Agriculture, Ecosystems and Environment, 39 (1992) 267-278
Elsevier Science Publishers B.V., Amsterdam

Denitrification and N_2O production in pasture soil: the influence of nitrogen supply and moisture

P. Colbourn
AFRC Institute of Grassland and Environmental Research, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 3EB, UK

(Accepted 17 July 1991)

ABSTRACT

Colbourn, P., 1992. Denitrification and N_2O production in pasture soil: the influence of nitrogen supply and moisture. *Agric. Ecosystems Environ.*, 39: 267-278.

Denitrification was studied in packed soil columns and in soil cores in a controlled environment facility. The soils were kept warm (10-15°C) and moist (70-90% water-filled porosity). Urine, urea, ammonium or nitrate alone were added at rates typical of pasture systems. The denitrification from native nitrogen was low (less than 0.02 kg N ha^{-1} day^{-1}) and addition of ammonium nitrate caused a rapid tenfold increase. All forms of nitrogen given at rates similar to urine deposits in the field gave much higher denitrification (up to 0.3-1.0 kg N ha^{-1} day^{-1}); the highest rate came from 80 kg N ha^{-1} as nitrate (4.8 kg N ha^{-1} day^{-1}). Nitrous oxide formed 8-76% of the product, but was always a smaller part of the product from ammonium nitrate (10-25%) than from urea (45-75%). Some of this extra nitrous oxide from urea probably came from nitrification not denitrification. This was calculated to be 50% in the controlled environment studies but was only 11% in a parallel field study. Field denitrification losses, based on these results, could range from 3 kg N ha^{-1} $year^{-1}$, with neither added nitrogen nor grazing, to 20 kg N ha^{-1} $year^{-1}$ or more from a grazed pasture with 200 kg N ha^{-1} $year^{-1}$ fertiliser nitrogen.

INTRODUCTION

Nitrogen is a key plant nutrient. The success of the farming industry in northwest Europe over the past 40 years has been due, to a great degree, to improving the supply of nitrogen to the crop. Although the agricultural benefits of supplying fertiliser nitrogen are self-evident, the consequences for the wider environment are not well quantified. We know that nitrogen is lost from the soil and that working the soil can increase that loss (Dowdell et al., 1987).

We need to know more about gaseous losses of nitrogen from the soil for several reasons. Losses cost the farmer money, take a valuable plant nutrient

---

Correspondence to: P. Colbourn, AFRC Institute of Grassland and Environmental Research, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 3EB, UK.

0167-8809/92/$05.00

out of the system as loss from the soil nitrogen cycle, and release it into the atmosphere. The denitrification (dN) process releases mainly nitrous oxide (N_2O) and nitrogen gas (N_2). Nitrous oxide becomes involved in the 'greenhouse effect' and may help to destroy ozone (Crutzen, 1981; Fillery, 1983). For all of these reasons we need to study dN losses.

Changes in nitrogen supply, soil moisture and warmth help to govern dN rates in a given soil (Groffman et al., 1988). These can all alter rapidly in the field, creating a complex dynamic which is hard to interpret. We can separate them in controlled environments (CE) so that their effects can be sorted out.

In pasture there is active cycling of nitrogen through the grass and the animal to the soil. Nitrogen is added to the soil from the bag and in plant residues, particularly in residues from legumes like white clover. The risk of dN is highest when nitrate appears in the soil, for example, when ammonium nitrate is added or when a urine deposit nitrifies.

In west Britain, rainfall is high so that the soils are often wet. The mild, wet weather, together with sheep grazing indicates a high chance of dN because there is a regular supply of nitrogen in excreta, especially in urine. The dN losses in these soils have not been studied before.

Studies using CE facilities provide new, basic data to help develop models to estimate losses in the field. The primary aim of the present study was to define the potential rates of dN in common pasture soil from fertiliser nitrogen or urine deposits.

MATERIALS AND METHODS

Columns of repacked soil (50 cm) and complete cores (20 cm) were contained in lengths of PVC pipe (15 cm diameter) with sealed bases that allowed excess water to be drawn off. A weighted metal plate on a silicone rubber collar sealed the top for measurement of gas fluxes. Small cores (5 cm) were held in steel rings (7.5 cm diameter) with metal base caps. The small cores were sealed in jars to measure gas fluxes. The soil columns and cores were kept in CE cabinets.

The main soil (CR), a silt loam acid brown earth (Denbigh association, East Keswick series; C. Rudeforth, personal communication, 1985; Rudeforth, 1970), was freely drained with a bulk density of 1.0 kg dm^{-3} and 60% of the volume as pore space. Four other soils were compared with it in some studies. They were another acid brown earth (BM), found on sandstone at over 300 m above sea level (Milford series); a silt loam with impeded drainage (DA) and two organic soils (PM1, PM2).

Soil moisture in the soil cores and columns was kept in the range 70–90% water-filled porosity (WFP) (0.3–0.4 kg kg^{-1} oven dry soil). Temperature in the CE cabinet was kept in the range 10–15 °C. In the field it was 10 ± 2 °C.

In Experiment 1, nitrogen was applied to the surface of soil columns as urea

(0.7–1.9 mg N cm^{-2}, or 70–190 kg N ha^{-1}), or ammonium nitrate (30–70 kg N ha^{-1}). In Experiment 2, nitrogen was added to the large cores as nitrate daily in water (1–5 kg N ha^{-1} day^{-1}), or as urine (200 kg N ha^{-1}) taken from penned sheep. In Experiment 3, small cores were treated in parallel with the large cores except that on the small cores two rates of urine were used (120 and 240 kg N ha^{-1}) and the nitrate was added to give a higher range (6, 12 and 18 kg N ha^{-1} day^{-1}). In Experiment 4, artificial urine (Doak, 1952) was applied to small cores at three rates (140, 280 and 560 kg N ha^{-1}) and nitrate was added to others (40, 80 and 120 kg N ha^{-1}).

The acetylene blocking method was used to estimate total dN rate (Colbourn et al., 1984). Acetylene was injected into the centre of the soil columns to give about 1% in the soil air, 3 h before the flux of N_2O was measured with the top closed. Acetylene was not used too often and not in all soils to avoid changes in the soil biology (Germon, 1980). The small cores were sealed in 500 ml gas tight jars with 2% acetylene in air. They were kept in the steel sleeves with the base closed.

Nitrous oxide flux from the soil surface was measured with and without acetylene after nitrogen addition. Samples of headspace air were taken 15 min after the top of the columns had been closed with the lids. Gas samples were analysed for oxygen, CO_2, N_2O and C_2H_2 with a gas chromatograph (Pye-Unicam PU4500) fitted with thermal conductivity detector for general use and an electron capture detector for nitrous oxide in small amounts (Hall and Dowdell, 1981).

A small field trial was run on the CR soil. Small plots (1 m×1 m) were treated with urine (200 kg N ha^{-1}), ammonium nitrate (70 kg N ha^{-1}) or zero nitrogen, in triplicate. The nitrous oxide flux from the surface was measured using a static cover method (Colbourn and Harper, 1987) and small cores were taken from the plots from time to time for jar incubations with acetylene. The site had a uniform ryegrass sward.

RESULTS

Denitrification

Denitrification (dN) from native nitrogen in soil CR, in Experiment 1, was only 0.02 kg N ha^{-1} day^{-1} (coefficient of variation, CV=90%), with acetylene. The loss of nitrous oxide alone from field soil was even slower: 0.01 kg N ha^{-1} day^{-1} (CV=100%). Both urea and ammonium nitrate speeded up dN, and at rates intended to simulate urine patches in the field they both gave rise to 0.3–0.7 kg N ha^{-1} day^{-1} (mean 0.4, CV=60%). When ammonium nitrate was used at rates chosen to simulate fertiliser in the field, dN was 0.02–0.2 kg N ha^{-1} day^{-1} (mean 0.12, CV=60%). There was a delay of several days after urea had been added to the soil before significant dN was measured.

In Experiment 2, CR and BM soils reacted to urine with a dN rate of up to 1.0 kg N ha^{-1} day^{-1} after 16 days. The peat soils did less, although the peat soil taken from within a sheep-grazed paddock (PM1) gave a greater response compared with PM2 from an area of rough grazing. Surprisingly, the soil with impeded drainage, DA, gave only a moderate response (Table 1). Small cores of soil CR, in Experiment 3, showed dN of 0.2–0.5 kg N ha^{-1} day^{-1} (for 5 cm depth of soil), 12 days after urine had been added. Daily addition of nitrate of 3–18 kg N ha^{-1} day^{-1} to the small cores did not achieve such high rates.

In Experiment 4, a range of nitrogen addition to cores of soil CR showed how rapid dN usually followed the addition of nitrate but losses of urine nitrogen were slow to start. The minimum delay for urea or urine was 6 days but consistent dN was recorded only after 21 days in some urine-treated soils (Table 2). The fastest individual dN rate was found in a small core of soil CR with 80 kg N ha^{-1} as nitrate: 4.8 kg N ha^{-1} day^{-1} on the eleventh day. High nitrate levels (N3, Table 2) clearly did inhibit dN significantly over the first 10 days but nitrogen release then went on for a longer period, at least 40 days. Urine also started later. In the third period compared in Table 2, dN from the two lower rates of nitrate (N1+N2) was significantly slower than from all other treatments ($P<0.050$). The highest rate (N3) and the middle rate of urine (U2) together, werc significantly faster than all others ($P<0.005$). The cause of the differences between the urine treatments was not clear, but the nitrate-treated soils had run out of nitrogen in the low nitrogen treatments by Day 20.

Soil moisture had a very clear effect on dN (Fig. 1). Analysis of variance

TABLE 1

Nitrous oxide flux and denitrification from urine (200 kg N ha^{-1}) added to five soils, summary of data from Experiment 2 (kg N ha^{-1} day^{-1})

Soil	CR	BM	DA	PM1	PM2
Nitrous oxide flux					
Mean	0.25	0.32	0.09	0.06	0.05
SD[1]	0.8	0.21	0.04	0.04	0.03
CV[2](%)	33	67	39	74	59
n[3]	6	6	6	6	6
Denitrification					
Mean	0.81	0.76	0.43	0.35	0.08
SD	0.20	0.23	0.06	0.24	0.09
CV(%)	25	30	14	69	104
n	5	5	5	5	5

[1] Standard deviation.
[2] Coefficient of variation.
[3] Number of samples.

TABLE 2

Denitrification (means, $n=3$) from cores of soil CR with nitrate or artificial urine at 85% WFP and 15 °C; summary of data from Experiment 4 (kg N ha^{-1} day^{-1})

	N added as[1]:					
	Potassium nitrate			Artificial urine		
	N1	N2	N3	U1	U2	U3
Days						
1–10	0.80	1.33	0.58	0.01	0.09	0.02
11–20	0.04	1.57	0.90	0.01	0.05	0.04
21–40	0.00	0.09	0.50	0.12	0.39	0.14
dN as % of added N	21	38	20	1.4	3.2	0.6
Total of dN (kg N ha^{-1})	9	30	24	2	9	3

Nitrogen treatments (kg N ha^{-1}) were as follows. Nitrate: N1, 40; N2, 80; N3, 120. Urine: U1, 140; U2, 280; U3, 560.

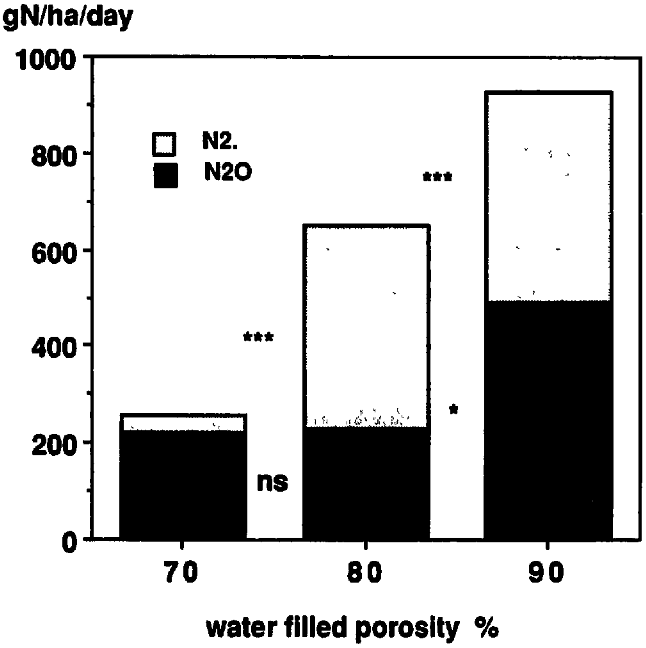

Fig. 1. Denitrification identified as nitrous oxide (N_2O) and nitrogen gas (N_2) from brown earth pasture soils (BM+CR) at three moisture levels and at 15 °C, 16 days after adding urine: $^*P<0.05$; $^{***}P<0.001$; NS, not significantly different.

of nitrous oxide production and of total denitrification for the combined brown earth soils (BM and CR) over three moisture levels showed the significant effect of soil water content. The probability of nitrous oxide output being

TABLE 3

Probabilities of mean denitrification or nitrous oxide production rates being the same for two moisture levels after the addition of urine (Experiment 2)

Moisture levels[1]	Soil CR	BM	DA	PM1	PM2	CR+BM
Nitrous oxide (probability less than)						
L vs. M	NS[2]	NS	NS	NS	NS	NS
M vs. H	NS	0.001	NS	NS	NS	0.025
L vs. H	NS	0.001	NS	NS	NS	0.010
Denitrification						
L vs. M	0.100	0.005	0.025	NS	NS	0.001
M vs. H	NS	0.010	NS	0.100	NS	0.001
L vs. H	0.005	0.005	NS	NS	NS	0.001

[1] Soil moisture levels (water-filled porosity): L, 70%; M, 80%; H, 90%.
[2] $P \geq 0.100$.

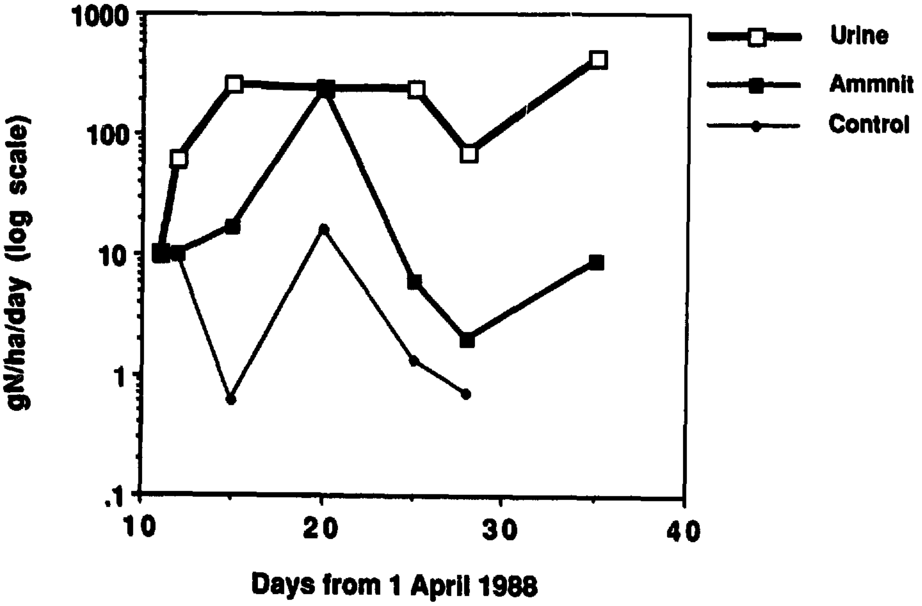

Fig. 2. Denitrification from field plots with urine, ammonium nitrate (Ammnit) or no nitrogen (Control); urine given on Day 8 at 200 kg N ha^{-1}, ammonium nitrate given on Day 12 at 70 kg N ha^{-1}. Note log scale.

the same for all moisture levels was less than 0.005; for denitrification, $P < 0.001$. However it was not possible to achieve uniform soil moisture conditions in replicate cores so statistical significance was not easy to establish for individual soils. Analysis of variance for all five soils together failed to establish a significant moisture effect. Individual comparison of means showed significant effects mainly in the brown earth soils (Table 3).

In the field, urine caused dN to climb to a maximum between 4 and 12 days later with soil moisture at 70–80% WFP. When the soil dried to 60% WFP, dN declined. Watering (3 mm) on Day 20 increased the rate from 0.06 to 0.4 kg N ha^{-1} day^{-1} and rainfall had a similar effect on Day 27 (Fig. 2). Ammonium nitrate treated plots had maximum dN at 8 days but then this declined rapidly and did not increase again after irrigation or rainfall. There was very little dN on control plots, with no added nitrogen, but it did respond clearly to changes in soil moisture (Fig. 2).

Nitrous oxide

From native nitrogen, nitrous oxide flux in the absence of C_2H_2 was 65% (CV=14%) of the total dN product from field soils. Urea gave a similar amount, 60% nitrous oxide (CV=10%), but ammonium nitrate gave less, 30% (CV=24%).

From urine, in CE, the nitrous oxide formed 38%, on average, of the total dN (range 8–76%, CV=9%). Soil BM gave more nitrous oxide, mean 53%; soil DA gave less, mean 23%. Soil CR with ammonium nitrate gave more nitrous oxide in drier soil: 60% at 70% WFP but only 26% at 80% WFP (Fig. 3).

In field plots, nitrous oxide made up 14% of the product (range 6–40%, CV=22%). Urine gave more (mean 20%, CV=27%); ammonium nitrate gave less (mean 10%, CV=35%).

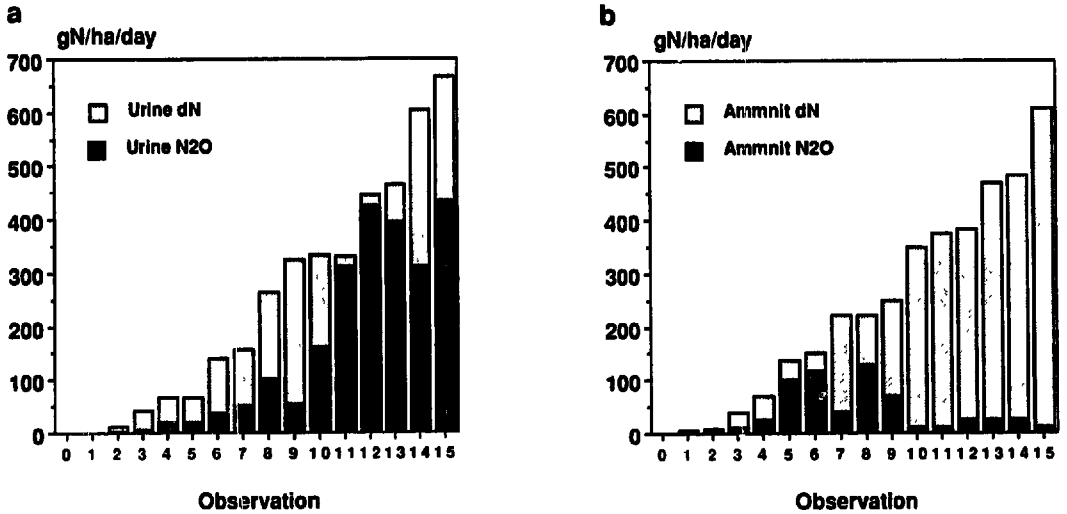

Fig. 3. Nitrous oxide (N_2O) released from (a) urine and (b) ammonium nitrate (Ammnit) compared with total denitrification (dN) measured with acetylene; observations ranked in ascending order of total dN. Cores of brown earth pasture soil (CR).

DISCUSSION

Model systems

Large applications of nitrogen, such as urine, onto warm wet grassland are known to increase the chance of big denitrification losses (Ryden, 1983; Thompson et al., 1987). The controlled environment study reported here using the brown earth soils gave similar results to the field measurements referred to above and therefore suggests that model systems may be useful in improving our understanding of factors influencing denitrification in the field. The value of these observations will of course, depend upon the extent to which they accurately represent the field. Checks were therefore carried out on soil pore geometry for the repacked soil treatments (see below). The fact that some soils did not respond strongly to nitrogen additions and that all soils responded less strongly to nitrate than to urine, suggests that soil acidity may have been an important constraint. Ammonium nitrate and urea proved to be adequate analogues of urine in as far as the rates of dN are concerned. The rates measured in Experiments 1 and 2 were broadly similar; the means were not significantly different for soil CR. Daily additions of nitrate did not prove to be a good analogue of nitrate supply for dN from urine in soil CR. A continuous, steady rate may be important to keep the dN process going, and the nitrification process itself may be important as discussed below. The artificial urine used in Experiment 4 delayed the onset of dN more than had been expected but the reason for this is not known.

Soil porosity and gas diffusion

Fick's law of diffusion relates gas concentration gradient to its flux using a diffusion coefficient (D) which is 0.14 $\mathrm{cm^2\ s^{-1}}$ for nitrous oxide in open air (Pritchard and Currie, 1982). Open airways are limited in soil. Values of D worked out from fluxes showed a change from a large value of D (0.021 $\mathrm{cm^2\ s^{-1}}$) representing a very open soil, to a much smaller value (0.0005 $\mathrm{cm^2\ s^{-1}}$) more typical of a field soil (Currie, 1965, 1983; Ball, 1981), caused by wetting and draining the soil. After the wetting/draining cycle, the soil did have a pore geometry like a field soil.

Another cause of concern comes from the methods used to measure dN. In a small study to check this, the small core method using jars was compared with the cover method used on large cores. Small cores, taken from the large cores of soils CR and BM, gave the same dN rates as the more direct method: 0.9 and 0.8 kg $\mathrm{ha^{-1}\ day^{-1}}$ (SD=0.3, CV=18%, n=4).

Field loss

Probable annual dN losses in the field have been worked out for a number of grassland systems from the dN rates seen in this study. With neither added nitrogen nor grazing, the losses would be expected to be less than 4 kg N ha^{-1} year^{-1}. Grazing, with the return of nitrogen in urine, speeds up the loss considerably. A high stocking rate is used in the calculation in Table 4 which relates to the typical fertiliser applications. At these rates, ammonium nitrate fertiliser by itself would lose less than urine in most years, mainly because the time available for dN is much more limited (Table 4). A lower stocking rate would be supported by a pasture receiving little or no fertiliser nitrogen, perhaps ten sheep ha^{-1}, and denitrification losses would be reduced accordingly.

These tentative predictions of field dN must be tested in the field. A good, reliable field method is still needed and we need to know more about urine deposits on grassland because this would help us make a better estimate of field loss. More studies are also needed of the time course of nitrogen gas release from urine.

TABLE 4

Projected annual denitrification loss of nitrogen under field management systems

Nitrogen applied	Denitrification rate (kg N ha^{-1} day^{-1})	Part of year (%)	Part of field (%)	Loss (kg N ha^{-1} year^{-1})
Nil	0.00	20	100	0.0
	0.005	40	100	0.7
	0.01	30	100	1.1
	0.03	10	100	1.1
				2.9
Fertiliser-N[1]	0.00	28	100	0.0
	0.01	60	100	2.2
	0.10	10	100	3.7
	0.50	2	100	3.6
				9.5
Urine[2]	0.00	20	100	0.0
	0.01	80	60	1.8
	0.05	50	40[3]	3.7
	0.10	30	28	3.1
	0.30	30	10	3.3
	0.50	30	2	1.1
				13.0

[1] Ammonium nitrate at 200 kg N ha^{-1} in four applications.

[2] Grazing approximately 30 sheep ha^{-1} for 200 days.

[3] Urine-affected area of 40% (P. Goodman, personal communication, 1988).

Based on rainfall and temperature for WPBS Aberystwyth (R. Scurlock, personal communication, 1989).

Nitrous oxide release

This study did not attempt to distinguish between nitrous oxide released as a first stage product of dN and nitrous oxide released during nitrification (Nf). Acetylene blocks Nf as well as nitrous oxide reduction (dN), so the total nitrogen gas loss (Nf+dN) could have been greater than that found in this study using acetylene. Nitrous oxide measured without acetylene includes both sources, dN and Nf.

The bigger amounts of N_2O seen in the nitrogen gas loss from urine-treated soils (Fig. 3) must be a result of a process other than the dN of nitrate. It is most likely that N_2O was released during the Nf of ammonium. We can assume that the N_2:N_2O ratio from nitrate reduction (dN) would have been the same for all sources of nitrate under the same conditions. The excess N_2O from urine can be estimated from the ratio measured with nitrate and this excess N_2O can be attributed to Nf.

This suggests that up to 50% of the N loss from the urine system can be as N_2O from Nf so this process could be a major source of N_2O to the atmosphere. However, the field measurements showed a smaller amount from Nf, about 11% of the total N loss. The reason for this difference between field and laboratory measurements needs further investigation, it may be simply one of temperature.

CONCLUSIONS

Denitrification frequently ran up to 1 kg N ha^{-1} day^{-1} in the two agricultural brown earths when there was an adequate source of nitrogen. The mean rate for measurements made at 70–90% water-filled porosity and 10–15°C was 0.3 kg N ha^{-1} day^{-1}. Denitrification was restricted in the organic soils, probably by low pH. The mean rate at 70–90% water-filled porosity and 10–15°C was 0.06 kg N ha^{-1} day^{-1}. Denitrification rate can be related to soil moisture content but more measurements are needed to establish the true effect, mostly because soil moisture content was difficult to control in a properly replicated way. The linear regression for dN from the two brown earths (Fig. 1) was

$$y = -1884.7 + 33.2\,W, \quad r^2 = 0.991$$

where W is water-filled porosity (%) and y is measured in g N ha^{-1} day^{-1}.

The proportion of nitrous oxide in the product seemed to be smaller when ammonium nitrate was applied than when urea was used. This might reward further study.

There was a delay of several days before N_2O and dN losses from urea/urine treated soils could be measured and the losses continued right up to the end of the study periods. More information is needed on the time course of denitrification loss from urine.

Denitrification and related losses might accrue to as much as 20 kg N ha^{-1} from an intensive, sheep grazed pasture system but would be as little as 7 kg N ha^{-1} from a low input system.

ACKNOWLEDGEMENTS

The technical support of D.H. Williams is gratefully acknowledged as is the encouragement of Dr. P.J. Goodman, Professor R.Q. Cannell, Professor J.L. Stoddart and Dr. L.R. Mytton. The manuscript was typed in the Publications Section at WPBS.

REFERENCES

Ball, B.C., 1981. Pore characteristics of soils from two cultivation experiments as shown by gas diffusivities and permeabilities and air-filled porosities. J. Sci. Food Agric., 32: 483–498.

Colbourn, P. and Harper, I.W., 1987. Denitrification in drained and undrained arable clay soil. J. Soil Sci., 38: 531–539.

Colbourn, P., Iqbal, M.M. and Harper, I.W., 1984. Estimation of total gaseous nitrogen losses from clay soils under laboratory and field conditions. J. Soil Sci., 35: 11–22.

Crutzen, P., 1981. Atmosphere chemical processes of the oxides of nitrogen, including nitrous oxide. In: C.C. Delwiche (Editor), Denitrification, Nitrification and Atmospheric Nitrous Oxide. Wiley, New York, pp. 17–44.

Currie, J.A., 1965. Diffusion within soil microstructure, a structural parameter for soils. J. Soil Sci., 16: 279–289.

Currie, J.A., 1983. Gas diffusion through soil crumbs: the effects of wetting and swelling. J. Soil Sci., 34: 217–232.

Doak, B.W., 1952. Some chemical changes in the nitrogenous constituents of urine when voided to pasture. Agric. Sci., 42: 162–171.

Dowdell, R.J., Colbourn, P. and Cannell, R.Q., 1987. A study of mole drainage with simplified cultivation for autumn sown crops on a clay soil. 5. Losses of nitrite-N in surface runoff and drain water. Soil Tillage Res., 9: 317–331.

Fillery, I.R.P., 1983. Biological denitrification. In: Gaseous loss of nitrogen from plant-soil systems. In: J.R. Freney and J.R. Simpson (Editors), Martinus Nijhoff/W. Junk, The Hague, pp. 33–64.

Germon, J.C., 1980. Etude quantitative de la denitrification biologique dans le sol a l'aide de l'acetylene. Ann. Microbiol. (Paris), 131B: 69–80.

Groffman, P.M., Tiedje, J.M., Robertson, G.P. and Christensen, S., 1988. Denitrification at different temporal and geographical scales: proximal and distal controls. In: J.R. Wilson (Editor), Advances in Nitrogen Cycling in Agricultural Ecosystems. CAB International, Wallingford, UK, pp. 174–192.

Hall, K.C. and Dowdell, R.J., 1981. An isothermal gas chromatographic method for simultaneous estimation of oxygen, nitrous oxide and carbon dioxide content of gases in soil. J Chromatogr. Sci., 19: 107–111.

Pritchard, D.T. and Currie, J.A., 1982. Diffusion coefficients of CO_2, N_2O, C_2H_4 and C_2H_6 in air and their measurement. J. Soil Sci., 33: 175–184.

Rudeforth, C., 1970. Soils of North Cardiganshire. Soil Survey of England and Wales, Harpenden, UK.

Ryden, J.C., 1983. Denitrification loss from grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. J. Soil Sci., 34: 355–363.

Thompson, R.B., Ryden, J.C. and Lockyer, D.R., 1987. Fate of nitrogen in cattle slurry following surface application or injection to grassland. J. Soil Sci., 38: 689–700.