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## Analysis of manure and soil nitrogen mineralization during incubation

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**Abstract** Understanding the N-cycling processes that ensue after manuring soil is essential in order to estimate the value of manure as an N fertilizer. A laboratory incubation of manured soil was carried out in order to study N mineralization, gas fluxes, denitrification, and microbial N immobilization after manure application. Four different manures were enclosed in mesh bags to allow for the separate analysis of manure and soil. The soils received 0.15 mg manure N g<sup>-1</sup> soil, and the microcosms were incubated aerobically and sampled throughout a 10-week period. Manure addition resulted in initial NH<sub>4</sub>-N concentrations of 22.1 to 36.6 mg kg<sup>-1</sup> in the microcosms. All manured microcosms had net declines in soil mineral N. Denitrification resulted in the loss of 14.7 to 39.2% of the added manure N, and the largest N losses occurred in manures with high NH<sub>4</sub>-N content. Increased soil microbial biomass N amounted to 6.0 to 8.6% of the added manure N. While the microcosms as a whole had negative N mineralization, all microcosms had positive net nitrification within the manure bags. Gas fluxes of N<sub>2</sub>O and CO<sub>2</sub> increased in all manured soils relative to the controls. Our results show that measurement of microbial biomass N and denitrification is important to understand the fate of manure N upon soil application.

**Keywords** Manure · Denitrification · Nitrification · Mineralization · Nitrogen

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### Introduction

Excessive use of animal manure as an N fertilizer has resulted in groundwater and atmospheric contamination. Denitrification is one of the main sinks of manure N during manure decomposition (Calderón et al. 2004), and N<sub>2</sub>O emissions from animal waste management in 2001 were estimated to be 230,000 tons in the USA (US Department of Energy 2002).

The N mineralization potential of manures, composts, and soils has conventionally been estimated using laboratory incubations (Hadas and Portnoy 1994; Jedidi et al. 1995; Sørensen 1998). During manure decomposition, the mineralized manure N may take several routes: (1) the mineral N may remain in the soil and become part of the net mineralized N pool; (2) the mineral N may be immobilized by microbes and become part of the microbial biomass pool; and (3) NO<sub>3</sub>-N derived from manure may be denitrified and lost from the soil as either N<sub>2</sub>O or N<sub>2</sub>. Within this set of conditions, a manure with high N content should result in high net N mineralization, increased soil microbial biomass N, high denitrification, or a combination of these outcomes.

Previous studies of manure incubation in soil did not measure microbial biomass N directly, yet referred to negative N mineralization as N immobilization (Jedidi et al. 1995; Sørensen and Jensen 1995; Hadas and Portnoy 1994). In addition, Hart et al. (1994) assume negligible denitrification for their definition of net N immobilization. We hypothesize that it is important to obtain direct measurements of microbial biomass N, denitrified N, as well as soil extractable mineral N, in order to make a correct estimate of the N supplying capacity of manure. For example, manure potentially mineralizable N may be underestimated if there is high immobilization of N during manure decomposition. In addition, we hypothesize that negative manure mineralizable N does not necessarily be related to N immobilization by soil microbes because there may be high denitrification N losses.

Data is lacking about the differences in N-cycling dynamics between decomposing manure and the surrounding soil. This is fundamental since a large fraction of the

nitrogen oxides produced during manure decomposition occurs in “hot spots” where manure N, microbes, and mineral N come together. Manure is often injected or disked into soil, leading to uneven distribution within the soil matrix. In these situations, the total N mineralization and N<sub>2</sub>O fluxes are determined by the interplay between the N pools in the manure and soil. For example, it has been shown that nitrification and denitrification may be closely linked across a soil–manure boundary (Nielsen and Revsbech 1998; Meyer et al. 2002).

Placing manure inside mesh bags should allow us to differentiate between the N-cycling dynamics in manures and soils within the same microcosm. Recently, this approach has been applied to the study of manure decomposition rates in soils (Somda and Powell 1998; Lupwayi and Haque 1999). The retrieval of the manure at different stages of decomposition should allow us to study the N-cycling dynamics of soil and manure separately and in that way better understand the processes that take place during manure N mineralization.

The objective of this research was to analyze the nutrient-cycling dynamics of manure and soil during a manure mineralizable N laboratory assay. Four different manures and a control were included to determine effects of manure composition. Several C and N pools and gas fluxes were measured during a 10-week laboratory incubation, and the net nitrification, denitrification, and immobilization were measured and compared.

## Materials and methods

The Beltsville silt loam soil (fine loamy, mixed, mesic Typic Fragiuudults) used for this experiment was collected from a corn field located on the USDA–Agricultural Research Service (ARS) Beltsville Agricultural Research Center. The soil was obtained from 0- to 10-cm depth and was sifted (4.76-mm mesh) to eliminate coarse rocks and plant material. The soil had a pH of 5.7 (saturated paste method). The soil organic matter content (40.0 mg g<sup>-1</sup>) and particle-size distribution (60% sand, 27% silt, and 13% clay) were determined at the University of Maryland Cooperative Extension Laboratory. Soil samples were dried at 55°C and total soil C (1.98%) and N (0.16%) were measured with a CNS-2000 LECO Elemental Analyzer (LECO Corporation, St. Joseph, MI).

Four different manures were collected and used for this experiment. The manures varied in the age, sex, and breed of the animals, as well as the diet: (1) UMB was collected at the University of Maryland from yearling Hereford–Angus beef steers fed with Timothy hay and prepared feed (see below). (2) USB was collected from Hereford–Angus yearling beef heifers. The animals were allowed to graze on a pasture and the diet was supplemented with prepared feed. (3) USD was obtained from a confined milking herd of 4-year-old Holsteins on a high-protein diet. (4) USH was collected from a herd of 2-year-old Holstein heifers. Besides the items described above, the animals from all the manure treatments received feeds prepared by the USDA Feed Center, Beltsville, MD. The feeds varied among treat-

ments and included mixtures of grains, meal, seeds, fruit pulp, silages, molasses, salt, minerals, calcium carbonate, and vitamins. The manures were collected in September 2002. All manures were obtained from the ground with care to avoid any bedding or soil, and the manure was stored at 5°C until homogenization. The USD manure may have also included some urine since it was collected from the gutter at the dairy facility. Each manure was mixed with dry ice (1:2 manure/dry ice, v/v), and the mixture was homogenized at high speed in a Vita Mix 3600 Plus blender (Vita Mix Corp., Cleveland, OH). The CO<sub>2</sub> was allowed to sublimate from the samples at 5°C. The manures were then stored frozen until the preparation of the manure bags (see below).

Before the start of the incubation experiment, manure samples (3 g) were extracted by shaking in 300 ml of 2 M KCl for 30 min on a wrist-action shaker. The solids were allowed to settle overnight at 4°C, then the supernatants were transferred to 20 ml screw-capped vials for storage at 4°C. The extracts were filtered (Fisherbrand Serum Filter System, I.B. model, Fisher Scientific, Pittsburg, PA), and the filtrates were analyzed for NO<sub>3</sub>-N, NO<sub>2</sub>-N, and NH<sub>4</sub>-N on a AutoAnalyzer 3 (Bran+Luebbe, Hamburg, Germany). Subsamples of fresh manure were analyzed for total C and N as with the soils (see above).

All manures were placed inside mesh bags prior to addition to soil in order to allow for the separate sampling of manures and soils. The bags were made of polyethylene/polyester filter material (Unibond HK-250-N, Midwest Filtration Co., Fairfield, OH), and the seams were welded with a Kwik Seal sealer (Cole Parmer Instrument Co., Chicago, IL). The bag material allowed for the exchange of dissolved nutrients and water between the manure and the soil, while it retained the manure solids in the bag for future analysis. Immediately before the start of the experiment, the prescribed amount of manure was added to each bag (ranging from 3.73 to 3.96 g), and the bags were sealed. Exactly 0.15 mg of manure N g<sup>-1</sup> soil were added to each microcosm in all manure treatments, comparable to a field application of 397.5 kg ha<sup>-1</sup> of manure N. The use of the manure bags allowed us to measure extractable mineral N in the manure as it decomposed, as well as in the surrounding soil. Hereafter, we will use microcosm concentrations of NH<sub>4</sub>-N, NO<sub>2</sub>-N, and NO<sub>3</sub>-N to denote the combined manure plus soil concentration of the respective N pools.

Each bag was placed vertically in a 120-ml specimen cup, and 125 g of soil was placed into the cup completely covering the manure bag. The soil was packed lightly in order to ensure adequate manure–soil contact. Water was added as necessary to each manure treatment and control to ensure uniform initial moisture (25% gravimetric moisture, dry soil basis) in all microcosms. Each microcosm was placed in a separate screw-capped jar (3.8 l) fitted for headspace gas sampling. Time zero was set at the time when the jars were closed after loading the soil microcosms. The jars were opened every week during the experiment in order to aerate the microcosms. Two milliliters of water were added to the jars (but outside the microcosms) every week in order to minimize moisture loss. The size of the jars was large

enough to avoid the complete exhaustion of oxygen in the microcosms, and the seal prevented NH<sub>3</sub> losses through volatilization.

There were five manure treatments comprised of the four manure types (see above) plus an empty bag control. A total of 30 microcosms were made for each manure treatment, and the total number of microcosms in the experiment was 150. Five replicate microcosms from each manure treatment were sampled destructively at weeks 0, 1, 2, 4, 7, and 10.

During each destructive sampling, four soil subsamples and four manure bags from each manure treatment were extracted for mineral N analysis. The soil samples (10 g) were extracted with 50 ml, while 300 ml of 2 M KCl were used to extract each manure bag. The extraction and analysis of mineral N was carried out using the same method and instruments used for the manures (see above). In this study, the term net N mineralization is used as the change in the soil inorganic N concentration over the 10-week incubation.

One week prior to each destructive sampling, the jars received 40 ml of acetylene in order to measure weekly N losses through denitrification. The microcosms were then sampled for headspace CO<sub>2</sub> and N<sub>2</sub>O immediately before each destructive sampling. Weekly gas sampling of the microcosms allotted for the week 10 destructive sampling allowed for a comparison of gas fluxes from microcosms receiving acetylene and microcosms receiving no acetylene. The N<sub>2</sub>O fluxes of microcosms receiving acetylene, indicative of denitrified N, is hereafter referred to as N<sub>2</sub>O<sub>a</sub>. The N<sub>2</sub>O fluxes from microcosms receiving no acetylene are hereafter referred to as N<sub>2</sub>O<sub>n</sub>. The cumulative N<sub>2</sub>O<sub>a</sub> was calculated by the area under the curve, while the cumulative CO<sub>2</sub> and N<sub>2</sub>O<sub>n</sub> were calculated by adding the weekly fluxes. Ammonia volatilization is deemed negligible during this experiment since the manure was covered by the soil, and it has been shown that NH<sub>3</sub> volatilization is markedly reduced when the manure is incorporated into the soil (Thompson and Meisinger 2002). In addition, the microcosms were in closed jars, and any volatilized NH<sub>3</sub> was available for re-absorption by the slightly acid soil.

In order to sample the headspace N<sub>2</sub>O, 2 ml of the jar headspace were obtained with a syringe and injected into 22-ml vials with butyl rubber septa flushed with He beforehand. The N<sub>2</sub>O concentration of the samples was determined using a Tekmar 7000 HT headspace autosampler (Tekmar Co., Cincinnati, OH) in line with a Shimadzu GC-ECD (GC-8A, Shimadzu Scientific Instruments Inc., Columbia, MD).

Gas samples for CO<sub>2</sub> analysis were obtained in the same manner as the N<sub>2</sub>O samples. The CO<sub>2</sub> concentration was analyzed using a Tekmar 7000 HT headspace autosampler (Tekmar Co.) in line with a Tremeetrics Model 540 gas chromatograph fitted with an ultrasonic detector (Tremeetrics Inc., Austin, TX), using the methods described in McCarty and Blicher-Mathiesen (1996).

At the last sampling time (week 10), four soil samples (20 g) from each manure treatment were analyzed for microbial biomass C (MBC) and microbial biomass N (MBN) using the fumigation-incubation method of Horwath and Paul (1994). To calculate the MBC, the CO<sub>2</sub>-C flush from the

fumigated sample was multiplied by 2.44 (Horwath and Paul 1994). To calculate the MBN, the NH<sub>3</sub> flush from the fumigated sample was multiplied by 1.47 (Horwath and Paul 1994).

The analysis of variance (ANOVA) was used to test the effects of manure treatment and time. We used the proc GLM procedure of SAS version 8.2 (Cary, NC). To determine the statistical significance of the mean differences, we carried out least significant difference (LSD) tests based on a *t*-test. In addition, we carried out correlation analyses using the proc CORR procedure of SAS. While regression analysis is often used to test hypotheses of direct (cause and effect) associations, the use of correlation analysis allowed us to examine relationships without the assumption that a direct effect exists between the variables or treatments. We carried out the correlations in two ways. First, weekly gas flux and soil data within each manure treatment was used for correlation analysis. This was done separately for each treatment. Second, analysis of cumulative data as well data from specific weeks was carried out. In this case, the data of all manured treatments was combined in order to find general patterns. Controls were excluded in order to avoid a bimodal distribution of the data, as well as to limit the comparisons to manured soils.

## Results

The soil pH remained favorable for nitrification throughout the incubation. Initial soil pH was 5.7 for all the treatments. After the 10-week incubation, the soil pH of the controls had risen to 6.4, while the pH of the manured treatments ranged between 6.5 and 6.8.

The soils received a range of manure C from 2.4 mg g<sup>-1</sup> soil for USD to 3.3 mg g<sup>-1</sup> soil for UMB (Table 1). The C/N ratios of the manures ranged from 16.2 for USD to UMB with a C/N ratio of 22.2 (Table 1). The amount of added manure N was equivalent for all the manured treatments. However, different amounts of manure NO<sub>3</sub>-N, NO<sub>2</sub>-N, and NH<sub>4</sub>-N were added because of compositional differences between the four manures (Table 2). The predominant mineral N form in the four manures before addition to soil was NH<sub>4</sub>-N, followed by NO<sub>2</sub>-N, then NO<sub>3</sub>-N (Table 2). Manure contributed a range of NH<sub>4</sub>-N from 18.0 mg kg<sup>-1</sup> soil in UMB to 32.5 mg kg<sup>-1</sup> in USD. All four manures had lower amounts of NO<sub>2</sub>-N, contributing less than 1.0 mg kg<sup>-1</sup>

**Table 1** Total carbon and total nitrogen content of the manures at day 0 of the incubation

Manure	Dry matter (%)	Total N (%)	Total C (%)	C/N <sub>total</sub> ratio	Added manure C (mg g <sup>-1</sup> soil)
UMB	18.35	0.38	8.45	22.23	3.33
USB	16.45	0.39	7.05	18.21	2.73
USD	15.50	0.47	7.54	16.15	2.42
USH	18.18	0.43	8.44	19.63	2.94

The dry matter, total N, and total C are expressed as percent of fresh weight. Each number is the mean; *n*=3

**Table 2** Mineral N added with the manures at day 0 of the incubation

	mg NO <sub>3</sub> -N kg <sup>-1</sup>	mg NO <sub>2</sub> -N kg <sup>-1</sup>	mg NH <sub>4</sub> -N kg <sup>-1</sup>
UMB	0.21 (0.01)	0.55 (0.03)	17.98 (0.17)
USB	0.44 (0.14)	0.74 (0.01)	19.86 (0.63)
USD	0.53 (0.13)	0.60 (0.01)	32.47 (0.41)
USH	0.55 (0.05)	0.99 (0.04)	20.32 (0.64)

Data are expressed per unit mass of dry soil. Each number is the mean;  $n=4$ . Data are averages (standard error of the mean)

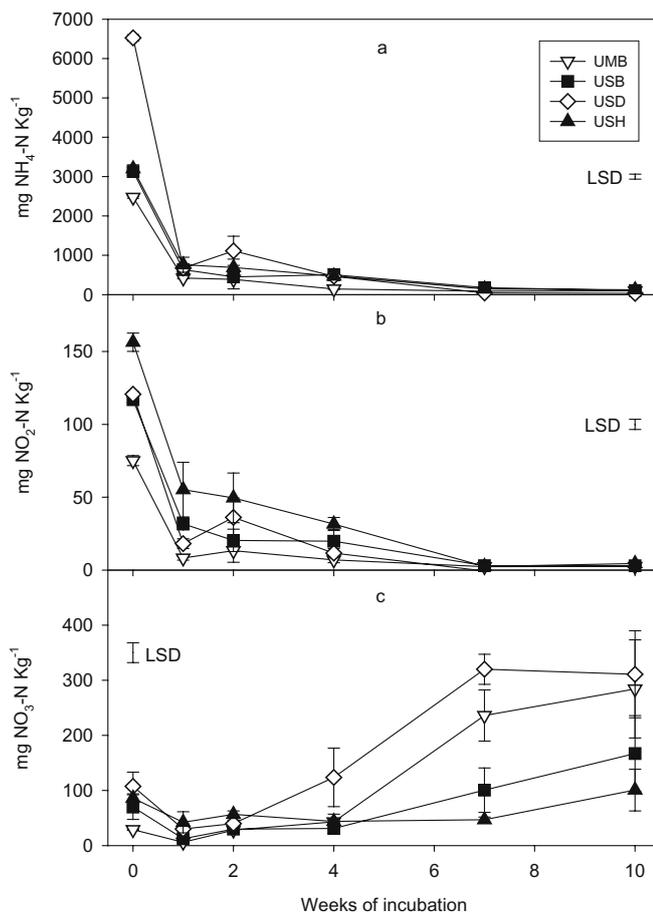
soil. NO<sub>3</sub>-N was the least abundant form of mineral N in all manures contributing below 0.6 mg kg<sup>-1</sup> to the manured soils.

The ANOVA for both manure and soil NO<sub>3</sub>-N and NH<sub>4</sub>-N showed significant manure, time, as well as manure×time effects during the incubation ( $p<0.001$ ). Manure NO<sub>2</sub>-N had significant manure, time, as well as manure×time effects ( $p<0.001$ ), whereas soil NO<sub>2</sub>-N only had a significant time effect ( $p<0.001$ ) with statistically indistinguishable manure treatment means.

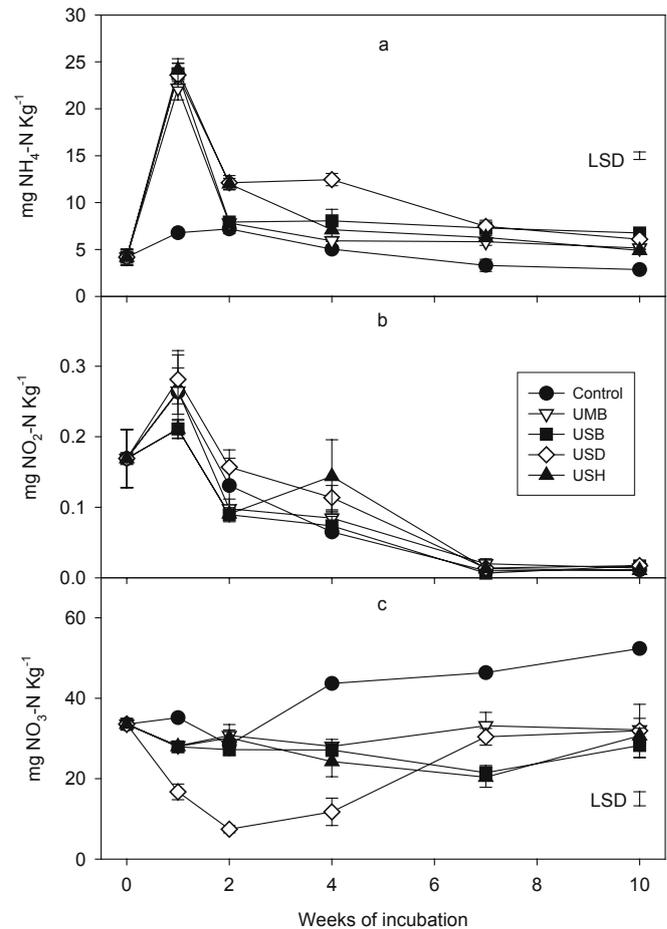
NH<sub>4</sub>-N was initially high in all the manure bags, but decreased markedly during week 1 of the incubation (Fig. 1a). Concurrently with the decrease in NH<sub>4</sub>-N within the manure, NH<sub>4</sub>-N increased in the surrounding soil (Fig. 2a). Soil NH<sub>4</sub>-N was initially 4.2 mg kg<sup>-1</sup> in all soils since the same soil homogenate was used for all manure treatments. Thereafter, soil NH<sub>4</sub>-N increased markedly at week 1 in all manured treatments.

Initially, manure NO<sub>2</sub>-N ranged from 75.1 to 156.4 mg kg<sup>-1</sup>, but declined to trace amounts after the fourth week of the incubation (Fig. 1b). In the manured soils, NO<sub>2</sub>-N was always below 0.3 mg kg<sup>-1</sup> (Fig. 2b).

The soil had initially higher NO<sub>3</sub>-N concentrations than the manures, with an average concentration of 33.6 mg kg<sup>-1</sup> (Fig. 2c). Manure NO<sub>3</sub>-N was initially low then increased to between 101 and 311 after week 7 (Fig. 1c). Between time zero and week 2 of the incubation, total mineral N declined in all the manured microcosms (Fig. 3a), while denitrification reached the highest values of the incubation for all manured treatments (Fig. 4c), suggesting a source–sink relationship. The decline in denitrification activity from weeks 2 to 4 (Fig 4c) coincided with the progressive accu-



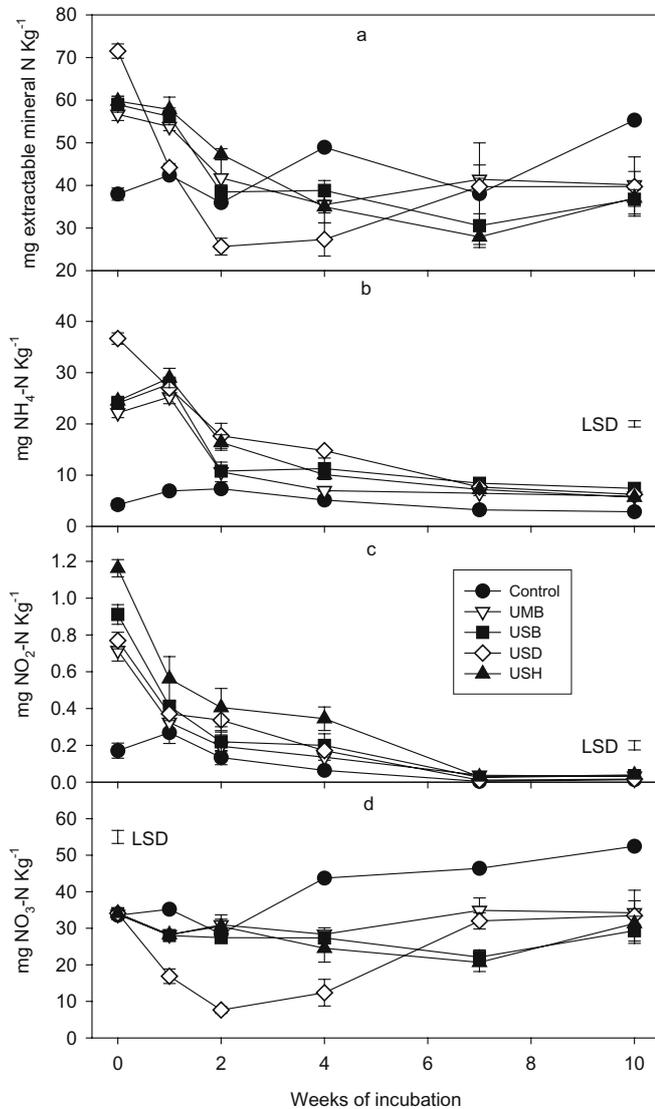
**Fig. 1** Extractable manure mineral N measured during the 10-week incubation of four different manures in soil. **a** NH<sub>4</sub>-N, **b** NO<sub>2</sub>-N, and **c** NO<sub>3</sub>-N. The data is expressed per unit weight of dry manure. Error bars are SEM values;  $n=4$ . The least significant difference (LSD) between manure treatment means according to a  $t$ -test is shown



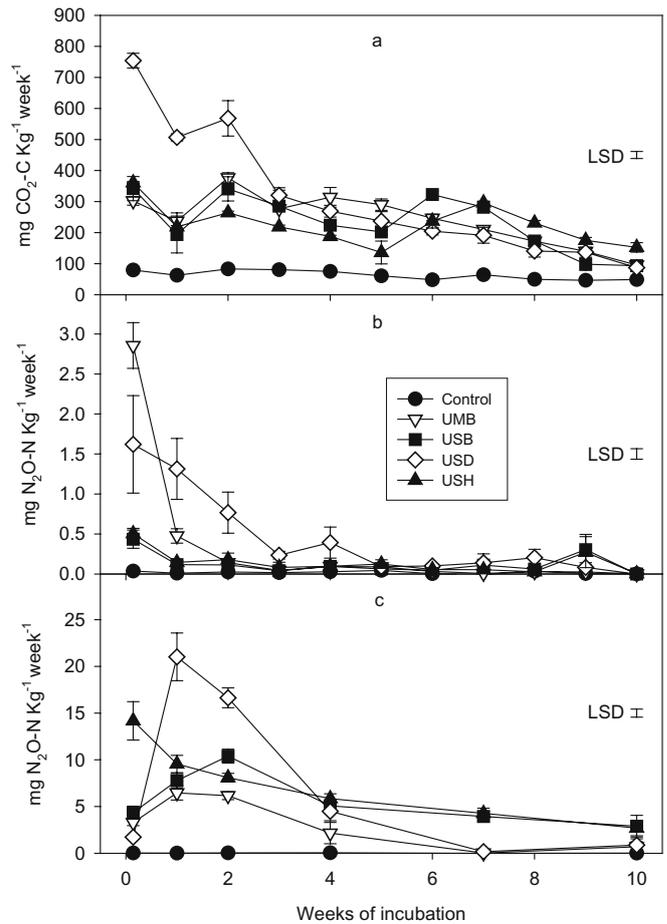
**Fig. 2** Extractable soil mineral N measured during the 10-week incubation of four different manures in soil. **a** NH<sub>4</sub>-N, **b** NO<sub>2</sub>-N, and **c** NO<sub>3</sub>-N. The data is expressed per unit weight of dry soil. Error bars are SEM values;  $n=4$ . The least significant difference (LSD) between manure treatment means according to a  $t$ -test is shown

mination of  $\text{NO}_3\text{-N}$  in the manures after week 4 (Fig. 1c).  $\text{NO}_3\text{-N}$  dynamics in soils and manures were very different. There was positive net nitrification within the manure bags during the 10-week incubation, while no positive net nitrification was observed in soils for the same time period (Figs. 1, 2).

The manured soils had negative N mineralization for the 10-week incubation, mainly because of the disappearance of the initially high  $\text{NH}_4\text{-N}$  (Figs. 2, 3). Average N mineralization values in  $\text{mg N kg}^{-1}$  were:  $-16.5$  for UMB,  $-22.2$  for USB,  $-31.8$  for USD, and  $-22.7$  for USH. Manured microcosms had higher amounts of total mineral N relative to the controls only during the first 2 weeks of the incubation (Fig. 3a). However, mineral N analyses done on extra manured samples from all four manured treatments showed



**Fig. 3** Extractable mineral N measured during the 10-week incubation of control and manured microcosms. This is the combined data of manure and soil extractable N. **a** Total mineral N, **b**  $\text{NH}_4\text{-N}$ , **c**  $\text{NO}_2\text{-N}$ , and **d**  $\text{NO}_3\text{-N}$ . The data is expressed per unit weight of dry soil+manure. Error bars are SEM values;  $n=4$ . The least significant difference (*LSD*) according to a *t*-test between manure treatment means is shown



**Fig. 4** Weekly gas fluxes measured during the 10-week incubation of control and manured soils. **a**  $\text{CO}_2\text{-C}$ , **b**  $\text{N}_2\text{O-N}$ , and **c**  $\text{N}_2\text{O-N}$  after acetylene block ( $\text{N}_2\text{Oa}$ ). The data is expressed per unit mass of soil. Error bars are SEM values;  $n=5$ . The least significant difference (*LSD*) between manure treatment means according to a *t*-test is shown

slightly positive N mineralization by week 12 (data not shown).

All four manures had higher cumulative  $\text{N}_2\text{Oa}$  than the control, indicative of higher denitrification rates (Fig. 4c, Table 3). Manure, time, as well as manure $\times$ time had significant  $\text{N}_2\text{Oa}$  effects according to ANOVA ( $p<0.001$ ). The difference in  $\text{N}_2\text{Oa}$  between manured soils and controls was pronounced between weeks 1 and 4 of the incubation (Fig. 4c). The  $\text{N}_2\text{Oa}$  dynamics during the incubation varied markedly between the manure treatments. While USD had the highest  $\text{N}_2\text{Oa}$  fluxes between weeks 1 and 2 followed by a steep decline, the rest of the treatments had more gradual fluctuations (Fig. 4c). Weekly,  $\text{N}_2\text{Oa}$  correlated positively with  $\text{CO}_2$  flux for all manured treatments ( $r>0.51$ ;  $p<0.01$ ), showing that high  $\text{CO}_2$  fluxes correspond with high denitrification activity. In the USD and USH treatments, which had the highest cumulative  $\text{N}_2\text{Oa}$  fluxes (Table 3), the weekly  $\text{N}_2\text{Oa}$  correlated positively with  $\text{N}_2\text{O-N}$  ( $r>0.51$ ;  $p<0.01$ ), suggesting that  $\text{N}_2\text{O}$  fluxes may be explained by denitrification activity. In the USD treatment, weekly patterns of soil and manure  $\text{NO}_3\text{-N}$  were negatively correlated to  $\text{N}_2\text{Oa}$ , suggesting that soil  $\text{NO}_3\text{-N}$  accumulated when



supplied with the manure stimulated microbial activity during the incubation.

At the end of the 10-week incubation, all of the manured microcosms had 22–31% higher MBN than the control (Table 5). Increased MBN accounted for 6.0% (UMB) to 8.6% (USD) of the added manure N. Initial  $\text{NH}_4\text{-N}$  in the manure correlated positively with MBN (Table 4). MBC increased 11–27% in the manured treatments (Table 5). The ratio of added manure C to net manure C immobilized during the incubation ranged from 1.2% for USH to 3.2% for USD.

## Discussion

In this study, we show that microbial denitrification first, then immobilization, are important sinks of N after manure application to soil. We have also shown that there are marked differences in the N mineralization dynamics between manure and the surrounding soil. While  $\text{NO}_3\text{-N}$  was depleted in soil during the incubation, net nitrification was observed within the manure volume. Initial manure  $\text{NH}_4\text{-N}$  may be an important predictor of total C mineralization, total immobilized N, and total denitrified N during the incubation. This study also illustrates that the quality of manure C, not just the quantity, is important in determining the  $\text{CO}_2$  fluxes, as well as the amounts of denitrification in manured soils. Our hypothesis that negative N mineralization values are not equivalent to immobilization is supported by our observation of high denitrification N losses.

In all four manure treatments, total soil mineral N declined during the incubation. In general, manure provided readily available C, which in turn diverted potentially available N to denitrification and immobilization. In all soils, the high  $\text{NH}_4\text{-N}$  supplied by the manure was offset by demand for  $\text{NO}_3\text{-N}$  throughout the incubation; thus, no increase in soil  $\text{NO}_3\text{-N}$  was observed. In contrast, the control soils did show positive soil net nitrification possibly because the limited supply of available C was not enough to fuel net assimilation of N or denitrification by microbes.

This study simulates a scenario of manure application to a relatively moist soil with moderately high  $\text{NO}_3\text{-N}$  content, making the soil conditions conducive to denitrification. However, the range of denitrification N losses of 14.7 to 39.2% of added manure N reported in this study are within the values reported by Lowrance et al. (1998). Denitrification was a stronger sink of mineral N than immobilization during the incubation of the manured soils. However, we did not measure MBN inside the manure bags; thus, the MBN data may be an underestimate when extrapolated to the microcosm level. Available C seems to play a role in the amount of N immobilized since the USD manure, with high cumulative  $\text{CO}_2$  production, resulted in relatively high N immobilization.

The presence of readily assimilable C may favor  $\text{N}_2\text{O}$  fluxes from manure slurries via denitrification (Petersen 1999). The USD treatment supplied the least amount of total C to the microcosms relative to the rest of the manures, but

the high  $\text{CO}_2$  production suggests that USD contained more easily degradable C compared with the rest of the manures. We hypothesize that high C availability in the USD treatment promoted high denitrification, preventing significant accumulation of  $\text{NO}_3\text{-N}$  in the manure and reducing the  $\text{NO}_3\text{-N}$  concentration in soil. Because of this, the USD treatment illustrates how the quality of the manure carbon, besides the quantity, determines the nutrient-cycling dynamics after manure addition to soil. Our conclusion that USD manure has greater available C is supported by the fact that the USD manure had a large (net C mineralization) / (C added ratio), as well as the high, microbial C immobilization during the experiment. In contrast, the UMB treatment provided the most amount of total C to the microcosms, but had only moderately high cumulative C mineralization. The  $\text{CO}_2$  fluxes in the UMB treatment did not have wide fluctuations during the incubation. The lack of a distinct peak in microbial activity in the UMB treatment suggests that relatively few hot spots of oxygen consumption and anaerobiosis occurred. We hypothesize that manures such as UMB, which have a slow and gradual release of available C, will favor microbial N immobilization rather than denitrification through the indirect effect on the aerobicity of soil. Conversely, manures rich in readily available C such as USH and USD will favor denitrification because high microbial activity leads to oxygen depletion in microsites for limited periods.

Manure application to soil may result in a period of high  $\text{N}_2\text{O}$  production that may last for days or weeks (Petersen 1999; Clemens and Huschka 2001; Meyer et al. 2002). This experiment also shows that the effects of manuring on  $\text{N}_2\text{O}$  flux can be prolonged. The USB and USH treatments had high denitrification rates after week 4 of the incubation. These results suggest that some manures can supply a sustained amount of available C and can supplement soil with additional  $\text{NO}_3\text{-N}$  for denitrifiers for several weeks. This is supported by the  $\text{NO}_3\text{-N}$  concentration within the manure bag, which increased when soil  $\text{NO}_3\text{-N}$  remained stable or declined in the surrounding soil.

Manuring soils is sometimes followed by an extended period where N immobilization limits N availability (Flowers and Arnold 1983; Beauchamp 1986; Kirchmann and Lundvall 1993; Paul and Beauchamp 1994). In this study, all the manured treatments resulted in net negative N mineralization at 10 weeks of incubation. Others have found that laboratory incubations of manured soil lasting for weeks may result in negative N mineralization values (Eneji et al. 2002), while longer incubations result in positive values (Hadas and Portnoy 1994). Waiting for more than 10 weeks for positive N mineralization would miss the period of high N demand of most crops had the soils been planted soon after manuring the field. However, separating the N-cycling dynamics within and outside the decomposing manure allowed us to measure positive net nitrification inside the manures even when  $\text{NO}_3\text{-N}$  was being depleted in the adjacent soil. This suggests that in manured soils, nitrate may be available even when the bulk of the soil nitrate is declining. Previous studies have associated high C/N ratios to reduced N mineralization in sludges (Barbarika et al. 1985). Our

results agree with previous studies which suggest that manure C/N ratios greater than 15 result in net N immobilization (Paul and Beauchamp 1989; Van Kessel et al. 2000).

The fluctuations of  $\text{NH}_4\text{-N}$  during the first 2 weeks in the incubation suggests a movement of  $\text{NH}_4\text{-N}$  from the manure bag to the outside soil, possibly by  $\text{NH}_3$  volatilization from the manure and dissolution in the adjacent soil solution. Alternatively, these fluctuations in  $\text{NH}_4\text{-N}$  may be explained by net N mineralization in the soil, concurrent with nitrification–denitrification of  $\text{NH}_4\text{-N}$  within the manure. The decline in soil  $\text{NH}_4\text{-N}$  between weeks 1 and 2 can be explained by an event of rapid nitrification combined with denitrification. The fact that the soil  $\text{NO}_3\text{-N}$  did not increase between weeks 1 and 2 of the incubation suggests that the  $\text{NO}_3\text{-N}$  was taken up by the denitrifiers as soon as it was produced, preventing an increase in the standing  $\text{NO}_3\text{-N}$  pool. This close coupling between nitrification and denitrification has been observed by others and results on a high proportion of the mineralized N to be lost as N gas (Nielsen and Revsbech 1998; Meyer et al. 2002). The high correlation between initial manure  $\text{NH}_4\text{-N}$  and cumulative  $\text{N}_2\text{O}$  further confirms the important role of initially available N on the nitrification and denitrification dynamics during manure mineralization. There was a significant correlation between initial manure  $\text{NH}_4\text{-N}$  and MBN, which suggests that part of the manure N is eventually allocated to soil microbial biomass. The net increase in microbial biomass N may result in future net mineralization by providing enzymes for degradation of organic molecules as well as the potential remineralization of the immobilized N.

Nitrite was relatively high within the manures at the start of the incubation, ranging from 75 to 120  $\text{mg kg}^{-1}$ . These concentrations have been deemed toxic to root growth (Sawyer et al. 1990). However, the high  $\text{NO}_2\text{-N}$  is ephemeral and diffusion to soil is limited since only a small increment in soil  $\text{NO}_2\text{-N}$  was observed at week 2.

Tenuta et al. (2001) observed that  $\text{N}_2\text{O}$  emissions from manure increased with  $\text{NO}_3\text{-N}$  content, suggesting that denitrification is an important source of  $\text{N}_2\text{O}$  in manures. In this experiment, however,  $\text{NO}_3\text{-N}$  was the least abundant form of mineral N in the manures, and soil may have contributed most of the  $\text{NO}_3\text{-N}$  for denitrifiers during the first 2 weeks of the incubation. Field studies that show increased denitrification when fertilizer  $\text{NO}_3\text{-N}$  is applied soon after manuring soil support the idea that denitrification in manured soils is initially limited by  $\text{NO}_3\text{-N}$  (Stevens and Laughlin 2002).

McCarty et al. (unpublished data) found a correlation between  $\text{N}_2\text{O}$  flux and denitrification activity on manure-amended soils. In this experiment, we found a slight, but significant, correlation between the cumulative  $\text{N}_2\text{O}$  flux and denitrification at week 2, when the manure treatments had the highest denitrification values (data not shown). These results support the ‘hole-in-the-pipe’ concept introduced by Firestone and Davidson (1989), which states that the emissions of  $\text{N}_2\text{O}$  increase with increasing rates of nitrification and denitrification.

In this study, 51–85% of the added manure C was mineralized during the incubation. This range is similar to the 54 to 63% values reported for a 12-week incubation of animal slurries (Sørensen 1998). We found a significant correlation between cumulative  $\text{CO}_2$  flux and cumulative  $\text{N}_2\text{O}$  flux, suggesting that soil microbial activity, in general, is conducive to denitrification.

We hypothesize that increased respiration may indirectly favor  $\text{N}_2\text{O}$  fluxes by favoring  $\text{O}_2$  consumption and anaerobiosis on soil microsites. Manure C leads to increased microbial activity, and oxygen consumption results in the formation of anaerobic microsites (Clemens and Huschka 2001). Other studies, however, have found a negative correlation between  $\text{N}_2\text{O}$  flux and  $\text{CO}_2$  flux on manured soils (McCarty et al. unpublished data), showing that this pattern is not universal across different manure or incubation conditions.

Future experiments should address whether denitrified N should be included as part of the net manure mineralizable N calculations. Analysis of specific manure chemical components, as well as separate analysis of manure and soil, will be necessary to fully understand the value of manure as an N fertilizer. Similarly, future studies should examine the quantity and quality of readily available C in manures, with particular attention to water-soluble C. We hypothesize that knowledge of the amount and degradability of manure C will be essential to understand the fate of manure N after soil application. Manure applications may promote the growth of soil microfaunal populations (Edmeades 2003). Microfaunal organisms such as nematodes and microarthropods may in turn affect soil nitrogen cycling by mixing the organic matter and promoting the turnover of soil microorganisms (Vreeken-Buijs and Brussaard 1996; Savin et al. 2001; Cole et al. 2004). Future experiments should address how factors such as the microfauna may affect the fate of manure N after application.

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