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NITROGEN CYCLING DYNAMICS IN AGRICULTURAL FIELDS FERTILIZED WITH LIQUID SWINE WASTE – MICROBIAL NITRIFICATION AND DENITRIFICATION

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ABSTRACT

The effects of temperature, moisture and effluent dose on rates of microbial nitrification and denitrification were assessed in time course experiments involving intact soil cores and homogenized soil samples collected from agricultural fields regularly fertilized with liquid swine waste. Additionally, the influences of fertilization history and substrate concentration (NO^{3-}-N and organic-C) on rates of nitrification and denitrification, respectively, were determined using similar methodology. Nitrifying activity was concentrated in the 0 to 5 cm zone where NO_3^--N increased linearly following waste application and accounted for roughly 80% of the total effluent-N applied. Temperature, substrate availability and fertilization history significantly affected net nitrification rates, while the moisture levels used in these experiments had no impact. Short-term immobilization of effluent-NH_4^+-N and subsequent remineralization may be important in determining long-term availability of NO_3^--N for plant assimilation and denitrificaton while microbial immobilization was an inconsequential sink for NO_3^--N. Waste application immediately stimulated denitrification rates due to increases in anaerobic soil volume, labile-C, and coupled nitrification, which supplied NO_3^--N for denitrifiers. However, elevated rates only lasted 3 to 5 d, until soil moisture content and the availability of labile-C became limiting. Strong relationships were observed between rates of denitrification and environmental factors (soil moisture, organic-C, NO_3^--N, and temperature), pointing to the importance of these controls on denitrification in these soils. Waste application resulted in fractional denitrification losses of 1.6 to 4.5% of the applied N over a <2 week observational period.

(nitrification, denitrification, liquid swine waste, organic fertilizer, nitrogen cycling)

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SUMMARY AND CONCLUSIONS

Nitrification Experiments

Swine lagoon effluent has little mineralization potential compared to other wastes and is a source of immediately available NH_4^+-N for nitrifying bacteria in receiving soils. On average, about 90% of total-N in effluent was found as NH_4^+-N. Applied effluent NH_4^+-N remained largely in the top 0 to 5 cm soil zone where it was rapidly nitrified to NO_3^--N in 100 h.

Temperature, effluent dose and field type (i.e., fertilization history) significantly affected net nitrification rates while moisture levels used in these experiments had no statistically significant influence. Nitrate increased and reached a steady state within several days of waste application at all loading rates in these soils. Roughly 80% of the total-N in applied waste was accounted for as NO_3^--N within 10 d of application. The remainder was presumably lost to volatilization and dentrification. Short-term immobilization of effluent NH_4^+-N and subsequent remineralization may be important in determining the long-term accumulation of NO_3^--N. High net nitrification rates in previously fertilized soils suggest a viable indigenous nitrifier population capable of rapidly responding to changes in nutrient loads, while in fallow soils a lag in NO_3^--N accumulation indicates that nitrifiers required an induction period.

Denitrification Experiments

In agreement with data from nitrification experiments, the primary N species of swine waste in the lagoon sampled in denitrification experiments was NH_4^+-N, which comprised 80 to 90% of the total-N content.

Waste addition to intact soil cores rapidly promoted elevated rates of N_2O emission and denitrification. However, the immediate influence of waste application was short-lived (<5d). Although the addition of large amounts of NH_4^+-N and TOC in liquid form immediately created conditions optimal for coupled nitrification-denitrification, rates of N-gas production soon returned to background levels as %WFPS gradually declined and became limiting to denitrification. As nitrification of added NH_4^+-N continued, soil concentrations of NO_3^--N increased dramatically and NO_3^--N became the largest component of the soil N pool. By comparison, gaseous losses of N via N_2O and N_2 via denitrification were much less significant; fractional loss of applied N via denitrification was 1.6 to 4.5%.

At the process-level, soil moisture content, concentrations of organic-C and NO_3^--N, and soil temperature were found to be important variables regulating denitrification. Both N_2O emission and denitrification increased dramatically at threshold soil moisture contents near saturation. Denitrification enzyme activity was stimulated by individual treatments of both glucose-C and NO_3^--N, with the combined effect of the two substrates having a greater stimulatory effect than each individual treatment. Denitrification enzyme activity was strongly dependent on soil temperature, increasing by a factor of nearly 2 with a temperature increase of 10^oC.

RECOMMENDATIONS

These laboratory studies demonstrated that vigorous populations of nitrifying and denitrifying bacteria become established in spray fields repeatedly fertilized with liquid swine waste and that these microbes respond rapidly to fertilization with liquid effluent at rates representative of the industry practices. Moreover, comparison of results of nitrification experiments between the aged and fallow fields suggests that populations of nitrifiers develop with time in response to years of fertilization. This is consistent with our previous study (Whalen and Nelson 2000), where we reported that denitrifying enzyme activity and nitrifying enzyme activity were significantly greater in a spray field that had been repeatedly fertilized for ten years compared to a spray field that had been in use for one year. Insofar as nitrification and denitrification are processes central to offsite transport of land-applied swine waste by increasing mobility of inorganic forms (oxidation of NH_4^+ to NO^{3-} by nitrification) or by transforming NO_3^- to benign N_2 gas, we recommend further study to determine if these limited observations that aged spray fields more rapidly and actively process swine effluent are simply fortuitous or represent a regional pattern. This information has clear implications for determining permissible loading rates.

A relatively small fraction of the land-applied waste was lost to denitrification. This microbial process represents a source of N loss from receiving fields that does not impact adjoining terrestrial and aquatic ecosystems, although a minor endproduct (N_2O) is radiatively and chemically active. Rates of denitrification are strongly linked to soil type, as clays are more susceptible to the formation and persistence of anaerobic microzones than sands when soils are amended with liquid effluent. Accordingly, we recommend that studies of denitrification be expanded to include the entire spectrum of soil textures common regionally to firmly establish the expected rate of loss of fertilizer N to this microbial process.

Our studies of denitrification were of short duration (< two weeks) and measured the immediate loss of N_2 and N_2O to coupled nitrification-denitrification following a spray event. Denitrification diminishes or ceases once oxic conditions are restored to soils following fertilization. However, considerable NO_3^--N has accumulated by this time and this is available as a substrate for further denitrification if soils again become anaerobic following rainfall. Hence, it is clear that our experiments underestimate total fertilizer loss to denitrification. We recommend additional field experiments of seasonal duration or laboratory experiments where post-fertilization rainfall is simulated to estimate total fertilizer loss to denitrification.

1. INTRODUCTION

Growth of the Swine Industry in North Carolina

Continuous growth of the human population increases the demand for food. Consequently, crop and animal agricultural operations worldwide have expanded and become increasingly intensified and consolidated. Following this global trend, North Carolina has recently experienced both explosive growth in the number of swine produced and a decrease in the number of production facilities. In the 1991 to 1995 period, the state realized a 124% increase in total marketing share, while Iowa, the industry leader, recorded an 8% loss (Vansickle 1997). From 1993 to 1997, the total number of swine production facilities has decreased 23%, such that over 70% of North Carolina's 10 million swine reside in facilities with greater than 5000 animals and only 2% live at locations with less than 500 (NCDACS 2000). North Carolina currently ranks second among states in swine production.

Potential Environmental Impact of Swine Production

Growth of North Carolina's swine production industry is geographically localized in the Coastal Plain and Piedmont, largely in Duplin, Sampson, Wayne and Bladen Counties. This presents a formidable challenge with regard to disposal of swine waste which annually includes 48 Gg N (Crouse 1995). With the intensification and consolidation of all forms of agriculture, the fate of N in land-applied fertilizers, including organic wastes, has become of increasing concern because N is a primary limiting nutrient to terrestrial and aquatic ecosystems (Vitousek and Howarth 1991). Uninformed or improper waste management may encourage offsite transport, leading to enhanced N loading in adjoining ecosystems, particularly rivers, estuaries and coastal waterways. Surface water loss to estuarine and coastal waters is the most well-documented fate of land-applied inorganic and organic N fertilizers that are unassimilated by the host crop (Correll and Ford 1982; Fleischer et al. 1987; Brockmann et al. 1988; Smetacek 1991; Billen et al. 1991), although aeolian transport of volatilized NH_3 and other reactive N compounds is increasingly recognized as an important external source of aquatic N loading (Fisher and Oppenheimer 1991; Hinga et al. 1991; Paerl and Fogel 1994; Paerl 1995; Paerl et al. 1995, 1998). Agricultural N input has been identified as a major cause of accelerated eutrophication of estuarine and coastal waters worldwide (Nixon 1995). In North Carolina, agricultural activities, including swine production, have been implicated in the general deterioration of water quality in the Neuse River Estuary (Copeland and Gray 1989; Paerl et al. 1995, 1998; Burkholder et al. 1997; Mallin et al. 1997; Mallin 2000) and the Albemarle-Pamlico watershed in general (Stanley 1992).

Swine Waste Management

Water quality nondischarge rules for livestock farms in North Carolina (2H.0200 rule) require that swine farms with waste management systems designed to serve 250 or more animals follow registration, approval and certification procedures which generally include a plan for storage of swine wastes in anaerobic lagoons and land application of liquid phase waste as an organic fertilizer (Barker et al. 1994). The nitrogenous component of waste stored in anaerobic lagoons consists entirely of organic-N (dissolved and particulate) and NH_4^+-N (Kirchmann 1994). This nutrient has several potential fates following land application (Figure 1.1). Briefly, organic-N

can be microbially decomposed to NH_4^+. Ammonium can be incorporated into the soil cation exchange complex, lost to the atmosphere via volatilization (NH_3) or oxidized to NO_3^--N by nitrifying bacteria in aerobic soil zones. Nitrate can be reduced to the inert gas, N_2, (and to a much lesser extent N_2O) by denitrifying bacteria in anaerobic microzones, with the gaseous end products again lost to the atmosphere. Dinitrogen gas is available as a plant and microbial nutrient only to a few specialized groups of N-fixers and symbionts. Among other factors, rates of denitrification are dependent on the microbial release of NH_4^+-N from decomposition of organic materials and subsequent nitrification of NH_4^+-N to NO_3^--N. Inorganic-N (NO_3^- and NH_4^+) is available for plant consumption and all organic and inorganic species are subject to export from the ecosystem via surface and ground waters, although NO_3^- shows the most mobility.

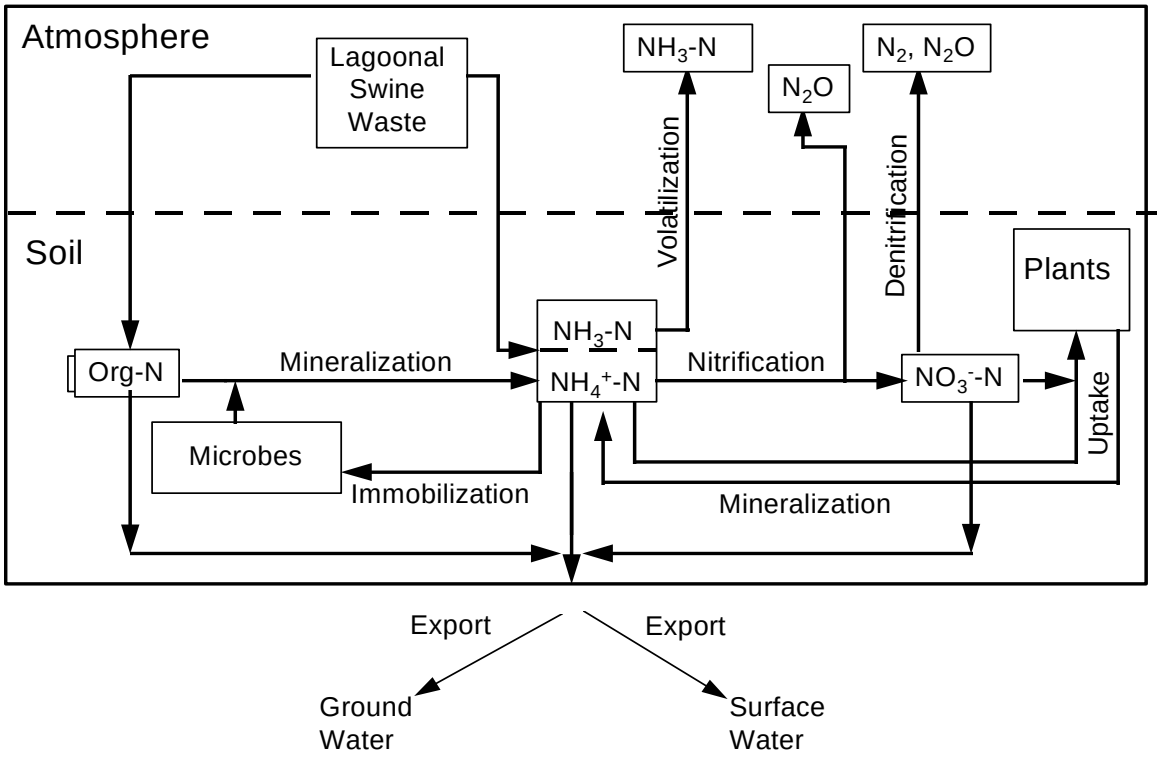

Figure 1.1. Potential fate of land-applied liquid lagoonal swine effluent.

Various aspects of the fate of N in land-applied liquid lagoonal swine effluent (Figure 1.1) have been individually analyzed in detail, including efficiency of plant utilization (Burns et al. 1990), long-term soil accumulation (King et al. 1990), NH_3 loss to volatilization during application (Safley et al. 1992; Walker et al. 2000) and export to groundwater (Gilliam et al. 1996; Westerman et al. 1996). Considerably less research effort has focused on microbial N transformations in spray field soils. Nonetheless, development and refinement of Best Management Practices (BMPs) for waste disposal in large scale swine production facilities in the

Southeast requires a complete understanding of environmental controls on rates of microbial N transformations in spray fields. Microbial nitrification and denitrification play central roles in determining the fate of land-applied liquid swine effluent. First, oxidation of NH_4^+-N to NO_3^--N by nitrification reduces undesirable N loss to volatilization. Second, the end product (NO_3^--N) can be denitrified and beneficially removed from the ecosystem as inert N_2 gas. Third, the end product is also an easily leached anion.

Scope and Objectives of Research

The overall objective of this research was to assess *in situ* rates of nitrification and denitrification in representative spray field soils and further, to determine the influence of application volume and environmental factors (soil moisture and temperature) on these rates. To this end, we utilized intact soil cores and homogenized soil samples in a controlled laboratory environment. Specifically, we followed time courses for change in the accumulation of endproducts of nitrification or denitrification in response to manipulation of single or multiple influencing variables. Chapters 2 through 4 respectively, give the Methods, Results and Discussion for our studies on nitrification. Chapters 5 through 7 are similarly organized with respect to studies on denitrification. Information given here will prove useful for modeling N transformations in spray field soils and refining and developing BMPs that simultaneously reduce the environmental impacts of offsite fertilizer transport and meet the nutritional requirements of receiving crops.

2. MATERIALS AND METHODS - NITRIFICATION EXPERIMENTS

Study Site Description

The study site is located in Sampson County, North Carolina (Figure 2.1) and is owned by a major corporate swine producer. The swine operation is a farrow to half-finish facility and includes approximately 2200 animals at any one time. Waste management is conducted in accordance with standard industry practices. Swine waste is stored in a lagoon prior to application (with fixed sprinkler system) on a host crop throughout the year.

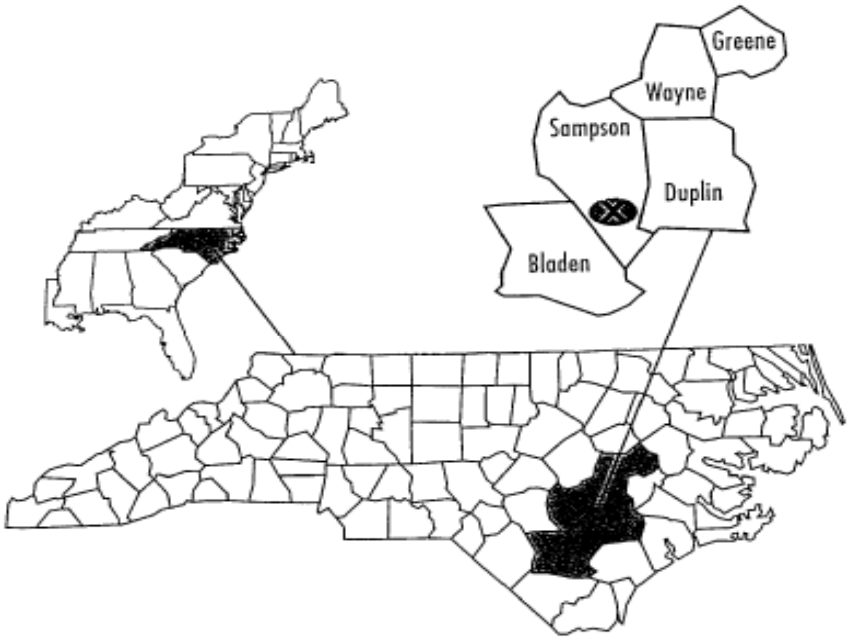

Figure 2.1. Location of the study site in Eastern North Carolina.

Site A is planted with hybrid coastal Bermuda grass, overseeded with fescue (*Festuca sp*.) in winter. Lagoon effluent is applied as a fertilizer in accordance with the agronomic N requirement of these species 20 to 25 kg N t_{dry}^{-1} (Zublena et al. 1995). Site A has been irrigated with effluent for ten years, generally on a once per month basis. Site B is a fallow agricultural field recently purchased by the producer to accommodate future growth. The field did not received any lagoon effluent before or during the study.

Research focused on the surface to 20 cm of soil since measurement of short-term nitrification potential in soil profiles to 50 cm at both sites showed that 90% of activity occurred in this zone (data not shown).

Soil Core Collection

An area was randomly selected for soil core collection within the agricultural field at Site A or B on each sampling date. Soil cores were collected in a confined area (1.5 m^2) to minimize submeter-scale variability in soil physiochemical properties and characteristics of microbial community (Robertson et al. 1988). Intact soil cores were collected by driving acrylic tubing (7 cm ID) twenty centimeters into the ground and gently excavating around each tube. This method minimized disturbance of the soil profile. Occasionally, acrylic core tubes were damaged and the 15 to 20 cm soil zone was disrupted. The integrity of any damaged cores was assessed visually and cores were salvaged when soil disruption was minimal. Cracks on damaged cores were sealed with duct tape to prevent exposure to air and drying. After collection, the cores were capped on both ends, packed on ice, transported to the laboratory and refrigerated overnight at 4^oC.

Temperature Response Experiment – Site A

On March 24, 1998, sixty soil cores were collected from Site A for analysis of the effect of temperature on nitrification. The cores were removed from refrigeration and the top caps were removed. The cores were randomly divided into three groups of twenty and equilibrated for 10 h at 10, 20, and 30^oC, respectively, in controlled temperature incubators. Two cores from each incubation were then sectioned (0 to 5, 5 to 10, 10 to 15, and 15 to 20 cm zones) for time zero analyses. The remaining cores were amended with 50 ml of lagoon effluent, replaced in the incubators, and two cores from each incubation temperature were removed and sectioned for analyses at 0.5, 3, 6, 12, 24, 48, 96, 144, and 240 h.

In an effort to maintain constant soil moisture during the incubation period, deionized water was periodically added to the soil cores remaining in incubation. The amount of water added was based on the volume of water evaporated from an open beaker within each incubator. Loss of water from the open beaker in each incubator was periodically quantified and an amount of water equal to 33% or 50% of evaporational loss was added to the cores. The water additions were timed such that any core receiving water would not be sectioned for at least 24 h, and in most instances 48 h or more.

Moisture Response Experiment – Site A

On May 20, 1998, soil cores were collected from Site A for analysis of the effect of moisture on nitrification. The field was irrigated with lagoon effluent less than 24 hours prior to core collection. Therefore, to reduce the amount of nitrogenous nutrient in the cores prior to amendment with additional effluent, the cores were stored at room temperature (23^oC) for five additional days except for a 12-h period during which cores were exposed to natural sunlight to promote photosynthesis and N-nutrient uptake. Based on previous experiments, this five day time period should have allowed for nitrification of most of the NH_4^+ applied during the irrigation with waste effluent.

The cores were randomly divided into three groups of 23 after the five day pre-incubation period. Soil core moisture content was designated as low, medium and high. Moisture in the medium

and high groups was increased by adding 50 mL and 100 mL of deionized H_2O, respectively, while the low moisture cores experienced no H_2O addition. All cores were equilibrated at 25°C for 10 h in a controlled temperature incubator. Two cores from each group were sectioned (0 to 5, 5 to10, and 10 to 20 cm) for time zero analyses and two cores from each group were identified as control cores (no effluent addition), while the remaining cores in each group were amended with 50 ml of effluent. Two cores from each group were sectioned as described above for analyses at 0.5, 3, 10, 24, 48, 96, 144, 240, and 480 h. Soil moisture was maintained during the incubation as described previously except that the beaker of water and all of the soil cores were covered with aluminum foil with two 1.5-cm diameter holes punched in the top. This decreased water evaporation while allowing gas exchange between the core headspace and incubator atmosphere.

Dose Response Experiment - Site A

On April 21, 1998, soil cores were collected from Site A for analysis of the influence of effluent loading on nitrification. The cores were removed from refrigeration and top caps removed. Cores were randomly divided into three groups of eighteen and one group of two and were equilibrated for 10 h at 25°C in a controlled temperature incubator. The group of two cores were sectioned for time zero analyses as described above.

The three groups of 18 cores were amended with low [0.65 cm (2.4 g N m^{-2})], medium [1.3 cm (4.9 g N m^{-2})] and high [2.6 cm (9.7 g N m^{-2})] doses of lagoon effluent, respectively. Effluent application of 0.5 to 2.5 cm is common for the industry. Two cores from each group were then sectioned for analyses at 0.5, 3, 6, 12, 24, 48, 96, 144, and 240 h. Soil moisture was maintained during the incubation as described above.

Dose Response – Site B: Field Type Experiment

On May 11, 1998, soil cores were collected from Site B for analysis of the combined effect of soil type and spray history on nitrification. The cores were randomly divided into three groups of 20 and two cores were labeled as control cores (no effluent additions). All cores were equilibrated at 25°C for 10 h in a controlled temperature incubator.

The three groups of cores were to be amended with low [0.65 cm (2.4 g N m^{-2})], medium [1.3 cm (4.9 g N m^{-2})] and high [2.6 cm (9.7 g N m^{-2})] doses of lagoon effluent. However, during the high dose addition, liquid migrated through the soil profile and leaked from the bottom cap. When this leakage was detected, it was quantified by placing cores in secondary containers and measuring the accumulated volume of effluent, or by measuring the increase in mass of a sorbant positioned beneath the core. Ponding of effluent was also observed in several cores receiving the high dose. After 6 h, ponded effluent was removed, quantified, and included in the leakage estimate.

Based on the observation that over half of the high dose leaked through the soil profile, the planned medium dose was abandoned. Only four cores receiving the low dose had visible leakage that was collected in secondary containers. However, some of the cores in this group had an accumulation of liquid confined in the bottom cap.

The cores amended with lagoon effluent were incubated at 25°C and two cores from the group amended with 100 ml lagoon effluent were sectioned (0 to 5, 5 to 10, 10 to 20 cm) for analyses at 3, 10, 24, 48, 96, 144, 216, 336, and 504 h. Two cores from the group amended with 25 mL lagoon effluent were sectioned for analyses at 0.5, 3, 10, 24, 96, 144, 216, 336, and 504 h.

Waste Lagoon Effluent Collection

Lagoon effluent was collected on the same day that soil cores were collected for each experiment. Effluent was collected with a plastic grab bucket attached to an expandable 4 m rod. Four samples of approximately 2-L volume were randomly collected from the shore of the lagoon, composited in a 10-L polystyrene jug, and transported on ice to the lab.

Soil Analyses

Each soil core section was sieved through a US Standard Sieve #4 (4 mm), homogenized in a plastic tub, transferred to a sealable plastic bag and the mass of soil plus bag was recorded. Soil moisture was determined gravimetrically (Topp 1993) and pH (H_2O) was measured potentiometrically (Hendershot et al. 1993). Particle size distribution (sand, silt, clay) was measured hydrometrically (Sheldrick & Wang 1993), particle density was measured pycnometrically (Culley 1993), water-holding capacity was determined by the soak and drain method (Parent & Caron 1993), and air and water filled pore space were calculated from the above measurements (Carter & Ball 1993).

Ammonium, NO_2^--N, and NO_3^--N were extracted in 2 M KCl (10:1 volume/soil wet wt.) for 1 h on a rotary shaker (150 rpm). Soil extracts were filtered (Whatman #42) and stored frozen. Ammonium and NO_2^--N + NO_3^--N (hereafter referred to as NO_3^--N) were quantified colormetrically on a Shimadzu UV-1201V spectrophotometer by the phenol-hypochlorite and copper-cadmium reduction methods respectively (Bundy & Meisinger 1994; Maynard & Kalra 1993). Soil nutrient data are expressed on a dry mass (dw) basis. The coefficients of variation for replicate (n = 10) NH_4^+ and NO_3^- determinations at soil concentrations of 6 µg g_{dw}^{-1} and 73 µg g_{dw}^{-1}, respectively, were 4% and 2%, respectively. Net nitrification rates were calculated using the methods of Hart et al. (1994).

Soil Respiration

Respiratory activity was quantified by analysis of accumulated CO_2 in the static headspace of briefly capped cores. One half-hour prior to sectioning, cores were capped and headspace gas was syringe-sampled (5-cc nylon SESI syringe) immediately after capping and at 15 and 30 minutes. Gas samples were analyzed for CO_2 concentration on a thermal conductivity gas chromatograph (Shimadzu TCD-GC-8A). Gases were separated on a $^1/_8''$ x 2 m porapak N (60/80) column with a He carrier (30 mL min^{-1}). Injector/detector and column temperatures were 90 and 50°C and the detector current was 140 mA. The coefficient of variation for replicate (n = 10) determinations at CO_2 concentration of 1936 ppm was 0.5%. The CO_2 flux was calculated from the linear increase in concentrations with time and the headspace volume and surface area of the cores (Zibilske 1994).

Lagoon Effluent Analysis

A sample of the lagoon effluent applied to the soil cores was analyzed for NH_4^+-N using the phenol-hypochlorite method (Bundy & Meisinger 1994). The coefficient of variation for replicate (n = 7) determinations at effluent concentration of 334 µg NH_4^+-N L^{-1} was 2%. Effluent total-N was determined using the persulfate oxidation method (Solorzano & Sharp 1980). The coefficient of variation for replicate (n = 5) determinations at effluent concentration of 397 µg N L^{-1} was 9%.

Net Nitrification Rate Calculations and Statistical Analysis

Short-term net nitrification rates (µg NO_3^--N g_{dw}^{-1} soil h^{-1}) were determined by linear regression of the increase in soil NO_3^--N concentration in the 0 to 5 cm zone with time from 0.5 to 96 h. Increases in NO_3^--N concentration were most rapid during this 96 h period and, on average, accounted for more than 60% of the total NO_3^--N. Area based total net nitrification rates (mg NO_3^--N m^{-2} d^{-1}) were calculated using the increase in NO_3^--N in the entire soil core (0 to 20 cm) over the duration of the experiment.

The influence of treatments on short-term (96 h) net nitrification rates was evaluated by analysis of covariance as modified by Zar (1984) for comparison of regression coefficients. The modified Tukey "honestly significant difference" test procedure was conducted to determine which slopes significantly differed from which others (Zar 1984). A significance level of α=0.05 was used for all tests.

3. RESULTS - NITRIFICATION EXPERIMENTS

Soils at Sites A and B are roughly comparable with respect to physicochemical characteristics (Table 3.1). Both soils are light textured, showing a sand content of around 80% in the 0 to 20 cm zone. The clay content of both soils is low, about 9% for Site B and 3% for Site A. Organic content and water holding capacity (WHC) decrease markedly with depth at site A, while this trend is absent (WHC) or less pronounced (organic content) at Site B. The generally higher WHC and organic content at Site A relative to Site B is consistent with the fact that Site A has been fertilized for the past ten years with swine waste lagoon effluent while Site B has been fallow.

Table 3.1. Selected soil properties from Sites A and B.

	Soil Zone	Particle Density (g/cm^3)	Water-Holding Capacity (%)	% Silt	% Clay	% Sand	Organic Content (wt. %)
Site A	0-20 cm			12.19	2.84	84.79	
	0-5 cm	2.36	50.40				6.32
	5-10 cm	2.31	35.80				2.64
	10-20 cm	2.66	28.00				1.46
Site B	0-20 cm			11.89	8.68	79.43	
	0-5 cm	2.30	31.40				2.60
	5-10 cm	2.64	33.60				2.40
	10-20 cm	2.64	31.40				1.40

Ammonium and total-N content of the swine lagoon effluent collected for each of the four experiments (temperature, effluent loading, moisture and soil type) is comparable (Table 3.2). The average NH_4^+-N and total-N content was 337 mg L^{-1} and 367 mg L^{-1} respectively. Ammonium accounted for 90 to 96 % of the total-N.

Table 3.2. Ammonium and total-N concentrations in liquid swine lagoon effluent used in soil core experiments to assess the effect of temperature, moisture, dose response or field type on N transformations.

Experiment	NH_4^+-N (mg L^{-1})	Total N (mg L^{-1})	% Total N as NH_4^+
Temperature Response	285	298	96
Moisture Response	380	422	90
Dose Response	337	375	90
Field Type (Dose Response Site B)	347	374	93
Average	337	367	92
SD	39	51	

Most of the NH_4^+-N in effluent applied to soil cores remained in the 0 to 5 cm zone of the soil profile (Figure 3.1a) where it was nitrified to NO_3^--N (Figure 3.1b). This pattern was observed in all experiments and, on average, 76% of net nitrification in the entire 0 to 20 cm soil core occurred in the 0 to 5 cm zone (Table 3.3). Consequently, the time course results of all experiments (Figures 3.2 to 3.5) include only the 0 to 5 cm soil zone.

Short-term (96 h) net nitrification rates (μg NO_3^--N g_{dw}^{-1} soil h^{-1}) were determined by linear regression of the increase in soil NO_3^--N concentration in the 0 to 5 cm zone with time from 0.5 to 96 h. Increases in NO_3^--N concentration were most rapid during this 96 h period and, on average, accounted for more than 60% of the total NO_3^--N (Table 3.3). Total net nitrification rates (mg NO_3^--N m^{-2} d^{-1}) were calculated using the increase in NO_3^--N in the entire soil core (0 to 20 cm) over the duration of the experiment.

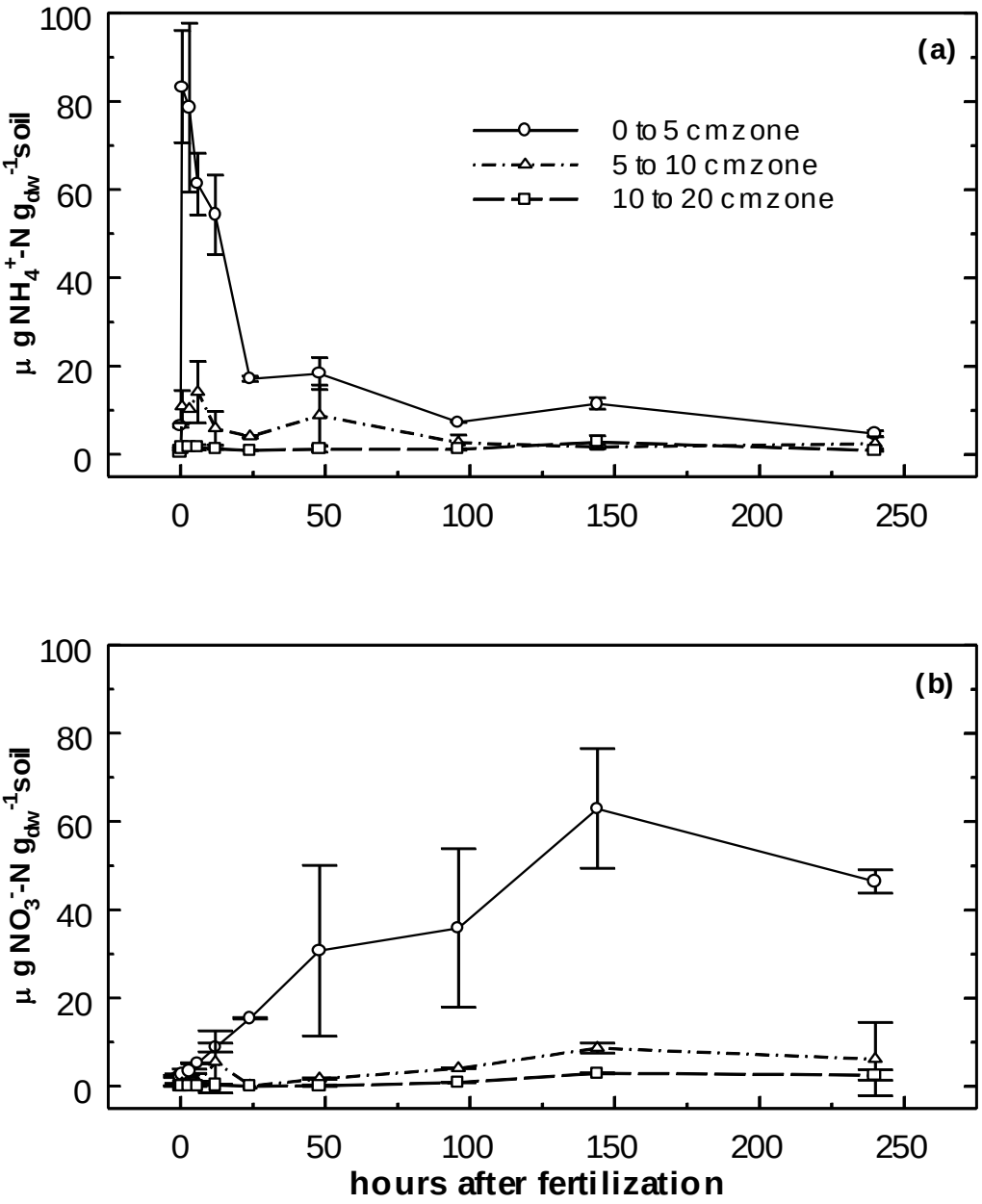

Figure 3.1. Time course for changes in (a) NH_4^+-N and (b) NO_3^--N concentrations in soil cores to a depth of 20 cm following fertilization with 1.3 cm (4.9 g N m^{-2}) of liquid swine lagoon effluent. Soil cores from the dose response experiment on Site A were chosen to provide a representative depth profile of inorganic N. Error bars denote ±1 SD for duplicate cores.

Table 3.3. Nitrate accumulation and net nitrification rates in soils following amendment with swine lagoon effluent in experiments assessing the influence of environmental variables on net nitrification.

Experiment	Mass of NO_3^--N in the 0-20 cm Zone (mg N)		Percent of Final NO_3^--N Mass Present at 96h (%)	Short-Term Net Nitrification Rate (r^2) in the 0-5 cm Zone [b] ($mg\ g_{dw}\ soil^{-1}\ h^{-1}$)	Percent of Nitrification Contained in the 0-5 cm Zone (%)	Total Net Nitrification in the 0-20 cm Zone [c] ($mg\ m^{-2}\ d^{-1}$)
	96 h	Final [a]				
Temperature Response—Site A						
10°C	3.4	3.6	96	0.16 a (0.90)	88	89
20°C	3.9	4.9	79	0.16 ab (0.69)	90	110
30°C	6.4	15.0	43	0.26 b (0.84)	82	383
Moisture Response—Site A [d]						
Low	17.8	22.8	78	0.40 a (0.78)	62	480
Medium	19.0	25.7	74	0.42 a (0.87)	68	516
High	16.5	23.6	70	0.54 a (0.83)	86	463
Dose Response—Site A [e]						
Low	6.2	10.1	62	0.25 a (0.85)	88	250
Medium	9.5	12.7	74	0.37 ab (0.68)	87	318
High	12.6	25.5	50	0.59 b (0.92)	85	650
Dose Response—Site B: Field Type [e]						
Low	6.1	10.3	59	0.08 a (0.98)	37	122
High	0.6	31.6	2	0.01 b (0.12)	67	386

[a] Final core soil (0 to 20 cm) NO_3^--N mass measured at the end of each experiment: 240 h (temperature and dose, Site A); 264 h (moisture, Site A); and 504 h (dose/field type, Site B).

[b] Slope of linear increase in NO_3^--N concentration with time from 0.5 to 96 h in the 0-5 cm zone. Rates followed by the same letter within each experimental group are not significantly different.

[c] Based on final net NO_3^--N accumulation in the entire 20-cm soil core at the termination of the experiment.

[d] Low, medium, high soil moistures correspond to 0.0, 1.3, and 2.6 cm H_2O applied to soil surface to achieve an approximate water-filled pore space of 27, 40, and 50%, respectively.

[e] Low, medium, and high doses correspond to 0.65, 1.3, and 2.6 cm of effluent applied to the soil surface, respectively.

Temperature Response Experiment—Site A

Soil NH_4^+-N concentrations in temperature response experiments increased abruptly from a prefertilization concentration of about 4 μg g_{dw}^{-1} to roughly 60 μg g_{dw}^{-1} immediately following amendment with liquid swine lagoon effluent (3.9 g N m^{-2}). Thereafter, NH_4^+-N concentrations rapidly decreased, dropping below 20 μg g_{dw}^{-1} by about 50 h (Figure 3.2a). Nitrate concentrations increased more gradually than NH_4^+-N concentrations decreased (Figure 3.2b).

Nitrate concentrations in low temperature (10 and 20°C) soils increased from a prefertilization concentration of about 1 μg g_{dw}^{-1} to 20 μg g_{dw}^{-1} by 100 h and then stabilized at this level. In contrast, NO_3^--N concentration in soils incubated at 30° increased for the duration of the experiment, reaching a final concentration of about 55 μg g_{dw}^{-1}. This was nearly equal to the NH_4^+-N concentration realized immediately after fertilization. Short-term (96 h) and total net nitrification rates increased with increasing temperature (Table 3.3). The short-term net nitrification rate in soils at 30°C (0.26 μg NO_3^--N g_{dw}^{-1} h^{-1}) was significantly higher than the rate in soils at 10°C (0.16 μg NO_3^--N g_{dw}^{-1} h^{-1}).

Carbon dioxide emissions increased briefly after effluent application (within 3 to12 h), then gradually declined (Figure 3.2c). Secondary spikes were observed at 100 h for the 30°C soil and at 50 h for the lower temperature soils. Carbon dioxide efflux again declined after the secondary spikes. In general, CO_2 emission increased with temperature. At all temperatures, the percent WFPS remained relatively constant at about 53% following fluctuations during the first 50 h (Figure 3.2d).

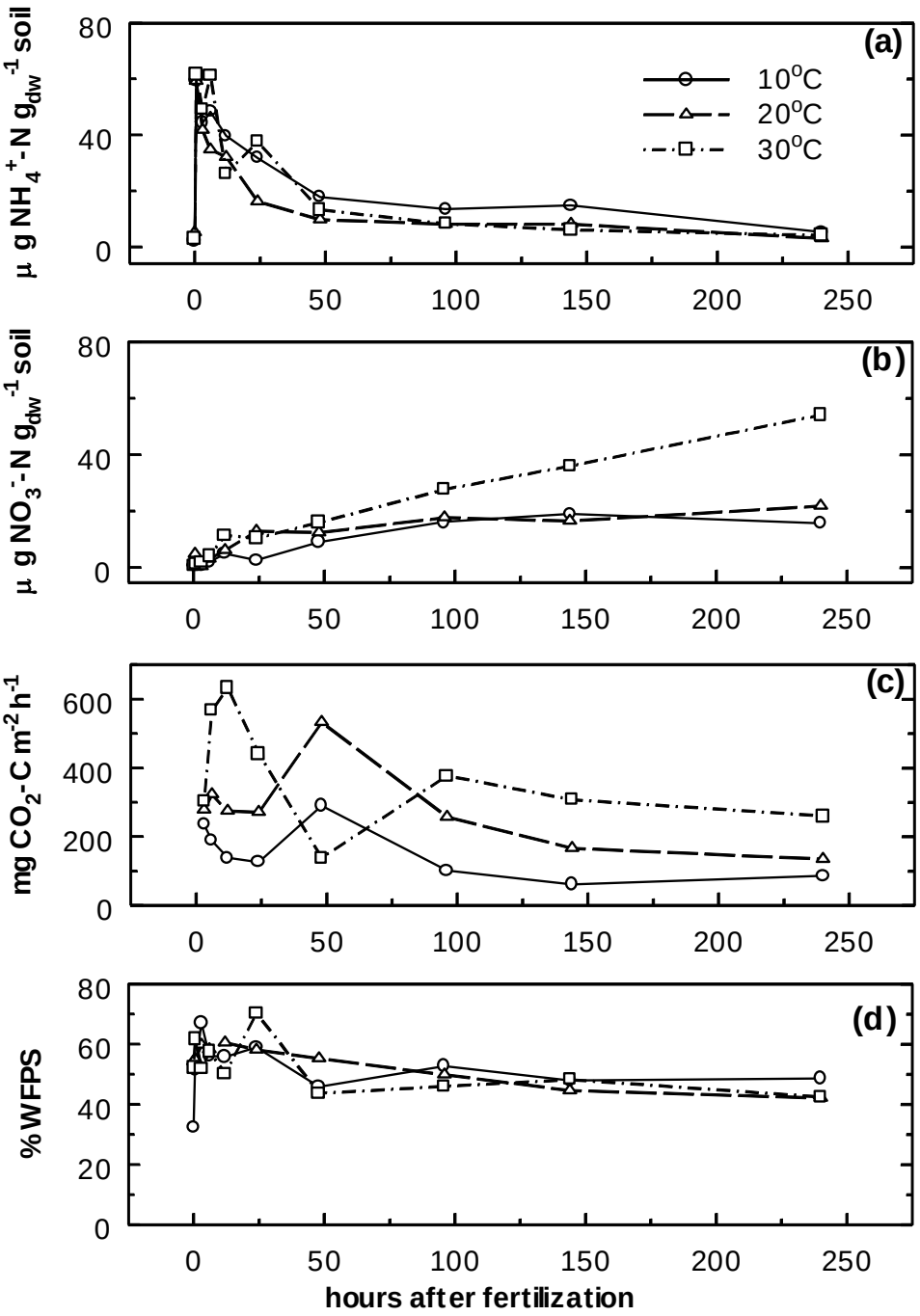

Figure 3.2. Temperature Response Site A. Time course for change in (a) NH_4^+-N; (b) NO_3^--N; (c) CO_2 efflux; and (d) percent water filled pore space (%WFPS) in soil cores from Site A amended with 1.3 cm (3.9 g N m^{-2}) of liquid lagoon effluent and incubated at 10^o, 20^o or 30^oC. Data points are the average of duplicate cores and data for NH_4^+-N, NO_3^--N and %WFPS are from the 0 to 5 cm zone of soil cores taken to a total depth of 20 cm. Data for CO_2 efflux are for the entire 20 cm core. The SD for the duplicate cores at each time point averaged: 2.4 and 3.0 μg N g_{dw} $soil^{-1}$ for NH_4^+-N and NO_3^--N; 28 mg CO_2-C m^{-2} h^{-1}; and 6% WFPS. Error bars are eliminated for clarity.

Moisture Response Experiment—Site A

Soil NH_4^+-N concentrations in moisture response experiments increased abruptly from a prefertilization concentration of about 6 µg g_{dw}^{-1} to 68 µg g_{dw}^{-1} immediately following amendment with swine lagoon effluent of 5.5 g N m^{-2} (Figure 3.3a). Thereafter, NH_4^+-N concentrations rapidly decreased, dropping below 20 µg g_{dw}^{-1} by 100 h. Nitrate concentrations in all three moisture-adjusted soils increased from a pre-fertilization concentration of about 9 µg g_{dw}^{-1} to 55 µg g_{dw}^{-1} as the concentration of NH_4^+-N decreased proportionally (Figure 3.3b). Net nitrification rates generally increased with increasing moisture but differences were not significant (Table 3.3). Total net nitrification varied from 462 to 516 mg NO_3^--N m^{-2} d^{-1} and did not show a consistent relationship to soil moisture.

Carbon dioxide efflux increased from about 70 to 600 mg CO_2-C m^{-2} h^{-1} immediately after effluent additions. Thereafter, CO_2 efflux rapidly decreased to about 200 mg $m^{-2}h^{-1}$ by about 10 h, but tended to be higher with higher initial H_2O additions (Figure 3.3c). The initial WFPS of 11% increased and stabilized at about 28, 41, and 51% WFPS after H_2O and effluent additions for the low, medium and high moisture treatments, respectively (Figure 3.3d).

Dose Response Experiment—Site A

Soil NH_4^+-N concentrations in the Site A dose response experiments increased from about 6 µg g_{dw}^{-1} to 44, 83, and 129 µg g_{dw}^{-1} immediately following low, medium and high effluent-N doses, respectively (Figure 3.4a). Ammonium concentrations decreased rapidly to near background levels by 100 h in soils with the low and medium dose while background concentrations were not reached until about 250 h in high dose soils. Nitrate concentrations increased more gradually than NH_4^+-N concentrations decreased (Figure 3.4b). The NO_3^--N concentration in low dose soils increased steadily from a pre-fertilization concentration of about 2 µg g_{dw}^{-1} to 22 µg g_{dw}^{-1} in 50 h. The NO_3^--N concentration remained constant from 50 to 150 h but then increased from 24 to 40 µg g_{dw}^{-1} between 150 and 250 h. Similarly, NO_3^--N concentrations in high dose soils increased from a prefertilization concentration of 2 µg g_{dw}^{-1} to 56 µg g_{dw}^{-1} in 100 h, and remained at this concentration until 150 h when an increase from 56 to 83 µg g_{dw}^{-1} occurred between 150 and 250 h. In contrast, the NO_3^--N concentration in the medium dose soil increased from 2 µg g_{dw}^{-1} to about 60 µg g_{dw}^{-1} in 150 h and then showed an apparent decrease to about 46 µg g_{dw}^{-1} at 250 h. Net nitrification rates increased with dose and were significantly higher at the high dose than the low dose (Table 3.3). Total net nitrification rates also increased with dose.

Carbon dioxide efflux increased from a prefertilization rate of about 180 mg CO_2-C m^{-2} h^{-1} to 375, 575 and 540 mg CO_2-C m^{-2} h^{-1} immediately after effluent additions for the low, medium and high doses, respectively (Figure 3.4c). Thereafter CO_2 efflux rapidly decreased to about 300 mg $m^{-2}h^{-1}$ by about 10 h. Secondary spikes were observed at 24 h for the medium and high dose soils and at 50 h for the low dose soil. Carbon dioxide emissions again declined after the secondary spikes. Overall, CO_2 efflux increased with dose. Percent WFPS stabilized at about 40, 48, and 52 % by 50 h for the low, medium and high effluent dose, respectively (Figure 3.4d).

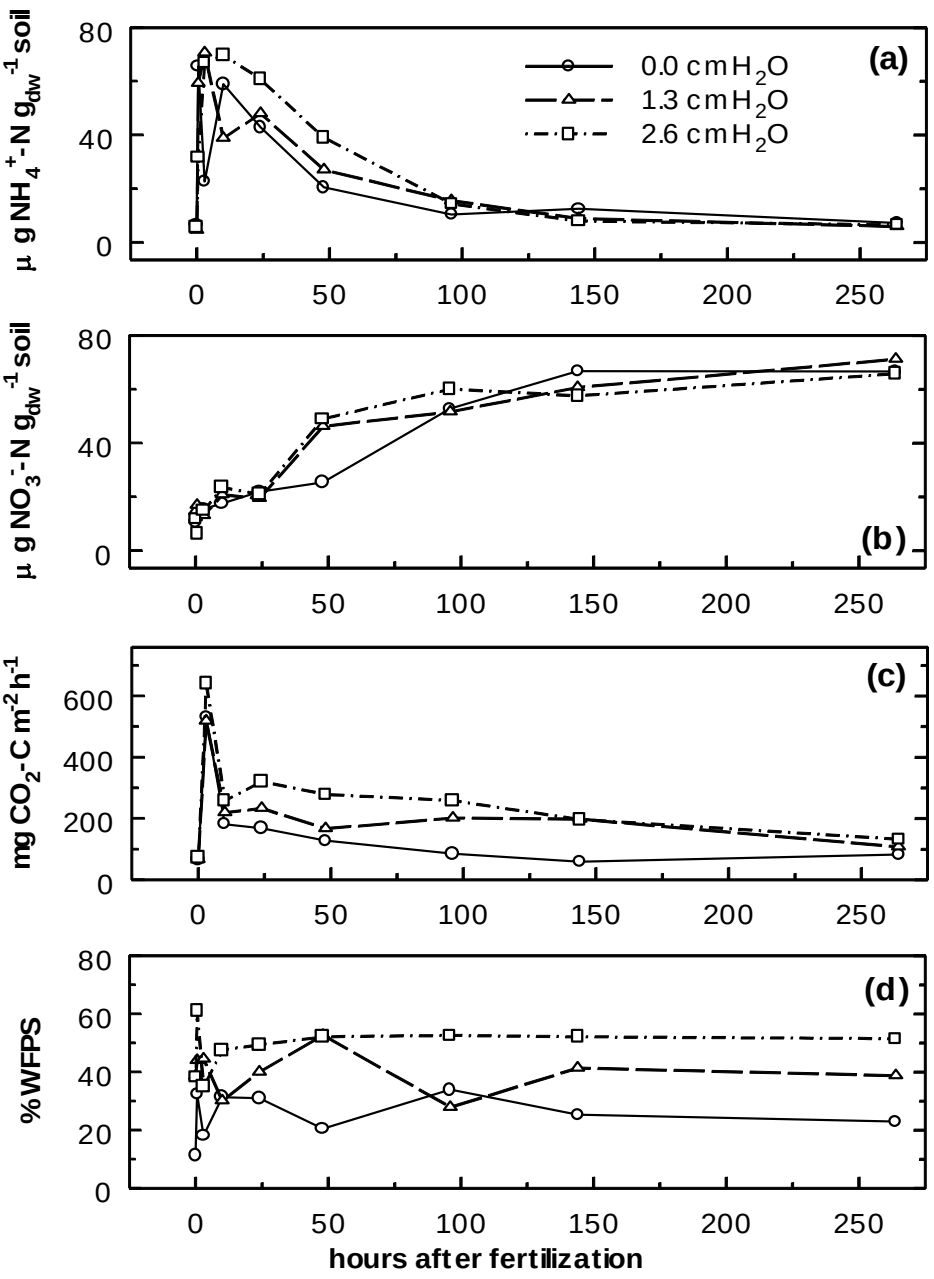

Figure 3.3. Moisture Response Site A. Time course for change in (a) NH_4^+-N; (b) NO_3^--N; (c) CO_2 efflux; and (d) percent water filled pore space (%WFPS) in soil cores from Site A amended with 0, 1.3, and 2.6 cm de-ionized H_2O to adjust soil moisture. Cores were subsequently fertilized with 1.3 cm (5.5 g N m^{-2}) of liquid lagoon effluent and incubated at 25°C. Data points are the average of duplicate cores and data for NH_4^+-N, NO_3^--N and %WFPS are from the 0 to 5 cm zone of soil cores taken to a total depth of 20 cm. Data for CO_2 efflux are for the entire 20 cm core. The SD for the duplicate cores at each time point averaged: 4.2 and 5.7 μg N g_{dw}^{-1} soil for NH_4^+-N and NO_3^--N; 25 mg CO_2-C $m^{-2}h^{-1}$; and 7% WFPS. Error bars are eliminated for clarity.

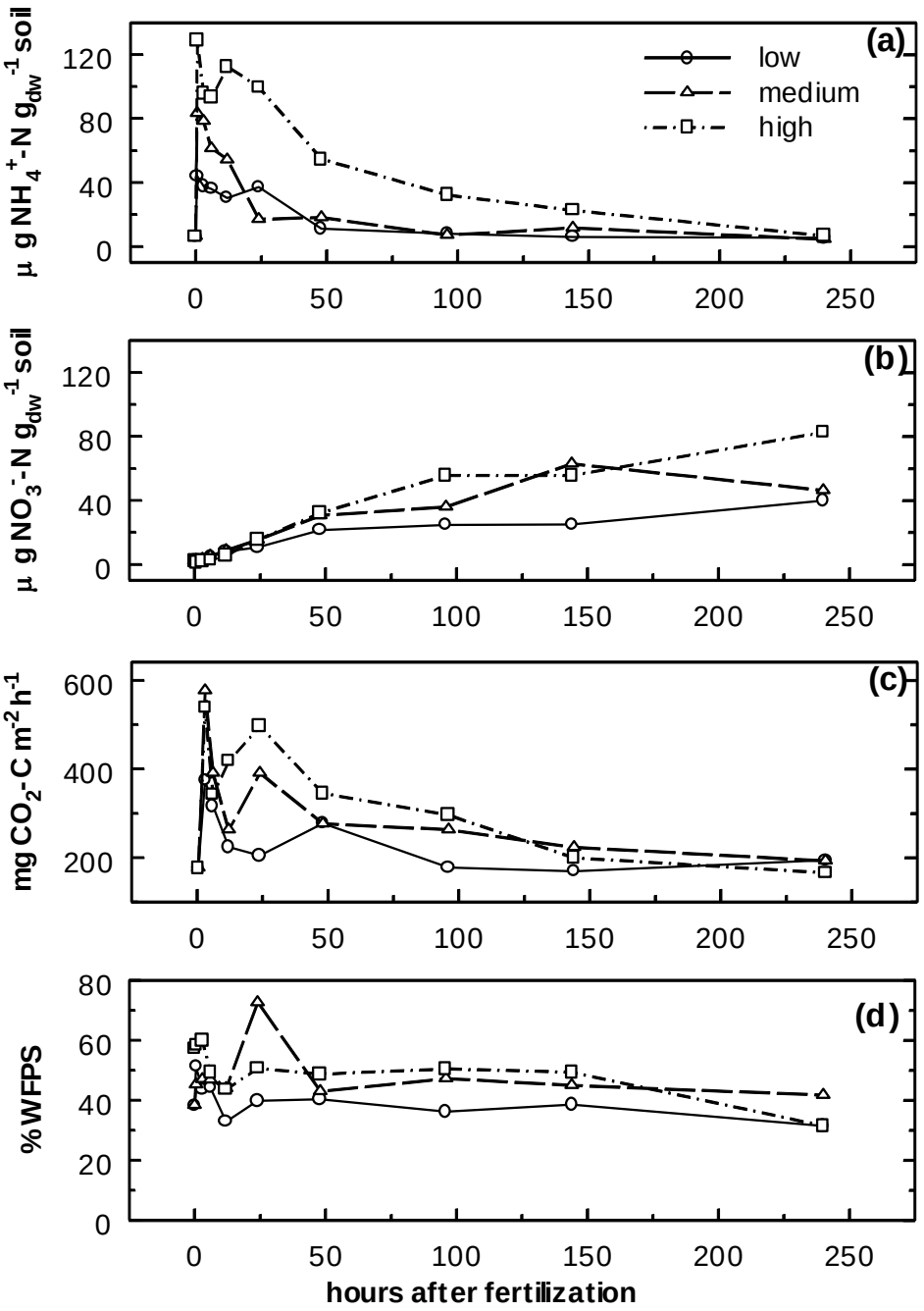

Figure 3.4. Dose Response Site A. Time course for change in (a) NH_4^+-N; (b) NO_3^--N; (c) CO_2 efflux; and (d) percent water filled pore space (%WFPS) in soil cores from Site A amended with low [0.65 cm (2.5 g N m^{-2})], medium [1.3 cm (4.9 g N m^{-2})] and high [2.6 cm (9.9 g N m^{-2})] doses of liquid lagoon effluent and incubated at 25°C. Data points are the average of duplicate cores and data for NH_4^+-N, NO_3^--N and %WFPS are from the 0 to 5 cm zone of soil cores taken to a total depth of 20 cm. Data for CO_2 efflux are for the entire 20 cm core. The SD for the duplicate cores at each time point averaged: 5.6 and 4.2 μg N g_{dw}^{-1} soil for NH_4^+-N and NO_3^--N; 48 mg CO_2-C m^{-2} h^{-1}; and 6% WFPS. Error bars are eliminated for clarity.

Dose Response Experiment—Site B: Field Type

Soil NH_4^+-N concentrations in Site B dose response experiments increased from a pre-fertilization level of about 2 µg g_{dw}^{-1} to 17 and 61 µg g_{dw}^{-1} immediately following low and high effluent-N doses, respectively (Figure 3.5a). The decrease in NH_4^+-N concentration over 216 h in high dose Site B (from 61 to 28 µg g_{dw}^{-1}) was much less than the decrease over 240 h in similarly treated soils at Site A (from 129 to 7µg g_{dw}^{-1}) (cf. Figures 3.4a and 3.5a). Similarly, the concentration of NO_3^--N in Site B soils did not increase as rapidly compared to Site A. Nitrate concentrations increased from 0.2 to 11 µg NO_3^--N g_{dw}^{-1} (low dose) and 0.2 to 19 µg NO_3^--N g_{dw}^{-1} (high dose) after 200 h at Site B (Figure 3.5b), while similarly treated Site A soils increased from 2.2 to 40 µg NO_3^--N g_{dw}^{-1} (low dose) and from 2.2 to 82.8 µg NO_3^--N g_{dw}^{-1} (high dose) over 240 h.

The net nitrification rates in Site B (fallow field) soils were significantly lower than in Site A (fertilized cropped field) soils receiving the same dose. Interestingly, the short-term (96 h) net nitrification rate in Site B soil with low dose (0.08 µg NO_3^--N g_{dw}^{-1} soil h^{-1}) was significantly higher than the rate with a high dose (0.01 µg NO_3^--N g_{dw}^{-1} soil h^{-1}). The reverse was true with the total area based net nitrification rates of 311 and 188 mg NO_3^--N m^{-2} d^{-1} for the high and low doses, respectively.

Carbon dioxide emissions peaked at 117 and 236 mg CO_2-C m^{-2} h^{-1} 10 h after low and high effluent doses, respectively (Figure 3.5c). Carbon dioxide efflux was highest in soils with high effluent loading but remained much lower than CO_2 emissions from Site A soils, which peaked at 540 mg CO_2-C m^{-2} h^{-1} within 3 h of fertilization. Soil WFPS increased from a prefertilization level of 43% to about 66% and was comparable under both low and high effluent doses (Figure 3.5d). The 100 mL effluent dose resulted in liquid leaching from the bottom of the soil cores, which indicated that the water holding capacity of Site B soil had been exceeded. The leachate contained 8.6 mg L^{-1} NH_4^+-N compared to the 347 mg L^{-1} in the applied effluent.

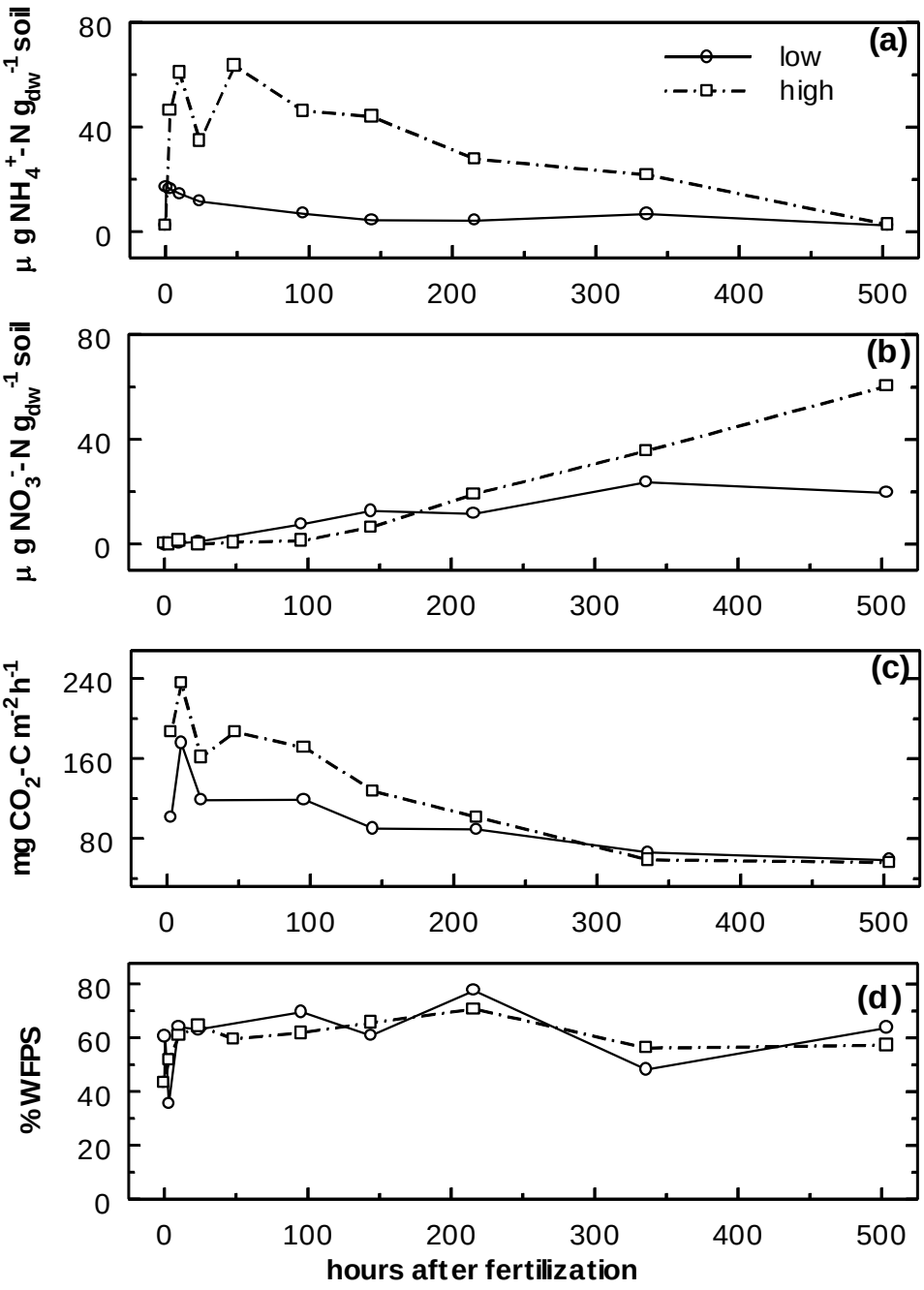

Figure 3.5. Dose Response Site B: Field Type. Time course for change in (a) NH_4^+-N; (b) NO_3^--N; (c) CO_2 efflux; and (d) percent water filled pore space (%WFPS) in soil cores from Site B amended with low [0.65 cm (2.5 g N m^{-2})] and high [2.6 cm (9.8 g N m^{-2})] doses of liquid lagoon effluent and incubated at 25°C. Data points are the average of duplicate cores and data for NH_4^+-N, NO_3^--N and %WFPS are from the 0 to 5 cm zone of soil cores taken to a total depth of 20 cm. Data for CO_2 efflux are for the entire 20 cm core. The SD for the duplicate cores at each time point averaged: 7.1 and 1.6 μg N g_{dw}^{-1} soil for NH_4^+-N and NO_3^--N; 18 mg CO_2-C $m^{-2}h^{-1}$; and 10% WFPS. Error bars are eliminated for clarity.

Soil pH Changes with Dose

Post fertilization soil pH increased with increasing dose in both Site A and B soils (Table 3.4). The pH of Site A soil increased from 0.3 to 1.4 pH units following fertilization, with larger increases associated with higher doses. Similarly, the pH of Site B soil increased 0.1 and 1.1 pH units with the low and high effluent doses, respectively.

Table 3.4. Changes in soil pH following application of swine lagoon effluent.

Experiment	Pre-fertilization Soil pH[a]	Post-fertilization Soil pH
Dose Response Site A[b]		
Low	6.2	6.5
Medium	6.2	6.6
High	6.2	7.6
Dose Response Site B: Field Type[b]		
Low	6.6	6.7
High	6.6	7.7

[a] Soil pH determined with 20g soil in 40ml H_2O. Post-fertilization measured 24 h after effluent amendment.

[b] Low, medium and high doses are 0.65, 1.3, and 2.6 cm of effluent applied to soil surface, respectively.

Soil Inorganic-N Balance

A mass balance of soil inorganic-N in the entire soil core (0 to 20 cm) was conducted 24 h after amendment with lagoon effluent and at the end of the experiment to gain insight into the fate of added N (Table 3.5). Inorganic-N in the temperature response soils at 24 h accounted for up to 81% of effluent inorganic-N applied. No clear pattern was evident for the 24 h mass balances in temperature response experiments as the mid-range temperature (20^{o}C) showed the highest percent of missing inorganic-N. The mass balance conducted at the end of the temperature experiment indicated a net loss of around 60% of the inorganic-N applied to 10 and 20^o C soils, while essentially all of the inorganic-N applied to 30^o C soil was recovered. Data at 24 h for the moisture response experiment indicated essentially no change in inorganic-N in soil with low moisture and losses of nearly 15% in soils with medium and high moisture. Increases equal to 4 to 10% of the inorganic-N applied were found at the termination of the experiment. There was a 25 % gain of inorganic-N at 24 h in Site A soil with a low effluent dose while losses of 28 to 51% were observed for other doses. Recovery of applied inorganic-N at termination of the dose response experiment at Site A ranged from 77 to 108%. A net loss of nearly 20% of inorganic-N applied to Site B soil was realized with the high dose at 24 h while no change was observed at the low dose. A net loss of roughly half of the inorganic-N applied was observed in both the low and high dose soils at the termination of the experiment.

Table 3.5. Mass balance for inorganic-N in soil cores (0 to 20 cm) for each experiment at 24h and end of experiment.

Experiment	Pre-fertilization Soil Inorganic-N (mg N)		Effluent-N Applied (mg N)		Soil Inorganic-N at 24 h (mg N)		Percent of Initial N Remaining at 24 h [a] (%)	Soil Inorganic-N at End (mg N)		Percent of Initial N Remaining at End [b] (%)
	NH_4^+-N	NO_3^--N	NH_4^+-N	Total-N	NH_4^+-N	NO_3^--N		NH_4^+-N	NO_3^--N	
Temperature Response Site A										
10°C	1.0	0.2	14.3	14.9	9.2	0.6	61	2.6	3.6	38
20°C	1.7	0.7	14.3	14.9	5.2	2.9	47	0.9	4.9	34
30°C	1.7	0.2	14.3	14.9	10.5	2.6	78	1.1	15.0	96
Moisture Response Site A [c]										
Low	3.8	2.5	19.0	21.1	19.3	6.6	95	4.7	22.8	100
Medium	2.7	3.8	19.0	21.1	16.4	5.5	79	2.4	25.7	102
High	2.7	4.0	19.0	21.1	15.1	7.2	80	3.2	23.6	96
Dose Response Site A [d]										
Low	2.2	0.5	8.4	9.4	11.4	2.6	116	1.9	10.1	100
Medium	2.2	0.5	16.9	18.8	5.8	3.8	44	2.3	12.7	70
High	2.2	0.5	33.7	37.5	23.4	2.9	65	5.3	25.5	77
Dose Response Site B: Field Type [d]										
Low	1.7	0.4	8.7	9.4	10.3	0.6	94	0.7	5.8	56
High	1.7	0.4	34.7	37.4	29.9	0.0	76	0.8	16.5	44

[a] Relative amount of initial N (pre-fertilization + applied) present in the 0 to 20 cm zone at 24 h.

[b] Relative amount of initial N (pre-fertilization + applied) present in the 0 to 20 cm zone at end of experiment: 240 h (temperature and dose, Site A); 264 h (moisture, Site A); and 205 h (dose/field type, Site B).

[c] Low, medium, high soil moistures correspond to 0.0, 1.3, and 2.6 cm H_2O applied to soil surface to achieve an approximate water-filled pore space of 27, 40, and 50%, respectively.

[d] Low, medium, and high doses correspond to 0.65, 1.3, and 2.6 cm of effluent applied to the soil surface, respectively.

4. DISCUSSION - NITRIFICATION EXPERIMENTS

Soil Net Nitrification Rates

Stratification of soil microorganisms in the absence of conventional field tillage may influence microbial processing of fertilizer-N (Kandeler & Bohm 1996). Conventional tillage had not been practiced on either study site for several years. Consequently, care was taken during collection of 20 cm cores to maintain the integrity of the soil structure at field conditions to accurately assess zonation and rates of nitrification.

Applied effluent-N generally did not penetrate below the 0 to 5 cm zone (Figure 3.1) which retained on average 63% of the NH_4^+-N applied. Other studies involving organic fertilizers also show little migration of added NH_4^+-N. For example, McCormick et al. (1983) found the greatest concentrations of NH_4^+-N at 2.5 and 5 cm from the center of the injection zone for liquid swine manure, while Petersen (1992) observed that NH_4^+-N in liquid cattle manure remained within 5 cm of the injection zone 21 d after application. Further, Yadvinder-Singh and Beauchamp (1988) showed that NH_4^+-N ions from hydrolyzed urea moved only 6 cm from the localized source in 35 d and that nitrifier activity was greatest in zone 3 to 6 cm from the source.

Nitrifier activity was concentrated in the 0 to 5 cm horizon, consistent with the localization of effluent NH_4^+-N in this zone. Similarly, Staley et al. (1990) reported highest nitrification activity in the 0 to 3.8 cm zone. The percent of total net nitrification occurring in this zone was higher in previously fertilized soils (80%) than in fallow soils (52%).

Nitrate accumulation was linear with little or no lag time in both of these soils. Linear NO_3^--N increases have been reported following pig slurry amendments and zero order rate constants described the data (Flowers & O'Callaghan 1983; Boyle & Paul 1989). Myrold and Tiedje (1986) found that both zero order and first order models could describe nitrification but that in some experiments only a portion of the data set could be fit well by either model. Flowers & O'Callaghan (1983) evaluated both zero order and first order equations and found nitrification of pig slurry was well represented by zero order rate constants. Zero-order nitrification rate constants were calculated from the slope of the NO_3^--N accumulation in the first 100 h, as this tended to be the time frame with the greatest response at all ranges of temperature, moisture and effluent dose analyzed. These short-term (96h) rate constants were used for comparisons among treatments in this study (Table 3.3). Rate constants ranged from 0.16 to 0.59 μg N g_{dw}^{-1} soil h^{-1} for Site A soils which is within the range reported for other agricultural soils (Flowers & O'Callaghan 1983; Myrold & Tiedje 1986).

Net nitrification rates increased with increasing temperature, consistent with pervious studies (Sabey et al. 1956; Frederick 1956; DeLeval & Remacle 1975; Macduff & White 1985). Short-term (96 h) net nitrification rates were significantly lower in soils at 10°C (0.16 μg NO_3^--N g_{dw}^{-1} soil h^{-1}) than at 30°C (0.26 μg NO_3^--N g_{dw}^{-1} soil h^{-1}). Flowers & O'Callaghan (1983) found higher nitrification rates of pig slurry with higher temperature and noted that the temperature response varied considerably between different soil types receiving similar NH_4^+-N loading. The authors observed nitrification rates as low as 0.1 μg N g^{-1} soil h^{-1} in soils at 5°C, while nitrification rates as high as 0.29 μg N g^{-1} soil h^{-1} were achieved in 15°C soils. Similar

temperature responses have been observed for cattle slurry, but with a pronounced lag time in nitrification at low (5^oC) temperature (Opperman et al. 1989).

Net nitrification rates increased with increasing soil moistures from 28 to 51 % WFPS, but differences in rates were not significant. Increases in the rate of nitrification with increasing soil moisture have been reported previously (Reichman et al. 1966; Sabey 1969; Malhi & McGill 1982; Robertson 1982; Macduff & White 1985; Yadvinder-Singh & Beauchamp 1988; Davidson et al. 1990; Hutchinson et al. 1993; Tate 1995) but comparison among studies is confounded by varying and non-interconvertible measures of soil moisture (e.g. water potential, gravimetric moisture, % water holding capacity). In a comparable study, Reichman et al. (1966) showed little NO_3^--N accumulation in fertilized soils at less than 15% WHC, a sharp increase with moisture ranging from about 15 to 25 % WHC, and a less dramatic, linear increase from about 25 to 55% WHC. In addition, Flowers & O'Callaghan (1983) reported a nitrification rate of about 0.58 µg N g_{dw} $soil^{-1}$ h^{-1} in soil at 60% WHC after a pig slurry amendment of 250 µg NH_4^+-N g^{-1} soil. Hence, the short-term (96 h) net nitrification rate increase observed here from 0.4 to 0.54 µg NO_3^--N g_{dw}^{-1} soil h^{-1} with increasing moisture from 26 to 52% WHC (28 to 51% WFPS) in soils receiving lagoon effluent at roughly 95 µg NH_4^+-N g_{dw} $soil^{-1}$ appears consistent with these earlier findings.

Effluent dose affected net nitrification rates in both Site A and Site B soils. Rates were significantly lower for the low than the high doses, which ranged from 0.65 to 2.6 cm effluent (2.2 to 8.9 g NH_4^+-N m^{-2}). Soil moisture increased concurrently with increasing effluent dose but the 5 to 26% increase above prefertilization WFPS did not significantly affect nitrification rates. Increases in NO_3^--N accumulation rates with increased NH_4^+-N dose have been reported previously (Cooper 1975; Malhi & McGill 1982; Flowers & O'Callaghan 1983; Lindemann & Cardenas 1984). Cooper (1975) reported faster NO_3^--N accumulation with increasing pig slurry for doses of 60, 115 and 260 µg N g_{dw} $soil^{-1}$ but did not calculate nitrification rates. Flowers & O'Callaghan (1983) showed an increase in nitrification rate from about 0.3 to 0.6 µg N g_{dw} $soil^{-1}$ h^{-1} as pig slurry-N increased from 50 to 250 µg NH_4^+-N g_{dw} $soil^{-1}$. Short-term (96 h) net nitrification rates in the present study are in reasonable concordance, ranging from 0.25 to 0.59 µg NO_3^--N g_{dw} $soil^{-1}$ h^{-1} for doses ranging from roughly 42 to 168 µg NH_4^+-N g_{dw} $soil^{-1}$.

Short-term (96 h) net nitrification rates in Site A soils were significantly higher than Site B soils for the same effluent dose. These data point to a more active nitrifier population in Site A soils. Site A has a 10-yr history of irrigation with swine lagoon effluent that likely supports a viable indigenous population of nitrifiers capable of rapidly responding to changes in nutrient levels. Increased biological activity in general has been reported in soils receiving repeated manure applications (Chang et al. 1998). More specifically, Ceccherini et al. (1998) showed that soils receiving repeated pig slurry fertilization over time had a greater indigenous population of nitrifiers and a greater nitrification potential compared to unfertilized soils.

In contrast to Site A, Site B is a fallow field and the pronounced lag time in soil NO_3^--N accumulation indicates nitrifier populations likely required an induction period. The lag time was most obvious for the high dose where NO_3^--N concentrations did not begin to increase until 100 h after effluent addition (Figure 3.5). Similar lag times in NO_3^--N accumulation following

NH_4^+-N fertilization have been attributed to development of a viable population of nitrifiers (Petersen 1992; Nielsen & Revsbech 1998).

Soil Respiration and pH

Respiration of labile-C in pig waste is the likely source of immediate, and transient bursts of CO_2 following effluent addition (Comfort et al. 1988; Paul & Beauchamp 1989b). Lovell & Jarvis (1996) found increased soil respiration following manure amendments. Increased CO_2 efflux with increases in temperature and soil moisture have also been reported (Hutchinson et al. 1993; MacDonald et al. 1995). Consistent with these earlier findings, our results show CO_2 efflux generally increased with increasing temperature, moisture and effluent dose in all experiments. Soil cores collected for the temperature response experiment were collected in the spring and visibly had more organic matter on the soil surface. This likely served as the source of organic-C accounting for protracted CO_2 efflux during the temperature response experiment (Figure 3.2c).

Water soluble-C in effluent is likely composed of several labile-C components. Volatile fatty acids (VFA; e.g., acetic, propionic and butyric acids) accounted for up to 85% of water soluble-C in swine slurry and an apparent hierarchy for the utilization of VFA's has been demonstrated (Cooper & Cornforth 1978; Paul & Beauchamp 1989b). An ordered utilization of labile-C components may provide an explanation for the secondary CO_2 spikes occurring between 24 and 100 h as were most pronounced in the temperature and dose response experiments (Figures 3.2c and 3.4c).

Volatile fatty acids in anaerobically stored manure slurry may also explain the observed increase in soil pH with increasing effluent dose. Paul & Beauchamp (1989a) suggest that microbial oxidation of VFAs occurs rapidly upon application to soil resulting in a pH increase. Short-term increases in soil pH immediately following manure applications have been reported previously (Cooper 1975; Crane et al. 1981; Flowers & Arnold 1983; Comfort et al. 1988).

An increase in soil pH shifts the $NH_3 \boxtimes NH_4^+$ equilibrium in favor of NH_3 volatilization. Crane et al. (1981) showed soil pH increases from 1.3 to 2.2 pH units when increasing manure applications from 22 to 105 g m^{-2} and observed losses of 31 to 75% of NH_4^+-N applied during application. We found pH increases from 0.3 to 1.4 pH units with effluent doses of 2.4 to 9.7 g N m^{-2} but the inorganic-N balance (Table 3.5) did not indicate substantial losses of NH_4^+-H to volatilization. Alternatively, loss may have been compensated by mineralization of native organic-N.

Net nitrification rates in Site A soils (pH = 6.2) increased with increasing effluent dose and were seemingly not affected by an increase of up to 1.4 pH units. In contrast, net nitrification rates in Site B soils (pH = 6.6) may have been affected by the increase of up to 1.1 pH units with high effluent dose as rates in the low dose soil (increase of 0.1 pH units) were higher (Table 3.3). A previous study with pig slurry addition (Cooper 1975) showed nitrification in neutral (pH = 7.1) soils was inhibited and nitrification in acidic (pH = 5.8) soils increased following an increase of up to 1.3 pH units with pig slurry applications.

Immobilization of Effluent-N

Temperature appeared to influence immobilization of inorganic-N. Inorganic-N decreased for the duration of the experiment in 10^o and 20^o C soils. In 30^o C soils inorganic-N decreased only temporarily (48 h) followed by a linear increase and ultimately accounted for most of the effluent inorganic-N applied (Table 3.5). These observations suggest that missing inorganic-N in all soils may not be entirely lost from the soil, but rather immobilized for a period of time dependent on temperature. Flowers & Arnold (1983) reported longer immobilization of NH_4^+-N in pig slurry at lower temperatures, consistent with observations here.

Moisture also influenced immobilization, and soils receiving H_2O treatments had more inorganic-N missing 24 h after effluent addition (Table 5.5) than soils receiving no H_2O. Flowers & Arnold (1983) reported more rapid losses of pig slurry-N at higher moistures and attributed the loss to microbial immobilization. As much as 40% of the applied NH_4^+-N in pig slurry was immobilized with no clear evidence of remineralization during a 40-d incubation. In contrast, this study showed a net increase of inorganic-N during only 10 d of incubation. This net increase of inorganic-N in the moisture response experiment could be explained by immobilization and remineralization of N from a previous effluent application. The field was irrigated with effluent only 6 d prior to sampling and microbial stimulation associated with the previous fertilization could have immobilized N that was subsequently released during the experiment.

Missing N at 24 h in the dose response experiments is also likely due to immobilization since much of the missing inorganic-N returned as NO_3^--N by the end of the experiment (Table 5.5). Likewise, Cooper (1975) reported an increase in soil NO_3^--N at 5 weeks, following an initial loss of pig-slurry inorganic-N.

Fate of Effluent-N

The change in inorganic-N concentration with time indicates the importance of immobilization and remineralization in determining long-term N availability to plants. Soil inorganic-N fluctuations (Table 5.5) indicate that some effluent NH_4^+-N is rapidly immobilized and later remineralized. As much as 52% of effluent NH_4^+-N was immobilized within 24 h, and in many of the treatments this was remineralized by 250 h. Net increases of inorganic-N were observed in some of the response experiments and could be due to coupled mineralization-nitrification of the organic-N of the pig waste. Alternatively, mineralization of organic-N resident in the soil has been demonstrated (Flowers & Arnold 1983; Cooper 1975) to contribute to post-fertilization increases in soil inorganic-N. Contributions of initial soil-N and effluent-N to mineralization and net changes in soil inorganic-N were not analyzed separately, however, and no clear indication of the source of remineralized N could be determined from this study.

Increases in NO_3^--N concentration did not always correspond quantitatively with decreases in NH_4^+-N concentration, consistent with the immobilization of effluent NH_4^+-N indicated from the inorganic-N mass balance (Table 5.5). The soil NO_3^--N lag phase was most pronounced in soil cores where CO_2 efflux remained high after effluent applications (Figures 3.2 & 3.4) suggesting immobilization of NH_4^+-N is initially greater than nitrification when a labile source of organic-C

is present. Davidson et al. (1990), found that nitrifying bacteria were not at a competitive disadvantage in their experiments of monthly sampling in grassland soils amended with NH_4^+-N as $(^{15}NH_4)_2SO_4$ but no C source. In contrast, Jones & Richards (1977) found that heterotrophic bacteria outcompeted nitrifiers for NH_4^+-N when readily available C was present for energy. Collectively, these studies suggest that swine lagoon effluent provides a source of labile-C which can be used by heterotrophic bacteria to outcompete nitrifiers in the short-term (i.e., 50 h). After initially high soil CO_2 efflux declines, concentrations of NO_3^--N increase as heterotrophs exhaust labile-C and nitrifiers gain a competitive advantage for effluent NH_4^+-N and remineralized N.

Losses of NH_4^+-N in excess of NO_3^--N increases have also been attributed to a combination of NH_3 volatilization, immobilization, and coupled nitrification-denitrification (Comfort et al. 1988; Petersen 1992; Sommer 1996). Ammonia losses of 13% and 69% of NH_4^+-N in swine effluent have been reported during irrigation and within 24 h of application to field, respectively (Sharpe & Harper 1997). Recovery of most, if not all, inorganic-N suggests little volatilization here, although some of the inorganic-N pool at the end of experiment could have been from native-N. Ammonia volatilization was likely limited in this experiment as effluent was poured onto cores, affording little opportunity for volatilization during application. Further, soil cores were incubated in the laboratory with restricted air flow across soil surfaces.

While immobilization and remineralization of effluent NH_4^+-N seems to be of importance in these short-term experiments, microbial immobilization of NO_3^--N is of comparatively little significance. Soil NO_3^--N concentrations generally increased with time without any subsequent losses. This corroborates well earlier reports (Jones & Richards 1977; Flowers & Arnold 1982) that microbial immobilization of NO_3^--N does not occur as readily as immobilization of NH_4^+-N. Jones & Richards (1977) concluded that NO_3^--N does not participate actively in the soil internal-cycle of N.

The accumulation of NO_3^--N indicates that denitrification is not an important sink for effluent-N in these soils. The soils used in these experiments were sandy and not likely to remain saturated (anaerobic) very long. Hence, conditions are only transiently favorable for coupled nitrification-denitrification. Immediately prior to fertilization, soil NO_3^--N is below the threshold of 5 μg N g_{dw} $soil^{-1}$ generally considered necessary for denitrification (Ryden 1983; Goulding et al. 1993). Nitrification is necessary before denitrification becomes active, but coupled nitrification-denitrification is possible only during the short post-fertilization period when soils are moist enough to allow persistence of anaerobic microzones. Thereafter, denitrification ceases and conditions become increasingly favorable (oxic) for nitrification and NO_3^--N accumulates. Since NO_3^--N is highly mobile and NH_4^+-N is not, several fates are possible including crop-uptake, leaching and lateral movement (Evans et al. 1984). Additionally, denitrification of some accumulated NO_3^--N may occur following rainfall events (Sommer et al. 1996; Chang et al. 1998; Whalen 1999). Chang et al. (1998) reported bursts of N_2O flux from denitrification in manured soils following rain events. Whalen (1999) reported similar N_2O bursts and attributed their occurrence to increased soil moisture and reduced soil O_2 following rain.

Evans et al. (1984) conducted a five year study on fields irrigated with swine lagoon effluent and found that crop-uptake, surface runoff and soil accumulation of NO_3^--N increased with increasing dose. At effluent-N rates of 347 kg N ha^{-1}, roughly equivalent to the crop utilization

rate recommended by Barker (1996), crop uptake, surface runoff and subsurface drainage accounted for 75, 1, and 5 % of N applied. Percolation of NO_3^--N through the soil following rain fall transports nutrient below the rooted zone and the coinciding zone of maximum microbial activity (0 to 20 cm; Figure 3.1) where it may accumulate at depth. Regionally, NO_3^--N pollution of ground water has been reported at effluent fertilization rates exceeding recommendations for crop utilization (Evans et al. 1984; King et al. 1985).

Comparisons to Other Wastes

Comparison of nitrification rates reported for agroecosystems is confounded by differences in environmental and soil physiochemical variables among studies. Nitrification rates are affected by the fertilization (Petersen 1992; Nielsen & Revsbech 1998) and tillage (Staley et al. 1990; Kandeler & Bohm 1996) history of the soil. Nitrification rates can also vary due to the time frame in which data are collected to calculate the rates (hourly, daily, weekly, or monthly). In this study, short-term (96 h) net nitrification rates were greater than total net nitrification rates by as much as a factor of 2 (Table 3.3). Hence, nitrification rates of different types and forms of waste are only broadly comparable and it may be more appropriate to compare potential rates based on waste composition.

Ammonium accounted for about 92% of total-N in the liquid swine lagoon effluent used in these studies, which was comparable to other regional studies (Westerman et al. 1985, 1990; Safley et al. 1992). This high NH_4^+-N/total-N ratio indicates lagoon effluent has little mineralization potential compared to raw swine manure and slurry which has NH_4^+-N/total-N ratios of 0.51 and 0.61 respectively (Zublena et al. 1995). Other animal manure slurries have NH_4^+-N/total-N ratios of 0.41 (cow) and 0.64 (poultry) (Crane et al. 1981; Barker 1996). The slow breakdown and release of nutrients from manure is not sufficiently concentrated to cause any measurable localized effects (Lovell & Jarvis 1996). Manure and slurries generally have medium to long term effects (e.g. months) on NO_3^--N accumulation due to gradual decomposition of organics and reliance on rainfall to bring waste into contact with soil microorganisms (Lovell & Jarvis 1996; Beauchamp 1997; Ellis et al. 1998). Reductions in soil NO_3^--N have been reported to result from denitrification in anaerobic zones created by the oxygen demand exerted by slurry (Crane et al. 1981). In contrast, we found rapid increases in NO_3^--N attributable to liquid-phase fertilization with an immediately available form of N (NH_4^+) to a viable nitrifier population in the 0 to 5 cm zone of sandy, aerobic soils.

5. MATERIALS AND METHODS - DENITRIFICATION EXPERIMENTS

Description of Study Site

The study site is a corporate, farrow-to-finish swine production facility located in Sampson County (35°06'N, 78°11'W), about 30 km northeast of the sites employed in nitrification studies (Figure 4.1). The facility maintains a herd of 1200 breeding sows and an on-site population of approximately 10,000 head. The liquid phase is periodically land-applied to 63 ha of spray fields via a traveling big gun sprinkler irrigation system. The spray field selected for this study had been fertilized with effluent for four years and cropped in a continuous rotation of soybeans (*Glycine* sp.) and winter wheat (*Triticum* sp.). Soils of the study site (0 to 20 cm zone) were similar to those used for nitrification experiments (Chapter 3). Soil pH averaged 6.3 units, while bulk and particle densities averaged 1.44 and 2.61 g cm^{-3}. The water holding capacity was 37% and soil texture was 64% sand, 23% silt and 13% clay. Soil organic and total-N contents averaged 4.0 and 0.08%, respectively.

Sample Collection and Preparation

Intact soil cores were collected using a hammer-driven core device containing stainless steel inserts (4.65 cm diameter x 30 cm height). The sampling depth was 20 cm. Previous studies at this site showed that >90% of the depth-integrated potential nitrifying and denitrifying activity was localized in the surface 20 cm.of soil (Whalen et al. 2000). Cores were capped at one end and transported on ice to the laboratory. Cores were refrigerated at 4°C until use.

Bulk soil samples were obtained by combining 10 to 15 cores of 20-cm depth in a plastic tub. The tub was covered with aluminum foil and transported on ice to the laboratory. Soil composites were prepared by sieving the bulk samples through a 4.76-mm mesh and gently mixing prior to experimental manipulation. In order to minimize the effect of plot-scale heterogeneity (Parkin 1993) on repeated experiments using soil composites, core collection was restricted to a randomly defined, 3-m^2 section of the field.

Lagoon liquid samples were collected and stored as described in Chapter 3.

Physicochemical Properties of Soils and Liquid Lagoon Effluent

Analysis of soil physicochemical properties and total-N and NH_4^+-N concentrations in liquid lagoon effluent followed procedures outlined previously (Chapter 3). Total organic carbon (TOC) in liquid lagoon waste was determined by high temperature catalytic oxidation using a Shimadzu Model 5000 Total Organic Carbon Analyzer.

Nitrous Oxide Flux Determinations

The same methodology was used to determine nitrous oxide fluxes in both intact cores and soil composites. Static chambers were produced by sealing intact soil cores in steel liners or soil composites in 4-oz. (~133 mL) Mason jars with plastic caps or screw-on lids, respectively. All caps and lids were fitted with 1/8 in. Swagelok O-seal fittings and rubber septa for syringe

sampling. Soils were incubated aerobically in the static chambers at a fixed temperature while four headspace samples were collected at selected intervals for analysis of N_2O. Fluxes were calculated based on the time-linear rate of N_2O accumulation in the headspace during incubation. Fluxes were expressed on an areal basis for intact cores and a dry mass basis for composites.

Headspace samples were collected in 5-mL SESI nylon syringes fitted with hypodermic needles and containing pistons that were modified with larger diameter O-rings for an improved seal. Samples were stored for analysis by inserting the hypodermic needles into rubber stoppers. Samples were typically analyzed within 3 to 4 h of collection. Nitrous oxide concentration was measured by gas chromatography using a Shimadzu GC-14A equipped with a ^{63}Ni electron capture detector. Gases were separated on a 1-m precolumn and a 3-m analytical column, both packed with 50/80 mesh Porapak Q. The flow rate of carrier gas (5% CH_4 - 95% Ar) to the detector was 25 mL min^{-1}. Column and detector temperatures were 40°C and 325°C, respectively. The coefficients of variation for 10 replicate analyses of two different N_2O standards (301 and 8042 ppb) were less than 2% (Whalen et al. 2000). Calibration gases are traceable to National Institute for Standards and Technology standards.

For experiments in which soils were amended with water, N_2O concentrations were corrected to include dissolved N_2O using Bunsen solubility coefficients (Gevantman 1996). Although coefficients are only specified over the range 0 to 40°C, values were extrapolated for temperatures up to 60°C and found to be in close agreement with Bunsen solubility coefficients extrapolated from other sources (Tiedje 1994; Weiss & Price 1980; Wilhelm et al. 1977).

Denitrification Rate Measurements

Denitrification rates were measured by the acetylene (C_2H_2) inhibition method (Klemedtsson et al. 1990; Yoshinari et al. 1977). In this method a chamber headspace is amended with C_2H_2 to achieve a partial pressure of 10 kPa C_2H_2. At this concentration, C_2H_2 inhibits the microbial reduction of N_2O to N_2 (Balderston et al. 1976). Therefore, the time-linear rate of N_2O production in the presence of 10 kPa C_2H_2 corresponds to the rate of production of gaseous denitrification end-products ($N_2O + N_2$) and, effectively, represents the *in situ* rate of denitrification. This method was adapted for use with both intact cores as well as homogenized soil composites.

For measurements on intact cores, a static chamber was prepared by sealing both ends of a steel core liner with the plastic end-caps described above. Prior to use, C_2H_2 was scrubbed with concentrated sulfuric acid to remove acetone (Paul & Zebarth 1997a) and other impurities. With the top septum momentarily removed to avoid pressurization, the appropriate volume of purified C_2H_2 was injected into the soil core through the bottom septum using a 20-cm long perforated needle. Acetylene was allowed to diffuse for 2 h before samples were collected. In preliminary experiments (data not shown), 2 h proved adequate to allow homogeneous distribution of an introduced gas in the soil pore space. In the third hour, four headspace samples were removed at 20-min intervals and analyzed for N_2O.

For measurements on soil composites, soils were incubated in the Mason jar/static chamber apparatus described earlier. Acetylene was injected through the septum to achieve 10 kPa C_2H_2.

One hour was allowed for the C_2H_2 to diffuse and equilibrate in the aqueous phase. During the next 3 h, four headspace samples were collected at 1-h intervals and analyzed for N_2O.

Denitrification Enzyme Activity (DEA)

The DEA method (Tiedje 1994) is founded on the principle that the rate of denitrification is directly proportional to the concentration of denitrifying enzymes when all environmental factors limiting denitrification are removed and further microbial growth is inhibited. Therefore, DEA serves as an estimate of denitrification potential under optimal conditions rather than a direct measure of denitrification rate under field conditions.

In 4-oz. Mason jars, a soil slurry was prepared by combining 25 g wet weight of homogenized soil with 25 mL of solution. The standard solution contained 40 mg glucose-C L^{-1}, 100 mg NO_3^--N L^{-1}, and 10 mg chloramphenicol L^{-1} (to inhibit *de novo* enzyme synthesis); however, as noted below, adjustments were made to tailor substrate availability as desired in specific experiments. Sealed jars were repeatedly evacuated, filled with N_2, and vigorously shaken to eliminate dissolved O_2. Excess pressure inside the jars was removed by venting through the septum with a needle.

The anaerobic jars were preincubated on a rotary shaker at 175 rpm at a specified temperature for approximately 30 min. After preincubation, the jars were assayed for denitrification rate by adaptation of the 10 kPa C_2H_2 inhibition method. Acetylene was injected through the septum to achieve a partial pressure of 10 kPa and the jars were shaken by hand to equilibrate headspace C_2H_2 in the aqueous phase. Incubation on the rotary shaker was resumed and four 5-mL samples of headspace gas were collected at regular intervals and analyzed for N_2O.

Experimental Design

Two sets of laboratory experiments were conducted. First, simulated fertilization experiments were conducted to evaluate the time course for changes in soil physicochemical parameters, rates of denitrification, and associated microbial processes following fertilization at representative lagoonal effluent loading rates. Second, a series of individual experiments was carried out to characterize the process-level response of denitrifying bacteria to individual environmental variables, including soil moisture, organic-C, NO_3^--N, and temperature. Intact cores were employed in the first set of experiments to simulate field conditions, while homogenized soil composites were used in the second set to reduce spatial heterogeneity in an effort to better assess the impact of the selected variables on rates of denitrification. With the exception of the moisture response experiments, all process-level experiments were based on adaptations of the denitrification enzyme activity assay of Tiedje (1994).

Time Course Intact Core Experiments

Two closely related fertilization experiments were conducted. They are herein referred to as time course experiments 1 and 2, or simply TC-1 and TC-2. These experiments focus on nitrification, denitrification, and the effect of these processes on soil concentrations of inorganic-N (NH_4^+ and NO_3^-) and emission of gaseous N (N_2O and N_2) in response to swine waste

fertilization. TC-1 involved three fertilization rates and a 12-d time course, while TC-2 was limited to two fertilization rates and an 8-d time course to increase the level of replication. The intact cores and lagoon samples used in these fertilization experiments were collected on two separate occasions during the spring while the field was cropped in mature wheat: May 8, 1998 and June 6, 1998 (TC-1 and TC-2, respectively). In both experiments, all cores were preincubated overnight at 25^{o}C and maintained at this temperature throughout experimentation. Losses of fertilizer N to N_2O emission and denitrification were calculated based on the rate of total-N applied and linear integration of N_2O fluxes and denitrification rates, respectively, according to the trapezoidal rule.

In TC-1, 63 cores were randomly assigned to receive liquid lagoonal swine waste at loading rates of 0.62, 1.25, or 2.5 cm (41, 82, and 164 kg N ha^{-1}, respectively). At selected intervals after fertilization (6, 18, 30, 54, 120, 192, and 285 h), three cores per treatment were chosen at random for analysis. Nitrous oxide flux was determined in one core, while denitrification rate was measured in the other two by the C_2H_2 inhibition method. Immediately following the flux and rate measurements, all cores were dismantled into three fractions (0 to 5 cm, 5 to 10 cm, and 10 to 20 cm depth intervals) and each fraction was assayed to determine %WFPS and the concentrations of NH_4^+-N and NO_3^--N. Baseline measurements of N_2O flux, denitrification rate, and the physicochemical parameters listed above were made in triplicate prior to fertilization.

TC-2 followed the same experimental design with slight alterations: two fertilization rates were used to increase replication and the N_2O flux determinations were omitted. In TC-2, 48 cores were randomly assigned to receive liquid lagoonal swine waste at a rate of either 1.25 or 2.5 cm (71 and 142 kg N ha^{-1}, respectively). At selected intervals after fertilization (2, 26, 50, 74, 122.5, and 194 h), four cores per treatment were chosen at random for analysis. All cores were assayed to determine the rate of denitrification according to the C_2H_2 inhibition method and subsequently partitioned into three fractions to measure %WFPS and the concentrations of NH_4^+-N and NO_3^--N with respect to depth. Baseline measurements for all parameters were made in quadruplicate prior to fertilization.

Effect of Moisture Content under Aerobic Headspace

Samples of homogenized soil were subjected to a series of moisture treatments in order to investigate the effect of soil moisture content on net N_2O flux and the rate of denitrification in otherwise unamended soils. Approximately 30 g of field-moist soil (10.3% gravimetric moisture content) were added to each of thirty-six 4-oz. Mason jars. Each jar was randomly assigned to one of six different moisture treatments: 0, 1, 3, 5, 7, or 9 mL deionized H_2O, achieving soil moisture contents of 27, 37, 57, 77, 96, and 118% of maximum water-holding capacity, respectively. Treatments were made using volumetric pipets to dispense the water dropwise and ensure uniform application. Jars were sealed and preincubated at 25 $\pm$ 1^{o}C for 9 h prior to sampling. In preliminary experiments, this preincubation period was found to be sufficient for allowing the microbial population time to adjust to its new moisture regime while not depleting denitrification capacity. Although the preliminary experiments were not necessarily designed to corroborate past research, the findings were consistent with studies in the literature which have described the short-term response of denitrification and N_2O emission to changes in soil O_2 status (Freney et al. 1979; Sexstone et al. 1985a; Smith & Tiedje 1979b).

After preincubation, the jar lids were removed for 10 min in order to vent the headspace and restore the headspace N_2O concentration to ambient levels. Jars were resealed and the gas flux determinations were begun. For each moisture treatment, three jars were amended with C_2H_2 and three were left unamended for determination of net N_2O flux and denitrification rate, respectively. Jar headspaces were sampled at 1-h intervals over the next 4 h and analyzed for N_2O concentration. At the end of the experiment, a portion of the initial soil composite was extracted for analysis of NH_4^+-N and NO_3^--N concentrations.

Organic-C and NO_3^--N Limitations of DEA

The factors limiting denitrification enzyme activity were investigated by comparing responses to four substrate treatments: (1) water (i.e. the control); (2) organic-C; (3) NO_3^--N; and (4) organic-C + NO_3^--N. The methodology that follows represents a basic adaptation of the DEA assay described above in Section 4.3.5. Approximately 25 g of field-moist soil (14.9% gravimetric moisture content) were added to each of twenty-four 4-oz. Mason jars. Soils were saturated (1:1 water volume/soil wet weight) with one of four treatments: (1) deionized water; (2) 40 mg glucose-C L^{-1}; (3) 100 mg NO_3^--N L^{-1} as KNO_3; and (4) 40 mg glucose-C L^{-1} + 100 mg NO_3^--N L^{-1}, respectively. These concentrations of glucose and nitrate were considered uptake saturating. Jars were sealed and the contents rendered anaerobic through repeated evacuation, filling with N_2, and vigorous shaking. Acetylene was added to the anaerobic jars for determination of denitrification rate using the C_2H_2 inhibition method and headspace samples were collected at 20, 50, 80, and 110 min for analysis of N_2O concentration and calculation of DEA. Jars were maintained at 23 $\pm$ 1^{o}C throughout the experiment.

Effect of Organic-C and NO_3^--N Availability on DEA

The dose response of DEA to various concentrations of organic-C and NO_3^--N was investigated in greater detail using a completely crossed factorial design. There were four levels for each of the two factors. Organic-C was added as glucose at concentrations of 0, 40, 250, and 500 mg C L^{-1}, while NO_3^--N was added as KNO_3 at concentrations of 0, 25, 50, and 100 mg NO_3^--N L^{-1}. These levels were selected according to the following rationale: The highest level for organic-C was roughly equivalent to the level of TOC found in a typical sample of lagoon liquid (see Table 6.1), whereas the highest level of NO_3^--N concentration, when applied at a rate of 1:1 volume/soil mass, approximated the maximum NO_3^--N concentration measured in the soil profile after laboratory fertilization of cores with liquid lagoonal swine waste (see Results section). Thus, the levels chosen for each factor were intended to comprise the range of concentrations presumably encountered by denitrifiers in the field upon fertilization with liquid lagoonal waste.

In accordance with the factorial design, 16 solutions were prepared and applied in triplicate to 25 g samples of homogenized, field-moist soil (15.0% gravimetric moisture content). The rate of application was 1:1 volume/soil wet weight. Soils were maintained at 24 $\pm$ 1^{o}C and assayed for DEA as described above.

Temperature Dependence of DEA

The effect of temperature on DEA was investigated by incubating soil slurries in triplicate at eleven temperatures: 1, 7, 15, 20, 25, 30, 35, 42, 48, 54, and 60^{o}C. Again, the methodology deviated slightly from the DEA assay described in Section 4.3.5. First, jars were sampled (5 mL) five times during the incubation period and the headspace gas was replaced each time with an equal volume of a 1:9 mixture of C_2H_2 + N_2 to maintain atmospheric pressure. Subsequent calculations of headspace N_2O concentrations were corrected for this dilution. Second, soil slurries were incubated statically and gently shaken by hand for 5 to 10 s on each sampling occasion in order to assist diffusion of gases and nutrients during incubation.

Sampling frequencies were tailored to each temperature regime to ensure that the N_2O concentrations analyzed would be within the range of detection. For example, samples were collected at 20-, 15-, or 10-min intervals for temperatures in the ranges 1 to 15^{o}C, 20 to 42^{o}C, and 48 to 60^{o}C, respectively. Nitrous oxide accumulation was time-linear ($R^2 > 0.90$) and within detectable ranges during the period of analysis.

The temperature coefficient (Q_{10}) for DEA was calculated based on an exponential fit to rates of DEA over the range 7 to 35^{o}C, where rates increased monotonically with temperature (Whalen et al. 1990). A third-order polynomial function was fit to the mean DEA values over the entire temperature range using the General Linear Model procedure in SYSTAT 7.0 (SPSS Inc., Chicago, IL). The optimal temperature (T_{opt}) for DEA was calculated based on this polynomial function.

Statistical Analyses

Statistical analyses were performed using SYSTAT 7.0 (SPSS Inc., Chicago, IL). Data were log-transformed to homogenize variances when determined necessary according to Bartlett's test for heteroscadasticity (Zar 1984). Significant differences between means were determined using Student's t-test or parametric one-factor or two-factor analysis of variance (ANOVA) with equal replication. Post-hoc multiple comparison of means was performed using Bonferonni's test. All statistical analyses were performed at a significance level of $\alpha = 0.05$ unless otherwise noted.

6. RESULTS - DENITRIFICATION EXPERIMENTS

Time Course Fertilization Experiments

Fertilizer Composition. The total-N content of liquid waste samples collected during this study ranged from 470 to 630 mg L^{-1} (Table 6.1). Variability among dates likely reflects temporal differences in the interacting influences of hydraulic residence time, evaporation, rainfall, and age profile of the resident swine population. Anaerobic decomposition was highly efficient as NH_4^+-N comprised 82 to 86% of the total-N and the total organic-C:total-N ratio was near unity. A single pH determination showed that the lagoon was slightly basic.

Table 6.1 Chemical characteristics of anaerobic lagoon liquid samples.

Parameter	Mean ± Standard Deviation		
	May 8, 1998	June 6, 1998	October 1, 1998
pH	n/d[a]	n/d	7.86 ± 0.01
NH_4^+-N (mg L^{-1})	540 ± 80	450 ± 110	n/d
Total-N (mg L^{-1})	630 ± 60	550 ± 20	470 ± 5
TOC (mg L^{-1})	580	550	550
NH_4^+-N:Total-N	0.86	0.82	n/d
TOC:Total-N	0.92	1.01	1.17

[a] n/d = not determined.

Fertilization Experiment 1. Nitrous oxide emission during TC-1 increased markedly within 0.25 d following fertilization at all loading rates except the highest (2.5 cm), in which fluxes were initially depressed relative to pre-fertilization levels (Figure 6.1a). Nitrous oxide fluxes peaked at 1700 to 2280 μg N m^{-2} h^{-1} within 0.75 to 5 d following fertilization. Qualitatively, maximum hourly fluxes occurred increasingly later relative to time of waste application as the loading rate was increased. Elevated fluxes were maintained at or near maximum levels for 2 to

4.25 d before returning to pre-fertilization values. During this period, N_2O fluxes averaged a factor of 6 $\pm$ 4 greater than pre-fertilization values.

Transient post-fertilization increases in N_2O emission were accompanied by similar, immediate increases in rates of denitrification, which persisted for up to 5 d (Figure 6.1b). In general accord with N_2O emissions, maximum rates of denitrification (1730 to 7560 μg N m^{-2} h^{-1}) increased with increasing loading rate. However, in contrast to the N_2O fluxes, denitrification rates did not peak later relative to time of waste application as loading rate increased. The highest single rate of denitrification (11,120 μg N m^{-2} h^{-1}) was observed 2.25 d following amendment with 2.5 cm of waste and exceeded the average pre-fertilization rate by a factor of 28. Elevated post-fertilization denitrification rates averaged 7 $\pm$ 3 times higher than pre-fertilization values, in good agreement with the time course data for N_2O emission.

Soil moisture content (%WFPS) was assessed from 1.25 d after fertilization until the termination of the experiment at 12 d (Figures 6.2a-c). Three trends are apparent. First, higher loading rates corresponded to higher %WFPS. For instance, mean %WFPS in the 0 to 5 cm depth interval for the measurement period was 47, 53 and 66% for the 0.62, 1.25, and 2.5 cm applications, respectively. Second, %WFPS decreased with increasing depth below the soil surface. Third, %WFPS generally decreased at each depth interval as the experiment progressed. For example, decreases of 8 to 26% were observed for the 0 to 5 cm depth interval. This latter trend is not readily apparent due to the influence of high submeter-scale spatial variability in soil organic content on %WFPS.

Time courses for changes in soil inorganic-N concentrations following fertilization varied with depth and loading rate (Figures 6.3a-c). Immediately after fertilization (<0.25 d), soil NH_4^+-N concentrations increased over pre-fertilization levels (2 to 4 μg N g_{dw} $soil^{-1}$) for all depth intervals and rates of fertilization. The increase was directly proportional to fertilizer loading rate in the 0 to 5 cm zone, where soil NH_4^+-N concentrations increased 6-, 13- and 26-fold in response to waste amendments of 0.62, 1.25, and 2.5 cm, respectively. Resultant NH_4^+-N concentrations in the 0 to 5 cm zone were 25 to 100 μg N g_{dw} $soil^{-1}$. Soil NO_3^--N concentrations also increased relative to pre-fertilization values (0.7 to 1 μg N g_{dw} $soil^{-1}$) immediately following fertilizer application at all depths and loading rates, but to a lesser extent. For instance, soil NO_3^--N concentrations in the 0 to 5 cm horizon at 0.25 d following fertilization increased by factors of 4, 8, and 2 relative to pre-fertilization values for the 0.62, 1.25, and 2.5 cm applications, respectively. The initial post-fertilization increase in soil NH_4^+-N concentrations was followed by a rapid decline in concentrations to day 5, when concentrations returned to near pre-fertilization levels. In contrast, soil NO_3^--N generally continued to increase to day 12, when concentrations in the 0 to 5 cm zone reached 58, 68, and 68 μg N g_{dw} $soil^{-1}$ for the 0.62, 1.25, and 2.5 cm applications, respectively. The maximum average NO_3^--N concentration of 96 μg N g_{dw} $soil^{-1}$ in this depth interval was observed 8 d after the 2.5 cm application. Nitrate concentrations consistently decreased with depth, but were clearly elevated relative to pre-fertilization values by the end of the experiment. Nitrate concentrations were 5, 11, and 12 μg N g_{dw} $soil^{-1}$ in the 10 to 20 cm depth interval at 12 d for cores amended with 0.62, 1.25, and 2.5 cm of waste, respectively.

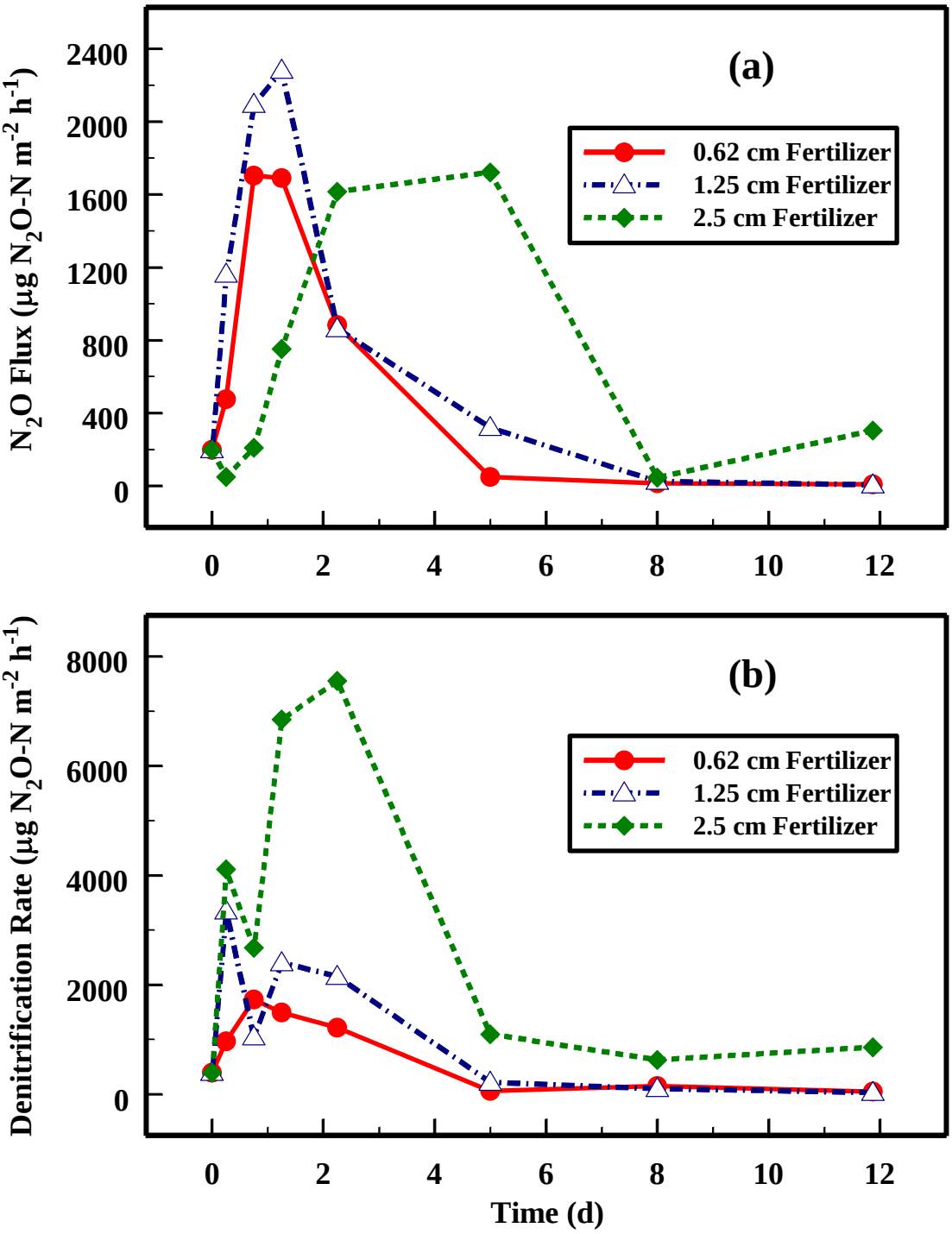

Figure 6.1 Time course for (a) net N_2O-N flux and (b) denitrification rate following fertilization with liquid lagoonal waste at three different application rates in TC-1. Application rates of 0.62, 1.25, and 2.5 cm of fertilizer correspond to loading rates of 41, 82, and 164 kg N ha^{-1}, respectively.

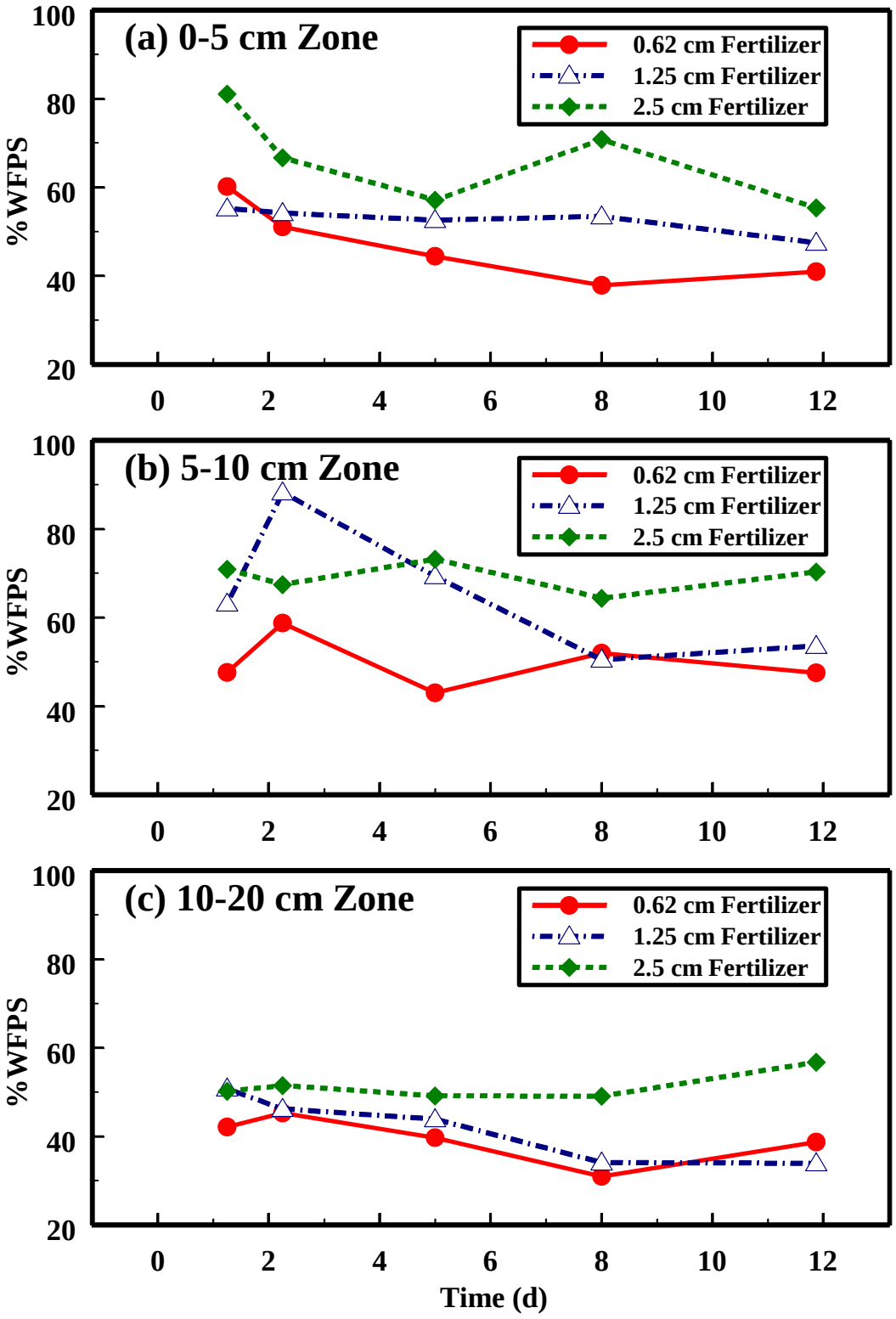

Figure 6.2 Percent water-filled pore space (%WFPS) in the (a) 0 to 5 cm, (b) 5 to 10 cm, and (c) 10 to 20 cm zones following waste application in TC-1.

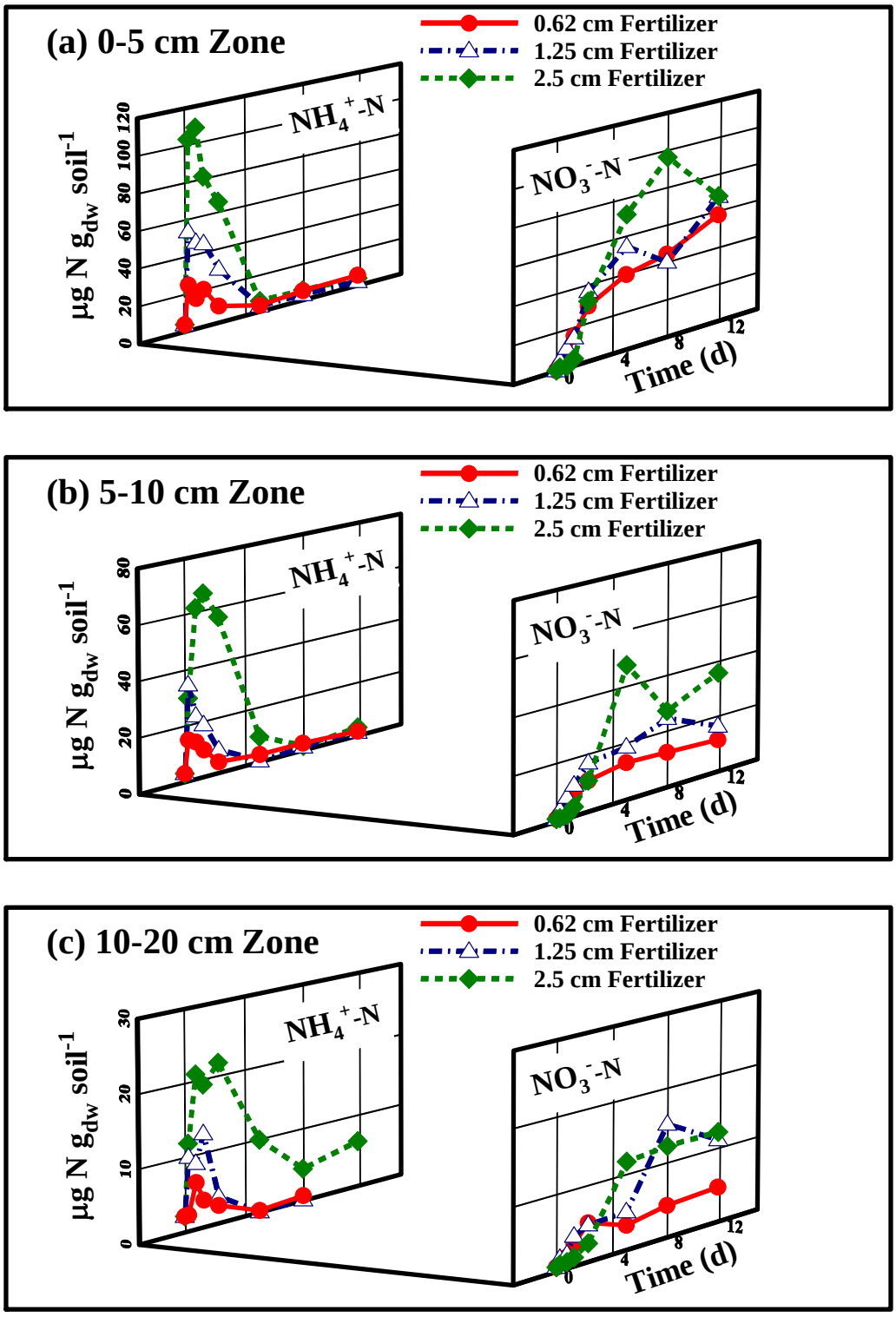

Figure 6.3 Soil inorganic-N concentrations in the (a) 0 to 5 cm, (b) 5 to 10 cm, and (c) 10 to 20 cm zones following waste application in TC-1.

Fertilization Experiment 2. The results of TC-1 were generally corroborated in TC-2. Rates of denitrification in TC-2 experienced immediate (<0.08 d) increases upon fertilizer application and remained elevated for up to 3 d (Figure 6.4). During this period, denitrification rates generally increased with increasing loading rates. Maximum rates of denitrification were 1480 and 4280 $\mu g\ N\ m^{-2}\ h^{-1}$ for the 1.25 and 2.5 cm waste applications, respectively. These values represented 3- and 8-fold increases, respectively, over pre-fertilization levels (550 $\mu g\ N\ m^{-2}\ h^{-1}$). Elevated post-fertilization denitrification rates averaged 3 $\pm$ 2 times higher than pre-fertilization values. Replicate denitrification rate measurements (n = 4) were highly variable in TC-2, with coefficients of variation ranging from 39 to 130%.

Percent WFPS prior to fertilization averaged 38, 46, and 47% in the 0 to 5 cm, 5 to 10 cm, and 10 to 20 cm zones, respectively (Figures 6.5a-c). As expected, application of liquid lagoonal swine waste immediately increased %WFPS throughout the soil profile. For example, at 0.08 d after fertilization, %WFPS in the 0 to 5 cm zone averaged 51 and 63% for the 1.25 and 2.5 cm applications, respectively. As the experiment progressed, %WFPS steadily decreased throughout the soil profile. Nevertheless, %WFPS at the termination of the experiment (day 8) remained above pre-fertilization values at all depth intervals except 10 to 20 cm, where %WFPS had returned to pre-fertilization levels.

Soil inorganic-N concentrations during TC-2 displayed many of the same trends observed in TC-1 with respect to depth and loading rate (Figures 6.6a-c). Soil NH_4^+-N concentrations experienced immediate (<0.08 d) increases over pre-fertilization levels (0.4 to 5 $\mu g\ N\ g_{dw}\ soil^{-1}$) for all depth intervals and rates of fertilization. For instance, at 0.08 d after fertilization, NH_4^+-N concentrations in the 0 to 5 cm zone reached 90 and 100 $\mu g\ N\ g_{dw}\ soil^{-1}$ with waste amendments of 1.25 and 2.5 cm, respectively. This initial post-fertilization increase in NH_4^+-N concentrations was followed by a steady decline in concentrations until the end of the experiment at day 8, when soil NH_4^+-N concentrations returned to near pre-fertilization levels. Soil NO_3^--N concentrations also increased relative to pre-fertilization values (3 to 7 $\mu g\ N\ g_{dw}\ soil^{-1}$) immediately following waste amendment, but again the initial changes were much less pronounced for NO_3^--N than NH_4^+-N. Soil NO_3^--N gradually increased to day 8, when concentrations in the 0 to 5 cm zone measured 55 and 83 $\mu g\ N\ g_{dw}\ soil^{-1}$ for the 1.25 and 2.5 cm applications, respectively. As in TC-1, nitrate concentrations in TC-2 decreased with depth, but were still higher than pre-fertilization values at the end of the experiment. For example, soil NO_3^--N concentrations had reached 13 and 21 $\mu g\ N\ g_{dw}\ soil^{-1}$ in the 10 to 20 cm horizon by day 8 in cores amended with 1.25 and 2.5 cm of waste, respectively.

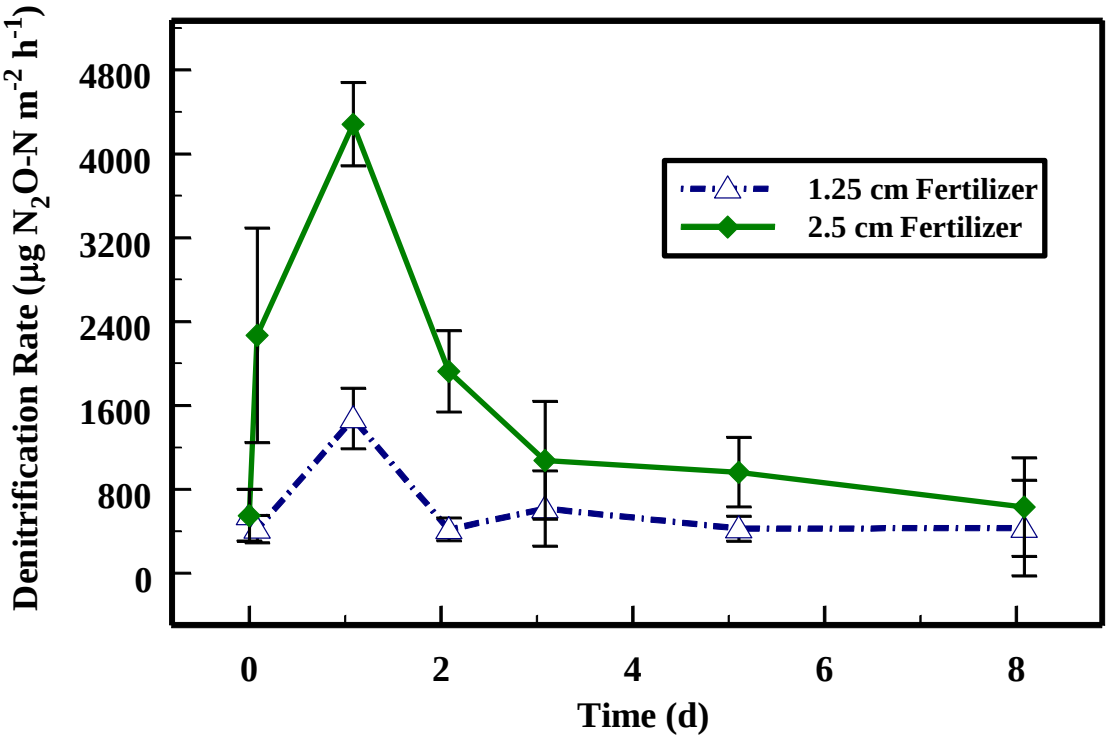

Figure 6.4 Time course for mean denitrification rate following fertilization with liquid lagoonal waste at two different application rates. Application rates of 1.25 and 2.5 cm of fertilizer correspond to loading rates of 71 and 142 kg N ha^{-1}, respectively.

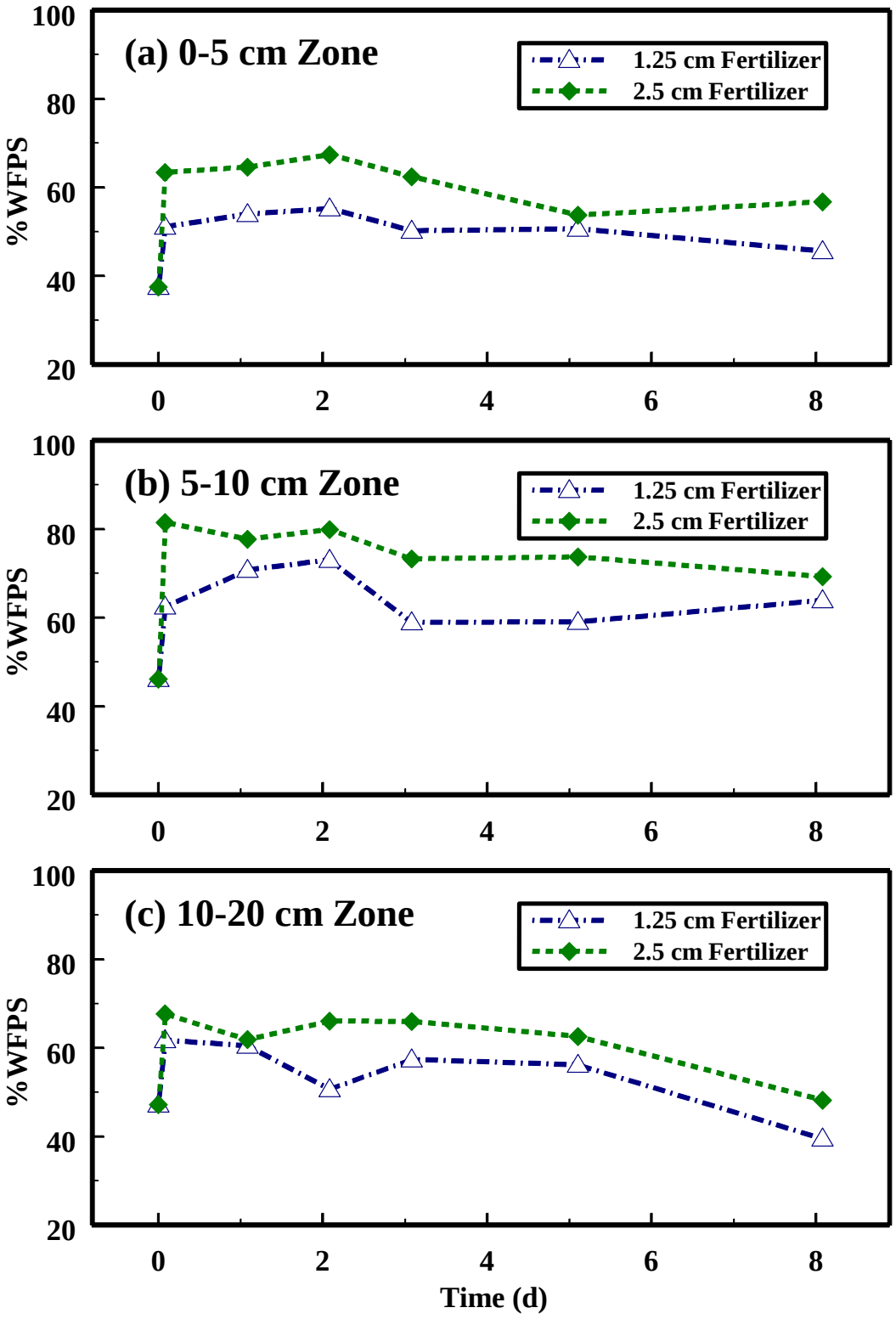

Figure 6.5 Percent water-filled pore space (%WFPS) in the (a) 0 to 5 cm, (b) 5 to 10 cm, and (c) 10 to 20 cm zones following waste application in TC-2.

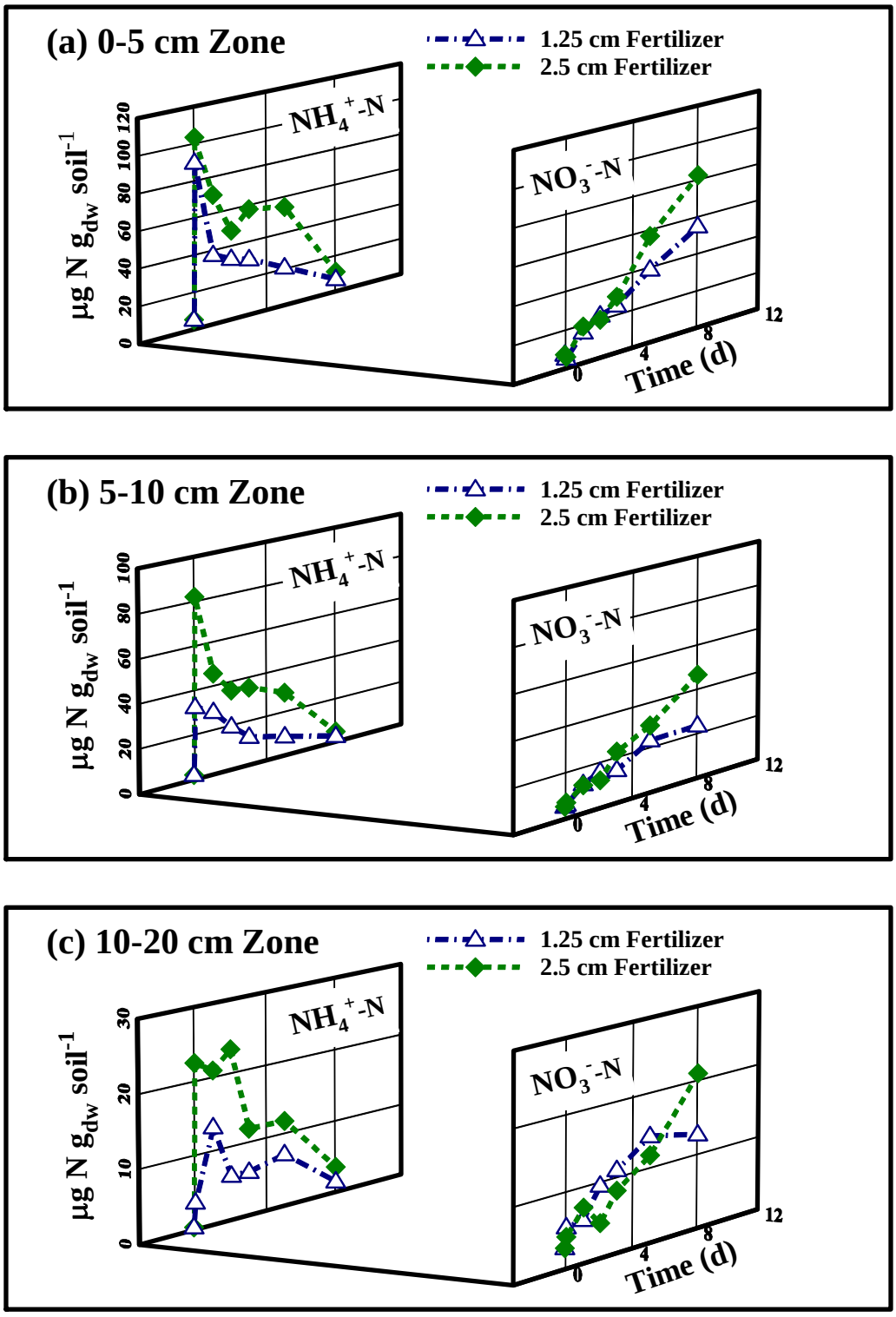

Figure 6.6 Soil inorganic-N concentrations in the (a) 0 to 5 cm, (b) 5 to 10 cm, and (c) 10 to 20 cm zones following waste application in TC-2.

Gaseous Losses of Applied N in TC-1 and TC-2. Post-fertilization emissions of N_2O (potentially from both nitrification and denitrification) resulted in time-integrated losses of 1.4 to 2.5% of the applied fertilizer N in TC-1 (Table 6.2). The *in situ* flux of N_2O was not determined in TC-2. Denitrification losses (comprising both N_2O-N and N_2-N) totalled 2.6 to 4.5% and 1.8 to 2.1% of the applied fertilizer N in TC-1 and TC-2, respectively (Table 6.2). No statistically significant correlations were observed between fertilizer loading rate and the relative proportion of gaseous N losses via either N_2O emission or denitrification.

Table 6.2 Time-integrated gaseous losses of applied N to denitrification and N_2O emission. Losses were integrated over 11.875 d and 8.083 d for TC-1 and TC-2, respectively.

Experiment	Fertilizer Loading Rate (cm)	% N Loss	
		Via Emission of N_2O-N	Via Denitrification
TC-1	0.62	2.5	3.2
	1.25	1.7	2.6
	2.5	1.4	4.5
TC-2	1.25	n/d[a]	1.8
	2.5	n/d	2.1

[a] n/d = not determined.

Effect of Soil Moisture Content on N_2O Emissions and Denitrification Rate

Both N_2O emissions and rates of denitrification exhibited a binary response to gravimetric moisture content, expressed in terms of %WHC (Figures 6.7a-b). That is, at high (i.e. near saturation) moisture contents, changes in %WHC strongly affected N_2O emissions and denitrification rates, whereas at low to intermediate moisture levels, these gaseous N fluxes were independent of %WHC. For instance, N_2O fluxes at 96% WHC were 5-fold higher than the pooled average of fluxes at all lower moisture contents and 33-fold higher above saturation. Similarly, the mean denitrification rate above saturation was 39-fold higher than the pooled average of rates below saturation. However, no moisture response was apparent for N_2O fluxes and denitrification rates below 77 and 96% WHC, respectively.

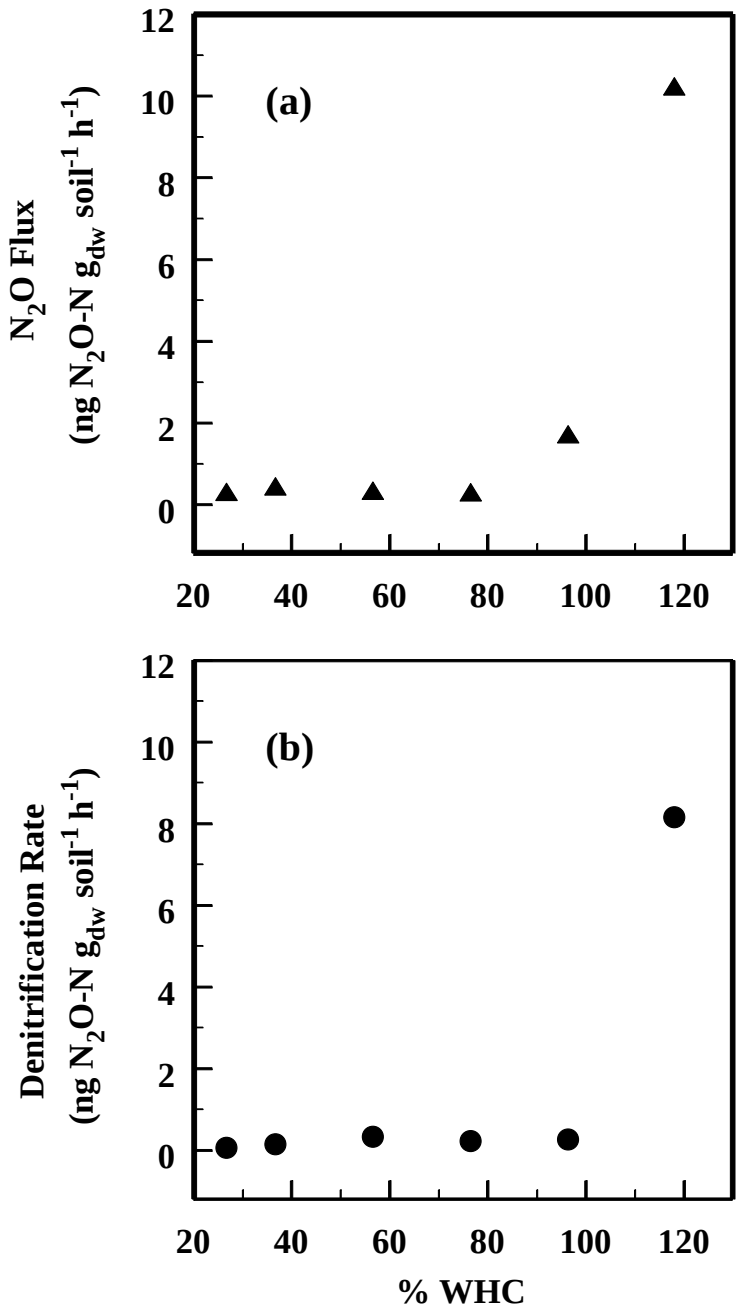

Figure 6.7 (a) Net N_2O-N flux and (b) denitrification rate as functions of percent maximum water-holding capacity (%WHC) under an aerobic headspace.

Effects of Added Organic Carbon and Nitrate on DEA

Treatments with glucose-C and NO_3^--N at levels generally considered uptake-saturating (40 mg C L^{-1} and 100 mg N L^{-1}, respectively) showed that these denitrification substrates stimulated DEA when added individually or in combination (Figure 6.8). Denitrification enzyme activity with no added substrate was 1.2 ng N_20-N g_{dw} $soil^{-1}$ h^{-1}, but increased 5- and 6-fold with individual treatments of glucose-C and NO_3^--N, respectively, and 16-fold when the treatments were combined. The increase from the combined treatment exceeded the additive effects of the individual treatments.

Factorial treatments of glucose-C and NO_3^--N confirmed the individual and combined effects of these two denitrification substrates on DEA and elucidated the substrate response over a wide range of concentrations (Table 6.3). With no added substrate (water only), DEA was 1.3 ng N_20-N g_{dw} $soil^{-1}$ h^{-1}. Following single substrate treatments, fluxes ranged from 12 to 14 ng N_20-N g_{dw} $soil^{-1}$ h^{-1} with NO_3^--N (25, 50, and 100 mg N L^{-1}) and 16 to 25 ng N_20-N g_{dw} $soil^{-1}$ h^{-1} with glucose-C (40, 250, and 500 mg C L^{-1}). However, combined treatments resulted in fluxes that ranged from 39 to 59 ng N_20-N g_{dw} $soil^{-1}$ h^{-1}. Multiple comparisons of treatment means revealed three notable features of the DEA response to substrate concentrations. First, fluxes were invariably higher with individual treatments of either glucose-C or NO_3^--N than without any treatment. Second, with the exception of one value (39 ng N_20-N g_{dw} $soil^{-1}$ h^{-1}), fluxes for all combined treatments were consistently higher than for individual glucose-C treatments, but not necessarily higher than those for individual NO_3^--N treatments. Third, there were no characteristic differences among the combined treatments at the concentrations investigated.

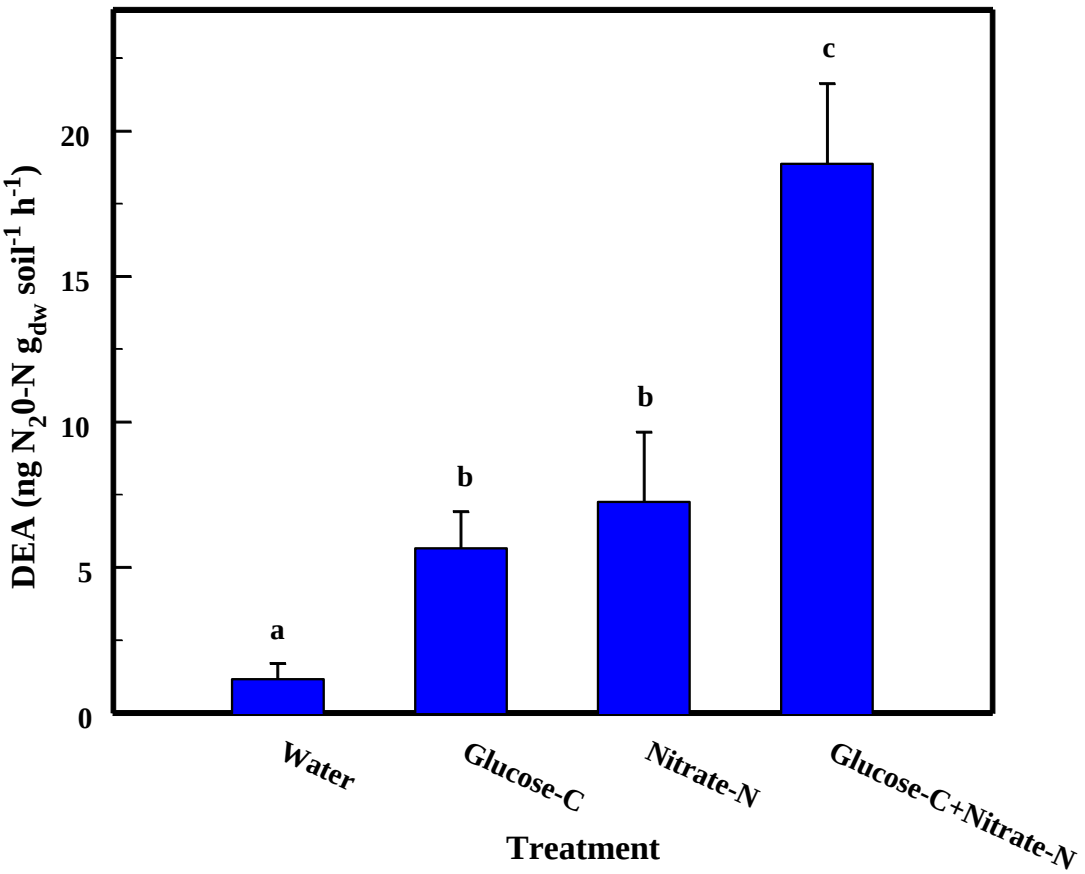

Figure 6.8 Effect of substrate treatment on denitrification enzyme activity (DEA). The concentrations of glucose and NO_3^- used were 40 mg C L^{-1} and 100 mg N L^{-1}, respectively. Treatments accompanied by the same letter do not differ significantly at $P<0.01$.

Table 6.3 Mean denitrification enzyme activity (DEA) for factorial glucose-C and nitrate-N treatments. Values followed by the same letter do not differ significantly at $P<0.05$.

Treatment		Mean DEA[†]		
Glucose-C ($mg\ C\ L^{-1}$)	NO_3^--N ($mg\ N\ L^{-1}$)	($ng\ N_2O\text{-}N\ g_{dw}\ soil^{-1}\ h^{-1}$)		
0	0	1	(1)	a
0	25	16	(7)	bcd
0	50	25	(10)	bcdef
0	100	21	(9)	bcde
40	0	13	(5)	b
40	25	40	(12)	cdef
40	50	62	(5)	f
40	100	59	(23)	ef
250	0	14	(0.3)	bc
250	25	46	(0.7)	ef
250	50	47	(4)	ef
250	100	52	(2)	ef
500	0	12	(4)	b
500	25	39	(10)	cdef
500	50	42	(7)	def
500	100	49	(7)	ef

[†] Numbers in parentheses denote one standard deviation (n = 3).

Temperature Dependence of DEA

Denitrification enzyme activity varied greatly over the range of temperatures investigated (Figure 6.9). This temperature dependence was best described by a third-order polynomial (R^2 = 0.94). Based on this function, the optimal temperature for DEA was 50°C. An exponential model was fitted (R^2 = 0.96) to the data over the range where DEA increased monotonically with temperature (7 to 35°C). Using this model, an average Q_{10} of 1.9 was calculated for DEA over this temperature range. Interestingly, DEA was still appreciable at near freezing temperatures. In fact, a rate of 28 ± 4 $ng\ N_2O\text{-}N\ g_{dw}\ soil^{-1}\ h^{-1}$ was observed at 1°C.

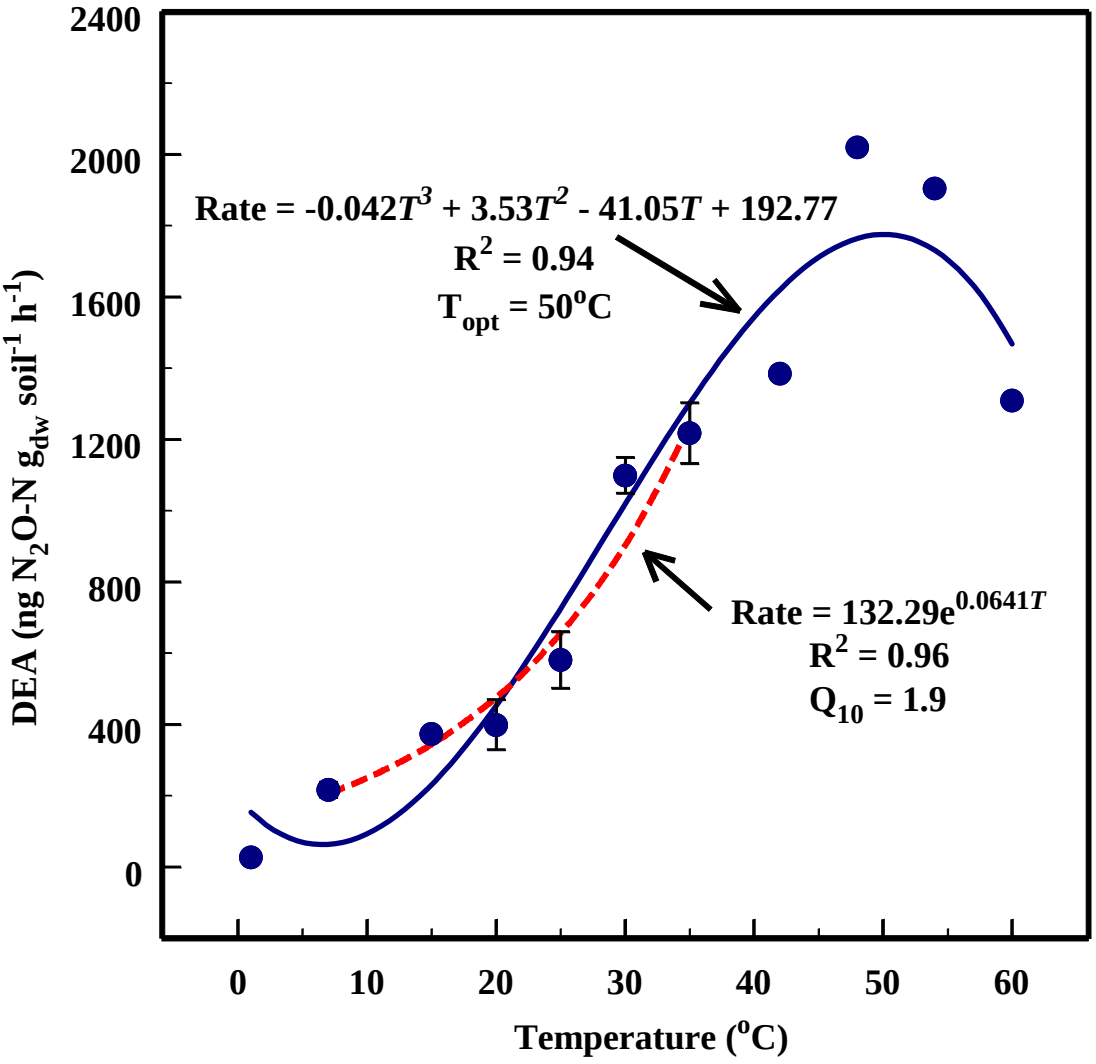

Figure 6.9 Temperature dependence of denitrification enzyme activity (DEA). Q_{10} is the temperature coefficient representing the factor by which DEA increases by raising the temperature 10°C. T_{opt} is the optimal temperature as determined from the best-fit polynomial curve.

7. DISCUSSION - DENITRIFICATION EXPERIMENTS

Fertilizer Composition

The concentrations of total-N and NH_4^+-N in lagoon liquid samples collected in the present study (Table 6.1) are consistent with data reported by other researchers (Barker et al. 1998; Barker & Zublena 1995; Westerman et al. 1985b), which show concentrations ranging from 530 to 600 mg total-N L^{-1} and 430 to 490 mg NH_4^+-N L^{-1}. Ammonium comprised 82 to 86% of the total-N in swine waste in this study, which is consistent with the 76 to 86% reported for liquid lagoonal waste elsewhere (Barker et al. 1998; Barker & Zublena 1995; Safley et al. 1992; Westerman et al. 1985b). However, the relative percentage of NH_4^+-N in liquid waste is less than the values of 52 to 58%, 28 to 53%, and 61 to 64% reported for fresh, scraped lot, and slurried swine manure, respectively (Barker & Zublena 1995; Westerman et al. 1985b; Zublena et al. 1995). Microbial decomposition in anaerobic lagoons is clearly more efficient at mineralizing organic-N to NH_4^+-N than in other animal waste storage systems. Consequently, most of the N in field-applied liquid swine waste is in a form readily available for plant assimilation, microbial immobilization, and nitrification.

Ratios of TOC:total-N for the lagoon samples in this study remained close to unity (Table 6.1), in agreement with ratios of about 1.8:1 reported for soluble organic-C:total-N in liquid swine waste from a four lagoon system in Georgia (Sharpe & Harper 1997). These values are 10-fold lower than the ratios of 11:1 to 15:1 reported for fresh swine manure (Kirchmann 1994; Reddy et al. 1980). The difference may result from a combination of chemical and physical processes that take place during waste storage in lagoons. For instance, under anaerobic conditions, complex C compounds in animal waste are rapidly decomposed into short-chain volatile fatty acids and later into CO_2 or reduced to CH_4 by methanogenic bacteria (Guenzi & Beard 1981). Accordingly, the reduced TOC:total-N ratio in stored liquid waste relative to fresh waste may stem from a longer residence time of waste in the lagoon and volatilization of C species. In a 42-d redox-controlled experiment by Guenzi & Beard (1981), 42% of the total-C in a manure slurry was evolved as CO_2. Additionally, C-rich particulate matter settles during lagoon storage, leaving the more readily decomposed soluble fraction in the liquid phase.

Time Course for N_2O Emission in Fertilized Cores

Fertilization of intact cores with liquid lagoonal swine waste in TC-1 brought about a rapid (<0.25 d) increase in N_2O emissions at all fertilizer loading rates except the highest, where fluxes did not increase until 0.75 d (Figure 6.1a). Emissions at the low and medium loading rates remained elevated for 2 d before returning to background levels, compared to 4.25 d at the high loading rate. This pattern of immediate, but short-lived increase in N_2O efflux is consistent with results from the two published studies of N_2O emission in soils receiving regular applications of liquid lagoonal swine waste (Sharpe & Harper 1997; Whalen et al. 2000). In these studies, large effluxes of N_2O occurred within 0.5 d of sprinkler irrigation and persisted for approximately 2 d before returning to background levels. That enhanced emissions at the high loading rate in the present study occurred slightly later and lasted roughly twice as long as observed in other cases involving liquid swine waste is likely due to diffusional constraints on surface fluxes imposed by a comparatively higher soil moisture content (Davidson 1991).

Nitrous oxide production in soils may result from both nitrification and denitrification, with each process contributing variably under different conditions (Davidson et al. 1986; Kester et al. 1997; Klemedtsson et al. 1988; Rudaz et al. 1991; Webster & Hopkins 1996). Whereas N_2O is a by-product of nitrification, it is either an intermediate or end-product of the denitrification pathway (Firestone & Davidson 1989). Fertilization with liquid lagoonal swine waste simultaneously satisfies many of the prerequisites for both nitrification and denitrification and can thus be expected to produce an immediate increase in soil N_2O emissions. For example, liquid swine waste provides an immediate source of NH_4^+-N for nitrifiers. Rapid nitrification makes NO_3^--N readily available and can lead to a close coupling of nitrification and denitrification (Nielsen & Revsbech 1994; Petersen et al. 1991). Liquid swine waste is also rich in labile C (Sommer et al. 1996) that can be utilized directly by denitrifiers (Paul et al. 1989) or decomposed by other heterotrophs, creating local zones of anaerobiosis and indirectly promoting denitrification (Parkin 1987; Petersen et al. 1996). Finally, addition of swine waste as a liquid increases WFPS, optimizing the conditions for denitrification, and provides an effective medium for the overall diffusion of microbial substrates and products.

Maximum post-fertilization N_2O-N fluxes for the intact cores investigated here ranged from 1700 to 2280 $\mu g\ N\ m^{-2}\ h^{-1}$ (Figure 6.1a). Such values fall well within the range of 1040 to 2500 $\mu g\ N\ m^{-2}\ h^{-1}$ observed for post-fertilization maxima in the field study by Sharpe & Harper (1997), but are significantly lower than the average post-fertilization flux of 3900 $\mu g\ N\ m^{-2}\ h^{-1}$ reported by Whalen et al. (2000). On the whole, peak fertilizer-induced N_2O fluxes for liquid swine waste tend to exceed peak values reported for solid and slurried animal wastes. Maximum values for N_2O-N emission in soils receiving solid manures or slurries typically range from 170 to 2400 $\mu g\ N\ m^{-2}\ h^{-1}$ (Cates & Keeney 1987; Christensen 1983; Clayton et al. 1997; Goodroad et al. 1984; Lessard et al. 1996). Unlike liquid swine waste, these fertilizer types do not necessarily optimize conditions for N_2O production immediately upon application. For instance, solid manures and slurries contain a lower percentage of total-N as NH_4^+-N, less readily-decomposable organic-C, and a reduced moisture content. Thus, N_2O emissions from solids and slurries often depend additionally on rates of mineralization and rainfall.

Time Course for Denitrification and Relationships with Soil Physicochemical Variables in Fertilized Cores

In a pattern similar to N_2O emissions, post-fertilization rates of denitrification were also immediate and short-lived (Figures 6.1b and 6.4). For intact cores in both TC-1 and TC-2, elevated denitrification rates were observed within 1 d of fertilization, and on some occasions increases occurred as soon as 0.08 d after fertilization. Such rapid increases in denitrification rates following waste application indicate the presence of a viable population of denitrifiers, rapid conversion of added NH_4^+-N to NO_3^--N by nitrifiers, and a close association between nitrifier and denitrifier populations in this agricultural soil. With a 4-year history of lagoon effluent application at this site, development of a microbial population adapted to processing inorganic-N is not surprising. In a previously fallow field, Christensen (1985) observed notable increases in the most-probable number count of denitrifying bacteria within 1 month of applying an ammonium-rich cattle slurry and also isolated a diverse array of N_2-, N_2O-, and NO_2^--producing species that were not present beforehand.

Given established nitrifier and denitrifier populations in the spray field soil, denitrification rates remain steady and unpronounced at background levels until microaerophilic conditions (such as high %WFPS) are brought about and denitrification substrates (organic-C and NO_3^--N) become available. This convergence of environmental factors is achieved rapidly with the application of liquid swine waste (as it immediately penetrates the soil surface to occupy soil pore space and supplies labile C compounds and NH_4^+-N at the microsite) and the activity of closely coupled nitrification (which converts the newly available NH_4^+-N to NO_3^--N). As evident from the dramatic post-fertilization increases at all loading rates, the results are highly favorable for denitrification. However, these results are short-lived (lasting only 3 to 5 d) and suggest that certain factors become limiting in the near-term. Among the possible candidates, the most probable is soil moisture content. Although the initial effects of liquid fertilizer application on %WFPS are dramatic, soil moisture content at most loading rates and depths gradually decreases over time (Figures 6.5a-c). In fact, based on the complete data set for %WFPS in TC-2, average soil moisture content levels remained very close to or below the critical value of 60% WFPS for the experiment as a whole. Under such conditions, soil moisture contents can quickly become sub-optimal or fully limiting to denitrification throughout much of the soil profile.

Additional limitation in the near-term may also be due to a deficit of labile organic-C. Recognizing that the overall denitrification process (i.e. reduction of NO_3^- to N_2) requires a stoichiometric C:N ratio of 1.25:1, the TOC:total N ratios near unity observed here for lagoon liquid samples raise concern that the supply of C substrate relative to N should become rate-limiting over time if no other convenient sources of organic-C are made available. Moreover, it is not known precisely what percentage of the TOC in liquid swine waste is readily-available but it is unlikely to be 100%. Finally, denitrifiers must compete with other heterotrophs for labile-C, and although competition is certainly minimal under microaerophilic conditions, the heterogenous nature of the soil environment, the fact that all of the common soil denitrifying species are facultative anaerobes, and the thermodynamic advantages of aerobic over anaerobic respiration help ensure that aerobic processes reserve a high priority for labile-C sources.

Previous research at the same site rejected the idea of a possible C limitation, concluding instead that denitrification in this soil was limited primarily by soil moisture content and the availability of N (Whalen 2000). However, C availability has been found to limit denitrification in other manure-amended soils (Loro et al. 1997; Reddy et al. 1980), including a similar Coastal Plain soil in Georgia (Lowrance et al. 1998; Lowrance & Smittle 1988). Based on the results of this study, a deficit of labile-C remains a possible explanation for denitrification limitations. Perhaps the best way to resolve the issue would be to monitor the rate of soil respiration (i.e. CO_2 evolution) as well as denitrification following waste application in order to determine if the former process, a rough index of readily metabolizable C (Burford & Bremner 1975), also experiences a transient pulse of activity. Also, it would be useful to investigate the effects of a simulated rainfall event after NO_3^--N concentrations have peaked to observe the extent of denitrification in the absence of additional C amendments. Nevertheless, the evidence presented in this study indicates that the primary factor limiting denitrification rates in this waste-amended soil is soil moisture content.

Although denitrification rates in TC-1 and TC-2 returned to pre-fertilization levels after a short pulse of activity, nitrification continued seemingly unabated. As a result, NO_3^--N quickly accumulated in the soil profile (Figures 6.3a-c & 6.6a-c). In fact, by the end of both time courses, soil NO_3^--N concentrations reached potentially hazardous levels throughout the 0 to 20 cm zone. Certainly, this outcome cannot be extrapolated entirely to field situations. In reality, NH_3 volatilization, although undesirable, is sure to be much more prominent and could constitute losses of up to 69% of the applied NH_4^+-N (Sharpe & Harper 1997). Plant uptake of N, passed over here by the exclusion of plants from all intact cores, should also moderate soil N concentrations, while respiration in the rhizosphere potentially creates its own local zone of anaerobiosis favorable to denitrification (Knowles 1981; Smith & Tiedje 1979a). Nonetheless, the results of several past studies involving liquid swine waste amendments at representative loading rates have revealed a high potential for NO_3^--N accumulation and export via groundwater or surface runoff (Evans et al. 1984; King et al. 1990; King et al. 1985; Westerman et al. 1996; Westerman et al. 1985a). These results are better explained now with evidence of the active nitrification of applied NH_4^+-N and the highly periodic nature of denitrification.

Denitrification rates in soil cores were highly variable. In TC-2, coefficients of variation for denitrification rates in four replicate cores ranged from 39 to 130%. High coefficients of variation are characteristic for denitrification measurements in soil cores and very often exceed 100% (Parkin & Robinson 1989). Very little of this variability is directly due to the dynamics of denitrifier populations, since denitrification enzyme activity and counts of denitrifying bacteria are typically uniform over the landscape in agricultural soils (Parkin et al. 1987; Parsons et al. 1991). Rather, most variability is attributable to microscale variations in the distribution of O_2 (Sexstone et al. 1985b), organic-C (Parkin 1987; Seech & Beauchamp 1988), and occasionally NO_3^--N (Myrold & Tiedje 1985) that are brought about by the natural heterogeneity of soil aggregate structure. Having preserved soil structure through the use of intact cores, and considering the addition of a N-rich fertilizer and the high concentrations of NO_3^--N remaining in the bulk soil at the end of the experiment, variations in the former two physicochemical factors are the most probable sources of the denitrification variability observed here. Denitrification rate variability is typically described by a log-normal distribution (Parkin & Robinson 1994). However, given the low level of replication in this study, log-transformation of the data was not practical.

Nonetheless, the large differences in denitrification rates observed here during periods of elevated denitrification allowed for useful comparisons. For instance, it was easily discernible that maximum rates of denitrification correlated positively with fertilizer loading rates in TC-1 and TC-2. Paul et al. (1993) observed identical trends for soils receiving different rates of liquid and solid dairy cattle manures, but adjusted to the same moisture content. Such results appear to be the combined effect of varying loads of C and N. Although the effects of these substrates certainly contribute to the denitrification rates in this study, varying soil moisture contents is probably the key factor in determining maximum rates and the fertilizer dose response noted here.

Although the microbiological source of N_2O was not determined directly in this study, denitrification appeared to be a major producer of N_2O following waste application. This is supported by two reasonable lines of evidence. First, N_2O emission tracked denitrification rates

extremely well during TC-1 (Figures 6.1a and 6.1b) despite continued production of NO_3^--N by nitrification throughout the experimental period. In addition, although magnitudes of N_2O emission and denitrification (N_2O+ N_2) flux during peak intervals differed roughly by a factor of 3, the average ratio of peak to pre-fertilization fluxes were very similar in both cases: 6 ± 4 and 7 ± 3 for N_2O emission and denitrification flux, respectively. Second, both N_2O flux and denitrification rate responded positively to increasing fertilizer loading rates, presumably due to the effects of increasing soil moisture content rather than the total load of organic-C or NH_4^+-N supplied by the fertilizer source. Increased %WFPS clearly promotes anaerobic conditions, which are required for denitrification yet inhibiting to nitrification. However, as WFPS increases over 80%, in theory the proportion of denitrification end-products represented by N_2O (i.e. the mole fraction of N_2O) declines until denitrification itself becomes a negligible source of N_2O (Davidson 1991). In practice, denitrification frequently contributes significant fluxes of N_2O even as saturating conditions are approached (Klemedtsson et al. 1988; Webster & Hopkins 1996). At the medium to high levels of WFPS generally observed here (roughly 60 to 80%), moisture/aeration conditions after waste application can be considered optimal for the N_2O mole fraction for denitrification.

Gaseous Losses of Applied N

Time-integrated losses of N_2O-N following fertilization in TC-1, which ranged from 1.4 to 2.5% of the applied N (Table 6.2), compare very well with fractional N_2O-N losses reported in two previous studies involving amendments of liquid swine waste at the same study site, where losses ranged from 0.5 to 2.3% (Whalen 2000; Whalen et al. 2000). This general agreement is interesting to note even though the two previous studies involved field measurements using static chambers as opposed to intact cores and suggests that the two techniques give consistent estimates of the relative magnitude of N_2O-N losses. However, on the whole, the contribution of N_2O-N emissions to fertilizer N losses appears limited to a narrow range, making it difficult to discern significant differences. In a review of 87 agroecosystems amended with mineral and organic fertilizers, Bouwman (1994) reported losses ranging from 0.0 to 6.8% of the applied N. Nonetheless, the relative contribution of N_2O emissions to fertilizer N losses observed in the present study is consistent with past reports for liquid swine waste as well as the assembled data in the literature.

Measurements of denitrification include fluxes of both N_2O-N and N_2-N. Altogether the two time course fertilization experiments, TC-1 and TC-2, indicate denitrification losses ranging from 1.8 to 4.5% of the applied N (Table 6.2). These are the first known estimates of the relative contribution of denitrification to gaseous N losses from an agricultural soil fertilized with liquid lagoonal swine waste. These values lie towards the lower end of estimates for denitrification losses from other animal-derived fertilizers. For instance, reported losses range from 3 to 20% for slurried dairy cattle waste (Loro et al. 1997; Paul & Zebarth 1997a; Paul & Zebarth 1997b; Thompson 1989), 3 to 31% for slurried swine waste (Maag 1989), and as high as 11 to 37% for liquid dairy cattle waste (Lowrance et al. 1998). However, these estimates typically involved field denitrification measurements over periods of months or years and inevitably include the effects of subsequent rainfall and freeze/thaw cycles, events which are likely to promote subsequent pulses of denitrification in the same way that they have been shown to renew N_2O emissions in fertilized soils (Cates & Keeney 1987; Coyne et al. 1995; Goodroad et al. 1984;

Lessard et al. 1996). Because denitrification losses in the present study are derived solely from the one-time effect of fertilizer application, the relative contribution of denitrification to seasonal or annual gaseous N losses is likely to be underestimated here.

Process Level Control of Physicochemical Variables

It is evident from the results of the time course fertilization experiments that soil physicochemical variables such as %WFPS and the concentrations of NO_3^--N and NH_4^+-N vary dramatically following the one-time application of liquid swine waste. These variables, along with organic-C and temperature, are important as proximal controls on rates of denitrification. However, due to the complexity of interactions in intact soil cores, these experiments do not do well to predict the individual effects of proximal variables. These individual variables were investigated here using homogenized soil samples and anaerobic soil slurries to better understand their process-level effects.

The effect of soil moisture content on microbiological N-gas production has typically been described by an exponential model with a critical value around 60% (Linn & Doran 1984). The data presented here for N_2O emission and denitrification rates under varying soil moisture conditions (Figures 6.7a-b) indicate the existence of threshold moisture content, but displayed a simple binary rather than exponential response. That is, there was a distinct moisture content below which neither N_2O emission nor denitrification rates exceeded background levels and increasing moisture content above this threshold produced 33- to 39-fold increases in N-gas fluxes. Without the addition of C or N substrates, this threshold was very near complete saturation (i.e. 100% WHC). Although it is very difficult to interpret the magnitude of this threshold in terms of the moisture parameter used in the time course experiments (i.e. %WFPS), it does indicate that anaerobic conditions are extremely important to the occurrence of denitrification and supports the conclusion that soil moisture content is the principal factor controlling denitrification in this fertilized soil. Furthermore, assuming that soil moisture/aeration is evenly distributed in these sieved and mixed soil samples, the fact that appreciable N_2O fluxes occurred only at moisture contents above saturation suggest that denitrification is a potentially significant source of N_2O.

The effects of glucose-C and NO_3^--N on denitrification enzyme activity are also described by a simple relationship. Essentially, the substrate dose response at the concentrations investigated here is merely a function of the presence or absence of the two substrates, while the combined effects are consistently greater than the individual effects (Figure 6.8; Table 6.3). These results raise a number of interesting points. First, a shortage of either labile-C or NO_3^--N has great potential to diminish the capacity for denitrification. Also, it appears that given an adequate supply of complementary substrate, the concentrations of fertilizer-derived organic-C and NO_3^--N observed in the field far exceed enzyme-saturating levels and are unlikely to become denitrification-limiting. However, this may be misleading because DEA experiments involve conditions that optimize the distribution and diffusion of denitrification substrates, while substrate availability under natural conditions is complicated by numerous factors.

Although soils were incubated at constant temperature during the time course fertilization experiments, diurnal and seasonal temperature variations are sure to affect denitrification rates in

the field. The results of temperature-controlled DEA experiments indicate that these variations can have significant impacts on denitrification of fertilizer N. According to the average Q_{10} of 1.9 calculated over the range 7 to 35 ^{o}C, potential denitrification rates roughly double with every 10 ^{o}C increase in temperature (Figure 6.9). Therefore, the difference between mean daily temperatures during winter (6 ^{o}C) and summer (25^{o}C) in the Coastal Plain of North Carolina (Brandon 1986) implies a 4-fold difference in potential denitrification rates between winter and summer. This could be significant in the southeast United States, where it is common practice to land-apply lagoonal swine effluent year-round. Because estimates of denitrification losses of fertilizer N in this study were made at 25^{o}C, it is possible (disregarding the effects of seasonal differences in rainfall and average soil moisture content) that denitrification losses will be much lower during winter months.

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