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Denitrification and the evolution of nitrous oxide after the application of cattle slurry to a peat soil

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Abstract

The impact of cattle slurry on denitrification losses and nitrous oxide emission was determined on a peat soil in the Netherlands. As well as measuring losses on a day-to-day basis after three different methods of slurry application, two methods for estimating denitrification and nitrous oxide emissions were compared, i.e. coring/incubation and enclosure techniques. Slurry was applied either in a conventional way, diluted 1:3 or acidified with nitric acid. There was much variation with time, method of assessment and method of slurry application in both apparent denitrification rates and N_2O fluxes: it was not always possible to provide adequate explanation for all of the effects demonstrated. Major proportions of the variation in denitrification and N_2O emission could be accounted for by variation in soil moisture, soil temperature and NH_4^+ (but not NO_3^-) content. It was suggested that nitrification was playing a key role in maintaining an adequate substrate supply (NO_3^-) for denitrification and perhaps contributing directly to an unknown extent to N_2O emissions. There were overall differences in the extent of losses with the different methods of slurry application but these were highly dependent upon interactions with current soil and weather conditions.

Introduction

Nitrous oxide (N_2O) concentrations in the atmosphere are increasing and there are growing concerns over the implications of this for global warming effects (Bouwman, 1990). Although having a concentration that is three orders of magnitude lower than CO_2 (the major greenhouse gas), the potential per N_2O molecule for adsorbing longwave radiation is up to 200 times greater (Warneck, 1988). Agricultural soils, because of high inputs of nitrogen (N) fertilizer, are considered to be a major source of N_2O largely through the microbiological process of denitrification of excess nitrate (NO_3^-) under anaerobic conditions. Denitrification makes an important contribution to the inefficient use of N on farms (Jarvis, 1993). The products of denitrification are nitrogen (N_2) as well as N_2O (and NO) but there is yet insufficient information to be able to predict either the rates or the proportions in which the emissions occur. The situation is further complicated by the likelihood that there is another, as yet unquantified, source of N_2O (Bremner and Blackmer, 1980) from nitrification (i.e. the microbial oxidation of ammonium NH_4^+ to NO_3^-). Intensively managed grasslands receive large inputs of substrates for both processes (i.e. NO_3^- and NH_4^+) from fertilizer, excreta and mineralization of soil organic matter. Because of their geographic distribution, grassland soils are also often subject to rapidly changing moisture conditions and aerobicity. It is possible that the two processes may be coupled through common intermediates or occur sequentially or simultaneously in adjacent soil pores of different aerobicity for example with impact on the release of N_2O.

The disposal of waste from housed livestock as slurry onto agricultural land is important in this respect in a number of ways. Firstly, slurries contain much NH_4^+ and therefore increase the potential for nitrification followed by denitrification. Secondly, significant volumes

of water are added and thirdly much labile carbon (C) is returned. All of these factors increase the potential for denitrification. Other studies have indicated that, even when soil temperatures are relatively low, denitrification after slurry application is significant (Thompson, 1989) because of an enhanced supply of soluble carbon to provide an energy source. Furthermore, whereas spreading slurry by conventional methods invariably results in large releases of ammonia (NH_3) (Pain et al., 1990), current legislative and environmental pressures to reduce NH_3 volatilization are prompting the development of alternative methods to apply slurry (Pain et al., 1990) which may result in an enhanced denitrification loss.

There are difficulties in measuring denitrification and/or N_2O loss, especially under field conditions (Smith and Arah, 1990). As well as much spatial, especially in grazed grassland soils, and temporal variability (Jarvis et al., 1991; Scholefield et al., 1990) there are technical problems with current methods. In the present studies we measured, after the application of slurry to a peat soil (with much readily available carbon), N_2O and denitrification losses and determined the effects of different slurry treatments. As well as measuring losses on a day-to-day basis after three different methods of slurry application, we also compared two methods for measuring denitrification and N_2O emission.

Materials and methods

Experimental site

The measurements were made on grassland at the Regional Research Centre for Cattle Husbandry at Zegveld, 20 km west of Utrecht in the Netherlands. The farm is 2.15 m below sea level and is situated on a low peat soil derived from wood sedge peat (6–7 m deep) which had an organic content of 38% (oven dry basis) and a pH (KCl) of 5.0 (De Klein, pers. comm.). The grassland was intensively managed and was rotationally grazed by dairy cattle: the swards used in the present studies had been cut to a height of 5 cm prior to slurry application.

Slurry application

The characteristics of the cattle slurry and the various treatments used in this study are shown in Table 1. The treatments were designed to reduce NH_3 loss and may therefore have subsequent effects on other N transformation including nitrification and denitrification. Measurements were made in three treatments during two phases of an experiment which started during the last week in August. In week 1 the slurry treatments were A, conventionally surface spread, no-amendments; B as A, but diluted (1:3) with water, and C, conventionally surface spread and acidified (with sufficient concentrated nitric acid added to the slurry to reduce pH to below 5.0). At the beginning of week 2 slurry was spread onto new areas and two treatments were compared: D, conventionally surface spread and E, acidified slurries, as before. The area receiving treatment D, had been irrigated with water (10 m^3 ha^{-1}) during the 24 h before the treatments were applied. All treatments were applied with a tractor powered tanker at rates of approximately 10 m^3 ha^{-1} (except treatment B, see Table 1) over a circular area of 25 m diameter. Spreading started at 09.00 h on day 1 of each week and measurements were started immediately afterwards.

Denitrification and N_2O measurements

Two methods of estimating total denitrification (TDN) and N_2O losses were used in the field. The first was a core incubation method based on that described by Ryden et al. (1987) and which was used for all of the present treatments. Cores were taken at random over the whole of each treatment circle to a depth of 10 cm and were packed (without any protective sleeving) in 1 L glass containers with airtight screw tops each fitted with a septum. During week 1 each jar contained 8 × 25 mm diameter cores: for later measurements the technique changed slightly and 4 × 35 mm cores were taken to provide a similar total volume of soil. Four jars were prepared from each treatment and after sealing the tops, 50 mL of the head space in two jars were removed and replaced by 50 mL of acetylene. This was supplied to inhibit the reduction of N_2O to N_2 during denitrification and thus allow estimation of TDN by measurement of the accumulated N_2O. The remaining two jars from each treatment were incubated without acetylene so that N_2O emission alone could be determined. Immediately after sealing, the jars were placed in 10 cm deep holes to be at ambient soil temperatures over a 24 h period. Details of the sampling schedule from the jars prepared from each treatment are shown in Table 2.

For analysis, duplicate 2 mL samples of the head space gases in each jar were removed and stored in evacuated glass tubes (100 × 16 mm Vacutainers: Bec-

Table 1. Some characteristics of the applied slurries

Treatment	N applied (kg ha^{-1})		Slurry added (kg ha^{-1} × 10^3)	Dry matter (g kg^{-1})	pH
	NH_4^+ - N	Total N			
Week 1					
A: Surface spread	25.6	50.1	12.7	72.9	7.1
B: Diluted (1:3)	20.8	42.8	42.5	17.0	7.5
C: Acidified	17.8	85.6[a]	13.2	80.7	4.4
Week 2					
D: Surface spread	24.3	45.1	9.7	77.1	7.5
E: Acidified	11.6	54.0[a]	8.2	80.9	4.5

[a]Acidified with nitric acid to give 42.2 kg NO_3^--N ha^{-1}: no detectable NO_3^- - N was present in the other treatments.

Table 2. Details of sampling schedule (days after slurry application) for measurement of denitrification and nitrous oxide losses after slurry application to grassland

Treatment	Duration of measurement (h)	Method	
		Core/incubation (I)	Enclosure (BE)
Week 1 (30 August)			
A	4	1–5	1–5
	24	1–7, 9, 11, 14, 16	—
B	24	1–7, 9, 11, 14	—
C	24	1–7	—
Week 2 (6 September)			
D	4	1–5	1–5
	24	1–5	—
E	24	1–5	—

ton Dickinson) sealed with a rubber septum. Each tube had been previously opened, flushed with helium and re-evacuated. Head space gas samples were taken 4 and 24 hours after the start of the incubation over a five day period in treatments A and D (Table 2). For the remainder of the treatments (see Table 2) samples were taken only after 24 h. On each sampling occasion, a standard gas sample containing 100 ppm N_2O was stored in an evacuated tube in exactly the same way as the head space samples. All tubes were stored in a refrigerated cool box at 4°C before analysis.

At the same time as the soil cores were being incubated, an enclosure method based on that described by Ryden and Dawson (1982) was also used to measure losses from treatments A and D (Table 2). Each day, six 15 × 50 × 20 cm rectangular open topped steel boxes were inserted into new areas of ground to a depth of 5 cm: perspex lids were clamped onto sealing strips on the tops of the boxes immediately prior to sampling. Each lid had a 3-cm diameter hole at one end and an air line at the other which was attached, in series, to 3 replaceable cartridges containing, in turn, drierite (500 g, in 58 diameter/ 25 cm long tube), carbosorb (30 g in 1.6 diameter × 20 cm long tube), and 60 g of 5 Å molecular sieve (1.6 diameter × 50 cm long tube) to remove, respectively, H_2O, CO_2 and N_2O from the air

stream being sampled. With three of the boxes, three 2-mm internal diameter gas supply lines were inserted into the soil to a depth of 35–40 cm along 2 sides of the box (i.e. 6 per box) and at an angle so that they were directed immediately below the block of soil enclosed by the box. The tubes were cleared of any obstruction and connected to an acetylene (C_2H_2) supply from a cylinder. There was no C_2H_2 supply to the remaining 3 boxes. The objective was again to provide estimates of TDN and N_2O losses.

On day 1 six boxes were positioned immediately after slurry was spread on the upwind edge of the treated area: the boxes were separated from each other by a distance of 1.5 m. For measurements on subsequent days, another six boxes without lids were positioned during each preceding afternoon so that new areas were sampled each day. Before measurements started, C_2H_2 was infused into the soil under three enclosures at 600 cm^3 min^{-1} for 2 h, the supply was then stopped and the system allowed to equilibrate for 1 hour. The perspex lids were then positioned and clamped to each box and an air line attached. Air from within the enclosed head space of each box was sampled at a rate of 200 cm^3 min^{-1} and drawn, in turn, through each of the three cartridges by an air pump. Each line had an adjustable flowmeter to maintain flow rates and a small gas meter attached to provide accurate assessment of the total air volume sampled. After 4 h, which was coincident with the incubation period for the soil cores in the jars, air sampling was stopped and the cartridge containing the molecular sieve was sealed immediately with tight fitting rubber bungs for transport to the laboratory for desorption of N_2O. This procedure was repeated with a new molecular sieve cartridge on each of the first five days after application for treatments A and D (Table 2). The Carbosorb cartridge was also replaced daily and the drierite after the measurements on Treatment A had been competed (i.e. 5 days). Background atmospheric N_2O concentrations were estimated each day by absorbing N_2O from a similar monitored volume of air taken over the same periods through air lines with a sampling height of 50 cm above the soil surface and positioned upwind of the treatment area. The sample air was scrubbed of H_2O and CO_2 and N_2O collected on molecular sieve as before.

Other field sampling and measurement procedures

Soils. Six 2.5 cm diameter cores were taken at random from each treatment area to 10 cm depth at the same time as those taken for incubation. After collection the soil was bulked and stored at 4°C until extraction later during the same day when it was thoroughly mixed, stones and coarse organic remains removed and duplicate 100 g samples dispersed in 200 mL of 1 *M* KCl and stood overnight. The suspension was then filtered and, after discarding the first 2 mL the filtrate was collected and stored under refrigeration for later analysis of NO_3^- and NH_4^+. Soil moisture contents were determined gravimetrically after drying a further subsample from the bulked soil at 100°C.

Environmental measurements. Soil temperatures were measured with thermistors at the surface and at 5 and 10 cm depth: air temperature was also measured.

Analysis

NH_4^+ and NO_3^- contents of the KCl extracts were determined by automated colorimetry using the reaction with sodium phenate and sodium hypochlorite for NH_4^+ and the reaction of NO_2^- with the azo dye Orange 1 following the reduction of NO_3^- to NO_2^- on a cadmium column for NO_3^-.

The N_2O collected onto molecular sieves from the air samples from the enclosure boxes was desorbed as described by Ryden and Dawson (1982). In brief, duplicate 7 g sub-samples of molecular sieve from each day's cartridge were placed in stoppered glass vessels which were evacuated and 50 cm^3 water was then added to release N_2O into the head space. A subsample of the head space was analysed for N_2O by ECD gas chromatography. The total N_2O absorbed on the sieve was then estimated from the concentration in the gas and water phases (using the Bunsen coefficient). Emission of N_2O and total denitrification losses were then calculated from a knowledge of the volume of air sampled and taking account of ambient aerial N_2O concentrations.

The gas samples stored in the Vacutainers taken from the head space of the incubation jars were analysed directly for N_2O using ECD gas chromatography. Analysis of the stored gas standards gave recoveries in the range 95–105% and appropriate corrections for this were applied as necessary.

Results

Data are presented without transformation as this had no beneficial effect on the relationships under discussion.

Table 3. Total denitrification and N_2O losses, expressed on a 24 h basis (g N ha^{-1} d^{-1}) when determined by 4 h or 24 h assessments using core/incubation (I) or box enclosure (BE) methods after spreading cattle slurry on to a peat grassland soil

Time (days) after spreading slurry	Total denitrification (TDN)						N_2O					
	4 h				24 h		4 h				24 h	
	I[a]		BE[b]		I[a]		I[a]		BE[b]		I[a]	
Treatment A (Week 1)												
1	50	(8.0)	20	(7.5)	27	(12.0)	33	(4.0)	7	(4.5)	49	(29.6)
2	180	(107.0)	80	(34.1)	70	(45.0)	62	(0.1)	30	(14.5)	30	(3.5)
3	125	(28.1)	260	(63.1)	25	(3.0)	55	(11.1)	54	(29.9)	15	(0.4)
4	49	(6.0)	46	(15.9)	15	(0.5)	33	(0.1)	24	(6.6)	11	(0.1)
5	102	(12.0)	86	(51.0)	33	(14.1)	34	(0.1)	47	(19.9)	17	(1.1)
Treatment D (Week 2)												
1	299	(79.0)	66	(31.7)	60	(15.0)	110	(14.9)	67	(5.4)	25	(1.1)
2	102	(16.0)	15	(3.7)	31	(2.5)	86	(0.1)	29	(5.7)	22	(1.0)
3	102	(8.0)	31	(10.5)	39	(15.0)	95	(0.5)	34	(14.9)	32	(0.9)
4	213	(33.0)	167	(16.9)	78	(21.0)	78	(11.9)	49	(2.6)	27	(1.4)
5	205	(66.5)	86	(9.5)	55	(29.5)	380	(273.1)	34	(11.5)	73	(51.4)

[a]Values are means (+/- s.e.) from duplicate incubation jars.

[b]Values are means (+/- s.e.) from three replicate enclosure boxes.

Core/incubation method: 4 h vs. 24 h assessments

As the data for conventionally spread slurry in Table 3 show, there were substantial TDN and N_2O losses throughout the whole of the measurement period. TDN rates on neighbouring swards (which had received 220 kg fertilizer N ha^{-1}, but no slurry) using a similar core incubation technique ranged from 0–7.6 (mean 1.3, s.d. +/- 2.7) g N ha^{-1} d^{-1} on August 28th and from 0–24.6 (mean 6.8, s.d. +/- 9.2) g N ha^{-1} d^{-1} on September 18th (de Klein, pers. comm), i.e. always, and often much, lower than recorded on the slurry treated areas. Even though the technique involves some spatial integration because of the number of cores taken with each measurement there was a large degree of variability. Similar variability was shown with both the 24 and the 4 hourly incubations, and for estimates of TDN and N_2O losses.

There was a major effect of incubation time on estimated TDN and N_2O losses. The 24 h measurements resulted in values which were, on average, over each 5 day comparison (i.e. with treatments A and D), over 3 times smaller than those which were extrapolated from the 4 h measurements for both TDN and N_2O losses (Table 3).

Core/incubation vs. enclosure methods

Except on two occasions, estimates of TDN and N_2O losses from the conventionally spread slurry in week 1 were lower (on average, by 22 and 45%, respectively), with the enclosure method than with the concurrent 4 hour incubation method (Table 3). The differences were even greater during the second week when the respective estimates with the enclosures were over 3 and 4 times lower on average than with the incubation method. Despite these differences, the pattern of temporal variation was similar for both methods especially during week 2 (Treatment D).

Effects of application treatments on total denitrification loss

The effects of slurry treatments on TDN and N_2O losses as determined by the incubation method are shown in Table 4. For the conventionally spread slurry (A) during week 1, the average daily TDN loss was 109 g N ha^{-1} compared with 79 and 37 g N ha^{-1} d^{-1}, respectively, from treatments B and C. The average rate of denitrification loss from slurry applied during week 2 was 53 and 252 g ha^{-1} d^{-1} for treatments D and E, respectively. Total denitrification losses over

Table 4. Total denitrification and N_2O losses (g ha^{-1} d^{-1}) as determined by the core/incubation method and as affected by method of application of cattle slurry to a peat grassland soil

Days after spreading	Total loss (N_2 + N_2O)						N_2O					
Treatment:	A		B		C		A		B		C	
Week 1												
1	27	(12.0)	35	(11.5)	61	(7.0)	49	(28.7)	23	(—)	107	(80.5)
2	70	(45.0)	17	(1.5)	30	(14.6)	30	(3.4)	23	(1.6)	23	(1.4)
3	25	(3.0)	69	(27.5)	23	(4.6)	15	(0.5)	30	(11.9)	14	(0.1)
4	15	(0.5)	11	(0.1)	11	(0.1)	11	(0.1)	13	(1.4)	8	(0.1)
5	33	(14.1)	11	(6.4)	27	(6.4)	17	(1.0)	23	(3.6)	18	(0.9)
6	40	(16.0)	35	(2.0)	23	—	26	(0.1)	39	(—)	22	(—)
7	139	(92.5)	39	(11.5)	87	(1.4)	56	(9.6)	18	(2.9)	23	(9.5)
9	368	(334.0)	300	(179.5)	nd	—	40	(13.5)	23	(2.4)	nd	—
11	264	(176.5)	208	(44.0)	"	—	63	(8.5)	nd	—	"	—
14	79	(37.5)	62	(28.0)	"	—	198	(77.0)	76	(2.1)	"	—
16	138	(65.5)	nd	—	"	—	37	(18.9)	nd	—	"	—
Treatment:	D		E				D		E			
Week 2												
1	60	(15.0)	288	(121.0)			25	(1.1)	59	(23.6)		
2	31	(2.5)	68	(23.0)			22	(1.1)	54	(17.5)		
3	39	(15.1)	488	(256.0)			32	(1.1)	194	(62.2)		
4	78	(20.5)	196	(16.5)			27	(1.5)	75	(9.9)		
5	55	(29.4)	220	(157.5)			73	(51.5)	58	(19.0)		

Values are mean (+/- s.e.) from duplicate incubation jars.

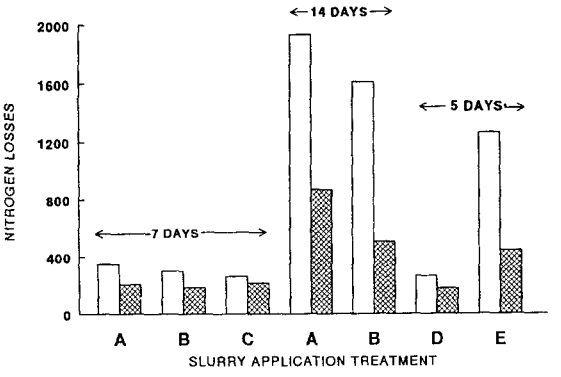

Fig. 1. Total apparent denitrification (□) and nitrous oxide (▨) losses (g N ha^{-1}) from a grassland peat soil after application of conventional (A and D), diluted (B) or acidified slurries (C and E). Data are shown for after 7 and 14 days for treatments A, B and C (applied week 1), and after 5 days for treatments D and E (applied week 2).

the first 7 days from treatments A, B and C were all less than 400 g N ha^{-1} and there was no difference between the treatments (Fig. 1) at this time. However, over the following 7 days there was a five fold increase in overall loss from treatments A and B. When the new treatments were applied in the week 2, the loss from conventionally applied slurry (D) was similar to that during the first 7 days after spreading in week 1, but losses from the acidified slurry (E) were nearly 5 times greater (Fig. 1, Table 4).

During the first week, application treatment had little effect on the ratio of TDN-N: N_2O - N (Fig. 1, Table 5): the mean values for the proportion of N_2O for treatments A, B and C were 59, 78 and 68%, respectively. The average proportion of N_2O (61%) during the first 5 days after applying treatment D was also similar to those for A, B and C, but was smaller with treatment E (32%) which had larger overall TDN losses than treatment D. All the average values represent a wide range of ratios.

There were also differences in the ratio obtained by the two measurement techniques; whereas those from incubations after either 4 or 24 h were similar on each occasion, there were often marked differences between assessments with incubation/coring or box enclosures

Table 5. Apparent proportions (%) of total denitrification released as nitrous oxide as determined by the core/incubation method (I) with 4 or 24 h incubation or the box enclosure method (BE) and as affected by the method of application of cattle slurry to a peat grassland soil

Time (days) after slurry spreading		Measurement method				
		I (4 h)	I (24 h)	BE(4 h)	I (24 h)	I (24 h)
Week 1	Treatment:		A		B	C
1		66	>100	37	66	>100
2		34	43	37	>100	77
3		44	60	21	44	61
4		67	73	51	>100	73
5		33	51	55	>100	67
6			56		>100	100
7			40		48	27
9			11		8	-
11			24		-	-
14			>100		>100	-
16			27		-	-
Week 2	Treatment:		D		E	
1		37	42	100	20	
2		84	71	>100	79	
3		93	82	>100	39	
4		37	35	29	38	
5		>100	>100	39	26	

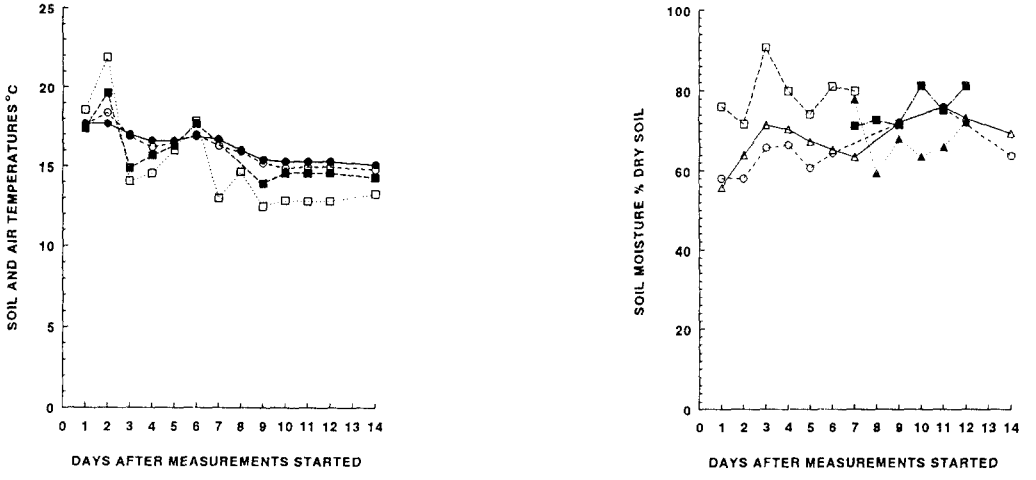

Fig. 2. Changes in (**a**) air (□) and soil temperature (■ surface: ○ 5 cm: ● 10 cm) and (**b**) soil moisture in a grassland peat soil after application of conventional (A Δ and D ▲), diluted (B ○) or acidified (C □ and E ■) farm slurry. Treatments A, B and C were applied at the start of week 1, and D and E at the start of week 2.

over 4 h (Table 5). On some occasions TDN was apparently less than N_2O losses.

Variation in daily rates

Daily TDN loss was generally not large, but the range was wide, i.e. from 8–488 g N ha^{-1} d^{-1}. All of the major measured variables thought to influence denitrification i.e. soil moisture (or aerobicity), temperature and NO_3^- changed appreciably over the measurement period (Fig. 2). Over the experimental period there was a decline in air and soil temperatures; soil temperature at 5 cm depth fell from 17.5 to 14.6°C over the 14 days. Soil moisture contents were high, as expected on this site, but also fluctuated widely and rapidly. During the first week, soil from treatment C (diluted slurry) had a higher moisture content than that from A or B. In the second week, despite the application of water to treatment D prior to spreading, this soil was drier than that from treatment E except for the first day after application.

Soil NO_3^- contents were very variable, both within and between treatments (Fig. 3a) and the patterns of change were complex. Whilst some of this may result from sampling variability, multiple regression analysis over all of the treatments indicated that much of the variation could be accounted for by the additive effects of the variation in soil temperature, moisture and NH_4^+ contents (adjusted $R^2 = 0.813$). Other factors will also have contributed, especially the various processes competing for the removal of NO_3^-, i.e. by uptake into soil or plant biomass, denitrification or leaching. With soil NH_4^+ content, the general trend in treatments A, B and C was for an initial increase during the first seven days and a decrease during the second (Fig. 3b). The changes with treatment E were not so marked but there was also a sharp increase and fall in treatment D.

Daily variability in TDN and N_2O losses and in their ratio was reasonably well accounted for by the variation in soil moisture, temperature, NO_3^- and NH_4^+. Multiple correlation analysis of the data for 24 h incubation of soil from all treatments indicated that 63.5% of the variability in TDN was due to changes in soil moisture and NH_4^+, i.e. increasing with increased soil water and decreased NH_4^+ contents. Inclusion of NO_3^- and soil temperature in the regression model did not improve the adjusted R^2 value. When only treatment A over a longer timespan was considered, more of the variability (80.6%) in TDN was accounted for when all four soil variables were included. Day to day variation in N_2O losses from all treatments was also correlated with moisture and NH_4^+, although to a lesser extent than TDN (adjusted $R^2 = 0.498$): a similar pattern was shown when treatment A was considered on its own. In all treatments less than 60% of the variation in the ratio TDN: N_2O could be explained by the measured variables with soil moisture usually having by far the biggest impact. In general, it was possible to explain greater proportions of the variation when measurements were made with the core/incubation rather than the enclosure method.

Discussion

In almost all environments, the problems associated with obtaining reliable estimates of denitrification rates are substantial (Smith and Arah, 1990; Tiedje et al.,1989): no one technique has been developed which avoids all of the problems likely to be encountered. The nature of the present peat soil should have reduced variability since it was relatively homogeneous (compared with mineral soils) with uniform porosity, background C and NO_3^- distribution. Application of livestock slurries may also reduce variability by providing more uniform distributions of NO_3^- and available C. However, the large volumes of water in the slurry may create localized changes in soil moisture/aeration status and consequently promote differential potentials for denitrification and/or nitrification over small spatial scales. As well as direct effects on denitrifier populations, other interactions with microbiological and biological/physical processes will also be important.

There is a need for measurements which contain a large component of spatial integration. The present results also demonstrate the extent of temporal changes. Other studies of denitrification losses from fertilized, grazed swards have demonstrated the 'spikey' nature of temporal responses in denitrification rates (Jarvis et al., 1991; Scholefield et al., 1990). Rates of loss ranging from 30–470 g N ha^{-1} d^{-1} have been recorded during the first 10 weeks after cattle slurry application to a sandy loam soil (Thompson et al., 1987). Our measurements relate to short-term responses only and there may be long lasting effects; significant denitrification has occurred for at least 2 months after slurry application (Thompson et al., 1987).

Although the trends and patterns recorded were similar, the estimates with the two methods for TDN and N_2O loss, respectively, differed on average, by 22 and 40% in week 1 and 78 and 77% in week 2. Both

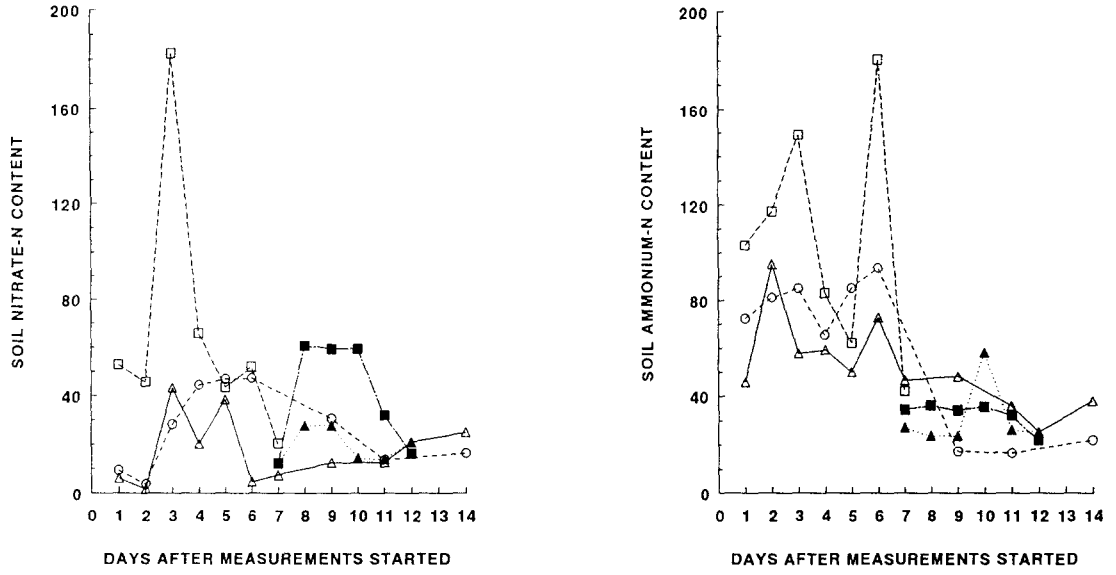

Fig. 3. Changes in soil (**a**) NO_3^--N ($\mu g\ g^{-1}$ dry soil) and (**b**) NH_4^+-N ($\mu g\ g^{-1}$ dry soil) with time in a grassland peat soil after application of conventional (A Δ and D ▲), diluted (B ○) or acidified (C □ and E ■) farm slurry. Treatments A, B and C were applied at the start of week 1, and D and E at the start of week 2.

methods are likely to influence conditions affecting denitrification (Smith and Arah, 1990). Enclosure systems influence the local micro-environment and for the determination of TDN there can be no assurance that all the pores within the soil block had been permeated with acetylene. Measurements were also restricted to relatively short periods.

There are also major problems with coring/incubation methods, through effects of disturbance and changes in the composition of the soil atmosphere in the cores (Smith and Arah, 1990). Whilst the effect of slurry on the core surface on the O_2 content of the vessel headspace is unlikely to influence denitrification rates (Thompson, 1989), the initial exposure of the sides of the cores to a relatively O_2-rich environment may have significant effects. Conversely, there may be effects of reducing O_2 concentration during incubation. Furthermore, the method provides an assessment for the top 10-cm of soil only and takes no account of denitrification below this (Jarvis et al., 1991).

It is perhaps not surprising that although trends were reproduced, there were significant differences between the methods. It is difficult, however, to explain the greater apparent denitrification rates with the core/incubation method because this estimates loss from a restricted depth and would have been expected to have a reduced potential because of enhanced aeration. Other comparisons between chamber and coring methods in permanent grassland soils (Ryden et al., 1987) have shown good agreement, but there has been much poorer agreement with arable soils (Webster and Goulding, 1989). Previous studies (Ryden et al.,1987) and trials before the present experiment indicated that collection, recovery and analytical procedures for the N_2O from both methods were efficient so that any difference is most likely to be related to inherent differences of the sampling methodology. The fact that N_2O loss (without acetylene) was also smaller with the enclosure method indicates either reduced microbial activity and/or restricted transport from the enclosed compared with the incubated soil rather than any difference in the effectiveness of the acetylene in inhibiting reduction process in either method. It is possible that with additional soluble C supplied in the slurry to an already highly organic soil there may have been considerable sensitivity to differences in temperature in the two methods. The proportions of TDN apparently released as N_2O were very similar with both methods.

With the coring method, the differences in estimates made after either 4 or 24 hours were often substantial and are of some importance. There may have been diurnal patterns in the processes involved and N_2O release, i.e. an initial high rate at higher ambient temperatures and the lower 24 h rate reflecting subsequent lower temperatures and a depletion of NO_3^- but the methodology may have had effects. The involvement of NH_4^+ as an important variable is indicative that the NO_3^- pool size was restricted and dependent

on nitrification. Soil moisture contents were unlikely to have changed significantly during incubation, but soil atmosphere O_2 concentration will have decreased from start of incubation, i.e. a trend that follows the measured TDN rates and N_2O release but opposite to the expected effects on denitrification.

Because of lack of consistency between methods it is difficult to draw conclusions about the absolute rates TDN and N_2O release. Nevertheless, distinct trends were shown. Multiple correlations indicated that major proportions of the daily variability in TDN could be accounted for by differences in soil moisture, temperature and NH_4^+ contents. It was also clear that soil NO_3^- contents had very little apparent direct effect on denitrification: this indicates that either substrate levels were generally non-limiting, that there may have been significant distributions of NO_3^- in locations not readily accessible to microbial utilization or that NO_3^- was limiting and denitrification was dependent upon pool replenishment by nitrification. The latter seems probable because of the generally low NO_3^- content and underlines the importance of NH_4^+ oxidation as a major determinant of the fate of excess N in soil systems as indicated by the strong negative correlation between NH_4^+ contents and denitrification. Nitrification, however, confounds interpretation of the present results in at least two ways. As indicated, the conditions in the present soil were such that both denitrification and nitrification could have occurred simultaneously leading to a direct coupling of the two processes. Understanding the controls over denitrification, because of this interaction, becomes even more complex. Secondly, determination of the fluxes of N_2O are further complicated by nitrification acting as a source (Bremner and Blackmer, 1980). In the present organic soil mineralization as well as addition of farm wastes will have increased the potential for nitrification. Changes in nitrification rate, changing soil conditions during the period of measurement and the effects of acetylene in inhibiting nitrification as well as denitrification, make interpretation of effects on denitrification and N_2O loss difficult.

However, there can be little doubt that denitrification occurred and that slurry additions increased the rate of this and of N_2O losses. The trends in the present study showed considerable changes in the ratio of TDN: N_2O over time and with treatment. Studies with fertilized grassland have indicated that N_2O represented 25% of the total loss (Ryden, 1981) and recent estimates of N_2O losses after slurry addition assumed that the ratio of denitrification products, i.e. N_2: N_2O was 3: 1 (Jarvis and Pain, 1994). The rates of N_2O losses over all treatments in our study were very variable but increased as denitrification increased. The proportions of N_2O were higher on this soil with added slurry and equivalent to, on average, between 40–74% of the total losses. Those occasions when N_2O losses were apparently greater than total losses are at least in part the result of the wide variability experienced but may also be the result of changes in the balance of denitrification and nitrification.

It is clear that application of livestock slurries has impact on losses and N_2O emissions. All of the present techniques of applying cattle slurry increased denitrification and N_2O losses. Of those used, the conventional method of applying slurry should have had the lowest potential for denitrification since it offered the greatest opportunity for the escape of mobile N (and hence potential substrate) by ammonia volatilization. Other studies with cattle and pig slurries have reported increased denitrification rates when steps have been taken to reduce NH_3 loss (i.e. by injection or acidification) (Pain et al., 1990; Thompson et al., 1987). Although denitrification losses from our conventional slurry treatment were lower than with acidified slurry applied in week 2 there were only a small differences for slurries applied during week 1. Diluting the slurry reduced losses slightly in the second week of measurements, presumably through a reduced substrate and carbon input. The increase in loss with acidification in week 2 can be attributed to reduced NH_3 loss and the extra NO_3^- added as acid. Differences with the same treatments in the two different weeks are most probably related to changes in environmental conditions and their interactive effects on all the processes involved.

The interactions involved are clearly complex and if appropriate management measures are to be taken to minimize losses and reduce leakage of gaseous N into the atmosphere, research at a fundamental level is required. This will require the development of more appropriate methodology for field measurement of fluxes and also detailed investigation of the coupling of denitrification and nitrification processes in a range of soils with precisely defined conditions. An increased understanding about the sites of microbial activity and distribution of substrates at a fine scale should help to provide the bases on which to develop models to allow prediction of the impact of new approaches to better management of soils and waste materials.

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