PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fate of Nitrogen Applied to Agricultural Crops with Particular Reference to Denitrification [and Discussion]

R. J. Dowdell and M. H. Mian

Phil. Trans. R. Soc. Lond. B 1982 **296**, 363-373
doi: 10.1098/rstb.1982.0012

Phil. Trans. R. Soc. Lond. B **296**, 363–373 (1982)
Printed in Great Britain

Fate of nitrogen applied to agricultural crops with particular reference to denitrification

By R. J. Dowdell

Agricultural Research Council Letcombe Laboratory, Wantage, Oxon. OX12 9JT, U.K.

The effectiveness of N fertilizer depends on the ability of crops to compete with microorganisms for the available N in the soil. Adverse growth conditions (e.g. drought and waterlogging) diminish the capacity of the plants for N uptake, while N supply may be limited by transient assimilation by microorganisms, by longer-term immobilization into soil humus and by losses through leaching and denitrification. Conversely, mineralization of soil organic matter contributes to the availability of N. Significant alteration of soil management methods (e.g. improvement of drainage, use of simplifed cultivation systems, or rotation of arable land with grass) may change the balance of processes within the nitrogen cycle.

The results of field and lysimeter experiments (some with ^{15}N-labelled fertilizer) are used to discuss these aspects of the fate of applied N, with particular attention to the role of denitrification.

1. Introduction

Denitrification is important in agriculture as a route whereby inorganic N is lost from soil, thus diminishing the amount available to crop plants. Not all of the N applied as fertilizer is utilized by the crop in creating harvestable dry matter. Nitrogen may be temporarily retained in roots, immobilized into the soil organic matter by soil microorganisms, or lost by leaching or denitrification. However, the vigour of the crop can greatly influence the magnitude of these processes. Unfavourable growing conditions (e.g. drought or waterlogging) or major changes in management of the crop–soil system (e.g. improvements in drainage or use of simplified cultivation methods) can profoundly affect the interrelations of the nitrogen cycle.

2. Crop uptake of applied nitrogen

Many agronomic experiments have suggested that crops typically use only about half of the applied fertilizer N (Allison 1966). In most of these experiments the efficiency of N utilization is estimated by comparing the N contents of crops with and without fertilizer and expressing the difference as a proportion of the total fertilizer N applied (the 'apparent' recovery). This disregards whether the absorbed N comes from the fertilizer or from other sources in the soil. Unambiguous information on the contribution made by fertilizer to the nutrition of the crop plants can be obtained by use of ^{15}N-labelled fertilizer (see Broadbent & Carlton (1978) and Hauck & Bremner (1976) for discussions of the assumptions and problems associated with the technique).

Results from experiments at Letcombe Laboratory show that the efficiency of N fertilizer use, expressed as the percentage recovery of applied ^{15}N in the above-ground parts of the plants, averaged around 45–50%, with a range of 39–68% (Table 1). The 'apparent' recovery of

N by the plants in the same experiments as those receiving ^{15}N always exceeded the recovery estimated using ^{15}N, being in the range of 51–111%. The estimates differ because in the 'apparent' recovery calculation the N used by the crop is taken to have come only from the fertilizer applied. Other studies have shown that fertilizer contributes only about two-third of the N assimilated, with most of the remainder coming from mineralization of soil organic N (see § 3).

Table 1. Recovery of fertilizer N into the above-ground parts of crops in the growing season that the N was applied

crop	experimental system	soil series and texture	nitrogen form	application rate kg N ha^{-1}	recovery of ^{15}N (% of applied)	apparent recovery† (% of applied)
perennial ryegrass	lysimeters (1974)	Rowlands sandy loam	$Ca(NO_3)_2$	400	43–54	59–69
permanent grassland	lysimeters (1977)	Salop clay loam	$Ca(NO_3)_2$	400	45–47	51–55
permanent grassland	lysimeters (1977)	Bromyard silt loam	$Ca(NO_3)_2$	400	39–52	53–54
winter wheat	lysimeters (1975)	Skipwith sandy loam	NH_4NO_3	100	54–63	n.a.
winter wheat	lysimeters (1976)	Evesham clay loam	NH_4NO_3	95	39–54	n.a.
spring barley	lysimeters (1977)	Andover silt loam over chalk	$Ca(NO_3)_2$	120	41–50	71–83
winter wheat	field (1977)	Evesham clay loam	$Ca(NO_3)_2$	80	60–68	78–111
winter wheat	field (1980)	Denchworth clay loam	$Ca(NO_3)_2$	80	69–72	n.a.

† $100 \times \frac{\text{(N in fertilized crop} - \text{N in crop without fertilizer)}}{\text{(N in fertilizer)}}$.

Adverse environmental conditions for crop growth can markedly diminish the efficiency of N fertilizer utilization (table 2). On a grass sward, drought conditions either for 4 weeks before each cut (treatment B) or for 2 weeks before and after each cut (treatment C) significantly decreased the recovery of ^{15}N when compared with lysimeters subjected to average distribution of rainfall (treatment A) or irrigated to maintain the soil at field capacity (treatment D). This effect was particularly marked in treatment B where the combination of a period of water stress together with defoliation diminished the plants' ability to recover N applied at the beginning of a short period of heavy precipitation.

Waterlogging has also been shown to depress the N content of the plants. Midwinter waterlogging of winter wheat on a clay soil diminished the N content to 66% of that in unwaterlogged controls (Cannell *et al.* 1980) and in a similar treatment (Cannell *et al.* 1977) on a sandy soil, recovery of labelled fertilizer was less (54%) than in controls (68%).

The effect of contrasting cultivation methods on the absorption of fertilizer N has not been so marked. In winter wheat grown on a clay soil, 60–67% of the applied ^{15}N was recovered in the crop, but there were no significant differences between the effects of direct drilling and ploughing (Dowdell & Crees 1980). Other observations made on the surrounding plots in this experiment revealed no significant interaction between cultivation and response to N fertilizer applications ranging from 40 to 160 kg N ha^{-1}. In other field experiments on a clay soil in eastern England, with the use of isotopically labelled N in the same year, recovery of applied N was greater in direct drilled crops (46 %) than in crops grown after tine cultivation (37 %) (Vaidyanathan & Leitch 1980).

Table 2. Recovery of ^{15}N-labelled fertilizer in herbage and in drainage water (percentage of ^{15}N applied in year 1)

(silt loam (Bromyard series), Perennial ryegrass sward.)

		drought		
	average rainfall (A)	4 wks before each harvest (B)	2 wks before and 2 wks after each harvest (C)	no water stress (D)
recovery in herbage				
year 1	48.7	38.9	44.8	51.8
year 2	9.6	6.9	9.3	8.0
year 3	2.3	1.5	2.3	2.0
recovery in leachate				
year 1	2.5	11.4	5.4	2.9
year 2	1.0	5.1	2.2	0.7
year 3	0.1	0.9	0.5	0.1
totals	**64.2**	**64.7**	**64.5**	**65.5**

Table 3. Proportions of immobilized N (104 kg N ha^{-1}, equivalent to 26.7 % of that applied) contained within various horizons of a sandy loam soil under perennial ryegrass

depth/cm	0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80
fertilizer N recovered/(kg ha^{-1})	44.9	23.8	16.3	6.2	2.6	2.9	3.0	2.4	1.9
percentage of immobilized N	40.9	22.9	15.7	5.9	2.5	2.7	2.8	2.3	1.7

(From Dowdell *et al.* (1980).)

3. Immobilization and mineralization

The temporary assimilation of applied inorganic N into plant roots and microorganisms, the more permanent immobilization into the soil humus and subsequent remineralization all influence the availability of N to the crop and the magnitude of leaching and denitrification losses. Some of the N contained in the living plant roots must, of course, be considered available to the plant for production of dry matter, but once roots die, the organic N enters the internal N cycle of the soil through the action of the growth and decay of soil microorganisms.

In the growing season after that when fertilizer was applied, amounts of labelled N found in herbage were small and ranged between 0.5 and 9 % of the initial dressing (Dowdell & Webster 1980). In the second year of a winter wheat experiment the crop recovered less than 1 kg/ha of the N applied in the first year (Dowdell & Crees 1980). Likewise in another study with winter wheat (Vaidyanathan & Leitch 1980) the second crop recovered only small amounts (about 3 %) of the fertilizer N that had been applied to the first crop. The N remaining unused from one year's inorganic N application to winter wheat thus seems to make only a very small contribution to the N nutrition of a succeeding crop of the same kind. This N, however, is unlikely to have remained in its original, inorganic chemical form, but to have been immobilized into the soil from organic matter and later remineralized. Analysis of soil and roots from lysimeters showed (table 3) that of the NO_3-N applied to the grass sward at the beginning of the experiment (4 years earlier) 25 % was found in roots and soil, and of this, two-thirds remained in the top 10 cm of the profile. Thus in an arable soil sown to grass, a sizeable fraction of the applied inorganic N can be immobilized in the soil organic matter and is largely unavailable for crop growth. How far such N may be released by mineralization after ploughing the sward is the subject of new experiments at Letcombe Laboratory.

Table 4. The uptake of labelled and unlabelled N (kilograms per hectare) by crops during the year in which fertilizer had been applied

	grassland			winter wheat
	sandy loam	clay	silt loam	clay
total N applied	400	400	400	80
N uptake of fertilized crop				
labelled N	201	206	247	52
unlabelled N	143	194	152	87
total	344	400	399	139
N uptake of unfertilized crop	72	121	138	63

The N requirement of direct drilled crops may be different from that of crops grown on ploughed land (Davies & Cannell 1975; Hodgson *et al.* 1977; Davies *et al.* 1976). In experiments on Evesham clay loam with winter wheat the smaller mineral N content in direct drilled soil than in ploughed soil was probably attributable to diminished mineralization rates (Dowdell & Cannell 1975). However, in another similar experiment only 8 % of ^{15}N-labelled fertilizer remained in the 0–15 cm layer and tillage did not significantly affect the amount of immobilized ^{15}N or its remineralization (Dowdell & Crees 1980). In lysimeter studies on grassland on a sandy loam (Dowdell & Webster 1980) the amount of N in the herbage derived from non-fertilizer sources in the soil and in rainfall was 135–145 kg N ha^{-1} during the summer when fertilizer was added. In comparison the amount of N absorbed by unfertilized grass was 72 kg N ha^{-1}, and this is close to the mean of 60 kg N ha^{-1} reported for a national experiment on N utilization by grass (Morrison *et al.* 1980). However, in unfertilized permanent grass swards growing on silt loam or clay soils (table 4) the values for N content of the herbage were greater (120–140 kg N ha^{-1}). In a winter wheat crop grown without N fertilizer, the absorption of N was 51–63 kg N ha^{-1} (Dowdell & Crees 1980). However, we do not know how well an unfertilized crop utilizes the naturally produced mineral N. Under conditions of N deficiency all of the N is probably used, so that the results from a zero-N treatment may be good estimates of the total mineral N available.

The contribution of soil-derived N to the total N assimilated by the plant may be changed by applying fertilizer. Consideration of the ratio of labelled to unlabelled N in a fertilized crop suggests that the production of inorganic N in the soil may be greater than that estimated from the quantity in unfertilized crops. In an experiment on ryegrass growing on a sandy loam soil

(Dowdell & Webster 1980), 140 kg N ha^{-1} of unlabelled N was absorbed by the crop in the first growing season when fertilizer was applied (table 4). In a winter wheat crop (Dowdell & Crees 1980) 80–92 kg N ha^{-1} of unlabelled N was assimilated. Thus if the plants absorb only half of the fertilizer applied (estimated by ^{15}N studies), these amounts of unlabelled (non-fertilizer) N represent only about half of the mineral N present (i.e. a total of 120–180 kg N ha^{-1}). These changes in the proportion of fertilizer N to soil N used by the plants could result from the fertilizer's increasing the vigour of the crop and the extent of exploration of the soil by roots. Stimulation of the activity of soil microorganisms by the supply of fertilizer nitrogen and by readily metabolizable exudates from plant roots may also be important. However, these results should be interpreted cautiously because dilution of the labelled mineral N pool by turnover of unlabelled soil organic N could result in apparent increases in uptake of soil N (Hauck & Bremner 1976).

4. Losses by leaching

During the first winter following application of ^{15}N-labelled fertilizer N to perennial ryegrass swards (Dowdell & Webster 1980) the losses of fertilizer N in water draining through the soil were quite small (2.5–11 % of that applied). Although the losses of fertilizer N in drainage were a small fraction of the N applied, they represent about 60 % of the total N dissolved in the water (almost exclusively nitrate). Losses of fertilizer N from grass swards (table 2) were greatest when irrigation was applied immediately after a defoliation and N fertilizer application (treatment B), and were also enhanced by drought at the time of defoliation (treatment C). The effect of the pattern of summer rainfall on the amount of N lost by leaching in the subsequent winter presumably resulted not only from deep penetration of fertilizer N during periods of irrigation and rainfall, but also from the diminished ability of the sward to take up N during the recovery period after the herbage had been cut.

In the second winter after fertilizer application, the amount of fertilizer-derived N found in the drainage water had declined to 1–5 % of that applied, and after three winters to less than 1 %. Thus in grassland, losses of fertilizer N by leaching are essentially confined to the winter period after the summer in which it had been applied.

In recent experiments (Cannell *et al.* 1977, 1980; Dowdell *et al.* 1980) with arable crops at Letcombe Laboratory (winter wheat grown on sandy loam and clay loam soils or spring barley on silt loam over chalk in lysimeters), only 1–7 % of the fertilizer N applied to the crops was lost in drainage water during the next winter. Total annual N losses in drainage were 15–105 kg N ha^{-1}. These results compare well with recent measurements of losses of N in drainage water from field plots of Denchworth clay with or without a mole drainage system; all plots had systems to collect surface runoff and water moving through the cultivated layer. Losses of N (almost entirely in the form of nitrate) were 50–66 kg N ha^{-1} from the mole-drained plots and 5–18 kg N ha^{-1} from the plots without mole drains.

5. Losses by denitrification

The study of denitrification in field or lysimeter soils has centred on the detection and estimation of nitrous oxide (an intermediate gaseous product in the reduction of nitrate to molecular nitrogen). High concentrations of nitrous oxide in the soil atmosphere were first measured by infrared absorption spectroscopy of large (1 l) samples (Arnold 1954). Subsequently the development of improved sampling techniques (Dowdell *et al.* 1972; Dowdell &

Crees 1974) and gas chromatographic methods (Burford 1969; Smith & Dowdell 1973; Hall 1980; Hall & Dowdell 1981; Wentworth & Freeman 1973) has allowed rapid, accurate and sensitive analyses of nitrous oxide content of small samples (1–5 ml). Measurements on heavy-textured soils during the past 10 years have shown that nitrous oxide can be found in the soil atmosphere in the wetter, cooler months of every year (table 5). The duration of this evolution seems to be between 2 and 6 months, and is particularly sensitive to extremes of winter rainfall below (1975/6) or above (1976/7) the average.

Table 5. Nitrous oxide content of the atmosphere of clay soils (published and unpublished results from Letcombe Laboratory)

soil series	year	rainfall, July–June (mm)	highest mean nitrous oxide concentration (μl/l)	period detected (weeks)
Denchworth	1970–1	833	800	6 (Jan.–Feb.)
Denchworth	1971–2	694	48	30 (Dec.–July)
Evesham	1971–2	694	520	32 (Dec.–July)
	1972–3	550	370	15 (Nov.–Apl)
	1973–4	578	460	14 (Nov.–Apl)
	1974–5	814	325	15 (Jan.–May)
Denchworth	1975–6	413	9	6 (Jan.–Feb.)
Denchworth	1976–7	811	2283	27 (Dec.–June)
Denchworth	1977–8	638	536	28 (Nov.–May)
Lawford	1977–8	540	133	28 (Nov.–May)
Denchworth	1978–9	616	1405	37 (Oct.–June)
Lawford	1978–9	631	81	26 (Dec.–June)

(From Dowdell (1981).)

Although estimates of nitrous oxide emission have been attempted from considerations of the concentration gradient and diffusion coefficient of the gas in the soil atmosphere (Rolston *et al.* 1976), the method is not reliably accurate because heterogeneity in the air-filled pore space results in large variability in the concentrations (Dowdell & Smith 1974) and rates of transport of the gas. Thus most workers have measured nitrous oxide emission by using sealed chambers that cover a small area of soil (Dowdell & Webster 1976; Denmead 1979; Hutchinson & Mosier 1979; Ryden *et al.* 1979*a*, *b*; Burford *et al.* 1981). From measurements of the increase in nitrous oxide concentration within the chamber over a short period of time, the rate of emission can be calculated.

Burford *et al.* (1981) detected emission of nitrous oxide from arable heavy clay soils at most times of year. On a Denchworth series soil, the emission from direct-drilled plots during the period November 1977 to June 1979 was nearly always greater than from ploughed plots, by factors ranging up to 15-fold (figure 1). During periods of greatest evolution (late autumn and spring) the difference between the treatments often exceeded one order of magnitude and the peak hourly rates of nitrous oxide loss from the direct-drilled plots were 2.5–3.0 mg N m^{-2}, equivalent to a daily rate of 0.6–0.7 kg N ha^{-1}. A similar pattern of nitrous oxide emission was observed on a Lawford soil, but rates were only 10–20% of those from the Denchworth soil.

An estimate of the total amount of N loss from the soil as nitrous oxide has been made by integrating the areas under the temporal curves (table 6). In both years on both soils the amounts of nitrogen lost were greatest from direct-drilled treatments, with the largest quantity

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

being evolved from the Denchworth soil in 1979. In an 8 day period between 31 May and 7 June 1979 the ploughed plots lost an amount of nitrous oxide equivalent to 2.2 kg N ha^{-1}, while the direct-drilled plots lost 3.3 kg N ha^{-1}. The smaller rates of emission from the Lawford soil probably reflected the relative lack of availability of organic substrates to microorganisms associated with a smaller organic matter content (4%) compared with the Denchworth soil (6–8%). The larger clay content of the Denchworth soil (50%) compared with the Lawford soil (35%) also contributed to the possibility of more extensive anaerobic conditions developing in wet weather on the Denchworth soil.

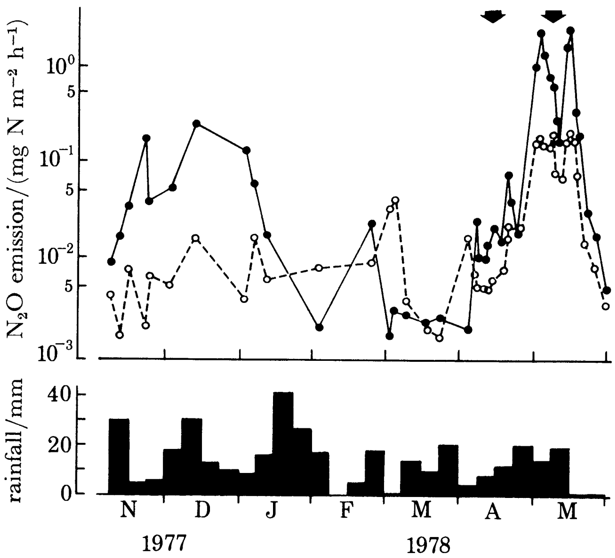

Figure 1. Emission of nitrous oxide from the surface of Denchworth clay soil under winter wheat. The arrows indicate applications of N fertilizers. ●——●, Direct drill; ○- - -○, plough. (From Burford *et al.* (1981).)

Table 6. Total nitrous oxide emission (kilograms N per hectare) from two clay soils after direct drilling or ploughing

	direct drilled	ploughed
Denchworth series		
Nov. 1977–June 1978	5.4	0.9
Nov. 1978–June 1979	8.6	5.6
Lawford series		
Nov. 1977–June 1978	1.5	0.5
Nov. 1978–June 1979	2.1	1.0

(From Burford *et al.* (1981).)

In grassland on a sandy loam soil, annual nitrous oxide emission did not exceed 0.04 kg N ha^{-1} (Dowdell & Webster 1976), but in an experiment on clay and silt loam soils (Webster & Dowdell 1981) the daily emission was consistently greater from the clay, with peak rates reaching 0.012 kg N ha^{-1} in the autumn and 0.002 kg N ha^{-1} in winter. during the summer months, peak daily rates often reached 0.25 kg N ha^{-1}, but these did not persist for more than 2–5 days. Peaks were observed in both soils, particularly when N applications coincided with significant irrigation or rainfall events (figure 2). Nitrous oxide and N_2 are usually thought to be lost only by movement in the gaseous phase toward the soil surface, where they escape to the atmosphere. However, nitrous oxide can leave the soil dissolved in drainage water in amounts comparable with those emitted in the gas phase from the soil surface (Dowdell *et al.* 1979). The quantity of nitrous oxide lost during the winter is estimated as about 0.25 kg N ha^{-1}, this compares with emissions of nitrous oxide from the surface during the same period of 0.15–0.9 kg N ha^{-1}.

Daily emissions of nitrous oxide from lysimeters not treated with fertilizer were generally less than 0.0024 kg N ha^{-1}, but increased in wet weather to 0.007 kg N ha^{-1}. In Australia, Denmead *et al.* (1979) found similarly small daily emission rates from unfertilized closely mown swards, but increases to 0.024 kg N ha^{-1} in moist conditions, with peaks of 0.22 kg N ha^{-1}. These high values compare well with the peaks found on fertilized swards (figure 2).

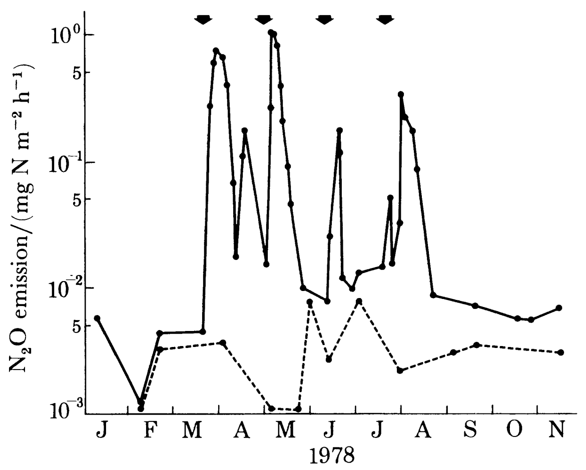

FIGURE 2. Emission of nitrous oxide from a grass sward growing in a clay loam in lysimeters. The arrows indicate additions of N to the fertilized sward (totalling 400 kg N ha^{-1} as NO_3-N). ----, Control; ——, fertilized. (From Dowdell (1981).)

These estimates suggest that losses of plant-available N by nitrous oxide emission are only rarely of agricultural significance. However, nitrous oxide is not the only product of denitrification. Dinitrogen (N_2) is also produced and may be evolved in much larger amounts. A compilation of results from about 20 studies of gaseous compounds of N produced by denitrification in soil (CAST 1976) suggests that an N_2:N_2O ratio of 16 (about 6% N_2O) would be typical for conditions usually found in agricultural soils during denitrification episodes. However, in the presence of abundant nitrate, such as after a fertilizer application, nitrous oxide production is favoured (Blackmer & Bremner 1978). Nitrous oxide may also be formed during nitrification in an aerobic soil (Bremner & Blackmer 1978), and this process may be an important source of nitrous oxide (Denmead *et al.* 1979). Nitrification and denitrification possibly proceed simultaneously in different components of the same soil, but in our studies discussed here, the contribution of nitrous oxide formed by the nitrification process was probably small, as the fertilizer was applied solely in the nitrate form.

Direct field estimation of the evolution of N_2 from soil has been difficult in the past because of the large concentration of nitrogen in the atmosphere. The use of the isotopes ^{13}N and ^{15}N (Smith *et al.* 1978; Tiedje *et al.* 1979) is helpful only in certain limited circumstances. However, acetylene has been shown to inhibit the reduction of nitrous oxide to N_2 by denitrifying bacteria

(Federova *et al.* 1973; Yoshinari & Knowles 1976; Yoshinari *et al.* 1977), resulting in the accumulation of readily measured nitrous oxide. Field measurements of nitrous oxide emissions after injecting acetylene into the soil have been made in California (Ryden *et al.* 1979*b*), and laboratory evaluations of the method demonstrated the stoichiometric recovery of N as nitrous oxide in the presence of 0.1–1 % acetylene (Yoshinari *et al.* 1977; Klemedtsson *et al.* 1977; Smith *et al.* 1978; Ryden *et al.* 1979*a*). Denitrification rate and overall soil respiration were apparently unaffected by acetylene but the inhibition remained effective for only 7 days in one trial (Yeomans & Beauchamp 1978). Also it is unclear as to whether the extent of inhibition is complete in heavy-textured, well structured soils with large inhomogeneities in air-filled pore space.

We are evaluating the utility of this indirect method of assessing the total losses of nitrogenous gases by denitrification in the humid conditions of the United Kingdom in an experiment where effects of direct drilling and ploughing are compared on a clay soil with or without mole drainage. In December 1980, on undrained plots, the peak hourly rate of nitrous oxide emission from areas of soil not treated with acetylene was 7 μg N m^{-2} compared with 180 μg N m^{-2} on the drained plots. In comparable areas treated with acetylene (assuming 100 % inhibition), the hourly emission was markedly increased on the undrained plots (270 μg N m^{-2}), whereas on the drained plots the increase was only small (200 μg N m^{-2}). These results suggest that although the rates of denitrification ($N_2O + N_2$) were similar on both drainage treatments, on the undrained plots the gas emitted was largely nitrogen (N_2:N_2O = 38), while on the drained plots it was largely nitrous oxide (N_2:N_2O = 0.1). Thus measurements of nitrous oxide emissions alone do not provide a good estimate of the extent of denitrification. To establish the size of each component in the total denitrification gas emissions, frequent measurements of both N_2O and N_2 will be needed, as the ratio of the two gases cannot be taken as a constant while soil management practice or weather are changing.

I acknowledge the contributions made to this research by my colleagues, Dr R. Scott Russell, Dr R. Q. Cannell, Mr E. R. Mercer, Dr P. Colbourn, Mr K. C. Hall, Mr C. P. Webster and Miss R. Crees.

References (Dowdell)

Allison, F. E 1966 The fate of nitrogen applied to soils. *Adv. Agron.* **18**, 219–258.

Arnold, P. W. 1954 Losses of nitrous oxide from soil. *J. Soil Sci.* **5**, 116–128.

Blackmer, A. M. & Bremner, J. M. 1978 Inhibitory effect of nitrate on reduction of nitrous oxide to N_2 by soil micro-organisms. *Soil Biol. Biochem.* **10**, 187–191.

Bremner, J. M. & Blackmer, A. M. 1978 Nitrous oxide emission from soils during nitrification of fertilizer nitrogen. *Science, N.Y.* **199**, 295–296.

Broadbent, F. E. & Carlton, A. B 1978 Field trials with isotopically labelled nitrogen fertilizer. In *Nitrogen in the environment* (ed. D. R. Nielsen & J. G. MacDonald), pp. 1–41. New York: Academic Press.

Burford, J. R. 1969 Single sample analysis of N_2, N_2O, CO_2, A, O_2 mixtures by gas chromatography. *J. chromatogr. Sci.* **7**, 760–762.

Burford, J. R., Dowdell, R. J. & Crees, R. 1981 Emission of nitrous oxide to the atmosphere from direct-drilled and ploughed clay soils. *J. Sci. Fd Agric.* **32**, 219–223.

Cannell, R. Q., Belford, R. K. & Beetlestone, G. R. 1977 Uptake of fertilizer nitrogen by winter wheat and losses of nitrogen by leaching. In *Agricultural Research Council Letcombe Laboratory Annual Report, 1976*, pp. 88–90.

Cannell, R. Q., Belford, R. K., Gales, K., Dennis, D. W. & Prew, R. D. 1980 Effects of waterlogging at different stages of development on the growth and yield of winter wheat. *J. Sci. Fd Agric.* **31**, 117–132.

Cast 1976 *Effect of increased nitrogen fixation on stratospheric ozone.* (Council for Agricultural Science and Technology, report no. 53.) Ames: Iowa State University.

Davies, D. B. & Cannell, R. Q. 1975 Review of experiments on reduced cultivation and direct drilling in the United Kingdom, 1954–1974. *Outlook Agric.* **8**, 216–220.

Davies, D. B., Vaidynathan, L. V. & Bradley, P. L. 1976 Efficiency of nitrogen use by winter wheat established in cultivated and uncultivated soil. In *ADAS Experiments and Development in Eastern Region, Ministry of Agriculture, Fisheries and Food*, pp. 42–47.

Denmead, O. T. 1979 Chamber systems for measuring nitrous oxide emission from soils in the field. *J. Soil Sci. Soc. Am.* **43**, 89–95.

Denmead, O. T., Freney, J. R. & Simpson, J. R. 1979 Studies of nitrous oxide emission from a grass sward. *J. Soil Sci. Soc. Am.* **43**, 726–728.

Dowdell, R. J. 1981 Denitrification in soils treated with animal slurry. In *Nitrogen losses and surface runoff from land spreading of manures* (ed. J. C. Brogan), pp. 397–408. The Hague: Martinus Nijhoff.

Dowdell, R. J., Burford, J. R. & Crees, R. 1979 Losses of nitrous oxide dissolved in water from agricultural land. *Nature, Lond.* **278**, 342–343.

Dowdell, R. J. & Cannell, R. Q. 1975 Effect of ploughing and direct drilling on soil nitrate content. *J. Soil Sci.* **26**, 53–61.

Dowdell, R. J. & Crees, R. 1974 Measurement of nitrous oxide content of the atmosphere. *Lab. Pract.* **23**, 488–489.

Dowdell, R. J. & Crees, R. 1980 The uptake of ^{15}N-labelled fertilizer by winter wheat and its immobilisation in a clay soil after direct drilling or ploughing. *J. Sci. Fd Agric.* **31**, 992–996.

Dowdell, R. J., Morrison, J. & Hood, A. E. M. 1980 The fate of fertilizer nitrogen applied to grassland: uptake by plants, immobilization into soil organic matter and losses by leaching and denitrification. In *Proc. Int. Symp Eur. Grassland Fed. on the Role of Nitrogen in Intensive Grassland Production*, pp. 129–136. Wageningen: Pudoc.

Dowdell, R. J. & Smith, K. A. 1974 Field studies of the soil atmosphere. II. Occurrence of nitrous oxide. *J. Soil Sci.* **25**, 231–238.

Dowdell, R. J., Smith, K. A., Crees, R. & Restall, S. W. F. 1972 Field studies of ethylene in the soil atmosphere – equipment and preliminary results. *Soil Biol. Biochem.* **4**, 325–331.

Dowdell, R. J. & Webster, C. P. 1976 Denitrification and leaching of nitrogen fertilizer. In *Agriculture and water quality* (*M.A.F.F. tech. bull.* no. 32), pp. 163–173. London: H.M.S.O.

Dowdell, R. J. & Webster, C. P. 1980 A lysimeter study using nitrogen-15 on the uptake of fertilizer nitrogen by perennial ryegrass swards and losses by leaching. *J. Soil Sci.* **31**, 65–75.

Dowdell, R. J., Webster, C. P., Mercer, E. R. & Hill, D. 1980 Lysimeter studies of the fate of fertilizer nitrogen in a shallow arable soil overlying chalk. In *Agricultural Research Council Letcombe Laboratory Annual Report, 1979*, pp. 40–42.

Fedorova, R. I., Milekhina, E. I. & Il'Yukhina, N. I. 1973 Evaluation of the method of 'gas metabolism' for detecting extra-terrestrial life. Identification of nitrogen-fixing micro-organisms. *Izv. Adad. Nauk SSSR. Ser. Biol.* **6**, 797–806.

Hall, K. C. 1980 Gas chromatographic measurement of nitrous oxide dissolved in water using a headspace analysis technique. *J. chromatogr. Sci.* **18**, 22–24.

Hall, K. C. & Dowdell, R. J. 1981 An iso-thermal gas chromatographic method for the simultaneous estimation of oxygen nitrous oxide and carbon dioxide content of gases in the soil. *J. chromatogr. Sci.* **19**, 107–111.

Hauck, R. D. & Bremner, J. M. 1976 The use of tracers for soil and nitrogen research. *Adv. Agron.* **28**, 219–266.

Hodgson, D. R., Proud, J. R. & Browne, S. 1977 Cultivation systems for spring barley with special reference to direct drilling (1971–1974). *J. agric. Sci., Camb.* **88**, 631–644.

Hutchinson, G. L. & Mosier, A. R. 1979 Nitrous oxide emissions from an irrigated cornfield. *Science, N.Y.* **205**, 1125–1127.

Klemedtsson, L., Svensson, B. H., Lindberg, T. & Rosswall, T. 1977 The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. *Swed. J. agric. Res.* **7**, 179–185.

Morrison, J., Jackson, M. V. & Sparrow, P. E. 1980 The response of perennial ryegrass to fertilizer nitrogen in relation to climate and soil. *Report of the joint A.D.A.S./G.R.I. Grassland Manuring Trail–GM*20. (Grassland Research Institute Technical Report no. 27.)

Rolston, D. E., Fried, M. & Goldhamer, D. A. 1976 Denitrification measured directly from nitrogen and nitrous oxide fluxes. *J. Soil Sci. Soc. Am.* **40**, 259–266.

Ryden, J. C., Lund, L. J. & Focht, D. D. 1979*a* Direct measurement of denitrification loss from soils. I. Laboratory evaluation of acetylene inhibition of nitrous oxide reduction. *J. Soil Sci. Soc. Am.* **43**, 104–110.

Ryden, J. C., Lund, L. J., Letey, J. & Focht, D. D. 1979*b* Direct measurements of denitrification loss from soils. II. Development and application of field methods. *J. Soil Sci. Soc. Am.* **43**, 110–118.

Smith, K. A. & Dowdell, R. J. 1973 Gas chromatographic analysis of the soil atmosphere – automatic analysis of gas samples for O_2, N_2, Ar, CO_2, N_2O and C_1–C_4 hydrocarbons. *J. chromatogr. Sci.* **11**, 655–685.

Smith, M. S., Firestone, M. K. & Tiedje, J. M. 1978 The acetylene inhibition method for short-term measurement of soil denitrification and its evaluation using nitrogen-13. *J. Soil Sci. Soc. Am.* **42**, 611–615.

Tiedje, J. M., Firestone, R. B., Firestone, M. B., Betlach, M. R., Smith, M. S. & Caskey, W. H. 1979 Methods for the production and use of nitrogen-13 in studies of denitrification. *J. Soil Sci. Soc. Am.* **43**, 709–716.

Vaidyanathan, L. V. & Leitch, M. H. 1980 Use of fertilizer and soil nitrogen by winter wheat established with and without soil cultivation prior to drilling. *J. Sci. Fd Agric.* **31**, 852–853.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Webster, C. P. & Dowdell, R. J. 1981 Nitrous oxide emission from permanent grass swards. *J. Sci. Fd Agric.* (In the press.)
Wentworth, W. W. & Freeman, R. R. 1973 Measurement of atmospheric nitrous oxide using an electron capture detector in conjunction with gas chromatography. *J. Chromatogr.* **79**, 322–324.
Yeomans, J. C. & Beauchamp, E. G. 1978 Limited inhibition of nitrous oxide reduction in soil in the presence of acetylene. *Soil Biol. Biochem.* **10**, 517–519.
Yoshinari, R. & Knowles, R. 1976 Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochem. biophys. Res. Commun.* **69**, 705–710.
Yoshinari, T., Hynes, R. & Knowles, R. 1977 Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biol. Biochem.* **9**, 177–183.

Discussion

M. H. Mian (*Department of Biological Sciences, The University, Dundee, U.K.*). Dr Dowdell found that nitrous oxide concentrations varied from 9 μl/l to 2283 μl/l. What caused such variation? Was nitrous oxide further reduced to N_2 later on?

R. J. Dowdell. The measured concentrations do not give information on the production rate of N_2O nor of its escape from the soil, nor of its conversion into N_2. Large differences in nitrous oxide concentrations may be due in part to variation in the extent of anaerobic conditions and of supply of nitrate in the soil arising from differences in rainfall distribution from year to year. In wet conditions, high nitrous oxide concentrations may be found, but these rapidly decline as nitrate is exhausted and nitrous oxide reduced to N_2.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES