Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory

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Paul, J. W., Beauchamp, E. G. and Zhang, X. 1993. **Nitrous and nitric oxide emissions during nitrification and denitrification from manure-amended soil in the laboratory.** Can. J. Soil Sci. **73**: 539–553. Denitrification and nitrification processes in soil produce significant amounts of atmospheric N_2O and NO. Laboratory experiments were designed to measure N_2O and NO emissions from an agricultural soil shortly after manure addition. Nitrous oxide emissions were higher from soil following addition of manure slurries than following addition of composted manure. Emissions of both N_2O and NO were highest between 1 and 4 d after manure addition. Nitrous oxide emission following manure application was the result of both denitrification and nitrification, which occurred simultaneously in soil. Denitrification was a major producer of N_2O because both denitrification rates and N_2O emission increased dramatically at higher soil-moisture contents and increased manure concentration. Nitric oxide production occurred during nitrification. Nitrous oxide emitted during the 6 d after manure addition ranged from 0.025 to 0.85% of the manure N. Nitric oxide emissions were approximately 0.26% of the amount of added manure N.

Key words: Nitrous oxide, nitric oxide, manure, denitrification, nitrification

Paul, J. W., Beauchamp, E. G. et Zhang, X. 1993. **Emissions d'oxyde nitreux et d'oxyde nitrique durant les processus de nitrification et de dénitrification à partir d'un sol amendé au fumier en laboratoire** Can. J. Soil Sci. **73**: 539–553. Les mécanismes de dénitrification et de nitrification dans le sol sont des sources importantes d'émissions de N_2O et de NO dans l'atmosphère. Une série d'essais en laboratoire avait pour objet de mesurer ces émissions à partir d'un sol agricole peu de temps après l'incorporation du fumier. Les émissions d'oxyde nitreux (N_2O) étaient plus fortes après apport de lisier qu'après incorporation de fumier complet composté. Les émissions de N_2O et de No atteingnaient leur maximum un à quatre jours après l'incorporation du fumier. Alors que les émissions de NO se faisaient durant la nitrification, celles d'oxyde nitreux étaient le produit à la fois de la démitrification et de la nitrification, les deux phénomènes intervenant simultanément dans le sol. La dénitrification était une source importante de N_2O, à en juger par l'augmentation spectaculaire des taux de dénitrification et des émissions de N_2O observée aux teneurs en eau du sol élevées et en présence de quantités de fumier plus abondantes. Les émissions d'oxyde nitreux dans les 6 jours suivant l'incorporation du fumier repéresentaient de 0,025 à 0,85% de l'azote du fumier, alors que les émissions d'oxyde nitrique correspondaient à environ 0.26% du N du fumier.

Mots clés: Oxyde itreux, N_2O, oxyde nitrique, NO, fumier, dénitrification, nitrification

Soils are a significant source of N_2O (Arnold 1954) and NO (Galbally and Roy 1978). Nitrous oxide is an object of environmental concern because of its destructive effect on stratospheric ozone (Crutzen 1974) and its contribution as a greenhouse gas (Yung et al. 1976; Lashof and Ahuja 1990). Nitric oxide

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is involved in the production and destruction of trophospheric ozone and contributes to the formation of acid rain (Galloway and Likens 1981; Logan et al. 1981).

Nitrous and nitric oxides are products of both denitrification (Crutzen and Ehhalt 1977; Payne 1981) and nitrification (Hahn and Junge 1977; Lipschultz et al. 1981). Emissions of both N_2O and NO have been shown to increase following applications of various N fertilizers (Slemr and Seiler 1984; Breitenbeck and Bremner 1986; Anderson and Levine 1987; Nagele and Conrad 1990). Application of manure also increases N_2O emissions (Christensen 1983; Goodroad et al. 1984; Maag 1990). There is little information on NO emissions following manure application.

Conditions in the soil environment may encourage large emissions of N as N_2O and NO shortly after manure application to soil. In a sandy loam soil amended with pig manure, Maag (1990) measured the highest N_2O production rates within 7 d after application of manure. Bremner et al. (1978) observed large N_2O emissions shortly after the addition of nitrogenous organic material to the soil. It is not clear whether N_2O and NO emissions result primarily from denitrification, nitrification or both processes simultaneously. Both nitrification and denitrification processes in soil are enhanced following manure application (Paul and Beauchamp 1989a).

Laboratory experiments were conducted to determine N_2O and NO emission rates in relation to denitrification and nitrification processes in soil amended with manure. The effects of soil moisture and additional NO_3^- were also investigated.

MATERIALS AND METHODS

Characteristics of the Soil and the Manures Used in the Experiments

Soil used in this study was obtained from sites where the soil was identified as a Conestogo silt loam (Gleyed Melanic Brunisol). Total organic C and N concentrations were 21 and 2.1 g kg^{-1} soil, respectively. The soil pH (1:1 water) was 7.4. The soil used for the first experiment was taken in May from a field cropped to corn the previous year. The soil contained 156 g H_2O kg^{-1} and was stored for 2 mo at 4°C before initiation of the experiment. Extractable NH_4^+ and NO_3^- concentrations in the soil were 0.8 and 25.4 mg N kg^{-1} soil, respectively. The soil used for the remaining experiments was taken in September from a field cropped to corn. The extractable NH_4^+ and NO_3^- concentrations were 0.8 and 7.2 mg N kg^{-1} soil, respectively. The soil was dried in the greenhouse to a moisture content of 128 g kg^{-1} and stored at 4°C until initiation of the experiments.

The manures used in the first experiment were obtained from local farms during early spring and stored in sealed containers for 2 mo at 4°C before initiation of the experiment. The solid beef-cattle manure used in exp. 2 and the liquid dairy-cattle manure used in exps. 2–4 were obtained in October from the University of Guelph research station at Elora, Ontario (20 km north of Guelph). Chemical characteristics of the manures are presented in Table 1. Total N was determined colorimetrically after Kjeldahl digestion; NH_4^+ and NO_3^- were determined colorimetrically after extraction with 2*M* KCl (Tel and Heseltine 1990); moisture was determined by freeze-drying the manure to avoid C and N losses; and total C was measured on a Leco induction furnace after freeze-drying the sample. Volatile fatty acids in the manure were measured using the method of Paul and Beauchamp (1989b), modified for manure analysis. The modification included a higher water/manure ratio to increase the dilution factor and a higher centrifugation speed to obtain a clearer supernatant.

The quantities of manure added to the soil in the experiments were equivalent to field applications of 40–200 t ha^{-1} (80 g kg^{-1} soil is approximately 160 t ha^{-1}), incorporated in the top 15 cm of soil. This manure application is higher than that applied on most farms; however, the manure is rarely homogeneously mixed in the top 6 cm of soil. Therefore, the concentrations of manure used in these experiments are probably typical of those found in localized zones in manured soils.

Nitric Oxide Analysis

Nitric oxide was measured by a 1-mL injection of headspace gas into the airflow entering a chemiluminescent NO-NO_2-NO_x analyzer (Model 14B/E, Thermo Electron Corp., Hopkinton, MA). The airflow tubing was fitted with a T-junction containing a septum through which the gas samples were injected. The flow rate of the air entering the analyzer was 0.5 L min^{-1}. Peak heights were measured by an integrator connected to the output meter of the analyzer after

Table 1. Chemical characteristics of manures used in the experiments

Manure	Total N	NH_4^+	NO_3^-	Dry matter ($g\ kg^{-1}$)	C/N ratio	Volatile fatty acids[z] ($g\ kg^{-1}$)	pH
	– (mg N kg^{-1} manure, as is) –						
Experiment 1							
Liquid dairy cattle manure 1	3400	1950	neg[x]	60	7.1	12.8	7.0
Solid beef cattle manure	4530	1183	neg	175	16.7	neg	7.8
Liquid dairy cattle manure 2	4400	2500	neg	80	7.1	11.6	7.3
Liquid beef cattle manure	4000	2533	neg	80	7.7	7.2	7.6
Liquid swine manure 1	4000	2667	neg	50	4.1	11.1	7.3
Liquid swine manure 2	2600	2000	neg	30	3.8	6.2	7.5
Composted manure	4370	125	10	158	14.9	neg	8.2
Experiments 2 through 4							
Liquid dairy cattle	3467	1720	neg	95	11.0[y]	ND[y]	7.5
Fresh solid beef	3900	677	neg	170	17.7[y]	ND	8.7

[z] Acetic, propionic and butyric acids.

[y] The amount of C in these manures was assumed to be 40% of the dry matter (based on analysis of similar manures).

[x] neg = negligible.

ND = not determined.

the analyzer was switched to measure NO only. The response of NO standards was linear to the highest concentration measured in the experiments.

The NO emission rate was calculated from the amount of NO accumulated 20 min after flushing and sealing of the bottles. Preliminary analysis showed that NO accumulation reached a plateau after 60 min, when NO consumption equalled NO production (Fig. 1). The NO accumulated during the first 20 min was considered the best approximation of NO production as determined by repeated flushing and measurement of NO emission from NH_4^+- and liquid dairy manure-amended soil (Fig. 2).

The rate of NO production during denitrification could not be measured in these experiments, perhaps because NO is not stable in the presence of C_2H_2. Nitric oxide standards injected into bottles containing 10 kPa C_2H_2 could not be recovered by measurement on the NO analyzer,

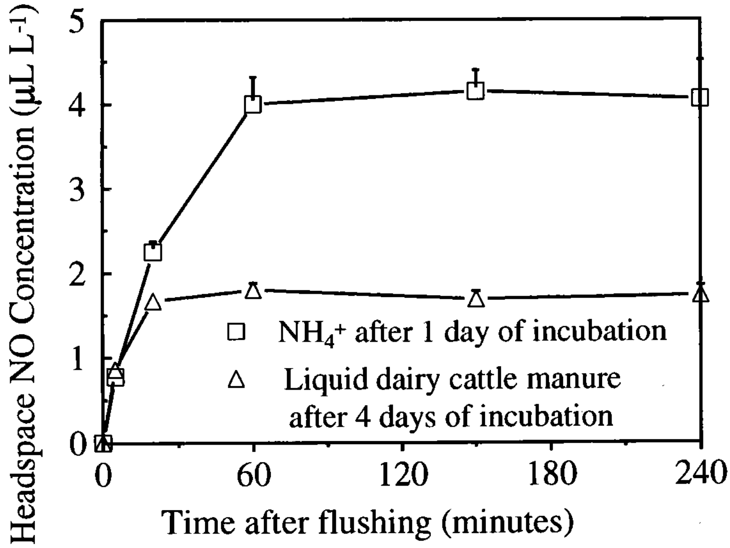

Fig. 1. Nitric oxide (NO) concentration measured from the headspace of sealed bottles containing $NH_4^{\pm}$ and manure-amended soils. Bars above data points indicate 1 SE (n = 3).

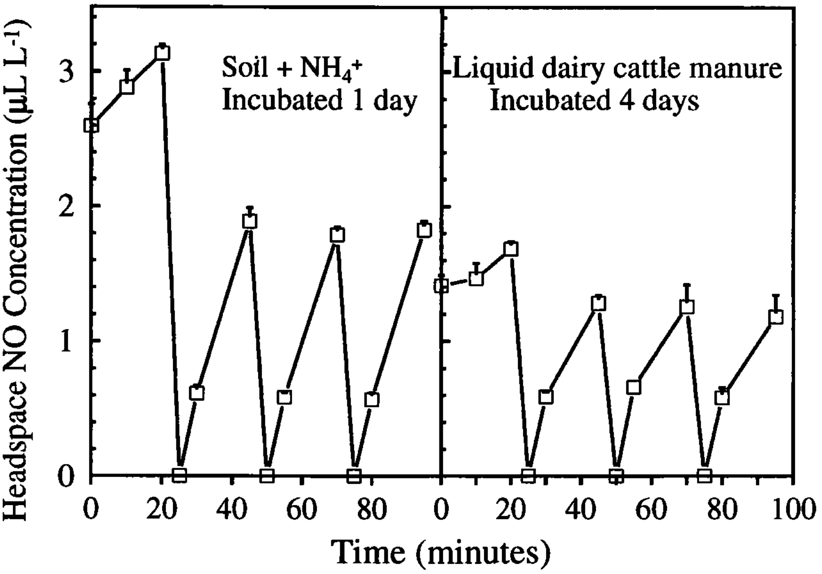

Fig. 2. Headspace NO accumulation at time of bottle sealing (without flushing, at time 0) and after flushing with air at 25, 50 and 75 min. Bars above data points indicate 1 SE ($n = 3$).

within a few seconds of injection, suggesting a chemical reaction between NO and C_2H_2 transforming NO into another compound. Davidson (1992) observed that the presence of high concentrations of C_2H_2 interfered with NO analysis.

Nitrous Oxide Emission from Various Manure-amended Soils (Exp. 1)

The purpose of exp. 1 was to compare N_2O emissions from unamended soil with those from soil amended with manure or NH_4^+. The experiment consisted of 10 treatments with four replicates in a completely randomized block design. The experimental unit consisted of a 250-mL centrifuge bottle containing 57.8 g soil (50.0 g, oven-dry basis). The treatments included soil amended with each of seven manures (80 g manure kg^{-1} soil), $(NH_4)_2SO_4$ (200 mg N kg^{-1} soil), $(NH_4)_2SO_4$ + CH_3COOH (neutralized to pH 7.0 with KOH; 200 mg N and 150 mg C kg^{-1} soil), and a control. Water was added to all bottles as required to bring the moisture content to 235 g kg^{-1} soil (−100 kPa). The soil in each of the bottles was thoroughly mixed with a spatula after the treatments were added. The bottles were covered with parafilm, which was punctured with several small holes to enhance gas exchange, and the bottles were then incubated at 25°C and 99% humidity.

Nitrous oxide was measured daily for the first 5 d, then again on days 7, 10 and 14. On each sampling day, the headspace of the bottles was flushed with air to ensure background levels of N_2O; the bottles were sealed for 6 h; then accumulated N_2O in the headspace was measured. The lids used to seal the bottles were fitted with a serum stopper to allow gas sampling (Suba Seal, Barnesley, U.K.). Nitrous oxide was analyzed on a Hewlett-Packard 3850A gas chromatograph equipped with a ^{63}Ni electron-capture detector. Background concentrations of N_2O were subtracted from the measurements.

The amount of N lost from the soil to N_2O emission or denitrification was estimated by summing the daily averages for the 14 d of the experiment. The amount of N lost from the control treatment was subtracted from each of the manured treatments to estimate N lost as a percentage of N added. This method gave results that were very similar to those obtained by fitting a polynomial curve to the data and solving to obtain the area under the curve.

Effect of Manure Concentration on N_2O Emission and Denitrification (Exp. 2)

This experiment focused on the change in N_2O emission and denitrification rates with three concentrations of manure in soil. The experiment consisted of seven treatments, which included liquid dairy-cattle and solid beef-cattle manure at rates of 20, 50 and 100 g manure kg^{-1} soil, as well as a control. The experimental unit was a 250-mL amber bottle containing 56.4 g soil (50.0 g, oven-dry weight). Water was added to each of the bottles to bring the moisture content to 235 g kg^{-1} soil (−100 kPa). The soil was mixed with a spatula after the treatments were prepared; the bottles covered with parafilm; then they were incubated at 25°C. Forty-four bottles of each treatment were prepared: four for measurement of extractable NH_4^+ and NO_3^- immediately after manure addition and eight for measurement of N_2O as well as NH_4^+ and NO_3 after each of 1, 2, 3, 4 and 6 d of incubation. The bottles were flushed with air and sealed with a serum stopper on each of the sampling days as described for exp. 1. For measurement of denitrification rates, four replicates received C_2H_2 (10 kPa) after the bottles were sealed (Yoshinari et al. 1977). For measurement of net N_2O emission rates, the other four replicates did not receive C_2H_2. Nitrous oxide concentration in the headspace was measured 6 h after the bottles were sealed. Ammonium and nitrate in the soil were extracted with 2*M* KCl, immediately after gas sampling, and analyzed using the method of Tel and Heseltine (1990).

The amount of N lost from the treatments to N_2O emissions or denitrification was estimated by summing the daily averages for the 6-d period as described for exp. 1.

Effect of Soil-moisture Content on N_2O and NO Emissions and Denitrification (Exp. 3)

The purpose of exp. 3 was to measure the change in N_2O and NO emissions from soil amended with liquid dairy-cattle manure (100 g manure kg^{-1} soil) and incubated at moisture contents of 180 g kg^{-1} (−250 kPa), 235 g kg^{-1} (−100 kPa) and 280 g kg^{-1} soil (−20 kPa). The three treatments represented soil incubated at 30% below field capacity, 10% below field capacity and 10% above field capacity, respectively. The experimental unit and the preparation of the treatments were as described for exp. 2. Sixty-four bottles of each treatment were prepared: four for measurement of 2*M* KCl-extractable NH_4^+, NO_3^- and NO_2^- immediately after manure addition and 12 for measurement of N_2O on each of day 1, 2, 3, 4 and 6. For measurement of denitrification rates, four replicates received C_2H_2 (10 kPa) after the bottles were sealed. For measurement of N_2O emission rates, four replicates did not receive C_2H_2. The other four replicates were flushed with Ar and received C_2H_2 (10 kPa) for measurement of potential denitrification rates under completely anaerobic conditions. Nitric oxide emission rates and extractable NH_4^+, NO_3^- and NO_2^- were measured from the four replicates receiving no C_2H_2 on each of the sampling days. Nitrous oxide and extractable NH_4^+, NO_3^- and NO_2^- were analyzed as they were for exp. 2. Nitric oxide was measured and analyzed as described earlier in the Nitric Oxide Analysis section.

Total N_2O and NO emissions and N lost by denitrification were estimated by summing the daily average rates for the 6 d of the experiment.

Effect of Soil Saturation and Added Nitrate on N_2O and NO Emissions and Denitrification (Exp. 4)

The objective of exp. 4 was to measure NO and N_2O emissions from saturated soil and soil receiving additional NO_3^-. The two treatments consisted of liquid dairy cattle manure (100 g manure kg^{-1} soil) incubated at soil-moisture contents of 235 and 400 g kg^{-1} soil (−100 kPa and saturation, respectively). A third treatment included soil amended with manure and 150 mg NO_3^--N at 235 g H_2O kg^{-1} soil. The experimental unit and the treatment preparation were as described for exp. 2. Nitrous and nitric oxide emissions were measured on days 1, 2, 3, 4, 5 and 6; denitrification rates were measured on days 1, 4 and 5.

Nitrous and Nitric Oxide Emissions from Soil Following Amendment with Nitrate, Ammonium or Carbon (Exp. 5)

The purpose of exp. 5 was to determine the relative importance of nitrification and denitrification on N_2O and NO emissions by amending soil with either NH_4^+ or NO_3^-, with or without a carbon source. The six treatments were soil amended with 0 and 150 mg N as $(NH_4)_2SO_4$ or KNO_3, each with and without 400 mg C kg^{-1} as glucose. The C and N were added in solution to bring the soil-moisture content to 235 g kg^{-1} soil. No manure was added to the soil in this experiment. The experimental unit and method of preparation of the treatments were similar to those of the previous experiments. Twenty-eight replicates of each treatment were prepared: four for analysis of extractable NH_4^+ and NO_3^- at the beginning of the experiment and eight for measurement of N_2O, NO and extractable NH_4^+, NO_3^- and NO_2^- on each of days 1, 2 and 3. Denitrification rates and N_2O emission rates were measured on four replicates on each day as described in exp. 2. Nitric oxide emissions were measured on the replicates not receiving C_2H_2. Nitrous oxide, nitric oxide and extractable NH_4^+, NO_3^- and NO_2^- were measured as described in the previous experiments. Carbon dioxide was analyzed on a Gow-Mac gas chromatograph equipped with a thermal-conductivity detector.

Statistical Analysis

Statistical analyses of N_2O for the experiments were performed after a log transformation of the data because the standard deviation increased with the treatment mean, a failure of one of the assumptions of ANOVA (Snedecor and Cochran 1980). The experiments were analyzed using ANOVA for each sampling day or as a split plot experiment using days as main plots and the treatments as the split plot. Statistical significance was set at $P < 0.05$. Standard error bars were included on the graphs to show the variation of the data.

RESULTS

Nitrous Oxide Emission from Various Manure Amendments (Exp. 1)

During the first 4 d of exp. 1, the N_2O emission rate from each of the manure-amended soils was higher than that of the $(NH_4)_2SO_4$-amended soil or the control, with the exception of the compost-amended soil (Fig. 3). Nitrous oxide emission from the

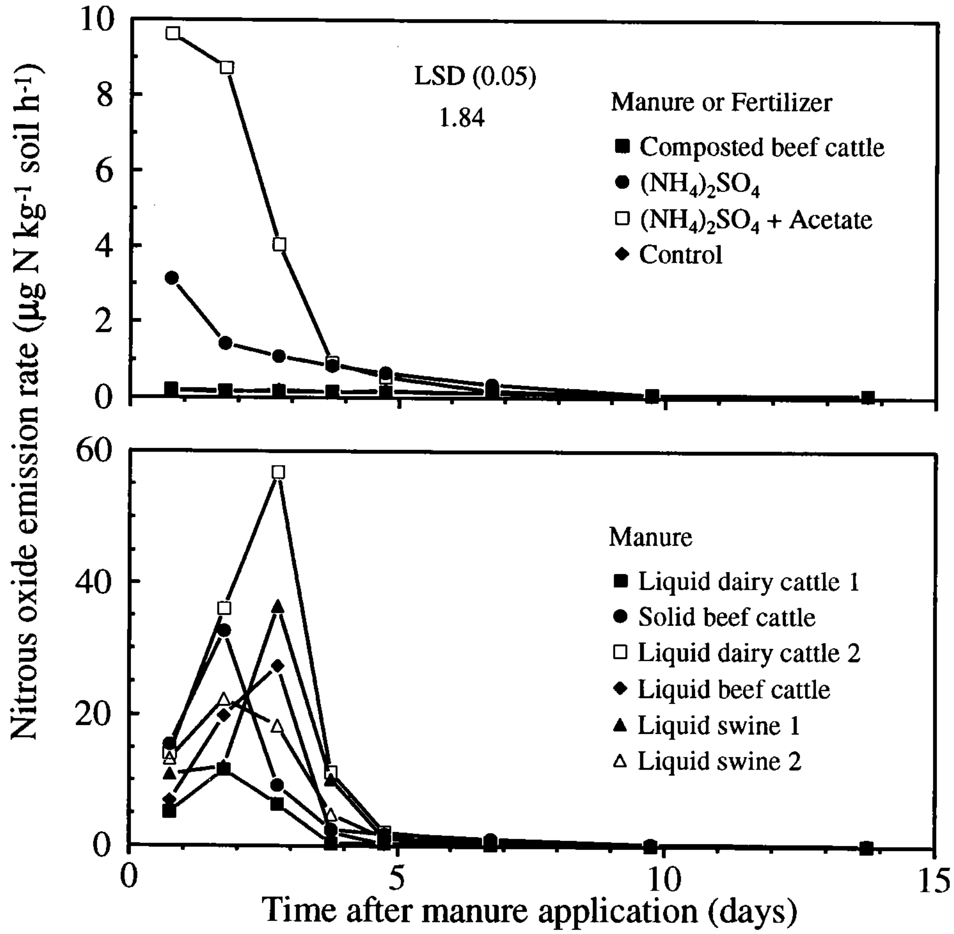

Fig. 3. Nitrous oxide (N_2O) emission rate from soil following application of various manures and $(NH_4)_2SO_4$ (exp. 1).

compost-amended soil was not significantly different from that of the control during the entire experiment. Soil amended with solid beef-cattle manure produced significantly higher N_2O emission rates than any other treatment on days 7 and 10. Differences between treatments on day 14 were not significant. In the soil amended with $(NH_4)_2SO_4$ and $(NH_4)_2SO_4$ + acetate, the highest N_2O emission rates were measured on day 1. Addition of C with the NH_4^+ resulted in significantly higher N_2O emission rates during the first 3 d.

Total N_2O emissions during the 14-d period were 0.09–2.22% of NH_4^+-N added or 0.09–0.85% of the total N added in manure or fertilizer.

Effect of Manure Concentration on N_2O Emission and Denitrification (Exp. 2)

Nitrous oxide emission rates increased significantly with increasing manure concentration and were not different between manure types at each manure concentration, except at the highest manure concentration, where liquid dairy-cattle manure produced the highest N_2O emission rate (Fig. 4). In soil amended with 100 g kg^{-1} manure, the highest N_2O emission rates occurred on days 2 and 3. Lower concentrations of solid beef-cattle

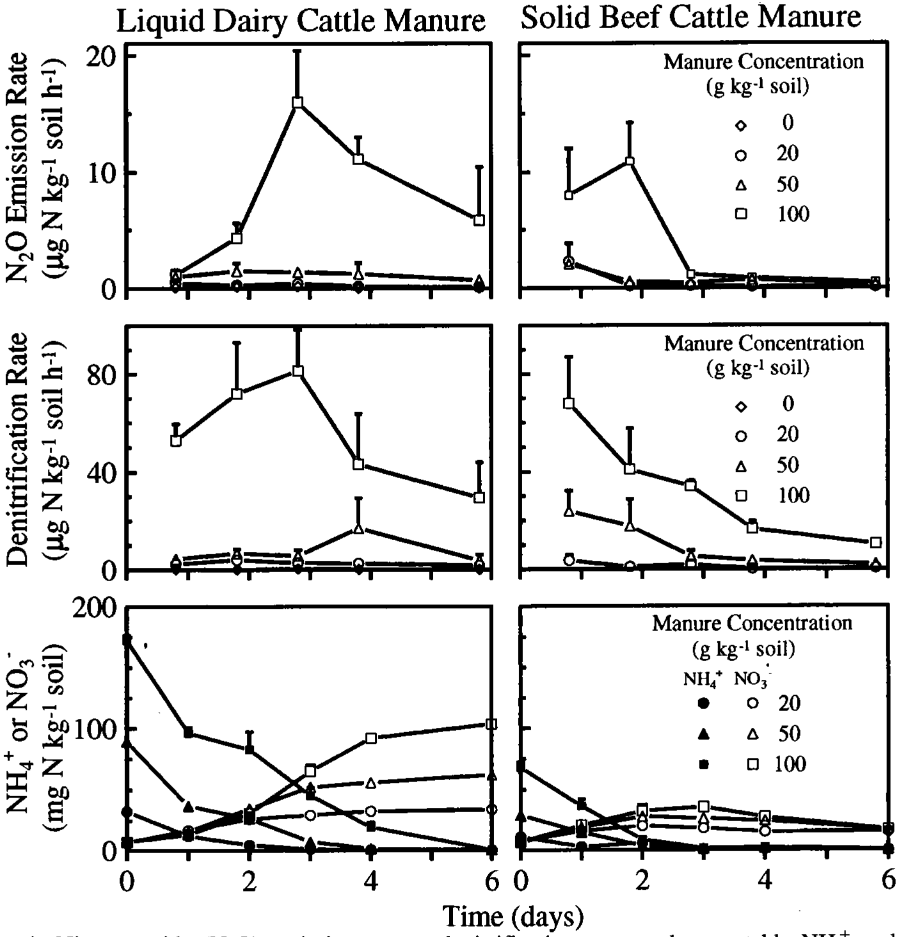

Fig. 4. Nitrous oxide (N_2O) emissions rates, denitrification rates and extractable NH_4^+ and NO_3^- concentrations in soil amended with liquid dairy-cattle manure or solid beef-cattle manure at concentrations of 20, 50, or 100 g kg^{-1} soil (exp. 2). Bars above data points indicate 1 SE (*n* = 4).

manure resulted in maximum N_2O emission rates on day 1.

Denitrification rates also increased significantly with increasing manure concentration. Denitrification rates were higher than the N_2O emission rates but followed a similar pattern. Notable exceptions were in the soils amended with 100 g kg^{-1} manure, where denitrification rates were proportionately larger than N_2O emissions on day 1 than on day 2 or 3.

Total denitrification losses were higher with the highest concentration of liquid dairy-cattle manure than with the highest concentration of solid beef-cattle manure. Denitrification losses during the experiment were 0.2–2.1% of the total N applied. Emission of N as N_2O constituted 0.05–0.30% of the total N applied.

Nitrification of NH_4^+ was complete after 2 d with the lowest concentration of manure and after 6 d with the highest concentration of liquid dairy-cattle manure. Maximum NO_3^- production rates occurred on days 1, 2 and 3 for the low, intermediate and high concentrations of liquid dairy-cattle manure, respectively.

Effect of Soil-moisture Content on N_2O and NO Emissions (Exp. 3)

Nitrous oxide emission rates were significantly higher at a soil moisture content of 280 g kg^{-1} than at 235 or 180 g kg^{-1} (Fig. 5). Nitrous oxide emissions peaked on day 4 with all treatments. Denitrification rates increased significantly with increasing soil-moisture contents and were highest on day 2 at moisture contents of 180 and 235 g kg^{-1} and on day 4 at 280 g H_2O kg^{-1} soil.

The potential for denitrification was highest on day 2 and decreased during the next 4 d. The potential for denitrification was approximately 100 times higher than the actual denitrification rates. Soil-moisture content did not significantly affect potential denitrification rate on any of the days. The amount of N denitrified under total anaerobic conditions on day 2 was slightly greater than the amount of NO_3^- extractable on that day (42 mg N_2O-N kg^{-1} soil vs. 35 mg NO_3^--N -kg^{-1} soil).

Nitric oxide emission also peaked on day 4 and was lowest on days 1 and 6. There was no significant effect of soil-moisture content on NO emission. There was also no significant effect of soil-moisture content on the rates of NH_4^+ decrease and NO_3^- increase. Nitrification of the NH_4^+ was complete by day 6. Nitrite concentrations of up to 5.5 mg N kg^{-1} soil were measured during the experiment. Nitrite concentration peaked on day 4, the same time as the peak N_2O and NO emissions and the peak denitrification rate.

The total amount of NO emitted during the experiment ranged from 0.9 to 1.3 mg N kg^{-1} soil. The amount of N_2O emitted was lower, ranging from 0.1 to 0.6 mg N kg^{-1} soil. Emission of N_2O was 0.025–0.13% of the NH_4^+-N applied, and emission of NO averaged 0.26% of the total manure N applied.

Effect of Soil Saturation and Added Nitrate on N_2O and NO Emissions (Exp. 4)

Nitrous oxide emission rates were much higher at soil-moisture contents of 400 g kg^{-1} than at 235 g kg^{-1} (Fig. 6). This reflected the higher denitrification rates in the soil incubated at 400 g H_2O kg^{-1}. Both denitrification rates and N_2O emission rates were significantly higher in soil amended with manure + NO_3^- than in soil amended with manure alone. Nitric oxide emission rates were lowest from soil incubated at 400 g H_2O kg^{-1} and highest from soil amended with NO_3^- and incubated at 235 g H_2O kg^{-1}.

Nitrous and Nitric Oxide Emissions from Soil Following Amendment with Nitrate, Ammonium or Carbon (Exp. 5)

Nitrous oxide emission rates were highest in soil amended with NH_4^+ (Fig. 7). Addition of C with the NH_4^+ resulted in an additional significant increase in N_2O emission.

Denitrification rates were also highest in soil amended with C and were highest in the NH_4^+-amended soil, intermediate in the NO_3^--amended soil, and low in the control. Denitrification in the three treatments without added C were very low.

Nitric oxide evolution rates were highest in soil amended with NH_4^+. Nitric oxide

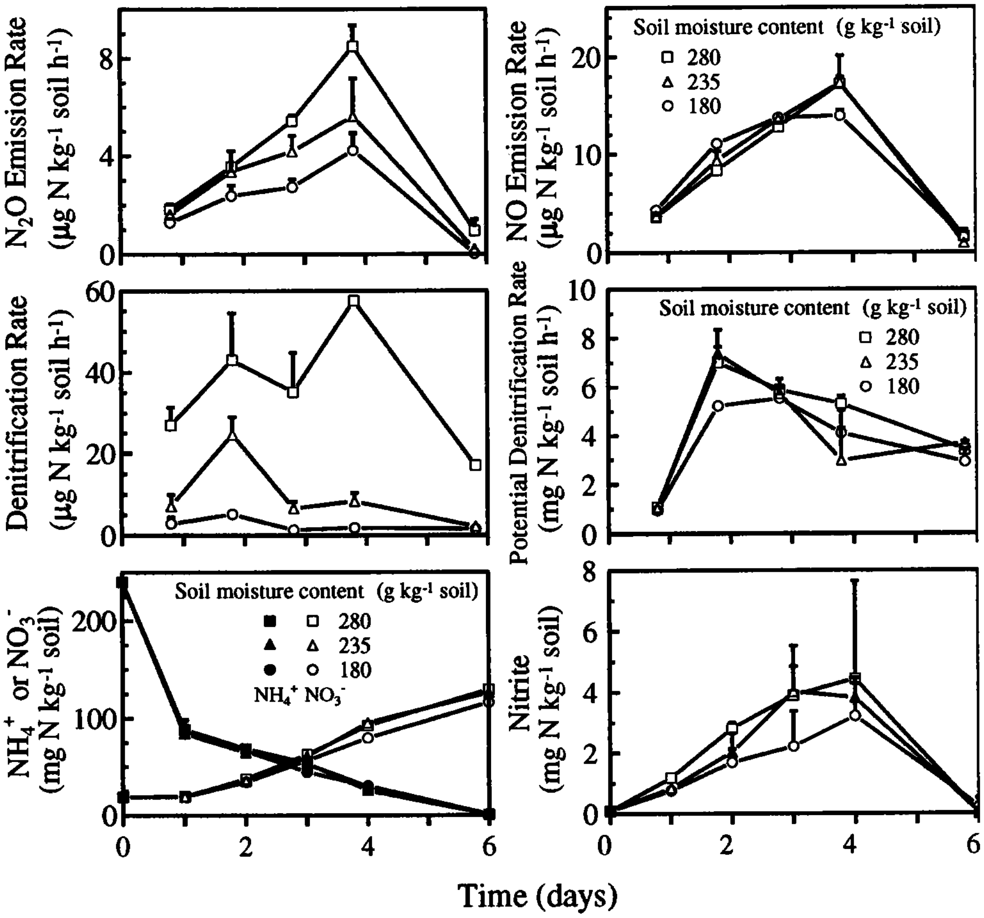

Fig. 5. Nitrous oxide (N_2O) emission rate, denitrification rate, NO emission rate, potential denitrification rate, and extractable NH_4^+, NO_3^- and NO_2^- concentrations in soil amended with liquid dairy-cattle manure at soil-moisture contents of 280, 235 and 180 g kg^{-1} soil (exp. 3). Bars above data points indicate 1 SE (*n* = 3).

evolution was negligible in both the NO_3^--amended soil and the control, whether or not C was added.

Disappearance of NH_4^+ was more rapid in the presence of C, probably as a result of immobilization by soil microorganisms. Production of NO_3^- was higher in soil amended with NH_4^+ alone than in soil amended with NH_4^+ + C. In the soils amended with NO_3^-, some of the N was probably immobilized during C oxidation. There was little NO_2^- accumulation in soil in any of the treatments. The highest concentration of NO_2^- was 0.2 mg N kg^{-1} soil on day 3 in soil amended with NH_4^+ alone.

Carbon dioxide emission from glucose-amended treatments was similar to that of the non-amended soils by day 3, suggesting that the microorganisms had completed decomposition of the glucose by this time. The peak CO_2 evolution may have occurred before the first measurement after 18 h.

DISCUSSION

Nitrous oxide emissions from soil began less than 1 d after manure or fertilizer addition,

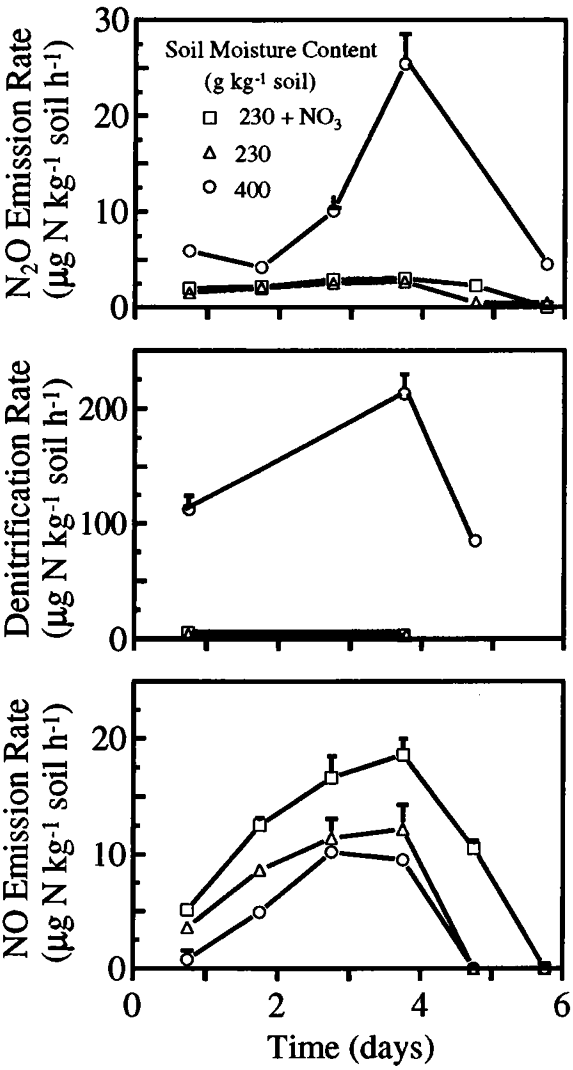

Fig. 6. Nitrous oxide (N_2O) and NO emission and denitrification rates in soil amended with liquid dairy-cattle manure at soil-moisture contents of 235 and 400 g kg^{-1} soil and in soil amended with liquid dairy-cattle manure and NO_3^- at a soil moisture content of 235 g kg^{-1} soil (exp. 4). Bars above data points indicate 1 SD (*n* = 3).

peaked between 1 and 4 d, and decreased to almost background levels within 6 d. Total emission of N_2O from manure-amended soil ranged from 0.025 to 0.85% of the total N applied in manure and up to 2% of the NH_4^+ applied in manure. The emission of N_2O may be significant in terms of its effect on ozone in the stratosphere, but it was not significant in terms of a loss of plant-available N.

The N_2O emission rates were comparable to those measured by Maag (1990) in a sandy loam soil amended with 20 g pig slurry kg^{-1} soil in the laboratory. He measured N_2O emission rates of up to 0.7 and 1.9 g N kg^{-1} soil h^{-1} in soil incubated at 50 and 100%

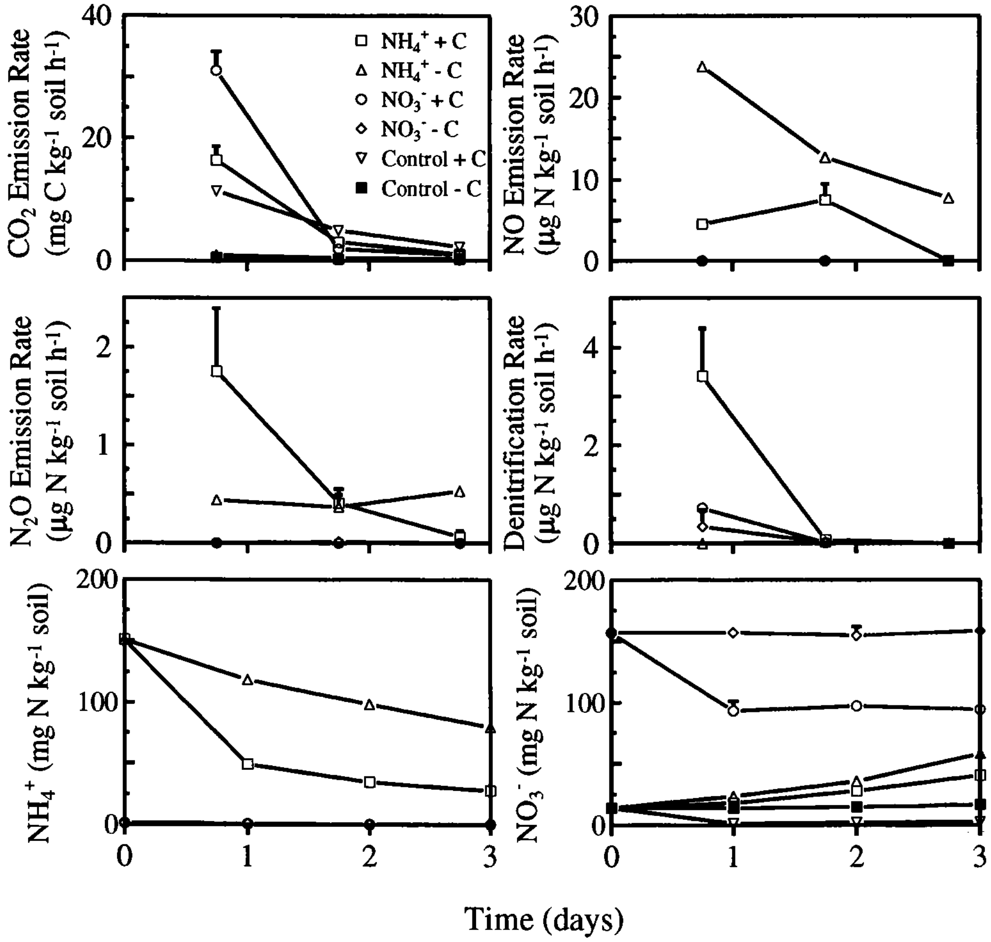

Fig. 7. Nitrous oxide (N_2O) NO and CO_2 emission rates, denitrification rate and NH_4^+ and NO_3^- concentrations in unamended soil or soil amended with NH_4^+, NO_3^-, with and without glucose (exp. 5). Bars above data points indicate 1 SD (*n* = 3).

of field capacity, respectively. This compares to N_2O emission rates of up to 0.5 and 1.5 µg N kg^{-1} soil h^{-1} in soil incubated at 85% of field capacity amended with 20 and 50 g kg^{-1} as liquid dairy-cattle manure (exp. 2).

Nitrous oxide emissions from $(NH_4)_2SO_4$-amended soils ranged from 0.09 to 0.28% of the N applied (exp. 1), which is within the range reported by other researchers. Breitenbeck et al. (1980) concluded that N_2O emission after fertilization with most NH_4^+ sources was <0.1–0.2% of that applied. Tortoso and Hutchinson (1990) calculated that 0.02% of $(NH_4)_2SO_4$ applied was emitted as N_2O. Breitenbeck and Bremner (1986) calculated N_2O emissions of 1.2% of added anhydrous NH_3 and concluded that the higher N_2O emissions resulted from higher soil NH_4^+ concentrations in an alkaline zone because of banding the fertilizer.

The total amount of N_2O emitted in soil amended with NH_4^+ (exps. 1–5) may have been underestimated in this study. Tortoso and Hutchinson (1990) showed that maximum N_2O emission rates in $(NH_4)_2SO_4$-amended soil occurred within 10 h of fertilizer application. In the experiments reported here,

N_2O emissions were not measured until 18 h after NH_4^+ addition.

Nitrous oxide emissions increased with soil-moisture contents. Nitrous oxide emission increased 600% as the soil-moisture content increased from below field capacity to saturation. Anderson and Levine (1987) also observed dramatic increases in N_2O emissions as soil-moisture content exceeded field capacity.

Denitrification was probably the major contributor to N_2O emissions, especially at the higher soil-moisture contents, as well as with increased manure concentrations. The increase in denitrification rate and N_2O emission with increasing soil-moisture contents and increased manure concentrations may be attributed to lower O_2 concentrations in the soil atmosphere due to the high O_2 demand during oxidation of readily available C compounds in manure at higher manure concentrations and decreased O_2 movement at higher soil-moisture contents.

Nitrification was also an important contributor to N_2O emissions. The highest rates of N_2O emission were measured at the time of the highest rates of NO_3^- production. In exps. 2 and 3, N_2O emission decreased to almost background levels by day 6, which was also when nitrification was complete. Experiment 5 also provided relevant information on the role of nitrification on N_2O emissions. Nitrous oxide emissions from soil at moisture contents of 80% of field capacity were negligible when NO_3^- was added to soil, even when glucose was added. In the presence of NH_4^+, both with and without glucose, N_2O evolution was significant.

The high denitrification rates in soil amended with NH_4^+ + C in the presence of C_2H_2 (exp. 5) are consistent with activity of heterotrophic nitrifiers that also have the ability to denitrify (Castignetti and Hollocher 1984; Robertson and Kuenen 1991). Anderson and Levine (1986) and Papen et al. (1989) showed that *Alcaligenes faecalis* was both a heterotrophic nitrifier and an aerobic denitrifier. Robertson and Kuenen (1990) studied the link between aerobic denitrification and heterotrophic nitrification and suggested that bacteria that were able to keep up denitrifying ability even under air saturation may have ecological advantages. The large amount of N denitrified within 6 h of total anaerobiosis (>30 mg N kg^{-1} soil h^{-1}) supports the suggestion that denitrifying enzymes of the microbial population must have been active. Bacteria having capabilities for both aerobic denitrification and heterotrophic nitrification have been isolated from sewage sludges, an environment similar to stored manures (Krul 1976; Robertson and Kuenen 1983).

The potential denitrification loss in manured soils shortly after manure application was high after an onset of anaerobiosis. The denitrification rate in exp. 3 was 7 mg N kg^{-1} soil h^{-1}, which is similar to the rate of 150 mg N kg^{-1} soil d^{-1} (6 mg N kg^{-1} soil h^{-1}) measured by Paul and Beauchamp (1989c). The potential for denitrification was high shortly after manure application because of the rapidly degradable short-chain fatty acids (Paul and Beauchamp 1989c).

Nitric oxide was emitted primarily during nitrification, not during denitrification. Both NO emission and nitrification were not affected by soil-moisture contents ranging from 180 to 280 g kg^{-1} soil (−250 to −20 kPa, exp. 3), and NO was produced from NH_4^+-amended soil but not from NO_3^--amended soil (exp. 5). Slemr and Seiler (1984) concluded that nitrification was much more efficient at producing NO_x than denitrification because NO was not emitted from saturated soils and more NO was emitted from NH_4^+-amended soils than from NO_3^--amended soils. Hutchinson et al. (1993) concluded, from a study using nitrification inhibitors, that NO was produced primarily during nitrification. Davidson (1992) concluded that NO was produced during nitrification, on the basis of observations of negligible NO production following treatment of the soil with C_2H_2 as a nitrification inhibitor. In the experiments of Davidson (1992), C_2H_2 was vented from flasks containing soil before NO measurements were made because he observed that the presence of C_2H_2 at high concentrations interfered with NO analysis.

Nitric oxide emission rates of up to 25 g N kg^{-1} soil h^{-1} measured in this study were comparable to those reported in other studies. Tortoso and Hutchinson (1990) measured NO emission rates of up to 18 g N kg^{-1} soil h^{-1} within 10 h of NH_4^+ application. Hutchinson et al. (1993) measured rates of up to 4 g N kg^{-1} soil -h^{-1}.

Denitrification did not contribute to NO emission; rather, it may have encouraged NO consumption. The NO-emission data in Fig. 1 suggest that NO may have been more rapidly consumed in the manure-amended soil, which supported higher denitrification rates. This may have caused the lower equilibrium NO concentration, despite similar NO production rates (Fig. 1). Davidson (1991) discussed the importance of denitrification as a sink for NO and suggested that denitrifying bacteria may utilize NO in preference to N_2O.

The pattern of NO and N_2O emissions measured in this study are consistent with the model presented by Davidson (1991) on the relationship between water-filled pore space of soil and the relative fluxes of N gases. He suggested that with lower water-filled pore space in soil, NO produced during nitrification is the major gas emitted. As the amount of water-filled pore space increases, more N_2O is produced during both denitrification and nitrification and NO emission decreases as a result of decreased nitrification rates and increased consumption of NO. In soil containing more than 80% water-filled pore space, N_2 is the major N gas emitted because NO or N_2O that was produced is consumed in the soil. In this study, NO emissions decreased when the soil-moisture content approached saturation. As the soil-moisture content increased, the denitrification rate and N_2O emissions rate increased, but the N_2O emission rate / denitrification rate ratio decreased. Although the pattern of NO and N_2O emission was consistent with the model presented by Davidson (1991), both NO and N_2O emissions occurred at 100% water-filled pore space. This may be in part due to the nature of the laboratory incubation, where a 2-cm layer of soil is exposed to the atmosphere, allowing greater diffusion of gaseous N products out of the soil than would occur in the field, even at higher soil-moisture contents.

Nitric oxide is usually measured with a flow-through gas analyzer (Nagele and Conrad 1990; Tortoso and Hutchinson 1990). In the experiments reported in this study, NO was analyzed by an injection of headspace gas as in the experiments of Davidson (1992). Figures 1 and 2 show that the 0–20-min time period was the best for measuring NO production rates because NO concentrations appeared to reach an equilibrium 60 min after the sealing of the flasks.

The observations of negligible N_2O emission rates after application of composted manure compared with the other manures (exp. 1) suggests implications for manure-management strategies for environmental sustainability. The entire manure-management system must be evaluated, however, from the time of excretion through to crop harvest. Significant quantities of N_2O are released during the composting process (Vogtmann and Besson 1978), compared with negligible release during anaerobic manure storage (unpubl. data). The question remaining is whether one type of manure-management system is more environmentally sustainable than another with respect to N_2O pollution of the atmosphere.

ACKNOWLEDGMENTS

This research was supported by the Ontario Ministry of Agriculture and Food as well as a Natural Sciences and Engineering Research Council of Canada postgraduate scholarship to the senior author.

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