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Denitrification in nitric-acid-treated cattle slurry during storage

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Abstract

Treatment of cattle slurry with HNO_3 to lower the pH is a measure to reduce NH_3 volatilization from this slurry during storage and after surface application. Moreover, the addition of HNO_3-N increases the fertilizer value of the slurry. Incubation studies were carried out to examine possible NO_3^- losses and N_2O emission from HNO_3 treated slurry during storage. Batches of cattle slurry were treated with various amounts of HNO_3 to obtain a pH range of 6.0 to 3.0. The slurries were stirred once or twice a week and stored for 6 months at 15 °C. Changes in pH, Eh, concentrations of NO_3^- and NH_4^+, and emissions of N_2O, CO_2 and CH_4 were monitored. The loss of NO_3^- and the emission of N_2O were related to slurry pH, being lowest at low pH. Cumulative loss of NO_3^- ranged from about 40 mmol kg^{-1} for slurries of target pH ≤ 5.0 to about 400 mmol kg^{-1} for slurries of target pH 6.0. Homogenization of the slurries via stirring and addition of H_2O_2 decreased the NO_3^- loss and H^+ consumption. The strong pH-dependance of the NO_3^- loss, the production of N_2O and the stoichiometry of H^+ consumption and NO_3^- loss indicated that the loss of NO_3^- was mainly due to microbiological denitrification. Similar N_2O production rates in the presence and absence of C_2H_2 indicated that nitrification was not an important source of N_2O. It is concluded that lowering the pH to values ≤ 4.5 as well as regular and complete homogenization of the slurry via stirring are important for the success of the on-farm treatment of slurry with HNO_3.

Keywords: nitrous oxide, ammonia volatilization, proton consumption, redox potential, pH

Introduction

In the past few years a number of measures has been introduced to reduce ammonia (NH_3) emission from cattle slurries on Dutch dairy farms. Such measures include immediate storage of slurry in proper covered basins, application through injection into the soil and feeding the cattle an adjusted ration (e.g. Den Boer et al., 1990). In practice, a number of measures often have to be combined, to ensure that all entrapped NH_3 can be used for plant growth and to prevent that entrapped N is lost via other pathways in the N cycle.

An alternative measure that may integrate the effect of a number of measures is the treatment of cattle slurry with acid to pH 4.0-4.5, immediately after its deposition by the animal in the livestock building. Due to the shift in the NH_3:NH_4^+ equilibrium, the emission of NH_3 from acid treated slurry is reduced during storage, and during and after surface application. Field experiments have shown indeed that NH_3 losses from such slurries are much lower than from untreated slurry after surface application (e.g. Stevens et al., 1989). If treated with nitric acid (HNO_3), the fertilizer value of the slurry also increases, strongly reducing the need for the additional use of mineral N fertilizers.

Unfortunately, a mixture of cattle slurry and HNO_3 is not very stable. Cattle slurries are highly anoxic and under such conditions the added NO_3^- may be denitrified to nitrous oxide (N_2O) and N_2, even though the low pH will strongly limit the rate of the microbial denitrification (Knowles, 1982). The trace gas N_2O possibly contribute to global warming (Wang et al., 1976) and the catalytic destruction of stratospheric ozone (Crutzen, 1976). Denitrification also increases the pH of the slurry, so that NH_4^+ may be deprotonated into NH_3° and then still be emitted into the atmosphere. Control of denitrification seems therefore crucial for a successful on-farm implementation of the treatment of cattle slurries with HNO_3.

Thus far, pilot farms have faced variable NO_3^- losses from HNO_3 treated slurries of pH 4.0-4.5 during storage. Variations in slurry composition, storage time and temperature, and insufficient mixing leading to variations in slurry pH have been put forward as major factors accounting for the variable NO_3^- loss. This prompted us to study the effects of pH, slurry mixing and storage time on denitrification and trace gas emissions from HNO_3 treated slurries during storage and after surface application. This paper mainly deals with the NO_3^- losses from HNO_3 treated slurries during storage under semicontrolled conditions. Emissions of N_2O after surface application of treated and untreated slurries are summarized by Velthof & Oenema (1993).

We examined the effect of pH values ranging from 3.0 to 6.0 on NO_3^- loss from slurry during storage. This range is much wider than the range of pH 4.0 to 4.5 which has been proposed for the on-farm treatment and storage of slurry.

Materials and methods

Titration curves of cattle slurries

Titration curves were established by adding 0.25 to 0.5 ml of 10.3 M HNO_3 to 200 g slurry under continuous stirring. Once the pH stabilized, usually after 5 to 10 min, another 0.25 to 0.5 ml HNO_3 was added until the pH was 3.0. Reverse titration was carried out by intermittently adding 0.25 to 0.5 ml of 4.0 M NaOH to 200 g slurry under continuous stirring.

Incubation of cattle slurries

Effects of pH, mixing and storage time on NO_3 losses and N_2O emission from HNO_3 treated cattle slurry were studied in a batch experiment with an incomplete randomized block design.

Fresh cattle slurry of less than one week old was obtained from a local dairy farm. Homogenized samples of 12 kg each in 38 PVC containers (26 l) were acidified to pH 6.0, 5.0, 4.5, 4.0, 3.5 and 3.0 by slow addition of 10.3 M HNO_3 under moderate stirring. A control treatment with no addition of HNO_3 was included. At all 7 pH levels, there were three stirring treatments in duplicate, except for pH levels 3.5 and 3.0, which were combined with two stirring treatments only. Slurries of stirring treatment A were gently stirred with a rudder blade at 90 revolutions per min, for 2 min once a week. This allowed sampling from homogeneous slurries. Slurries of treatment B were heavily stirred at 240 revolutions per min, for 5 min twice a week. Slurries of treatment C received as oxidant 7.5 mmol H_2O_2 kg^{-1} twice a week and were stirred as B. Oxidation of some of the easily metabolizable organic compounds in the slurry by H_2O_2 was assumed to diminish the amount of substrate for denitrifiers and hence the loss of NO_3^-. Combinations of treatment C and pH levels 3.5 and 3.0 were excluded. The purpose-designed rudder blades allowed a rapid and complete mixing of all slurry. Containers with slurry were incubated in a climate-conditioned room at 15±0.5 °C for 6 months from January to August 1991.

Denitrification rates in the slurries were deduced from the loss of NO_3^- and consumption of protons (H^+) in the course of time. Additional measurements of denitrification rates were carried out, using the acetylene inhibition technique (Ryden & Rolston, 1983). Acetylene (C_2H_2) is an inhibitor of nitrification (Bremner & Blackmer, 1979) and of the reduction of N_2O to N_2, the last step in the denitrification process (Yoshinari & Knowles, 1976). Portions of 10 ml slurry were incubated under ambient air and under ambient air with 10 % acetylene in glass vials with rubber septa. Gas samples of 0.5 ml were taken every 30 min from the head space, and were analyzed for N_2O.

The pH of the incubated slurries was monitored once or twice a week, and the Eh once every two weeks. HNO_3 was added to the slurries if the actual pH exceeded the target pH by more than 0.15 units. Concentrations of dissolved and exchangeable NH_4^+ and NO_3^- in the slurries were measured in 20-ml subsamples, after homogenization by stirring and correction of slurry pH if necessary. The subsamples were transferred to 50-ml polypropylene tubes containing 25 ml 1.0 M KCl. After shaking for 15 min, the slurries were centrifuged at 4000 revolutions per min for 15 min. The supernatant was diluted 100 to 250 times and analyzed by means of Technicon autoanalyzer. N_2O concentrations in the slurries were determined in 5-ml subsamples transferred into 70-ml glass vials with rubber caps. Vials were strongly agitated for 5 min and the concentration increase in the headspace was analyzed via gas chromatography.

Fluxes of N_2O and CO_2 from incubated slurries were calculated from the changes in the N_2O and CO_2 concentrations in the headspace after the containers were sealed, usually for 0.5 to 2 h. Gas samples were taken with 12-ml polypropylene syringes through rubber septa in the caps at regular time intervals. Samples were analyzed by gas chromatography. During the last month we also used a photo acoustic infra-red Multi-gas Monitor 1302 from Brüel & Kjær, directly attached to the containers (cf. Velthof & Oenema, 1993).

Analytical procedures

Redox potential (Eh) and pH were measured electrochemically. NO_3^- and NH_4^+ were analyzed by standard auto-analyzer methods (Technicon; US no 824-87T and US no 795-86T, respectively). The NO_3^- analyses were reasonably accurate; the linear relationship between the amount of NO_3^- added (x) via HNO_3 and the amount of NO_3^- analyzed (y) in 42 samples was: $y = 0.03 + 0.99x$ with $r^2 = 0.995$. Total C, N, P and K in the slurry were analyzed using standard methods (Vierveijzer et al., 1979). N_2O and CO_2 in 0.5 ml gas samples were analyzed via a PU 4400 gas chromatograph using a 27.5 m Porapak Q wide bore column and an electron capture detector (ECD).

Results

Changes in overall slurry composition

The fresh slurry, a mixture of urine and dung, had a relatively high pH and high total N and K contents compared to average values for cattle slurry (Table 1). Organic C content of the solids was about 42% and the molar ratio of total C:N:P was about 101:13:1. During storage, the weight of the slurries decreased by about 14% due to evaporation. To facilitate comparison, contents of incubated slurries were corrected for weight loss by evaporation. Total solids, organic C, total N and NH_4^+ contents of untreated slurry decreased during storage due to decompostion of organic matter and subsequent volatilization of CO_2, NH_3 and possible other volatile compounds like fatty acids and H_2S. The pH of the untreated slurries gradually decreased from 8.4 to about 7.5 within 30 days, possibly as the result of NH_3 volatilization and precipitation of carbonate and struvite (Bril & Salomons, 1990), and remained at that level for the remaining 145 days.

Addition of HNO_3 slightly increased the total volume and the content of total solids of the slurry. The treatment with HNO_3 had a significant effect on the C con-

Table 1. Average composition of the cattle slurry at the start of the experiment and after incubation for six months at various pH levels (g kg^{-1}). The composition of incubated slurries was corrected for weight loss due to evaporation.

	Fresh slurry	Slurries after six month incubation			
		Untreated	HNO_3-treated		
pH	8.4	7.5	6.0	4.5	3.0
Total solids	113	88	118	132	133
Organic C	47	38	38	42	45
NH_4^+-N	4.3	3.0	3.4	4.1	4.1
NO_3^--N	0.0	0.0	4.9	6.5	7.0
Total N	7.3	*	–	–	–
Total P	1.2	–	–	–	–
Total K	7.1	–	–	–	–

* Not determined.

tent of the slurry after incubation for 6 months. Stirring did not have a distinct effect, so that averaged values of treatments A, B and C are given in Table 1 for selected pH-levels only. After storage for 6 months, the organic C and NH_4^+-N contents of slurries of pH ≤5.0 were only slightly decreased relative to the fresh slurry, suggesting that rates of organic matter decompostion and NH_3 volatilization were low. However, organic C and NH_4^+-N contents were significantly decreased in the HNO_3 treated slurry of target pH 6.0.

Effects of HNO_3 addition on slurry pH and Eh

The relationship between added amounts of acid (H^+) and base (OH^-) and the pH of the slurry is shown in Fig. 1. The slurry had a high acid neutralizing capacity (ANC). The ANC of the slurry was highest within the range of pH 7.0 to 6.0. Ageing of untreated slurries slightly decreased the ANC at pH ≥6.0, possibly as a result of NH_3 volatilization, and increased the ANC in the range of pH 6.0-5.0. Total ANC was little affected during storage. The base neutralizing capacity (BNC) of slurry that was acidified to pH 2.5 was less than half of the ANC of untreated slurry (Fig. 1B). This suggests that a large fraction of the ANC was associated with (bi)carbonate and anions of fatty acids (Husted et al., 1991; Japenga & Harmsen, 1990), which volatilized as CO_2 and volatile fatty acids after H^+ addition. The BNC was about 42 mmol kg^{-1} per unit pH change and was essentially constant between pH 2.5 and 7. Once the ANC associated with volatile compounds had escaped, the titration and reverse titration curves were similar (Fig. 1C), provided that care was taken not to trap CO_2 in the slurry at high pH.

Addition of HNO_3 increased the Eh of the slurry. Untreated slurry was highly anoxic and had a mean Eh of about –400 mV (Fig. 2). The Eh of HNO_3 treated slurries was ranging from 0 to 300 mV. More intensive stirring (treatment B) and H_2O_2 addition (treatment C) had no distinct effect on the Eh of untreated slurry and HNO_3 treated slurry of target pH 6, but significantly increased the Eh of HNO_3 treated slurries of pH ≤ 5.0. Effects of stirring became most clear after about 40 days (Fig. 2); after the initial 'adaption phase' the Eh remained relatively constant. Even though Eh was increased by stirring and the addition of HNO_3 and H_2O_2, it did not surpass 400 mV, a redox potential which others have found as an upper limit for denitrification (e.g. Letey et al., 1982).

Rates of H^+ and NO_3^- consumption

A typical course of the actual slurry pH during storage is shown in Fig. 3. The pH of HNO_3 treated slurries of target pH < 5.0 remained relatively constant for most of the storage time. Addition of H_2O_2 (treatment C) slightly decreased the pH, suggesting the release of H^+ through the oxidation of reduced inorganic compounds, like H_2S and NH_4^+. A notable increase in pH was observed after day 125, irrespective of stirring. This increase was probably associated with the gradual increase of the incubation temperature from 15±0.5 °C until day 125 to about 22 °C during the last month. The reason for this increase was that the climate conditioner could not cope with the high out-

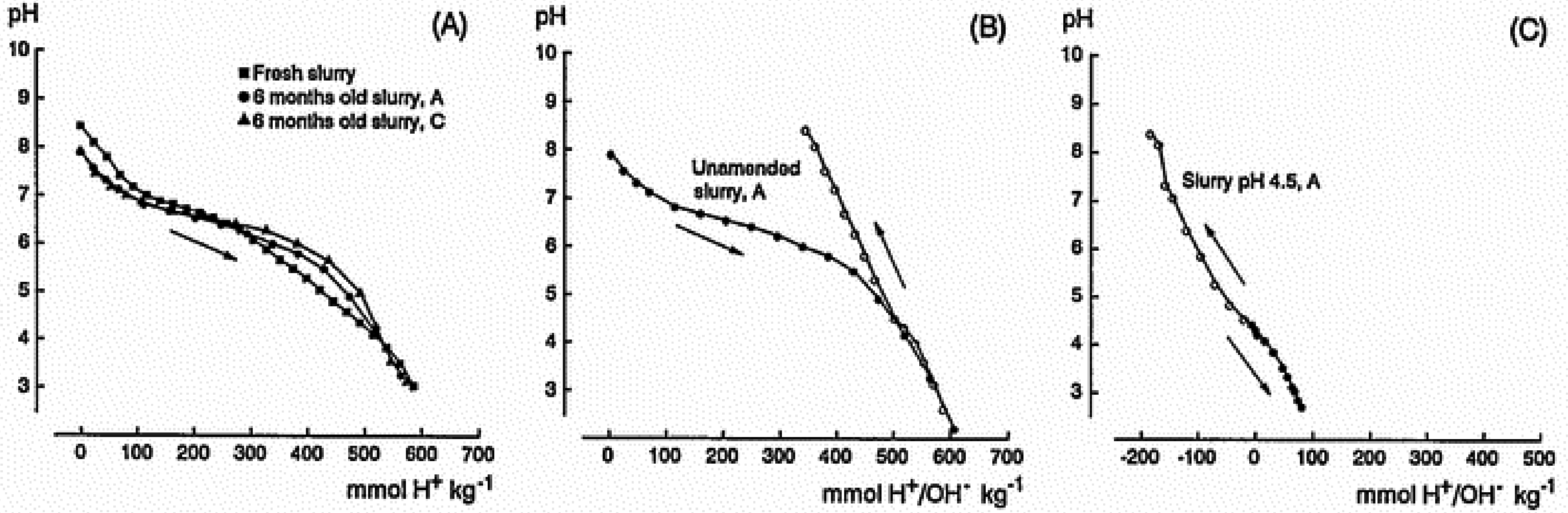

Fig. 1. Titration curves of cattle slurries. A) Fresh and 6 months incubated but untreated slurries of stirring treatment A and C. B) Untreated slurry, which was incubated for 6 months, titrated with HNO_3 to pH 2.5 and reverse with NaOH. C) Titration and reverse titration of HNO_3 treated slurry of pH 4.5, which was incubated for 6 months.

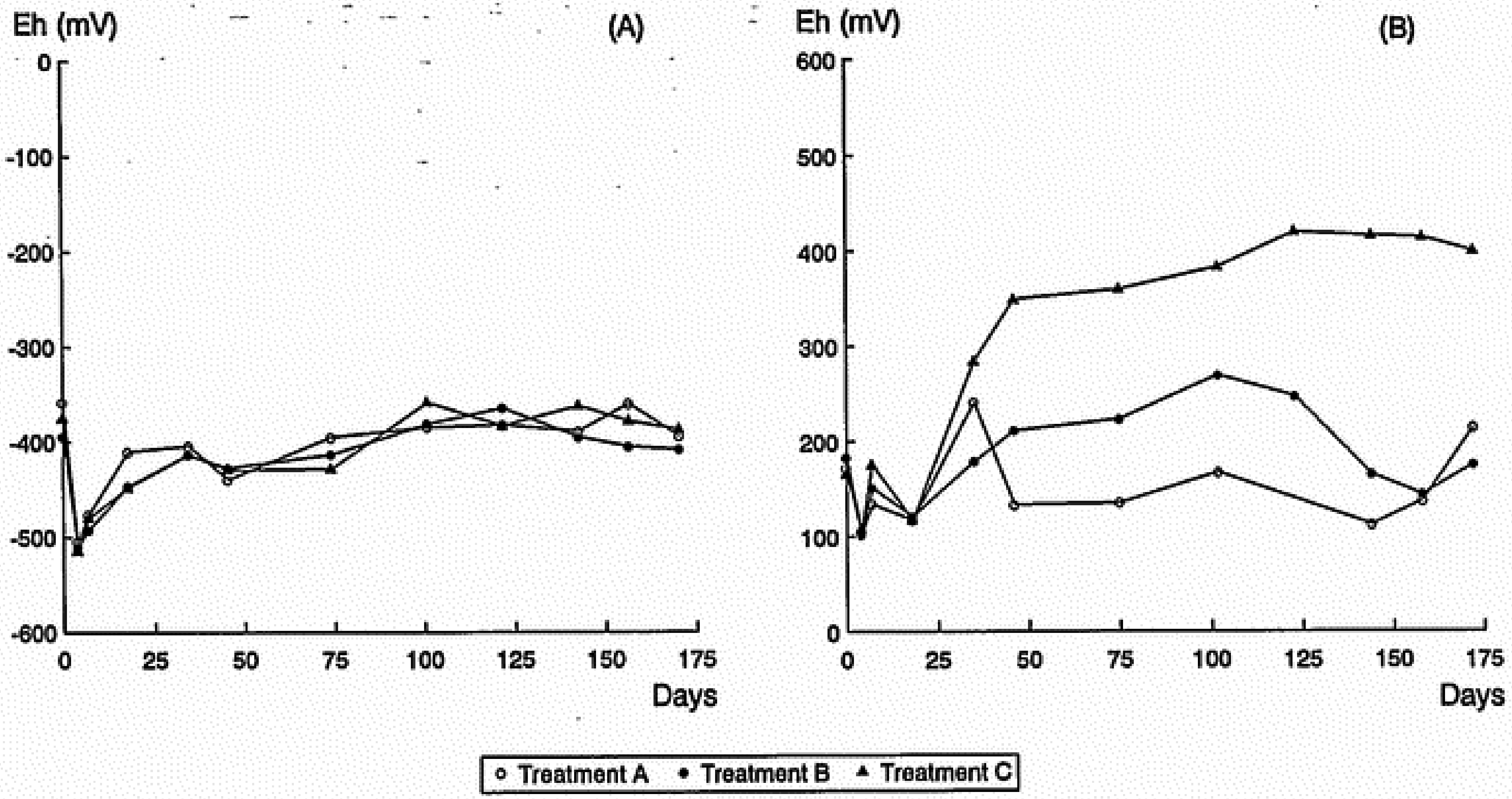

Fig. 2. Changes in redox potential (Eh) of slurry during incubation for 6 months. A) Untreated slurry. B) HNO_3 treated slurry of pH 4.5. Note the scale differences of the y-axis.

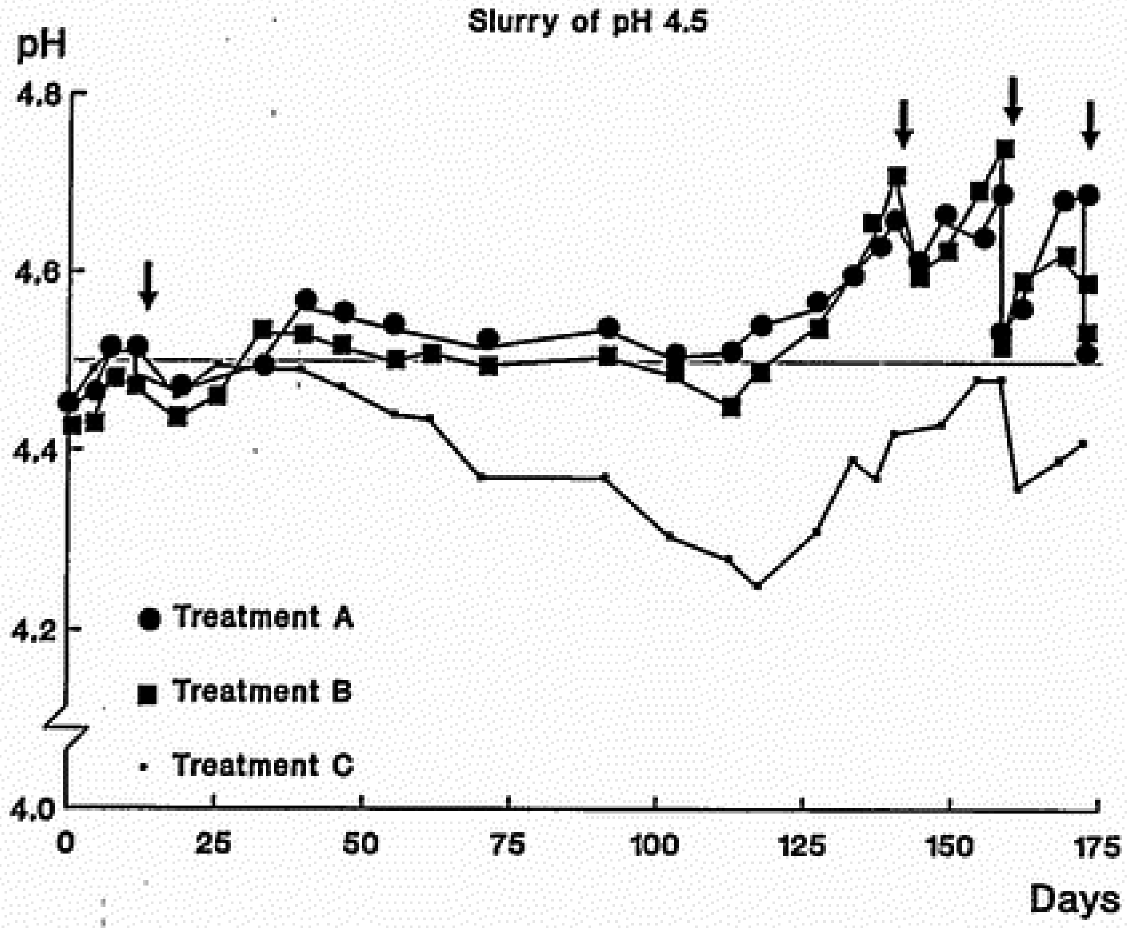

Fig. 3. Changes in pH of HNO_3 treated slurry of target pH 4.5, as a function of stirring treatments A, B and C, and incubation time. Arrows indicate pH correction by HNO_3 addition.

side temperature during the hot summer months of 1991. As a result, more than 75% of the total H^+ consumption in slurries with target pH < 5.0 occurred after day 125.

Slurries of target pH 6.0 had a high and nearly constant H^+ consumption rate throughout the incubation period (Fig. 4). The mean H^+ consumption rate was more than 2 mmol kg^{-1} d^{-1} for slurries of stirring treatment A. More intensive stirring and addition of H_2O_2 decreased the rate of H^+ consumption. The increase in H^+ consumption rate of slurries of stirring treatment A and B between day 112 and 138 was due to the fact that the pH was allowed to rise to about 8 between day 112 and 138. Such a rise in pH may have accelerated H^+ consumption rate. The temperature rise noted before may also have contributed. Table 2 summarizes the total H^+ consumption rate during incubation. Evidently, pH had a strong effect on H^+ consumption. H^+ consumption rate was doubled between pH ≤ 4.5 and 5.0 but increased tenfold between pH 5.0 and 6.0. Differences between stirring treatments A and B were relatively small, but addition of H_2O_2 in treatment C significantly decreased H^+ consumption.

Concentrations of NO_3^- were measured when the actual pH was equal to the target pH, after adjustment via HNO_3 addition if necessary. There were statistically

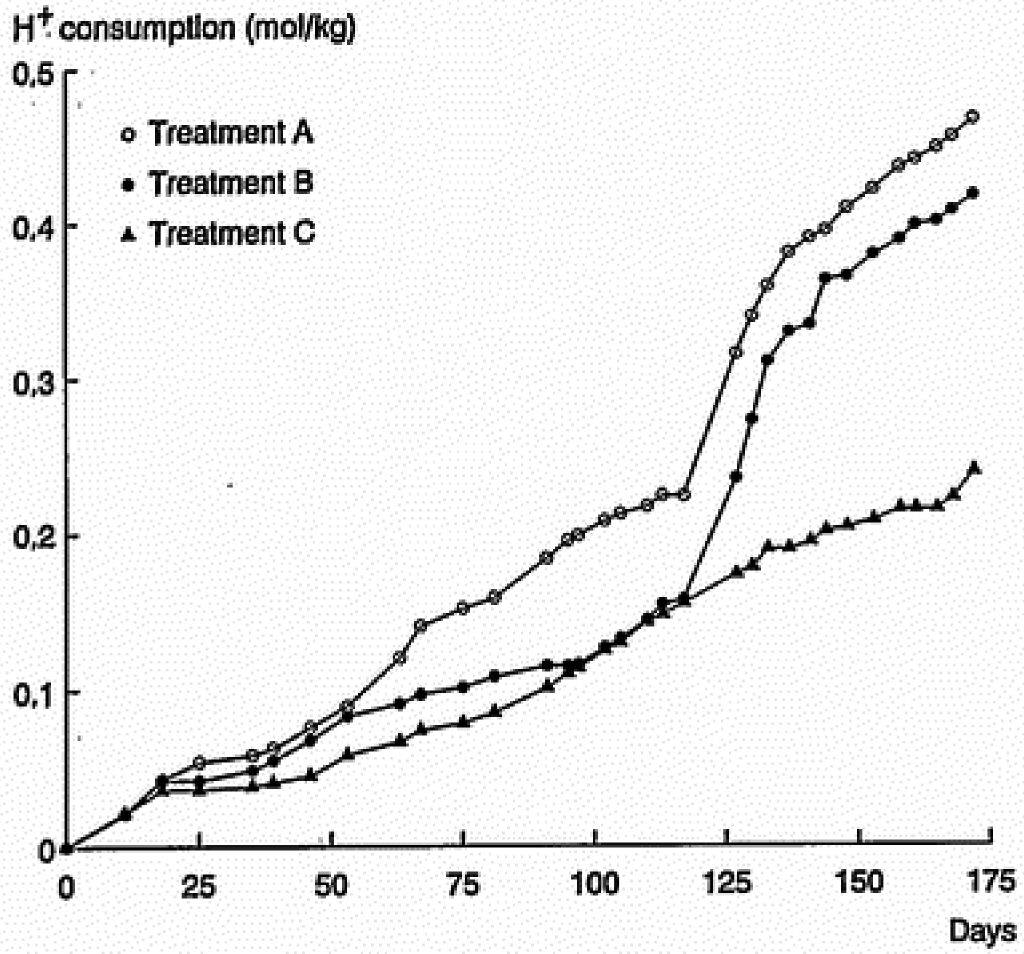

Fig. 4. Cumulative H^+ consumption by HNO_3 treated slurries of target pH 6.0 and stirring treatments A, B and C during incubation for 6 months.

significant effects of target pH and date on the NO_3^- and NH_4^+ concentrations and there was a significant date × pH interaction (Table 2). Stirring had no significant effect on NO_3^- and NH_4^+ concentrations. During incubation, NO_3^- concentrations increased in slurries of pH 6.0 and decreased in slurries of pH $\leq$ 4.0. Irregular variations were measured in slurries of pH 4.5 and 5.0. Changes in NO_3^- concentration were most apparent from the second month onwards. The overall mean NO_3^- loss was 8 mmol kg^{-1} between the first and last samplings. The significant effect of the date and the significant date × pH interaction for the NO_3^- data suggest that the H^+:NO_3^- stoichiometry in the slurries slightly changed during incubation. However, as it follows from the Least Significant Difference (L.S.D.) in Table 2, the standard error of difference (s.e.d.) was rather large for the NO_3^- data. NH_4^+ concentrations decreased in untreated slurries and in HNO_3 treated slurries of pH 6.0 during incubation. This decrease was probably due to NH_3 volatilization; it occurred predominantly during the first month, concomitant with the pH decrease in untreated slurry. The slightly higher NH_4^+ losses in stirring treatment B than in treatment A suggest that the more intensive stirring in B has stimulated NH_3 volatilization. The total mean NH_4^+ loss was 17 mmol kg^{-1}.

Flux of N_2O, CO_2 and CH_4

Fluxes of CO_2 and CH_4 strongly decreased with a decrease in pH (Fig. 5), suggesting

Table 2. Cumulative mean H^+ (HNO_3) consumption and mean net changes in NO_3^- and NH_4^+ concentrations (mmol kg^{-1}) during incubation for 172 days, as a function of slurry pH and stirring treatments A, B, C. A summary of the analysis of variance of NO_3^- and NH_4^+ concentrations is given in the second part of the table.

pH	H^+ consumption			Change in [NO_3^-]			Change in [NH_4^+]		
	A	B	C	A	B	C	A	B	C
~ 7.5	n.d.	n.d.	n.d.	0	0	0	–80	–109	–89
6.0	467	417	240	+40	+55	+36	–61	–74	–49
5.0	41	29	8	+20	+7	–23	+5	+1	–3
4.5	23	25	0	–31	+40	+22	–13	+24	+27
4.0	15	21	0	–29	–46	–62	0	–11	–7
3.5	23	14	n.d.	–36	–46	n.d.	–17	–2	n.d.
3.0	18	23	n.d.	–35	–36	n.d.	–18	–31	n.d.

	NO_3^- concentration		NH_4^+ concentration	
	P	L.S.D.	P	L.S.D.
DATE	0.059	11	<0.001	6
STIRRING	0.862	9	0.281	6
PH	<0.001	16	<0.001	7
DATE × STIRRING	0.777	16	0.591	10
DATE × PH	<0.001	28	<0.001	13
STIRRING × PH	0.280	23	0.293	13
DATE × STIRRING × PH	0.569	39	0.140	22

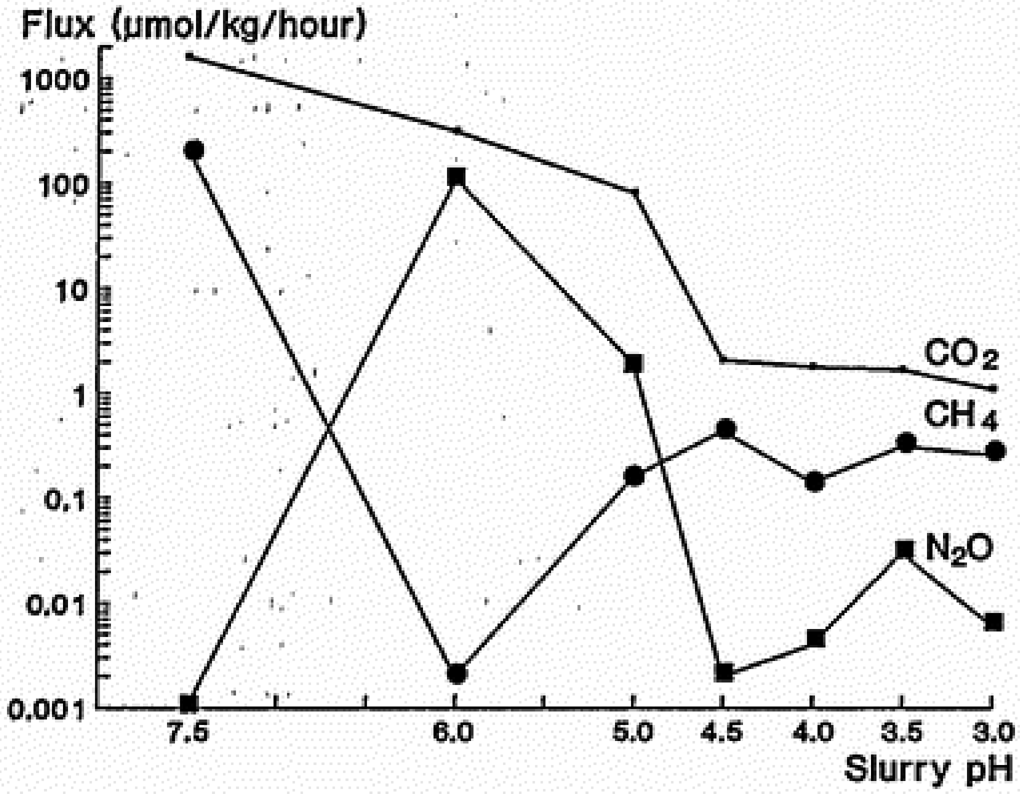

Fig. 5. Fluxes of CO_2, CH_4 and N_2O from untreated slurry of pH 7.5 and HNO_3 treated slurries of pH 6.0, 5.0, 4.5, 4.0, 3.5 and 3.0, all from treatment A, on July 7 1991. Note the log scale of the y-axis.

that lowering the pH via the addition of HNO_3 strongly decreased organic C respiration. For CO_2, the effect was strongest between pH 5.0 and 4.5, whereas even small additions of HNO_3 almost completely inhibited methanogenesis. Flux of N_2O peaked at pH 6.0. Mean N_2O fluxes from slurries of pH 6 were about two orders of magnitude higher than those from slurries of pH ≤ 5.0 (Oenema et al., 1993). At pH 6.0 the molar C/N ratio of the CO_2 and N_2O fluxes ranged from 2 to 4, being lowest with stirring treatment A and highest with C. At pH ≤ 5.0, the molar C/N ratio was one to two orders of magnitude higher than at pH 6.0.

The possible role of nitrification as source of N_2O was examined in additional batch experiments. Vials containing HNO_3 treated slurry of pH 4.5 and 10 % C_2H_2 in the headspace produced 22.9±0.9 and those without C_2H_2 21.5±1.9 μg N_2O-N kg^{-1} d^{-1} ($n = 5$). The similar N_2O production, in the presence and absence of C_2H_2, indicates that N_2O was not produced via nitrification but probably via denitrification.

Profiles of pH and N_2O in slurry

Measurements of pH and N_2O concentration in unstirred slurries at various depths may provide insight into a possible depth-dependent rate of H^+ consumption and N_2O production. In slurries of target pH ≤ 4.5 the pH remained relatively constant with depth for more than 50 days (Fig. 6). In some treatments with slurry of pH ≤ 4.5 small and irregular pH increases were measured in the surface layer (data not shown). The overall pH was increased by about 0.1-0.2 pH units in 55 days. The straight pH profiles suggest that the rate of H^+ consumption was essentially constant with depth or that diffusion could level out differences which may result from a depth-dependent consumption rate. Concentration of N_2O in slurries of pH 4.5 increased from less than 15 μg N kg^{-1} after 3 days to about 150 μg N kg^{-1} after 55 days of incubation.

Significant and rather sudden increases in pH were observed in the surface layer of slurries of pH 5.0 between day 43 (data not shown in Fig. 6) and day 55. This pH increase coincided with an increase in the N_2O concentration in the surface layer (Fig. 6), suggesting concomitant H^+ consumption and N_2O production. Because the resolution of our measurements was only about 1 cm, the N_2O profile in the slurry of pH 5.0 differed from a steady-state diffusion reaction profile of a dissolved constituent typically found in porous media. The high concentrations near the surface and the decreasing concentrations with depth indicate that N_2O diffused from the surface layers to the atmosphere and to the subsurface. Downward diffusion will continue until the concentration in the subsurface has increased to levels similar to those in the surface layers. Thenceforth, diffusion will be directed to the atmosphere only. This proposed sequence of diffusion, as it follows from the depth and time dependent excursions of pH and N_2O, also adds to the explanation of the variations in N_2O flux with time (Oenema et al., 1993).

In slurries of pH 6.0, depth-dependent excursions of pH were measured after 3 and often after only 1 day. After 12 days the pH had increased to about 8.0 irrespective of depth (Fig. 6) and large amounts of N_2O had accumulated in the slurry. The N_2O profile of the slurry of pH 6.0 also indicates that a quasi steady state was reached already after 12 days with upward directed diffusion only.

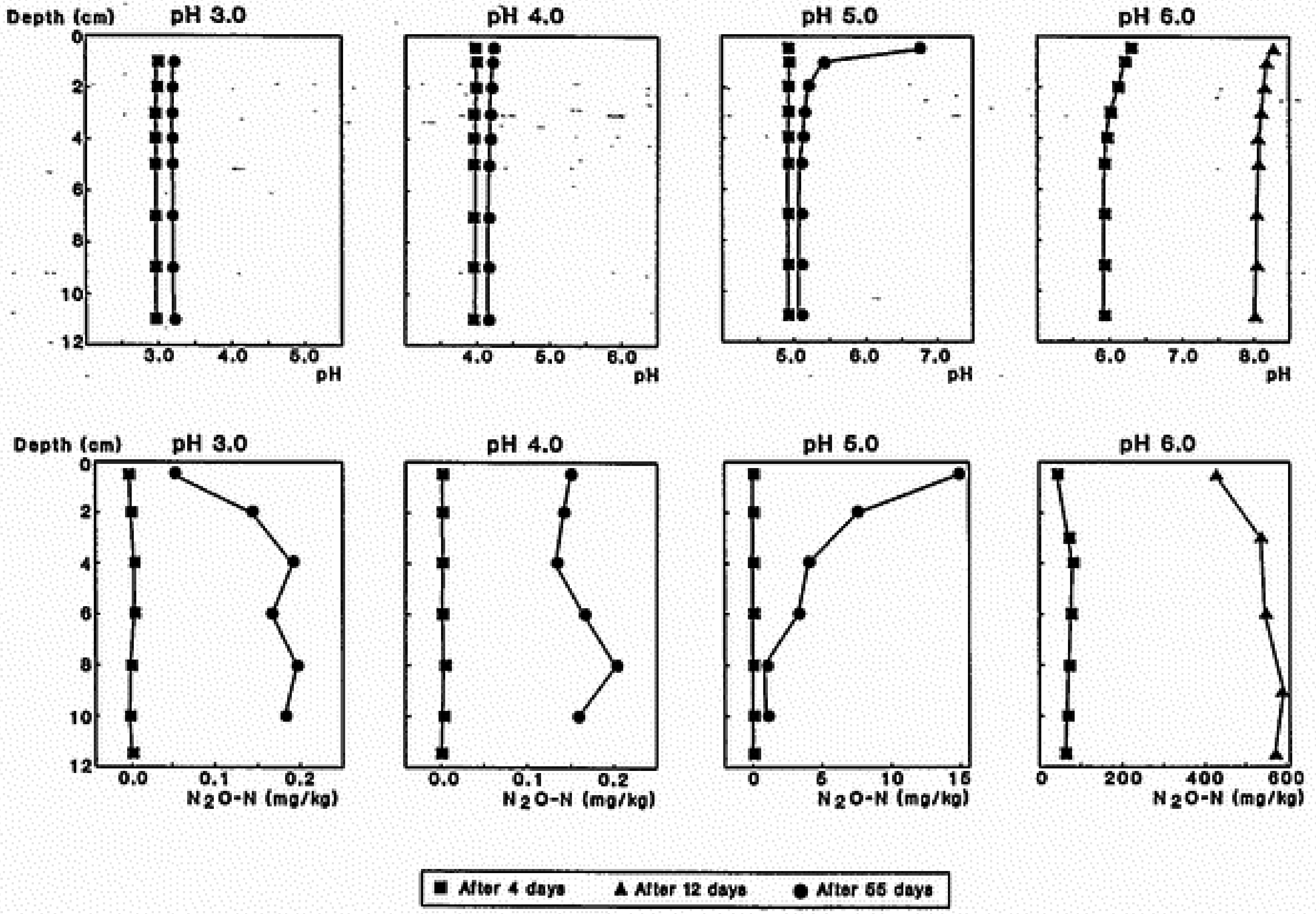

Fig. 6. Depth profiles of pH (upper half) and N_2O (lower half) in HNO_3 treated slurries op pH 3.0, 4.0, 5.0 and 6.0 and stirring treatment A. Measurements were carried out 4 and 55 days after homogenization of the slurries by stirring. Note the scale differences of the x-axis of the N_2O profiles and also that data from slurries of target pH 6.0 are from measurements carried out 4 and 12 days after stirring.

Discussion

Cause of the NO_3^- loss

Basically, there are three possible pathways by which NO_3^- was removed from the solute in HNO_3 treated slurry: 1) microbiological denitrification; 2) dissimilatory reduction of NO_3^- to NH_4^+; and 3) chemodenitrification. Precipitation of NO_3^- is not included, because of its high solubility. Immobilization of NO_3^-, i.e. the assimilatory reduction of NO_3^- to amines, in biomass is also highly unlikely, because of the low C/N ratio in the slurry (Table 1). Our results provide evidence that microbiological denitrification was a major pathway. The NO_3^- loss and the N_2O emission were highest at pH 6.0 and lowest at pH $\leq$ 4.5. The pH-dependance of the NO_3^- loss is in accordance with the fact that the optimum pH for microbiological denitrification is around 7 and that lowering the pH strongly decreases denitrifying activity (Knowles, 1982).

The HNO_3 treated cattle slurry provides all ingredients needed by denitrifiers: organic C, NO_3^- and the (near) absence of O_2 (Tiedje, 1988). The metabolizable organic matter in the slurry serves as energy source and H^+ and electron (e^-) donor for microbiological denitrification. The overall stoichiometry of the organic matter oxidation via denitrification is given in reactions 1a and 1b, and 2a and 2b of Table 3, with N_2O and N_2 as the end products, respectively. Such reactions can be used to examine the consistency between H^+ consumption and the NO_3^- loss.

Table 3. Stoichiometry of organic matter oxidation in cattle slurry. In the first series of reactions, the molar C:N:P ratio of the organic matter was set at 101:13:1 and the pH of the slurry at about 7. In the second simplified reactions organic matter consisted of CH_2O only.

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1. Nitrate consumption reactions
   a. $(CH_2O)_{101}(NH_3)_{13}H_3PO_4 + 101HNO_3 = 90CO_2 + 11HCO_3^- + 13NH_4^+ + HPO_4^{2-} + 50.5N_2O + 140.5H_2O$

   b. $(CH_2O)_{101}(NH_3)_{13}H_3PO_4 + 80.8HNO_3 = 90CO_2 + 11HCO_3^- + 13NH_4^+ + HPO_4^{2-} + 40.4N_2 + 130.4H_2O$

   c. $(CH_2O)_{101}(NH_3)_{13}H_3PO_4 + 50.5HNO_3 + 11H_2O = 39.5CO_2 + 61.5HCO_3^- + 63.5NH_4^+ + HPO_4^{2-}$

2. Nitrate consumption reaction; simplified reactions
   a. $CH_2O + HNO_3 = CO_2 + 1.5H_2O + 0.5N_2O$

   b. $CH_2O + 0.8HNO_3 = CO_2 + 1.4H_2O + 0.4N_2$

   c. $CH_2O + 0.5HNO_3 = 0.5CO_2 + 0.5HCO_3^- + 0.5NH_4^+$

3. Aerobic oxidition
   $CH_2O + O_2 = CO_2 + H_2O$

4. Methanogenesis
   $CH_2O = 0.5CO_2 + 0.5CH_4$

If we assume that the molar C:N:P ratio of the slurry was 101:13:1, as may follow from its composition (Table 1), the overal molar ratio of H^+ consumption and NO_3 loss will vary between 1:0.90 in reaction 1a to 1:0.88 in reaction 1b. The molar ratio of H^+ consumption and NO_3^- loss is slightly less than 1:1, because of the mineralization of ammonium and phosphate; the ammonification of 1 mol amine N produces 1 mol alkalinity and the dissociation of 1 mol H_3PO_4 to HPO_4^{2-} consumes 2 mol alkalinity. However, a large fraction of total N and P was present in inorganic form (data not shown). This suggests that the molar ratios of C/N and C/P of the decomposing organic matter is higher than 7.8 and 101, respectively, as assumed in reactions 1a and 1b of Table 3. Thus, as another extreme, the stochiometry of the decomposition of N- and P-free organic matter via denitrification is given in the simplified reactions 2a and 2b of Table 3. In these reactions, equivalent amounts of H^+ and NO_3^- are consumed. Hence, the molar ratio of H^+ consumption and NO_3 loss will vary between 1:0.88 and 1:1, provided that no other reactions take place than organic matter oxidation via denitrification and mineralization of ammonium and phosphate.

In slurries of pH 6.0 and stirring treatments A, B and C, total NO_3^- loss was 427, 362 and 204 mmol kg^{-1}, respectively, as follows from the HNO_3 consumption, corrected for the net change in NO_3^- concentration (Table 2). The molar ratio of H^+ consumption and NO_3^- loss was 1:0.91, 1:0.88 and 1:0.85, respectively. This is close to the overall H^+:NO_3^- ratio of 1:0.90 in reaction 1a and 1:0.88 in reaction 1b of Table 3, assuming that the decomposing organic matter had a molar C:N:P ratio of 101:13:1. However, the changes in NH_4^+ concentration with time (Table 2) suggest that NH_3 volatilization will also have contributed to H^+ consumption. Volatilization of NH_3 will diminish the total H^+ consumption. The total H^+ consumption, due to organic matter oxidation via denitrification, ammonification and NH_3 volatilization may than be approximated by (all in mmol kg^{-1}):

$$H^+ \text{ consumption} = \text{total } NO_3^- \text{ loss} + \Delta NH_4^+ \quad (1)$$

Because H^+ consumption due to mineralization and subsequent dissociation of phosphate is of minor importance, these processes were disregarded in Equation (1). The H^+ consumption unaccounted for ($\#H^+$) by denitrification, ammonification and NH_3 volatilization may than be approximated by:

$$\#H^+ = \Delta NO_3^- - \Delta NH_4^+ \quad (2)$$

where ΔNH_4^+ and ΔNO_3^- stand for the changes in the NH_4^+ and NO_3^- concentrations, respectively, between the first and last sampling according to Table 2. In slurries of target pH 6.0, H^+ consumption was larger than accounted for by denitrification, ammonification and NH_3 volatilization (Table 4). In slurries of target pH ≤5.0, $\#H^+$ was relatively small and, although mostly negative, may be neglected if we consider the possible variations introduced by all assumptions and analyses involved.

The $\#H^+$ in slurries of target pH 6.0 is puzzling. Total H^+ consumption was more than 25% larger than follows from microbiological denitrification, ammonification and NH_3 volatilization. It suggests that there must have been additional processes

Table 4. Proton consumption unaccounted for according to Equation 2, as a function of pH and stirring treatments A, B and C (mmol kg^{-1}). A surplus indicates that more H^+ was consumed than follows from the total NO_3 consumption and the net change in the NH_4^+ concentration in Table 2.

pH	A	B	C
~7.5			
6.0	102	129	85
5.0	15	6	–20
4.5	–18	–16	–4
4.0	–29	–35	–55
3.5	–19	–44	n.d.
3.0	–17	–5	n.d.

that contribute to a ratio of H^+ consumption/NO_3 consumption > 1 and/or processes that have a ratio of H^+ production/NO_3 production < 1. Our study was not designed to address these possibilities, but they deserve some discussion. Nitrification does not meet the contraints, because 2 mol H^+ is produced for each mol NH_4^+ oxidized to NO_3^-. It is also unlikely that an initial delay in the establishment of an equilibrium, due to the difference in solubility between CO_2 and NH_3 (Husted et al., 1991) has significantly contributed to #H^+, because #H^+ was rather small during the first month of the incubation period. Dissolution of previously formed minerals, e.g. carbonates and struvite (Bril & Salomons, 1990) consumes H^+ and thus may have contributed to #H^+, although the fresh slurry that we used will not have contained large amounts of mineral precipitates yet. Basically, dissimilatory reduction of NO_3^- to NH_4^+ could also explain that the ratio of H^+ consumption and NO_3^- consumption is larger than 1 (reactions 1c and 2c of Table 3). The dissimilatory reduction of NO_3^- to NH_4^+ has been shown to occur in highly anoxic and NO_3^--poor environments, such as digested sludge (Kaspar et al., 1981) and the bovine rumen (Kaspar & Tiedje, 1981). Because NH_4^+ concentrations decreased rather than increased (Table 2), and treated slurries are NO_3^--rich, dissimilatory reduction of NO_3^- to NH was probably not an important pathway in these slurries. Chemodenitrification has been put forward in a number of studies to explain unaccountable NO_3^- losses. Nitrite (NO_2^-), and its undissociated form nitrous acid (HNO_2), are key compounds in chemodenitrification, and their chemical decomposition into various volatile N compounds is stimulated generally by acidic conditions (Chalk & Smith, 1983). A possible reaction is: $2\ HNO_2 = NO + NO_2 + H_2O$, indicating a 1:1 molar ratio of H^+ consumption and NO_2^- consumption. If chemodenitrification occurs concomitant with the oxidation of reduced inorganic compounds (e.g. Sørensen & Thorling, 1991), than the ratio is lower than 1. Relatively high but varying NO_2^- concentrations were found in slurries of pH 6.0 and low concentrations in slurries of pH ≤ 5.0 (data not shown), which could be explained by the facts that the rate of NO_2^- formation is related positively to pH and the rate of NO_2^- decomposition is related negatively to pH (e.g. Van Cleemput & Baert, 1984). This suggests that chemodenitrification cannot be excluded as a possible pathway for NO_3^- loss from treated slurries. However, we conclude that the large H^+ consumption unaccounted for in slurries of pH 6.0 can not be explained satisfactorily by any of the aforementioned possible processes.

Denitrification and N_2O production

Generally, the ratio of the possible end products of denitrification, N_2O/N_2, increases with a decrease in pH and with an increase in NO_3^- concentration (Koskinen & Keeney, 1982). This suggests that more N_2O in proportion to N_2 will be emitted from slurries of pH ≤ 5.0 than from slurries of pH 6.0. If we assume that the total NO_3^- loss (Table 2) was emitted as N_2O and N_2, and combine this with the estimated mean diffusive N_2O fluxes given by Oenema et al. (1993), it follows that the mean $N_2O/(N_2O+N_2)$ ratio was 0.5 for slurries of pH 6 and ≤ 0.01 for slurries of pH ≤ 5.0. This is a surprising result. The relatively low accuracy of the mean N_2O fluxes and of the NO_3^- loss for slurries of pH ≤ 5.0, because of the relatively low and variable fluxes and NO_3^- losses, respectively, may have contributed to the apparently reverse effect of pH on the N_2O/N_2 ratio. Emission of other volatile N compounds, such as NO and NO_2, may also have contributed to the NO_3^- loss, especially in low-pH slurries. Evidently, the effect of pH on the N_2O/N_2 ratio and on the possible emission of NO and NO_2 from HNO_3 treated cattle slurry needs further study.

As shown in Fig. 5, the molar C/N ratio of the CO_2 and N_2O emissions was low for slurries of pH 6.0 and much higher for slurries of target pH ≤ 5.0. A CO_2-C/N_2O-N ratio of about 2 for slurry of pH 6.0 and stirring treatment A would also suggest that the $N_2O/(N_2+N_2O)$ ratio was about 0.5, if it is assumed that organic carbon was predominantly respired by denitrifiers. An increase in stirring intensity and addition of H_2O_2 (treatment B and C, respectively) increased the CO_2/N_2O ratio (data not shown), suggesting that aerobic oxidation (reaction 3 of Table 3) became relatively more important, or that the N_2O/N_2 ratio of denitrification decreased. Lowering the pH also increased the CO_2/N_2O ratio. The apparently positive effect of pH on the N_2O/N_2 ratio and the possible emission of NO and NO_2, as discussed before, may help to explain that the ratio of CO_2/N_2O increased as pH decreased. Nonetheless, these findings highlight the need for a better understanding of the effect of pH on the N_2O/N_2 ratio of denitrification in slurry.

Absence of O_2 is generally regarded as a prerequisite for denitrification, even though a number of studies have indicated the persistence of denitrification under the presence of molecular O_2 (Robertson & Kuenen, 1984; Lloyd et al., 1987). In heterogeneous substrates, denitrification seems to be controlled by the rate of O_2 transport to the anaerobic and NO_3^- and metabolizable C containing sites of denitrification (Cho, 1982). Surprisingly, in many treated slurries denitrification and N_2O production were highest at or near the interface of slurry and atmosphere (e.g. Fig. 6). The background of this depth-dependent reaction rate is not yet clear. Apparently, O_2 directly or indirectly stimulated denitrification. It has been suggested that some denitrifiers withstand acidic conditions more easiliy in the presence of small amounts of molecular O_2 than in its absence (Oenema et al., 1993). The denitrifying activity in the subsurface slurry may be also partly inhibited by NO_2^-, because NO_2^- is highly toxic to denitrifiers at relatively low concentrations (Payne, 1981; p. 48-49). Albeit still unclear, the depth-dependant rate of denitrification in HNO_3 treated slurries has important implications for the on-farm treatment of slurry. Regular and complete homogenization of the slurry via stirring appears to be of crucial importance for the control of denitrification. Due to the concomitant consumption of H^+ and NO_3^- and its strong pH-dependance, denitrification accelerates, first in the surface layers but subsequently also downwards, when the slurry is not stirred. The accelerating H^+ consumption combined with the relatively low BNC of treated cattle slurry (Fig. 1) may quickly increase the pH to values conducive to NH_3 volatilization, if pH is not quickly adjusted. Regular stirring will level out any gradients in pH and denitrifying activity and will limit the overall NO_3^- losses. Local spots with a somewhat elevated pH may also serve for the onset of rapid denitrification. Such 'hot spots' with high N_2O emissions have also been observed locally in slurry storage basins on dairy farms (unpublished results).

Conclusions

Treatment of cattle slurry with HNO_3 to lower the pH strongly diminished NH_4^+ losses and CH_4 and CO_2 fluxes during storage. NO_3^--N losses and flux of N_2O from these HNO_3 treated slurries had increased compared to untreated slurry. The pH-dependance of the NO_3^- loss and the coupling of NO_3^- loss, H^+ consumption and N_2O production (e.g. Table 2 and Fig. 6) indicate that microbiological denitrification was the dominant cause of the NO_3 loss. The H^+ consumption unaccounted for ($\#H^+$) by denitrification, ammonification and NH_3 volatilization in slurries of pH 6.0 cannot be explained satisfactorily yet with the data now available. This and the depth-dependent denitrification rate in the treated slurries, and the ratio of the flux of N_2O and N_2 need further study. Lowering of the pH to a value of 4.0-4.5 and regular homogenization of the slurry via stirring are of crucial importance for the succes of the on farm treatment of slurry with HNO_3.

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