving lagoon effluent. The purpose of this study was to compare the amount and rate of inorganic N released from an agricultural soil that received either aerobic compost or anaerobic lagoon effluent of dairy wastes. A 70-day laboratory incubation was conducted to measure the accumulated inorganic N with time. A modified first-order model was used to derive the N mineralization potentials and rate constants. The results showed that soils receiving dairy-waste compost had higher N mineralization potentials and lower rate constants than those receiving dairy-waste lagoon effluent. After subtracting the N mineralization potential of soil alone, the amount of mineralizable N from dairy-waste compost or dairy-waste lagoon effluent was expressed as the percentage of their organic N. Dairy-waste compost was more stable and approximately 5% of organic N was mineralized; in contrast, up to 90% of the organic N in lagoon effluent was mineralized

under our experimental conditions. The results indicate that the N release from anaerobic lagoon effluent acted like a mineral N fertilizer with immediately available N, whereas dairy-waste compost acted like a slow-release organic N fertilizer. The results suggest that dairy-waste lagoon effluent can be applied during the growing season when crops require a large amount of N, while dairy-waste compost must be applied earlier than the growing season to allow enough time for N mineralization prior to crop N demand.

Introduction

Aerobic composting and anaerobic lagoon digestion are two common practices for collection, storage, and biological stabilization of dairy wastes. Although aerobic compost and anaerobic lagoon effluent are different in their forms of solid versus liquid, they have the same ultimate fate: disposal to agricultural land as organic N fertilizers. The accepted practice for waste disposal is to apply as much waste as possible without posing potential risk to soil, ground water, or crop quality. Because $\text{NH}_4^+\text{-N}$ is rapidly nitrified in most agricultural soils, available N in excess of crop demand generally accumulates in soil as $\text{NO}_3^-\text{-N}$. The accumulated $\text{NO}_3^-\text{-N}$ may leach to ground water, denitrify to the atmosphere or remain in soil. High levels of $\text{NO}_3^-\text{-N}$ in soil can lead to its accumulation in crops, which may be undesirable, especially for forage (Burns et al., 1990). Application rate and timing are keys to environmentally sound animal-waste N management. As animal-waste N is mainly organic N, understanding its N mineralization

dynamics will provide important information for deciding at the timing and rate of animal waste application.

Once animal wastes are applied to agricultural soils, their carbon (C) and N qualities decide their inorganic N-release characteristics. In addition to the generalization that net N mineralization occurs at or below a C:N ratio of 20 to 25, more detailed information on the C:N ratio at which agricultural wastes will mineralize has been reported (Aoyama and Nozawa, 1993). Differently treated animal wastes will differ in the quality of organic C and N. Therefore, the N-release characteristics of these animal wastes may vary with management. Some of these factors have been previously examined, including composted versus non-composted (Castellanos and Pratt, 1981; García et al., 1992), aerobically versus anaerobically treated solid wastes (King, 1984; Kirchmann, 1991; Bernal and Kirchmann, 1992), and liquid versus solid untreated wastes (Beauchamp, 1986). The authors of these studies tried to provide qualitative or semi-quantitative information for the environmentally sound disposal of animal wastes.

The main goal of aerobic composting and anaerobic lagoon digestion is the same, to stabilize organic wastes. However, the treatment conditions and management strategies are very different, which leads to different decomposition processes, and therefore different end products. Aerobic composting produces stabilized solid organic matter along with the release of CO₂. The majority of inorganic N released during aerobic composting can be assimilated by microorganisms and transformed to more stable organic N, since the initial C:N ratio has often been adjusted to above 35:1 through adding wheat straw, wood chips, or sawdust. In contrast, anaerobic lagoon

digestion forms stabilized organic matter along with the release of methane (CH_4). The majority of the inorganic N released during anaerobic digestion remains in the lagoon; therefore, the proportion of organic N to total N can significantly decrease. Limited data are available for the N-release characteristics of dairy-waste compost, and even less are available for dairy-waste lagoon effluent (Castellanos and Pratt, 1981; Liang et al., 1995; Hadas et al., 1996). Usually, plant-available N in lagoon effluent is estimated by summation of inorganic N in the liquid fraction and mineralizable organic N in the solid fraction (EPA, 1983). However, King (1984) suggested that mineralizable organic N in the solid fraction was not equal to mineralizable organic N in the lagoon effluent. Therefore, it may be better not to separate the solid fraction from the liquid fraction to estimate the mineralizable organic N in dairy-waste lagoon effluent.

Incubation experiments combined with first-order mathematical modeling have been used to assess soil N availability (Stanford and Smith, 1972; Stanford et al., 1974), and this method has been extended to monitor the N-release characteristics of organic wastes added to soil (King, 1984; Bitzer and Sims, 1988; Kirchmann, 1991; Bernal and Kirchmann, 1992; Nugroho et al., 1992; Aoyama and Nozawa, 1993; Chèneby et al., 1994). Although researchers used long-term incubation experiments of 16-30 weeks to estimate the mineralizable organic N, i.e., N mineralization potential (N_0) (Stanford and Smith, 1972; King, 1984; O'Keefe et al., 1986), relatively short-term incubation experiments (< 10 weeks) have been used to compare the N availability among different organic N sources (Castellanos and Pratt, 1981; Kirchmann, 1991; Bernal and Kirchmann, 1992; Beloso et al., 1993). First-order models with one or multiple pools

have been used to describe the inorganic N-release characteristics of soils or soil-waste mixtures (Stanford and Smith, 1972; Stanford et al., 1974; Lindemann and Cardenas, 1984; Sierra, 1990; Hadas and Portnoy, 1994). However, multiple pool models of N mineralization are often over-parameterized for the available data (Richter and Benbi, 1996). Therefore, we used a single pool first-order model to describe the inorganic N-release characteristics of aerobic compost or anaerobic lagoon effluent.

The purpose of this study was to compare the N mineralization dynamics of soils with fresh addition of either aerobic dairy-waste compost or anaerobic dairy-waste lagoon effluent in a short-term incubation experiment.

Materials and Methods

Dairy-Waste Compost

Dairy-waste compost was sampled from windrow piles that were frequently turned and watered. The composting material was feces and urine with bedding materials and additional wheat straw to form the initial C:N ratio of 38:1. After a 2-month composting, the compost was collected from the different zones (upper vs. lower, and inner vs. outer) of windrow piles to form a composite sample that was passed through 2-mm screen and kept at 4 °C until incubation with soil. The characteristics of the dairy-waste compost are given in Table 3.1.

Dairy-Waste Lagoon Effluent

Dairy-waste lagoon effluent was collected from the anaerobic pond of a two-stage anaerobic and aerobic lagoon (Caine Dairy Farm of Utah State Agricultural

Table 3.1. The selected characteristics of soil, aerobic dairy-waste compost, and anaerobic dairy waste lagoon effluent.

Properties	Soil	Dairy-waste compost	Dairy-waste lagoon effluent [†]
Organic C (g kg ⁻¹)	16	230	845
Organic N (g kg ⁻¹)	1.6	19	132
C:N ratio	10:1	12:1	6:1
NH ₄ ⁺ -N (mg kg ⁻¹)	0.1	61	100
NO ₃ ⁻ -N (mg kg ⁻¹)	37	661	53
EC (ds m ⁻¹) [‡]	0.8	17.9	5.2
pH [‡]	8.4	8.7	9.3
Total solids (mg L ⁻¹)	—	—	2800

[†] Organic C, organic N, NH₄⁺- and NO₃⁻-N of lagoon effluent are expressed as mg L⁻¹.

[‡] Soil in 1:1 H₂O, compost in 1:10 H₂O, lagoon effluent in 1:0 H₂O.

[§] Soil in 1:2 H₂O, compost in 1:5 H₂O, lagoon effluent in 1:0 H₂O.

Experiment Station). The recycling between the aerobic and the anaerobic pond accelerated the inorganic N loss through either ammonia (NH₃) volatilization or NO₃⁻ denitrification. The raw materials loaded into the anaerobic pond were milking parlor waste water, feces and urine, and bedding material. Before sampling, the anaerobic pond was agitated for about 2 days. After sampling, the lagoon effluent was kept at 4°C until incubation with soil. The properties of the dairy-waste anaerobic lagoon effluent are given in Table 3.1.

Soil Sample

The Nibley silty clay loam soil (fine, mixed, superactive, mesic Aquic Argiustoll) was collected in fall 1994 from Caine Dairy Farm of Utah State Agricultural Experiment Station. The soil was sampled from 0-15 cm depth at two fields; one was cropped with corn in the spring and the other with alfalfa. After sampling, the soil was sieved through

a 2-mm screen, partially air-dried to avoid excessive moisture after the addition of lagoon effluent, then kept at 4°C until incubation. Chemical properties of soil composited from the alfalfa and corn fields are given in Table 3.1.

Incubation Experiment

The soil and soil with the amendment of dairy-waste compost or dairy-waste lagoon effluent were incubated at 20°C for 70 days. Dairy-waste additions in this laboratory experiment were calculated from the recommended field application rates in Utah. The incubation treatments for both corn and alfalfa soils were as follows: 1) control, soil without addition of either compost or lagoon effluent; 2) compost, soil with addition of the compost at 3.3 g (dry wt.) per 100 g soil (corresponding to 66 Mg dry wt. ha⁻¹); 3) low lagoon, soil with addition of the lagoon effluent at 10 ml per 100 g soil (corresponding to 2×10⁵ L ha⁻¹) as low level; 4) high lagoon, soil with addition of the lagoon effluent at 20 ml per 100 g soil (corresponding to 4×10⁵ L ha⁻¹) as high level. The soils or soil-dairy waste mixtures (equivalent to 20 g dry wt.) were weighed into 120-ml specimen containers. Soil gravimetric moisture contents were adjusted to 23% (about 60% of the field capacity) every 3 days. The sample containers were covered by lids with a small hole (2-mm diameter) for gas exchange to maintain aerobic conditions and to minimize water loss. Twenty-one cups were prepared for each treatment. Three cups from each treatment were withdrawn at each sampling date of 0, 7, 14, 28, 42, 56, and 70 days and extracted with 2 M KCl (1:5, soil wt.:KCl vol.) by shaking for 1 h. Extracts were filtered through pre-rinsed Whatman #1 filter papers, and the filtrates were

frozen until analyzed for inorganic NH_4^+ - and $(\text{NO}_3^- + \text{NO}_2^-)$ -N by colorimetric analysis (Lachat Flow Autoanalyzer, QuikChem Systems, 1992; 1993).

Data Analysis

A nonlinear regression (SigmaPlot 3.0, 1995, Jandel Scientific, San Rafael, CA) was used to derive the best fit of data to the modified first-order model given by $N_m = N_i + N_0(1 - e^{-kt})$, where N_m was the mineralizable N at time t , N_i was the initial inorganic N and was assigned as the mean of inorganic N at time zero, N_0 is the potentially mineralizable organic N defined as N the mineralization potential, and K is the N mineralization rate constant. For comparing the initial inorganic N among the treatments, we used one-way ANOVA (SuperANOVA, 1989, Abacas Concepts, Berkeley, CA). For comparing the N_0 and K between treatments, we used the method of Motulsky (1996). In brief, we compared the N_0 or K between treatments by t -values calculated from the best fit values of variables and their standard errors. The number of degrees of freedom (df) equaled the number of data points minus the number of variables fit. See Appendix B for the details of statistical analysis.

Results and Discussion

Throughout the 70-day incubation period, NO_3^- -N was the dominant form of inorganic N. The inorganic N mineralized from the control soil and from the soil receiving compost or lagoon effluent was fit to the modified first-order model and is presented in Fig. 3.1. Curves of inorganic N accumulation in the alfalfa soil were similar to those in the corn soil. However, these curves among the control soil, the soil

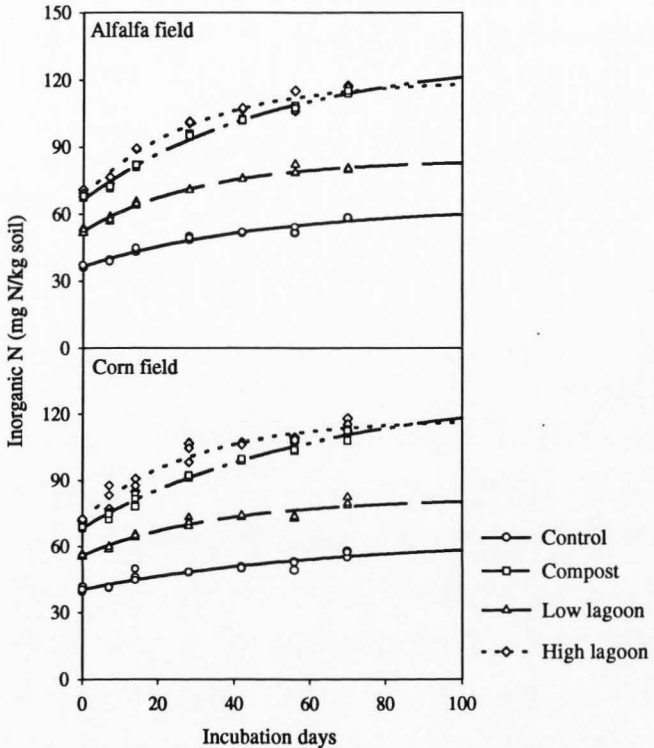


Fig. 3.1. Experimental (symbols, $n = 3$) and simulated (lines) inorganic N accumulation in the control soil (Control), the soil receiving compost (Compost), the soil receiving lagoon effluent at low level (Low lagoon), and the soil receiving lagoon effluent at high level (High lagoon).

receiving compost and the soil receiving lagoon effluent were different. The three parameters, N_i , N_0 , and K for characterizing the N mineralization, are shown in Table 3.2. The N_i was highest for the soil receiving high-level lagoon effluent followed closely by the soil receiving compost, intermediate for the soil receiving low-level lagoon effluent, and lowest for the control soil. The N_0 were significantly higher for the soil with compost, intermediate for the soil with high-level lagoon effluent, and lowest for the soil alone or the soil with low-level lagoon effluent. The values of K were significantly higher for the soil with lagoon effluent at both low and high levels than for the soil alone or the soil with compost.

Table 3.2. First-order parameters of N mineralization in the control soil (Control), the soil receiving compost (Compost), the soil receiving lagoon effluent at low level (Low lagoon), and the soil receiving lagoon effluent at high level (High lagoon).

Soil type	Treatments	N_i [†]	N_0	K ($\times 10^{-3}$) day ⁻¹	$R^{2\ddagger}$
		mg N kg ⁻¹ soil			
Alfalfa	Control	36.7 (0.3) a [§]	27.1 (4.1) a	19 (5) ab	0.960
	Compost	68.1 (0.3) c	67.2 (6.2) c	16 (2) a	0.989
	Low lagoon	52.8 (0.6) b	31.9 (1.4) a	31 (3) c	0.987
	High Lagoon	69.4 (0.9) c	51.9 (3.2) b	31 (4) bc	0.972
Corn	Control	40.6 (0.7) a	22.5 (7.6) a	15 (8) a	0.898
	Compost	68.7 (0.3) c	62.8 (6.5) c	16 (3) a	0.988
	Low Lagoon	56.1 (0.4) b	25.9 (2.8) a	27 (6) ab	0.946
	High Lagoon	72.2 (0.3) d	45.5 (3.1) b	36 (6) b	0.954

[†] N_i , initial inorganic N; N_0 , N mineralization potential; K , mineralization rate constant.

[‡] For nonlinear regression.

[§] Values are parameters and (standard errors). Values in a column and within one soil type followed by the different letters are significantly different at $p < 0.05$.

In the absence of net N immobilization, initial inorganic N comprises the amount of N immediately available for crop growth. After subtracting the soil initial inorganic N from that of soil with compost or with lagoon effluent (see Table 3.2), we may express the added initial inorganic N from compost or lagoon effluent in terms of the percentage of the total N added by either compost or lagoon effluent. Compost inorganic N comprised approximately 4% of the total added N, while inorganic N in lagoon effluent was about 50% of the total added N. If we calculate the proportion of inorganic N in compost or lagoon effluent according to their chemical properties (Table 3.1), we can get the same results. The different balance of the N transformations in aerobic composting and anaerobic lagoon digestion produces these significantly different proportions of inorganic N to total N in aerobic compost versus anaerobic lagoon effluent. As indicated by Sutton (1994), lagoon effluent usually has less organic N than inorganic N. The inorganic N may be several times the organic N (Safley and Westerman, 1994; Sweeten and Wolfe, 1994). Such different N characteristics in aerobic compost versus anaerobic lagoon effluent are important considerations for determining the application rate and timing for environmentally sound management of treated dairy wastes.

Assuming that the fresh addition of dairy-waste compost or dairy-waste lagoon effluent had no effects on the decomposition of soil endogenous organic matter, we can express the mineralizable N from compost or lagoon effluent in terms of the added organic N, i.e., the ratio of difference of N mineralization potentials between treated soil and control soil to the added organic N. We found that the N mineralized from compost

in this short-term incubation experiment comprised about 5% of its organic N. In contrast, the N mineralized from lagoon effluent comprised 30% and 90% of the added organic N for the low and high level addition, respectively. These results showed that the application rates of lagoon effluent affected the percentage of the added organic N transformed to inorganic N. Several conflicting observations have been reported on the effects of application rate on the recovery percentage of inorganic N to added organic N. Lindemann and Cardenas (1984) showed that the percentage of N mineralized from added sludge organic N tended to increase with increasing sludge addition. However, Hadas et al. (1996) reported that the percentage of compost N recovered as inorganic N was independent of the compost application rates. The discrepancy between the recovery percentages of the mineralizable organic N that were found for the two levels of lagoon effluent (30% versus 90%) has several possible explanations. There may be an interaction such as adsorption between soil clays and organic compounds in lagoon effluent, which may prevent the utilization of adsorbed organic compounds by soil microorganisms. Due to the finite adsorption capacity of the soil clays, a greater mass of organic compounds may be in the free state in the high level versus low level of lagoon effluent treatment. Therefore, a higher percentage of organic N was converted to inorganic N at the high-level addition than at the low-level addition. Another possible explanation is that the C use efficiency of soil microorganisms may vary with the waste application rates. Microbial C use efficiency is the proportion of the total decomposed organic C that is converted into microbial biomass C. High-level addition of the lagoon effluent may decrease the C use efficiency, thereby decreasing the microbial N

requirement per unit of waste. As a result, a higher recovery of added organic N in the inorganic N in high-level addition would be expected. Thirdly, the discrepancy of mineralizable N between high-level and low-level addition of the lagoon effluent may be explained by a saturation of the capacity for microbial N immobilization. The mineralizable N in this experiment was determined from net N mineralization, which is the difference of actually mineralized N (gross N mineralization) and microbial utilization of N (microbial N immobilization). The high-level addition of lagoon effluent may lead to higher gross N mineralization than low-level addition of lagoon effluent, while the two levels of additions of lagoon effluent may have the similar microbial N immobilization. Thus, high-level addition of lagoon effluent would result in greater net N mineralization (i.e., more inorganic N) than low-level addition.

The percentage of added organic N recovered as inorganic N is very important in determining N fertilizer values and in predicting the effects of residual organic N on soil. Studies have reported that 4-20% of dairy-waste compost N can generally be mineralized during a several-month incubation period or in a growing season (Castellanos and Pratt, 1981; Hadas and Portnoy, 1994; Hadas et al., 1996). Our result of 5% mineralizable dairy-waste compost N in 70 days was within that range. The low recovery percentage indicated that more dairy-waste compost N remained in soil, which would increase soil organic N. In contrast, lagoon effluent would have less effect on increasing soil organic N, because most lagoon organic N was mineralized to inorganic N. While laboratory incubations analyzed with first-order models do not simulate field conditions, they represent the quality of N source in the organic materials. The different characteristics

of N mineralization suggest that the quality of aerobic compost differs from that of anaerobic lagoon effluent.

Aerobic composting or anaerobic lagoon digestion is a microbial decomposition process, which yields partially stabilized organic matter. The formed organic matter will decompose slowly even if conditions are favorable to microbial activity. However, the aerobic versus anaerobic condition may produce organic matter with different stability and varying decay rates. Hadas and Portnoy (1994) determined the N mineralization rate constants for the soluble and insoluble components of compost. They found that the N mineralization rate constant was much higher for the soluble than for the insoluble components. Generally, liquid wastes have higher proportion of soluble to insoluble components than solid wastes. In our study, the higher N mineralization rate constant observed in soil with addition of dairy-waste lagoon effluent may be due to the higher amount of soluble components in the effluent.

The high N percentage of dairy-waste lagoon effluent that was mineralized could also be explained as a priming effect, which is an interaction between the soil and the added animal wastes that results in the increased mineralization of the native soil organic N. There are conflicting reports on the N priming effect of fresh addition of organic wastes. Dalenberg and Jager (1989) reported that an N priming effect did not occur in soil with addition of organic wastes. Bernal and Kirchmann (1992) indicated that there was no N priming effect after the addition of aerobically or anaerobically treated manure. However, Dumontet et al. (1985) observed the N priming effect in soil with the addition of aerobically digested sludge slurry, where over 200% of the added organic N was

mineralized. Liang et al. (1995) also observed over 100% of the added organic N in the mineral pool when water soluble organic matter extracted from composted dairy waste was added to a clay soil. Several mechanisms of N priming effect have been proposed (Smith, 1979; Jenkinson et al., 1985; Woods et al., 1987); however, in our study we could not identify the source of increase in the mineralizable N between the low and high rates of lagoon effluent, i.e., from added organic N and/or from soil endogenous organic N.

Conclusions

The N-release characteristics of the organic matter in aerobic compost and anaerobic lagoon effluent are different. Dairy-waste compost is more stable than dairy-waste lagoon effluent. Approximately 5% of the organic N in compost can mineralize, while up to 90% of the organic N in lagoon effluent can mineralize, which may include some contribution from increased decomposition of endogenous soil organic N. The high proportion of mineralizable N plus the high proportion of initial inorganic N suggests that the N release from dairy-waste lagoon effluent is more like mineral N fertilizers. In contrast, the low proportion of organic N that is mineralizable along with the low proportion of initial inorganic N indicates that the dairy-waste compost should be managed as a slow-release N fertilizer. The anaerobically treated dairy-waste lagoon effluent is more appropriately utilized during the growing season when crops require a high amount of available N for their growth, while the aerobic compost should be applied earlier than the growing season to leave enough time for soil microorganisms to

transform its stabilized organic N to inorganic N. The application of composts may result in the accumulation of organic N in soils, while lagoon effluent has only a short-term seasonal impact on soil organic N.

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CHAPTER 4

MICROBIAL CONTROL OF NITRATE CONCENTRATIONS IN AN
AGRICULTURAL SOIL TREATED WITH DAIRY WASTE
COMPOST OR AMMONIUM FERTILIZER

Abstract

We conducted a 112-day laboratory incubation of an agricultural soil treated with dairy-waste compost or ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) to examine the role of microbial NO_3^- production and consumption in controlling soil NO_3^- concentrations. Inorganic N, net N process rates, and nitrification potentials were measured at various time periods in the treated soils. Gross N process rates were measured at day 7, 40, 70, and 112 of the incubation by ^{15}N pool dilution techniques. The $^{15}\text{NO}_3^-$ recoveries determined one day after ^{15}N injections were not lower than those determined shortly after injections for all the three soil treatments and at all four labeling dates. The 100% recovery of $^{15}\text{NO}_3^-$ indicates that microbial NO_3^- consumption was not an important process in controlling soil NO_3^- concentrations in these soil systems during the incubation period. Nitrification rates were significantly correlated with and comprised about 50% of the gross N mineralization rates. This suggests that nitrifying bacteria were not weaker competitors for soil NH_4^+ than heterotrophs in these systems during the incubation period. Nitrification rates were highly correlated with net N mineralization rates in the control soil and in the soil receiving the compost. Near 1:1 relationship along with zero of the intercept value reflects that the source of the NH_4^+ available to nitrifiers depended solely

on N mineralization rates. Nitrification rates were not significantly correlated with net N mineralization rates in the soil receiving the NH_4^+ fertilizer, but were correlated with soil NH_4^+ concentrations. This observation indicates that the primary source of the NH_4^+ available to nitrifiers was from the added mineral NH_4^+ in the case of NH_4^+ fertilization. During the first 40 days of the incubation when soil receiving the $(\text{NH}_4)_2\text{SO}_4$ had significantly higher NH_4^+ concentrations than soil receiving the compost, nitrification rates and potentials were also higher, and nitrifier populations increased in response to the added $(\text{NH}_4)_2\text{SO}_4$. Our observations suggest that the use of dairy-waste compost as a N source replacing NH_4^+ fertilizers may decrease early season NO_3^- loss from agricultural soils.

Introduction

Control of soil NO_3^- concentrations has both agricultural and environmental importance. Appropriate agricultural N management should control NO_3^- concentrations at levels meeting crop N requirements without excessive NO_3^- accumulation in soil, because excess NO_3^- is susceptible to loss by leaching or denitrification, and NO_3^- loss decreases N-fertilizer use efficiency. Microbial NO_3^- production and consumption occur simultaneously and their relationship controls soil NO_3^- concentrations. Nitrification is the process of microbial NH_4^+ oxidation producing soil NO_3^- , while microbial NO_3^- consumption is the process of microbial NO_3^- assimilation decreasing soil NO_3^- .

Microbial NO_3^- assimilation has not been considered an important process in controlling soil NO_3^- concentrations in most agricultural soils. This concept has been

incorporated into mathematical models (Myrold and Tiedje, 1986). The basis for the assumption that microbial NO_3^- assimilation can be ignored in controlling soil NO_3^- concentrations is that microorganisms generally prefer NH_4^+ for their growth (Jansson et al., 1955; Jansson 1958; Jones and Richards, 1977), and that NH_4^+ even at relatively low concentrations (i.e., $< 1 \mu\text{g N g}^{-1}$ soil) may decrease microbial utilization of NO_3^- (Rice and Tiedje, 1989). Nitrate accumulation is often observed in many agricultural soils. One explanation is that microbial NO_3^- assimilation is negligible in those soil systems, and that nitrification is the dominant process controlling soil NO_3^- concentrations. The other explanation may be that significant microbial NO_3^- assimilation does occur, but that the microbial NO_3^- production greatly exceeds NO_3^- consumption by both microorganisms and plants. Our experimental approach tested explicitly the role of microbial NO_3^- assimilation in agricultural soils.

Animal waste is being increasingly used as an organic N fertilizer in agricultural soils. Unlike one-time application of a large amount of inorganic N, organic N is slowly transformed to inorganic N through ammonification and subsequent nitrification. Fertilization of organic versus inorganic N may lead to very different C and N availabilities to soil microorganisms. We are interested in whether the assumptions made for agricultural soils receiving inorganic N apply to soils receiving animal waste.

The functional groups of soil microorganisms act variously as producers, consumers, and competitors of the different forms of soil N. For example, heterotrophs have dual impacts on soil nitrifiers. They decompose soil organic matter, transforming organic N to NH_4^+ , which may benefit soil nitrifiers. On the other hand, heterotrophs

may strongly compete with nitrifiers for soil NH_4^+ needed for their growth, which may limit nitrifier populations and activities (Verhagen and Laanbroek, 1991). Although organic amendments significantly increase the activities of soil heterotrophs (Schnürer et al., 1985; Fauci and Dick, 1994), the effects of this enhanced microbial activity on soil N processes of ammonification, nitrification, microbial N assimilation, and the interactions of these processes have not been well characterized.

Our objectives were to examine the importance of microbial NO_3^- consumption in controlling soil NO_3^- concentrations under different N-fertilization treatments, to determine the relationships between nitrification rates and net or gross N mineralization rates for evaluating the source and amount of NH_4^+ available to nitrifiers, and to compare nitrification rates and potentials in soils receiving organic N versus mineral NH_4^+ fertilizers.

Materials and Methods

Soil and N Source

Soil was collected from the 0-15 cm surface layer in bulk (approximately 30 kg) from the Blue Creek Farm of Utah State University. The soil is Timpanogos silt loam (fine-loamy, mixed, superactive, mesic Calcic Argixeroll). Ammonium sulfate was used as the inorganic N source, while mature dairy-waste compost (see Chapter 2 TW-treated compost) was used as the organic N source. Soil and dairy-waste compost were freshly sieved through 2-mm screen and stored in 4°C for later use. Selected properties of the soil and the compost are given in Table 4.1.

Table 4.1. The selected properties of Timpanogos soil and dairy-waste compost.

	Organic C	Organic N	C:N ratio	EC	pH
	----- g kg ⁻¹ -----			ds m ⁻¹	
Soil	14	1.6	9.0	0.2	6.8
Compost	237	19.4	12.2	7.0	8.8

Incubation Procedure

Three soil treatments in this laboratory incubation experiment were 1) Control, soil without additions; 2) SN, soil with addition of the $(\text{NH}_4)_2\text{SO}_4$ at 50 mg N kg⁻¹ soil (equivalent to 100 kg N ha⁻¹); and 3) SC, soil with addition of the dairy-waste compost at 2.0 g (dry wt.) per 100 g soil (equivalent to 40 Mg dry wt. ha⁻¹). The application rates of the $(\text{NH}_4)_2\text{SO}_4$ or the compost in this laboratory experiment were based on their field application rates in UT. Differently treated soil samples of 20 g (dry wt. equivalent) were weighed into 120-ml specimen containers and placed into an incubator at 20 °C. The gravimetric water content of soil samples was adjusted to 19% (60% of field capacity) every 4-6 days.

Measurement of Inorganic N

Three samples of each treatment were randomly withdrawn at day 0, 7, 25, 40, 70, and 112. Soil samples were extracted with 2M KCl (1:5, soil wt.:KCl vol.) and shaken for 1 h. The extracts were filtered through pre-washed Whatman #1 filter papers. The filtrates were frozen until analysis for NH_4^+ - and $(\text{NO}_3^- + \text{NO}_2^-)$ -N by colorimetric methods of Lachat Autoanalyzer (QuikChem Systems, 1992; 1993).

Measurement of Gross N Transformation Rates by ^{15}N Pool Dilution

Gross rates were measured by ^{15}N pool dilution techniques (Hart et al., 1994) for the three soil treatments, at the four labeling dates (incubation-day 7, 40, 70, and 112). Soil samples labeled with K^{15}NO_3 were used to measure gross nitrification rates and microbial NO_3^- assimilation rates, while soil samples labeled with $^{15}\text{NH}_4\text{Cl}$ were used to measure gross N mineralization rates and microbial NH_4^+ assimilation rates. For measuring gross nitrification and microbial NO_3^- assimilation rates of each soil treatment at each labeling date, three pairs of soil samples per treatment (as three replications) were withdrawn, and each soil sample received 1.0 ml of the $\text{K}^{15}\text{NO}_3^-$ solution (99% enrichment, $20 \text{ mg NO}_3^- \cdot \text{N L}^{-1}$) in 10 aliquots of 0.1 ml each. The rate of N addition was $1 \text{ mg N kg}^{-1} \text{ soil}$. For measuring gross N mineralization and microbial NH_4^+ assimilation rates, we used the same procedure as described above, except that the amounts of $^{15}\text{NH}_4\text{Cl}$ injected varied depending on the labeling dates. At day 7, the soil samples were labeled with $^{15}\text{NH}_4\text{Cl}$ of 99% enrichment at $1 \text{ mg N kg}^{-1} \text{ soil}$. The concentration of $^{15}\text{NH}_4\text{Cl}$ solution was 20 mg N L^{-1} . One day after the injection, however, the $\text{NH}_4^+ \cdot \text{N}$ in 100 ml of 2M KCl soil extraction was too low ($5\text{-}8 \mu\text{g N}$) for accurately determining isotope ratio. Therefore, we increased the injection amount of $^{15}\text{NH}_4\text{Cl}$ to improve the precision of the measurement of ^{15}N enrichments. At day 40, the soil samples were labeled with $^{15}\text{NH}_4\text{Cl}$ of 99% enrichment at $2 \text{ mg N kg}^{-1} \text{ soil}$. Each soil sample received twenty 0.1-ml injections. Because the $\text{NH}_4^+ \cdot \text{N}$ in 100 ml of 2M KCl soil extraction 1 day after the injection was still low ($10\text{-}15 \mu\text{g N}$), we decided to label soil samples with $^{15}\text{NH}_4\text{Cl}$ of 50% enrichment at $5 \text{ mg N kg}^{-1} \text{ soil}$ for the following two labeling dates of

day 70 and 112. The concentration of $^{15}\text{NH}_4\text{Cl}$ solution was 100 mg N L^{-1} , and a total of 1 ml was injected in 10 aliquots. The injection of K^{15}NO_3 or $^{15}\text{NH}_4\text{Cl}$ solution increased the soil gravimetric water content by 3-6%. For each pair of labeled soil samples, one was extracted with 100 ml of 2 M KCl 15 minutes after the injection (defined as T_0 sample). The other was extracted with 100 ml of 2 M KCl after 24.25 hours of the injection (defined as T_1 sample). A diffusion procedure (Brooks et al., 1989; Stark and Hart, 1996) was used to prepare samples for ^{15}N analysis. The ^{15}N enrichments in the NH_4^+ or NO_3^- pools were analyzed by continuous-flow direct combustion and mass spectrometry with a ANCA 2020 system (Europa Scientific, Cincinnati, OH).

Measurement of Nitrification Potentials

Nitrification potentials were measured by the method of Hart et al. (1994). Triplicate samples of each soil treatment were randomly withdrawn at day 0, 7, 25, 40, 70, and 112. Fifteen-gram samples of the moist soils were weighed into 250-ml Erlenmeyer flasks and 100-ml phosphate buffer containing 1.5 mM $\text{NH}_4^+\text{-N}$ was added into the flasks. The flasks were continuously shaken for 24 h at a speed of 200 rpm (Stark, 1996). The pH of the soil slurries was monitored and adjusted four times to maintain the pH near 7.5. About 9-ml aliquots of the slurry were taken at 2, 4, 22, and 24 h after shaking began. The aliquots were centrifuged at $8,000 \times g$ for 10 minutes. The $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ in liquids was analyzed by the colorimetric method as previously described. Nitrification potential was determined from the slope of the linear regression of the values of $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ with the sampling times, and was expressed as $\text{mg N kg}^{-1} \text{ soil day}^{-1}$.

Data Analysis

The amounts of $^{15}\text{N-NH}_4^+$ or $-\text{NO}_3^-$ in soil samples of T_0 and T_1 were calculated by multiplying the ^{15}N % excesses (^{15}N % enrichments minus the background, which was assumed to be 0.37%) by the concentrations of the NH_4^+ or NO_3^- -N, and expressed as mg N kg^{-1} soil. The recoveries of ^{15}N in soil samples of T_0 and T_1 were expressed as the percentage of the added ^{15}N . The ^{15}N excesses or recoveries for soil samples at T_0 and T_1 were compared by two-way ANOVA with the labeling dates and treatments as factors.

If the ^{15}N excesses in soil samples of T_1 were significantly lower than those of T_0 , the gross N rates were calculated by the equations of Kirkham and Bartholomew (1954). The net mineralization rates and net nitrification rates were calculated by the changes of inorganic N pool size and $(\text{NO}_3^- + \text{NO}_2^-)$ -N pool size over time, respectively. For soil samples labeled with $^{15}\text{NH}_4\text{Cl}$, the $(\text{NO}_3^- + \text{NO}_2^-)$ -N was also measured. Nitrification rates were calculated and related to the NH_4^+ consumption rates by a linear regression.

Effects of treatments and incubation days on soil process rates of N mineralization, nitrification, and microbial N assimilation, and the ratios of these rates were analyzed using a repeated measurement method. All statistical analyses were performed by a SuperANOVA software (Abacus Concepts, 1995, Berkeley, CA). See Appendix C for the details of statistical analysis.

Results

Dynamics of Inorganic N

Throughout the 112-day incubation, NH_4^+ -N concentrations in the control soil were very low ($0.1\text{-}0.5 \text{ mg kg}^{-1}$ soil), whereas NO_3^- -N concentrations were tens to hundreds times NH_4^+ -N concentrations and increased almost linearly with the incubation days (Fig. 4.1). The NH_4^+ - and NO_3^- -N concentrations in the soil amended with the compost followed the same trends as in the control soil, except that at the beginning of the incubation, NH_4^+ -N ($\approx 1 \text{ mg kg}^{-1}$ soil) and NO_3^- -N ($\approx 30 \text{ mg kg}^{-1}$ soil) were higher than those of the control soil. The NH_4^+ -N concentrations in the soil amended with the $(\text{NH}_4)_2\text{SO}_4$ were significantly higher than those of the control soil or the soil amended with the compost during the first 40 days of the incubation. However, NH_4^+ -N concentrations rapidly decreased with the incubation days, and they were at the same levels as the control soil or the soil amended with the compost after 40 days of the incubation. The NO_3^- -N concentrations increased rapidly along with the decrease of the NH_4^+ -N, and this increase was nonlinear with time (Fig. 4.1).

Recovery and Excess of Inorganic ^{15}N

The recoveries of $^{15}\text{N-NH}_4^+$ and $^{15}\text{NO}_3^-$ in soil samples of T_0 and T_1 are shown in Fig. 4.2. The recoveries of KCl extractable $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ in T_0 soil samples ranged from 60% to 107% and from 72% to 103%, respectively. The $^{15}\text{NO}_3^-$ recoveries of T_1 samples were not significantly different ($p = 0.26$) from those of T_0 samples in the three soil treatments and at the four labeling dates. Therefore, the ratios of $^{15}\text{NO}_3^-$ recoveries

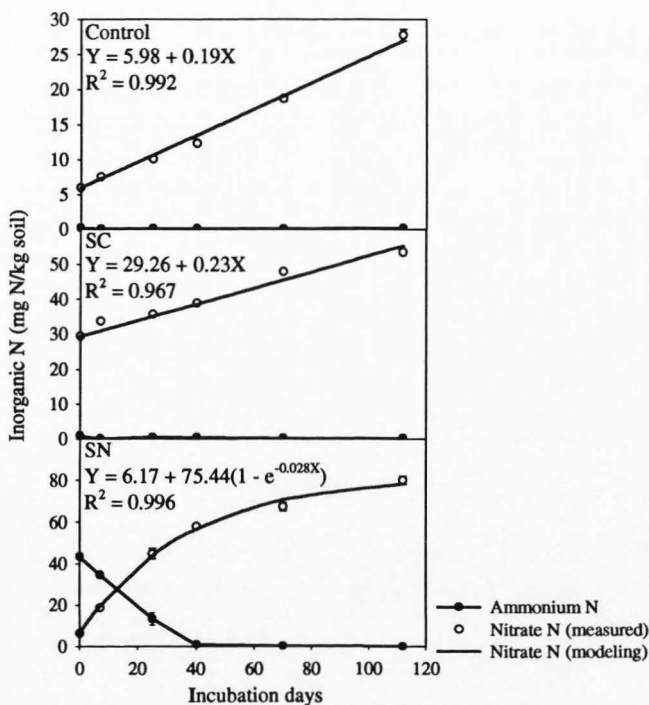


Fig. 4.1. Inorganic N accumulation patterns in the control soil (Control), soil with addition of the compost (SC), and soil with addition of the $(\text{NH}_4)_2\text{SO}_4$ (SN). Nitrate N in the control and SC treatments was fit to a linear model. Nitrate N in the SN treatment was fit to a first-order model. Note different Y axis scales.

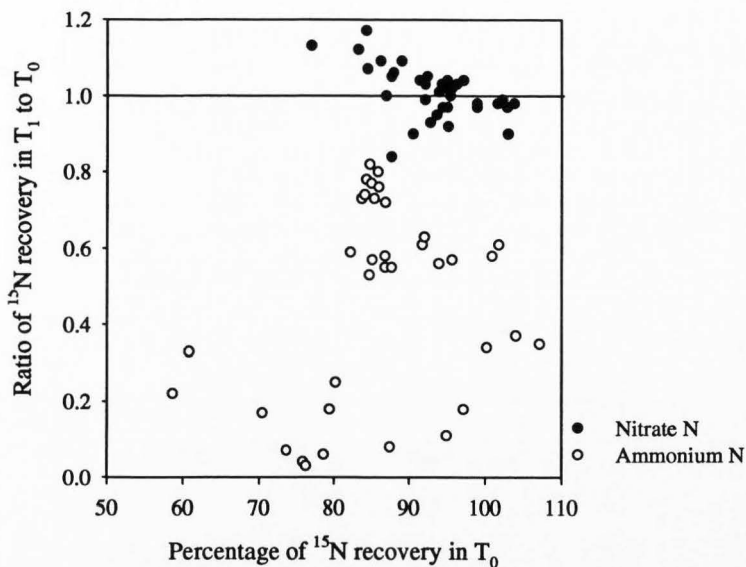


Fig. 4.2. Comparison of $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ recoveries between T_1 (one day after ^{15}N injections) and T_0 (immediately after ^{15}N injections) in the three soil treatments and at the four labeling dates.

of T_1 to T_0 soil samples were about equal to one (Fig. 4.2). However, the recoveries of $^{15}\text{NH}_4^+$ of T_1 samples were significantly lower than those of T_0 samples ($p < 0.01$), and the ratios of $^{15}\text{NH}_4^+$ recoveries of T_1 to T_0 soil samples were less than one in all the three soil treatments and at all the four labeling dates. The ^{15}N excess of NH_4^+ for T_1 samples was significantly lower than that for T_0 samples ($p < 0.01$) in the three soil treatments and at the four sampling dates. In contrast, there was no significant difference in ^{15}N excess of NO_3^- between T_0 and T_1 soil samples ($p = 0.10$).

Relationship of Mineralization Rates and Nitrification Rates

Nitrification rates were highly correlated with net N mineralization rates in the control soil or the soil amended with the compost (Fig. 4.3). The nitrification rates were almost equal to the net N mineralization rates in those soils. Nitrification rates in the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ were not equal to and poorly correlated with net N mineralization rates. However, they were significantly correlated with soil NH_4^+ concentrations ($Y = 0.57 + 0.04X$, $R^2 = 0.716$, $p < 0.001$). The relationships between nitrification rates and gross mineralization rates are presented in Fig. 4.4. Nitrification rates were significantly correlated with and were about 50% of gross N mineralization rates in the three soil treatments.

Gross N mineralization rates were significantly different among the incubation times ($p < 0.01$) and among the three soil treatments ($p < 0.01$) (Table 4.2). Generally, gross mineralization rates decreased with the incubation days, and the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ had the highest gross mineralization rates. However, there was an interaction

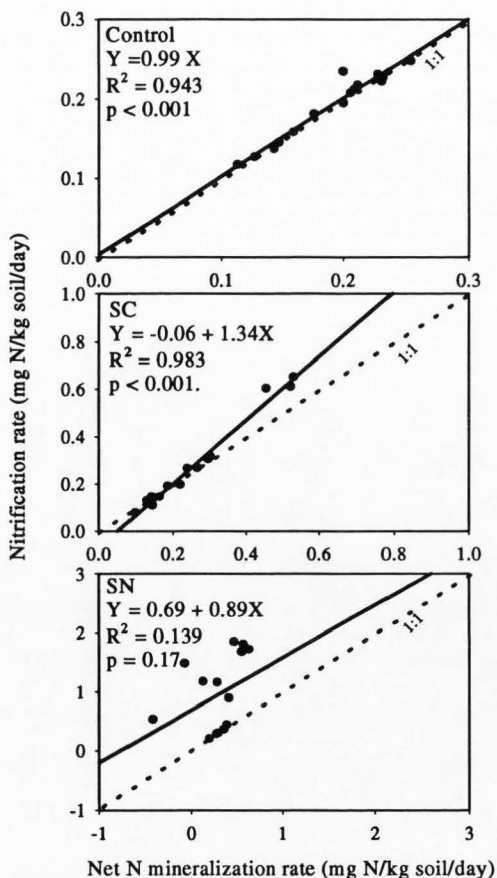


Fig. 4.3. Relationship between nitrification rates and net N mineralization rates in the control soil (Control), soil with addition of the compost (SC), and soil with addition of the $(\text{NH}_4)_2\text{SO}_4$ (SN). Symbols (\bullet) represent the actual values of three replications in the different incubation days. Solid lines (—) are the linear regressions of the actual values. Dotted lines (---) represent a 1:1 relationship of nitrification rates to net N mineralization rates. Note different X- and Y-axis scales for each treatment.

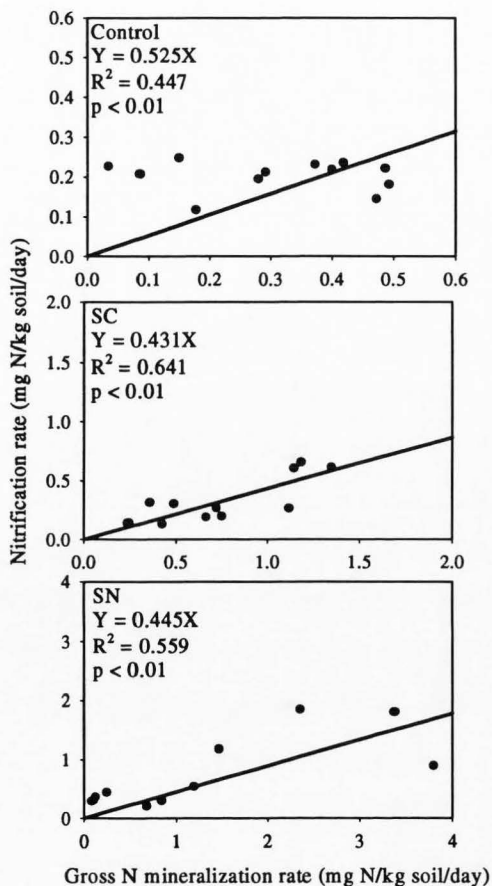


Fig. 4.4. Relationship between nitrification rates and gross N mineralization rates in the control soil (Control), soil with addition of the compost (SC), and soil with addition of the $(\text{NH}_4)_2\text{SO}_4$ (SN). Symbols (•) represent the actual values of three replications in the four incubation times. Solid lines (—) are the linear regressions of the actual values. Note different X- and Y-axis scales for each treatment.

Table 4.2. Rates of gross N mineralization and NH_4^+ consumption in the control soil (Control), the soil with addition of the compost (SC), and the soil with addition of the $(\text{NH}_4)_2\text{SO}_4$ (SN).

Incubation days	Gross N mineralization rate (mg N kg^{-1} soil day^{-1})			NH_4^+ consumption rate (mg N kg^{-1} soil day^{-1})		
	Control	SC	SN	Control	SC	SN
7	0.44 (0.03) [†]	1.23 (0.06)	3.30 (0.53)	1.10 (0.07)	2.00 (0.06)	8.56 (0.62)
40	0.38 (0.10)	0.85 (0.14)	2.15 (0.82)	1.66 (0.19)	2.45 (0.13)	3.52 (0.57)
70	0.32 (0.03)	0.52 (0.11)	0.60 (0.18)	2.27 (0.10)	2.25 (0.06)	2.24 (0.10)
112	0.09 (0.03)	0.30 (0.06)	0.11 (0.01)	1.26 (0.02)	2.07 (0.11)	1.11 (0.04)

[†] Values are means (SE) for n = 3.

between the treatments and the incubation times ($p < 0.01$). The soil receiving the compost had the highest gross mineralization rates at day 112 of the incubation. The NH_4^+ consumption rates were generally higher in the soil receiving $(\text{NH}_4)_2\text{SO}_4$ than in the control soil or the soil receiving the compost ($p < 0.01$) (Table 4.2).

Nitrification Potentials and Nitrification Rates in the Three Soil Treatments

The dynamic patterns of nitrification potentials were significantly different among the three soil treatments ($p < 0.01$). In the NH_4^+ fertilized treatment, nitrification potentials increased after the addition of $(\text{NH}_4)_2\text{SO}_4$ and peaked at day 40 (Fig. 4.5). Thereafter nitrification potentials decreased, however they were still significantly higher than those of the control soil or the soil amended with the compost before day 70. Nitrification potentials in the soil amended with the compost followed a similar pattern as to that of the control soil during the first 70 days of the incubation. At the end of the incubation (day 112), however, nitrification potentials in the soil amended with the compost were significantly higher than those of the control soil or the soil receiving the $(\text{NH}_4)_2\text{SO}_4$.

The patterns of nitrification rates with the incubation days in the three soil treatments are given in Fig. 4.5. Nitrification rates in the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ were highest at day 7 and thereafter decreased. But they were significantly higher than those of the control soil or the soil receiving the compost until day 70 of the incubation. The nitrification rates in the soil amended with the compost were similar to those of the control soils throughout the 112-day incubation.

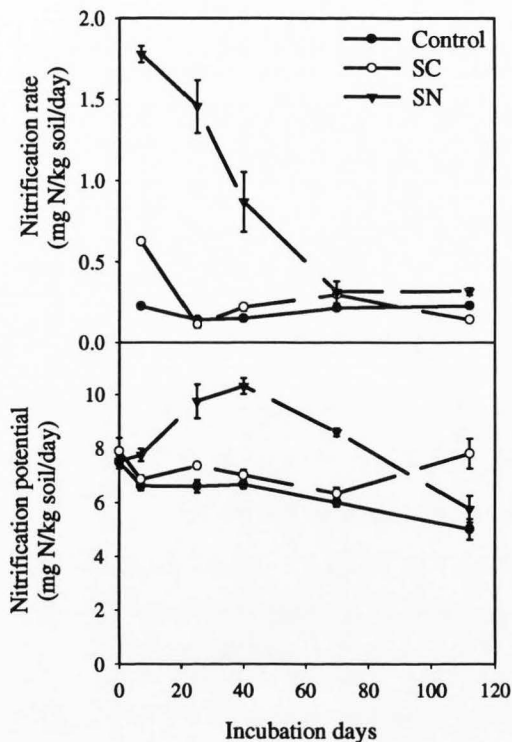


Fig. 4.5. The patterns of nitrification rates and potentials with the incubation days in the control soil (Control), the soil receiving the compost (SC), and the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ (SN). Values are means and SE for $n = 3$.

The ratios of gross N process rates in the three soil treatments are given in Table 4.3. The ratios of nitrification rates to gross N mineralization rates were not significantly different among the three soil treatment ($p > 0.05$) (Table 4.3). Except for the control soil and the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ at day 112, nitrification rates were about 50% of the gross N mineralization rates in the three soil treatments during the incubation. The ratios of nitrification rates to nitrification potentials were significantly different among the three soil treatments ($p < 0.01$) (Table 4.3). There was an interaction between the treatments and incubation days ($p < 0.01$). The ratios of nitrification rates to nitrification potentials were much higher in the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ than in the control soil or the soil receiving the compost during the first 40 days of the incubation ($p < 0.01$). Thereafter the ratios of nitrification rates to nitrification potentials were similar in the three soil treatments.

Discussion

Microbial NO_3^- Assimilation

Microbial NO_3^- assimilation has recently been documented as an important process for controlling soil NO_3^- concentrations in natural forest and grassland ecosystems (Jackson et al., 1989; Schimel et al., 1989; Davidson et al., 1990; Stark and Hart, 1997). The high rates and the high spatial variability of C inputs, and the prevailing fungal populations in those systems have been considered as the major factors leading to microsite heterogeneity of inorganic N availability, and therefore to substantial microbial NO_3^- assimilation (Stark and Hart, 1997). High rates of microbial NO_3^-

Table 4.3. Ratios of gross N process rates in the control soil (Control), the soil receiving the compost (SC), and the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ (SN).

Incubation days	Nitrification rate/gross N mineralization rate			Nitrification rate/nitrification potential		
	Control	SC	SN	Control	SC	SN
7	0.52 (0.03) [†]	0.51 (0.03)	0.57 (0.11)	0.03 (0.00)	0.08 (0.01)	0.24 (0.01)
40	0.45 (0.11)	0.26 (0.02)	0.50 (0.16)	0.02 (0.00)	0.03 (0.00)	0.09 (0.02)
70	0.68 (0.03)	0.63 (0.15)	0.50 (0.48)	0.04 (0.00)	0.05 (0.00)	0.03 (0.01)
112	3.53 (1.50)	0.50 (0.09)	3.06 (0.17)	0.05 (0.01)	0.02 (0.00)	0.06 (0.00)

[†] Values are means (SE) for n = 3.

assimilation accelerate the turnover of soil NO_3^- and lead to low NO_3^- concentrations, while NH_4^+ concentrations are often sizable in those systems (Jackson et al., 1988; Schimel and Firestone, 1989; Davidson et al., 1990). In contrast, NO_3^- usually accumulates in agricultural soils, and often at levels several to hundreds times soil NH_4^+ .

Denitrification and microbial NO_3^- assimilation are two processes that may decrease soil NO_3^- concentrations in laboratory incubation experiments. We measured the recoveries of $^{15}\text{NO}_3^-$ one day after the ^{15}N injections and found that they did not differ from those measured immediately after the ^{15}N injections (Fig. 4.2). This result was consistent for the three soil treatments and for the four labeling dates. No difference in $^{15}\text{NO}_3^-$ recoveries between T_0 and T_1 soil samples (Fig. 4.2) combined with the accumulation of soil NO_3^- (Fig. 4.1) suggests that microbial NO_3^- assimilation and denitrification were both very low, and that they can be ignored as important processes controlling NO_3^- concentrations during the incubation period for these soils. Rice and Tiedje (1989) documented that NH_4^+ could decrease microbial NO_3^- assimilation even at relatively low concentrations ($< 1 \mu\text{g N g}^{-1}$ soil). They suggested that microbial NO_3^- assimilation would not be an important process in most agricultural soils. In contrast, in forest soils under the conditions of sufficient C and limited N, substantial microbial NO_3^- assimilation has been observed (Norton and Firestone, 1996). The absence of microbial NO_3^- assimilation in our experiment may indicate C limitation even in the soil amended with the compost. Wichramasinghe et al. (1985) showed that there was no microbial NO_3^- assimilation in agricultural soils even with 4% organic C but with C:N ratios of 13. Recous and Mary (1990) also reported that microbial NO_3^- assimilation in cultivated soil

was negligible when KNO_3 was added at $50 \mu\text{g N g}^{-1}$ soil but without the addition of glucose C. In contrast, when glucose at $500 \mu\text{g C g}^{-1}$ soil was added along with the same amount of KNO_3 , microbial NO_3^- assimilation occurred. These authors suggest that available C is a dominant factor in regulating the microbial immobilization of NO_3^- -N.

Because of the low NH_4^+ concentrations in the control soil or the soil amended with the compost, the addition of NH_4^+ from ^{15}N injections even at 1 mg N kg^{-1} soil would enhance the rates of those N processes that utilize NH_4^+ . Our data (Table 4.2) showed that the NH_4^+ consumption rates were much higher than the gross N mineralization rates, implying that the NH_4^+ enhanced nitrification and microbial NH_4^+ immobilization. Heterotrophs may be stronger competitors for NH_4^+ than nitrifiers (Jones and Richards, 1977). Under the condition of sufficient available C, more NH_4^+ will be utilized by heterotrophs. We could not examine the effects of the added NH_4^+ from ^{15}N injections on the rates of nitrification and NH_4^+ consumption in the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ due to the high soil NH_4^+ concentrations. However, soil nitrifiers oxidized most of the NH_4^+ added from ^{15}N injections in the control soil or the soil receiving the compost. The enhanced nitrification rates were almost equal to the enhanced NH_4^+ consumption rates (Fig. 4.6), which suggests that nitrifiers were very competitive for NH_4^+ in these systems. High ratios of nitrification rates to gross N mineralization rates in the three soil treatments throughout the 112-day incubation (Table 4.3) also indicate that nitrifiers are not weak competitors for NH_4^+ in these systems. One plausible explanation is that available C limits NH_4^+ assimilation by soil heterotrophs.

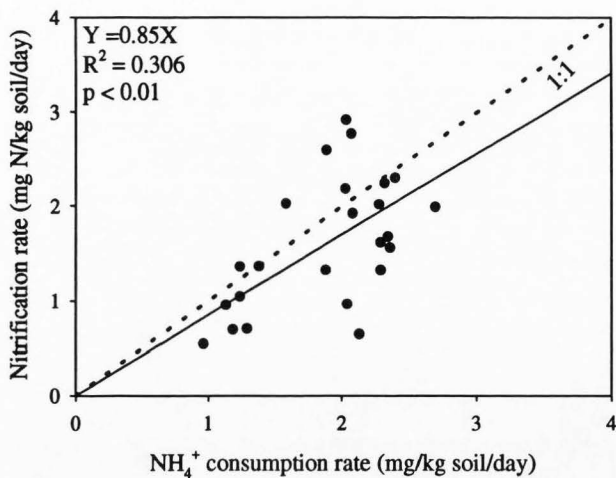


Fig. 4.6. Relationship between nitrification rates and NH₄⁺ consumption rates in the control soil and the soil receiving the compost. The symbols (•) are the actual values for the three replications and the four labeling dates in the two soil treatments. The solid line (—) is the linear regression of the actual values. The dotted line (---) represents the 1:1 relationship between nitrification rate and NH₄⁺ consumption rate.

Control of Nitrification Rates

No difference in the ^{15}N excess in the NO_3^- pool between T_0 and T_1 soil samples indicated that gross nitrification rates could not be measured by ^{15}N pool dilution, partially due to the high background of NO_3^- concentrations. The 100% recovery of $^{15}\text{NO}_3^-$ (Fig. 4.2) implies that the net nitrification rates were equal to the gross nitrification rates. Therefore, we did not differentiate between the gross and net nitrification rates in this experiment, and the nitrification rates were determined by the net rate method. The nitrification rates of day 112 in the control soil or the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ may be overestimated by measuring the NO_3^- pool size changes over the longer time period of 40 days. As a result, the ratios of the nitrification rates to the gross N mineralization rates exceeded one (Table 4.3).

Soil nitrifiers get their energy solely from the oxidation of NH_4^+ to NO_3^- . The low NH_4^+ concentrations in most agricultural soils may suggest that available NH_4^+ is a limiting factor for nitrification rates. Increased nitrification rates with increasing additions of mineral NH_4^+ have been reported in the studies of nitrification kinetics (Darrah et al., 1985; Nishio and Fujimoto, 1990). In the present study, NO_3^- linearly accumulated in the control soil or the soil amended with compost throughout the 112-day incubation. This linear function of NO_3^- accumulation was in contrast to the nonlinear function observed in the soil with addition of the mineral NH_4^+ (Fig. 4.1). In this case the NO_3^- accumulations were best described by a first-order model. The first-order response of soil nitrifiers to the added NH_4^+ reflected the rapid increase of nitrification rates. The increased nitrification rates along with addition of the $(\text{NH}_4)_2\text{SO}_4$

supports the observation that available NH_4^+ limits the nitrification rates in this soil system. Soil NH_4^+ comes from the mineralization of soil organic matter, or directly from mineral NH_4^+ fertilizers. In the control soil or the soil receiving the compost (Figs. 4.3 and 4.4), N mineralization was the primary source of the NH_4^+ available to soil nitrifiers. Therefore, N mineralization was a rate limiting process for the subsequent nitrification. When soil received $(\text{NH}_4)_2\text{SO}_4$ at 50 mg N kg^{-1} soil, both N mineralization and soil NH_4^+ pools contributed to the control of nitrification rates (Figs. 4.3 and 4.4).

Nitrification potential is an index of nitrifier population size (Belser, 1979). Increased nitrification potentials along with the addition of mineral NH_4^+ reflected the growth of nitrifier population. Specific growth rate is commonly used to describe population growth and can be determined from the exponential increases of product concentrations (Powell and Prosser, 1986), from the exponential increases of cell concentrations (Powell and Prosser, 1992), or from mathematical modeling (Darrah et al., 1985; Nishio and Fujimoto, 1990). In this study, we considered nitrification as a one-step transformation of NH_4^+ to NO_3^- , because NO_2^- was not detectable. We assumed that nitrifier population grew exponentially during the period of day 7 to day 25 (Fig. 4.1, Fig. 4.5). The apparent specific growth rate is then described by the equation: $\mu = [\ln X_t - \ln X_0]/t$, where μ is the apparent specific growth rate, X_0 is the initial NO_3^- concentration or nitrification potential, and X_t is NO_3^- concentration or nitrification potential over time t . The apparent specific growth rate was 0.05 day^{-1} , equivalent to a doubling time of 14 days based on the NO_3^- concentrations, and was 0.01 day^{-1} , equivalent to a doubling time of 69 days based on the nitrification potentials. The 0.05

day⁻¹ value is similar to the result of Darrah et al. (1985), i.e., 0.07 day⁻¹ value for the specific growth rate in a sandy loam soil, based on NO₃⁻ concentrations. However, the 0.05 day⁻¹ value for the apparent specific growth rate may be an overestimate, because the assumption that the growth of soil nitrifiers coincides the exponential increase of NO₃⁻ concentrations may not be completely valid. We found that the increase of NO₃⁻ concentrations in the control soil was not accompanied by an increase of nitrification potentials (Fig. 4.5). This may be interpreted as a baseline level of nitrification activity necessary for population maintenance.

Maximum specific growth rates, which are determined when NH₄⁺ oxidation is independent of NH₄⁺ concentrations, have been reported from pure or mixed cultures (Keen and Prosser, 1987; Prosser, 1989) or from various soils (Darrah et al., 1985; Nishio and Fujimoto, 1990). The relationship between apparent and maximum specific growth rate is described by the equation: $\mu = \mu_{\max} S / (K_s + S)$, where S is the NH₄⁺ concentration and K_s is the half saturation constant (Belser, 1979; Darrah et al., 1985). Although the KCl-extractable NH₄⁺ concentrations were much higher than the half saturation constant for this soil (see Chapter 6), the available NH₄⁺ concentrations in soil solution may not be higher. The NH₄⁺ diffusion in soil may further limit the NH₄⁺ supply to nitrifiers. Therefore, the observed specific growth rate may be much lower than the maximum specific growth rate.

Growth of nitrifiers indicated that NH₄⁺ oxidation was limited by the population size. This result was consistent with that of Nishio and Fujimoto (1990). These authors found that increased nitrification rate was attributed to the growth of nitrifiers when

NH_4^+ was added at levels $> 50 \mu\text{g N g}^{-1}$ soil. When $(\text{NH}_4)_2\text{SO}_4$ was added to soil, the existing nitrifier population responded to it quickly, which led to increased nitrification rates (Fig. 4.5) and therefore increased ratios of nitrification rate to nitrification potential (Table 4.3). In the following time period, nitrifier populations grew. However, the larger nitrifier populations require additional maintenance energy. With the depletion of the added NH_4^+ , nitrification rates began to decrease (Fig. 4.5) along with decreases in the ratios of nitrification rate to nitrification potential (Table 4.3). The energy produced by the oxidation of mineralized NH_4^+ could not maintain the enlarged nitrifier population. Nitrification potentials began decreasing at day 40 until they were equivalent to those of the control soil (Fig. 4.5). This transient change of nitrifier population further indicates that NH_4^+ availability is the primary factor limiting nitrification in this soil system.

Gross and Net N Transformation Rates in the Three Soil Treatments

Gross N mineralization rates and NH_4^+ consumption rates were significantly higher in the soil receiving the $(\text{NH}_4)_2\text{SO}_4$ than in the control soil or the soil receiving the compost during the first 40 days (Table 4.2). However, the net N mineralization rates were not different from those of the control soil or the soil receiving compost (Fig. 4.3). It seems that the addition of mineral NH_4^+ accelerated the rates for both mineralization and immobilization. Several explanations for the effects of added N have been proposed, including priming effect of fertilizer N on organic N mineralization, and added N interactions by pool substitution (Smith, 1979; Jenkinson et al., 1985; Woods et al., 1987; Molina et al., 1990). However, we do not have the available information to

substantiate their explanations. With the depletion of added NH_4^+ , its effects on mineralization and immobilization rates subsided. At the end of the incubation, gross N mineralization rates decreased to those of the control soil, which were lower than those of the soil receiving the compost (Table 4.2).

Generally, there is a period when crops have low N uptake rates following fertilization. It is often in this period that NO_3^- may accumulate in soil and is susceptible to loss by leaching or denitrification. We observed lower nitrification rates and nitrification potentials in the soils receiving compost than in the soils receiving $(\text{NH}_4)_2\text{SO}_4$ (Fig. 4.5), suggesting that use of dairy-waste compost as a N source compared to NH_4^+ fertilizers could decrease potential for NO_3^- loss. However, the effect of composts and mineral NH_4^+ fertilizers on nitrification should be further investigated under field conditions with different application rates of NH_4^+ fertilizers and compost.

Conclusions

Microbial NO_3^- assimilation did not occur in the well-mixed laboratory soils treated with dairy-waste compost or $(\text{NH}_4)_2\text{SO}_4$. Therefore the net nitrification rates were equal to the gross nitrification rates. Available NH_4^+ was the primary factor controlling nitrification rates and soil NO_3^- concentrations. When soils received dairy-waste compost, N mineralization rates determined the NH_4^+ available to soil nitrifiers, and therefore the nitrification rates. When soils received fertilizer NH_4^+ , nitrification rates increased quickly and the nitrifier population grew. However, the growth of nitrifiers was transient. Once the available NH_4^+ was depleted, the growth of nitrifiers

ceased and the nitrifier population decreased to the size that could be maintained by the available NH_4^+ produced from N mineralization of soil organic matter.

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CHAPTER 5

NITRIFICATION RATES AND POTENTIALS IN A CORN FIELD
TREATED WITH LIQUID OR COMPOSTED DAIRY WASTE

Abstract

Nitrification rates and potentials were evaluated in a silage corn field fertilized with dairy wastes or ammonium fertilizers. Ammonium sulfate, dairy-waste liquid, or dairy-waste compost were applied at rates approximately equivalent to 100 or 200 kg N ha⁻¹. We determined gross rates of N mineralization, nitrification, and microbial N consumption by ¹⁵N isotope dilution techniques. Gross N process rates and nitrification potentials were determined 90 days after silage corn planting, while inorganic N pool sizes were measured over the course of the growing season. Silage corn yield and plant N content were also evaluated. The recoveries of ¹⁵NO₃⁻ measured one day after the ¹⁵N injections were not different from those measured immediately. This result was independent of the soil treatments, which suggests that microbial NO₃⁻ immobilization was negligible at this time in this agricultural soil no matter what N fertilization was performed. Soil treated with high-rate compost had the highest N mineralization rate (1.7 mg N kg⁻¹ soil day⁻¹), nitrification rate (2.9 mg N kg⁻¹ soil day⁻¹), and nitrification potential (8.1 mg N kg⁻¹ soil day⁻¹). Silage corn yields were not significantly different in the soils treated with the various N fertilizers and the application rates, but ear leaf N and whole silage corn N were significantly higher in the soils treated with compost. Although silage corn removed more N from soils with high-rate compost, the NO₃⁻

production in excess of plant demand resulted in the accumulation of soil NO_3^- during the growing season and after the harvest. The high level of NO_3^- in soil treated with high-rate compost suggests that the appropriate application rate is the low-rate of compost ($50 \text{ Mg dry wt. ha}^{-1}$) evaluated in this study.

Introduction

Autotrophic nitrification is an important biological process in agricultural soils. Nitrate, the product of nitrification, comprises the major N form for the growth of many crops. Crops utilize both NH_4^+ - and NO_3^- -N, but many crops grow better with a high proportion of NO_3^- (Adriaanse and Human, 1990; Below and Gentry, 1992; Crawford and Chalk, 1993). However, surplus NO_3^- may accumulate in soils, and the accumulated NO_3^- is susceptible to loss by leaching or denitrification. The NO_3^- leached from agricultural soils may pollute ground water (Power and Scheper, 1989; Greenwood, 1990), and the N_2O emitted from agricultural soils may destructively affect the ozone layer (Paul et al., 1993; Skiba et al., 1993). In most soils, microbial NO_3^- production is the major contributor to soil NO_3^- . Understanding microbial NO_3^- production will provide information for better utilizing NO_3^- and avoiding the potential risk of excess NO_3^- to the environment.

Production of NO_3^- by autotrophic nitrification has been investigated in differently managed agricultural soils (Hadas et al., 1986; Laanbroek and Gerards, 1991). Net nitrification rate, which is determined by the change of NO_3^- pool size over time, is usually measured because microbial NO_3^- immobilization is suggested to be trivial

in most agricultural soils (Jansson et al., 1955; Winsor and Pollard, 1956; Rice and Tiedje, 1989). However, studies in natural forests or grasslands (Jackson et al., 1989; Schimel et al., 1989; Davidson et al., 1990; Stark and Hart, 1997) have shown that microbial NO_3^- immobilization should not be overlooked in the systems with high C availability. Because environmental factors may act differently on various microbial N processes (Davidson et al., 1992; Low et al., 1997), we should be very careful in interpreting experimental results based on net rate measurements. Direct measurement of individual N process rate, especially for agricultural soils with organic amendments, is therefore very important for understanding soil NO_3^- dynamics.

Ammonium availability to soil nitrifiers has been assumed to depend on the utilization of NH_4^+ by crops and by soil heterotrophs because nitrifiers have been considered weak competitors for NH_4^+ (Jones and Richards, 1977; Rosswall, 1982). Application of organic wastes to agricultural soils has been advocated and practiced for utilizing their N fertilizer value and for improving soil physical properties (Golueke, 1973; Buchanan and Gliessman, 1991; García et al., 1992). These organic amendments may concurrently change microbial NO_3^- production for two reasons. Firstly, unlike mineral N, organic N provides crop-available N slowly by ammonification and subsequent nitrification. The rate at which NH_4^+ is produced may coincide with the N uptake by crops, and the synchrony of crop NH_4^+ uptake with NH_4^+ supply may decrease soil nitrification rates and nitrifier population activities (Verhagen et al., 1994). Secondly, organic amendments add organic C to soil and may significantly increase soil microbial biomass and activity (Schnürer et al., 1985; Fauci and Dick, 1994). The

enhanced heterotrophic activity may accelerate microbial NH_4^+ immobilization, which can lead to the decrease of NH_4^+ availability to nitrifiers.

Various rates of N fertilizers have been used in agricultural soils to achieve high crop yields, but crop yields do not always respond linearly to the increased addition of N fertilizers (Greenwood, 1990). Instead, various rates of N fertilization may result in changes of N resource availability, leading to the change of the relationship among soil heterotrophs, nitrifiers and crops. The supply of available N from organic wastes is dependent on microbial decomposition and the quality of the waste. Hence agricultural management of organic N sources is more complex and more challenging than that of mineral N fertilizers.

Our objectives were to determine the effects of organic wastes and fertilizer N and their application rates on soil nitrification rates and potentials, to understand the mechanisms of microbial controls of soil NO_3^- concentrations, and to evaluate the effects of various N sources and their application rates on silage corn yield and plant N content.

Materials and Methods

Study Site

The study was located in the Greenville Farm of Utah State University. The soil is the very strongly calcareous Millville silt loam (coarse-silty, carbonatic, mesic Typic Haploxeroll). The average annual precipitation was 17 inches. The average annual temperature was 9 °C, and the frost-free season was 156 days (Utah Climate Center,

personal communication). We conducted the experiment using the inorganic N fertilizer of ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ and the organic N fertilizers of dairy-waste compost and dairy-waste liquid. Mature dairy-waste compost treated with frequent turning and watering (Shi et al., In press) was donated by the Department of Agricultural Systems and Technology Education at Utah State University. Dairy-waste liquid was urine, feces, and milking parlor waste water, which were liquid/solid separated and stored in a holding pond for a short time. The selected properties of the soil, dairy-waste compost, and dairy-waste liquid are given in Table 5.1.

Organic-waste amendments were applied at two rates of 100 and 200 kg N ha⁻¹ based on the assumptions that all N in dairy-waste liquid is available for crop growth, while only 10% total N in dairy-waste compost is available. These assumptions were based on the previous work (Chapter 2 and 3) and waste analysis. Therefore, the seven treatments were 1) control, no added N; 2) AS-100, $(\text{NH}_4)_2\text{SO}_4$ at 100 kg N ha⁻¹; 3) AS-200, $(\text{NH}_4)_2\text{SO}_4$ at 200 kg N ha⁻¹; 4) LS-100, dairy-waste liquid at 100 m³ ha⁻¹; 5) LS-200, dairy-waste liquid at 200 m³ ha⁻¹; 6) DC-100, dairy-waste compost at 50 Mg dry wt. ha⁻¹; and 7) DC-200, dairy-waste compost at 100 Mg dry wt. ha⁻¹. Because the N

Table 5.1. The selected properties of the soil (0-15 cm depth), dairy-waste compost, and dairy-waste liquid.

Properties	Soil	Dairy-waste compost	Dairy-waste liquid
Organic C	10 g kg ⁻¹	237 g kg ⁻¹	4.3 g L ⁻¹
Kjeldahl N	1.0 g kg ⁻¹	19.0 g kg ⁻¹	0.6 g L ⁻¹
C:N ratio [†]	10.0	12.5	14.5
pH	8.4	8.8	8.5

[†] Inorganic NH_4^+ -N was subtracted from Kjeldahl N for calculating C:N ratio.

content of the liquid waste was overestimated before application, the actual application rates for the LS-100 and the LS-200 treatments were 65 and 130 kg N ha⁻¹, respectively. These treatments were arranged in a completely randomized block design with four replications. The 28 plots were each 3.0 m wide and 9.1 m long with four rows of corn in each plot. Between each block was a 1.0 m alley, and no alley was between each plot in a single block. Nitrogen fertilizers were broadcast on the soil surface in the middle of May 1997, then tilled into the 0-15 cm soil layer. Silage corn (variety DK- 656) was planted at 82,000 plants ha⁻¹ on May 28, 1997. Corn was irrigated and maintained according to the standard agricultural practices for Cache Valley, Utah.

Soil Inorganic N

Effects of the various N fertilizers and their application rates on soil inorganic N were examined in the early growing season (June 26) and after the harvest (November 4). We collected the variously treated soils from 0-30 and 30-60 cm depths. About 15-g samples of moist soils were immediately placed in 120-ml specimen containers with 2 M KCl (1:5, soil wt.:KCl vol.) and stored in a cooler. After we came back to the laboratory, the soil samples were shaken for 1 h, and the extracts were filtered through pre-rinsed Whatman #1 filter papers. The filtrates were frozen until analysis for NH₄⁺ and (NO₃⁻+NO₂⁻)-N by the Lachat N Autoanalyzer (QuikChem Systems, 1992; 1993).

Measurement of Gross N Process Rates

We conducted field ¹⁵N labeling to measure gross N transformation rates in late August (90 days after planting) when we expected that considerable N would be

required by the corn. Soil inorganic N at 0-15 cm depth was also measured. We selected soils in the middle of each plot and between corn rows for ^{15}N labeling. Four small PVC cylinders (5 cm dia. \times 15 cm long) were driven into the soil at each plot. Large PVC cylinders (10 cm dia. \times 20 cm long) were then driven into the soil around each small cylinders. The pair of a large and a small cylinder was removed and the soil between the two cylinders was placed into a plastic bag, mixed, and immediately subsampled for extraction with 2M KCl (about 15 g dry soil in 75 ml). The remaining mixed soil was used later for measuring soil gravimetric water content and nitrification potentials. Two of the small cylinders received K^{15}NO_3 injections and other two received $^{15}\text{NH}_4\text{Cl}$ injections. The solutions contained N at 50 mg L^{-1} at 50% ^{15}N enrichment. Twenty-ml $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+$ solution was injected by an 18-gauge side-port spinal needle into each small cylinder to provide about 2 mg N kg^{-1} dry soil. For ensuring uniform labeling in each small cylinder, we covered the top with aluminum foil and injected the ^{15}N solution from the bottom with eight 1.25-ml injections. Then we covered the bottom with aluminum foil and the cylinder was turned upright. We injected the remaining 10-ml ^{15}N solution from the top with eight 1.25-ml injections. The soil moisture was increased by about 4% following the injections of ^{15}N solution.

In each pair of the small cylinders injected with $^{15}\text{NO}_3^-$ or $^{15}\text{NH}_4^+$ solution, one cylinder (T_0 cylinder) was immediately (within 15 minutes after labeling) broken up, mixed, and extracted with 2 M KCl to determine the ^{15}N extraction efficiency (Stark, In press). The other cylinder (T_1 cylinder) covered at the bottom with aluminum foil was placed into a 1-L Mason jar that was capped and buried in the original location. After

24.25 h, the T_1 cylinder was broken up, mixed, and extracted with 2 M KCl (about 20 g dry soil in 100 ml). Inorganic N was prepared and analyzed using the method described above. A ^{15}N diffusion procedure (Stark and Hart, 1996) was used to prepared ^{15}N samples. The ^{15}N enrichment of NH_4^+ and NO_3^- pools was measured by continuous-flow direct dry combustion and mass spectrometry with an ANCA 2020 system (Europa Scientific, Cincinnati, OH).

The amount of $^{15}\text{N-NH}_4^+$ or $^{-}\text{NO}_3^-$ in the T_0 and T_1 cylinders was calculated by multiplying the ^{15}N excess (^{15}N enrichment % minus the background 0.37%) by the NH_4^+ or NO_3^- pool size, and was expressed as $\text{mg } ^{15}\text{N kg}^{-1}$ soil. The recovery of ^{15}N was expressed as a percentage of the added ^{15}N . Gross rates of N mineralization and nitrification were calculated by the equation of Kirkham and Bartholomew (1954). The initial $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ pool size and its ^{15}N excess were calculated by the method of Stark (In press). Gross immobilization rate of NH_4^+ was calculated by subtracting the gross nitrification rate from the NH_4^+ consumption rate. Gross immobilization rate of NO_3^- was calculated by subtracting the net nitrification rate from gross nitrification rate.

Nitrification Potentials

Nitrifier population activity was determined by the shaken soil slurry method (Hart et al., 1994). After passed through a 2-mm screen, soil samples (about 15-g moist soils) were placed into 250-ml Erlenmeyer flasks and 100-ml phosphate buffer of $(\text{NH}_4)_2\text{SO}_4$ was added to these flasks. The flasks were continuously shaken for 24 hours at 200 rpm (Stark, 1996). At 2, 4, 22, and 24 h after the beginning of shaking, 9-ml aliquots were removed. The aliquots were centrifuged at $8,000 \times g$ for 10 minutes. The

$(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ was analyzed by the colorimetric method described above. Nitrification potential was the slope of linear regression of concentrations of $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ versus time, and expressed as mg N kg^{-1} dry soil day^{-1} .

Carbon Mineralization Rates

Carbon mineralization rates were measured simultaneously with the field ^{15}N labeling experiment. A 20-ml vial containing 1 ml 1M NaOH was placed into the 1-L Mason jar along with a T_1 cylinder. After 24.25 h, the vial was removed from the Mason jar and capped tightly for later analysis of CO_2 trapped in the base. A Mason jar containing only the base trap was used as a blank. The rate of CO_2 production was determined by titration with standardized 0.2 M HCl (Zibilsik, 1994).

Silage Corn Yield and Plant N Content

Silage corn yield was determined by harvesting aboveground plants from 5.3 m of the middle two rows in each plot, and was expressed as $\text{Mg dry wt. ha}^{-1}$. The N content of the plant tissue was determined in eight ear leaves at silking phase and in one chopped whole corn at mature phase from each plot. After drying at $80\text{ }^\circ\text{C}$ for 24 h, the ear leaves and chopped corn were finely ground and N content was determined by Kjeldahl digestion and distillation method (Jones et al., 1991).

Statistical Analysis

All statistical analyses were performed with SuperANOVA statistical software for Macintosh computer (Abacus Concepts, 1995, Berkeley, CA). The recovery and excess of $^{15}\text{N-NH}_4^+$ or $-\text{NO}_3^-$ at T_0 and T_1 soil samples were analyzed by a split plot

method with treatments as a main plot and labeling days as a subplot. Treatment effects on inorganic N were also analyzed by a split plot method with treatments as a main plot and soil depths as a subplot. Treatment effects on rates of C and N processes, silage corn yield, and plant N content were analyzed by a complete randomized block method. See Appendix D for the details of statistical analysis.

Results

Soil Inorganic N

Effects of the various N sources and their application rates on soil NH_4^+ and NO_3^- concentrations in the early growing season and after the harvest are presented in Figs. 5.1 and 5.2. During the early growing season, soil NH_4^+ and NO_3^- pool sizes were at the highest levels (Fig. 5.1). Among the soils treated with the various fertilizers and application rates, soil NH_4^+ and NO_3^- concentrations at the 0-30 cm depth were significantly different, while soil NH_4^+ and NO_3^- concentrations at the 30-60 cm depth were not different (Fig. 5.1). Except for the soil treated with dairy-waste liquid, soil NO_3^- concentrations were significantly higher with the high rate of N fertilization than with the low rate. After the harvest, only soil treated with the high-rate dairy-waste compost had sizable NH_4^+ and NO_3^- pools at both 0-30 and 30-60 cm depths (Fig. 5.2). This trend of inorganic N accumulation was observed at the 0-15 cm depth in soils treated with the high-rate compost during the ^{15}N experiment (rapid growth of corn) (Table 5.2).

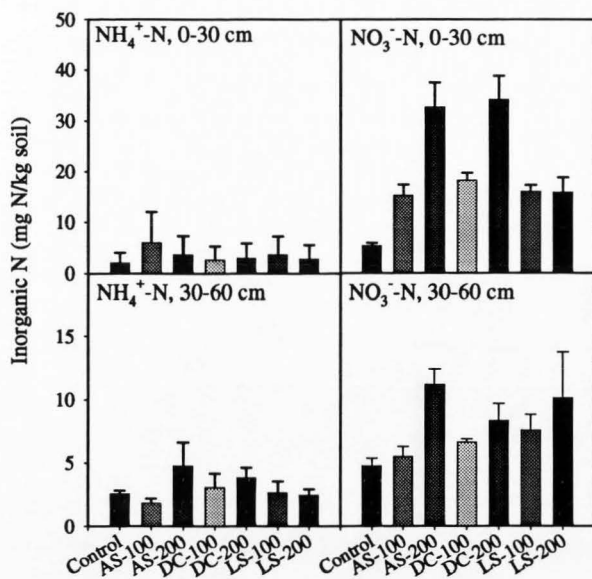


Fig. 5.1. The NH_4^+ - and NO_3^- -N pool sizes of two depth intervals in the variously treated soils in the early growing season (June 26). Values are means and standard errors for $n = 4$. Control, soil without N fertilization; AS, soil fertilized with $(\text{NH}_4)_2\text{SO}_4$; DC, soil fertilized with dairy-waste compost; LS, soil fertilized with dairy-waste liquid; 100, N application rate at 100 kg ha^{-1} ; and 200, N application rate at 200 kg ha^{-1} . See Materials and Methods for details.

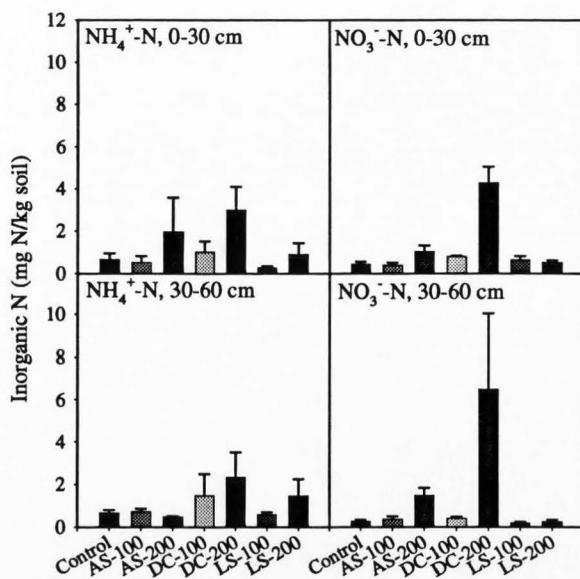


Fig. 5.2. The NH_4^+ - and NO_3^- -N pool sizes of two depth intervals in the variously treated soils after harvest (November 4). Values are means and standard errors for $n = 4$. See Fig. 5.1 for the treatments.

Table 5.2. Comparison of inorganic N pool sizes at the 0-15 cm depth and rates of C mineralization, N mineralization, and microbial NH_4^+ immobilization in the variously treated soils 90 days after planting.

Treatments [†]	NH_4^+ -N	NO_3^- -N	C mineralization	Gross N mineralization	Microbial NH_4^+ immobilization
	mg kg^{-1} soil		mg C kg^{-1} soil day ⁻¹	mg N kg^{-1} soil day ⁻¹	
Control	0.57 a [‡]	0.16 a	5.66 a	0.05 a	0.51 a
AS-100	0.65 a	0.21 a	6.24 ab	0.12 a	0.46 a
AS-200	0.62 a	0.72 a	5.60 a	0.01 a	0.43 a
DC-100	0.82 a	0.42 a	8.68 c	0.24 a	0.69 a
DC-200	1.15 b	17.27 b	12.89 d	1.65 b	0.69 a
LS-100	0.68 a	0.28 a	7.04 b	0.38 a	0.68 a
LS-200	0.66 a	0.31 a	7.08 b	0.38 a	1.02 a

[†] Control, soil without N fertilization; AS, soil fertilized with $(\text{NH}_4)_2\text{SO}_4$; DC, soil fertilized with dairy-waste compost; LS, soil fertilized with dairy-waste slurry; 100, N application rate at 100 kg ha^{-1} ; and 200, N application rate at 200 kg ha^{-1} . See Materials and Methods for detail.

[‡] Values in a column followed by the same letters are not significantly different ($p > 0.05$).

Gross N Transformation Rates and C Mineralization Rates

The recoveries of $^{15}\text{NO}_3^-$ determined immediately after ^{15}N injections (T_0 cylinders) ranged from 70% to 94% (Fig. 5.3). The recoveries of $^{15}\text{NO}_3^-$ determined one day after ^{15}N injections (T_1 cylinders) were not significantly different from those (T_0 cylinders) for the variously treated soils ($p = 0.96$). The ratios of $^{15}\text{NO}_3^-$ recoveries in T_1 to T_0 cylinders were almost equal to one (Fig. 5.3). In contrast, the $^{15}\text{NH}_4^+$ recoveries of T_1 cylinders were significantly lower than those of T_0 for the variously treated soils ($p < 0.001$). Therefore, the ratios of $^{15}\text{NH}_4^+$ recoveries in T_1 to T_0 cylinders were less than one (Fig. 5.3). The ^{15}N excesses of T_0 cylinders were significantly higher than those of T_1 cylinders for both $^{15}\text{NO}_3^-$ ($p < 0.01$) and $^{15}\text{NH}_4^+$ ($p < 0.01$). Hence, the ^{15}N pool dilution calculations can be used to determine the gross N mineralization rates and gross nitrification rates.

Microbial NO_3^- immobilization in the various treatments was determined from two different methods. Firstly, we compared the recoveries of $^{15}\text{NO}_3^-$ in T_0 cylinders with those in T_1 cylinders. If there were any sinks of soil NO_3^- present inside the soil cores, the $^{15}\text{NO}_3^-$ recoveries in T_1 should be lower than those in T_0 . The $^{15}\text{NO}_3^-$ recoveries in T_1 did not differ from those in T_0 in the variously treated soils (Fig. 5.3), thus indicating that there was no microbial NO_3^- consumption (i.e., immobilization or denitrification). Secondly, we calculated the microbial NO_3^- immobilization rates by ^{15}N pool dilution calculations (Stark, In press), then tested whether these calculated rates were greater than zero (by t-test). The t-values for the variously treated soils were less than the critical t-value at $p = 0.05$, and therefore we accepted the null hypothesis that

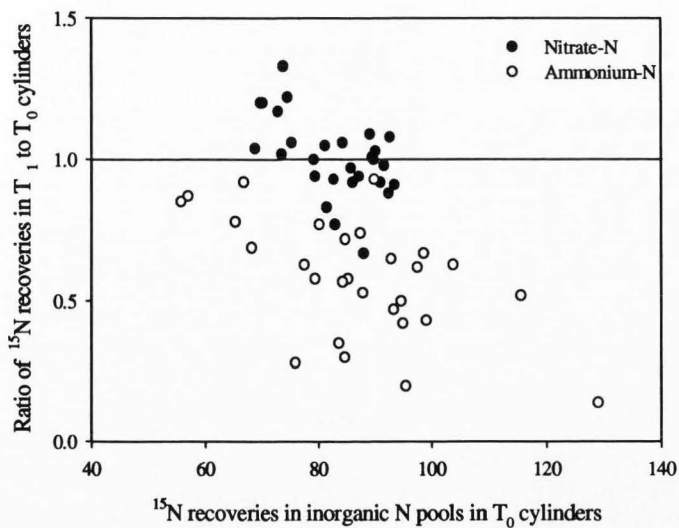


Fig. 5.3. Recoveries of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ in T_0 and T_1 cylinders in the four blocks and the seven soil treatments.

microbial NO_3^- immobilization did not occur in this agricultural soil regardless of the N fertilization treatments.

Effects of the various N fertilizers and application rates on C and N mineralization rates and microbial NH_4^+ immobilization rates are given in Table 5.2. Carbon mineralization rates ranged from 5.6 to 12.9 $\text{mg C kg}^{-1} \text{ soil day}^{-1}$. Various N fertilizers and application rates significantly affected the C mineralization rates ($p < 0.01$). Soils treated with dairy-waste compost or liquid had higher C mineralization rates than the control soil or the soil treated with $(\text{NH}_4)_2\text{SO}_4$. The highest C mineralization rate and the highest gross N mineralization rate were observed in the soil treated with high-rate dairy-waste compost, while microbial NH_4^+ immobilization rates were not significantly different in the variously treated soils (Table 5.2).

Generally, nitrification potentials were significantly affected by the N application rates ($p < 0.01$), but not by the various N fertilizers ($p = 0.50$). Nitrification potentials were higher with the high-rate N fertilization than with the low-rate N fertilization (Table 5.3). The control soil had the lowest nitrification potential of 2.3 $\text{mg N kg}^{-1} \text{ soil day}^{-1}$, and the soil treated with high-rate compost had the highest nitrification potential of 8.1 $\text{mg N kg}^{-1} \text{ soil day}^{-1}$. Gross nitrification rate was higher in the soil treated with high-rate compost than in the other treated soils (Table 5.3). The nitrification rate in the soil treated with high-rate compost was 2.9 $\text{mg N kg}^{-1} \text{ soil day}^{-1}$, whereas nitrification rates in the other treated soils were less than 1 $\text{mg N kg}^{-1} \text{ soil day}^{-1}$.

Silage Corn Yield and Plant N Content

Effects of the various N fertilizers and application rates on silage corn yield and

Table 5.3. Comparison of nitrification rates, potentials, and their ratios in the variously treated soils 90 days after planting.

Treatments [†]	Nitrification rate	Nitrification potential	Nitr. rate/Nitr. potential
	mg N kg ⁻¹ soil day ⁻¹		
Control	0.21 a [‡]	2.33 a	0.10 a
AS-100	0.50 a	4.61 ab	0.12 a
AS-200	0.43 a	6.08 bc	0.08 a
DC-100	0.69 a	4.58 ab	0.15 a
DC-200	2.86 b	8.12 c	0.39 b
LS-100	0.40 a	5.34 b	0.08 a
LS-200	0.31 a	6.99 bc	0.05 a

[†] See Table 5.2 for the treatments.

[‡] Values in a column followed by the same letter are not significantly different ($p > 0.05$).

plant N content are given in Table 5.4. Corn planted in the soil treated with high-rate compost had the highest ear leaf N and whole silage tissue N, followed by the soil with low-rate compost and high-rate ammonium sulfate. Although silage corn dry wt. yields were not significantly different in the soils treated with the various N fertilizers and application rates, yields in all fertilized treatments were higher than in the control soil (no fertilizer applied).

Discussion

Effects of N Fertilizers and Application Rates on Silage Corn Yield, Plant N Content, and Soil Inorganic N Pool Size

Various animal wastes and their application rates on crop yield, crop N uptake, soil chemical property, and ground-water quality have been evaluated in field experiments (Culley et al., 1981; Patni and Culley, 1989; Burns et al., 1990; King et al.,

Table 5.4. Effects of N fertilizers and their application rates on corn yields and corn N contents.

Treatments [†]	Corn yields Mg ha ⁻¹	Ear leaf N at day 82 %	Whole silage N at harvest %
Control	18.1 a [‡]	1.72 a	0.62 a
AS-100	23.1 ab	2.19 bc	0.64 a
AS-200	23.6 ab	2.57 de	0.90 cd
DC-100	26.9 b	2.38 cd	0.84 bc
DC-200	27.0 b	2.74 e	1.07 d
LS-100	22.1 ab	1.87 ab	0.58 a
LS-200	25.8 b	2.06 abc	0.70 ab

[†] See Table 5.2 for the treatments.

[‡] Values in a column followed by the same letter are not significantly different ($p > 0.05$).

1990; Zebarth et al., 1997). These authors tried to determine an appropriate application rate for animal waste that would improve crop yield and plant N uptake, while maintaining soil and ground-water quality. It has been observed that increasing the applications rates of animal wastes above a threshold level will not benefit crop yield. Instead, excessively high application rates elevate NO_3^- concentrations in the soil profiles and pose potential risk to ground-water quality. High application rates may also result in NO_3^- accumulations in crop tissue exceeding toxic levels especially for forage when feeding to ruminants (Burns et al., 1990). Hence, we need to consider crop yield, N uptake and soil residual NO_3^- when recommending the appropriate application rates for animal wastes.

Treatments of the various N fertilizers and application rates increased silage corn yields over the control soil (Table 5.4). This was expected because soil N fertility in the unfertilized control soil was low (Table 5.1). However, statistically significant

differences in silage corn yields were only observed between the control soil and those treated with dairy-waste compost (Table 5.4). Furthermore, only silage corn from the soil treated with dairy-waste compost or with the high-rate $(\text{NH}_4)_2\text{SO}_4$ had significantly higher ear leaf N and whole silage tissue N than those of the control soil (Table 5.4). Dairy-waste liquid at the low or high rates did not benefit silage corn yield or plant N content, which suggests that available N provided by dairy-waste liquid was not sufficient for silage corn growth. This N deficiency began to be observed 80 days after planting, and silage corn developed typical visual N deficiency symptoms. Several processes may explain the cause. Firstly, the actual N application rates of dairy-waste liquid were lower than the desired rates because of the overestimation of N content from pre-application samples. Secondly, the assumption that 100% of the total N in the dairy-waste liquid would be available during the growing season may have been unrealistic. Thirdly, some of the mobile plant-available N from dairy-waste liquid may have been lost during the early growing season by NO_3^- leaching or by denitrification (Zebarth et al., 1997).

Although considerable N is required during the corn reproductive phase, N fertilizers are often applied before corn seeding. The fate of inorganic N, especially NO_3^- during the growing season and after the harvest, should be given consideration for environmentally sound N management. Nitrate may significantly accumulate in soils during the early growing season when young corn plants require little N. The relatively high NO_3^- level versus NH_4^+ (Fig. 5.1) indicates that soil NH_4^+ either from $(\text{NH}_4)_2\text{SO}_4$ or from N mineralization of dairy wastes was rapidly oxidized to NO_3^- . Except for the soil

treated with dairy-waste liquid (Fig. 5.1), the soils treated with the high-rate N fertilizers had significantly higher NO_3^- levels than those with the low-rate N fertilizers at the 0-30 cm depth. The similar NO_3^- levels in the soil treated with low- or high-rate dairy-waste liquid implies NO_3^- losses, probably through both leaching and denitrification. While soil NO_3^- concentrations in 30-60 cm depth were increased by the application of high-rate liquid, no significant difference was measured between high and low rates (Fig. 5.1). When corn growth requires considerable N, soil inorganic N pool sizes would be expected to decrease. Indeed, soil inorganic N concentrations decreased to the very low level (Table 5.2), except for soil treated with high-rate compost. The accumulation of NO_3^- in soil treated with high-rate compost (Fig. 5.2) suggests that available N supplied exceeded the corn N requirement. The high level of NO_3^- remaining in soil after harvest may pose potential risk to the environment. This emphasizes that even animal wastes stabilized by composting will increase the potential for NO_3^- leaching if applied at excessive rates.

Microbial N Immobilization

Microbial N immobilization may immediately occur following the application of N fertilizers (Rice and Smith, 1984; Okereke and Meints, 1985). The rapid immobilization of inorganic N into organic forms would be important in protecting fertilizer N losses through leaching and denitrification during the early growing season. The amount of N immobilized by microorganisms, however, should be low enough so that microbial N immobilization does not deplete the soil inorganic N needed for crop growth. We have measured the microbial immobilization of NH_4^+ - and NO_3^- -N by ^{15}N

pool dilution techniques during a rapid N uptake phase for silage corn. Microbial NH_4^+ immobilization rates, which were independent of soil treatments, averaged at $0.64 \text{ mg N kg}^{-1} \text{ soil day}^{-1}$ (Table 5.2). Microbial NO_3^- immobilization rates were rarely detectable regardless of N fertilizer treatments.

In laboratory experiments using sieved agricultural soils, Recous and Mary (1990) documented that microbes prefer NH_4^+ to NO_3^- for their growth even in the case of high N ratio of NO_3^- to NH_4^+ (110:5). Their results are consistent with the previous studies in well-mixed agricultural soils (Jansson et al., 1955; Winsor and Pollard, 1956; Jansson, 1958; Broadbent and Tyler, 1962). Despite low rates of microbial NO_3^- immobilization in these laboratory experiments, significant microbial utilization of NO_3^- has been observed in field experiments (Aulakh and Rennie, 1984; Recous et al., 1988). In the field situations, soil heterogeneity may lead to depleted NH_4^+ zones where microbes can use NO_3^- for their growth (Davidson et al., 1990; Stark and Hart, 1997). However, microbial NO_3^- immobilization did not occur in our field experiment even with the amendment of dairy-waste compost. In our situation, lack of microbial NO_3^- immobilization may be due to the low C availability relative to the NH_4^+ availability in soil even after the amendment of dairy wastes.

In contrast to the grassland and forest ecosystems where available N is a limiting factor for various microbial N transformations, available C is generally a key factor in limiting microbial N processes in agricultural soils. Significant microbial NO_3^- immobilization has been observed only in sieved agricultural soils when readily available C such as glucose or sucrose is added (Winsor and Pollard, 1956; Okereke and Meints,

1985; Recous and Mary, 1990). Dairy-waste compost contained a high amount of organic C (Table 5.1); however, this organic C may be associated with organic N or be of limited biological availability. This was indicated by the lack of difference in microbial NH_4^+ immobilization rates (Table 5.2) for the different treatments. Even soil amended with a high rate of dairy-waste compost did not show stimulation of microbial immobilization of NH_4^+ (Table 5.2). The observation of higher C mineralization value for the soil that received high-rate compost suggests that there was an impact on C availability. However, the lack of stimulation of microbial NH_4^+ immobilization suggests that the readily available C may not be sufficient to support microbial growth with use of NO_3^- . The lack of microbial NO_3^- immobilization in this agricultural soil indicates that measurements of net nitrification rates (excluding plant roots) can give the same information as gross nitrification rates in directing the management of microbial NO_3^- production.

Nitrification Rates and Potentials

Nitrifier population activity has been considered to reflect the events occurring weeks to months before samplings (Berg and Rosswall, 1985). The higher nitrification potentials in soils with high-rate N fertilization (Table 5.3) may indicate that soil NH_4^+ concentrations were higher in the past when compared to those with low-rate N fertilization. Because soil NH_4^+ concentrations were not significantly different among the various treatments 40 days after N fertilization (Fig. 5.1), the higher nitrifier population activity in soil with high-rate N fertilization would be the residual effect of the higher NH_4^+ concentrations in the earlier days following fertilization. Except for soil

treated with high-rate compost, other treated soils had low NH_4^+ -N concentrations and N mineralization rates 100 days after N fertilization (Table 5.2), which suggests that nitrifier population activity would be limited by the NH_4^+ availability thereafter. However, the higher ratio of nitrification rate to nitrification potential in soil treated with high-rate compost (Table 5.3) implies that there is still relatively high available NH_4^+ for maintaining nitrifier population activity at a higher level.

The NH_4^+ available for nitrifiers in the variously treated soils 100 days after N fertilization was mainly provided through the microbial decomposition of organic matter. The mineralized NH_4^+ may be utilized by either heterotrophs or nitrifiers, but it has been previously assumed that heterotrophs are stronger competitors than nitrifiers for available NH_4^+ (Jones and Richards, 1977). Our data did not support this assumption (Tables 5.2 and 5.3); on the contrary, we observed that nitrifiers accounted for a large proportion of NH_4^+ consumption. For example, the nitrification rate in soil with the high-rate compost was about four times the microbial NH_4^+ immobilization rate (Tables 5.2 and 5.3), which may also indicate that readily available C limited microbial N assimilation rates in this agricultural soil. The highest C mineralization rate was associated with the highest N mineralization rate and nitrification rate in soil amended with high rate of dairy-waste compost (Tables 5.2 and 5.3), which indicates a higher rate of organic matter turnover in that soil.

Method Evaluation

Isotope pool dilution technique is a very powerful tool for determining short-term rates of N processes. The multiple rates of N mineralization, nitrification, and

microbial NH_4^+ and NO_3^- consumption have simultaneously been measured in this study by adding $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ to soils. Rate estimates, however, are fairly sensitive to the data variability. A small error in measured data can be amplified and a large error may be reflected in calculated rates (Myrold and Tiedje, 1986). Davidson et al. (1991) have evaluated the effects of errors in a variety of important factors, including initial N pool sizes and uneven distribution of added ^{15}N on isotope dilution calculations.

We estimated the initial NH_4^+ - or NO_3^- -N pool size following the injection of ^{15}N by the equation: initial $^{14}\text{N} + ^{15}\text{N}$ pool size in $T_0 = ^{14}\text{N}$ pool size outside $T_0 + \text{mass of } ^{15}\text{N added} \times ^{15}\text{N extraction efficiency}$ (Stark, In press). We corrected the mass of added ^{15}N by a factor of ^{15}N extraction efficiency because some abiotic processes such as clay fixation and organic adsorption can rapidly consume the added ^{15}N within a few minutes, which leads to less than 100% of the added ^{15}N recovered in 2M KCl extraction (Davidson et al., 1991). When using this equation, we substituted soil ^{14}N pool size in T_0 with that outside T_0 . But fine-scale spatial heterogeneity may introduce a large error when using this substitution. Higher fine-scale spatial heterogeneity was observed in NO_3^- pool size in soils treated with high-rate compost. Even in the same pair of small and large cylinders, the $^{14}\text{NO}_3^-$ -N pool sizes were sometimes three times different, which could not be explained by the addition of ^{15}N . Therefore, it is not surprising that we calculated some negative values in microbial NO_3^- immobilization.

When we prepared the ^{15}N samples by the diffusion procedure, the N recovered in filter paper disks were very low at 20-30% due to unknown reasons. The 100% recovery is not necessary for accurately estimating ^{15}N enrichment if it is corrected with

a calculated blank value (Stark and Hart, 1996). The low amount of N in filter paper disks (5-10 μg) may decrease the precision of measured ^{15}N enrichment because $> 20 \mu\text{g}$ N is usually recommended for analysis by diffusion techniques and direct combustion-mass spectrometry (Stark, In press). Consequently, we may attribute the negative values of gross rates partially to the low amount of N in the filter paper disks analyzed.

We injected $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ at 2 mg N kg^{-1} dry soil. Because the background levels of NH_4^+ and NO_3^- were very low except for those in soils amended with high-rate compost (Table 5.2), the addition of ^{15}N increased the N pool size by a factor of 2-3 for NH_4^+ and 5-8 for NO_3^- . Therefore, N consumption rates may be overestimated due to the addition of substrates. Microbial NH_4^+ immobilization exceeded the gross N mineralization rates except in soil treated with high-rate compost (Table 5.2), while microbial NO_3^- immobilization did not occur (Fig. 5.2). As mentioned above, microbial N immobilization may be limited by C availability. Even with the high level of inorganic N, microbial N immobilization was still low. Hence, the relationship between the added N amounts and consumption rates is not clear.

Conclusions

Silage corn removed more N from soil that received the high-rate N fertilization with compost and ammonium sulfate, leading to significantly high N contents in ear leaves and in aboveground plants, while silage corn yields were not different from those with low-rate N fertilization. Instead, application of compost at the high rate resulted in a large amount of NO_3^- remaining in the soil after harvest. The high concentration of

NO_3^- in the soil profile may suggest that the appropriate application rate is the low rate of compost (50 Mg dry wt. ha^{-1}) evaluated in this study. Microbial NO_3^- immobilization was not observed in this agricultural soil regardless of N fertilizer treatments, suggesting that NO_3^- assimilation was limited by low C availability. Higher C mineralization rates were associated with higher N mineralization and subsequent nitrification rates. In soils treated with compost at a high rate, N mineralization and subsequent nitrification exceeded NO_3^- consumption by soil microorganisms and plants, resulting in the accumulation of soil NO_3^- .

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CHAPTER 6

EFFECT OF LONG-TERM, BIENNIAL, FALL-APPLIED ANHYDROUS
AMMONIA AND NITRAPYRIN ON SOIL NITRIFICATION

Abstract

Long-term dryland wheat plots were established in northwestern Utah and maintained for 8 years in a 2-year wheat-fallow rotation. Nitrapyrin was applied with anhydrous ammonia (NH_3) in the fall preceding wheat growth to retard nitrification. Our objective was to determine the effects of long-term, biennial application of anhydrous NH_3 with and without nitrapyrin on soil nitrification. We were particularly interested in the potential residual effects of the long-term repeated applications of anhydrous NH_3 and nitrapyrin. Nitrification potentials were measured in control soil, or soil fertilized with anhydrous NH_3 with or without nitrapyrin for both rotation phases. Nitrification potentials were higher in soils receiving anhydrous NH_3 than in the control (no added N) soils during the cropped rotation. Nitrification potentials in soils receiving anhydrous NH_3 with nitrapyrin were similar to those of the control soils during the entire wheat-fallow rotation period. Further, nitrification potentials in soils with a history of nitrapyrin use were significantly lower than in soils without nitrapyrin use when measured after 2 years. We observed a transient increase in nitrification potentials with the application of anhydrous NH_3 that did not last in the fallow year, suggesting that the long-term, biennial application of anhydrous NH_3 had no detectable residual effect on soil

nitrification. In contrast, our results suggest that the long-term, biennial application of nitrapyrin did have a residual effect on soil nitrification which lasted at least 2 years.

Introduction

Ammonium-based N fertilizers combined with nitrification inhibitors are commonly applied to winter wheat in the fall. Anhydrous NH_3 , a major N fertilizer source, has been widely applied with nitrapyrin, a nitrification inhibitor, in winter wheat in the northwest region, USA (Papendick and Engibous, 1980). Nitrapyrin retards nitrification (Keeney, 1986), and thus the applied NH_4^+ -based N will be retained in NH_4^+ form, which is less susceptible to loss by leaching or denitrification than NO_3^- . Therefore, it is expected that N fertilizer use efficiency and crop yields will be increased for systems treated with nitrapyrin. The potential for long-term, repeated use of anhydrous NH_3 with nitrapyrin to have a residual effect on soil nitrification has not previously been investigated.

Since nitrapyrin was first introduced in 1962 by C.A.I. Goring of The Dow Chemical Company, its inhibition of nitrification has been extensively tested in laboratory and field experiments (Briggs, 1975; Gomes and Loyanachan, 1984; Powell and Prosser, 1986; Sahrawat et al., 1987; McCarty and Bremner, 1990; Walters and Malzer, 1990). Factors affecting the efficacy of nitrapyrin and other nitrification inhibitors have been reviewed by Keeney (1980; 1986). The general belief about nitrapyrin and other nitrification inhibitors is that their inhibition of nitrification is short term, usually lasting for a few days to a few months (Briggs, 1975; Gomes and Loyanachan, 1984; Malhi and

Nyborg, 1988; McCarty and Bremner, 1990; Glasscock et al., 1995; Rochester et al., 1996). The functional period of nitrapyrin depends on its bioactivity and persistence in soil; these are related to soil type, organic matter content, temperature, moisture, and soil management practice (Keeney, 1980; 1986). Once nitrification inhibitors are degraded, the nitrification rate may recover. Since the persistence and the efficacy of nitrification inhibitors are interrelated, the degradation of nitrification inhibitors has also been studied. The half-life of nitrapyrin was reported ranged from less than 2 weeks to 13 weeks (Keeney, 1986). In contrast to the accepted concept, Klemetsson and Mosier (1994) reported that long-term exposure of soil to acetylene, a nitrification inhibitor, had a long-lasting effect on soil nitrification; soil nitrification potential was lower than that of the control soil even one year later after a long-term exposure to acetylene.

Autotrophic ammonium oxidizers get their metabolic energy solely from the oxidation of NH_4^+ to NO_2^- . Nitrification rate and nitrifier populations respond to NH_4^+ -N availability (Belser, 1979). The short-term effect of NH_4^+ substrate concentration on increased nitrification rate and nitrifier populations has been studied in the laboratory (Darrah et al., 1985; Nishio and Fujimoto, 1990). However, relatively few studies (Eaton and Patriquin, 1988; Biederbeck et al., 1996) have documented the residual effect of long-term application of NH_4^+ -based fertilizers on soil nitrification. We have used a long-term dryland wheat experiment to investigate the residual effect of the repeated use of anhydrous NH_3 and nitrapyrin on soil nitrification.

Generally, NO_3^- or NH_4^+ pool sizes are used to evaluate the effects and efficacy

of nitrification inhibitors. The assumption is that if nitrification inhibitors block nitrification, the NH_4^+ pool size will be larger or the NO_3^- pool size will be smaller in soils treated with nitrification inhibitors than in those without nitrification inhibitors. Therefore, two general indices to evaluate nitrification inhibitors are 1) the percentage of difference of NH_4^+ - or NO_3^- -N pool size between soils with or without a nitrification inhibitor in relation to the NH_4^+ - or NO_3^- -N pool size of the respective control soil (McCarty and Bremner, 1990; Goos and Johnson, 1992), and 2) the recovery of applied NH_4^+ -N in soil (Gomes and Loyanachan, 1984; Zourarakis and Killom, 1990). However, we cannot differentiate the effect of NH_4^+ substrate concentration from that of changes in the nitrifier population by measuring the NH_4^+ - or NO_3^- -N pool size after the long term. Long-term residual effects of anhydrous NH_3 and nitrapyrin on soil nitrifiers need to be investigated by isolating the effect of NH_4^+ substrate concentration. In this study, we used nitrification potential as an index to evaluate a long-term residual effect of anhydrous NH_3 and nitrapyrin on soil nitrification.

The aim of this study was to test if a long-term (8 years), biennial, fall-applied anhydrous NH_3 and nitrapyrin has a residual effect on soil nitrification. We compared soils that were untreated (control) and treated with anhydrous NH_3 or anhydrous NH_3 plus nitrapyrin. The NH_4^+ - and NO_3^- -N pool sizes were used to evaluate short-term effects of anhydrous NH_3 and nitrapyrin. Nitrification potentials and nitrifier sensitivity to nitrapyrin were used to evaluate long-term, residual effects of anhydrous NH_3 and nitrapyrin.

Materials and Methods

Experimental Site

The experimental site was located at the Blue Creek Farm of Utah State University in northwestern Utah. The soil is Timpanogos silt loam (fine-loamy, mixed, superactive, mesic Calcic Argixeroll). Average annual precipitation was 15 inches. Average annual temperature was 7.4 °C (Utah Climate Center, personal communication).

Experimental Design

The experiment was set up in the late 1980's for testing the effects of fall-applied anhydrous NH_3 with nitrapyrin on winter wheat yields. The experiment involved a two-year wheat-fallow rotation and was carried out in two fields that were cropped in alternative years. Each field involved 14 treatments, which were arranged as a randomized complete block design with two replications. We sampled the soil from the following treatments: 1) Control, without application of anhydrous NH_3 and nitrapyrin; 2) 50 A, 50 kg N ha^{-1} of anhydrous NH_3 ; 3) 50AN, 50 kg N ha^{-1} of anhydrous NH_3 plus 0.56 kg nitrapyrin ha^{-1} ; and 4) 70AN, 70 kg N ha^{-1} of anhydrous NH_3 plus 0.56 kg nitrapyrin ha^{-1} . The treatment 70AN was changed to 70A (70 kg N ha^{-1} of anhydrous NH_3) in fall 1994. The plots for each treatment were 4 m wide and 180 m long. Anhydrous NH_3 with or without nitrapyrin was contained in a pressurized tank and injected in bands 30 cm apart and 8-10 cm deep to soil by an applicator equipped with

banding knife shanks. During each cropping year, soil was tilled three or four times to less than 15 cm depth.

Soil and Plant Sampling

The study was conducted in the fields from 1995 to 1997. The dates of fertilization, planting, harvesting, and soil and plant samplings are given in Table 6.1. The plots were divided into four subplots along their length with each about 90 m long to stratify sampling. The soil was collected by coring (5 cm dia.) from both 0-15 cm and 15-30 cm depths in each subplot. Wheat leaves for nitrate reductase measurements were also collected from each subplot.

Analysis Methods

Samples were kept on ice until processing later that day. Soil inorganic N was extracted with 2 M KCl (1:5 soil wt.:KCl vol.) and shaken for 1 h. The extracts were

Table 6.1. Information on dates of fertilization, sowing, harvesting, and sampling in dryland wheat fields of the Blue Creek farm.

Field	Fall'95-Summer'96		Fall'96-Summer'97		Fall'97 - Summer'98	
	I	II	I	II	I	II
Rotation phase	Cropped	Fallow	Fallow	Cropped	Cropped	Fallow
Fertilization	9/14/95			9/2/96	9/5/97	
Sowing	9/20/95			9/27/96	9/9/97	
Harvesting	7/31/96			8/19/97		
Soil sampling	9/29/95		10/3/96	10/1/96	9/29/97	9/29/97
			4/18/97	4/17/97		
			7/1/97	7/2/97		
Wheat sampling				4/15/97		

filtered through pre-rinsed Whatman #1 filter paper. The filtrates were frozen until analysis for inorganic N by colorimetric analysis (Lachat Flow Autoanalyzer, QuikChem Systems, 1992; 1993).

Nitrification potentials were measured by the soil shaken slurry method (Hart et al., 1994). Fresh soils were sieved (< 2 mm) and 15-g moist soils were weighed into 250-ml flasks. The flasks were added with 100-ml phosphate buffer and continuously shaken for 24 h at a high speed (200 rpm) (Stark, 1996). Ten-ml aliquots were sampled at 2, 4, 22, and 24 h and centrifuged at 8,000 g for 10 minutes. The $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ in the liquid was analyzed colorimetrically as described above. Soil nitrification potential was expressed on soil dry weight basis.

Nitrifier sensitivity to nitrapyrin was determined by a modified nitrification potential method. The soils were sampled from 0-15 cm depth on October 2, 1996, from the 50A and 50AN treatments in the fallow field. After the shaken soil slurries were sampled at 3, 6, and 18 h, different concentrations of nitrapyrin at 0, 0.1, 0.2, 0.5, and 1.0 mg kg⁻¹ were added to the individual flasks. Soil slurries were then sampled at 22, 27, 36, and 48 h. The $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ in soil slurries was analyzed by the method described as above.

Ammonium oxidation kinetics were determined by a modified nitrification potential method. Ammonium N at 0, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, or 2.0 mM in 100-ml phosphate buffer (Hart et al., 1994) was added to 250-ml flasks that contained 15-g fresh soils. Initial soil NH_4^+ expressed as mg N kg⁻¹ soil was converted to mM and summed to the NH_4^+ concentration in 100 ml buffer. The measured nitrification rates at different

NH_4^+ concentrations were fit to the nonlinear regression of the Michaelis-Menten equation (SigmaPlot 3.0, Jandel Scientific, 1995) for determining the apparent V_{max} (maximum nitrification rate, i.e., nitrification potential) and apparent K_m (Michaelis-Menten rate constant).

Nitrate reductase activity (NRA) of wheat leaves was measured by the *in vivo* method (Jaworski, 1971). The wheat leaves were cut to about 0.5 cm long. About 0.4-1.0 g fresh wt. wheat leaves were added to 20 ml reaction medium containing NO_3^- in vials and incubated in the dark for 2.5 h at about 23 °C. Then reduced NO_2^- -N was analyzed colorimetrically.

Degradation of nitrapyrin was measured in a laboratory incubation experiment. Composited soil was collected from the control plots of the fallow field on October 2, 1996. Ten-gram moist soils were placed into 20-ml vials, and 20 mg kg^{-1} nitrapyrin in emulsion was injected into the soil. Soils were incubated at 18 °C and soil moisture was adjusted to 10% every week. Three vials were withdrawn randomly at 0, 2, 7, 14, 30, 47, 64, and 93 days. The nitrapyrin was extracted using a solution containing 10 ml water, 1 g sodium sulfate, and 5 ml hexane. The nitrapyrin dissolved in hexane layer was determined by absorbance at 270 nm (Bremner et al., 1978). The measured nitrapyrin concentrations were fit to the exponential model, $\text{NI} = \text{NI}_0 e^{-kt}$, where NI_0 is initial nitrapyrin concentration, NI is nitrapyrin concentration at time t , k is the decomposition rate constant (Keeney, 1980). We used the nonlinear regression program (see above) to fit the data. The half-life of nitrapyrin was calculated from the equation $t_{1/2} = -k^{-1} \times \ln 0.5$.

The pH of soil shaken slurry in nitrification potential assay was measured for

convenience. Soil pH (1:2 H₂O) was measured only for soils sampled from the cropped field on October 2, 1996. The pH of soil shaken slurry was simply regressed with soil pH (1:2 H₂O).

Statistical Analysis

Inorganic N pool sizes, nitrification potentials, and pH of soil shaken slurry in different fields, blocks, treatments, sampling locations, soil depths and sampling times were statistically analyzed by a nested multiple split plot design, in which blocks were nested in the fields, treatments were the main plot, while sampling locations, soil depths and sampling times were multiple subplots.

The patterns of NO₃⁻-N accumulation with time in nitrapyrin sensitivity analyses were statistically analyzed by a multiple split plot design with treatments as the main plot, concentrations as the subplot, and sampling times as the sub-subplot.

The patterns of nitrification rates in NH₄⁺ oxidation kinetics analysis were analyzed by a split-plot design with treatment as main plot and NH₄⁺-N concentrations as subplot. The parameters of V_{max} and K_m were compared using t-values calculated from the best fit values and standard errors by the method of Motulsky (1996). See Appendix E for the details of statistical analysis.

Results

Soil NH₄⁺-N Pool Sizes as Affected by the Application of Anhydrous NH₃ and Nitrapyrin

Generally, NH₄⁺-N pool sizes were larger in soil fertilized with 50A or 50AN

than those of control soil ($p = 0.05$); this difference was only significant at the 0-15 cm depth. Fig. 6.1 shows the dynamic patterns of NH_4^+ -N pool sizes among the differently fertilized soils in the fallow and the cropped fields. The NH_4^+ -N pool size in the control soil was consistently small ($< 1 \text{ mg N kg}^{-1}$ soil) throughout all soil sampling dates, while the NH_4^+ -N pool size in the soil fertilized with anhydrous NH_3 significantly fluctuated with the soil sampling dates. The highest NH_4^+ -N concentrations were observed in the fall close to the application of anhydrous NH_3 , and then soil NH_4^+ -N concentrations decreased to the level of the control soil in the next spring and was maintained at that low level thereafter. However, NH_4^+ -N applied combined with nitrapyrin was significantly retained until the next spring.

Soil NO_3^- -N Pool Sizes as Affected by the Application of Anhydrous NH_3 and Nitrapyrin

In general, NO_3^- -N pool sizes were larger in the soil fertilized with 50A or 50AN than those of the control soil ($p = 0.05$). The difference of NO_3^- -N pool sizes between the fertilized and the unfertilized soils occurred at the 0-15 cm depth (Fig. 6.2). Generally, this difference was not observed at 15-30 cm depth, except that in the spring, in the cropped field, NO_3^- -N concentration was significantly higher in soil treated with 50A (6.4 mg N/kg soil) than in the control soil (2.6 mg N/kg soil). In contrast to the soil NH_4^+ -N, NO_3^- -N pool sizes in the control soil, like the soil fertilized with 50A or 50AN, significantly fluctuated with the sampling dates. In the cropped field, soil NO_3^- -N pool sizes were largest in the fall, then decreased to the smallest in the next summer. After wheat harvesting, soil NO_3^- -N pool sizes increased again. However, NO_3^- -N pool sizes

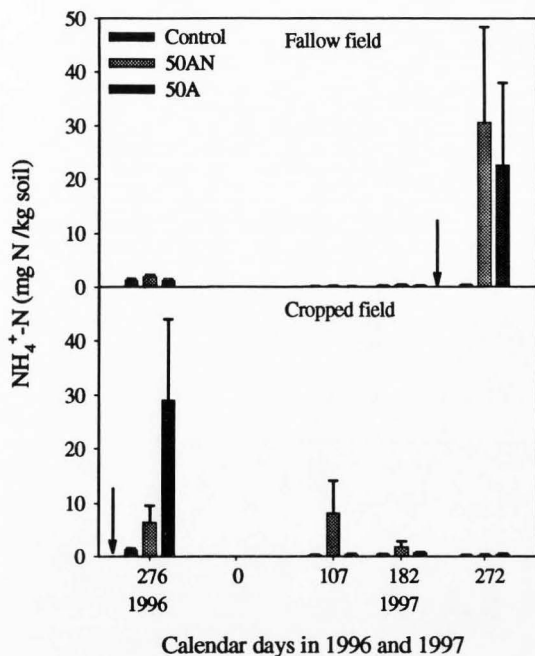


Fig. 6.1. Time course of $\text{NH}_4^+\text{-N}$ pool sizes at 0-15 cm soil depth in the control soil (Control), the soil fertilized with anhydrous NH_3 plus nitrapyrin (50AN), and the soil fertilized with anhydrous NH_3 (50A). Values are means and standard errors for $n = 8$. Arrows indicate the application time of anhydrous NH_3 and nitrapyrin.

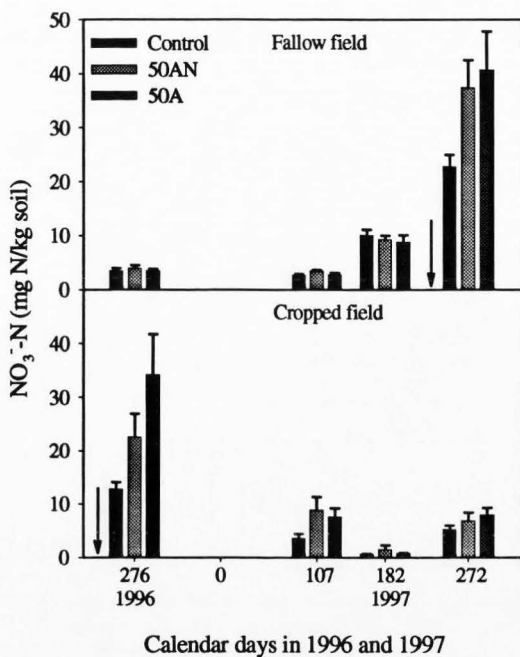


Fig. 6.2. Time course of $\text{NO}_3^- \text{-N}$ pool sizes at 0-15 cm soil depth in the control soil (Control), the soil fertilized with anhydrous NH_3 plus nitrapyrin (50AN), and the soil fertilized with anhydrous NH_3 (50A). Values are means and standard errors for $n = 8$. Arrows indicate the application time of anhydrous NH_3 and nitrapyrin.

of soil amended with nitrapyrin were not different from those without nitrapyrin. The NRA in wheat leaves ($=1.6 \mu\text{mol NO}_2^- \text{g}^{-1} \text{fresh wt hour}^{-1}$) was not significantly different among the three soil treatments.

Soil Nitrification Potentials as Affected by the Application of Anhydrous NH_3 and Nitrapyrin

Soil nitrification potentials were significantly higher at 0-15 cm soil depth than at 15-30 cm soil depth ($p = 0.01$) in both fallow and cropped fields. Anhydrous NH_3 and nitrapyrin effects on soil nitrification potentials were only observed at the 0-15 cm depth (Fig. 6.3). Fluctuations of soil nitrification potentials with time also occurred at 0-15 cm soil depth. Dynamic patterns of the nitrification potentials of the differently fertilized soils are shown in Fig. 6.4. In the cropped field, the highest nitrification potentials occurred in July and the lowest ones were in September after harvesting. The soils fertilized with 50A had the highest nitrification potentials for each sampling date when compared to the control soil or the soil fertilized with 50AN. In the fallow field, the nitrification potentials in soils fertilized with 50A one year before (October 2, 1996, sampling date) were still higher than those of the control soil or the soil fertilized with 50AN. Thereafter, nitrification potentials in the 50A treatment decreased to the level of the control soil and were maintained at that level until fall 1997 when anhydrous NH_3 was applied again. In contrast, nitrification potentials in the control soil or the soil fertilized with 50AN had smaller fluctuations with time; nitrification potentials of soil fertilized with 50AN were not significantly different from those of control soil at all the sampling dates.

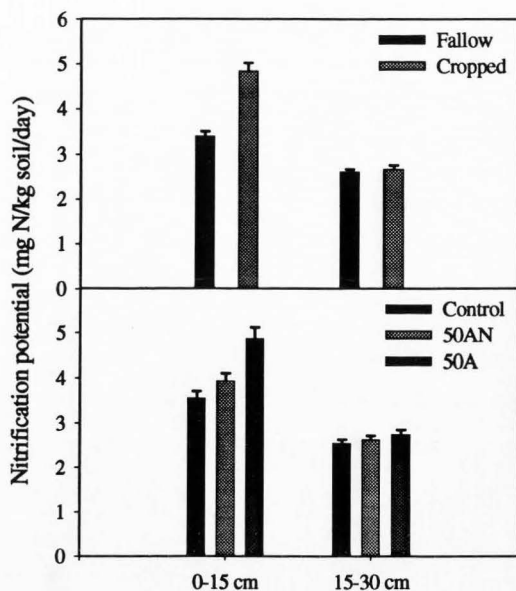


Fig. 6.3. Soil nitrification potentials at 0-15 cm and 15-30 cm soil depths. Nitrification potentials of the fallow field were compared with those of the cropped field by averaging the four sampling dates and the three soil treatments; values are means and standard errors for $n = 96$. Nitrification potentials were compared among the three soil treatments by averaging four sampling dates and two fields, values are means and standard errors for $n = 32$.

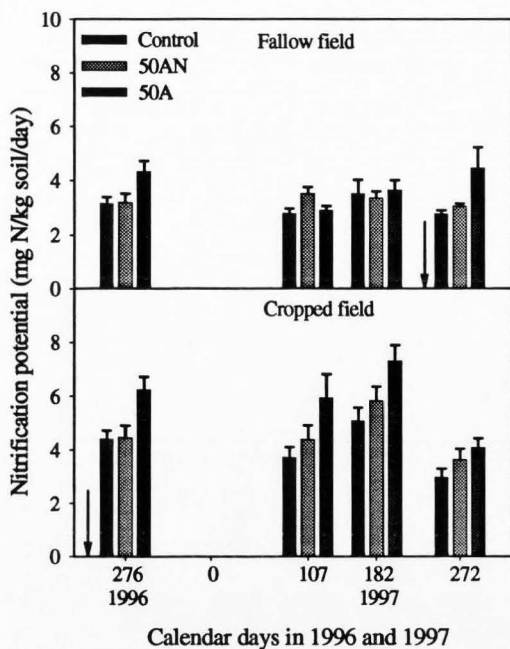


Fig. 6.4. Time course of nitrification potentials at 0-15 cm soil depth in the control soil (Control), the soil fertilized with anhydrous NH_3 and nitrapyrin (50AN), and the soil fertilized with anhydrous NH_3 (50A). Values are means and standard errors for $n = 8$. Arrows indicate the application time of anhydrous NH_3 and nitrapyrin.

Nitrifiers in soils that differed in their history of nitrapyrin use responded to the applied NH_4^+ -N differently (Table 6.2). Nitrification potential was significantly higher in the soil without a history of nitrapyrin use than in the soil with nitrapyrin use 2 years before, while it was similar to that of soil with nitrapyrin use 3 or 4 years before.

Soil Nitrifier Sensitivity to Nitrapyrin

In the soil slurry assay, the NO_3^- -N accumulation patterns in the presence of varying amounts of nitrapyrin were similar for soils fertilized with 50 AN or with 50A (Fig. 6.5). Soil nitrification was partially inhibited by nitrapyrin addition at 0.1 mg kg^{-1} soil, and completely inhibited by nitrapyrin addition at 1.0 mg kg^{-1} soil.

Soil Nitrifier Michaelis-Menten Kinetics as Affected by Anhydrous NH_3 and Nitrapyrin

Nitrate N accumulation patterns with a series of NH_4^+ concentrations from soil shaken slurry were marginally ($p = 0.10$) different among the three soil treatments. The highest nitrification rate was observed at NH_4^+ -N concentrations ranged from 0.8 to 1

Table 6.2. Response of nitrifier population in the soils with or without a history of nitrapyrin (NI) use to anhydrous ammonia (NH_3) applied most recently.

Years from NI use to soil sampling	Months from NH_3 use to soil sampling	Nitrification potential ($\text{mg N kg}^{-1} \text{ soil day}^{-1}$)	
		With NI-use history	No NI-use history
2	1	3.3 (0.4) a [†]	5.8 (1.6) b
3	12	3.7 (0.3) a	4.3 (0.4) a
4	1	6.1 (0.8) a	6.2 (0.5) a

[†] Values followed by the different letters in the same row indicate the significant difference at $p < 0.05$.

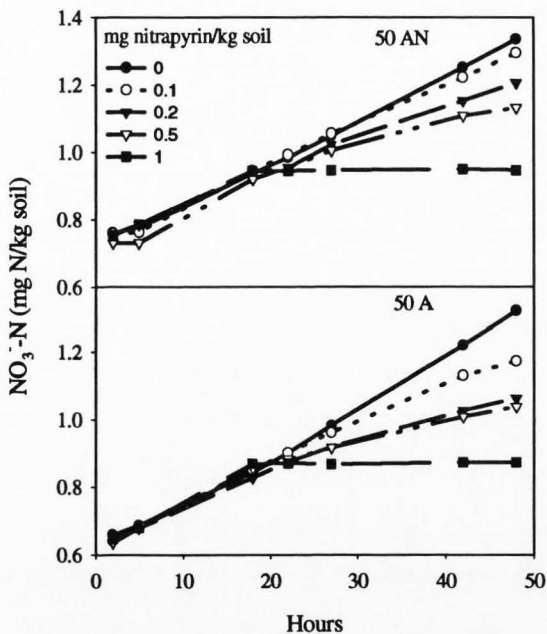


Fig. 6.5. The response of nitrifiers in the soil fertilized with anhydrous NH₃ plus nitrapyrin (50AN) or the soil fertilized with anhydrous NH₃ (50A) to the fresh addition of nitrapyrin at different concentrations.

mM. When $\text{NH}_4^+\text{-N}$ concentration was above 2 mM, nitrification rate decreased.

Michaelis-Menten kinetic parameters are given in Table 6.3. Nitrification potential (V_{\max}) was significantly higher in the soil fertilized with 50A than in the control soil or the soil fertilized with 50AN, while nitrifier affinities to NH_4^+ (K_m) were similar for the three soil treatments.

Discussion

Nitrification inhibitors are used with fall-applied NH_4^+ -based fertilizers for preventing N loss from late fall to early spring of next year, since fall-applied NH_4^+ may be transformed to NO_3^- by nitrifiers during this period (Malhi and McGill, 1982; Malhi and Nyborg, 1988). Our data (Fig. 6.1) showed that the applied NH_4^+ was rapidly transformed to NO_3^- in soil without the use of nitrapyrin from September to April, although it has previously been thought that the soil temperature in this region would not

Table 6.3. Kinetic parameters of nitrification in the control soil (Control), the soil fertilized with anhydrous NH_3 (50A), and the soil fertilized with anhydrous NH_3 plus nitrapyrin (50AN).

Soils	V_{\max} mg N kg ⁻¹ day ⁻¹	SE $_{V_{\max}}$ [†]	K_m mM	SE $_{K_m}$ [‡]	R [§]
Control	2.75 a [¶]	0.02	0.012 a	0.001	0.983
50A	3.33 b	0.08	0.019 a	0.004	0.938
50AN	2.78 a	0.07	0.010 a	0.003	0.833

[†] Standard error of V_{\max} .

[‡] Standard error of K_m .

[§] For nonlinear regression.

[¶] Values followed by the different letters in the same column indicate the significant difference at $p < 0.05$.

be favorable to nitrifier activity during this period (Papendick and Engibous, 1980).

Gomes and Loynachan (1984) suggested that NH_4^+ -based fertilizers should be applied in late fall when soil temperature was below 10 °C, because nitrification may proceed rapidly at soil temperature above 10 °C. The complete transformation of NH_4^+ to NO_3^- from September to April at our site may be the result of slow nitrification during winter and rapid nitrification in late fall or early spring.

Nitrapyrin successfully blocked nitrification from late fall to early spring, and the applied NH_4^+ was significantly retained in soil until next spring (Fig. 6.1). This short-term effect of nitrapyrin on soil nitrification was consistent with the field work of other researchers (Gomes and Loynachan, 1984; Malhi and Nyborg, 1988; Rao, 1996). However, NO_3^- -N pool size in soil fertilized with anhydrous NH_3 but with or without nitrapyrin was not different (Fig. 6.2). Wheat leaf NRA from the three soil treatments had no difference, either. Nitrate reductase is a substrate-inducible enzyme and its activity is sensitive and responsive to NO_3^- -N availability (Hall et al., 1990). Lodhi and Ruess (1988) indicated that NRA was a reliable index to soil mineral N status. Two studies (Barro et al., 1991; Stancheva and Dinev, 1995) showed that NRA was higher in wheat leaves grown in soil with NO_3^- supply versus NH_4^+ supply. In our study, wheat leaf NRA did not differ for the different soil treatments, further indicating the similarity of the NO_3^- pool sizes from the three soil treatments. Leaching of NO_3^- -N may be an explanation for this observation, since NO_3^- -N rapidly decreased from late fall to early spring when N uptake by winter wheat would likely be low. Papendick and Engibous (1980) indicated that drier upper soil layers in fall would favor water penetration, and

extensive leaching of NO_3^- -N might occur during winter. Nitrate N pool size in 15-30 cm depth in soil fertilized with anhydrous NH_3 was higher than the control soil, which may further indicate the occurrence of downward movement of NO_3^- -N.

Application of NH_4^+ will increase nitrification rate and nitrifier activity under the conditions of NH_4^+ limitation (Belser, 1979). The enhancement of nitrification by NH_4^+ has been reported in agricultural soils (Berg and Rosswall, 1985). In our study, the short-term effect of NH_4^+ on soil nitrification was obvious in the 0-15 cm soil depth where anhydrous NH_3 was placed (Fig. 6.1, Fig. 6.4). However, we did not observe a residual effect of repeated, biennial application of anhydrous NH_3 on soil nitrification. Nitrification potential is an index of active nitrifier population size (Belser, 1979). The established higher active nitrifier population by application of anhydrous NH_3 was not maintained in soil (Fig. 6.4). During the fallow period, the enhanced nitrifier activity decreased to that of the control soil. Davidson et al. (1996) reported that intensive repeated use of NH_4^+ -based N early in a single cropping season increased soil nitrifier activity, and this activity remained high even without further N fertilization. The residual effect of a 10-year, annual application of anhydrous NH_3 on soil nitrification was documented by Biederbeck et al. (1996). In their study, they found that the nitrifier populations were higher in the soil receiving anhydrous NH_3 (45 kg N ha^{-1}) than in the control soil until the next year's fertilization. Our observation that there was no residual effect of anhydrous NH_3 on soil nitrification may be due to the infrequent use of anhydrous NH_3 with every second year.

In contrast, repeated, biennial application of nitrapyrin had a residual effect on soil nitrification. Nitrification potential in soil fertilized with both anhydrous NH_3 and nitrapyrin was similar to that of the control soil through both cropped and fallow rotation phases (Fig. 6.4). Even without further use of nitrapyrin, nitrification potential was still lower in soil with a history of nitrapyrin use than in soil without this history (Table 6.2). However, this residual effect is not irreversible. Soil nitrifiers can finally recover after 3 or 4 years without nitrapyrin application (Table 6.2). Belser and Schmidt (1981) indicated that nitrifier communities had different sensitivities to nitrapyrin. They suggested that long-term repeated use of nitrapyrin might select for less sensitive strains. Our data (Fig. 6.5) showed that the dominant strains of nitrifiers in soils that received nitrapyrin and those that did not, had similar sensitivity to nitrapyrin. Therefore, the residual effect of nitrapyrin is not explained by changes in nitrifier sensitivity alone. The parameters of Michaelis-Menten kinetics also indicated that the residual effect depended on the differences in the active nitrifier populations (V_{\max}).

The degradation of nitrapyrin in this soil followed the exponential model of NI ($\text{mg kg}^{-1} \text{ soil}$) = $20.8e^{-0.016t}$. The half-life of nitrapyrin was calculated as 41 days, which is in the range previously reported (Keeney, 1980; 1986). With this high degradation rate, we do not expect that nitrapyrin itself stays in the soil in an amount high enough to directly block nitrification. Biederbeck et al. (1996) showed that a long-term, repeated application of anhydrous NH_3 decreased soil pH, which influenced on nitrifier activity. In our study, the repeated use of anhydrous ammonia did decrease soil pH to 6.9 when compared to the control soil of pH 7.0. However, the pH of the differently fertilized

soils was still near neutral, which should not significantly influence the nitrifier population activity.

Conclusions

Application of nitrapyrin with anhydrous ammonia in fall successfully retained applied NH_4^+ in soil until next spring. A long-term, biennial application of nitrapyrin had a residual effect on soil nitrification. After anhydrous ammonia was applied to soil, nitrification potential in soil with a history of nitrapyrin use was lower than in soil without this history. However, this effect is not irreversible; nitrification potentials recovered after 3 or 4 years without the use of nitrapyrin. In contrast, in our system, the long-term, biennial application of anhydrous ammonia had no residual effect on soil nitrification.

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CHAPTER 7

CONCLUSIONS

Despite the numerous studies on soil autotrophic nitrification, the control of microbial NO_3^- production in agricultural soils amended with animal wastes or nitrification inhibitors remains an important area for future progress. This dissertation contributes to the understanding of microbial NO_3^- production in relation to NH_4^+ availability and nitrifier population activity in agricultural soils after application of animal wastes or nitrification inhibitors. In our examination of microbial NO_3^- production in agricultural soils, this dissertation has differed from other studies in three areas. They are 1) comparison of N mineralization potentials in soil receiving differently treated dairy wastes; 2) simultaneous measurements of multiple gross rates of N mineralization, nitrification, and microbial N immobilization; and 3) effects of a long-term repeated use of nitrapyrin on soil nitrifier population activity.

Application rates and timing of dairy waste depend on the amount and rate of N mineralization. Few studies have assessed the N mineralization potential of dairy-waste compost, while even fewer have reported on the N mineralization potential of dairy waste digested in an anaerobic lagoon. We are not aware of any previous studies comparing N mineralization potentials in soils receiving composted or anaerobically digested dairy waste. In this project, N mineralization potentials in soils receiving the two types of dairy wastes were evaluated and compared.

Nitrogen mineralized from dairy waste depends on its quality and quantity. Variously treated dairy wastes may differ in their chemical, physical, or biological

properties. As a result, soils receiving these wastes may differ in their N availability. Windrow composting is one technique for treating dairy waste. Different aeration and moisture regimes constitute intensive or nonintensive composting. Dairy waste treated with frequent turning and watering (intensive composting) had the highest N mineralization potential in comparison to other treated compost (less intensive composting). Approximately 5% of the organic N in intensively managed dairy-waste compost was easily mineralized. Anaerobic lagoon digestion is another technique to collect and stabilize dairy waste. In contrast to dairy-waste compost, up to 90% of the organic N in the dairy waste digested in an anaerobic lagoon was mineralized. The different N mineralization potentials between the two types of dairy wastes suggest that the dairy waste digested in an anaerobic lagoon acted like a mineral N fertilizer that can quickly provide plant-available N, whereas dairy-waste compost was a slow-releasing organic N fertilizer. Thus, we recommend that dairy-waste anaerobic lagoon effluent may better be applied during the growing season, while dairy-waste compost should be applied before the growing season to allow enough time for N mineralization.

Net N process rates, which are determined by the changes of inorganic N pool sizes over time, confound the N processes of production and consumption. Even under conditions without NH_3 volatilization, plant N uptake, NO_3^- leaching, or denitrification, net rates may still confound the gross N production with microbial N consumption. Nitrogen management practices that use organic versus inorganic N sources and different N application rates may have various effects on gross N production and microbial N

consumption. As a consequence, gross rates may provide more detailed information than net rates for developing environmentally sound N management.

Gross rates of N mineralization, nitrification, and microbial N immobilization were determined by ^{15}N isotope dilution techniques in the laboratory and field experiments. In both experiments, microbial NO_3^- immobilization did not occur even in soil amended with dairy-waste compost or dairy-waste liquid. Low C availability is possibly the cause. No microbial NO_3^- immobilization in our system suggests that soil NO_3^- concentration is primarily controlled by nitrification. The extent of nitrification depends on NH_4^+ availability and nitrifier population activity. Without direct mineral NH_4^+ fertilization, N mineralization provided the NH_4^+ available to soil nitrifiers, and therefore controlled the subsequent nitrification. When mineral NH_4^+ was applied at 50 mg N kg^{-1} soil, however, it became the primary source of NH_4^+ available to soil nitrifiers for a period of 70 days. Therefore, the NH_4^+ supplied from this mineral N fertilization controlled the nitrification rates. Nitrification rates and potentials were higher in soil receiving the mineral NH_4^+ fertilizer than in soil receiving the dairy waste.

Gross N process rates have also been affected by the application rates of N fertilizers or animal wastes. We have determined nitrification rates and potentials in a corn field amended with ammonium sulfate, dairy-waste compost, and dairy-waste liquid at two application rates. High-rate N fertilizers increased nitrifier population activity. We have found that soil amended with high-rate compost (100 Mg dry wt. ha^{-1}) had the highest gross N mineralization rates (1.6 mg N kg^{-1} soil day^{-1}) and gross nitrification rates (2.9 mg N kg^{-1} soil day^{-1}). High NO_3^- concentrations were only observed in soils

receiving the high-rate compost, which indicates that N provided from the high-rate compost exceeded the N requirement of corn. Therefore, we recommend that the appropriate application rate of dairy-waste compost is the low rate (50 Mg dry wt. ha⁻¹) evaluated in this study.

The effect of nitrapyrin on soil nitrification has generally been evaluated by indices in relation to inorganic N pool sizes. However, inorganic N pool sizes are the confounding result of many N processes. Because we have not clearly understood the effect of nitrapyrin on other N processes, it may be unsuitable to use only inorganic N pool sizes for evaluating effect of nitrapyrin, especially after long-term repeated use. Nitrapyrin inactivates an essential enzyme required for the NH₄⁺ oxidation, thereby decreasing nitrifier populations. In this study, we used nitrification potential to evaluate a long-term, repeated use of nitrapyrin on soil nitrification.

Nitrifiers did not responded to the NH₄⁺ when nitrapyrin was simultaneously applied. Contrary to the accepted belief that nitrapyrin has only a short-term effect on soil nitrification, we have demonstrated that a long-term, biennial application of nitrapyrin did have a residual effect on soil nitrifier population activity. Nitrifier population activity was lower in soil with a history of nitrapyrin use than in soil without nitrapyrin use.

In conclusion, the application of dairy wastes and the long-term repeated use of nitrapyrin did have impacts on soil NH₄⁺ availability and nitrifier population activity, the two critical factors in controlling microbial NO₃⁻ production. The effect of dairy wastes on soil NH₄⁺ availability depends on the treatment systems of dairy wastes and their

application rates. In comparison to mineral NH_4^+ fertilization, dairy wastes can significantly decrease nitrification rates, potentials, and soil NO_3^- concentrations when the application rates are appropriate. However, even stabilized dairy-waste compost may lead to high nitrification rates and elevate soil NO_3^- concentrations when it is applied at an excessive rate, i.e., $100 \text{ Mg dry wt. ha}^{-1}$ evaluated in this study. We have demonstrated that long-term repeated use of nitrapyrin had a residual effect on soil nitrifier populations. Nitrifier population activity was significantly lower in soil with a history of nitrapyrin use than in soil without nitrapyrin use.

Long-term repeated use of dairy wastes and use of nitrapyrin with dairy wastes have been practiced in agriculture. We only investigated the soils with one-time use of dairy wastes or use of nitrapyrin with a mineral NH_4^+ fertilizer. Whether or not the conclusions in our studies can extend to the fields with long-term repeated applications of dairy wastes or with the use of nitrapyrin and dairy wastes combined needs to be demonstrated. The recommended future research includes the following two areas. Firstly, effects of dairy wastes on soil microbial NO_3^- production and consumption need to be assessed in soils with a long-term repeated use of dairy wastes. We are interested in the relationship of microbial NO_3^- production and consumption in regulating soil NO_3^- concentrations, the competition of nitrifiers and heterotrophs to soil NH_4^+ , and the application rates of dairy wastes for benefiting crop yields but without excessive soil NO_3^- accumulation. Secondly, effects of nitrapyrin on soil microbial NO_3^- production need to be evaluated in soils with a long-term repeated use of nitrapyrin and dairy wastes. The questions, in which we are specifically interested, include whether a long-

term repeated use of nitrapyrin has a residual effect on nitrification in soils receiving dairy wastes, and how a long-term use of dairy wastes influences the persistence and effectiveness of nitrapyrin.

APPENDICES

Appendix A

ANOVAs for Inorganic N Accumulation Patterns in Chapter 2

ANOVA for total C (%) of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	29.7	14.8	0.7	0.55
Treatments	3	57.5	19.2	0.8	0.52
Error	6	136.2	22.7		

ANOVA for total N (%) of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.044	0.022	0.215	0.81
Treatments	3	0.063	0.021	0.208	0.89
Error	6	0.609	0.102		

ANOVA for $\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$) of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	27652	13826	1.3	0.34
Treatments	3	13244	4415	0.4	0.75
Error	6	63987	10664		

ANOVA for $\text{NO}_3^-\text{-N}$ ($\mu\text{g g}^{-1}$) of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	1170224	585112	2.1	0.21
Treatments	3	380932	126977	0.4	0.75
Error	6	1704507	284085		

ANOVA for the C:N ratios of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	26.1	13.1	7.1	0.03
Treatments	3	10.3	3.4	1.9	0.24
Error	6	11.0	1.8		

ANOVA for pH of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.082	0.041	0.241	0.79
Treatments	3	0.182	0.061	0.357	0.79
Error	6	1.021	0.170		

ANOVA for optical density (OD) of the variously treated immature dairy-waste compost (Table 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.007	0.003	0.083	0.92
Treatments	3	0.147	0.049	1.211	0.38
Error	6	0.243	0.040		

ANOVA for total C (%) of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	27.9	13.9	1.0	0.44
Treatments	3	49.2	16.4	1.1	0.41
Error	6	87.6	14.6		

ANOVA for total N (%) of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.038	0.019	0.301	0.75
Treatments	3	0.125	0.042	0.655	0.61
Error	6	0.383	0.064		

ANOVA for $\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$) of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	68604	34302	1.8	0.25
Treatments	3	228789	76263	3.9	0.07
Error	6	115907	19318		

ANOVA for $\text{NO}_3\text{-N}$ ($\mu\text{g g}^{-1}$) of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	64164	32082	0.24	0.80
Treatments	3	545664	181888	1.33	0.35
Error	6	820086	136681		

ANOVA for C:N ratios of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	7.3	3.6	2.2	0.19
Treatments	3	7.7	2.6	1.5	0.30
Error	6	10.0	1.7		

ANOVA for pH of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.002	0.001	0.006	0.99
Treatments	3	0.276	0.092	0.543	0.67
Error	6	1.014	0.169		

ANOVA for optical density (OD) of the variously treated mature dairy-waste compost (Table 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.039	0.019	0.392	0.69
Treatments	3	0.532	0.177	3.603	0.09
Error	6	0.295	0.049		

ANOVA for inorganic N accumulation patterns in soil with additions of the various mature composts at a low level (Fig. 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	39.1	19.6	3.6	0.09
Treatments (Tr)	3	205.8	68.6	12.9	0.001
Error a	6	32.2	5.4		
Days (D)	3	2963.0	987.7	241.6	0.0001
Tr \times D	9	167.5	18.6	4.6	0.01
Error b	24	98.1	4.1		

ANOVA for inorganic N accumulation patterns in soil with additions of the various mature composts at a high level (Fig. 2.1).

Source	df	SS	MS	F-value	P-value
Blocks	2	143.4	71.7	1.1	0.40
Treatments (Tr)	3	648.1	216.0	3.2	0.10
Error a	6	399.8	66.6		
Days (D)	3	6991.0	2330.3	163.3	0.001
Tr \times D	9	343.8	38.2	2.7	0.03
Error b	24	342.5	14.3		

ANOVA for inorganic N in soil with additions of the various mature compost at a low level (factor effects) (Fig. 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	39.1	19.6	3.6	0.09
Turing (T)	1	163.8	163.8	30.5	0.002
Watering (W)	1	41.8	41.8	7.8	0.03
T \times W	1	0.2	0.2	0.04	0.99
Error a	6	32.2	5.4		
Days (D)	3	2963.0	987.7	241.6	0.0001
T \times D	3	83.7	27.9	6.8	0.002
W \times D	3	70.2	23.4	5.7	0.004
T \times W \times D	3	13.5	4.5	1.1	0.37
Error b	24	98.2	4.1		

ANOVA for inorganic N accumulation patterns in soil with additions of various mature composts at a high level (factor effects) (Fig. 2.2).

Source	df	SS	MS	F-value	P-value
Blocks	2	143.4	71.7	1.1	0.40
Turing (T)	1	597.2	597.3	9.0	0.02
Watering (W)	1	46.6	46.6	0.7	0.44
T \times W	1	4.3	4.3	0.1	0.81
Error a	6	399.8	66.6		
Days (D)	3	6991.2	2330.4	163.3	0.001
T \times D	3	227.7	75.9	5.3	0.006
W \times D	3	112.4	37.5	2.6	0.07
T \times W \times D	3	3.8	1.3	0.1	0.97
Error b	24	342.5	14.3		

ANOVA for inorganic N accumulation patterns in soil with additions of the various immature compost at a low level (Fig. 2.3).

Source	df	SS	MS	F-value	P-value
Blocks	2	102.1	51.1	2.4	0.17
Treatments (Tr)	3	61.6	20.5	1.0	0.46
Error a	6	126.3	21.1		
Days (D)	3	1331.9	444.0	39.3	0.001
Tr x D	9	80.5	8.9	0.8	0.63
Error b	24	271.1	11.3		

ANOVA for inorganic accumulation patterns in soil with additions of the various immature compost at a low level (factor effects) (Fig. 2.4).

Source	df	SS	MS	F-value	P-value
Blocks	2	102.4	51.2	2.4	0.17
Turing (T)	1	6.9	6.9	0.3	0.59
Watering (W)	1	26.5	26.5	1.3	0.30
T x W	1	28.0	28.0	1.3	0.29
Error a	6	126.0	21.0		
Days (D)	3	1331.0	443.6	39.3	0.001
T x D	3	14.4	4.8	0.4	0.74
W x D	3	51.2	17.1	1.5	0.24
T x W x D	3	14.5	4.8	0.4	0.74
Error b	24	271.2	11.3		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for treatment of NTNW (Fig. 2.5).

Source	df	SS	MS	F-value	P-value
Blocks	2	89.3	44.7	2.7	0.27
Mature types (M)	1	29.2	29.2	1.9	0.31
Error a	2	32.7	16.4		
Days (D)	3	615.9	205.3	23.5	0.001
D x M	3	17.2	5.7	0.7	0.52
Error b	12	104.9	8.7		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for treatment of NTW (Fig. 2.5).

Source	df	SS	MS	F-value	P-value
Blocks	2	0.6	0.3	0.1	0.91
Mature types (M)	1	5.1	5.1	1.5	0.35
Error a	2	6.9	3.4		
Days (D)	3	1257.6	419.2	19.7	0.001
D x M	3	13.7	4.6	0.2	0.82
Error b	12	127.5	21.3		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for treatment of TNW (Fig. 2.5).

Source	df	SS	MS	F-value	P-value
Blocks	2	69.2	34.6	3.3	0.23
Mature types (M)	1	150.2	150.2	14.5	0.06
Error a	2	20.8	10.4		
Days (D)	3	871.6	290.5	85.5	0.001
D x M	3	86.8	28.9	7.5	0.01
Error b	12	46.0	3.8		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for treatment of TW (Fig. 2.5).

Source	df	SS	MS	F-value	P-value
Blocks	2	37.4	18.7	0.9	0.53
Mature types (M)	1	297.4	297.4	13.9	0.07
Error a	2	42.9	21.5		
Days (D)	3	1500.8	500.3	66.1	0.001
D x M	3	179.2	59.7	7.9	0.01
Error b	12	90.9	7.6		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for factor of NT (Fig. 2.6).

Source	df	SS	MS	F-value	P-value
Blocks	2	37.7	18.9	0.9	0.44
Mature types (M)	1	29.4	29.4	1.4	0.27
Error a	8	164.6	20.6		
Days (D)	3	1813.3	604.4	6.0	0.0001
D x M	3	23.6	7.9	0.8	0.51
Error b	30	299.9	10.0		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for factor of T (Fig. 2.6).

Source	df	SS	MS	F-value	P-value
Blocks	2	92.8	46.4	3.7	0.074
Mature types (M)	1	435.1	435.1	34.4	0.0004
Error a	8	101.3	12.7		
Days (D)	3	2306.4	768.8	105.3	0.0001
D x M	3	249.8	83.3	11.5	0.0001
Error b	30	219.2	7.3		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for factor of NW (Fig. 2.6).

Source	df	SS	MS	F-value	P-value
Blocks	2	113.7	56.8	2.6	0.14
Mature types (M)	1	155.9	155.9	7.0	0.03
Error a	8	178.2	22.3		
Days (D)	3	1466.2	488.7	78.8	0.001
D × M	3	90.5	30.2	4.9	0.003
Error b	30	185.7	6.2		

ANOVA for inorganic N accumulation patterns in soil receiving immature vs. mature compost for factor of W (Fig. 2.6).

Source	df	SS	MS	F-value	P-value
Blocks	2	14.2	7.1	0.3	0.75
Mature types (M)	1	190.3	190.3	7.9	0.023
Error a	8	192.8	24.1		
Days (D)	3	2749.6	916.5	89.0	0.0001
D × M	3	110.2	36.7	3.6	0.02
Error b	30	309.9	10.3		

Appendix B

Statistical Analysis in Chapter 3

ANOVA for initial inorganic N (mg N kg^{-1} soil) in alfalfa soil with addition of the variously treated dairy-wastes (Table 3.2).

Source	df	SS	MS	F-value	P-value
Treatments	3	2116.0	705.3	719.7	0.0001
Error	8	7.8	1.0		

ANOVA for initial inorganic N (mg N kg^{-1} soil) in corn soil with addition of the variously treated dairy-wastes (Table 3.2).

Source	df	SS	MS	F-value	P-value
Treatments	3	1845.4	615.1	1092.3	0.0001
Error	8	4.5	0.6		

T-values with 38 of degrees of the freedom for N mineralization potential (mg N kg^{-1} soil) (Table 3.2).

Soil types	Treatments	Low lagoon	High lagoon	Compost
Corn	control	0.92	4.62	5.28
	Low lagoon		5.73	5.66
	High lagoon			2.29
Alfalfa	control	0.37	2.68	4.00
	Low lagoon		4.55	5.51
	High lagoon			2.50

T-values with 38 of degree of freedom for mineralization rate constant (day^{-1}) (Table 3.2).

Soil types	Treatments	Low lagoon	High lagoon	Compost
Corn	control	2.06	1.87	0.56
	Low lagoon		0	4.16
	High lagoon			3.35
Alfalfa	control	1.2	2.0	0.12
	Low lagoon		0.94	1.64
	High lagoon			2.83

Appendix C

ANOVAs for Inorganic ^{15}N and Various Rates of N Processes in Chapter 4ANOVA for gross N mineralization rates ($\text{mg N kg}^{-1} \text{ soil day}^{-1}$) (Table 4.2).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	9.4	4.7	15.2	0.0044
Error a	6	1.9	0.3		
Incubation days (D)	3	12.0	4.0	16.5	0.0001
I \times Tr	6	9.0	1.5	6.2	0.0012
Error b	18	4.4	0.2		

ANOVA for ratios of nitrification rates to gross N mineralization rates (Table 4.3).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	5.0	2.5	3.7	0.09
Error a	6	4.0	0.8		
Incubation days (D)	3	22.7	7.6	11.8	0.0002
I \times Tr	6	11.1	1.9	2.9	0.04
Error b	18	11.5	0.6		

ANOVA for ratios of nitrification rates to potentials (Table 4.3).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	0.033	0.016	98.7	0.0001
Error a	6	0.001	1.7e-4		
Incubation days (D)	3	0.036	0.012	111.4	0.0001
I \times Tr	6	0.046	0.008	72.0	0.0001
Error b	18	0.002	1.1e-4		

ANOVA for $^{15}\text{N-NH}_4^+$ recoveries (%) (Fig. 4.2).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	1.41	0.71	83.52	0.0001
Labeling days (L)	1	13.76	13.76	1625.53	0.0001
Tr \times L	2	0.29	0.14	17.08	0.0003
Error a	12	0.10	0.01		
Incubation days (I)	3	20.91	6.97	750.48	0.0001
I \times Tr	6	0.32	0.05	5.67	0.0003
I \times L	3	2.06	0.69	73.76	0.0001
I \times Tr \times L	6	0.22	0.04	3.91	0.0042
Error b	36	0.33	0.01		

ANOVA for $^{15}\text{N-NO}_3^-$ recoveries (%) (Fig. 4.2).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	0.043	0.021	10.985	0.0019
Labeling days (L)	1	0.003	0.003	1.383	0.2623
Tr \times L	2	0.007	0.004	1.830	0.2025
Error a	12	0.023	0.002		
Incubation days (I)	3	0.101	0.034	17.603	0.0001
I \times Tr	6	0.044	0.007	3.879	0.0044
I \times L	3	0.007	0.002	1.149	0.3425
I \times Tr \times L	6	0.004	0.001	0.312	0.9265
Error b	36	0.069	0.002		

ANOVA for $^{15}\text{N-NH}_4^+$ excesses (%).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	4864	2432	99	0.0001
Labeling days (L)	1	5238	5238	214	0.0001
Tr \times L	2	1492	746	30	0.0001
Error a	12	294	24		
Incubation days (I)	3	6607	2202	97	0.0001
I \times Tr	6	5557	926	41	0.0001
I \times L	3	3381	1127	49	0.0001
I \times Tr \times L	6	1730	288	13	0.0001
Error b	36	820	23		

ANOVA for $^{15}\text{N-NO}_3^-$ excesses (%).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	278.2	139.1	2250.1	0.0001
Labeling days (L)	1	0.2	0.2	3.5	0.08
Tr \times L	2	0.2	0.1	1.6	0.25
Error a	12	0.7	0.1		
Incubation days (I)	3	194.8	64.9	1251.9	0.0001
I \times Tr	6	98.0	16.3	314.8	0.0001
I \times L	3	0.6	0.2	4.1	0.01
I \times Tr \times L	6	0.5	0.1	1.6	0.18
Error b	36	1.9	0.1		

ANOVA for soil nitrification potentials ($\text{mg N kg}^{-1} \text{ soil day}^{-1}$) (Fig. 4.5).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	32.2	16.1	19.2	0.0025
Error a	6	5.0	0.8		
Incubation days (D)	5	21.0	4.2	21.0	0.0001
I \times Tr	10	35.3	3.5	17.7	0.0001
Error b	30	6.0	0.2		

ANOVA for nitrification rates ($\text{mg N kg}^{-1} \text{ soil day}^{-1}$) (Fig. 4.5).

Source	df	SS	MS	F-value	P-value
Treatments (Tr)	2	5.136	2.568	1130.9	0.0001
Error a	6	0.014	0.002		
Incubation days (D)	4	2.473	0.618	36.4	0.0001
I \times Tr	8	3.330	0.416	24.5	0.0001
Error b	24	0.408	0.017		

Appendix D

ANOVAs for Soil Inorganic N and Various N Process Rates in Chapter 5

ANOVA for soil NH_4^+ concentration (mg N kg^{-1} soil) 90 days after planting (Table 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	1.0	0.3	5.3	0.009
Treatments	6	1.9	0.3	5.1	0.003
Error	18	1.1	0.1		

ANOVA for soil NO_3^- concentration (mg N kg^{-1} soil) 90 days after planting (Table 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	126.9	42.3	0.9	0.48
Treatments	6	1965.2	327.6	6.7	0.0007
Error	18	879.4	48.9		

ANOVA for soil C mineralization rates (mg C kg^{-1} soil day^{-1}) 90 days after planting (Table 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	89.1	29.7	20.5	0.0001
Treatments	6	314.4	52.4	36.2	0.0001
Error	18	26.1	1.5		

ANOVA for soil N mineralization rates (mg N kg^{-1} soil day^{-1}) 90 days after planting (Table 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	0.9	0.3	1.1	0.36
Treatments	6	8.5	1.4	5.3	0.003
Error	18	4.8	0.3		

ANOVA for soil microbial NH_4^+ immobilization rates (mg N kg^{-1} soil day^{-1}) 90 days after planting (Table 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	8.1	2.7	2.1	0.14
Treatments	6	1.0	0.2	0.1	0.99
Error	18	23.3	1.3		

ANOVA for soil nitrification rates (mg N kg^{-1} soil day^{-1}) 90 days after planting (Table 5.3).

Source	df	SS	MS	F-value	P-value
Blocks	3	1.5	0.5	0.6	0.60
Treatments	6	20.8	3.5	4.5	0.006
Error	18	14.0	0.8		

ANOVA for soil nitrification potentials (mg N kg^{-1} soil day^{-1}) 90 days after planting (Table 5.3).

Source	df	SS	MS	F-value	P-value
Blocks	3	19.5	6.5	2.3	0.12
Treatments	6	84.3	14.1	4.9	0.004
Error	18	51.4	2.9		

ANOVA for the ratios of nitrification rates to nitrification potentials 90 days after planting (Table 5.3).

Source	df	SS	MS	F-value	P-value
Blocks	3	0.007	0.002	0.152	0.93
Treatments	6	0.325	0.054	3.726	0.014
Error	18	0.262	0.015		

ANOVA for silage corn dry wt. yields (Mg ha^{-1}) (Table 5.4).

Source	df	SS	MS	F-value	P-value
Blocks	3	55.5	18.5	1.8	0.18
Treatments	6	268.6	44.8	4.4	0.01
Error	18	182.6	10.1		

ANOVA for ear leaf N (%) 82 days after planting (Table 5.4).

Source	df	SS	MS	F-value	P-value
Blocks	3	0.17	0.06	1.08	0.38
Treatments	6	3.26	0.54	10.11	0.001
Error	18	0.97	0.05		

ANOVA for chopped corn tissue N (%) at harvest (Table 5.4).

Source	df	SS	MS	F-value	P-value
Blocks	3	0.03	0.01	0.70	0.57
Treatments	6	0.75	0.12	8.00	0.003
Error	18	0.28	0.02		

ANOVA for soil NH_4^+ -N concentrations (mg N kg^{-1} soil) in the early growth season (June 26) (Fig. 5.1).

Source	df	SS	MS	F-value	P-value
Blocks	3	107.4	35.8	7.8	0.002
Treatments (Tr)	6	22.7	3.8	0.8	0.56
Error a	18	82.4	4.6		
Depths (D)	1	1.9	1.9	0.4	0.54
Tr \times D	6	39.9	6.6	1.3	0.28
Error b	21	106.2	5.1		

ANOVA for soil NO_3^- -N concentrations (mg N kg^{-1} soil) in the early growth season (June 26) (Fig. 5.1).

Source	df	SS	MS	F-value	P-value
Blocks	3	7.0	2.3	0.1	0.96
Treatments (Tr)	6	17725.8	287.6	11.6	0.0001
Error a	18	446.1	24.8		
Depths (D)	1	1990.2	1990.2	78.3	0.0001
Tr \times D	6	933.2	155.5	6.1	0.0008
Error b	21	533.7	25.4		

ANOVA for soil NH_4^+ -N concentrations (mg N kg^{-1} soil) after harvest (Nov. 4) (Fig. 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	3.9	1.3	0.6	0.63
Treatments (Tr)	6	26.3	4.4	2.0	0.11
Error a	18	38.9	2.2		
Depths (D)	1	0.1	0.1	0.0	0.86
Tr \times D	6	6.4	1.1	0.4	0.84
Error b	21	50.3	2.4		

ANOVA for soil NO_3^- -N concentrations (mg N kg^{-1} soil) after harvest (Nov. 4) (Fig. 5.2).

Source	df	SS	MS	F-value	P-value
Blocks	3	15.0	5.0	0.9	0.44
Treatments (Tr)	6	164.1	27.4	5.1	0.003
Error a	18	96.1	5.3		
Depths (D)	1	0.5	0.5	0.2	0.64
Tr \times D	6	10.5	1.7	0.7	0.64
Error b	21	50.8	2.4		

ANOVA for $^{15}\text{NH}_4^+$ recoveries ($\mu\text{g N}$) (Fig. 5.3).

Source	df	SS	MS	F-value	P-value
Blocks	3	40668.5	13556.2	4.500	0.016
Treatments (Tr)	6	61608.5	10268.1	3.409	0.020
error a	18	54221.6	3012.3		
Labeling days (L)	1	473432.2	47342.6	78.263	0.001
Tr. \times L	6	90598.7	15099.8	2.496	0.056
error b	21	127033.6	6049.2		

ANOVA for $^{15}\text{NO}_3^-$ recoveries ($\mu\text{g N}$) (Fig. 5.3).

Source	df	SS	MS	F-value	P-value
Blocks	3	517.2	172.4	0.078	0.971
Treatments (Tr)	6	25114.5	4185.8	1.897	0.137
error a	18	39724.8	2206.9		
Labeling days (L)	1	3.5	3.5	0.003	0.959
Tr. \times L	6	15164.5	2527.4	1.944	0.121
error b	21	27308.0	1300.4		

ANOVA for $^{15}\text{N-NH}_4^+$ excesses (%).

Source	df	SS	MS	F-value	P-value
Blocks	3	170.8	56.9	3.143	0.051
Treatments (Tr)	6	966.7	161.1	8.896	0.001
error a	18	326.0	18.1		
Labeling days (L)	1	217.0	217.0	13.973	0.001
Tr. \times L	6	385.3	64.2	4.135	0.007
error b	21	326.1	15.5		

ANOVA for $^{15}\text{N-NO}_3^-$ excesses (%).

Source	df	SS	MS	F-value	P-value
Blocks	3	297.7	97.6	1.740	0.195
Treatments (Tr)	6	5798.9	966.5	17.231	0.001
error a	18	1009.6	56.1		
Labeling days (L)	1	588.4	588.4	100.625	0.001
Tr. \times L	6	69.3	11.6	1.976	0.115
error b	21	122.806	5.8		

Appendix E

ANOVAs for Inorganic N and Nitrification Potentials in Chapter 6

ANOVA for soil NH_4^+ concentrations in Blue Creek Farm

Source	df	SS	MS	F-value	P-value
Fields (F)	1	31.8	31.8	0.4	0.55
Blocks (B)/F	2	271.6	135.8	1.8	0.27
Treatments (Tr.)	2	999.0	499.5	6.8	0.05
F × Tr.	2	144.0	72.0	1.0	0.45
Error a	4	296.1	74.0		
Locations (L)	3	296.8	98.9	0.4	0.76
F × L	3	690.1	230.0	0.9	0.49
Error b	6	1509.8	251.6		
Tr. × L	6	1173.0	195.5	1.7	0.20
F × Tr. × L	6	549.0	91.5	0.8	0.59
Error c	12	1367.6	114.0		
Depths (D)	1	1224.0	1224.0	13.5	0.07
F × D	1	6.9	6.9	0.1	0.81
Error d	2	181.0	90.5		
Tr. × D	2	589.7	294.9	2.8	0.09
L × D	3	326.5	108.8	1.0	0.39
Tr. × L × D	6	980.0	163.5	1.6	0.32
F × Tr. × D	2	133.4	66.7	0.6	0.65
F × L × D	3	796.0	265.3	2.5	0.09
F × Tr. × L × D	6	775.7	129.3	1.2	0.42
Error e	22	2321.3	105.5		
Times (T)	3	1747.3	582.4	4.7	0.004
F × T	3	3314.0	1104.7	8.9	0.001
Tr. × T	6	1391.3	231.9	1.9	0.09
L × T	9	1824.2	202.7	1.6	0.11
D × T	3	791.6	263.9	2.1	0.10
Tr. × L × T	18	2298.3	127.7	1.0	0.44
L × D × T	9	2114.1	234.9	1.9	0.06
Tr. × D × T	6	952.0	158.7	1.3	0.27
Tr. × L × D × T	18	2406.7	133.7	1.1	0.39
F × Tr. × T	6	2371.1	395.2	3.2	0.006
F × L × T	9	1369.4	152.2	1.2	0.29
F × D × T	3	1963.6	654.5	5.3	0.002
F × Tr. × L × T	18	2998.3	166.6	1.3	0.17
F × L × D × T	9	1633.0	181.4	1.5	0.17
F × Tr. × D × T	6	1360.7	226.8	1.8	0.10
F × Tr. × L × D × T	18	2702.7	150.1	1.2	0.27
Error f	144	17955.9	124.7		

ANOVA for soil NO_3^- concentrations in Blue Creek Farm

Source	df	SS	MS	F-value	P-value
Fields (F)	1	577.1	577.1	16.4	0.02
Blocks (B)/F	2	406.4	203.2	5.8	0.07
Treatments (Tr.)	2	825.8	412.9	11.8	0.02
F × Tr.	2	110.6	55.3	1.6	0.31
Error a	4	140.6	35.1		
Locations (L)	3	122.7	40.9	0.9	0.49
F × L	3	69.4	23.1	0.5	0.68
Error b	6	267.7	44.6		
Tr. × L	6	139.9	23.3	1.5	0.26
F × Tr. × L	6	233.4	38.9	2.5	0.09
Error c	12	186.9	15.6		
Depths (D)	1	3089.6	3089.6	43.4	0.02
F × D	1	32.9	32.9	0.5	0.57
Error d	2	142.2	71.1		
Tr. × D	2	325.3	162.6	9.6	0.001
L × D	3	124.3	41.4	2.4	0.09
Tr. × L × D	6	181.4	30.3	1.9	0.16
F × Tr. × D	2	2.8	1.4	0.1	0.95
F × L × D	3	79.2	26.4	1.6	0.21
F × Tr. × L × D	6	128.7	21.4	1.3	0.31
Error e	22	373.6	17.0		
Times (T)	3	7583.9	2528.0	71.0	0.001
F × T	3	12175.7	4058.6	114.0	0.001
Tr. × T	6	604.9	100.8	2.8	0.01
L × T	9	185.8	20.6	0.6	0.81
D × T	3	1588.6	529.5	14.9	0.001
Tr. × L × T	18	808.4	44.9	1.3	0.22
L × D × T	9	238.5	26.5	0.7	0.68
Tr. × D × T	6	332.2	55.4	1.6	0.17
Tr. × L × D × T	18	435.1	24.2	0.7	0.83
F × Tr. × T	6	949.3	158.2	4.4	0.004
F × L × T	9	439.2	48.8	1.4	0.21
F × D × T	3	3281.0	1093.7	30.7	0.001
F × Tr. × L × T	18	809.1	45.0	1.3	0.22
F × L × D × T	9	365.2	40.6	1.1	0.34
F × Tr. × D × T	6	536.9	89.5	2.5	0.02
F × Tr. × L × D × T	18	474.3	26.3	0.7	0.77
Error f	144	5128.7	35.6		

ANOVA for soil nitrification potentials in Blue Creek Farm

Source	df	SS	MS	F-value	P-value
Fields (F)	1	54.1	54.1	21.3	0.01
Blocks (B)/F	2	8.7	4.3	1.7	0.29
Treatments (Tr.)	2	38.5	19.2	7.6	0.04
F × Tr.	2	7.6	3.8	1.5	0.33
Error a	4	10.1	2.5		
Locations (L)	3	34.7	11.6	28.6	0.001
F × L	3	51.8	17.3	42.7	0.000
Error b	6	2.4	0.4		
Tr. × L	6	4.0	0.7	1.2	0.36
F × Tr. × L	6	3.6	0.6	1.1	0.41
Error c	12	6.5	0.5		
Depths (D)	1	209.6	209.6	1574.0	0.001
F × D	1	45.8	45.8	344.0	0.003
Error d	2	0.3	0.15		
Tr. × D	2	20.9	10.4	14.4	0.001
L × D	3	9.3	3.1	4.3	0.02
Tr. × L × D	6	3.3	0.6	0.8	0.65
F × Tr. × D	2	2.9	1.5	2.0	0.17
F × L × D	3	4.6	1.5	2.1	0.15
F × Tr. × L × D	6	2.2	0.4	0.5	0.83
Error e	22	15.9	0.7		
Times (T)	3	35.4	11.8	17.9	0.001
F × T	3	13.6	4.5	6.9	0.002
Tr. × T	6	4.0	0.7	1.0	0.42
L × T	9	8.4	0.9	1.4	0.18
D × T	3	16.9	5.6	8.5	0.001
Tr. × L × T	18	7.9	0.4	0.7	0.84
L × D × T	9	6.0	0.7	1.0	0.43
Tr. × D × T	6	2.9	0.5	0.7	0.63
Tr. × L × D × T	18	6.2	0.3	0.5	0.94
F × Tr. × T	6	11.6	1.9	2.9	0.01
F × L × T	9	9.0	1.0	1.5	0.15
F × D × T	3	34.4	11.5	17.3	0.001
F × Tr. × L × T	18	11.1	0.6	0.9	0.54
F × L × D × T	9	6.3	0.7	1.1	0.40
F × Tr. × D × T	6	9.3	1.5	2.3	0.04
F × Tr. × L × D × T	18	12.2	0.7	1.0	0.44
Error f	144	95.2	0.7		

ANOVA for nitrifier sensitivity to additions of various amounts of nitrapyrin

Source	df	SS	MS	F-value	P-value
Blocks	1	0.081	0.081	22.61	0.13
Treatments (Tr)	1	0.272	0.272	75.71	0.07
Error a	1	0.004	0.004		
Concentration (C)	4	0.266	0.067	3.73	0.05
Tr × C	4	0.007	0.002	0.10	0.98
Error b	8	0.143	0.018		
Hours (H)	6	3.386	0.564	625.57	0.0001
Tr × H	6	0.002	2.6e-4	0.29	0.94
C × H	24	0.425	0.018	19.62	0.0001
Tr × C × H	24	0.011	4.5e-4	0.50	0.97
Error c	60	0.054	0.001		

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Major: Soil Science
Dissertation: Management of Microbial Nitrate Production in Agricultural Soils.
- M.S., 1990. Shenyang Agricultural University, P. R. China.
Major: Agricultural Ecology.
Thesis: Nitrogen Fixation and Supply of Sweet Clover in Corn/Sweet Clover Intercropping Ecosystem.
- B.S., 1987. Liaoning University. P. R. China.
Major: Biology

EXPERIENCE

- Research Assistant 1994-1998
Plants, Soils, and Biometeorology, Utah State University, Logan, Utah.
Research: microbial carbon and nitrogen transformations, autotrophic nitrification, microbial/plant interactions in animal waste management.
- Teaching Assistant 1995-1997
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Teaching: Soils and Plant Nutrition (laboratory).
- Assistant Research Professor 1990-1994
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 Liaoning Academy of Agricultural Sciences, P. R. China.
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Soil and environmental microbiology/biochemistry, Soil biogeochemistry and nutrient cycling, Animal waste management and soil fertility, Soil and environmental quality.

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Use of stable isotopes as tracer for N transformations, Biochemical methods for the determination of microbial population biomass and activity in soils, Molecular biology techniques, Statistical experimental designing, Computer modeling skills.

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HONORS AND AWARDS

- Student paper award 1998
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PUBLICATIONS

Referred publications

Shi, W., J.M. Norton, B.E. Miller, and M.G. Pace, Aeration and moisture effects during windrow composting on the N fertilizer values of composts. *Applied Soil Ecology*, in press.

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Shi, W., and J.M. Norton, Comparison of nitrogen mineralization dynamics of dairy wastes treated by aerobic composting or anaerobic lagoon digestion.

- Shi, W., and J.M. Norton, Effect of a long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification.
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