ABSTRACT

IYYEMPERUMAL, KANNAN. Soil Microbial Properties and Nitrogen Cycling in Forage Production Systems Receiving Swine Lagoon Effluent. (Under the direction of Dr. Wei Shi).

Land application of swine lagoon effluent (SLE) is widespread in the southeastern USA. As a surrogate of synthetic N fertilizer, SLE is applied to forage crops primarily based on requirement of plant yield and of dry matter quality such as protein and fiber contents. Although soil microorganisms play a central role in soil N turnover, retention and therefore the environmental fate of soil and fertilizer N, the response of soil microbial communities to SLE application has received little attention. In this study, soil microbial properties in forage production systems receiving swine lagoon effluent were examined at both organismal and process levels. The specific objectives of this research were to (1) determine microbial community structure and catabolic function; (2) assess soil enzyme activities involved in C, N and P cycling; (3) quantify soil N mineralization, immobilization and nitrification; and (4) link the changes in microbial community with soil properties as a consequence of contrasting N fertilization (SLE versus a synthetic N fertilizer) or of contrasting forage management practices (hay production versus cattle grazing). A holistic delineation may improve our understanding of microbial ecology in managed forage production systems and accordingly facilitate best management practices, in particular N fertilization.

Soil microbial biomass, activity and rates of N transformation were heterogeneous in a grazed pasture, due mainly to non-uniform distribution of animal excreta. While soil microbial biomass, respiration activity and net N mineralization were positively correlated with the concentration of animal excreta and associated changes in soil C and N ($P < 0.05$, Pearson correlation coefficient $r \approx 0.70$), gross N mineralization and

nitrification potential were not. This difference indicates that soil microbial community and its mediated processes may not respond linearly to N and C availability. Soil microbial community structure and functions were therefore examined in a hay production system supplied either SLE or ammonium nitrate (AN) at application rates of 0, 200, 400 or 600 kg plant available N ha^{-1} yr^{-1}. Microbial biomass, respiration activity and net N mineralization peaked at the application rate of 200 or 400 kg available N ha^{-1} yr^{-1}. Activities of several hydrolytic enzymes involved in C and N cycling were also highest at the application rate of 200 or 400 kg available N ha^{-1} yr^{-1}. However, oxidative soil enzymes including phenol oxidase and peroxidase activity were positively correlated with the application rates of SLE ($P < 0.05$), but not AN, suggesting that SLE and AN differed in their influence on soil microbial community or its mediated processes. Indeed, fingerprinting of phospholipid fatty acids and community-level physiology showed that microbial community composition differed between soils fertilized with SLE versus AN. The differences in microbial community and its mediated processes were highly correlated with soil pH. This research implies that as a consequence of changes in soil properties associated with a high application rate of animal waste, soil microbial community population size and community structure would adjust accordingly. However, this adjustment may not benefit soil fertility in the long run.

SOIL MICROBIAL PROPERTIES AND NITROGEN CYCLING IN FORAGE PRODUCTION SYSTEMS RECEIVING SWINE LAGOON EFFLUENT

by

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DEDICATION

This dissertation is dedicated to my family – my mom, Pasungili, dad, Iyyemperumal and my brothers, Selvakumar, Rajakumar, my wife, Kala and my sister-in-law, Padmapriya.

BIOGRAPHY

Kannan Iyyemperumal was born in 1977, the second of three children in the family of Pasungili and Iyyemperumal, in Solaiseri, Tamil Nadu, India. He received his elementary and high school education in Perunazhi, in southern Tamil Nadu. Following graduation from high school, Kannan attended the Tamil Nadu Agricultural University, and was voted as outstanding student when he received his B.S. (Ag) in 1998. In 1999 he enrolled in the Microbiology department at Tamil Nadu Agricultural University to pursue his M.S in Agricultural Microbiology where he was awarded again as Best Student and received his degree in August, 2001. After his graduation, he worked as research associate in Directorate of Oil seeds Research, Hyderabad where he carried out research on bio-pesticides until December, 2002. While working in Hyderabad, he decided to pursue his higher education and in January, 2003, he was admitted to North Carolina State University to begin his doctoral program in the department of Soil Science. Kannan Iyyemperumal is married to Kalamani Muthusamy in June, 2006.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction

Land application of swine lagoon effluent (SLE) is widespread in the southeastern USA. As a surrogate of synthetic nitrogen (N) fertilizers, SLE has been often applied to forage production systems managed for hay production or cattle grazing. While SLE can increase yield and nutritive value of crops (Adeli et al., 2005), there has been a great concern of SLE application on environmental quality and soil fertility. Research has been focused on monitoring the movement of elements through soil profiles and NO_3^- concentrations in ground and surface waters as a consequence of SLE application (King et al., 1985; Israel et al., 2005). However, despite their central role in organic matter decomposition and nutrient cycling, response of soil microorganisms to SLE application has received little attention in managed forage production systems.

Because soil microorganisms can respond more rapidly to management induced changes than many physical and chemical properties of soil, microbial properties may be better indicators of the impacts of SLE application on soil fertility and environmental quality. In general, the rates of N fertilization are recommended according to realistic yield expectation of crop and/or dry matter quality such as protein and fiber contents. However, soil microbial community responses to N fertilization have been considered little in developing N best management practices (Mulvaney et al., 2006). Informed management of N in SLE requires fundamental understanding of processes mediated by soil microorganisms.

Nitrogen fertilization is the most important management practice in boosting forage production. The source and level of nitrogen fertilization may alter the soil

microbial community through effects on plant productivity and/or soil physical and chemical properties. Liu et al. (1998) reported that in comparison to ammonium nitrate, 3-y SLE application reduced soil pH and base cations such as Ca^{2+} and Mg^{2+} and increased soluble salt concentration, suggesting that the soil environment was changed as a consequence of these two types of N fertilizers. Changes in soil properties as a result of SLE application likely influence the complex belowground biological systems. Rietz and Haynes (2003) demonstrated that acidity and high salt concentration in soil could reduce microbial biomass and microbially mediated nutrient transformations.

Nitrogen fertilization may also affect soil microbial community composition and in turn regulate nutrient transformation processes. Soils fertilized with dairy manure had 15 to 27% more Gram-negative bacteria PLFA biomarkers compared with control, while ammonium nitrate treatment resulted in a 15% decrease in these biomarkers (Peacock et al., 2001). The activities of soil enzymes can change with the fertilization practices. For example, synthetic N fertilizers enhanced the activity of cellulase, an enzyme involved in the degradation of polysaccharides, but reduced the activities of phenol oxidase and peroxidase, the oxidative enzymes involved in the degradation of lignin and polyphenols (Ajwa et al., 1999; Carreiro et al., 2000; Saiya-Cork et al., 2002; Sinsabaugh et al., 2005).

However, most information on the relationship between N fertilization and the soil microbial community was derived using synthetic fertilizers. Besides high N content, SLE also contains many other elements such as soluble C and heavy metals. These SLE-associated attributes may modify the relationship between soil microbial community and soil N availability. Therefore, a holistic approach to characterize the impacts of SLE application on the soil microbial community and N transformations mediated by this community is fundamental to understanding forage ecology and may help manage SLE to benefit soil fertility, while minimizing loss of N via leaching and denitrification.

Forage is managed for both hay production and cattle grazing. In contrast to hay production, grazing animals recycle a large fraction of ingested elements including C and N within a grazing pasture. But these elements are often distributed heterogeneously via animal excreta with highest concentrations in camping areas, i.e. near shade and watering sources (Whitehead, 1986; West et al., 1989; Haynes and Williams, 1993; Mathews et al., 1994). This non-uniform distribution of animal excreta modifies soil attributes, i.e. organic C and N, soluble salts and soil pH (Haynes and Williams, 1999; Franzluebbers et al., 2000) and thus creates a mosaic of environments for soil microorganisms. This heterogeneity in soil fertility and soil properties needs to be considered when SLE is applied to grazed pasture systems.

The purpose of this research was to assess the following hypotheses: (1) soil microbial community biomass, activity and rates of microbially mediated N transformations are positively correlated with the concentrations of animal excreta; (2) SLE application influences soil microbial community composition and catabolic functions, but degree of the impacts may differ with the application rates of SLE; (3) SLE application could restructure the relative abundance of soil enzymes involved in C, N and P cycling; and (4) changes in soil microbial community structure and function were correlated to soil properties induced by SLE application. Accordingly, the specific objectives of this research were to (1) determine soil microbial biomass, activity and N mineralization and nitrification in relation to animal excreta distribution; (2) characterize soil and microbial properties in a forage production system that received swine lagoon effluent (SLE) versus ammonium nitrate (AN); (3) assess the activities of soil enzymes involved in soil C, N and P cycling as a consequence of SLE versus AN application; and (4) compare soil microbial community composition and catabolic function in soils that received SLE versus AN applications. Therefore, this dissertation includes four chapters

reporting research related to microbial community characteristics as a consequence of animal waste application, each addressing one specific area not previously addressed in the literature. Chapter 2 reports the heterogeneity of soil microbial community biomass, activity and rates of N transformation in a grazed pasture; Chapter 3 compares microbial community biomass, activity and N transformations between soils fertilized with SLE versus AN; Chapter 4 characterizes the patterns of soil enzyme activities as a consequence of SLE application; and Chapter 5 fingerprints soil microbial community compositions and catabolic functions in soils receiving SLE versus AN. The overall goal of this dissertation research was to improve our understating of microbial ecology in managed forage production systems so that informed N management practices could be better formulated.

Literature Review

Non-uniform Distribution of Elements in a Grazing Pasture

Grazed pastures may influence global environment due to its high potential for C and N sequestration (Wright et al., 2004), production of greenhouse gases such as nitrous oxide and methane, and the losses of nutrients such as N and P into surface- and ground-water via runoff and leaching (Flessa et al., 1996; Owens and Bonta, 2004). Cattle grazing enhances soil C and N accumulation and accordingly is assumed to improve soil fertility as a result of nutrient recycling within a soil-plant-animal ecosystem (Haynes and Williams, 1993). Whitehead (1995) reported that grazing animals recycled a large fraction, i.e. 70 to 80% of ingested C and N, and thus animal excreta could redistribute 300 to 350 kg N ha^{-1} y^{-1} in a grazed pasture. In addition, elements other than C and N such as P, K, Cu and Zn also recycled and redistributed in a grazed pasture with highest concentrations in camping areas, i.e. near shade and watering sources (Whitehead, 1986;

West et al., 1989; Rowarth et al., 1992; Haynes and Williams, 1993; Mathews et al., 1994). This non-uniform distribution of nutrients may create a mosaic of environments for soil microorganisms (Jarvis et al., 1995).

Changes in Soil Properties Associated with Animal Waste Application

Swine population in North Carolina increased from 2.6 million head in 1989 to 9.6 million head in 1997, and has remained in the range of 9.6 to 10 million head since 1997 (National Agricultural Statistics Service, 2005). This large swine population generates a n enormous amount of waste which is often subjected to anaerobic lagoon treatment. On average, the nutrient concentration of swine lagoon effluent (SLE) from commercial facilities in North Carolina, is 565 mg L^{-1} of total Kjeldahl N, 61 mg L^{-1} P, 158 mg L^{-1} K and 238 mg L^{-1} total organic carbon (Personal communication with Dr. Westerman). Ammonium N typically accounts 80 to 85% of the total Kjeldahl N in SLE (Barker and Zublena, 1995). SLE also contains significant quantities of other elements, including base cations (Ca^{2+}, Mg^{2+}, Na^{+}) and heavy metals (Cu, Zn, Mn). Land application of SLE may have significant impacts on soil physical and chemical properties. For example, SLE application could reduce soil pH and base cations such as Ca^{2+} and Mg^{2+} and increased soluble salt concentration (Liu et al., 1998). However, some studies showed that SLE application at a high rate increased the concentration of many extractable elements including Zn and Cu (Adeli et al., 2002).

Soil Microbial Community Biomass and Composition

Soil microbial biomass is a small but dynamic entity that responds rapidly to changes in the soil environment including the quantity and quality of soil organic matter and soil nutrient availability. In general, soil microbial biomass C or N constitute a small

fraction, i.e. ~ 1 – 4% soil organic C and 2 – 6% soil organic N and is tightly correlated with soil C (Jenkinson and Ladd, 1981; Brookes et al., 1985). Microbial biomass serves as both source and sinks for nutrients including N, P, and S, regulates nutrient transformations and produces extracellular enzymes (Burn, 1978; Jenkinson and Ladd, 1981). Because soil organic matter is the major C and energy source for soil microbes, microbial community biomass is often considerably correlated to the content of soil C (Wardle, 1992). Application of animal waste to soil may stimulate microbial growth and activity via supply of bioavailable C (Lovell and Jarvis, 1996). However, change in soil properties associated with application of animal waste may considerably modify the relationship between microbial biomass and soil C and important soil properties including soil acidity, soluble salt and heavy metal concentrations (Tyler, 1981; Wardle, 1992; Witter et al., 1993; Bardgett and Leemans, 1995; Rietz and Haynes, 2003). In a fertilization trial, McAndrew and Malhi (1992) observed a ~ 40% or ~ 90% decline in microbial biomass C and N respectively from that of unfertilized controls due to ~ 1.5 units reduction in soil pH associated with fertilization. Several studies also reported that soil microbial biomass remained at a low level 20 years after waste application due mainly to high concentration of heavy metals (Brookes and McGarth, 1984; Brookes et al., 1986).

Microbial community composition, the relative abundance of different groups of microorganisms, including Gram-positive and Gram-negative bacteria, fungi and actinomycetes, that occupy a common habitat and interact with each-other and the environment, is considered to have regulatory role on N transformation processes in soil (Cavigelli and Robertson, 2000; Balser et al., 2002). Changes in microbial community composition are often related to management practices (Garbeva et al., 2004). Bossio et al. (1998) used membrane fatty acids fingerprint techniques and demonstrated that

microbial community compositions differed significantly in organic versus conventional farming practices. Certainly, source of N fertilizer may restructure soil microbial community via influences on soil properties. Soils fertilized with dairy manure or ammonium nitrate exhibited distinct fingerprints of membrane fatty acids and the shift in community composition was related to an increase in soluble organic C in manure fertilized soils and a decrease in soil pH in ammonium nitrate fertilized soils (Peacock et al., 2001; Marschner, et al., 2003; Larkin et al., 2006).

Activities of Soil Enzymes Affected by N Fertilization

Soil microbes are the major source of soil enzymes, which play an important role in decomposition of soil organic matter and nutrient cycling (Burns, 1978; Price and Morel, 1990; Sinsabaugh et al., 1991). Soil enzymes are very sensitive to soil N availability (Gianfreda and Bollag, 1996). For example, the activities of hydrolytic enzymes such as cellulase, acid phosphatase and chitinase are often increased with increasing soil N availability, but the activities of oxidative enzymes such as phenol oxidase are decreased with increasing soil N availability (Ajawa et al., 1999; Carreiro et al., 2000; Saiya-Cork et al., 2002; Matocha et al., 2004). In addition, soil enzymes involved in cycling of a particular nutrient are often negatively related to availability of that nutrient (Dick, 1992). For example, soil P availability was inversely correlated with acid phosphatase activity (Haussling and Marschner, 1989).

Soil pH can also affect the activities of soil enzymes via controls on the ionization and solubility of enzymes, concentration of substrates and enzyme cofactors, and bioavailability of elements that can inhibit enzymatic reactions (Dick et al., 1988; Gianfreda and Bollag, 1996). Tyler (1981) reported that heavy metals may adversely affect soil enzyme activities. Kandeler et al. (1996) observed that soil Cu at a

concentration of >100 μg g^{-1} soil and soil Zn at a concentration of >300 μg g^{-1} could significantly decrease soil enzyme activities. Again, several studies reported that acid phosphatase activity was negatively correlated with soil pH (Dick et al., 1988; Acosta-Martínez and Tabatabai, 2000) and β-glucosidase activity was positively correlated to soil pH (Acosta-Martínez and Tabatabai, 2000). In addition, soil pH also regulates microbial community biomass, a major source of soil enzymes (Burns, 1978) and thus could indirectly affect the enzyme production. DeForest et al. (2004) observed that soil enzyme activity declined with a reduction in soil microbial biomass in a hardwood forest soils and protease activity was positively correlated with microbial biomass (Alef et al., 1988; Zaman et al., 1999).

Nitrogen Transformations as Affected by Animal Waste

Net N mineralization is determined by a change of inorganic N pool size over time, which results from competing processes of microbial N production and consumptionIn contrast, gross N processes may provide more detailed information about controls of N transformations (Schimel and Bennett, 2004) and can be measured by ^{15}N pool dilution technique (Hart et al., 1994b). At the ecosystem level C-to-N ratio of soil organic matter is considered a key factor in determining the balance between microbial N mineralization and immobilization. However, this balance can be modified by soil microbial community eco-physiology (Bengtsson et al., 2003; Shi et al., 2006). Bengtsson et al. (2003) demonstrated that N mineralization and immobilization in forest soils were more related to microbial community activity than to the soil C-to-N ratio, and thus leaching of nitrate was largely dependent on the density and activity of the microbial community. In a recent study of turfgrass ecosystems, Shi et al. (2006) have also documented that N mineralization and immobilization differed considerably for turfgrass

ecosystems of different ages despite similar soil C-to-N ratios. It is well known that soil organic C is a primary factor regulating microbial N immobilization (Hart et al., 1994a) and a positive relationship between microbial N immobilization and soil organic C contents in many grassland soils has been reported (Barrett and Burke, 2000). However, Hatch et al. (2000) reported that input of animal waste, dung, into soil had little effect on gross N mineralization despite that organic C content was expected to be high. This suggests that factors other than organic C may exert controls on microbial N transformations in soils receiving animal wastes.

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CHAPTER TWO

MICROBIAL COMMUNITY ECO-PHYSIOLOGY AND SOIL NITROGEN CYCLING IN A PASTURE: IMPACT OF DUNG DISTRIBUTION

Abstract

Grazing animals recycle a large fraction of ingested C and N within a pasture ecosystem, but the redistribution of C and N via animal excreta is often heterogeneous, being highest in camping areas, i.e. near shade and watering sources. This non-uniform distribution of animal excreta may modify soil physical and chemical attributes, and likely affect microbial community eco-physiology and soil N cycling. A better understanding of microbial biomass and soil N transformations associated with the distribution of animal excreta is fundamental to pasture ecology and improved management decisions. We determined microbial population size, activity, N mineralization, and nitrification in areas of a pasture with different dung concentration. The pasture was cropped with coastal bermudagrass (*Cynodon dactylon* L.) and subjected to grazing by cattle for 4 y. Soil microbial biomass, activity and N transformations were significantly higher at 0 – 5 cm than at 5 – 15 cm soil depth, and the impacts of cattle dung were more pronounced in the uppermost soil layer. Microbial biomass, activity and net N mineralization were significantly greater in areas with high dung concentration, and were significantly ($P < 0.05$**)** correlated ($r^2 \approx 0.50$) with the associated changes in total soil C and N. However, gross N mineralization and nitrification potential were highest in areas with low dung concentration. The decrease in gross N mineralization, combined with greater net N mineralization in dung-concentrated areas, implied that microbial N assimilation was lower in those areas than in the other areas. Also, microbial biomass N

turned over more slowly in dung-concentrated areas than in other areas. The negative association between microbial N immobilization and soil C as a function of dung concentration is inconsistent with a bulk of publications showing that microbial N immobilization was positively related to the amount of soil C. We hypothesized that this negative correlation was due to changes in soil microbial community composition towards dominance of active fungi in dung-concentrated areas.

Introduction

Pasture ecosystems influence global environmental change through their strong potentials for C and N sequestration (Wright et al., 2004) and enhance the quality of life for humans through their provision of meat, milk and wool. Pastures cover ~ 20% of terrestrial land and constitute 10 – 30% of soil organic C (Haynes and Williams, 1993; Schuman et al., 2002). However, pastures may also be detrimental to environment by the production of greenhouse gases such as nitrous oxide and methane, and by the losses of nutrients such as N and P into surface- and ground-water via runoff and leaching (Flessa et al., 1996; Owens and Bonta, 2004). Intensive research has focused on soil fertility and the environmental consequence of pastures (During and Weeda, 1973; Haynes and Williams, 1993). Despite having key roles in the decomposition of soil organic matter and, thus, dictating the environmental fate of C and N, soil microbial biomass and activity have received less attention in pastures than in forests or grasslands.

Grazing animals recycle a large fraction of elements including C, N, P, K, Cu and Zn within a pasture ecosystem, but the redistribution of these elements via animal excreta is often heterogeneous, being highest in camping areas, i.e. near shade and watering sources (Whitehead, 1986; West et al., 1989; Haynes and Williams, 1993; Mathews et al., 1994). This non-uniform distribution of animal excreta will certainly modify soil

chemical and physical attributes. Studies have shown that soil organic C and N, soluble salts and soil pH changed along gradients of animal excreta (Haynes and Williams, 1999; Franzluebbers et al., 2000). These changes in soil properties represent a mosaic of environments for soil microbes and likely alter microbial eco-physiology and their mediated nutrient transformations.

Soil N cycling is driven predominantly by soil microbes, even though microbial biomass accounts for a small fraction of total soil C and N. Nitrogen mineralization and immobilization take place concurrently in a soil, and their balance often determines N available for plant uptake or lost via denitrification and leaching. At an ecosystem scale, C-to-N ratios of soil organic matter are considered a key factor determining the balance between N mineralization and immobilization. However, this balance can be modified by soil microbial community eco-physiology (Bengtsson et al., 2003; Shi et al., 2006). Bengtsson et al. (2003) demonstrated that N mineralization and immobilization in forest soils were more related to microbial community activity than to the soil C-to-N ratio, and thus leaching of nitrate was largely dependent on the density and activity of the microbial community. In a recent study of turfgrass ecosystems, Shi et al. (2006) have also documented that N mineralization and immobilization differed considerably for turfgrass ecosystems of different ages despite similar soil C-to-N ratios. We hypothesized that soil microbial community biomass and activity would be greater in areas of high dung concentration in a pasture ecosystem, which would lead to an increase in gross N mineralization, immobilization and nitrification.

Incorporation of animal dung into soil stimulates microbial growth and activity through the supply of microbially available C (Lovell and Jarvis, 1996). In a grazed pasture, dung is deposited on the soil surface but can become slowly incorporated by animal traffic. One study showed that surface deposition of dung and its consequent slow

decomposition did not affect soil microbial growth. Mineralized nutrients were, subsequently, lost or taken up by plants and thus dung nutrients available to the soil microbial community were not sufficient to cause any measurable effect for a short period (i.e. < 1 y after dung deposition) (Lovell and Jarvis, 1996). Bol et al. (2000) reported that only ~ 20% of cattle dung C could be recovered in soil and leachate, and the rest disappeared from the soil surface after a few months. Hatch et al. (2000b) further documented that short-term (i.e. ~ 3 months) effect of dung deposition on the surface of grasslands varied with soil fertility. They observed that soils with low initial microbial activities would respond positively to surface dung deposition. Taken together, surface-deposited animal excreta may not be directly involved in the fluctuation of microbial growth and activity, but may contribute to stabilized soil C pools, and thus indirectly affect soil microbes. In other words, although surface dung deposition may provide C, N and other nutrients to soil microbes and thus modify microbial biomass and associated N transformations, this impact would be more pronounced with long term (several years) animal grazing.

There is mounting interest in using movable shades and watering sources in pastures so that animal production would be enhanced in small and mid-size farms. This management practice also regulates livestock distribution and is believed to minimize adverse environmental impacts such as N losses to surface and groundwater. Another management practice for balancing animal production with environmental and regulatory pressures is to rotate grazed pasture with hay production system before undesirable environmental impacts take place in pastures. Soil microbiological characteristics and soil fertility are heterogeneous in a grazed pasture; understanding this heterogeneity will help to make informed management decisions. We hypothesized that soil microbial biomass and rates of N transformations would be greatest in animal excreta concentrated areas due

to high concentrations of soil C and nutrients. Our objectives were to determine soil microbial biomass, activity and rates of N mineralization and nitrification in areas of a pasture with different densities of animal excreta deposition. Relationships between microbiological attributes and soil chemical properties were used to assess changes in microbial community eco-physiology associated with dung distribution.

Materials and Methods

Study Site

We chose the study site as a well-managed pasture (~ 2 ha), located in a watershed along the upper reach of Six Runs Creek, Sampson County, North Carolina, USA. A hay production field on the same farm with no excreta was used for baseline comparisons. The pasture and hay production fields had been cultivated with corn (*Zea mays* L.), and were shifted to perennial coastal bermudagrass (*Cynodon dactylon* L.) in 1997 with rye (*Lolium multiflorum* L.) over seeded in winter. Nitrogen fertilization was performed on both fields with the application of swine lagoon effluent, equivalent to 250 kg ha^{-1} y^{-1} of plant available N. The hay production field was mechanically harvested 5 to 7 times y^{-1}. The pasture was grazed intensively since 1999 with ~ 100 feeder animals (beginning bodyweights of 160 – 180 kg) between July and September and with 15 to 20 cow/calve pairs between January and June, but occasionally harvested for hay. The animals were rotated between this pasture and a second pasture (2 ha) at a frequency that avoided over grazing. Soil types in both fields are loamy sand with classification of a Norfolk series (fine-loamy, kaolinitic thermic Typic Kandiudults) for hay production field and of a Wagram series (loamy, kaolinitic, thermic Arenic Kandiudults) for pasture field. A detailed site description was presented by Israel et al. (2005).

Soil Sampling

In general, animal excreta (i.e. dung and urine) are heterogeneously distributed in a pasture field, being highest in the camping areas, i.e. near shade and watering sources (Franzluebbers et al., 2000; White et al., 2001). While N is contained in both dung and urine, nutrients such as P, K, Ca, Mg, Cu and Zn are concentrated in the dung (Haynes and Williams, 1993). In October 2003, areas in the pasture with different densities of dung deposits were visually identified based on cattle grazing behavior. These consisted of two open grazing areas (DC_1, DC_2), one shade area (DC_3) and one area near the watering source (DC_4). A hay production field located ~ 500 m North of the pastures on the farm acted as the baseline (DC_0) (i.e. area without cattle dung) although this field differed considerably from the pasture with respect to aboveground plant dry matter output. The dung concentrated area (DC_3) was located along the fence in an area shaded by trees outside the pasture. The sampling area was a 20 m distance along the fence and 10 m distance away from the trees. This area was < 1% of pasture area. The second dung concentrated area (DC_4) was located approximately 100 m from the water source, near the entrance of a lane leading to the water source. The sampling area was < 2% of the pasture area.

Soil cores were collected in October 2003 from the areas of different dung concentration. Twenty-four cores (5 cm diameter × 15 cm depth) were randomly collected from each of the five sampling areas. Three composite sub-samples from each area were derived by randomly selecting and combining sets of 8 of the 24 soil cores. Soil samples were placed into ice-cold containers and transported to the laboratory. Since dung-derived C was documented to appear primarily at the 0 – 5 cm soil depth rather than the lower soil depth (Bol et al., 2000), we assumed that dung-associated changes in soil physical and chemical properties occurred mainly in the uppermost soil layer. Thus, soil

cores were sectioned into 0 – 5 cm and 5 – 15 cm soil depth, sieved (< 4 mm), roots were removed and stored at 4 °C for later analyses.

In November 2004, the density of dung deposits in the sampling areas was quantified as follows. A short plastic pipe was tossed 10 to 20 times in random directions within the sampling areas. After each toss, a 3 m^2 rectangular frame was centered over the pipe and the number of dung deposits within the frame was counted. The average number of cattle dung deposits in the study areas was as follows: DC_1 (2.4 ± 0.78), DC_2 (3.5 ± 1.03), DC_3 (4.9 ± 0.64) and DC_4 (6.7 ± 1.96) per 3 m^2. Absolute densities of dung deposits may have differed between October 2003 when soil samples were taken and November 2004 when dung densities in the same areas were measured. However, we interpret the measurements in November 2004 to indicate the relative differences in density of dung deposits in the different sampling areas.

Soil Chemical and Microbial Analyses

Total soil C and N were determined by dry combustion using a Perkin-Elmer Series II CHNS/O-2400 analyzer (Perkin Elmer Corp., Norwark, CT) from sub-samples dried at 105 ºC and ground to < 250 µm. Soil inorganic N was extracted with 2 M KCl (soil (g) to KCl (ml) = 1:6) and the filtrates were analyzed colorimetrically for NH_4^+- and (NO_3^- + NO_2^-)-N using a Lachat flow-injection auto-analyzer (Lachat Instruments, Mequon, WI). Soluble organic C was extracted with 0.5 M K_2SO_4 and analyzed with a total organic C analyzer (TOC-5000 Shimadzu). While 0.5 M K_2SO_4 extractable soil C in acidic and neutral soils may be 50% lower than the value of water extractable soil C, the treatment effects do not appear to be affected by the extraction methods (Haney et al., 2001). Soil pH was measured in water (soil (g) to H_2O (ml) = 1:2.5). We also determined soil bulk density at 0 – 7.5 cm soil depth in areas with different dung concentration. Bulk

density was similar in all areas, averaging 1.72 (± 0.05) g cm^{-3}. Other soil properties are given in Table 1.

Soil microbial biomass C and N were determined by the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Organic C in fumigated and unfumigated extracts was measured with a total organic C analyzer (TOC-5000 Shimadzu). Organic N in fumigated and unfumigated extracts was measured with the alkaline persulfate oxidation method (Cabrera and Beare, 1993). Soil microbial biomass C and N were calculated by dividing the difference of total extractable C (or N) between fumigated and unfumigated samples with conversion factors of 0.33 for biomass C and 0.54 for biomass N (Brookes et al., 1985; Sparling and West, 1988).

Potential Rates of C and N Mineralization and Nitrification

A 120-d laboratory incubation was conducted to determine the potential rates of C and N mineralization and nitrification. Mason jars (0.5 l) were used as incubation units; each unit consisted of four 20-ml scintillation vials containing ~ 15 g moist soil (~ 60% water holding capacity) and one scintillation vial containing 5-ml 0.5 M NaOH as a base trap to absorb the CO_2 evolved from microbial respiration. Mason jars containing only base trap were used as controls to determine any contribution from atmospheric CO_2. Periodically, once in a week, the base trap was removed from the Mason jar; the jar was flushed with air for ~ 30 min and a new base trap was placed into the jar. The base trap was titrated with 0.2 M HCl to determine the CO_2 production (Zibilske, 1994). Soil inorganic N was determined 4, 30, 60, and 120 d after the beginning of the incubation by extracting inorganic N with 2 M KCl (soil (g) to KCl (ml) = 1:6) and the filtrates were analyzed colorimetrically for NH_4^+- and (NO_3^- + NO_2^-)-N using a Lachat flow-injection auto-analyzer (Lachat Instruments, Mequon, WI). Potential C or N mineralization was

calculated as the difference between initial and final CO_2-C or soil inorganic N. Nitrification potential was measured by the shaken soil slurry method with 24 h of shaking (Hart et al., 1994b). Microbial biomass C and N were also determined during the incubation by the chloroform fumigation extraction method as described above.

Gross Rates of N Mineralization

Gross rates of N mineralization were measured by ^{15}N isotope pool dilution techniques. Again, 0.5-l Mason jars were used as incubation units; each unit contained two scintillation vials of ~ 12 g soil (equivalent to oven dry weight). Soils were labeled with 5 μg N g^{-1} soil of $^{15}NH_4Cl$ at ~ 50 atom %. The injected solution of $^{15}NH_4Cl$ (0.7 ml) brought the soil water content to ~ 60% water holding capacity. To enhance uniform distribution as well as to minimize soil disturbance, the ^{15}N solution was added as several small-volume injections with a needle and syringe. One scintillation vial was extracted with 100 ml of 2M KCl 30 min after the injection as time 0. The other was incubated for 24 h and then extracted with 100 ml 2 M KCl. The soil inorganic N was determined by the method described above. A diffusion procedure was used to prepare samples for the analysis of ^{15}N in the NH_4^+ pools (Stark and Hart, 1996), and ^{15}N enrichments were analyzed by a Thermo Finningan Delta Plus continuous flow isotope ratio mass spectrometer (CF-IRMS, Bremen, Germany). The rates of gross N mineralization were measured 4, 30, 60 and 120 d after the beginning of the incubation and calculated by the equation of Kirkham and Bartholomew (Hart et al., 1994a).

Data Analysis

Analysis of variance (ANOVA) of a split-plot design with restricted randomization was used to determine significant differences among the areas of dung

concentrations and soil depths; intact soil cores corresponding to individual areas of dung concentration (i.e. DC_0, DC_1, DC_2, DC_3, and DC_4) being the whole plot, soil depth being the split-plot, and three sub-samples being nested within the individual areas. Separation of means was performed with Tukey t-test (SAS Institute Inc. 2001, Cary, North Carolina, USA). To simply clarify the association of soil microbiological properties with dung concentration, we grouped soil samples into three areas: no dung (i.e. DC_0), low concentration of dung (i.e. DC_1 and DC_2), and high concentration of dung (i.e. DC_3 and DC_4) and analyzed treatment effects using a split-plot design with restricted randomization. Linear regression was also performed to examine the relationships of soil microbial properties and N transformations with dung concentration and the associated changes in soil chemical properties.

Results

Soil Microbial Biomass

Soil microbial biomass C and N increased with increasing dung concentration in the pasture ecosystem ($P < 0.05$) (Fig. 1) and were positively correlated with the associated changes in total soil C ($r^2 = 0.48$, $P < 0.05$) and N ($r^2 = 0.41$, $P < 0.05$). This increase was more pronounced at 0 – 5 cm than at 5 – 15 cm soil depth. However, microbial biomass C-to-N ratios were fairly stable throughout the areas of different dung concentration (Fig. 1).

Microbial biomass C or N as a fraction of total soil C or N did not change with the cattle dung concentration, averaging 1.4% of total soil C or 3.5% of total soil N at 0 – 5 cm depth (Table 2). This fraction was also greater at 0 – 5 cm than at 5 – 15 cm soil depth ($P < 0.05$) (Table 2).

Net C and N Mineralization

Net C mineralization, i.e. cumulative CO_2-C, increased with increasing dung concentration in the pasture ecosystem ($P < 0.05$); this increase appeared only at 0 – 5 cm (Fig. 2). The net C mineralization at 0 – 5 cm soil depth was ~ 2-fold greater in high dung-concentrated areas (i.e. DC_3 and DC_4) than in the others. The increase was also positively correlated with the associated changes in total soil C ($r^2 = 0.23$, $P = 0.06$).

Similarly, net N mineralization, i.e. cumulative inorganic N at 0 – 5 cm soil depth, increased with increasing dung concentration ($r^2 = 0.56$, $P < 0.05$) (Fig. 2). The potentially mineralized N during a 120-d incubation at 0 – 5 cm soil depth ranged from 100 μg N g^{-1} soil in high dung-concentrated areas (i.e. DC_3 and DC_4) to 60 μg N g^{-1} soil in the other areas. Again, the increase in the mineralized N was positively correlated with the associated changes in total soil N ($r^2 = 0.42$, $P < 0.05$).

However, net C or N mineralization as a fraction of the total soil C or N did not vary with the cattle dung concentration (Table 2). Microbial metabolic C and N quotients were also similar among areas with different rates of cattle excretal deposition (Table 2). While the qCO_2 values were greater at 0 – 5 cm than at 5 – 15 cm soil depth ($P < 0.05$), the qN values were similar at both depths.

Gross N Mineralization and Nitrification

Rates of gross N mineralization varied with the incubation time, but relative differences among the five areas of dung concentration did not change over time (data not shown). Thus, the values of gross N mineralization rates were averaged across dates and presented in Fig. 3. Since potential rates of nitrification remained constant over time (data not shown), the average rates are presented in Fig. 3.

Rates of gross N mineralization did not increase with increasing dung concentration (Fig. 3). Instead, rates of gross N mineralization in dung-concentrated areas (i.e. DC_3 and DC_4) tended to be lower than in the other areas at 0 – 5 cm soil depth. Also, potential rates of nitrification at 0 – 5 cm soil depth did not increase with the cattle dung concentration, with the highest rate being in the DC_2 area (Fig. 3). Changes in gross N mineralization and nitrification with increasing dung concentration were significantly different ($P < 0.05$) for the 0 – 5 cm soil depth (Fig. 3).

The relationships between net and gross rates of N mineralization were evaluated during the first 30-d of incubation. Ratios of net-to-gross N mineralization changed with the dung concentration at 0 – 5 cm soil depth (Table 3). Net N mineralization at 0 – 5 cm soil depth represented ~ 16% of gross N mineralization in areas with a low-level of dung deposition, but net N mineralization was ~ 30% of gross N mineralization in areas without dung or with high-level of dung deposition (Table 3). This ratio increased to ~ 50% at 5 – 15 cm soil depth. Ratios of biomass N-to-gross N mineralization at 0 – 5 cm soil depth were also higher ($P < 0.05$) in areas with high dung concentration and areas with no dung than in the others (Table 3). Again, these ratios were ~ 2-fold greater at 5 – 15 cm than at 0 – 5 cm soil depth. However, ratios of potential nitrification-to-gross N mineralization declined with increasing dung concentration ($P < 0.05$) (Table 3). These ratios were also ~ 2-fold greater at 5 – 15 cm than at 0 – 5 cm soil depth.

Discussion

Soil Microbial Biomass

Microbial biomass is a small but dynamic soil entity that responds rapidly to changes in the soil environment, including the quantity and quality of soil organic matter and soil nutrient availability. In general, soil microbial biomass C or N constitute a small

fraction, i.e. ~ 1 – 4% of soil organic C and 2 – 6% of soil organic N (Jenkinson and Ladd, 1981; Brookes et al., 1985). A study conducted in New Zealand on soils managed under pastures showed that soil microbial biomass C was ~ 0.3 – 3.9% of soil organic C (Sarathchandra et al., 1984). Our observation that soil microbial biomass C or N averaged 1.4% or 3.5% of total soil C or N at 0 – 5 cm soil depth (Table 2), is consistent with those studies. However, microbial biomass relative to total soil C or N was independent of intensity of animal excretal deposition (Table 2), indicating that any adjustment in soil properties (Table 1) did not alter the relative size of microbial community biomass. The significantly positive correlation of microbial biomass with soil C and N further suggests that soil organic C and N contents were the major factors that determined the population size of the soil microbial community. Soil microbial biomass in proportion to soil C or N differed significantly between the two soil depths (Table 2). The higher values at 0 – 5 cm than at 5 – 15 cm soil depth indicate that soil organic matter was more biodegradable in the surface soil layer. It may also result from soil abiotic conditions that were more favorable to the soil microbial community at the surface.

Soil microbial biomass C was positively correlated with K_2SO_4 extractable soil organic C ($r^2 = 0.24$, $P = 0.06$) and increased with increasing dung concentration. However, this correlation may not represent a direct causality between soluble C and soil microbial biomass. First, soluble C at 5 – 15 cm in stock camping areas (i.e. DC_3 and DC_4) was similar to the one at 0 – 5 cm in non-grazing and open grazing areas (i.e. DC_0, DC_1 and DC_2) (Table 1). However, microbial biomass was ~ 4-fold greater at 0 – 5 cm in non-grazing and open grazing areas (i.e. DC_0, DC_1 and DC_2) compared to stock camping areas (i.e. DC_3 and DC_4) at 5 – 15 cm depth (Fig. 1). Second, the K_2SO_4 extractable soil organic C was constant throughout the incubation period, but microbial biomass C declined over time and especially 30 d after initiating the incubation

(data not shown). Lundquist et al. (1999) also demonstrated that soluble soil organic C might not indicate C availability to soil microbes. Boyer and Groffman (1996) compared agricultural soils with forest soils and observed that while agricultural soils generally had lower amounts of total soil C and microbial biomass C than forest soils, the former support equal or greater rates of microbial activity than forest soils due to increased production of soluble soil C.

Soil microbial biomass serves as a sink and source of N and thus plays an important role in regulating soil available N and preventing undesirable N loss from soil systems. In poorly-drained pasture soils, microbial biomass N was observed to be 40 times greater than soil inorganic N in the top 10 cm soil depth, and at least 7 times greater than soil inorganic N in well-drained soils (Lovell et al., 1995). In our study, however, soil microbial biomass N was only ~ 2 times greater than the soil inorganic N at 0 – 5 cm soil depth and was equivalent to the soil inorganic N at 5 – 15 cm soil depth (Fig. 1 and Table 1). Our results suggest that the soil microbial biomass in the 4 y-old pasture has not reached its potential for assimilating N into soil.

Microbial Activities and their Associations with Soil Chemical Properties

While microbial activity (i.e. CO_2 respiration) was positively correlated with soil C and microbial biomass ($r^2 = 0.70$, $P < 0.05$), ratios of mineralized C to total soil C were constant with increasing dung concentration (Table 2). Haynes and Williams (1999) also reported that mineralized C in relation to soil organic C was independent of heterogeneous distribution of dung and urine, ranging from 1.6% to 3.0% for the six sites of established pastures. But the ratios of mineralized C to total soil C were significantly higher at 0 – 5 cm compared to 5 – 15 cm soil depth (Table 2), indicating that soil

microbial biomass was more active and/or that soil organic C was more degradable in the uppermost soil layer.

Integration of soil biological and chemical properties is considered to be more useful than individual variables in describing soil fertility and microbial community eco-physiology (Insam and Domsch, 1988). In this pasture ecosystem, relationships between several soil biological and chemical properties differed between soil depths, but not among cattle dung concentration (Table 2). For example, the ratio of mineralized to total soil N was constant with increasing dung concentration. Further, microbial community specific activity (i.e. qCO_2 and qN) did not differ among cattle dung concentration. These results were similar to the observation of Haynes and Williams (1999); in which microbial communities inhabiting dung-concentrated areas versus other areas expressed no differences in their microbial specific activities. The lack of differences in qCO_2 and qN reveals that microbial community C and N use efficiency was, in general, independent of dung concentration for a pasture ecosystem subjected to 4 y of grazing management.

Soil N Transformations

While net N mineralization increased with increasing dung concentration, gross N mineralization apparently did not support our hypothesis, as it was lower in dung-concentrated areas than in no dung or low dung concentration areas (Fig. 3). Since net N mineralization is the balance between gross N mineralization and microbial N immobilization, microbial N immobilization would be lower in dung-concentrated areas than in the other areas. It is well known that soil organic C is a primary factor regulating microbial N immobilization (Hart et al., 1994a). Barrett and Burke (2000) documented a positive relationship between microbial N immobilization and soil organic C contents in many grassland soils. In this pasture ecosystem, however, the derived microbial N

immobilization apparently declined with the increase in total soil C, which was unexpected and in contrast to the trend reported for natural, unmanaged ecosystems (Hart et al., 1994a; Barrett and Burke, 2000).

Our observation on gross N mineralization was consistent with those conducted in pasture ecosystems. Examination of gross N mineralization in soil directly beneath cattle dung patches verified that gross N mineralization was little affected by cattle dung (Hatch et al., 2000a). Also, soil N availabilities seemed to have no effect on gross N mineralization; but gross N immobilization varied with available soil N (Ledgard et al., 1998; Hatch et al., 2000a). Soils with the long-term N fertilization exhibited lower gross N immobilization than those without fertilization (Ledgard et al., 1998; Hatch et al., 2000a). Along with increasing dung concentration, the increased soil N availability might overwhelm soil C effects, and thus lead to the observed reduction in microbial N immobilization.

Reduction of microbial N immobilization with increasing dung concentration may result from the changes in microbial community composition. Bardgett et al. (1997) demonstrated that long-term grazing areas differed in soil microbial community composition from non-grazing areas. They observed that a ratio of active fungal to bacterial biomass was considerably higher in grazing compared to non-grazing areas, and this effect was most pronounced in intensively grazed fields. Since fungi contained less N in their biomass, microbial N assimilation was likely to be lower in the grazed areas, despite significant decomposition of soil organic C. However, we did not notice changes in biomass C-to-N ratios, a general indication of change in soil microbial community composition (Fig. 1). It is well known that the precision and accuracy of microbial biomass measurement by chloroform fumigation extraction are highly dependent on the extraction coefficient. A study showed that the extraction coefficient for microbial

biomass C (i.e. K_{ec} value) for manure-treated soils was lower than for untreated soil (Tessier et al., 1998). This suggests the C-to-N ratios of microbial biomass in the dung-concentrated areas may have been underestimated.

The potential rate of nitrification indicates the population size of soil nitrifiers. Soil nitrifiers are able to compete strongly with heterotrophic microbes for NH_4^+, the product of N mineralization, and thus potential rates of nitrification could correlate well with the rates of gross N mineralization. We also observed positive correlation between potential rates of nitrification and the gross N mineralization ($r^2 = 0.30$, $P < 0.05$), but not with net N mineralization. The lower potential rates of nitrification in dung-concentrated areas (Fig. 3) may have several explanations. First, available soil C was higher in dung-concentrated areas than in other areas (Table 1). Therefore, soil heterotrophs may out-compete nitrifiers for NH_4^+, leading to reduction in soil nitrifiers populations. However, this explanation was not consistent with our observation that gross N immobilization (gross N mineralization – net N mineralization) was low for dung-concentrated areas Secondly, soil properties associated with animal excretal deposition may affect soil microbial metabolism and thus N processes. For example, soil salt concentrations could considerably influence microbial N mineralization, immobilization and nitrification (Low et al., 1997). But soil electrical conductivity was not correlated with the potential rate of nitrification in areas with different concentration of dung. Third, dung-associated changes in soil properties may modify the soil microbial community and thus its eco-physiology. We hypothesize that high N availability in dung-concentrated areas may limit the production of extracellular enzymes involved in N mineralization. In addition, high levels of elements other than C and N at dung-concentrated areas may inhibit the functionality of extracellular enzymes. As we discussed previously, soil microbial community may also

change with increasing dung concentration, and community may be dominated by microbes with slow metabolic N activity.

Microbial biomass N turnover, i.e. ratio of microbial biomass N to the rate of gross N mineralization, tended to be slower in dung-concentrated areas than in the others (Table 3). This longer turnover time of microbial biomass N and the larger inorganic N pool in dung-concentrated areas suggest a potential for N loss via leaching and denitrification. In general, the camping areas constitute < 10% of the grazing land and, thus, for the young grazing land, the N loss may not pose serious threats to the environment. However, care should be taken to minimize the additive effect of dung concentration in the open grazing areas. Rotation of pastures with hay production fields may be a good practice to minimize the N loss-associated environmental issues.

In comparison to 0 – 5 cm soil depth, the 2-fold increase in ratios of net to gross N mineralization at 5 – 15 cm soil depth (Table 3) indicated a reduction in microbial N assimilation, possibly due to C limitation at deeper soil depth. Again, ~ 2-fold higher ratios of nitrification potential to gross N mineralization at 5 – 15 cm than at 0 – 5 cm soil depth implies that available soil C limited microbial N assimilation at the deeper soil depth. It also showed that ratios of microbial biomass N to gross N mineralization were ~ 2-fold higher at 5 – 15 cm than at 0 – 5 cm soil depth (Table 3). Longer turnover time further indicated that the microbial community at the lower depth was limited by soil C availability. As we discussed previously, the K_2SO_4 extractable soil C might not be a reliable indicator for soil C availability (Table 1).

Conclusion

The rates of soil N cycling processes were spatially heterogeneous within the pasture ecosystem and these differences were well correlated with changes in dung

concentration-associated soil properties. Soil microbial community biomass, microbial respiration and net N mineralization all increased with increasing dung concentration. However, the gross N mineralization and potential rates of nitrification were highest in areas with low level of dung concentration. In the investigated pasture ecosystem, mineralized C (or N) and microbial biomass C (or N) in proportion to total soil C (or N) did not vary with the history of animal excretal deposition. Again, microbial metabolic quotients, qCO_2 and qN values, were fairly stable across the areas of different dung concentration. But the ratios of net-to-gross N mineralization and microbial biomass N-to-gross N mineralization tended to be higher in dung-concentrated areas than in the low level of dung. This suggests a potential for increased N loss from dung-concentrated areas compared to less concentrated areas due to the reduction in microbial N assimilation and slower microbial biomass N turnover.

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Table 1. Selected soil properties at 0 – 5 cm and 5 – 15 cm soil depth in areas with different dung concentration in a pasture ecosystem. Values are means (standard errors) for n = 3.

	Soil C	Soil N	Soil C:N	Inorganic N	Soluble C[§]	Soil pH	EC[¶]
	(mg C or N g^{-1} soil)			(μg N or C g^{-1} soil)			(dS m^{-1})
0 – 5 cm depth							
DC_0[†]	11.4 (0.3)	1.0 (0.0)	11.4 (0.3)	19.1 (2.7)	36.4 (3.0)	6.47 (0.10)	0.15 (0.01)
DC_1	11.6 (0.9)	1.0 (0.1)	12.0 (0.4)	20.8 (2.6)	49.9 (2.4)	6.63 (0.36)	0.17 (0.03)
DC_2	17.6 (2.1)	1.4 (0.2)	12.3 (0.1)	21.0 (2.0)	45.7 (9.9)	6.81 (0.19)	0.33 (0.01)
DC_3	17.5 (1.4)	1.5 (0.1)	11.6 (0.0)	28.1 (5.2)	69.9 (3.7)	6.04 (0.34)	0.25 (0.01)
DC_4	21.4 (4.4)	1.8 (0.3)	12.0 (0.3)	35.8 (19.0)	62.9 (6.0)	6.61 (0.09)	0.27 (0.05)
5 – 15 cm depth							
DC_0	4.7 (0.4)	0.4 (0.0)	11.9 (1.0)	9.3 (2.0)	16.6 (2.0)	6.37 (0.05)	0.10 (0.01)
DC_1	5.2 (0.4)	0.4 (0.0)	12.0 (0.1)	10.0 (2.2)	22.9 (1.3)	6.48 (0.24)	0.09 (0.02)
DC_2	6.8 (0.7)	0.5 (0.1)	13.7 (0.6)	16.0 (1.2)	22.6 (4.1)	6.64 (0.20)	0.13 (0.03)
DC_3	6.1 (0.1)	0.6 (0.0)	10.9 (0.5)	5.7 (2.1)	41.3 (1.6)	5.42 (0.52)	0.10 (0.02)
DC_4	6.4 (0.5)	0.5 (0.0)	12.1 (0.9)	18.8 (11.3)	36.1 (3.1)	6.21 (0.04)	0.15 (0.04)

[§] 0.5 M K_2SO_4 soluble soil organic C
[¶] Electrical conductivity
[†] DC_0, DC_1, DC_2, DC_3 and DC_4 represent soil samples collected from the areas with no, low to high concentration of cattle dung, respectively. See details for soil samples in the Materials and methods.

Table 2.The amount of total soil C and N that was mineralized or that was in microbial biomass, and microbial C or N metabolic quotient (qCO_2 or qN)[§] for soils sampled from 0 – 5 cm and 5 – 15 cm soil depth in areas with different dung concentration in a pasture ecosystem. Values are means (standard errors) for n = 3.

	Mineralized C	Mineralized N	Biomass C	Biomass N	qCO_2	qN
	as a fraction of total soil C or N (%)				(d^{-1})	(d^{-1})
0 – 5 cm depth						
DC_0[†]	6.60 (0.40)	5.95 (0.48)	1.41 (0.09)	3.40 (0.28)	4.3 (0.2)	2.2 (0.2)
DC_1	6.43 (0.24)	5.63 (0.13)	1.23 (0.18)	3.96 (0.12)	4.9 (0.8)	1.7 (0.1)
DC_2	5.46 (0.71)	5.77 (0.80)	1.13 (0.15)	2.88 (0.15)	4.8 (0.8)	2.1 (0.2)
DC_3	8.32 (0.75)	6.35 (0.65)	1.66 (0.13)	3.99 (0.34)	4.2 (0.3)	1.3 (0.1)
DC_4	7.50 (2.10)	6.84 (1.59)	1.34 (0.23)	3.13 (0.88)	4.7 (0.5)	2.4 (0.9)
5 – 15 cm depth						
DC_0	2.74 (0.24)	4.37 (0.33)	0.78 (0.06)	1.39 (0.38)	2.5 (0.1)	3.8 (1.1)
DC_1	2.54 (0.15)	3.37 (0.23)	0.71 (0.06)	1.89 (0.47)	3.7 (0.9)	2.1 (0.7)
DC_2	1.86 (0.14)	3.01 (0.31)	0.63 (0.13)	1.57 (0.25)	3.4 (1.4)	3.0 (0.1)
DC_3	2.08 (0.13)	2.31 (0.26)	1.35 (0.19)	2.14 (0.04)	3.0 (0.3)	0.9 (0.2)
DC_4	1.91 (0.22)	3.41 (0.58)	0.63 (0.10)	2.15 (0.58)	1.1 (0.1)	2.1 (0.3)

[§] Actual values of qCO_2 and qN are the values in the table divided by 100.
[†] See Table 1 for sample labeling.

Table 3. Relationships of N transformation processes[§] at 0 – 5 cm and 5 – 15 cm soil depth in areas with different dung concentration in a pasture ecosystem. Values are means (standard errors) for n = 3.

	Net min./gross min	Pot. nitri./gross min	MBN/gross min (d)
0 – 5 cm depth			
DC_0[†]	0.27 (0.06)	4.80 (0.15)	12.1 (1.7)
DC_1	0.16 (0.01)	4.82 (0.56)	9.5 (0.7)
DC_2	0.16 (0.02)	4.06 (0.47)	7.7 (0.5)
DC_3	0.28 (0.04)	3.45 (0.80)	22.8 (4.7)
DC_4	0.29 (0.08)	2.92 (1.11)	13.6 (4.2)
5 – 15 cm depth			
DC_0	0.57 (0.01)	8.33 (0.97)	18.0 (5.2)
DC_1	0.53 (0.13)	10.17 (2.42)	28.7 (7.2)
DC_2	0.57 (0.01)	9.49 (3.30)	19.3 (1.2)
DC_3	0.29 (0.04)	3.18 (1.09)	33.7 (4.2)
DC_4	0.62 (0.07)	6.96 (2.34)	30.5 (3.9)

[§] Min. represents mineralization. Pot. nitri. is potential rate of nitrification. MBN represents microbial biomass N.
[†] See Table 1 for sample labeling.

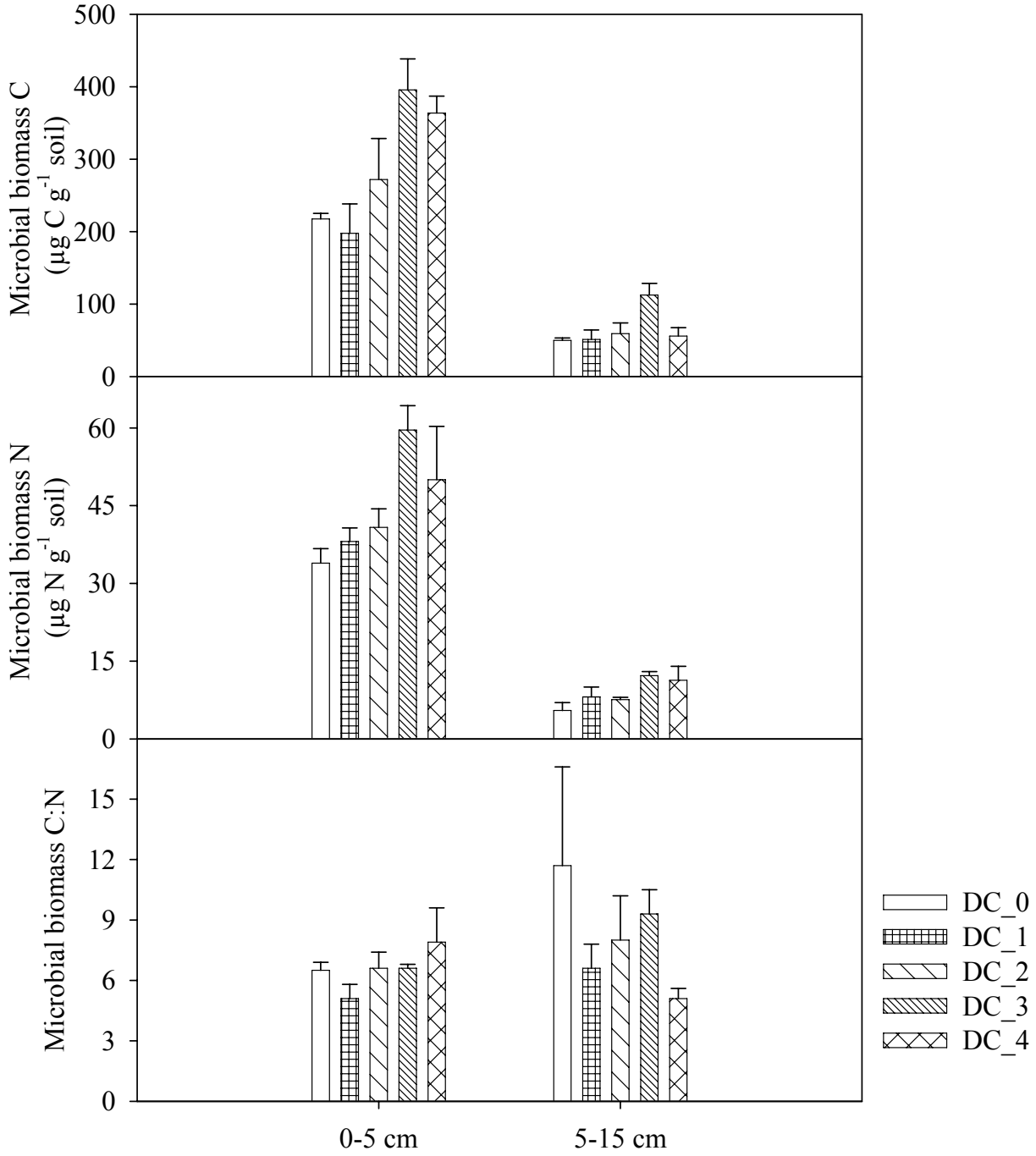

Fig. 1. Microbial biomass C, N and its C-to-N ratio in areas with different dung concentration in a pasture subjected to 4 y cattle grazing. Values are means and standard errors for n = 3. DC_0, DC_1, DC_2, DC_3 and DC_4 represent soil samples collected from the areas with low to high concentration of cattle dung, respectively. See details for soil samples in the Materials and methods.

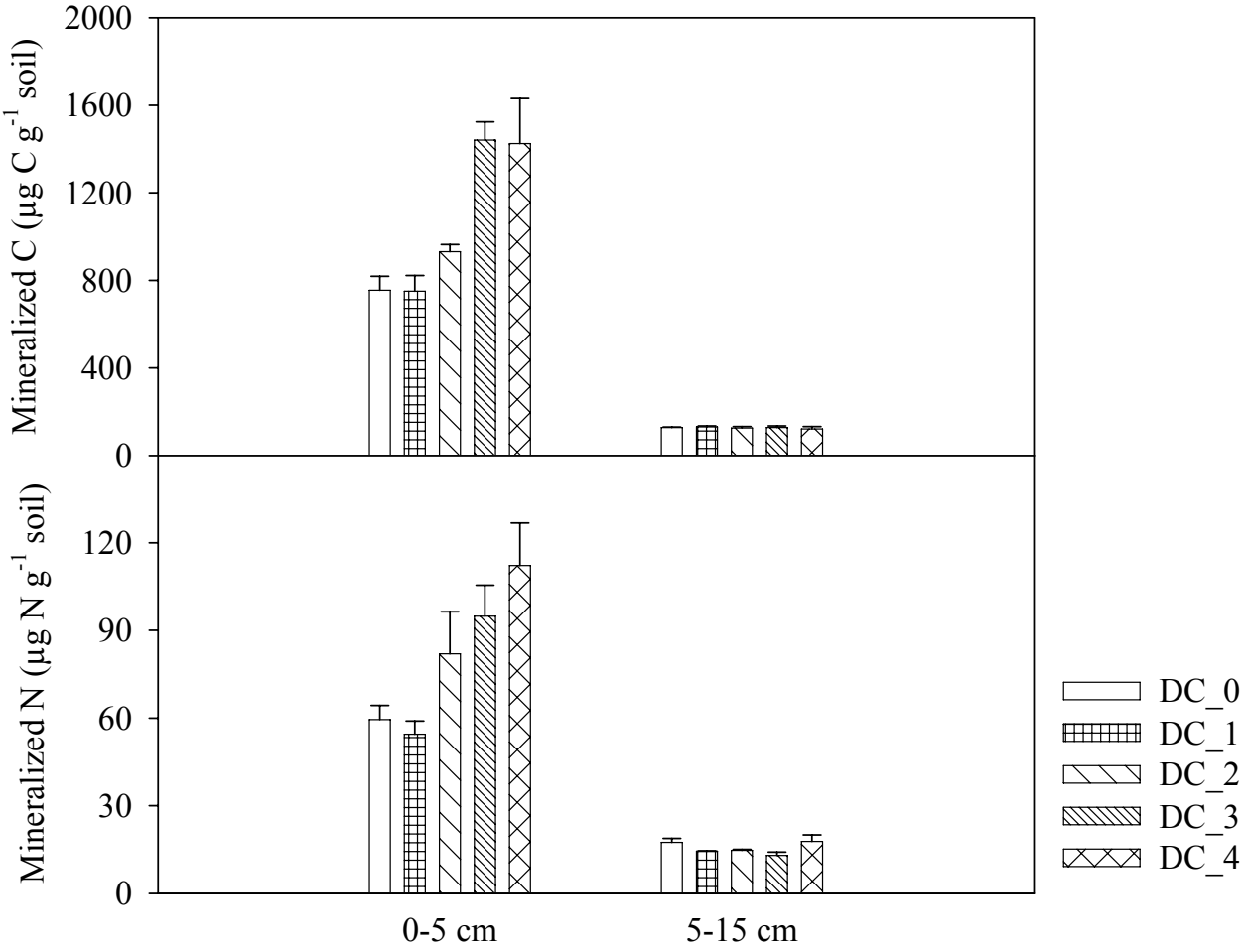

Fig. 2. Net C and N mineralization in areas with different dung concentration in a pasture subjected to 4 y cattle grazing. Values are means and standard errors for n = 3. See Fig. 1 for sample labeling.

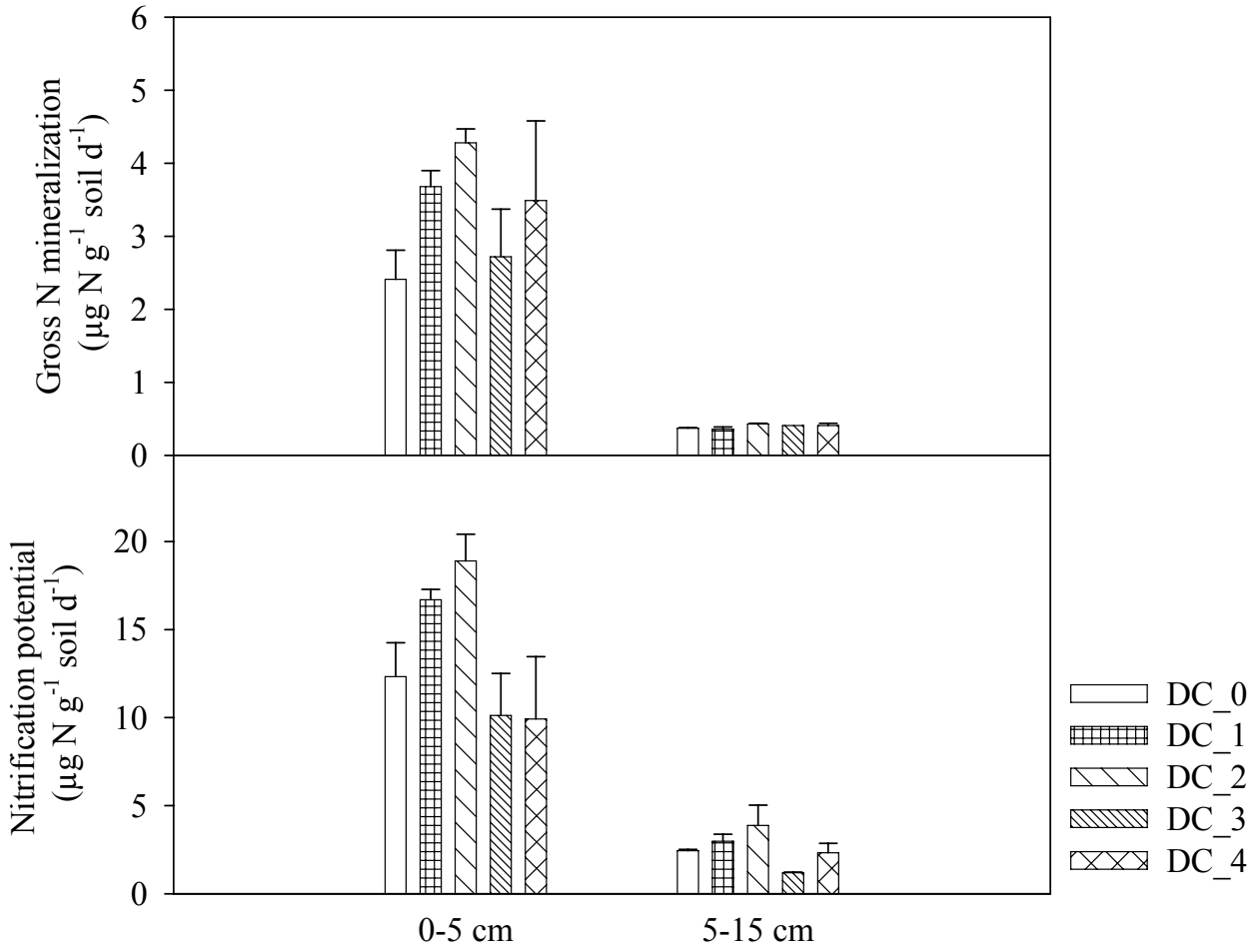

Fig. 3. Gross N mineralization and nitrification potential in areas with different dung concentration in a pasture subjected to 4 y cattle grazing. Values are means and standard errors for n = 3. See Fig. 1 for sample labeling.

CHAPTER THREE

SOIL MICROBIAL PROPERTIES IN FOARAGE SYSTEMS: CONSEQUENCES OF FERTILIZATION RATE AND SOURCE

Abstract

The level of nitrogen fertilization may alter the soil microbial community through effects on plant productivity and/or soil physical and chemical properties. This study characterized soil microbiological properties in a forage system that received from 0 to 600 kg plant available N ha^{-1} yr^{-1} from either swine lagoon effluent (SLE) or ammonium nitrate (AN). The forage system contained plots planted with bermudagrass (*Cynodon dactylon* L.) or endophyte-free tall fescue (*Festuca arundinaceae* Schreb.) and received N fertilization from 1999 to 2001. In March 2004, the plots were sampled for measurement of a suite of soil chemical and microbiological properties. Nitrogen fertilization rates were significantly correlated with soil pH and K_2SO_4 extractable soil C, but not with total soil C, soil C/N ratio, electrical conductivity, or Mehlich-3 extractable nutrients. Soil supplied with SLE had significantly lower Mehlich-3 extractable nutrients, than soil supplied with AN. In general, two indicators of soil N supplying capacity, i.e. potentially mineralizable N and amino sugar N content, peaked at an N application rate of 200 or 400 kg available N ha^{-1} yr^{-1}. The extent of this influence varied with plant species and types of N fertilizers. Soil microbial biomass C also peaked at an application rate of 200 or 400 kg available N ha^{-1}. Nitrification potential was significantly higher in soil supplied with AN than in the unfertilized control, but was similar between SLE-receiving and unfertilized soils. Our results indicate an application rate

as high as 600 kg available N ha^{-1} may not benefit soil microbial community proliferation and its mediated N transformation processes.

Introduction

Nitrogen fertilization is the most important management practice in boosting forage production. In the southeastern USA, expansion of the confined swine industry has led to widespread application of swine lagoon effluent (SLE) in managed forage systems. Like synthetic N fertilizer, SLE can increase yield and improve nutritive value of forage dry matter (Adeli et al., 2005). Additionally, SLE application may have profound impacts on soil physical and chemical properties. Liu et al. (1998) reported that in comparison to ammonium nitrate (AN), 3-yr SLE application reduced soil pH and base cations such as Ca^{2+} and Mg^{2+} and increased soluble salt concentration, indicating an altered soil environment between the two types of N fertilizer. Microbial growth and activity are very responsive to changes in the soil environment. For example, acidity and high salt concentration in soil reduced microbial biomass and its mediated nutrient transformations (Rietz and Haynes, 2003). A thorough assessment of the impacts of SLE application on soil microbiological properties may help manage SLE to benefit soil fertility but minimize loss of N via leaching and denitrification.

Recommendation of N fertilization is primarily based on N response of plant yield and of dry matter quality such as protein and fiber contents. Although soil microbes are vital in governing soil N turnover, retention and therefore the environmental fate of soil and fertilizer N, the response of soil microbial community to N fertilization has received little attention in developing N best management practices (Mulvaney et al., 2006). Nitrogen fertilization often stimulates primary productivity and, thus, improves soil C availability

through root exudates and inputs of dead roots and aboveground plant materials, which will certainly enhance soil microbial biomass and activity. However, plant yield does not increase linearly with N fertilization. In a forage production system, yield may reach a plateau at ~ 450 kg N ha^{-1} yr^{-1} (Harvey et al., 1996; Adeli et al, 2005). When N fertilizer is applied at a rate above which there is slight yield response, N itself and possibly resultant altered soil environment may serve as selective pressures that modify the soil microbial community. High N application rates can alter forage quality, such as increasing tissue NO_3^-concentration (Harvey et al, 1996; Adeli et al., 2005) and likely pose an environmental threat as a result of reduced N uptake efficiency by the crop and increased NO_3^- leaching (Burns et al, 1985; Adeli et al., 2001). However, the impacts of high N rates on belowground biological complexity are less understood. Microbial biomass and activity may not be linearly associated with N application rates. Indeed, there have been inconsistent or contradictory findings (i.e., positive, negative, or no effects) regarding the influence of N fertilization on soil microbiological properties (Biederbeck et al., 1996; Glending et al., 1996; Liebig et al., 2002). These conflicting results are likely attributable to the different rates and types of N fertilizers used in various studies. The magnitude of N fertilization and associated yield response are crucial to thoroughly address the influences of N fertilization on the soil microbial community.

Nitrogen fertilization likely has residual effects on soil N transformations (Shi and Norton, 2000). We believe that SLE application may also have residual effects on soil microbial community ~ 3 yr after the termination of fertilization. We hypothesize that the influence of SLE on the soil microbial community depends on application rate. When receiver crop yield increases with N application rate, SLE may positively affect the soil

microbial community, microbial biomass, and activity. However, when receiver crop yield does not respond to N applied above at optimum levels, SLE may have negative impacts on the soil microbial community biomass and activity due to an altered soil environment. In this study, we examined soil microbiological properties in forage systems 3-yr after applying different rates of SLE and synthetic N fertilizer for three growing seasons. Microbial biomass, respiration, and potential rates of N mineralization and nitrification were assessed in bermudagrass and tall fescue forage production systems.

Materials and Methods

Study Site and Experiment

The study site was located on the Caswell Farm, a research station of North Carolina State University, near Kinston, Lenoir County, NC. The soil was classified as Pocalla loamy sand (loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults). Field plots were established in fall 1998 to examine the responses of yield and N content of forage dry matter to different SLE application rates. Four application rates of 0, 200, 400, and 600 kg plant available N (PAN) ha^{-1} yr^{-1} were compared between SLE and AN across warm- and cool-season grass species of bermudagrass (overseeded with rye), tall fescue, gamagrass, and crabgrass (overseeded with rye), and rescue grass and we chose two forage species (bermudagrass and tall fescue) for this study. A partially stripped split plot design was used with three replications: grass species were stripped across fertilization source (i.e. SLE and AN); application rates were stripped across grass species and nested within fertilization source of each replication. A treatment split plot was ~ 3 m × 6 m, SLE was applied using a small plot effluent sprayer; the application rate was calculated as 60% of total N of SLE as

PAN and verified using collection cups placed on the plots. Chemical composition of SLE during ~ 3 yr application averaged ~ 293 mg total N L^{-1}, 87 mg P L^{-1}, 198 mg K L^{-1}, 154 mg Ca L^{-1}, 58 mg Mg L^{-1}, 28 mg S L^{-1}, 84 mg Na L^{-1} and trace amounts (i.e. < 10 mg L^{-1}) of Fe, Mn, Zn, and Cu. SLE was neutral with a value of pH 7.1. For plots that received broadcast application of AN and unfertilized control plots, P and K were also applied based on annual soil test recommendation. Because SLE was irrigated onto plots, the plots with AN application received irrigation water equivalent to that in plots with SLE. Both SLE and AN were applied every month from February to November; the split amounts of N were based on the growth physiologies of individual grass species. Forages were harvested in a manner that was consistent with the recommended practices for individual species. Fertilization treatments were implemented from April 1999 to November 2001. The yield and N content of forage dry matter are given in Table 1. Thereafter, the treatments were terminated but the forage was continually mowed and baled for hay until December 2004.

Soil Sampling and Chemical Analysis

Soil samples were collected from the uppermost 0-7.5 cm depth in March 2004 from the field plots planted with bermudagrass and tall fescue. Three soil cores (5 cm diameter) were sampled from the center of each plot and composited. Soils were sieved (< 4 mm) and stored at 4 °C for later analyses of soil chemical and microbiological properties.

Total soil C and N were determined by dry combustion using a Perkin-Elmer Series II CHNS/O-2400 analyzer (Perkin Elmer Corp., Norwark, CT) from sub-samples dried at 105 ºC and ground to < 250 µm. Soil inorganic N was extracted with 2 M KCl (soil (g):KCl (ml) = 1:6) and the filtrates were colorimetrically analyzed for NH_4^+- and (NO_3^- + NO_2^-)-N using

a Lachat flow-injection auto analyzer (Lachat Instruments, Mequon, WI). Soluble organic C was extracted with 0.5 M K_2SO_4 and analyzed with a total organic C analyzer (TOC-5000, Shimadzu). P, K, Ca, Mg, Mn, Cu, and Zn were extracted with Mehlich-3 solution (Mehlich, 1984) and analyzed by inductively coupled plasma (ICP) emission spectrophotometer (Perkin-Elmer ICP-OES 2000DV, Elmer Corp., Germany). Soil pH was measured in 1:1 soil/water slurry. Electrical conductivity (EC) was measured from a 1:5 soil/water extraction.

Soil Microbial Biomass and Respiration

Soil microbial biomass C and N were determined by the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Organic N in fumigated and unfumigated extracts was measured after the alkaline persulfate oxidation (Cabrera and Beare, 1993). Extraction coefficients were 0.33 and 0.54 for microbial biomass C and N calculation, respectively (Brookes et al., 1985; Sparling and West, 1988).

Soil microbial biomass respiration was determined by an alkaline base trap method (Zibilske, 1994) during a 30-d laboratory incubation. We used Mason jars (0.5 L) as incubation units; each unit contained 60 g moist soil at 60% apparent water holding capacity and a vial containing 5-ml 0.5 M NaOH to absorb CO_2 evolved from microbial respiration. Periodically, once in week, Mason jars were flushed with air for ~ 30 min to avoid anaerobic incubation and the base traps were replaced with new ones. The replaced base traps were titrated with 0.2 M HCl to determine the CO_2 production. Microbial respiration, i.e. C mineralization, was calculated as the cumulative CO_2-C during the 30-d incubation.

Soil N Supplying Capacity and Nitrification Potential

Nitrogen mineralization was used as an indicator of soil N supplying capacity. It was determined during the 30-d laboratory incubation for measuring CO_2 respiration. Soil inorganic N was determined at the beginning and end of the incubation via 2M KCl extraction and colorimetric analysis described above. Nitrogen mineralization was calculated as the difference between initial and final soil inorganic N. Amino sugar N content was used as an indicator of soil N supplying capacity. Amino sugar N was determined by the method of Khan et al. (2001). Nitrification potential was determined using the shaken soil slurry method (Hart et al., 1994).

Statistical Analysis

Since the objective of the study was not to directly compare the effect of forage species, data on soil chemical and microbial properties for bermudagrass and tall fescue systems were analyzed by individual forage species in a split-plot design, with N source as the whole plot factor and N rate as the sub-plot factor. The PROC MIXED in SAS 9 version (SAS Institute Inc. 2001, Cary, North Carolina, USA) was used to carry out the split-plot analysis and the differences among treatment means were compared by F-protected least significant differences (LSD) at $P < 0.05$.

Results

Soil Chemical Properties and Mehlich-3 Extractable Nutrients

Nitrogen fertilization effects were primarily reflected in soil N, soil pH, soluble C, and Mehlich-3 extractable element content of bermudagrass and tall fescue systems (Table 2). In bermudagrass and tall fescue systems, soil pH was significantly ($P < 0.05$) lower in soils supplied with SLE than those supplied with AN (Tables 2 and 4a, b). Across fertilization rates, soil pH was 0.5 units or 0.3 units lower in SLE- than AN-applied bermudagrass plots or tall fescue plots respectively (Tables 4a, b). Soil pH was also decreased significantly ($P < 0.05$) with increased fertilization rates (Table 2). In the bermudagrass system, soil pH was 0.8 units lower in plots supplied N at 600 kg ha^{-1} as AN or SLE than the unfertilized controls (Table 4a).

While fertilizer source had no significant effect on soil total N, and K_2SO_4 extractable soluble C content in bermudagrass and tall fescue plots, fertilization rate did ($P < 0.05$) (Table 2). In comparison with unfertilized controls, fertilization significantly ($P < 0.05$) increased soil total N, soluble C in bermudagrass and tall fescue plots (Tables 4a, b). The highest soil soluble C content occurred in soils supplied with N at 200 or 400 kg ha^{-1}. There was ~ 50% reduction from the highest soluble C content when soils received N at 600 kg ha^{-1}.

The significant interaction between N source and levels of N fertilization in tall fescue system (Table 2) indicated that more soil N was accumulated when increased N rates were used in conjunction with AN. Soil total N content increased with fertilization and accordingly soil C/N ratios were numerically lower in soils fertilized with AN or SLE than the unfertilized controls in bermudagrass and tall fescue systems (Tables 4a, b).

The significant interaction between N source and levels of N fertilization rate in bermudagrass plots indicated that Mehlich-3 extractable P and Ca were displaced to a greater extent when increased N rates from SLE were used (Table 2). Ca and Mg contents were ~ 30 and ~20% lower for soils receiving SLE versus receiving AN in bermudagrass and tall fescue plots respectively (Tables 5a, b).

Soil Microbial Biomass and Respiration

In bermudagrass and tall fescue plots, soil microbial biomass C was significantly ($P < 0.05$) affected by rate of fertilization, but biomass N was not (Table 3). The significant interaction between source and rate of N fertilization on microbial biomass C in bermudagrass plots indicated that microbial biomass C content was increased to a greater extent when increased N rates with AN as N source were used (Table 3). In general, microbial biomass C was highest at a fertilization rate of 200 or 400 kg N ha^{-1}, and was similar to the unfertilized control at the rate of 600 kg N ha^{-1} (Fig. 1). In bermudagrass plots, the highest microbial biomass C was ~ 3- (in 200 kg N ha^{-1}) and 1.8-fold (in 400 kg N ha^{-1}) greater in soils supplied with AN and SLE than in the unfertilized control, respectively.

Biomass C/N ratio showed tends similar to those of biomass C with the source and rate of fertilization in the bermudagrass system (Table 3, Fig. 1). In bermudagrass plots, microbial biomass C/N ratio was highest at an application of 200 or 400 kg N ha^{-1}. The highest biomass C/N ratio was 14 or 9 for soils supplied with AN or SLE, respectively, as compared to 4 in unfertilized controls. Again, in bermudagrass plots, biomass C/N ratio was also higher in soils supplied with AN than in soils supplied with SLE (Table 3). The significant interaction between source and rate of N fertilization on microbial biomass C/N in

bermudagrass plots indicated that microbial biomass C/N was greater when increased N rates were used with AN as N source (Table 3). Unlike microbial biomass, microbial respiration (i.e. microbial activity or potentially mineralized C) did not vary with the source and rate of fertilization in bermudagrass and tall fescue systems (Table 3, Fig. 2)

Soil N Supplying Capacity and Nitrification Potential

Nitrogen mineralization potential did not differ between soils supplied with SLE versus AN in bermudagrass and tall fescue systems (Table 3, Fig. 2). But in bermudagrass plots, N mineralization potential was significantly higher ($P < 0.05$) in fertilized soils than in unfertilized controls. In bermudagrass, N mineralization potential was ~ 50% greater in soils supplied with 200 kg N ha^{-1} than in the unfertilized control. A fertilization rate higher than 200 kg N ha^{-1} did not cause any additional increase N mineralization potential.

In bermudagrass and tall fescue, soil amino sugar N (ASN) content was not affected by N source but increased with fertilization rate, and the significant interaction between source and rate of N fertilization suggested ASN was conserved to a greater extent when increased N rates were used in conjunction with AN fertilization (Table 3). ASN in bermudagrass plots increased with fertilization up to 400 kg N ha^{-1}, but it only increased with fertilization up to 200 kg N ha^{-1} in tall fescue plots. The highest ASN content was ~ 100 µg N g^{-1} in fertilized soils compared to ~ 60 µg N g^{-1} in unfertilized controls.

Nitrification potential was significantly ($P < 0.05$) lower in soils supplied with SLE than with AN (Table 3). The significant interaction between source and rate of N fertilization suggested nitrification potential was lower to a greater extent when increased N rates from

SLE were used (Table 3). Nitrification potential increased with the AN application, but remained stable or decreased significantly at the highest rate with SLE application (Fig. 2).

Biological C or N Reservoir as a Fraction of Total Soil C or N

Microbial biomass C as a fraction of total soil C was increased by fertilization and the significant interaction between N source and fertilization rate indicated this fraction was greater in soils supplied with AN (Table 3, Fig. 4). The microbial biomass C fraction was greatest at a fertilization rate of 200 or 400 kg N ha^{-1}. In bermudagrass plots, the highest ratio of microbial biomass C to total soil C was ~ 4% for soils supplied with AN and ~ 3% for soils supplied with SLE compared to ~ 1.6% in unfertilized controls. The highest ratios in tall fescue plots were ~ 2.9% for soils supplied with AN and ~ 2.5% for soils supplied with SLE compared to ~ 1.9% in unfertilized controls.

ASN as a fraction of total soil N also varied with of fertilization in bermudagrass and tall fescue plots (Table 3, Fig. 3). This fraction was generally increased by fertilization, and the significant interaction of N source and rate in bermudagrass system suggested it depended on N source. The highest ratios of ASN to total soil N were ~ 14 and ~ 12% in fertilized soils in the bermudagrass and tall fescue plots, respectively, compared to 9% in unfertilized controls.

However, microbial biomass N as a fraction of total soil N did not vary with the source and rate of fertilization (Table 3, Fig. 4). Again, soil mineralized C or N as a fraction of soil C or N did not change significantly with source and rate of fertilization (Table 3, Fig. 4).

Discussion

Sampling for this study was conducted 3 yr after the termination of a 3-yr N fertilization trial. Accordingly, any change in soil microbial properties represented residual effects of fertilization. Such residual effects were possibly due to fertilization-associated alteration in soil environment. We observed significant modification in soil pH and base cation contents by fertilization (Tables 2, 4a, b and 5a, b).

Several previous studies showed that SLE application reduced soil pH (King et al., 1985; Liu et al., 1998). Our study also demonstrated that soils became more acidic with increased application rate of SLE and AN (Tables 4a, b). There are several possible explanations for such a change in soil pH following SLE application. First, since N in SLE is mainly NH_4 (Barker and Zublena, 1995), its application at a high rate likely enhances soil nitrification, a microbially mediated process of ammonia oxidation with H^+ as the by-product contributing to soil acidification. Secondly, plant uptake of NH_4^+ results in the discharge of H^+ to maintain the balance of plant internal electric charge (Killham, 1994). This H^+ release into soil may furthermore acidify the soil. However, SLE application reduced soil pH more than AN at an equivalent rate of N fertilization (Tables 4a, b). This was likely due to the greater proportion of NH_4^+ contained in SLE versus AN, and thereby greater amounts of nitrification and plant NH_4^+ uptake in SLE fertilized soil, causing more H^+ to be discharged into the soil.

In comparison to soils supplied with AN and unfertilized controls, soils supplied with SLE also had decreased Mehlich-3 extractable Ca^{2+} and Mg^{2+} in bermudagrass plots (Tables 2, 5a). This effect has been observed in other studies (King et al., 1985; Liu et al., 1998). As a consequence of increasing soil acidity, H^+ and hydroxyl-Al ions may compete well with

Ca^{2+} and Mg^{2+} for cation exchangeable sites. In addition, other cations including Na^{+} carried by SLE application may also compete with Ca^{2+} and Mg^{2+} for cation exchangeable sites (King et al., 1985). Consequently, displaced Ca^{2+} and Mg^{2+} are liable to leach out of top soils. Furthermore, leaching of NO_3^- produced via nitrification may enhance the loss of base cations (Raney, 1960), and loss of base cations likely accelerate soil acidity.

Because soils in the southeastern USA are highly weathered and often infertile for forage production (Robinson, 1996), fertilization usually greatly increases forage dry matter and N content. Indeed, both AN- and SLE-fertilization increased dry matter and N content of bermudagrass and tall fescue (Table 1). However, aboveground plant biomass was harvested as hay, and thus primary production-associated C and N input into soil was mainly through belowground plant materials including root exudates and rhizome and root biomass. Several studies reported that total soil C was increased in forage production systems after several years of AN application (Holt and Fisher, 1960; Franzluebbers and Stuedemann, 2005). We also observed an increase in total soil C by AN at the highest application rate in (Tables 4a, b). In comparison to total soil C, the residual effect of fertilization on total soil N was more pronounced likely because fertilization increased plant tissue N more than dry matter production (Table 1). In addition, fertilizer N may be directly incorporated into soil organic matter by biotic and abiotic reactions, leading to an increase in total soil N compared to unfertilized controls. At the highest fertilization rate, total soil C and N were significantly lower in soil supplied with SLE than in soil supplied with AN (Tables 4a, b), possibly due to some inhibition of root growth. For example, H^{+} and Al^{3+} toxicities due to soil acidity could decrease the root biomass (Ennik et al., 1980; Adams, 1984), causing reduced C and N input into soil.

Since soil microbial biomass is a small but active soil C pool. it is very sensitive to alterations in soil environment. Soil acidity is considered a key factor affecting soil microbial community proliferation (Wardle, 1992; Witter et al., 1993; Bardgett and Leemans, 1995). McAndrew and Malhi (1992) demonstrated that soil acidity associated with N fertilization significantly reduced soil microbial biomass C and N. They observed that soil microbial biomass C and N declined ~ 40% and ~ 90%, respectively, from that in unfertilized controls in response to 1.5 pH unit reduction in soil pH, determined 8 yrs after the termination of 17-yr of fertilization with AN at a rate of 305 kg N $ha^{-1}y^{r-1}$. Despite merely 3-yr of consecutive fertilization in our study, pH was reduced significantly by fertilization at a rate of 600 kg N ha^{-1} and thus the observed reduction in biomass C at this high N rate may have resulted partially from an associated decline in soil pH.

Soil organic C is a major source for microbial cytoplasmic components and for microbial energy requirement. Therefore, microbial biomass C is highly correlated with and generally accounts for 1 to 4% of soil organic C (Jenkinson and Ladd, 1981; Smith and Paul, 1990). Although soil microbial biomass as a fraction of soil C was consistent with what is generally expected, its great variation with source and rate of fertilization indicated that soil microbes did not use soil C at equal efficiencies. Challenged by environmental stress, soil microbes likely devote more C for energy production in order to survive, thereby leading to less C for microbial biomass production. While the quality of soil organic C may also regulate soil microbial growth efficiency, the changes in the ratio of microbial biomass C to soil C were probably due mainly to changes in soil pH. Again, lower microbial biomass in soils supplied with SLE than with AN likely resulted from the reduction in soil pH, although

heavy metal or salt content carried in SLE might have contributed to the reduction of soil microbial biomass (Tyler, 1981; Rietz and Haynes, 2003).

Another significant impact of fertilization was on microbial biomass C/N ratio, which can be used as coarse indicator for microbial community composition. Because soil microbial biomass N was relatively constant over different sources and rates of fertilization, changes in biomass C/N ratio resulted primarily from changes in soil microbial biomass C (Fig. 1). Therefore, the variation of biomass C/N ratio with fertilization was similar to that of biomass C. The highest biomass C/N ratio did not occur in unfertilized controls or soils supplied with N at the highest rate, but in soils with a low rate of N fertilization. Since fungi have a higher biomass C/N ratio than bacteria, the higher biomass C/N at appropriate N fertilization rates indicates an increased fungal presence in the microbial community.. Despite several studies that demonstrated N fertilization suppressed soil fungi as indicated by a low microbial biomass C/N ratio (Garcia and Rice, 1994; Bardgett et al., 1996), this phenomenon was not observed in this study. even at the highest rate of N fertilization

Soil microbes play vital roles in nutrient transformations. Changes in microbial biomass and possible microbial community composition due to fertilization may influence microbial community function. Nitrogen mineralization potential and C mineralization was independent of rate and source of fertilization (Fig. 2), which indicates that soil microbial community in such a diverse environment could maintain their function. Amino sugar, a chemical index of soil N availability (Parsons, 1981; Mulvaney et al., 2001) increased significantly with fertilization rate (Fig. 3), which is consistent with the observation of Mulvaney et al. (2001), who found that amino sugar N content was greater in fertilized than in unfertilized soils.

However, nitrification potential, an indicator of soil nitrifier population size, varied with source and rate of N fertilization (Fig. 2). While increased by AN, nitrification potential remained constant or decreased with increasing levels of SLE application. It is well known that nitrification is inhibited by soil acidity (Frederick 1956). The lowest nitrification potential by the highest SLE application and the lower nitrification potential in soils supplied with SLE than in soil supplied with AN were likely due to soil acidity. However, soil acidity cannot be the sole reason since nitrification potential was significantly higher for AN- versus SLE-fertilized soils despite the two soils having equivalent soil pH when AN and SLE were applied up to 400 kg N ha^{-1} (Fig. 2). The source for this difference could not be identified from the analyzed soil chemical properties because stress factors for nitrifiers such as salt concentration (Low et al., 1997) were similar among different source and rate of fertilization. Excess nutrients (Cu and Zn) supplied through SLE also does not account for this difference in nitrification potential (Tables 5a, b).

In conclusion, variation in N fertilization regimes for 3 years had significant residual effects on soil chemical properties and subsequently regulated soil microbiological properties. The most significant changes in soil chemical properties due to SLE fertilization were on soil pH and Ca^{2+} and Mg^{2+} content. Reduced soil pH likely limited microbial community proliferation and accordingly, soil microbial biomass declined. In addition, soil microbial biomass C/N ratio changed with fertilization rate, and was highest at an intermediate application rate, i.e. 200 kg N ha^{-1}. Despite the alterations in soil microbial community biomass and composition, soil heterotrophs managed to function similarly as indicated by constant potential C mineralization. Increased amino sugar N content due to fertilization indicated a possible change in the quality of soil organic matter. However, soil

nitrifiers were significantly affected by source and rate of fertilization, partially due to soil acidity. Since fertilization was implemented for only 3 yrs, the results might be different with longer-term experiments.

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Table 1. Yield and N content of forage dry matter in response to swine lagoon effluent (SLE) or ammonium nitrate (AN) at an application rate of 0, 200, 400, or 600 kg plant available N (PAN) ha^{-1} yr^{-1}. The yield and N content of forage dry matter were the average of bermudagrass and tall fescue for consecutive 3-yr fertilization.

	Forage yield		Forage N content	
Fertilization rate	SLE	AN	SLE	AN
kg PAN ha^{-1}	Mg dry matter ha^{-1}		kg N ha^{-1}	
0	3.1	3.4	44	52
200	7.3	7.5	131	143
400	10	7.3	199	171
600	11.9	8.6	259	221

Table 2. Analysis of variance for soil properties and Mehlich-3 extractable nutrients as affected by N fertilization source (S), fertilization rate (R) in bermudagrass and tall fescue forage systems.

Source of Variation	df	Total C	Total N	Soil C:N	Inorganic N	ASN[†]	pH	EC[‡]	Soluble C[§]	Mehlich-3 extractable nutrients						
										P	K	Ca	Mg	Mn	Cu	Zn
Bermudagrass																
Rep	2															
Source (S)	1	*	NS	NS	NS	NS	*	NS	NS	NS	NS	*	*	NS	NS	NS
Error a (S x Rep)	2															
N rate (R)	3	NS	*	NS	**	***	**	NS	***	NS	NS	NS	NS	*	NS	NS
R x S	3	NS	NS	NS	NS	**	NS	NS	NS	*	NS	*	NS	NS	NS	NS
Error b (R x Rep (S))	12															
Tall fescue																
Rep	2															
Source (S)	1	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS
Error a (S x Rep)	2															

N rate (R)	3	NS	*	NS	NS	***	**	NS	***	*	**	NS	NS	NS	NS	NS
R x S	3	NS	**	NS	NS	**	NS	NS	NS	**	NS	**	NS	NS	NS	NS
Error b (R x Rep (S))	12															

[†]ASN, Amino sugar nitrogen; [‡]EC, Electrical conductivity; [§] Soluble C, K_2SO_4 extractable soluble carbon.

*, **, *** and NS Significant at 0.05, 0.01 and 0.001 probability level and not significant respectively.

Table 3. Analysis of variance for microbial properties as affected by N fertilization source (S), fertilization rate (R) in bermudagrass and tall fescue forage systems.

Source of Variation	df	MBC[†]	MBN[‡]	Biomass C:N	PMC[§]	PMN[¶]	Nitrification potential	MBC	MBN	PMC	PMN	ASN[#]
								as a fraction of total C or N as (%)				
					Bermudagrass							
Rep	2											
Source (S)	1	**	NS	*	NS	NS	***	*	NS	NS	NS	NS
Error a (S x Rep)	2											
N rate (R)	3	**	NS	***	NS	*	**	***	NS	NS	NS	**
R x S	3	***	NS	*	NS	NS	**	**	NS	NS	NS	**
Error b (R x Rep (S))	12											
					Tall fescue							
Rep	2											
Source (S)	1	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
Error a (S x Rep)	2											

N rate (R)	3	**	NS	NS	NS	NS	NS	**	NS	NS	NS	**
R x S	3	NS	NS	NS	NS	NS	**	*	NS	NS	NS	NS
Error b (R x Rep (S))	12											

† MBC, Microbial Biomass Carbon; ‡ MBN, Microbial Biomass Nitrogen; § PMC, Potentially Mineralizable Carbon; ¶ PMN, Potentially Mineralizable Nitrogen; # ASN, Amino Sugar Nitrogen.

*, **, *** and NS Significant at 0.05, 0.01 and 0.001 probability level and not significant respectively.

Table 4a. Selected soil properties as a consequence of N fertilization in bermudagrass system received ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} yr^{-1} for consecutive 3 yr. Soils were sampled 3 yr later after N fertilization was terminated. Values are means for n = 3.

Source	Rate	Total Soil N	Total soil C	Soil C:N ratio	Soluble C†	Inorganic N	pH	EC‡
	kg N ha^{-1} yr^{-1}	mg N or C g^{-1} soil			µg C or N g^{-1} soil			dS m^{-1}
Control	0	0.70	9.8	14.0	35.5	16.4	6.1	0.1
AN	200	0.82	11.2	13.7	96.2	27.2	6.2	0.1
	400	0.77	9.9	12.8	73.1	22.9	6.3	0.1
	600	0.93	11.8	12.6	44.2	27.1	5.7	0.1
SLE	200	0.73	9.4	12.8	90.2	22.7	5.9	0.1
	400	0.78	10.4	13.3	73.1	24.5	6.1	0.1
	600	0.77	10.4	13.8	49.7	25.7	4.9	0.1
	$LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS
		----------N rate treatment means----------						
	0	0.70	9.8	14.0	35.5	16.4	6.1	0.1
	200	0.78	10.3	13.3	93.4	25.0	6.1	0.1
	400	0.78	10.2	13.1	73.1	23.7	6.2	0.1
	600	0.85	11.1	13.2	47.0	26.4	5.3	0.1
	$LSD_{0.05}$	0.03	NS	NS	20.5	6.4	0.43	NS
		----------N source treatment means----------						
	AN	0.84	11.0	13.0	71.2	25.7	6.1	0.1

SLE	0.76	10.1	13.3	71.0	24.3	5.6	0.1
$LSD_{0.05}$	NS	0.8	NS	NS	NS	0.4	NS

† Soluble C, K_2SO_4 extractable soluble carbon; ‡ EC, Electrical Conductivity.
NS, not significant at the 0.05 level.

Table 4b. Selected soil properties as a consequence of N fertilization in tall fescue system received ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} yr^{-1} for consecutive 3 yr. Soils were sampled 3 yr later after N fertilization was terminated. Values are means for n = 3.

Source	Rate	Total Soil N	Total soil C	Soil C:N ratio	Soluble C[†]	Inorganic N	pH	EC[‡]
	kg N ha^{-1} yr^{-1}	mg N or C g^{-1} soil			μg C or N g^{-1} soil			dS m^{-1}
Control	0	0.75	11.2	14.9	39.5	16.6	5.9	0.1
AN	200	0.80	11.4	14.2	86.8	26.3	6.1	0.1
	400	0.83	11.1	13.9	109.5	20.8	5.9	0.1
	600	0.93	12.7	13.7	44.5	24.4	5.6	0.1
SLE	200	0.80	11.5	14.3	85.6	20.8	5.9	0.1
	400	0.83	11.8	14.1	116.9	28.4	5.6	0.1
	600	0.77	11.1	14.5	52.1	16.8	5.3	0.1
	$LSD_{0.05}$	0.08[¶], 0.07[§]	NS	NS	NS	NS	NS	NS
			----------N rate treatment means----------					
	0		11.2	14.9	39.5	16.6	5.9	0.1
	200		11.5	14.3	86.2	23.6	6.0	0.1
	400		11.5	14.0	113.2	24.6	5.8	0.1
	600		11.9	14.1	48.3	20.6	5.5	0.1
	$LSD_{0.05}$		NS	NS	16.8	NS	0.3	NS
			----------N source treatment means----------					
	AN		11.7	13.9	80.3	23.8	5.9	0.1

SLE	11.5	14.3	84.9	22.0	5.6	0.1
$LSD_{0.05}$	NS	NS	NS	NS	0.2	NS

† Soluble C, K_2SO_4 extractable soluble carbon; ‡ EC, Electrical Conductivity.
NS, not significant at the 0.05 level.
¶ LSD for comparing N sources at the same N rate.
§ LSD for comparing N rates within N source.

Table 5a. Influence of N fertilization on Mehlich-3 extractable soil nutrients in bermudagrass system received ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} yr^{-1} for consecutive 3 yr. Soils were sampled 3 yr later after N fertilization was terminated. Values are means for n = 3.

Source	Rate	P	Ca	K	Mg	Mn	Cu	Zn
	kg N ha^{-1} yr^{-1}				µg g^{-1} soil			
Control	0	157	423	69	174	14.9	1.7	3.3
AN	200	221	487	65	196	13.4	1.0	5.3
	400	227	463	50	171	9.3	2.7	5.7
	600	262	490	80	182	9.4	2.0	5.7
SLE	200	135	324	53	132	6.1	0.6	3.0
	400	103	402	82	163	8.7	2.6	3.7
	600	129	215	38	91	4.5	3.1	1.9
	$LSD_{0.05}$	73.7¶, 56.2§	139¶, 120§	NS	NS	NS	NS	NS
				----------N rate treatment means----------				
	0			69	174	14.9	1.7	3.3
	200			59	164	9.8	0.8	4.2
	400			66	167	9.0	2.7	4.7
	600			59	137	11.7	2.6	3.8
	$LSD_{0.05}$			NS	NS	6	NS	NS
				----------N source treatment means----------				
	AN			65	183	10.7	1.9	5.6

SLE	58	129	6.4	2.1	2.9
$LSD_{0.05}$	NS	53	NS	NS	NS

NS, not significant at the 0.05 level.
[¶] LSD for comparing N sources at the same N rate.
[§] LSD for comparing N rates within N source.

Table 5b. Influence of N fertilization on Mehlich-3 extractable soil nutrients in tall fescue system received ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} yr^{-1} for consecutive 3 yr. Soils were sampled 3 yr later after N fertilization was terminated. Values are means for n = 3.

Source	Rate	P	Ca	K	Mg	Mn	Cu	Zn
	kg N ha^{-1} yr^{-1}				µg g^{-1} soil			
Control	0	140	378	66	162	4.4	0.3	2.8
AN	200	178	434	66	184	5.6	1.0	4.1
	400	179	349	51	146	4.4	0.5	3.4
	600	215	400	43	151	4.9	0.6	4.5
SLE	200	113	279	57	120	3.3	0.3	2.2
	400	94	423	50	174	1.9	0.2	3.7
	600	121	243	36	115	3.3	0.3	3.4
	$LSD_{0.05}$	35.9¶, 30.1§	107¶, 80.9§	NS	NS	NS	NS	NS
				N rate treatment means				
	0			66	162	4.4	0.3	2.8
	200			63	152	4.5	0.7	3.2
	400			51	160	3.2	0.4	3.6
	600			40	133	4.1	0.5	4.0
	$LSD_{0.05}$			25	NS	NS	NS	NS
				N source treatment means				
	AN			53	160	5.0	0.7	4.0

SLE	48	136	2.8	0.3	3.1
$LSD_{0.05}$	NS	NS	NS	NS	NS

NS, not significant at the 0.05 level.
[¶] LSD for comparing N sources at the same N rate.
[§] LSD for comparing N rates within N source.

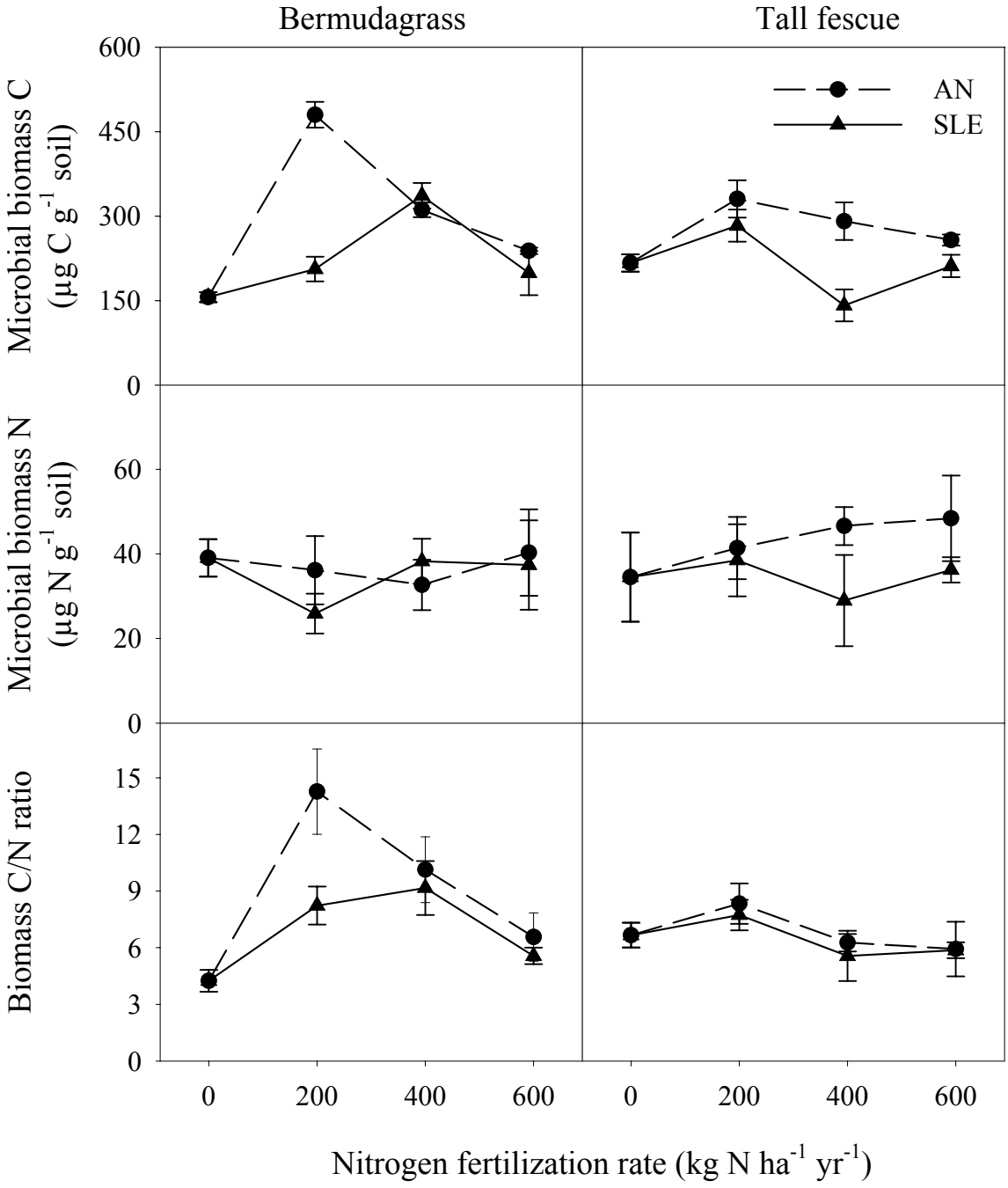

Fig. 1. Soil microbial biomass C, N and C/N ratio as a consequence of various application rates of swine lagoon effluent (SLE) versus ammonia nitrate (AN) in bermudagrass and tall fescue field plots.

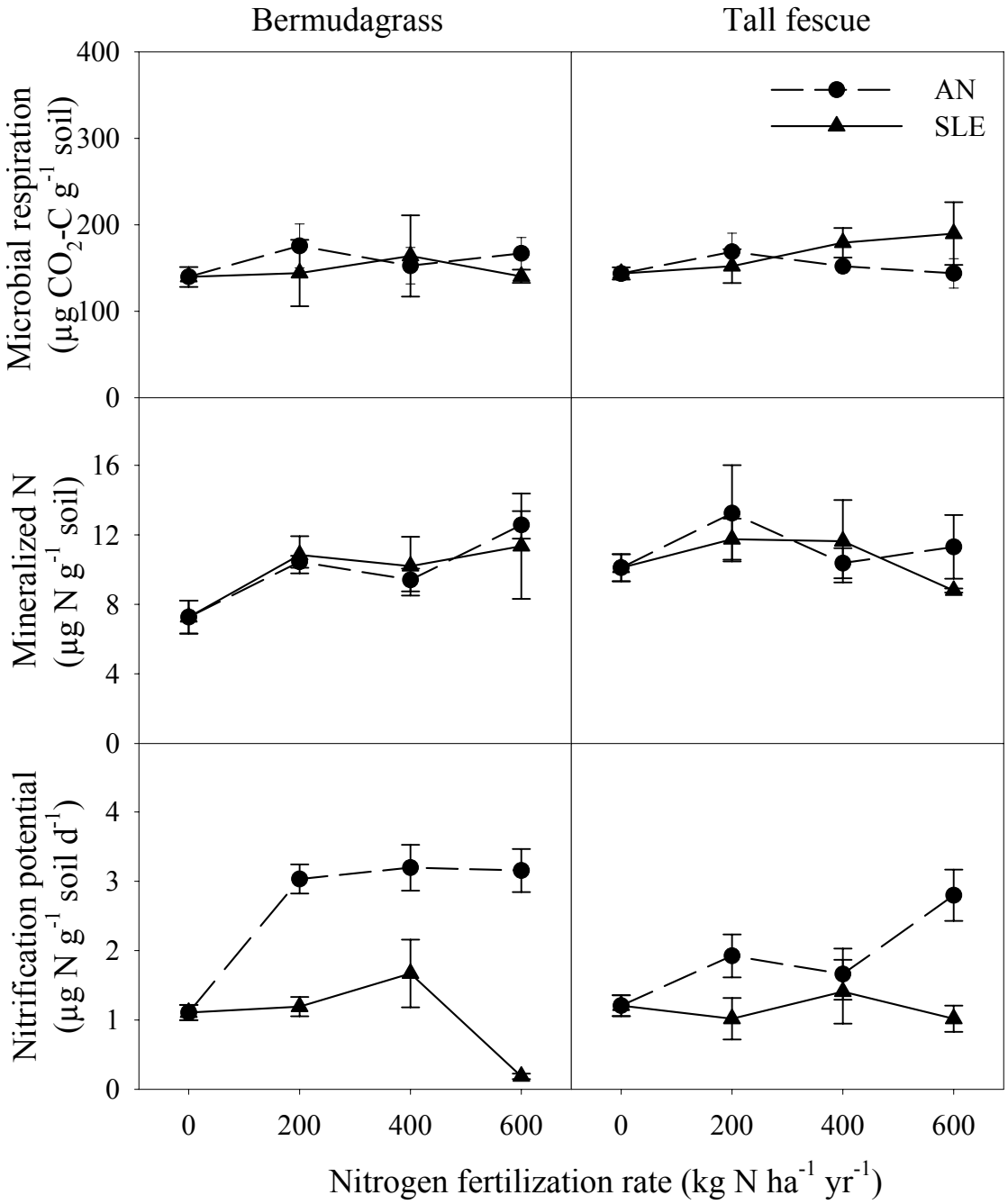

Fig. 2. Soil microbial respiration (i.e. microbial activity or C mineralization potential), N mineralization and nitrification potential as a consequence of various application rates of swine lagoon effluent (SLE) versus ammonia nitrate (AN) in bermudagrass and tall fescue field plots.

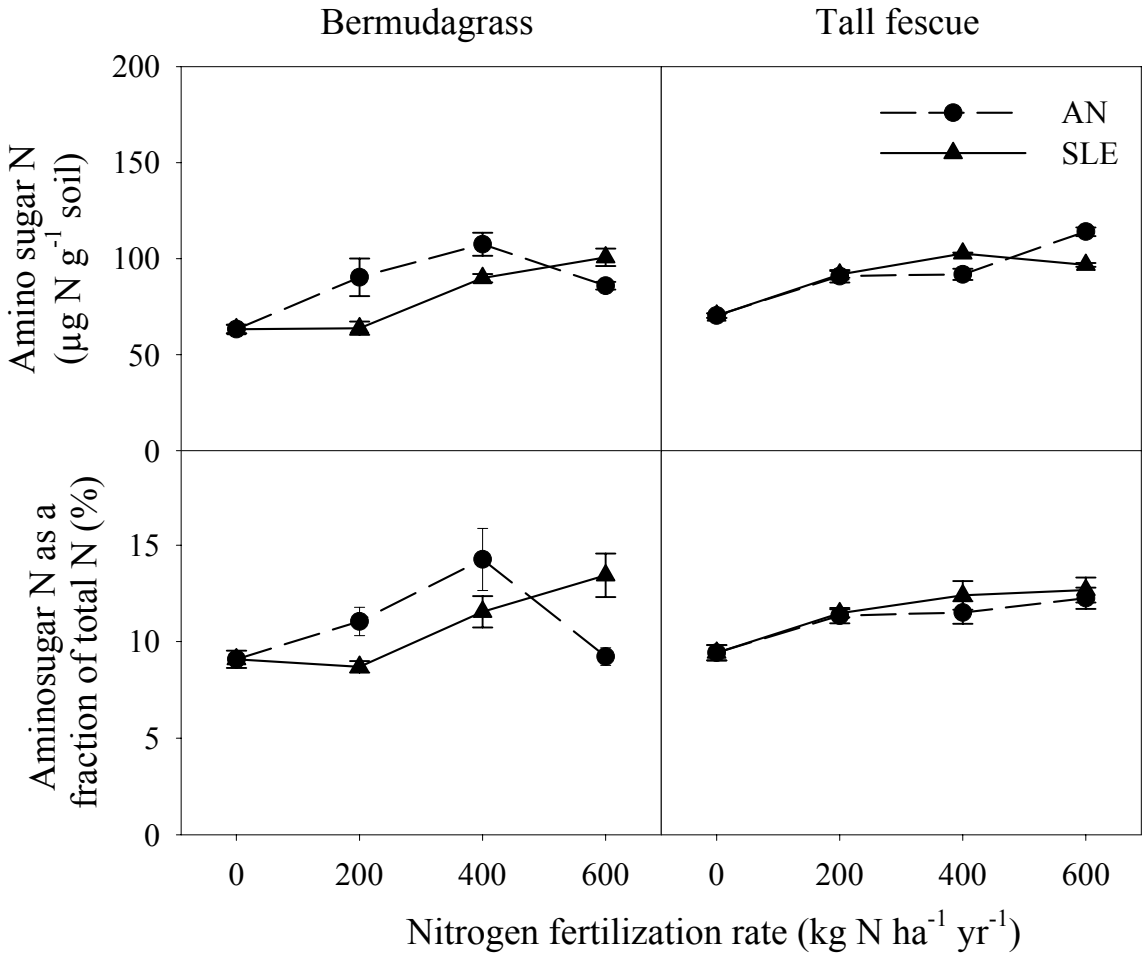

Fig. 3. Amino sugar N content and amino sugar N as a fraction of total soil N as a consequence of various application rates of swine lagoon effluent (SLE) versus ammonia nitrate (AN) in bermudagrass and tall fescue field plots.

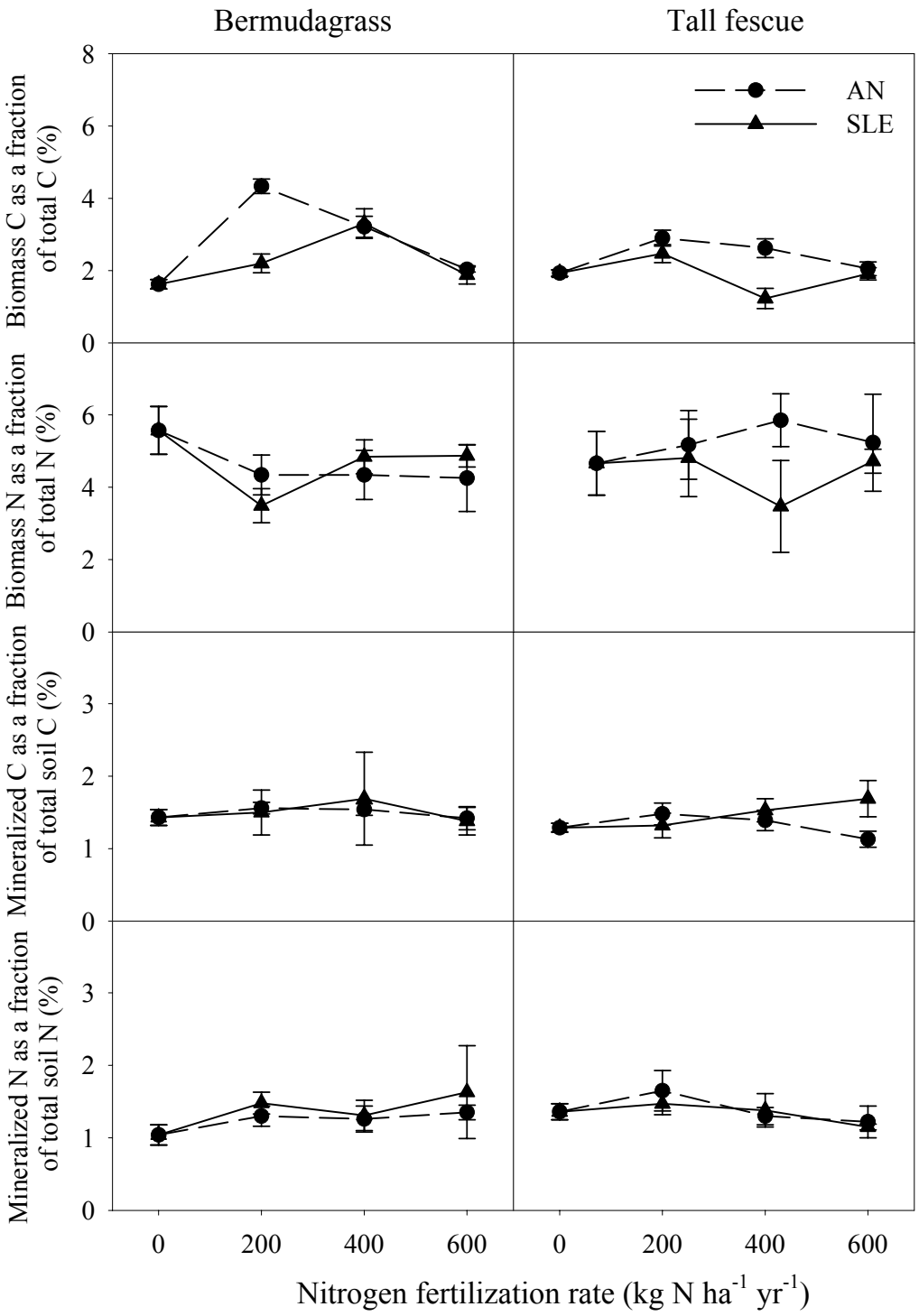

Fig. 4. Microbial biomass C (or N) and potentially mineralized C (or N) as a fraction of total soil C (or N) as a consequence of various application rates of swine lagoon effluent (SLE) versus ammonia nitrate (AN) in bermudagrass and tall fescue field plots.

CHAPTER FOUR

SOIL ENZYMES ACTIVITY IN FORAGE SYSTEMS: MODIFICATION BY SWINE LAGOON EFFLUENT VERSUS AMMONIUM NITRATE

Abstract

Land application of swine lagoon effluent (SLE) to forage production systems is common in the southeastern USA, which often results in changes in soil properties. Because soil enzymes are involved in degradation of soil organic matter and nutrient cycling, a better assessment of the relationship between activities of soil enzymes and SLE application may facilitate informed management decisions. The activities of soil enzymes important to soil C, N and P cycling were evaluated as influenced by 3 consecutive years of application of SLE versus ammonium nitrate (AN) at a rate equivalent to 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1}. The activities of enzymes varied differently for soils supplied with SLE versus AN. Enzymes involved in N and P cycling were very sensitive to reduction in soil pH due to application at a high rates of SLE or AN, The source of N had significant effects on oxidative enzymes involved in the C cycle. In soils supplied with AN, activities of phenol oxidase and peroxidase decreased or remained at the level of unfertilized controls. In contrast, those enzymes activity were stimulated by the SLE application. We attributed the differences activity of enzymes between soils supplied with SLE and AN to the changes in soil microbial community composition, caused in part by prolonged changes in soil pH. Non-metric multidimensional scaling analysis further showed that integrated soil enzyme activities differed significantly ($P < 0.05$) between fertilized soils and unfertilized controls and between soils supplied with SLE and AN. These differences were significantly correlated

with soil pH (Pearson's correlation coefficient $r = 0.76$, $P < 0.05$), indicating soil pH is a predominant factor in influencing soil enzyme activities.

Introduction

Land application of animal waste is common in places where there are large-scale confined domestic animal industries. In the southeastern USA, swine industry generates a large amount of swine waste annually. These wastes are treated in anaerobic lagoons and the effluent is irrigated onto adjacent forage production systems. As surrogates of synthetic N fertilizers, SLE can increase yield and improve nutritive value of forage dry matter (Adeli et al., 2005). However, SLE application also affects soil physical and chemical properties. In comparison to equivalent amount of N application as ammonium nitrate (AN), for example, application of SLE for 3 consecutive years significantly decreased soil pH and Ca^{2+}, Mg^{2+}but increased soil salt concentrations (Liu et al., 1998). These rapid adjustments in soil properties as a result of SLE application may influence the complex belowground biological systems and may subsequently constrain soil fertility. Because soil enzymes are involved in degradation of soil organic matter and nutrient cycling, and thus influence C sequestration in soils, a better assessment on the relationship of soil enzyme activities with source and rate of N fertilization may facilitate informed N management decisions.

Influences of synthetic N fertilizers on soil enzymes vary with the nature of catalytic reactions. It has been reported that N fertilization enhances the activity of cellulase, an enzyme involved in the degradation of polysaccharides, but decreases the activities of phenol oxidase and peroxidase, the oxidative enzymes involved in the degradation of lignin and polyphenols (Ajwa et al., 1999; Carreiro et al., 2000; Saiya-Cork et al., 2002; Sinsabaugh et

al., 2005). Fertilization-induced changes in soil pH can also affect the activities of soil enzymes through controls on enzyme ionization and solubility and on the availability of cofactors/inhibitors of enzymes (Dick et al., 1988; Gianfreda and Bollag, 1996). In addition, soil pH regulates microbial community biomass, a major source of soil enzymes, (Burns, 1978) and may thus controls the production of soil enzymes. Several studies reported that the activity of soil acid phosphatase was inversely correlated with soil pH and the activity of β-glucosidase was positively correlated with soil pH (Dick et al., 1988; Acosta-Martínez and Tabatabai, 2000). So far, the information on the relationship between N fertilization and activities of soil enzymes has been mainly derived using synthetic fertilizers. Besides high N content, SLE also contains many other elements such as soluble C and heavy metals. These SLE-associated attributes may modify the relationship between soil enzymes and soil N availability.

Our previous work (unpublished data) showed that both SLE and AN at a high rate (i.e. 600 kg plant available N ha^{-1} y^{-1}) led to soil acidity in forage production systems and accordingly reduced soil microbial biomass. These changes in soil chemical and microbiological properties may modify microbial community-level functions important to organic matter degradation and nutrient cycling. Activities of hydrolytic and oxidative enzymes vital to C, N and P cycling were assessed in this study. We hypothesize that (1) the activities of extracellular enzymes involved in C, N and P cycling would be influenced differently by application of SLE versus AN; (2) activities of oxidative enzymes and enzymes involved in N cycling would be decreased with increase in N fertilization rates; and (3) activities of hydrolytic enzymes involved in carbohydrate degradation would increase with the increase in N fertilization rates.

Materials and Methods

Study Site and Soil Sampling

The study site was located on the Caswell Farm, a research station of North Carolina State University, near Kinston, Lenoir County, NC, USA. The soil is classified as Pocalla loamy sand (loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults). Field plots were established in fall 1998 to examine the responses of yield and N content of forage dry matter to different SLE application rates. Four application rates of 0, 200, 400, and 600 kg plant available N (PAN) ha^{-1} y^{-1} were compared between SLE and AN across warm- and cool-season grass species of bermudagrass (overseeded with rye), tall fescue, gamagrass, crabgrass (overseeded with rye), and rescue grass and we chose two forage species (bermudagrass and tall fescue) for this study. A partially stripped split plot design was used with three replications: grass species were stripped across fertilization source (i.e. SLE and AN); application rates were stripped across grass species and nested within fertilization source of each replication. A treatment split plot was ~ 3 m × 6 m, SLE was applied using a small plot effluent sprayer. The application rate was calculated as 60% of total N of SLE as PAN and verified using collection cups placed on the plots. Chemical composition of SLE during ~ 3 y application averaged ~ 293 mg total N L^{-1}, 87 mg P L^{-1}, 198 mg K L^{-1}, 154 mg Ca L^{-1}, 58 mg Mg L^{-1}, 28 mg S L^{-1}, 84 mg Na L^{-1} and trace amounts (i.e. < 10 mg L^{-1}) of Fe, Mn, Zn and Cu. SLE was neutral with a value of pH 7.1. For plots that received broadcast application of AN and unfertilized controls, P and K were also applied based on annual soil test recommendation. Because SLE was irrigated onto plots, the plots with AN application received irrigation water equivalent to that in SLE. Both SLE and AN were applied every month from February to November; the split amounts of N were based on the growth

physiologies of individual grass species. Forages were harvested in a manner that was consistent with the recommended practices for individual species. Fertilization treatments were implemented from April 1999 to November 2001. Thereafter, the treatments were terminated but the forage was continually mowed and baled for hay until December 2004.

Soil samples were collected (0-7.5 cm depth) in March 2004 from the field plots cropped with bermudagrass and tall fescue. Three soil cores (5 cm diameter) were taken from the center of each plot and composited. Soils were sieved (< 4 mm) and stored at 4 °C for later analyses of soil chemical and microbiological properties. Total soil C was determined by dry combustion using Perkin-Elmer series II CHNS/O-2400 analyzer (Perkin Elmer Corp., Norwark, CT) from sub-samples dried at 105 ºC and ground to < 250 µm. Soil pH was measured in 1:1 soil/water slurry. Soil microbial biomass C was determined by the chloroform fumigation extraction methods (Brookes et al., 1985; Vance et al., 1987) and calculated using the extraction coefficients 0.33 (Sparling and West, 1988). Selected soil properties are given in Table 1.

Soil Enzyme Assays

Eight soil enzymes were examined including two oxidative enzymes (phenol oxidase and peroxidase) involved in C cycling; three hydrolytic enzymes (β-glucosidase, cellobiohydrolase and cellulase) involved in C cycling; two enzymes (N-acetyl-β-glucosaminidase and protease) involved in N cycling; and one enzyme (acid phosphatase) involved in P cycling. The duration of the assay time for each enzyme was chosen to be within the linear range of product formation or substrate disappearance.

The activity of soil phenol oxidase (EC 1.10.3.2) and peroxidase (EC 1.11.1.7) was determined by the method of Sinsabaugh et al. (1999). Phenol oxidase and peroxidase degrade L-dehydroxyphenylalanine (DOPA), and the disappearance of DOPA was calibrated against the DOPA extinction coefficient determined using mushroom tyrosinase (Sigma T3824) (Allison and Vitousek, 2004). For measurement of phenol oxidase activity, soil (~ 0.5 g dry weight equivalent) was incubated with 2-ml of 5 mM DOPA and 1.5 ml of 50 mM acetate buffer (pH 5.0) for 30 min with continuous shaking at 100 rev min^{-1}. The reaction was terminated by 10 min. centrifugation at ~ 5000 × *g*. The supernatant was subsequently measured colorimetrically at 460 nm using a UV spectrophotometer. For measurement of peroxidase activity, soil (~ 0.5 g dry weight equivalent) was incubated with 2-ml of 5 mM DOPA and 1.5 ml of 50 mM acetate buffer (pH 5.0) and 0.2 ml of 0.3% hydrogen peroxide for 30 min with continuous shaking at 100 rev min^{-1}. The reaction was terminated by 10 min. centrifugation at ~ 5000 × *g*. The supernatant was subsequently measured colorimetrically at 460 nm using a UV spectrophotometer. Peroxidase activity was calculated as the difference in activity between the phenol oxidase and peroxidase assay samples. The enzyme activities are expressed as μmol DOPA converted g^{-1} soil C h^{-1}.

Cellulase activity was determined by the method of von Meris and Schinner (1996). Soil (~ 10 g dry weight equivalent) was incubated with 0.7% of carboxymethyl-cellulose in 2 M acetate buffer (pH 5.5) at 50^{o}C for 24 h. The filtrates of soil-substrate slurries were subjected to color reaction and the produced Prussian blue was analyzed colorimetrically at 690 nm. The cellulase activity is expressed as μmol glucose produced g^{-1} soil C h^{-1}.

The activities of β-glucosidase (EC 3.2.1.21) and cellobiohydrolase (EC 3.21.91) were estimated by using the substrates of *p*-nitrophenyl-β-glucopyranoside and *p*-

nitrophenyl-cellobioside, respectively (Sinsabaugh et al., 1999). Soil (~ 1 g dry weight equivalent) was incubated with 4 ml of 50 mM acetate buffer (pH 5.0) and 1ml respective substrate solution for 1 or 3 h at 25ºC. Subsequently, 1ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 NaOH were added to terminate the reaction and to allow color development. The filtrates were measured colorimetrically at 410 nm. The enzyme activities are expressed as μmol *p*-nitrophenol produced g^{-1} soil C h^{-1}.

The activity of *N*-acetyl-β-glucosaminidase (EC 3.2.1.20) was determined by the method of Parham and Deng (2000). Soil (~ 0.5 g) was incubated with 4-ml of 50 mM acetate buffer (pH 5.5) and 1 ml of substrate *p*-nitrophenyl-N-acetyl-β-glucosaminide solution for 1 at 37ºC. Subsequently, 1ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 NaOH were added to terminate the reaction and to allow color development. The *p*-nitrophenol produced was subsequently measured colorimetrically at 410 nm. The enzyme activity is expressed as μmol of *p*-nitrophenol released g^{-1} soil C h^{-1}.

Protease activity was determined according to the method of Kandeler (1996). Soil (~ 1 g dry weight equivalent) was incubated for 2 h at 50^{o}C with the substrate casein solution buffered with 0.05M Tris of pH 8.1. The tyrosine released was measured colorimetrically at 700 nm. The enzyme activity is expressed as μmol tyrosine released g^{-1} soil C h^{-1}.

The activity of acid phosphatase (EC 3.1.3.2) was determined according to Sinsabaugh et al. (1999). Soil (~ 1 g dry weight equivalent) was incubated for 1 h at 25^{o} C with the substrate *p*-nitrophenol-phosphate. Subsequently, 1ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 NaOH were added to terminate the reaction and to allow color development. The filtrates were measured colorimetrically at 410 nm. The enzyme activity is expressed as μmol *p*-nitrophenol produced g^{-1} soil C h^{-1}.

Data Analysis

Since the objective of the study is not to directly compare the effect of forage species, data on soil enzymes activity of bermudagrass and tall fescue systems were analyzed by individual forage species in split-plot design, with N source as the whole plot factor and N rate as the sub-plot factor. The PROC MIXED in SAS 9 version (SAS Institute Inc. 2001, Cary, North Carolina, USA) was used to carry out the split-plot analysis and the differences among treatment means were compared by F-protected least significant differences (LSD) at $P < 0.05$.

Non-metric multidimensional scaling (NMS) was also used to integrate all the enzyme activities examined in this study, and to distinguish N treatments based on this integrated enzymatic function. The autopilot program of NMS (MjM Software, Gleneden Beach, Oregon, USA) was first run to determine the optimal dimensions. Subsequently, NMS was constructed with the selected dimensions. Furthermore, a multi-response permutation procedure (MRPP) was used to test whether or not N treatments and unfertilized controls differed with respect to integrated enzyme activities. The *A* value in the MRPP ranges from 0 to 1; a higher *A* value describes within-group homogeneity and *P* value evaluates how likely an observed difference is due to chance (McCune and Grace, 2002). 'Indicator species' analysis was performed when necessary to identify the enzymes that are responsible for the of treatment separation (McCune and Grace, 2002).The NMS axis that explained the largest variation was also subjected to Pearson correlation analysis with soil pH.

Results

In general, oxidative enzyme (peroxidase) activity differed significantly ($P < 0.05$) in their responses to SLE- versus AN-applications (Table 2, Fig. 1). Peroxidase activity in soils supplied with AN was similar to that in unfertilized control, but it was higher in soils received SLE than the unfertilized control. The most pronounced difference of soil peroxidase between SLE and AN was at the 600 kg N ha^{-1} y^{-1}. Phenol oxidase activity in soils supplied with AN was generally lower than that in unfertilized control; the magnitude of the decrease in activity increased as the fertilization rate increased (Fig. 1). However, phenol oxidase in soils that received SLE at the highest application rate remained unchanged or higher than that in the unfertilized control.

Hydrolytic enzymes of β-glucosidase, cellobiohydrolase and cellulase activity were similar in their responses to SLE- versus AN-applications (Table 2, Fig. 2). Soil cellulase activity tended to be higher in fertilized soils than in unfertilized control; but there was no difference in soil cellulase between SLE and AN sources. The activity of cellobiohydrolase tended to be higher in soils fertilized at 200 or 400 kg N ha^{-1} y^{-1} than the unfertilized control (Fig. 2). When application rate was 600 kg N ha^{-1} y^{-1}, cellobiohydrolase activity was similar to that in unfertilized control. Again, the activity of β-glucosidase was higher in fertilized than the unfertilized control except for the highest application rate of SLE (Fig. 2).

The activity of N-acetyl-β-glucosaminidase was increased by the fertilization, being highest at 600 or 400 kg N ha^{-1} y^{-1} for AN or SLE applications (Table 2, Fig. 3). While activity of protease increased with AN application, it was significantly ($P < 0.05$) lower in the highest application rate of SLE than in the unfertilized control (Fig 3). There was no

difference in soil acid phosphatase between SLE and AN treatments; the activity of acid phosphatase was increased by fertilization with either N source (Table 2, Fig. 3).

Based on the eight enzyme activities examined in the study, non-metric multidimensional scaling (NMS) grouped soils along the two dimensions explaining 68% (i.e. $r^2 = 0.68$) and 28% (i.e. $r^2 = 0.28$) of total variations, respectively (Fig. 4). Because integrated enzyme activities in soils cropped with bermudagrass was similar to those planted with tall fescue ($A = 0.023$, $P > 0.05$), soils cropped with different plants were not differentiated in Fig. 4. Soils fertilized with N were distinguished from soils without fertilization ($A = 0.165$, $P < 0.0001$, and $A = 0.222$, $P < 0.0001$ for comparing SLE and AN-applied soils with the unfertilized control, respectively). Furthermore, soils with SLE application were significantly different from those with AN applications for their integrated enzyme activities ($A = 0.081$, $P < 0.01$). This difference in integrated enzyme activities between soils supplied with SLE and AN was more pronounced at the application rate of 600 kg N ha^{-1} y^{-1} ($A = 0.249$, $P < 0.0001$).

Because soil pH was one of the significant changes after several years of SLE versus AN application, the integrated enzyme activities were correlated with soil pH (Fig. 5). Integrated enzyme activities were significantly correlated with soil pH (Pearson's correlation $r = 0.76$, $P < 0.05$).

Discussion

Because this study was conducted ~ 3 y after the termination of 3 consecutive years of application of SLE or AN at various rates, alterations in soil enzyme activities were likely due to residual effects of source and rate of N fertilization on soil properties. Activities of

eight soil enzymes that are important to C, N and P cycling were integrated using NMS so that enzymatic degradation of soil organic matter could be represented by a single parameter of soil microbial community. The integrated soil enzyme activity of soils supplied with SLE clustered together and separated from soils supplied with AN and unfertilized controls (Fig. 4). Indicator species analysis showed that increased activities of hydrolytic enzymes involved in C, N and P cycling in AN and SLE supplied soils separated the fertilized soils from unfertilized control in their integrated enzyme activities. However, the differences between soils supplied with SLE and AN were primarily attributed to the dissimilarities in the oxidative enzymes involved in C cycling as indicated by indicator species analysis and in Fig. 1.

Soil properties including organic matter content may considerably affect soil enzyme activities (Gianfreda and Bollag, 1996). Eight soil enzymes expressed on per unit soil mass were also integrated using NMS (data not shown). There was no significant correlation between such integrated soil enzyme activity and the content of soil organic matter (Pearson's correlation coefficient $r = 0.42$, $P > 0.05$), indicating that soil properties other than the quantity of soil organic matter may be more important in dictating soil enzyme activities in this fertilized forage production system.

Alteration in soil pH was one of the significant residual effects of SLE or AN application (Table 1). Fertilization decreased soil pH and the decline was more pronounced for SLE application at the highest rate of 600 kg N ha^{-1} y^{-1} (Table 1). To minimize the confounding effect of the quantity of soil organic matter with other soil properties, soil enzyme activities were normalized to soil organic matter and accordingly expressed on per unit soil C. The integrated soil enzyme activity calculated by NMS was significantly

correlated with soil pH (Fig. 5), indicating that soil pH was likely a key ecological factor regulating the decomposition of soil organic matter in this fertilized forage production systems.

Soil pH may directly or indirectly control the activity of soil enzymes. Directly, soil pH affects soil enzyme activities by altering their ionization and solubility, and concentrations of certain chemicals which may serve as the cofactors of enzymes or which may inhibit enzyme activity above a threshold value (Gianfreda and Bollag, 1996). For example, Kandeler et al. (1996) reported that soil Cu at a concentration of > 100 μg g^{-1} soil and soil Zn at a concentration of > 300 μg g^{-1} might adversely affect soil enzyme activities. However, soil (Mehlich-3 extractable) Cu and Zn concentrations after several years of SLE or AN application were in the range of 0.2 to 2.7 μg Cu g^{-1} soil and 1.9 to 5.7 μg Zn g^{-1} soil which was much lower than the reported values above which inhibition on soil enzyme activities occurred. Soil pH may also indirectly affect soil enzyme activities by altering soil microbial community biomass and/or compositions.

Soil microbes are very sensitive to soil pH; microbial growth is often limited under acidic soil condition (Alexander, 1977; Wardle, 1992; Bardgett and Leemans, 1995). Reduction in microbial population size likely affects the production of soil enzymes, thereby potential soil enzyme activities. DeForest et al. (2004) demonstrated that soil enzyme activity declined with the reduction in soil microbial biomass in a hardwood forest soil. In our study, the activities of several hydrolytic enzymes involved in C and N cycling were correlated with the microbial biomass (Table 1, Fig. 2 and 3), but the activity of oxidative enzymes of phenol oxidase and peroxidase did not (Table 1, Fig. 1), suggesting that other than biomass, microbial community compositions may also play important roles in regulating potential soil

enzyme activities. Indeed, integrated soil enzyme activity normalized to microbial biomass C (data not shown) was significantly correlated with the soil pH (Pearson's correlation coefficient $r = 0.60$, $P < 0.05$).

Soil enzymes involved in the cycling of a given nutrient are often negatively associated with the availability of that nutrient in the soil (Dick, 1992). Studies have demonstrated that soil P availability was inversely correlated with the phosphatase activity (Haussling and Marschner, 1989). With increase in the application rate of SLE or AN, soil became more acidic (Table 1). Accordingly, soil available P may become limiting due to sorption and/or precipitation reactions. The lack of soil available P for soil microorganisms likely stimulated the production of soil phosphatase. Soil phosphatase activity increased with fertilization rate (Fig. 3). This result was consistent with other reports that soil phosphatase was enhanced by N fertilization-induced decrease in soil pH (Johnson et al., 1998; Ajwa et al., 1999).

All soil microbes are capable of protein degradation (Alexander, 1977). Therefore, positive correlations between protease activity and soil microbial biomass have often been reported (Alef et al., 1988; Zaman et al., 1999). In this study protease activity was well correlated with the soil microbial biomass (Pearson correlation coefficient $r = 0.43$, $P < 0.05$) (Table 1, Fig. 3). At the highest application rate of N (i.e. 600 kg N ha^{-1} y^{-1}), soil microbial biomass was ~ 17% lower in soils supplied with SLE than AN (Table 1). Consequently, lower protease activity was associated with lower microbial biomass (Fig. 3).

N-acetyl-β-glucosaminidase is an enzyme involved in chitin degradation. Chitin is the major component of fungal cell wall and can serve as an inducer for the microbial production of N-acetyl-β-glucosaminidase (Smucker and Kim, 1987). The increase in N-acetyl-β-

glucosaminidase with the increase in the application rate of AN or SLE (Fig. 3) may suggest that turnover of active fungi was faster as soil acidity increased due to the high application rate of N fertilization. Accordingly, fungal necromass acts as an inducer to enhance the production of N-acetyl-β-glucosaminidase.

The activity of cellulase, a hydrolytic enzyme involved in C cycling was independent of source of N fertilization and tended to increase with the fertilization rate (Fig. 2). Nitrogen fertilization has been reported to stimulate cellulase activity (Carreiro et al., 2000; Sinsabaugh et al, 2002). Our result is consistent with those reports although soil became acidic due to a high rate of N fertilization (Table 1). Several factors may explain the observation that N fertilization stimulates soil cellulase activity. First, N fertilization may modify the composition of soil microbial community and thus cellulase production can be maintained even under the condition of lower microbial biomass as a consequence of a high rate of N fertilization (Table 1). Fungi are generally more tolerant to soil acidity than bacteria. The residual effect of fertilization on soil pH may have produced a distinctly active fungi community composition. Although a suite of bacteria and fungi can produce cellulase (Alexander, 1977), fungi are often the major bio-agent in producing cellulase.

The activities of cellobiohydrolase and β-glucosidase, hydrolytic enzymes involved in simple carbohydrate degradation, were related to fertilization-induced changes in soil properties. Activities of both enzymes were lowest at the highest application rate of SLE or AN (Fig. 2); the reduction of enzyme activities was paralleled with the significant reduction in soil pH and soil microbial biomass as well (Table 1). Eivazi and Tabatabai (1990) also reported that the activity of β-glucosidase was adversely affected by soil acidity.

Nitrogen fertilization has been shown to suppress the potential activity of oxidative enzymes of phenol oxidase and peroxidase involved in the degradation of lignin, polyphenols and humus (Carrerio et al., 2000; Matocha et al., 2004). This suppression is likely due to the selective pressure of N fertilization on certain soil microbes. For example, N fertilization may suppress white rot fungi (i.e. some basidiomycetes) (Fog, 1988; Miller and Lodge, 1997), which are vital bio-agents in producing oxidative enzymes of phenol oxidase and peroxidase (Krik and Farrell, 1987). Several studies showed that application of synthetic N fertilizers reduced the activities of phenol oxidase and peroxidase in forest and agricultural soils (DeForest et al., 2004; Matocha et al., 2004; Sinsabaugh, 2005). Our study also demonstrated that AN application caused reduction of phenol oxidase compared to unfertilized controls (Fig. 1). However, N application as SLE did not produce the expected results. Instead, peroxidase and phenol oxidase activities were stimulated by SLE application especially in the tall fescue system (Fig. 1).

Phenol oxidase and peroxidase activities can be stimulated by available Cu, Mn and Zn (Kirk and Farrell, 1987; Collins and Dobson, 1997). These micronutrients are often carried in animal waste and long-term application of animal waste may lead to their accumulation in soils (King et al., 1985). We speculate that 3-y consecutive application of SLE may increase soil available Cu, Mn and Zn. Measurement of Mehlich-3 extractable Cu, Mn and Zn did not support this speculation as concentrations were not different between SLE and AN application (data not shown). Although white rot fungi and other basidiomycetes, the key bio-agents in producing oxidative enzymes involved in the degradation of lignin and humus, could be suppressed by high availability of soil N (Kirk and Farrell, 1987), there are still some microbes not suppressed by higher available N (Collins and Dobson, 1997;

Hammel, 1997). The different consequence in soil enzyme production between SLE and AN application suggests that different source of N fertilization (i.e. SLE versus AN) may create conditions that favor different taxa of soil microbes. This shift in soil microbial community compositions may dictate dissimilar responses in the production of oxidative enzymes.

The difference in potential enzyme activities between SLE and AN application has very important implications in SLE management. As indicated by Sinsabaugh et al. (2005), the quantity of soil organic matter results from a suite of soil enzyme activities. Stimulated oxidative enzyme activities, in particular peroxidase, by SLE compared to AN application imply that long-term SLE application may not benefit C sequestration in soils. In fact, we did observe that at the highest N application rate, organic C was significantly lower in soils supplied with SLE than AN (Table 1).

In conclusion, soil enzymes involved in C, N and P cycling responded differently to fertilization-associated soil properties. While enzymes involved in N and P cycling were very sensitive to decreases in soil pH due to SLE or AN application at a high rate. .In accordance with a bulk of publications, activities of oxidative enzymes (phenol oxidase and peroxidase) decreased or remained at the level of unfertilized controls as a consequence of 3 consecutive years of fertilization with AN. However, activity of those enzymes was stimulated by the SLE application. Differences in soil enzyme activities between soils receiving SLE and AN are hypothesized to result from changes in soil microbial community composition, caused in part by prolonged changes in soil pH.

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Table 1. Selected soil chemical and microbiological properties in bermudagrass and tall fescue plots receiving either ammonium nitrate (AN) or swine lagoon effluent (SLE) at application rates of 0, 200, 400, or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Values are means of n = 3

Source	Rate	Bermudagrass field plots			Tall fescue field plots		
	(kg N ha^{-1} y^{-1})	Soil pH	Soil C (g C kg^{-1} soil)	Microbial biomass (µg C g^{-1} soil)	Soil pH	Soil C (g C kg^{-1}soils)	Microbial biomass (µg C g^{-1} soil)
Control	0	6.1	9.8	156.0	5.9	11.2	216.7
AN	200	6.2	11.2	479.9	6.1	11.4	330.6
	400	6.3	9.9	311.1	5.9	11.1	290.9
	600	5.7	11.8	238.3	5.6	12.7	257.6
SLE	200	5.9	9.4	205.9	5.9	11.5	283.0
	400	6.1	10.4	336.1	5.6	11.8	141.6
	600	4.9	10.4	198.4	5.3	11.1	211.4
	$LSD_{0.05}$	NS	NS	62.5¶, 54.1§	NS	NS	NS
		N rate treatment means			----------N rate treatment means----------		
	0	6.1	9.8		5.9	11.2	216.7

200	6.1	10.3	6.0	11.5	306.8
400	6.2	10.2	5.8	11.5	216.3
600	5.3	11.1	5.5	11.9	234.5
$LSD_{0.05}$	0.43	NS	0.3	NS	NS
	N source treatment means		----------N source treatment means----------		
AN	6.1	11.0	5.9	11.7	293.0
SLE	5.6	10.1	5.6	11.5	212.0
$LSD_{0.05}$	0.4	0.8	0.2	NS	NS

[¶] LSD for comparing N sources at the same N rate.
[§] LSD for comparing N rates within N source.
NS, not significant at the 0.05 probability level.

Table 2. Analysis of variance for activities of soil enzymes as affected by N fertilization source (S) and fertilization rate (R) in bermudagrass and tall fescue forage systems

Source of Variation	df	Phenol oxidase	Peroxidase	CBH†	β-Glucosidase	Cellulase	NGA‡	Protease	Acid phosphatase
				Bermudagrass					
Rep	2								
Source (S)	1	NS	*	NS	NS	NS	NS	NS	NS
Error a (S x Rep)	2								
N rate (R)	3	NS	NS	NS	*	NS	*	**	**
R x S	3	NS	NS	NS	NS	NS	NS	*	NS
Error b (R x Rep (S))	12								
				Tall fescue					
Rep	2								
Source (S)	1	NS	*	NS	NS	NS	NS	NS	NS
Error a (S x Rep)	2								
N rate (R)	3	NS	NS	*	NS	**	**	*	**
R x S	3	*	NS	NS	NS	NS	*	NS	NS
Error b (R x Rep (S))	12								

† CBH, Cellobiohydrolase; ‡ NGA, N-acetyl-β-D glucosaminidase.

*, **, *** and NS Significant at 0.05, 0.01 and 0.001 probability level and not significant respectively.

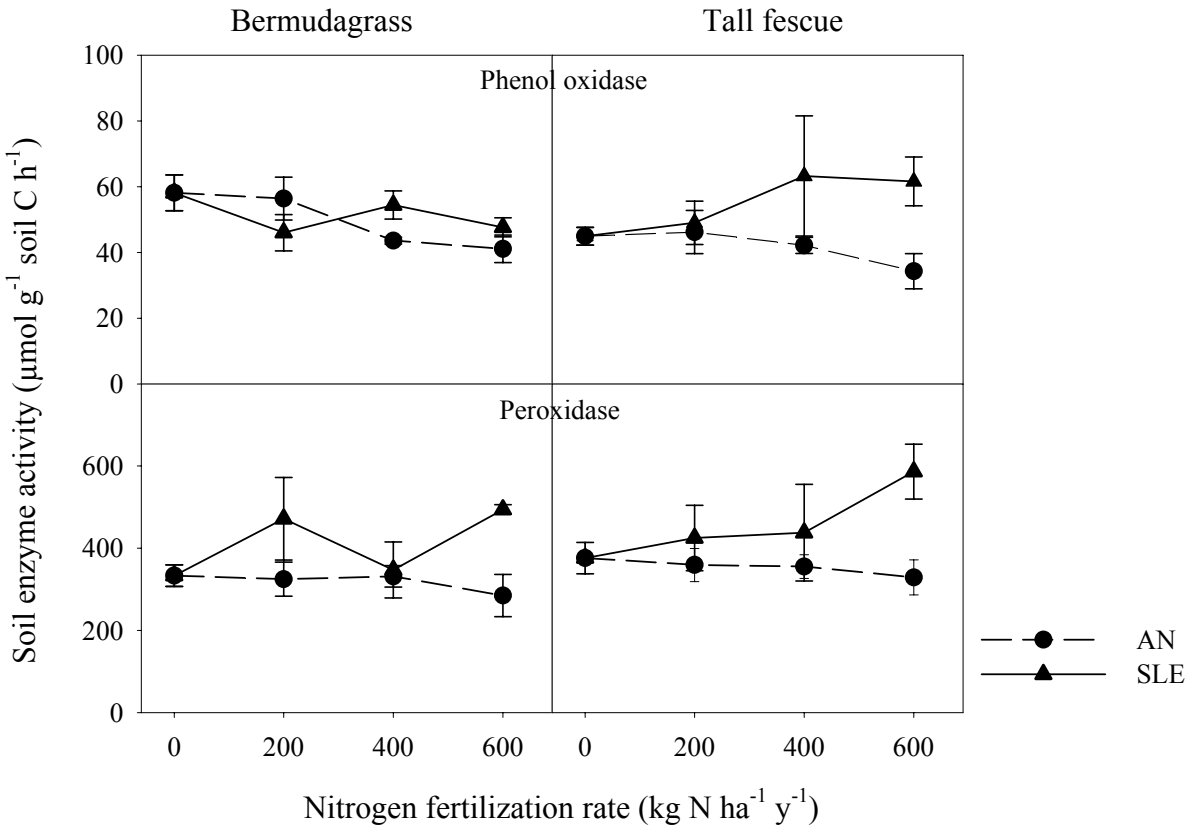

Fig. 1. Soil oxidative enzyme activities of phenol oxidase and peroxidase as a consequence of 3 consecutive years of swine lagoon effluent (SLE) or ammonium nitrate (AN) application at rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} in bermudagrass and tall fescue fields.

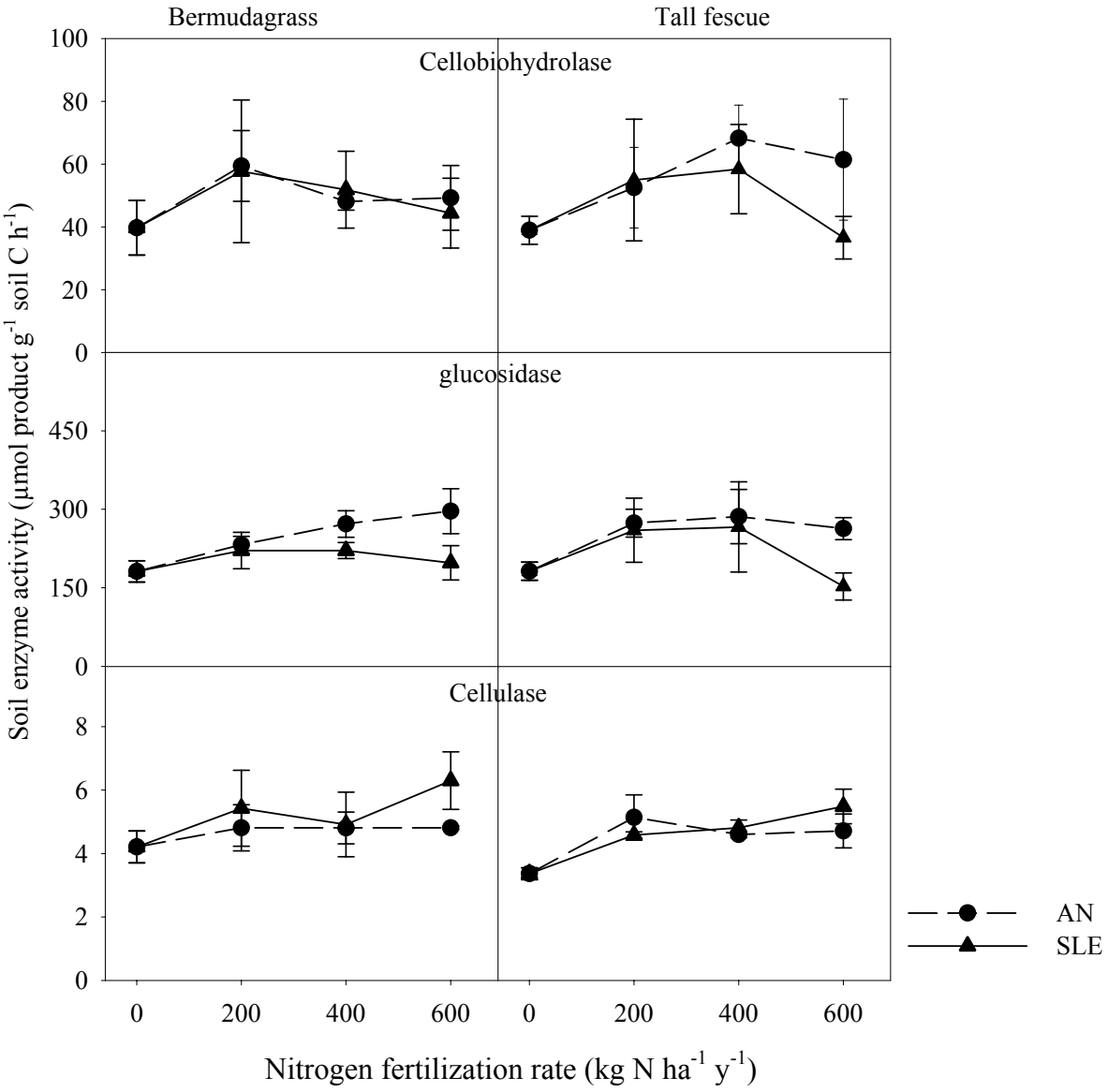

Fig. 2. Soil hydrolytic enzyme activities of β-glucosidase, cellobiohydrolase and cellulase as a consequence of 3 consecutive years of swine lagoon effluent (SLE) or ammonium nitrate (AN) application at rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} in bermudagrass and tall fescue fields.

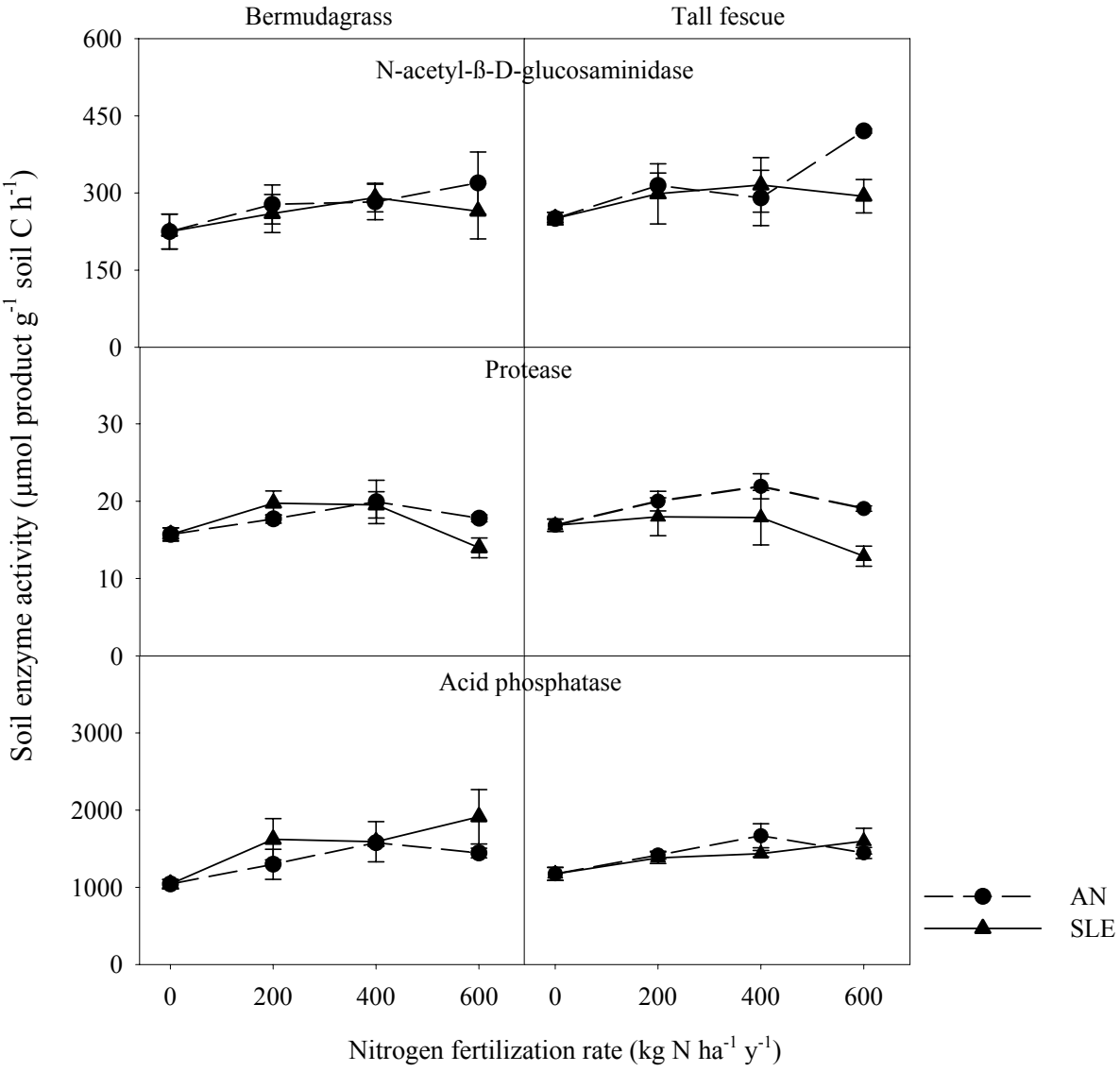

Fig. 3. The activities of soil enzymes involved in N and P cycling as a consequence of 3 consecutive years of swine lagoon effluent (SLE) or ammonium nitrate (AN) application at rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} in bermudagrass and tall fescue fields.

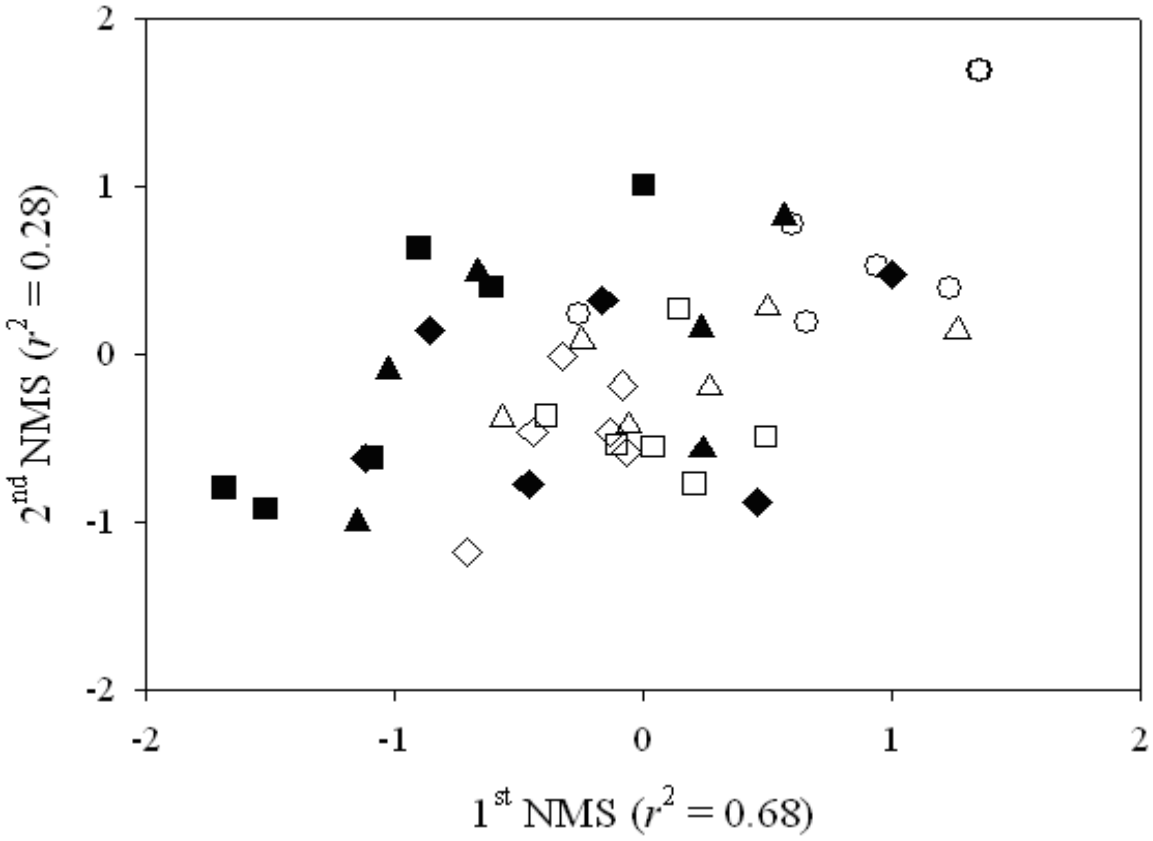

Fig. 4. Non-metric multidimensional scaling (NMS) integrated the eight soil enzyme activities in the study and accordingly distinguished soils. Symbol ○ represents unfertilized controls. Symbols ▲,♦, ■ represent the application rates of 200, 400, and 600 kg N ha^{-1} y^{-1} of swine lagoon effluent (SLE), respectively. The empty symbols are ammonium nitrate (AN)-applied soils.

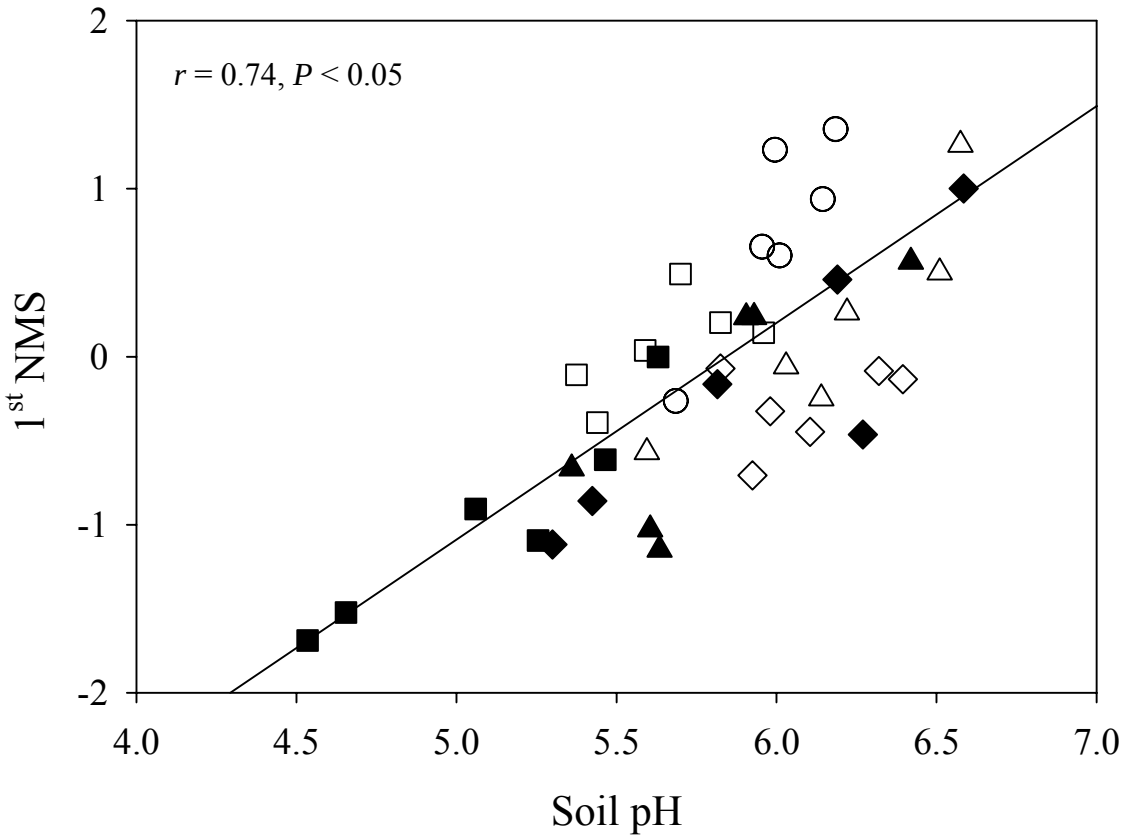

Fig. 5. Pearson correlation between the ordination score of the first axis of the NMS ordination (which explains 68% of the variance in original data) and soil pH.

APPENDIX

Appendix. Table of Pearson correlation (*r*) between the ordination score of the first axis of the non-metric multidimensional scaling ordination (which explains 68% of the total variance in the original integrated enzymes activity data) and key soil characteristics.

Variable	*R*	*P*
Soil pH	0.74	***
Soil C (mg g^{-1} soil)	0.14	ns
Soil N (mg g^{-1} soil)	-0.05	ns
ASN[§] (µg g^{-1} soil)	-0.47	**
EC[¶] (dS m^{-1})	0.06	ns
Soluble C[†] (µg g^{-1} soil)	-0.27	ns
Inorganic N (µg g^{-1} soil)	-0.07	ns

[§] Amino sugar nitrogen
[¶] Electrical conductivity
[†] 0.5 M K_2SO_4 soluble soil organic C
, * and ns significant at the 0.01 and 0.001 probability levels and not significant respectively.

CHAPTER FIVE

MICROBIAL COMMUNITY COMPOSITION AS A CONSEQUENCE OF CONTRASTING N FERTILIZATION: PROFILES OF PHOSPHOLIPID FATTY ACIDS AND COMMUNITY-LEVEL PHYSIOLOGY

Abstract

Our previous study illustrated that contrasting N fertilization of swine lagoon effluent (SLE) versus ammonium nitrate (AN) could alter the activities of soil enzymes involved in soil C, N and P cycling in different ways. These differences were proposed to result from shifts in soil microbial community composition and catabolic activities. In this study, microbial community structure and catabolic function were characterized using phospholipid fatty acids (PLFAs) and community-level physiological profile (CLPP). Soils (0 – 7.5 cm depth) were sampled in March 2004 from field plots cropped with bermudagrass or tall fescue that received 0 to 600 kg plant available N as SLE or AN ha^{-1} y^{-1} from 1999–2001. Analyses of PLFA profiles demonstrated that the relative abundance of 16:1ω7c, i17:0, 18:1ω7c PLFA biomarkers were ~15% lower and cy19:0 biomarker was 10% higher in soils supplied with SLE compared to soils supplied with AN indicating a significant ($P < 0.05$) shift in microbial community composition. These variations were strongly correlated with fertilization-induced changes in soil pH ($r = 0.80$, $P < 0.01$). Soil microbial community in acidic soils possessed relatively low concentrations of monounsaturated fatty acids but high ratios of saturated-to-unsaturated fatty acids and cyclopropyl fatty acids-to-their monoenoic precursors. CLPP showed increased utilization of lactose, glucose, glycogen and cellobiose C substrates by the bacterial community residing in soils supplied with SLE compared to those

supplied AN. Significant differences ($P < 0.05$) between soils fertilized with SLE and AN with respect to C utilization, suggests that changes in microbial community structure were manifested in microbial catabolic function. Again, the variations in CLPP were correlated with soil pH ($P < 0.01$). The significant correlation of PLFA or CLPP profile with soil pH denotes that fertilization-induced change in soil pH is the predominant factor in influencing soil microbial community composition and catabolic function in this managed forage production system.

Introduction

In the southeastern USA, large scale development of confined swine industry has produced large amount of swine waste. These wastes are often subjected to anaerobic lagoon treatment and the resultant swine lagoon effluent (SLE) is irrigated onto agricultural lands. As a surrogate of synthetic N fertilizer, SLE can increase yield and nutritive value of crops (Adeli et al., 2005). However, there has been a great concern that SLE application would have an adverse impact on environmental quality. Research had focused on monitoring chemical movement in soils and on NO_3^- concentrations in ground and surface waters (King et al., 1985; Israel et al., 2005). While soil microbial communities play central roles in organic matter decomposition and nutrient cycling, less information is available about impacts of SLE application on soil microbial properties. Given that microbes can respond to environmental changes faster than many physical and chemical soil properties, microbial properties may be better indicators to predict any future adverse impacts of SLE fertilization on soil fertility and environmental quality.

Our previous studies have demonstrated that SLE fertilization decreased soil microbial biomass, soil processes such as nitrification and increased oxidative enzyme activities involved in C cycling, and altered the activities of hydrolytic enzymes involved in N and P cycling; These alterations in soil enzyme activities were strongly correlated with the changes in soil pH caused by N fertilization (unpublished data). Because management practices may modify the soil microbial communities, an important component regulating soil processes (Balser et al., 2002), changes in soil processes and enzymatic activities may be associated with changes in microbial community composition

Many studies have demonstrated that a change in management practices can restructure soil microbial community composition. For example, Bossio et al. (1998) reported that microbial phospholipid fatty acid (PLFA) profiles differed between organic and conventional farming systems. Again, soils fertilized with dairy manure expressed a PLFA profile distinct from that observed for ammonium nitrate (AN) (Peacock et al., 2001) suggesting differences in microbial community composition (Marschner, et al., 2003; Larkin et al., 2006). In this study, PLFA and community level physiological profile (CLPP) were used to assess the impact of SLE fertilization on soil microbial community composition. We hypothesize that (1) fertilization of soils with SLE or AN could restructure soil microbial community compared to unfertilized soils, and microbial community composition would differ between soils fertilized with SLE versus AN; and (2) that the differences in microbial community composition would be associated adjustments in soil properties, in particular soil pH.

Materials and Methods

Study Site and Soil Sampling

The study site was located on the Caswell Farm, a research station of North Carolina State University, near Kinston, Lenoir County, NC. The soil was classified as Pocalla loamy sand (loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults). Field plots were established in fall 1998 to examine the responses of yield and N content of forage dry matter to different SLE application rates. Four rates of 0, 200, 400, and 600 kg plant available N (PAN) ha^{-1} yr^{-1} as SLE and AN were applied to warm- and cool-season grass species of bermudagrass (overseeded with rye), tall fescue, gamagrass, and crabgrass (overseeded with rye), and rescue grass. Two species (bermudagrass and tall fescue) were chosen for this study. A partially stripped split plot design was used with three replications: grass species were stripped across fertilization source (i.e. SLE and AN); application rates were stripped across grass species and nested within fertilization source of each replication. A treatment split plots were ~ 3 m × 6 m. Swine lagoon effluent was applied using a small plot effluent sprayer. The application rate was calculated using a PAN coefficient as 60% of total N and verified using collection cups placed on the plots. Chemical composition of SLE during ~ 3 yr application averaged ~ 293 mg total N L^{-1}, 87 mg P L^{-1}, 198 mg K L^{-1}, 154 mg Ca L^{-1}, 58 mg Mg L^{-1}, 28 mg S L^{-1}, 84 mg Na L^{-1} and trace amounts (i.e. < 10 mg L^{-1}) of Fe, Mn, Zn, and Cu. SLE was neutral with a value of pH 7.1. For plots that received broadcast application of AN and unfertilized control, P and K were also applied based on annual soil test recommendation. Because SLE was irrigated onto plots, the plots with AN application received irrigation water equivalent to that in SLE. Both SLE and AN were applied every month from February to November; the split amounts of N were based on the growth

physiologies of individual grass species. Forages were harvested in a manner that was consistent with the recommended practices for individual species. Fertilization treatments were implemented from April 1999 to November 2001. Thereafter, the treatments were terminated but the forage was continually mowed and baled for hay until December 2004.

Soil samples were collected (0-7.5 cm depth) in March 2004 from the field plots planted with bermudagrass and tall fescue. Three soil cores (5 cm diameter) were taken from the center of each plot and composited. Soils were sieved (< 4 mm) and sub-samples were freeze-dried after removing visible leaf and root residues. The remaining soils were stored at 4 °C for later analyses of soil chemical and microbiological properties. Total soil C and N were determined by dry combustion using Perkin-Elmer Series II CHNS/O-2400 analyzer (Perkin Elmer Corp., Norwark, CT) from sub-samples dried at 105 ºC and ground to < 250 μm. Soil pH was measured in 1:1 soil/water slurry. Soil microbial biomass C was determined by chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). See Table 1 for selected soil properties.

Assays on the Profiles of Phospholipid Fatty Acids

Soil microbial community composition was examined using profiles of phospholipid fatty acids. Phospholipid fatty acids (PLFA) of soil microbial community were extracted and subsequently derivatized into fatty acid methyl esters (FAME) which can be separated and identified using a gas chromatography (Yao et al., 2006). In brief, PLFA were extracted from freeze-dried soils (~ 5 g) in a single phase extraction mixture of 1-to-2-to-0.8 volume ratio of chloroform-to-methanol-to-phosphate buffer. Total lipids were then subjected to fractionation using silicic acid column and fatty acids were completely separated from

glycolipids and neutral lipids. The fatty acids were processed through alkaline methylation. The FAME were subsequently separated and identified using a gas chromatograph (Hewlett Packard 6890, San Fernando, CA, USA) equipped with the MIDI peak identification software (MIDI, Inc., Newark, DE, USA).

Fatty acid nomenclature is according to Frostegård et al. (1993). Unsaturated fatty acid 18:2ω6,9c was used to indicate fungal biomass, whereas the sum of fatty acids i14:0, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, cy17:0, 17:0, and 18:1ω7c was used to indicate bacterial biomass (Frostegård and Bååth, 1996). The ratios of relative abundance of cyclopropyl fatty acids/monoenoic precursors (cy17:0/16:1ω7c), (cy19:0/18:1ω7c) and total saturated/total monounsaturated fatty acids (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0/16:1ω11c + 16:1ω9c + 16:1ω7c + 16:1ω5c + 17:1ω9c + 17:1ω8c + 17:1ω7c + 17:1ω5c) were used to indicate stress in bacterial communities (Kieft et al., 1997; Bossio and Scow, 1998 and Fierer et al., 2003). A change in relative abundance monounsaturated fatty acids was used to indicate a shift in microbial community composition (Bossio and Scow, 1998).

Assays on Microbial Community-level Physiological Profiles (CLPP)

Bacterial community responses to an array of individual C substrates were examined and profiles of microbial community-level physiology were characterized. BIOLOG ECO micro-plates (BIOLOG Inc., Hayward, CA, USA), which contains three sets of 32 wells and each set consists of 31 individual C compounds and one control (i.e. no C compound), were inoculated with soil extracts. Soils (10 g) were first extracted with 90 ml of 50 mM phosphate buffer (pH 7.0) and then subjected to a series of 10-fold dilutions. Final soil extracts (150 µl) of 10^{-3} dilution were inoculated into the micro-plates and incubated at 25^{o}C.

The color development was measured immediately (time 0) after the inoculation of soil extracts and then every 24 h for 7 days by the absorbance at 590 nm using a μQuant micro-plate spectrophotometer (Bio-TEK instruments, Inc., Winooski, VT, USA). Because fine particles of soil in extracts might interfere with the measurement, we corrected the turbidity of soil extracts by the absorbance at 750 nm (Classen et al., 2003).

After subtraction of absorbance of control well and absorbance at time 0, the difference in absorbance between 590 nm and 750 nm was used to represent bacterial utilization of individual C compounds. If a value of absorbance was less than 0.1, it was considered as no response of bacterial community to this substrate. Accordingly, the value was set to zero for the analysis of microbial community-level physiology profile. Average well color development (AWCD) was calculated according to Garland and Mills (1991). The AWCD at day 7 was used to indicate the maximum microbial metabolic activity. However, the comparable AWCD among soil samples were chosen by running analysis of variance and then was used to determine the patterns of community-level physiological profiles.

Data Analysis

Non-metric multidimensional scaling (NMS) ordination (McCune and Mefford, 1999) was used to group soil microbial communities, and the analysis was performed using PC-ORD software (MjM Software, Gleneden Beach, Oregon, USA). The mol% of 29 PLFAs, which appeared in most soil samples, was used in NMS analysis. The fraction of absorbance of individual C compounds to the AWCD was used to construct the NMS so that the effects due to different inoculum density could be minimized (Garland, 1997). Furthermore, a multi-response permutation procedure (MRPP) was used to test if microbial

community profiles of PLFA or CLPP differed between forage species (bermudagrass versus tall fescue), between sources of N fertilizers (AN versus SLE), and among different rates of N fertilization. MRPP is a nonparametric procedure in which the *A* value ranges from 0 to 1 and a higher *A* value describes within-group homogeneity and *P* value evaluate how likely an observed difference is due to chance (McCune and Grace, 2002). 'Indicator species' analysis was performed when necessary to facilitate the identification of biomarkers and C substrates that are important descriptors of treatment effects in PLFA profiles and CLPP respectively (McCune and Grace, 2002).A univariate analysis was also performed on the CLPP data set to examine the diversity of soil microbial community-level physiology. According to Zak et al. (1994), Shannon's diversity (H) was calculated by the equation: $H = \Sigma\, p_i\, (\ln p_i)$, where p_i is the ratio of absorbance of *i*th substrate to sum of the absorbance of all substrates.

The data obtained from PLFA and CLPP assays were analyzed by individual forage species in split-plot design, with N source as the whole plot factor and N rate as the sub-plot factor. The PROC MIXED in SAS 9 version (SAS Institute Inc. 2001, Cary, North Carolina, USA) was used to carry out the split-plot analysis and the differences among treatment means were compared by F-protected least significant differences (LSD) at $P < 0.05$.

Results

Profiles of Phospholipid Fatty Acids

Microbial community composition in soils receiving contrasting N fertilization at different rates was substantially different (Fig. 1A). The r^2 values of axis 1 (0.78) and 2 (0.16) of NMS explained most of variation in PLFA (94%) in all the soil samples. Because microbial community composition in soils cropped with bermudagrass was similar to those

planted with tall fescue ($A = 0.013$, $P = 0.15$), soils cropped with different plants were not differentiated in Fig. 1A. However, there were significant differences in microbial community composition between soils fertilized with SLE ($A = 0.18$, $P < 0.05$) or AN ($A = 0.11$, $P < 0.05$) and unfertilized controls. Microbial community composition was also different between soils fertilized with SLE versus with AN ($A = 0.11$, $P < 0.05$). This difference between SLE- and AN-fertilized soils was more pronounced at N application rate of 600 kg N ha^{-1} y^{-1} ($A = 0.26$, $P < 0.05$) (Fig. 1A). The dimension of NMS that explained the largest variation of PLFA in all the soil samples (i.e. NMS axis 2) was significantly correlated with the soil pH ($r = 0.80$, $P < 0.01$) (Fig. 1B).

Individual PLFAs that greatly contributed to the separation of soil microbial communities between fertilized and unfertilized soils and between SLE- and AN-fertilized soils are listed in Table 4. Monounsaturated and branched saturated fatty acids differed for their roles in discriminating soil microbial communities. Monounsaturated fatty acids were positively associated with AN-fertilized soils, while branched saturated fatty acids were positively associated with SLE-fertilized soils. Again, the mol% of these individual PLFA was highly correlated with soil pH (Table 4).

Several biomarkers of individual or group PLFA also illustrated some differences between fertilized and unfertilized soils and between AN- and SLE-fertilized soils (Tables 2, and 5a, b). For example, the abundance of fungi,(18:2ω6,9c), was significantly affected by levels of N fertilization (Table 2) and tended to be higher in soils fertilized at 200 or 400 kg N ha^{-1} y^{-1} compared to that in unfertilized controls or soils fertilized at 600 kg N ha^{-1} y^{-1} (Tables 5a, 5b). Accordingly, ratio of fungi-to-bacteria changed with the rates of N fertilization (Table 2). Soils fertilized with SLE at 600 kg N ha^{-1} y^{-1} differed significantly

from others in the ratio of saturated-to-unsaturated fatty acids, cy17:0 or cy/19:0-to their corresponding precursor in tall fescue system (Table 5b). Again, the changes of these individual or group PLFA were correlated with soil pH (Fig. 2). The abundance of monounsaturated fatty acids increased with increase in soils pH ($r = 0.72$, $P < 0.01$). In contrast, ratios of saturated-to-monounsaturated fatty acids, cy17:0-to-its precursor and cy19:0-to-its precursor decreased significantly ($P < 0.01$) with increase in soil pH (Fig. 2).

Community-level Physiological Profiles (CLPP)

Shannon's diversity index did not differ between soils fertilized with SLE versus AN and the levels of N fertilization (Tables 3 and 6a, b). However, the numerically highest diversity occurred in soils fertilized with SLE despite maximum metabolic activity being much lower in soils fertilized with SLE at the rate of 400 or 600 kg N ha^{-1} y^{-1} than the other treated soils (Tables 6a, b). The maximum metabolic activity was not correlated with total microbial biomass C. For example, microbial biomass differed significantly between soils fertilized with AN at 200 versus 600 kg N ha^{-1} y^{-1} (Table 1), but the maximum metabolic activity in these soils was similar (Tables 6a, b). This indicates that there were differences in microbial community-level physiology, and these differences were not due to any difference in inoculum density. Differences in microbial community-level metabolic activity were clearly reflected in microbial community utilization of different groups of C substrates (Tables 3, and 7a, b). For example, while being independent of fertilization source, microbial utilization of amines and phenolic compounds were significantly altered by levels of N fertilization in bermudagrass system (Tables 3, and 7a). However, microbial community

utilization of amines varied with the source and rates of N fertilization in tall fescue plots (Tables 3, and 7b).

Non-metric multidimensional scaling (NMS) of CLPP data showed that microbial community physiological profiles were more clustered for soils fertilized with AN at different rates than those with SLE (Fig. 3A). Furthermore, MRPP analysis indicated that CLPP of microbes differed significantly between AN- and SLE-fertilized soils ($P < 0.05$), and this difference was more pronounced for soils that received above 400 kg N ha^{-1} y^{-1}. The variation in CLPP for soils receiving different source and rate of fertilization was also significantly ($r = 0.45$; $P < 0.01$) correlated with soil pH (Fig. 3B).

Discussion

Fertilization of forage production systems with SLE or AN for 3 consecutive years generated significant residual effects on the structure and function of soil microorganisms. This modification in soil microbial community was associated with fertilization-induced change in soil pH. Not only was PLFA strongly correlated with soil pH, CLPP also exhibited significant correlation with soil pH. However, grass species (warm- versus cool-season grasses) had little effect on the composition and function of soil microbial community.

It is well known that soil pH can exert a selection pressure on soil microorganisms since different microbes have varying degrees of biochemical capacities for surviving and proliferating as soil pH changes. As one example, some microbes only grow better under acid condition, i.e. acidophiles, neutral pH retard the growth of these acidophiles (Brock et al., 1994). Soil pH may also affect soil microbial community composition indirectly via controls on the availability of various nutrients (McLaren and Skujins, 1968). Crowley and Alvey

(2002) indicated that soil pH regulates the bioavailability of base cations, phosphorus, and heavy metals; most nutrients are available to soil microbes at pH of 6 to 7 (Sylvia et al., 2005). The impacts of soil pH on microbial community composition are possibly multi-faceted, and these complex interactions may magnify soil pH as a key factor regulating soil microbial community composition at a local, regional or even global scale. A recent study (Fierer and Jackson, 2006) has demonstrated that at a global scale, soil pH overwhelms other factors and is the most important factor dictating the diversity of soil microbial community. This study also showed that microbial community composition was strongly associated with soil pH in this forage production system.

The relationships, observed in this study, between soil pH and several PLFAs were similar to those of others (Frostegård et al., 1993; Schutter and Fuhrmann, 2001; Bååth and Anderson, 2003); the relative abundance of monounsaturated fatty acids significantly increased with the increase in soil pH, whereas the relative abundance of cyclopropane fatty acids declined (Tables 5a, b, Fig. 2). These types of changes in PLFA profiles were also observed for microbial communities under other stress conditions. For example, Bossio and Scow (1998) reported that monounsaturated fatty acids were reduced with low soil O_2 concentration as well as with the lack of C resource. Fierer et al. (2003) also showed that the ratio of cyclopropane fatty acids-to-their monoenoic precursors and ratio of saturated-to-monounsaturated fatty acids increased due to lack of C resource at deeper soil horizons. Therefore, it seems that regardless of the stress factor, the stress-induced changes in PLFA profiles of microbial community were similar.

However, changes in PLFA profiles may not simply result from the shifts in taxonomic species, but also from the changes in physiological status of microbes under stress

conditions (Bossio and Scow, 1998). In a earlier study using single species of *Escherichia coli*, Knivett and Cullen (1965) clearly demonstrated that the fatty acids of a bacteria could change with growth conditions and cyclopropane fatty acids were increased by acid media. Similarly, physiological status of a *Pseudomonas aureofacines* strain could be mirrored in its membrane PLFA profiles (Kieft et al., 1994, 1997); stresses caused an increase in ratios of saturated-to-unsaturated fatty acids and cyclopropyl fatty acids-to-their monoenoic precursors. Nevertheless, changes in relative abundance of monounsaturated fatty acids are often attributed to shifts in microbial community compositions (Bossio and Scow, 1998). We did observe a significant pH-associated change in monounsaturated fatty acids (Fig. 2), indicating authentic modification in soil microbial community composition by soil pH. It may be possible to use molecular biology techniques to elucidate changes in microbial community composition at a fine scale level.

It is very interesting to notice that relative abundance of fungi (i.e. 18:2ω6, 9c) and thus the ratio of fungi-to-bacteria were not correlated to soil pH ($r = 0.17$, $P = 0.24$; $r = 0.22$, $P = 0.12$ respectively) While unfertilized controls and soils fertilized with SLE or AN at the highest rate differed significantly in their soil pH (Table 1), their fungal abundance and thus the ratio of fungi-to-bacteria were similar (Table 5a, b). These results were also consistent with the conclusions obtained from the microbial C-to-N ratios in this forage production system (unpublished data). Bååth and Anderson (2003) found that relative abundance of fungi (i.e. 18:2ω6, 9c) was positively correlated with soil pH, but the correlation was much lower than other PLFA parameters, such as abundance of cy19:0 and monounsaturated fatty acids. In contrast, Clegg (2006) demonstrated that the relative abundance of soil fungi was negatively correlated with soil pH. These contradictory results, together with our

observations, suggest that relative abundance of soil fungi or the ratio of fungi-to-bacteria determined by PLFA may not be sensitive to changes in soil pH. By corollary, other soil factors may easily mask the changes of relative abundance of fungi and bacteria caused by soil pH. Accordingly, pH impacts on fungal community could be ecosystem-specific. All possible responses to soil pH (i.e. positive, negative or no relation) could be observed.

Microbial community catabolic activity and profile exhibited a significant correlation with soil pH (Tables 6a, b, Fig. 3b), indicating that microbial community structure, to some extent, determines its catabolic function. Our finding that microbial catabolic activity decreased with decreasing soil pH (Tables 6a, b) was similar to results from other studies (Bååth, 1998; White et al., 2005). However, microbial diversity of catabolic function increased slightly as soil pH decreased (Tables 6a, 6b). Yao et al. (2006) compared microbial catabolic function between two soils that had ~ 1.5 unit difference in soil pH and observed no difference in their Shannon diversity indexes calculated from Biolog ECO plates. By contrast, the authors observed significant variations in community catabolic function when a multivariate-analysis was performed. In this study, significant catabolic variations were observed when Biolog ECO plates were examined at a multivariate scale (Fig. 3). Our results together with Yao et al. (2006) imply that a multivariate analysis on Biolog ECO plates provides finer resolution for differentiating microbial community catabolic function.

Plants are considered an important determinant of soil microbial community structure and function (Grayston et al., 1998; Grayston et al., 2001; O'Donnell et al., 2001), due mainly to the differences in the quality and quantity of plant-derived organic matter input into the soil. However, a bulk of publications demonstrated that direct impacts of plant species on soil microbial community composition in many terrestrial ecosystems may not be

as great as once thought since soil properties may often overwhelm the influence of plant species on microbial communities (Bossio et al., 1998; Buckley and Schmidt, 2001). In some studies where plant species are considered as an important factor influencing soil microbial community, plant species effects may often be confounded with the impacts of soil properties. For example, shifts in soil microbial communities associated with land-use change are often due to both plant species and associated changes in soil properties (Nüsslein and Tiedje, 1999; Yao et al., 2006). In this forage production system; soil pH was clearly more powerful factor in influencing soil microbial community structure and function than plant species. White et al. (2005) examined soil microbial community catabolic function in boreal plain forest and concluded that soil pH rather than tree species or stand age was the most influential factor discriminating microbial communities. Collin and Cavigelli (2003) indicated that presence of plants rather than specific plant type was more important in dictating microbial catabolic function.

In our previous study (unpublished data), soil enzymatic profiles were correlated with fertilization-induced changes in soil pH. We hypothesized that soil enzymatic changes due to fertilization may partially result from changes in soil microbial community. In the present study, changes in microbial community composition and catabolic function were significantly correlated with changes in soil pH and accordingly may explain the variation in soil enzymatic activities. However, caution must be used in extrapolating differences in CLPP to in-situ microbial community function since CLPP may provide little information on in-situ microbial function (Konopka et al., 1998; Classen et al., 2003). Indeed, this significant difference in CLPP was not manifested in microbial C and N mineralization. There were

little or slight difference in C or N mineralization due to SLE or AN fertilization at rate > 200 kg N ha^{-1} y^{-1} (unpublished data).

In conclusion, microbial community composition and physiology can be significantly affected by changes of soil properties associated with fertilization practices. Significant correlations of microbial community composition (i.e. PLFA data) and physiology (i.e. CLPP data) with soil pH indicate that soil pH was the predominant factor influencing microbial community in this forage production system. However, microbial community composition, as measured by PLFA, responded to variation in soil pH more strongly than microbial community physiology. This is partially because the two methods examined different groups of the microbial community. While PFLA assessed all microbial groups including fungi, the CLPP only assessed the bacterial community. In addition, microbial communities commonly exhibit functional redundancy (Balser et al., 2002).Accordingly, loss of or reduction in certain microbial groups would not necessarily alter microbial physiology or function at a community level. Even though soil microbial community composition and physiology changed with soil pH, this change might not be manifested as a change in microbially-mediated processes such as C and N mineralization.

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Table 1. Selected soil chemical and microbiological properties in bermudagrass and tall fescue plots receiving either ammonium nitrate (AN) or swine lagoon effluent (SLE) at application rates of 0, 200, 400, or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 y. Values are means of n = 3

Source	Rate	Bermudagrass field plots			Tall fescue field plots		
	(kg N ha^{-1} y^{-1})	Soil pH	Soil C (g C kg^{-1} soil)	Microbial biomass (µg C g^{-1} soil)	Soil pH	Soil C (g C kg^{-1}soils)	Microbial biomass (µg C g^{-1} soil)
Control	0	6.1	9.8	156.0	5.9	11.2	216.7
AN	200	6.2	11.2	479.9	6.1	11.4	330.6
	400	6.3	9.9	311.1	5.9	11.1	290.9
	600	5.7	11.8	238.3	5.6	12.7	257.6
SLE	200	5.9	9.4	205.9	5.9	11.5	283.0
	400	6.1	10.4	336.1	5.6	11.8	141.6
	600	4.9	10.4	198.4	5.3	11.1	211.4
	$LSD_{0.05}$	NS	NS	62.5¶, 54.1§	NS	NS	NS
		N rate treatment means			----------N rate treatment means----------		
	0	6.1	9.8		5.9	11.2	216.7
	200	6.1	10.3		6.0	11.5	306.8

400	6.2	10.2		5.8	11.5	216.3
600	5.3	11.1		5.5	11.9	234.5
$LSD_{0.05}$	0.43	NS		0.3	NS	NS
	N source treatment means			----------N source treatment means----------		
AN	6.1	11.0		5.9	11.7	293.0
SLE	5.6	10.1		5.6	11.5	212.0
$LSD_{0.05}$	0.4	0.8		0.2	NS	NS

[¶] LSD for comparing N sources at the same N rate.
[§] LSD for comparing N rates within N source.
NS, not significant at the 0.05 probability.

Table 2. Analysis of variance for group parameters of fatty acids as influenced by N fertilization source (S) and N fertilization rate (R) in bermudagrass and tall fescue forage systems.

Source of Variation	df	Fungi	Fungi/bacteria	Saturated/ monounsaturated	cy17:0/ 16:1ω7c	cy 19:0/ 18:1ω7c
			Bermuda grass			
Rep	2					
Source (S)	1	NS	NS	NS	NS	NS
Error a (S x Rep)	2					
N rate (R)	3	*	*	NS	NS	NS
R x S	3	NS	NS	NS	NS	NS
Error b (R x Rep (S))	12					
			Tall fescue			
Rep	2					
Source (S)	1	NS	NS	NS	NS	NS
Error a (S x Rep)	2					
N rate (R)	3	*	*	NS	**	**
R x S	3	NS	NS	NS	*	*
Error b (R x Rep (S))	12					

*, **, *** and NS Significant at 0.05, 0.01 and 0.001 probability level and not significant respectively.

Table 3. Analysis of variance Shannon's diversity index (H'), maximum metabolic activity and metabolic activity (average well color development, AWCD) of microbial community over a group of carbon substrates as influenced by N fertilization source (S) and N fertilization rate (R) in bermudagrass and tall fescue forage systems.

Source of Variation	df	Shannon's Index (H')	Maximum Metabolic activity	Metabolic activity					
				Carbohydrates	Carboxylic acids	Amino acids	Amines	Phenolics	Polymers
				Bermudagrass					
Rep	2								
Source (S)	1	NS	NS	NS	NS	NS	NS	NS	NS
Error a (S x Rep)	2								
N rate (R)	3	NS	NS	NS	NS	NS	*	*	NS
R x S	3	NS	NS	NS	NS	NS	NS	NS	NS
Error b (R x Rep (S))	12								
				Tall fescue					
Rep	2								
Source (S)	1	NS	NS	NS	NS	NS	*	NS	NS
Error a (S x Rep)	2								
N rate (R)	3	NS	NS	NS	NS	NS	*	NS	NS
R x S	3	NS	*	NS	NS	NS	NS	NS	NS
Error b (R x Rep (S))	12								

*, **, *** and NS Significant at 0.05, 0.01 and 0.001 probability level and not significant respectively.

Table 4. Individual PLFAs most responsible for the separation of microbial communities in soils receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. The loading score was for the dimension of non-metric multidimensional scaling (NMS) that explained the largest variance of an array of identified fatty acids. Pearson correlation expresses the linear relationship between relative abundance of individual to total identified fatty acids (i.e. mol%) and soil pH. All correlation coefficient (*r)* values were significant at $P < 0.01$.

PLFA	Loading score	Pearson correlation (*r*)
16:1ω7c	0.92	0.79
18:1ω7c	0.81	0.75
16:1ω5c	0.65	0.50
11Me18:1ω7c	0.59	0.39
i15:1	0.56	0.34
18:1ω5c	0.56	0.41
i17:0	-0.86	-0.71
i16:0	-0.85	-0.71
18:0	-0.85	-0.72
cy19:0	-0.84	-0.76
16:0	-0.62	-0.37

Table 5a. Group parameters of fatty acids that discriminate microbial communities in bermudagrass system receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at 0, 200, 400, or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Individual groups are expressed as their abundances relative to total amount of fatty acids (mol%). Values are means for n = 3.

Source	Rate	Fungi	Fungi/bacteria	Saturated fatty acids /monounsaturated fatty acids	cy17:0/16:1ω7c	cy 19:0/18:1ω7c
Control	0	2.1	0.06	0.78	0.59	0.99
AN	200	3.1	0.08	0.71	0.53	0.84
	400	3.2	0.08	0.67	0.54	0.91
	600	1.9	0.05	0.74	0.55	1.0
SLE	200	3.2	0.08	0.77	0.62	1.3
	400	3.1	0.08	0.72	0.54	1.1
	600	2.2	0.06	1.10	0.93	1.9
	$LSD_{0.05}$	NS	NS	NS	NS	NS
	----------N rate treatment means----------					
	0	2.1	0.06	0.78	0.59	0.99
	200	3.2	0.08	0.74	0.58	1.07
	400	3.2	0.08	0.70	0.54	1.0
	600	2.1	0.06	0.92	0.74	1.45
	$LSD_{0.05}$	0.95	0.01	NS	NS	NS
	----------N source treatment means----------					
	AN	2.7	0.07	0.71	0.54	0.91
	SLE	2.8	0.07	0.86	0.70	1.43
	$LSD_{0.05}$	NS	NS	NS	NS	NS

Table 5b. Group parameters of fatty acids that discriminate microbial communities in tall fescue system receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at 0, 200, 400, or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Individual groups are expressed as their abundances relative to total amount of fatty acids (mol%). Values are means for n = 3.

Source	Rate	Fungi	Fungi/bacteria	Saturated fatty acids /monounsaturated fatty acids	cy17:0/16:1ω7c	cy 19:0/18:1ω7c
Control	0	2.3	0.06	0.77	0.55	1.1
AN	200	3.0	0.08	0.67	0.53	0.97
	400	3.7	0.10	0.68	0.57	1.0
	600	2.1	0.05	0.78	0.59	1.1
SLE	200	3.1	0.08	0.78	0.61	1.2
	400	3.3	0.08	0.77	0.63	1.3
	600	2.3	0.06	0.93	0.79	1.9
	$LSD_{0.05}$	NS	NS	NS	0.29¶, 0.24§	0.38¶, 0.31§
	----------N rate treatment means----------					
	0	2.3	0.06	0.77		
	200	3.1	0.08	0.73		
	400	3.5	0.09	0.73		
	600	2.2	0.06	0.86		
	$LSD_{0.05}$	0.74	0.02	NS		
	----------N source treatment means----------					
	AN	2.9	0.08	0.71		
	SLE	2.9	0.07	0.83		
	$LSD_{0.05}$	NS	NS	NS		

¶ LSD for comparing N sources at the same N rate.
§ LSD for comparing N rates within N source.
NS, not significant at the 0.05 probability level.

Table 6a. Maximum metabolic activity (i.e. average well color development, AWCD) and Shannon's diversity index (H) obtained from the profiles of microbial community-level physiology in bermudagrass system receiving ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Values are means for n = 3.

Source	Rate	Maximum metabolic activity (AWCD)	Shannon's Index (H')
Control	0	0.73	2.76
AN	200	0.88	2.77
	400	0.81	2.69
	600	0.85	2.74
SLE	200	0.98	2.78
	400	0.72	2.82
	600	0.77	2.88
	$LSD_{0.05}$	NS	NS
		----------N rate treatment means----------	
	0	0.73	2.76
	200	0.93	2.78
	400	0.77	2.76
	600	0.81	2.81
	$LSD_{0.05}$	NS	NS
		----------N source treatment means----------	
	AN	0.85	2.73
	SLE	0.82	2.83
	$LSD_{0.05}$	NS	NS

NS, not significant at the 0.05 probability level.

Table 6b. Maximum metabolic activity (i.e. average well color development, AWCD) and Shannon's diversity index (H) obtained from the profiles of microbial community-level physiology in tall fescue system receiving ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Values are means for n = 3.

Source	Rate	Maximum metabolic activity (AWCD)	Shannon's Index (H')
Control	0	0.77	2.81
AN	200	0.77	2.83
	400	0.78	2.86
	600	0.82	2.68
SLE	200	0.96	2.91
	400	0.69	2.83
	600	0.72	2.93
	$LSD_{0.05}$	0.19[¶], 0.17[§]	NS
			N rate treatment means
	0		2.81
	200		2.87
	400		2.84
	600		2.81
	$LSD_{0.05}$		NS
			N source treatment means
	AN		2.79
	SLE		2.89
	$LSD_{0.05}$		NS

[¶] LSD for comparing N sources at the same N rate.
[§] LSD for comparing N rates within N source.
NS, not significant at the 0.05 probability level.

Table 7a. Metabolic activity (i.e. average well color development, AWCD) of microbial community over a group of C substrates in soils receiving ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Values are means for n = 3.

Source	Rate	Carbohydrates	Carboxylic acids	Amino acids	Amines	Phenolics	Polymers
Control	0	0.19	0.67	0.91	0.51	0.38	0.49
AN	200	0.27	0.63	0.76	0.81	0.72	0.50
	400	0.23	0.67	0.84	0.44	0.47	0.52
	600	0.25	0.68	0.94	0.68	0.56	0.39
SLE	200	0.26	0.73	0.91	0.64	0.63	0.50
	400	0.30	0.77	0.70	0.27	0.27	0.61
	600	0.38	0.65	0.78	0.38	0.61	0.58
	$LSD_{0.05}$	NS	NS	NS	NS	NS	NS
		----------N rate treatment means----------					
	0	0.19	0.67	0.91	0.51	0.38	0.49
	200	0.27	0.68	0.84	0.73	0.68	0.50
	400	0.27	0.72	0.77	0.36	0.37	0.57
	600	0.32	0.67	0.86	0.53	0.59	0.49
	$LSD_{0.05}$	NS	NS	NS	0.21	0.24	NS
		----------N source treatment means----------					
	AN	0.25	0.66	0.85	0.64	0.58	0.47
	SLE	0.31	0.72	0.80	0.43	0.50	0.56
	$LSD_{0.05}$	NS	NS	NS	NS	NS	NS

Table 7b. Metabolic activity (i.e. average well color development, AWCD) of microbial community over a group of C substrates in tall fescue system receiving ammonium nitrate (AN) or swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for consecutive 3 years. Values are means for n = 3.

Source	Rate	Carbohydrates	Carboxylic acids	Amino acids	Amines	Phenolics	Polymers
Control	0	0.25	0.67	0.84	0.44	0.65	0.54
AN	200	0.22	0.74	0.72	0.76	0.37	0.54
	400	0.24	0.72	0.79	0.56	0.27	0.54
	600	0.16	0.73	0.76	0.72	0.54	0.53
SLE	200	0.27	0.67	0.91	0.62	0.75	0.57
	400	0.31	0.67	0.75	0.35	0.66	0.67
	600	0.28	0.55	0.74	0.46	0.52	0.71
	$LSD_{0.05}$	NS	NS	NS	NS	NS	NS
		----------N rate means----------					
	0	0.25	0.67	0.84	0.44	0.65	0.54
	200	0.25	0.71	0.82	0.69	0.56	0.56
	400	0.28	0.70	0.77	0.46	0.47	0.61
	600	0.22	0.64	0.75	0.59	0.53	0.62
	$LSD_{0.05}$	NS	NS	NS	0.19	NS	NS
		----------N source means----------					
	AN	0.21	0.73	0.76	0.68	0.39	0.54
	SLE	0.29	0.63	0.80	0.48	0.64	0.65
	$LSD_{0.05}$	NS	NS	NS	0.19	NS	NS

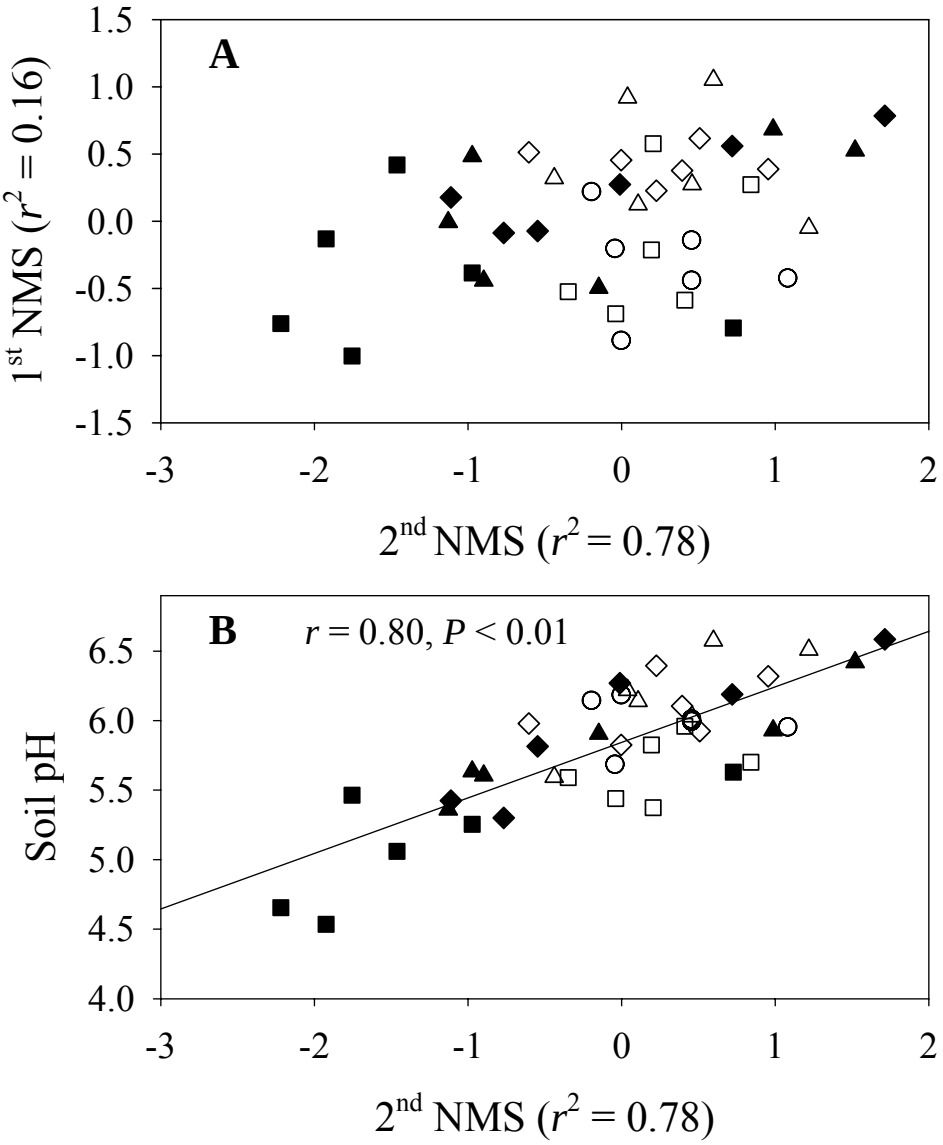

Fig. 1. (A) Microbial community composition differences revealed by non-metric multidimensional scaling (NMS) for soils receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at fertilization rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for 3 consecutive years. Symbols ○, ▲, ♦, and ■ represent the fertilization rates of 0, 200, 400, and 600 kg N ha^{-1} y^{-1}, respectively. Filled symbols are for SLE-fertilized soils, and empty symbols are AN-fertilized soils. (B) Correlation of soil pH and the dimension of NMS that explained the largest variation of the total community compositions.

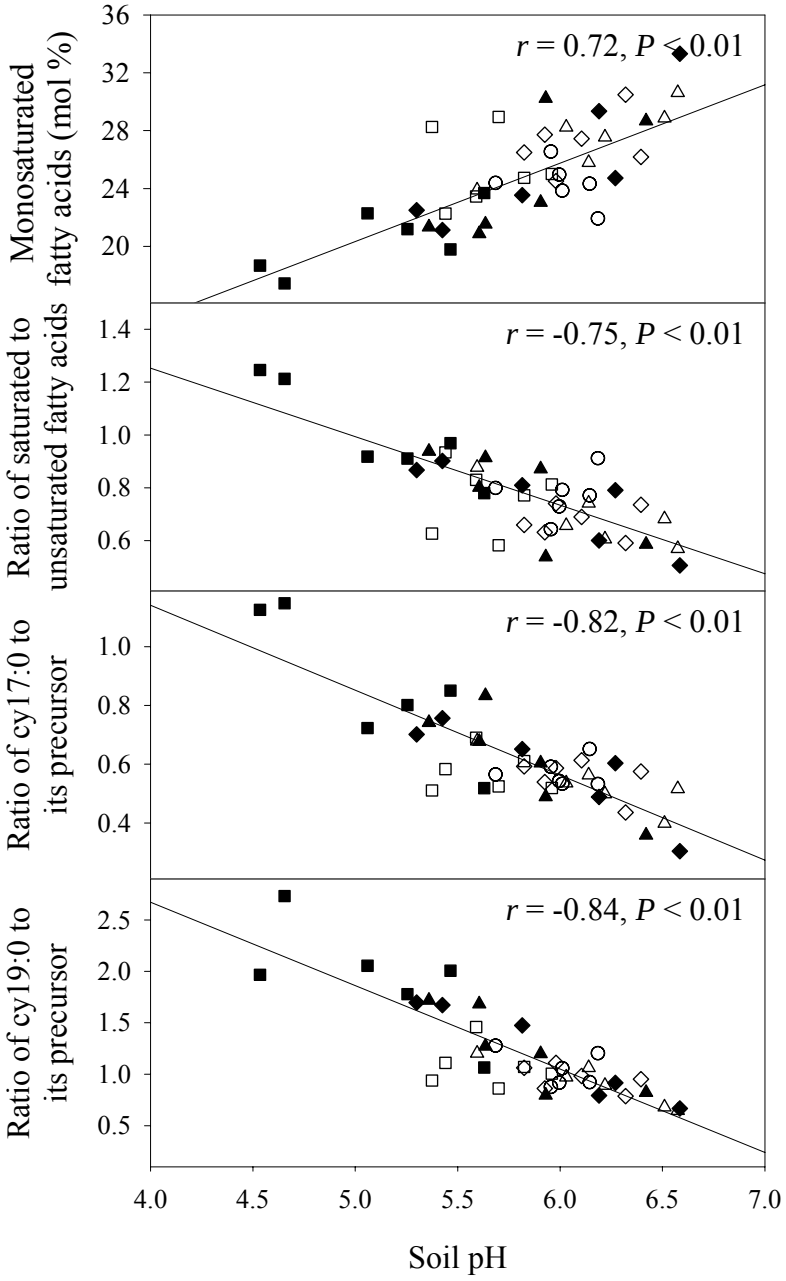

Fig. 2. Relationship of some group parameters of fatty acids with soil pH for soils receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at fertilization rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for 3 consecutive years. Symbols ○, ▲, ♦, and ■ represent the fertilization rates of 0, 200, 400, and 600 kg N ha^{-1} y^{-1}, respectively. Filled symbols are for SLE-fertilized soils, and empty symbols are AN-fertilized soils.

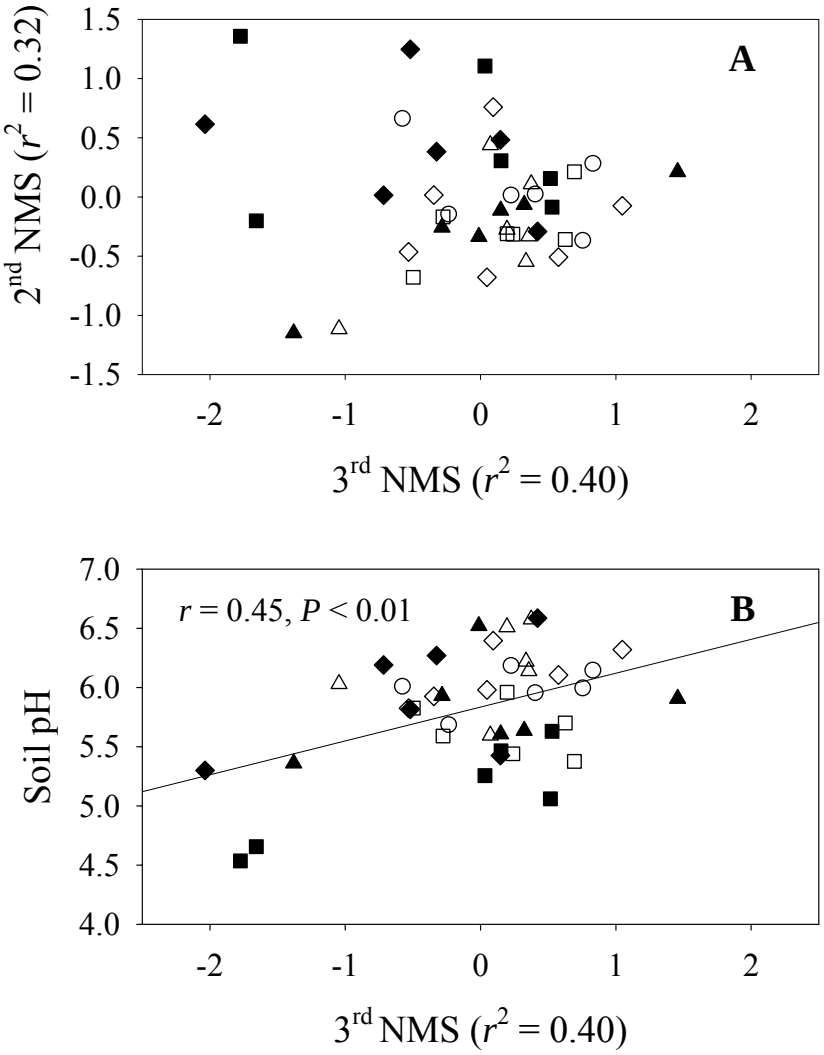

Fig. 3. (A) Microbial community-level physiological profiles revealed by non-metric multidimensional scaling (NMS) for soils receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at fertilization rates of 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for 3 consecutive years. Symbols ○, ▲, ♦, and ■ represent fertilization rates of 0, 200, 400, and 600 kg N ha^{-1} y^{-1}, respectively. Filled symbols are for SLE-fertilized soils, and empty symbols are AN-fertilized soils. (B) Correlation of soil pH and the dimension of NMS that explained the largest variation of the CLPP.

APPENDICES

Appendix A. Table of Pearson correlation (*r*) between key soil characteristics and relative abundance of individual to total identified fatty acids (mol%) which contribute significantly to the separation of microbial communities in soils receiving ammonium nitrate (AN) versus swine lagoon effluent (SLE) at 0, 200, 400 or 600 kg plant available N ha^{-1} y^{-1} for 3 consecutive years (n= 48).

PLFAs	Soil pH	Soil C (mg g^{-1} soil)	Soil N (mg g^{-1} soil)	ASN[§] (µg g^{-1} soil)	EC[¶] (dS m^{-1})	Soluble C[†] (µg g^{-1} soil)	Inorganic N (µg g^{-1} soil)
16:1ω7c	0.79^{***}	0.31^{*}	0.28^{ns}	-0.16^{ns}	0.27^{ns}	0.01^{ns}	0.17^{ns}
18:1 ω7c	0.75^{***}	0.26^{ns}	0.18^{ns}	-0.07^{ns}	0.19^{ns}	0.23^{ns}	0.19^{ns}
16:1 ω5c	0.50^{**}	0.33^{*}	0.20^{ns}	-0.05^{ns}	0.02^{ns}	-0.16^{ns}	-0.04^{ns}
11Me18:1 ω7c	0.39^{**}	0.39^{**}	0.30^{*}	0.13^{ns}	0.27^{ns}	0.13^{ns}	0.31^{*}
i15:1	0.34^{**}	0.41^{**}	0.45^{*}	0.27^{ns}	0.24^{ns}	0.06^{ns}	0.17^{ns}
18:1 ω5c	0.41^{**}	0.21^{ns}	0.17^{ns}	0.16^{ns}	0.07^{ns}	0.15^{ns}	0.12^{ns}
i17:0	-0.71^{***}	-0.14^{ns}	-0.09^{ns}	0.10^{ns}	-0.09^{ns}	0.06^{ns}	-0.09^{ns}
i16:0	-0.71^{***}	-0.33^{*}	-0.17^{ns}	0.13^{ns}	-0.10^{ns}	-0.05^{ns}	-0.10^{ns}
18:0	-0.72^{***}	-0.38^{**}	-0.32^{*}	0.10^{ns}	-0.23^{ns}	-0.19^{ns}	-0.19^{ns}
Cy19:0	0.76^{***}	0.01^{ns}	0.01^{ns}	0.23^{ns}	0.02^{ns}	0.07^{ns}	0.03^{ns}
16:0	-0.37^{**}	-0.32^{*}	-0.24^{ns}	-0.16^{ns}	-0.19^{ns}	-0.39^{*}	-0.14^{ns}

[§] Amino sugar nitrogen

¶ Electrical conductivity
† 0.5 M K_2SO_4 soluble soil organic C
*, **, *** and ns significant at the 0.05, 0.01 and 0.001 probability levels and not significant respectively.

Appendix B. Table of Pearson correlation (*r*) between the ordination score of the second axis of the non-metric multidimensional scaling ordination (which explains 78% of the total variance in the original PLFA data) and key soil properties.

Variable	*R*	*P*
Soil pH	0.80	***
Soil C (mg g^{-1} soil)	0.29	ns
Soil N (mg g^{-1} soil)	0.22	ns
ASN[§] (µg g^{-1} soil)	-0.16	ns
EC[¶] (dS m^{-1})	0.24	ns
Soluble C[†] (µg g^{-1} soil)	-0.01	ns
Inorganic N (µg g^{-1} soil)	0.14	ns

[§] Amino sugar nitrogen
[¶] Electrical conductivity
[†] 0.5 M K_2SO_4 soluble soil organic C
*** and ns significant at the 0.001 probability levels and not significant respectively.

CHAPTER SIX

CONCLUSION AND FUTURE RESEARCH

A holistic approach was used to characterize multiple facets of soil microbial communities in managed forage production systems receiving swine lagoon effluent (SLE). This research demonstrated that applications of swine lagoon effluent exceeding 400 kg N ha^{-1} yr^{-1} had negative impacts on soil microbial processes as a result of accelerated soil acidification. While anaerobic lagoon treatment of animal waste may be replaced by other waste treatment methods and current regulations do not allow application of 400 to 600 kg N $ha^{-1}$the general considerations and conclusions from this research provide new insight on how alterations in microbial community structure and function by low soil pH influence C and N transformations mediated by soil microbes.

Soil microbial community has a central role in regulating organic matter decomposition and nutrient cycling. Although soil microbes are small living component of soil, they are very active entities. Accordingly, soil microbes often respond to environmental changes more rapidly than many other soil properties such as total soil C. Consequently, soil microbial characteristics can be used as indicators to foresee ecosystem functions. This research showed that land application of swine lagoon effluent at a high rate could acidify soils, and subsequently reduce soil microbial biomass and alter soil microbial community composition and microbial catabolic function. In turn, the activities of soil enzymes involved in C, N and P cycling changed with the application rates of SLE. While most hydrolytic enzyme activities declined with increasing application rates of SLE, the activities of oxidative enzymes including peroxidase and phenol oxidase were generally increased with

application rates of SLE. Because oxidative enzymes are involved in decomposition of recalcitrant organic materials including lignin and humus, SLE application at a high rate may not promote the accumulation of soil organic matter. Neither did SLE application at a high rate further stimulate soil microbial activity and N transformations as compared to a low rate. Again, in a grazing pastoral soil microbial properties and N cycling processes varied with non-uniform distribution of animal excreta. It seems that at highly concentrated areas of animal excreta, microbial N immobilization rate were reduced, and thereby leading possibly to the accumulation of soil inorganic N. In conclusion, over application of animal waste doesn't benefit soil fertility but rather it is environmentally unsound. If a change in soil pH associated with the application of animal waste is the primary cause, periodic liming will alleviate unwanted side effects.

There are some emerging questions from this research. While we hypothesized that the active fungi were higher as a consequence of application of SLE at high rate, the phospholipid fatty acids fingerprinting did not support our assumptions. Another possibility is that fungal community may differ significantly. Fog (1988) indicated that over 20 species of basidiomycetes differed in their ability to produce oxidative enzymes in response to different soil N availabilities. Molecular biology techniques including 18s rDNA or rRNA may be more appropriate to characterize community composition of basidiomycetes. Another interesting finding is that potential rates of nitrification did not increase with the application rates of SLE but did with AN. This may due to a change in community composition of ammonium oxidizers. Again, molecular biology techniques will provide a finer resolution to identify the phylogenetic characteristics of ammonium oxidizers. Examining microbial community at a phylogenetic scale and link the community

composition at this finer scale to certain ecosystems functions will provide more insights on keystone microbial species and their ecological functions.