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Patterns of denitrification loss from grazed grassland: Effects of N fertilizer inputs at different sites

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Abstract

Factors influencing the seasonal and daily variation in denitrification rates in grazed swards were examined at 5 experimental sites in England with wide ranging environmental/geographic conditions. There was a wide range of fertilizer inputs at each site. Rates of denitrification were estimated by a coring and field incubation technique using acetylene to inhibit the reduction of N_2O to N_2. Major features of the detailed results from two of the sites were: (i) the large ranges in rates of loss, (ii) the relatively low contributions to total annual loss during autumn and winter, (iii) the apparent association of high rates of loss with fertilizer additions made when the soil was wet or immediately preceding a rainfall event, and (iv) significant losses from soil at 10–30 cm in the profile. Multiple quadratic regression analysis of the effects of soil NO_3^-–N, soil temperature and water was used to explain variability in rates of loss. When separate regressions were fitted within each site × year × season × fertilizer level subset, 51% of the variation in loss was explained on a poorly drained fine loam/silt but only 38% on a freely drained loam.

Introduction

Recent attempts to construct input/output balances for nitrogen (N) in grazed grassland systems (Garwood and Ryden, 1986; Whitehead *et al.*, 1988) indicate that in many circumstances the denitrification process is an important contributory mechanism in the overall release of N into the wider environment. As well as the resultant inefficiency in N utilization, the nitrous oxide (N_2O) component of denitrification loss contributes to the rapidly increasing concentration of this gas in the upper atmosphere which may play an important role in greenhouse effects and global warming (Ramanathan *et al.*, 1987) and may also be implicated in stratospheric ozone depletion. Denitrification losses from agricultural systems usually occur directly from applications of nitrate (NO_3^-) based fertilizers when these are coincident with the appropriate soil moisture and temperature status (Haynes and Sherlock, 1986); these effects have been well documented for grassland (Ryden, 1985; 1986). In grazed grassland, as well as inputs from fertilizers, accumulation of un-utilized NO_3^- from nitrified, excreted N returned to the sward by animals, can also provide a substantial supply of substrate to be denitrified, especially during those times of the year when uptake by the sward is reduced. Considerable differences in NO_3^- contents in soils in autumn have been demonstrated between cut and grazed swards on the one hand, and between areas of the sward which have or have not been affected by excreta on the other (Ball and Ryden, 1984).

Whilst differences in rates of denitrification have been demonstrated between high (fertilizer N) and low (clover-based) input grassland systems (Garwood and Ryden, 1986) there have been few attempts to establish the effects of a wide range of fertilizer N inputs to grazed swards. In another paper (Barraclough *et al.*, 1991) we describe, as part of a study to establish some of the outputs from different grazed swards, annual denitrification losses from a number of soils which received fertilizer applications which ranged from 0–800 kg ha^{-1}. Average annual denitrification losses represented 5 and 12%, respectively, of the fertilizer addition to a well or a poorly drained soil type. Although there is a need to establish the overall extent of losses under particular management regimes, in order to be better able to predict the effects of manipulating N supply on losses by denitrification, a greater understanding is also required of the mechanisms and the factors controlling this process. As well as gross differences between different sites and soils, the seasonal, daily and diurnal variations in denitrification rates which may reflect either relatively short-term fluctuations in environmental conditions and/or in supplies of substrate and available energy sources are also influential in determining overall loss. In the present paper therefore, we describe some of the factors influencing the seasonal and daily variation in denitrification rates in grazed swards with a wide range of N fertilizer inputs at 5 experimental sites in England with wide-ranging environmental/geographic conditions.

Methods

Sites and treatments

The sites, their soils and the experimental treat ments are described in detail elsewhere (Barra clough *et al.*, 1991). In brief, the measurement were taken on a grazing response trial (GM2 trial; Baker, 1985): five sites were involved an Table 1 gives details of their locations and resumé of some of the properties of their soil either measured in these or concurrent studies o abstracted from data from Soil Survey of Eng land and Wales. Measurements of denitrificatio were started during autumn of 1986 at all fiv sites and continued until the following sprin (1987) and aimed to provide estimates of lo every 7–10 days. After spring 1987 measur ments were discontinued at North Wyke an Drayton, but continued at Ravenscroft at th same frequency until the spring of the followin year (1988). Measurements also continued Hurley and Jealotts Hill, but the intensity sampling increased during the latter half of th investigation when measurements were ma every 3 or 4 days. The grazing and fertiliz managements were as described elsewhere (Ba raclough *et al.*, 1991); the rates of N fertiliz application varied with site. At Hurley, Jealot Hill, Ravenscroft and Drayton the treatmen prior to the present investigation period h been 100, 250, 450 and 750 kg N ha^{-1}; there w a further treatment of 350 kg N ha^{-1}

Table 1. Some characteristics of the soils at the experimental sites

Site	Series	Texture	Particle size distribution			Total pore space % by vol.	Available water % by vol
			Fine sand (60–200 μm)	Silt (2–60 μm)	Clay (<2 μm)		
Hurley	Frilsham	Loam (freely drained)	22	23	20	44.1	21.3
Jealotts Hill	Wickham	Fine loam/silt (poorly drained surface water gley)	7	66	22	54.3	22.6
Ravenscroft	Crewe	Clay (poorly drained gley)	27	37	36	56.1	20.1
North Wyke	Tedburn	Clay/silty clay loam (poorly drained surface water gley)	10	52	37	52.1	20.5
Drayton	Evesham	Clay (imperfectly drained)	7	36	49	57.1	16.8

Ravenscroft. The fertilizer rates at North Wyke were 0, 100, 200, 400 and 800 kg N ha^{-1}. These rates were applied in equal doses through the previous (1986) grazing season and were also repeated at Hurley, Jealotts Hill and Ravenscroft during 1987. At Hurley and Jealotts Hill, where measurements continued until early summer 1988, a series of uniform fertilizer dressings were also applied across all treatments during spring 1988.

Measurements of denitrification

Rates of denitrification were estimated by using acetylene to inhibit the reduction of N_2O to N_2 in a coring and incubation technique as described by Ryden *et al.* (1987). Briefly, a number of undisturbed soil cores (either eight of 25 mm diameter, or six of 35 mm diameter) were taken to a depth of 10 cm, at random, from within each experimental area. On some occasions at the Hurley and Jealotts Hill sites soil cores were also taken down to 10–20 and 20–30 cm. The cores (either 6 or 8) were quickly placed in a 1-litre glass container which was sealed with a top which incorporated two septum seal stoppers (Subaseal No. 20). Before incubation, 50 mL of the air in the head space of the jar were removed with a syringe and replaced by 50 mL C_2H_2. The sealed jars were then incubated for 24 h in an adjacent 10 cm (or 20 or 30 cm, as appropriate) deep hole of similar dimensions to the jar. After 24 h, samples of the head space gases were removed from the jars with 2.5 mL disposable, gas-tight syringes fitted with taps. Initial laboratory tests showed that known concentrations of N_2O could be stored in the syringes with fitted taps in the laboratory under ambient conditions without significant loss for at least 10–21 days. Nevertheless, whenever possible, the N_2O contents of the samples were determined quickly after collection. This was easily accomplished with samples taken at the Hurley and Jealotts Hill sites, but on occasion there were lags of a few days between collection and analysis of gas samples from the other sites. N_2O concentrations in the gas samples were determined with a Pye-Unicam PU 4500 gas chromatograph with a thermal conductivity detector.

Soil analyses

All soils were analysed for NO_3^-–N and water contents; for estimation of NO_3^-–N contents 100 g moist soil were thoroughly dispersed in 200 mL 1 *M* KCl and shaken for two hours, the suspension filtered and the NO_3^- contents of the filtrate determined with a standard auto-analytical procedure. Soil moisture content was determined gravimetrically on further samples of the remaining soil. Water-soluble carbon contents were determined on a limited number of samples using a method based on that of Burford and Bremner (1975), in which 10 g dried soil were shaken with 50 mL H_2O for 30 min, centrifuged and the supernatants filtered through 0.2 μm membrane filters. The carbon contents of the filtered extracts were then determined by dicromate oxidation.

Environmental variables

Soil temperatures were either taken routinely within the experimental areas or obtained, along with rainfall data from the routine collection of meterological data made at each site, at corresponding depths to those of the denitrification measurements.

Results and discussion

Methodology

The use of methods involving the inhibition of N_2O reduction has allowed measurements of total denitrification loss to be made in the field under a wide range of circumstances (Ryden, 1986; Tiedje *et al.*, 1989). All available techniques *i.e.* field enclosure chambers, large single intact cores for laboratory or field incubation, or the present method, have weaknesses which have been outlined recently (Tiedje *et al.*, 1989). For all soils there is enormous spatial variability in denitrification rates in the field (Tiedje *et al.*, 1989). Measurements in grazed swards are particularly difficult because the often considerable natural soil heterogeneity is confounded by the variability resulting from the grazing and excretion patterns of the animal which will also further interact with temporal variation. The extensive spatial variation considerably reduces the usefulness of an enclosure method, and for regular routine sampling in situations where animals are involved the present technique provides the most reasonable alternative. It has been shown that, despite the possibility of changes in aeration status of the soil samples, the method has provided reliable measurements of loss in a well-drained soil and has overcome some of the problems that occur when attempting to use enclosure methods in poorly drained soils with low air-filled porosities (Ryden *et al.*, 1987). However, whilst excellent agreement between chamber and core methods exists for measurements made in permanent grassland soils (Ryden *et al.*, 1987), much poorer agreement was found recently when the comparison was made with arable soils (Webster and Goulding, 1989). Other recent studies (Scholefield *et al.*, 1990) have demonstrated very large spatial and temporal variation in denitrification in long-term grazed grassland and these workers concluded that greater frequency of sampling rather than greater replication at each occasion provides for the greater accuracy.

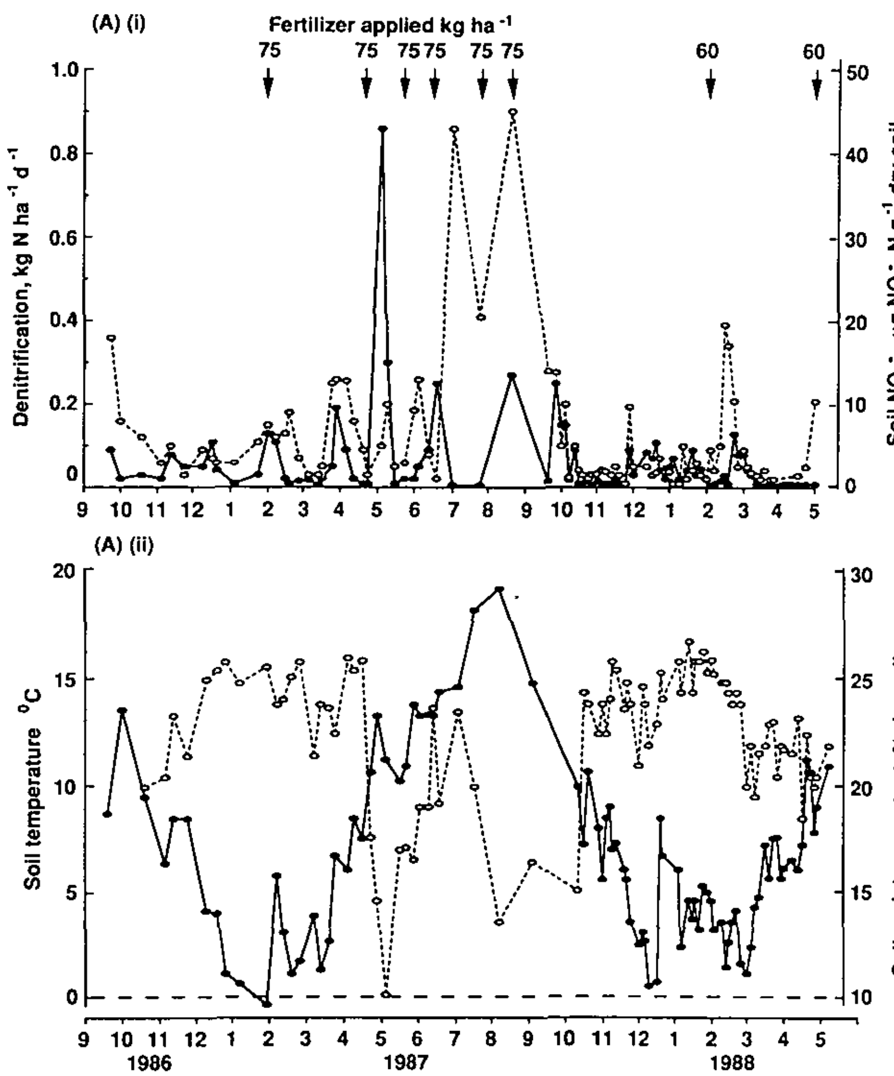

Fig. 1. (i) daily denitrification rates (●——●) and soil NO_3^--N contents (○---○) and (ii) soil temperature (●——●) and moisture contents (○---○) over the period September 1986–May 1988. Values are for cores taken to 10 cm depth in grazed swards receiving 450 kg N ha^{-1} per year at (A) Hurley and (B) Jealotts Hill.

Rates of denitrification: seasonal variation

The total annual losses as determined by extrapolation between the various sampling dates at each site are described elsewhere (Barraclough *et al.*, 1991): these demonstrated wide ranging differences between sites and between treatments. A major feature of the present results was the large range in the rate of loss at a particular site throughout the year; the extent of the variation is demonstrated for the 450 kg N ha^{-1} treatments at Hurley and Jealotts Hill in Fig. 1: total annual loss from this treatment was 19.2 and 47.5 kg N ha^{-1}, respectively (Barraclough *et al.*, 1991).

However, when denitrification rates are averaged over three-monthly periods, site, treatment and seasonal differences and trends can then be distinguished as shown in Fig. 2 for Hurley and Jealotts Hill. Although there were considerable accumulations of NO_3^-–N in the profiles of both soils in autumn after grazing (Barraclough *et al.*, 1991; Jarvis and Barraclough, 1990), the overall rates of loss were generally low (*i.e.* <0.1 kg N ha^{-1} day^{-1}) during September–November, except from the 750 kg N treatments. Secondly,

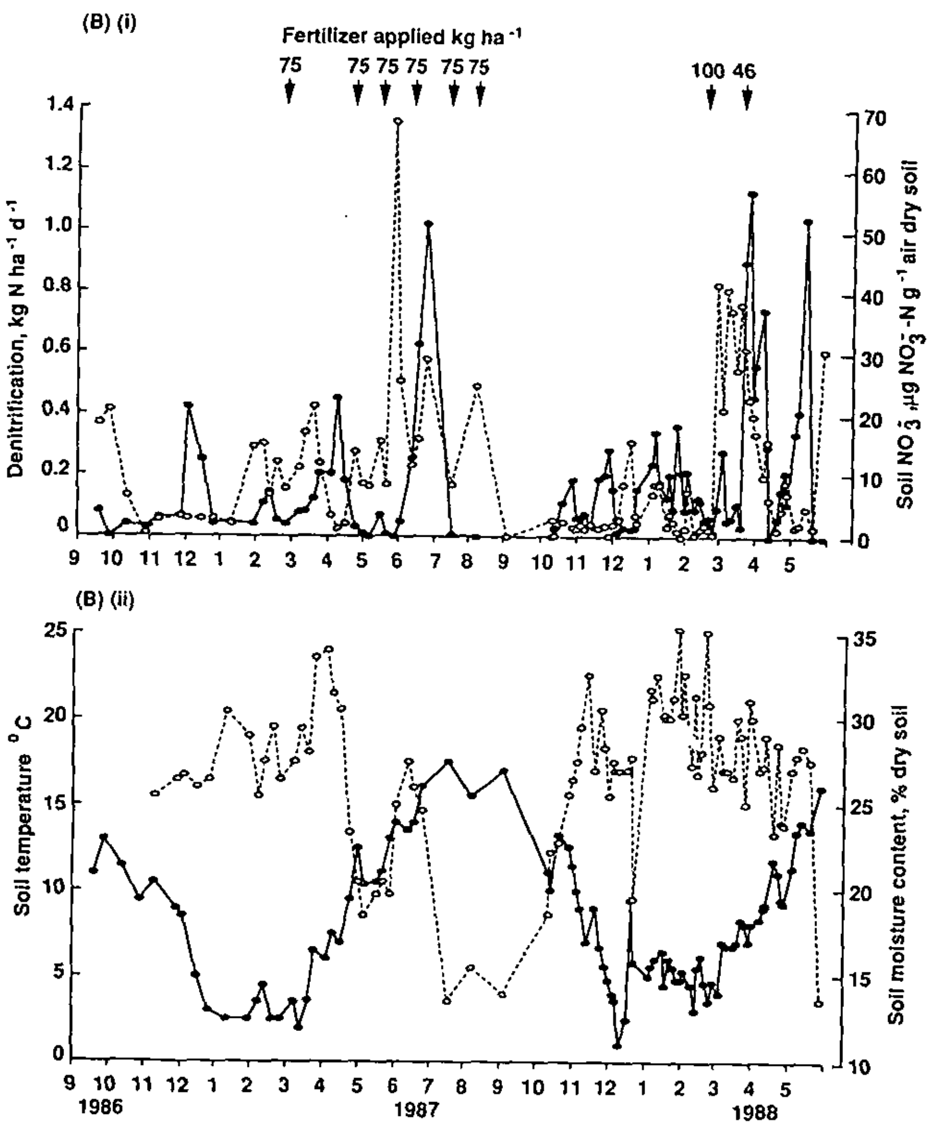

Fig. 1. (*continued*)

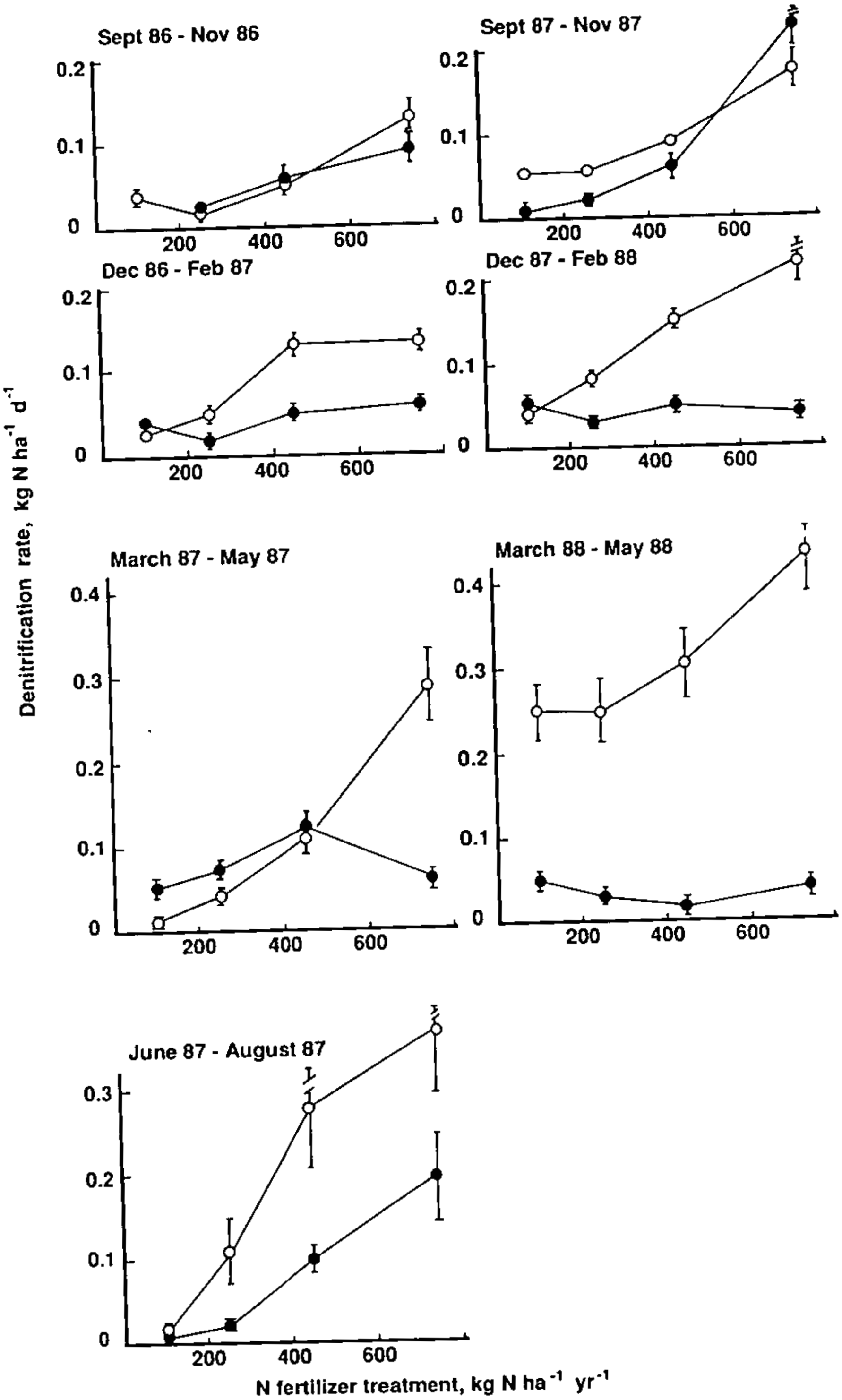

Fig. 2. Mean seasonal denitrification rates in grazed swards at Hurley (●——●) and Jealotts Hill (○---○) with from 100–750 kg fertilizer N ha^{-1} applied: s.e. of means indicated as vertical bars.

although there were substantial differences in total loss over a year between the sites, as would be expected from the physical characteristics of their soils, these differences were not evident during autumn. Thirdly, there was a trend for increasing denitrification loss with increasing N fertilizer rate at all times of the year at Jealotts Hill: this was also true during the summer and autumn periods at Hurley but at other times at this site there was either little difference or an irregular pattern with respect to fertilizer addition.

It is also of interest to note that during spring 1988 at Jealotts Hill when all treatments received a common level of fertilizer addition, denitrification rates increased in accordance with the level of fertilizer addition during the previous year: this did not occur at Hurley. The reasons for this 'carry-over' effect at Jealotts Hill are not known, but did not arise from different contents of residual NO_3^--N over the winter in the top 10-cm (Jarvis and Barraclough, 1990). It is possible that fertilizer addition may have stimulated the release of easily mineralized N at rates which depended upon previous inputs as temperatures increased in the following spring.

Low rates of denitrification loss after grazing had stopped were also recorded at the three other sites (Table 2), again indicating the relatively small contribution autumn and winter losses make to annual loss. Although the soils at North Wyke and Drayton have restricted aerobicity, the average rates of loss were low and showed little relationship to previous N fertilizer treatments. At North Wyke very low contents of NO_3^--N were present throughout in all fertilizer treatments (either because of restricted nitrification or through loss of accumulated NO_3^- in lateral surface and sub-surface movement of water prior to the present measurements), and

Table 2. Mean over-winter (Sept.–May) denitrification losses (kg N ha^{-1} d^{-1}) from grazed grassland at North Wyke, Drayton (1986–87) and Ravenscroft (1986–87 and 1987–88): numbers in brackets are numbers of samples; those in parentheses are s.e.

Site	N fertilizer treatment (kg N ha^{-1})	Measurement period		
		Sept.–Nov.	Dec.–Feb.	March–May
		[10]	[11]	[7]
North Wyke	0	0.02 (0.009)	<0.01	<0.001
	100	0.02 (0.009)	<0.01	<0.001
	200	0.03 (0.010)	0.01 (0.004)	<0.001
	400	0.03 (0.003)	0.02 (0.005)	<0.001
	800	0.12 (0.043)	0.03 (0.006)	<0.001
		[5]	[10]	[12]
Drayton	100	0.01 (0.010)	<0.01	<0.01
	250	0.03 (0.009)	0.01 (0.005)	0.06 (0.031)
	450	0.07 (0.027)	0.05 (0.014)	0.12 (0.040)
	750	0.07 (0.017)	0.15 (0.045)	0.13 (0.041)
		[2]	[12]	[12]
Ravenscroft (1986–87)	100	0.05 (0.034)	0.01 (0.006)	<0.01
	250	0.04 (0.025)	0.01 (0.023)	<0.01
	350	0.02 (0.017)	0.02 (0.018)	<0.01
	450	0.02 (0.001)	0.03 (0.014)	<0.01
	750	0.05 (0.023)	0.03 (0.010)	<0.01
		[6]	[13]	[11]
(1987–88)	100	<0.01	<0.01	<0.01
	250	0.01 (0.004)	<0.01	<0.01
	350	0.02 (0.009)	0.01 (0.003)	<0.01
	450	0.07 (0.021)	0.01 (0.004)	0.03 (0.016)
	750	0.10 (0.033)	0.02 (0.006)	0.10 (0.069)

little denitrification would therefore have been expected. At Drayton, even though average NO_3^-–N contents during September–November 1986 were 3.1, 3.6, 8.0 and 27.0 μg g^{-1} in the top 10-cm for 100, 250, 450 and 750 kg N ha^{-1} treatments, respectively, only low rates of denitrification were detected, even at 750 kg N ha^{-1}. The high clay content and highly plastic nature of this soil may have prevented useful measurements of denitrification by the present technique, which is dependent upon adequate gaseous diffusion to and from the zones of activity. The problem would have been increased through smearing of surfaces when core samples were taken.

Autumn and winter denitrification rates were also low at Ravenscroft (Table 2). At this site the exent of leaching (especially during the first winter) (Barraclough *et al.*, 1991) was such that denitrification potential would have been reduced markedly. Applications of fertilizer N continued at their original rates in 1988 at this site, but in contrast to Hurley and Jealotts Hill, there were no large increases in denitrification rates during spring and summer. The average total losses by denitrification over the autumn-spring period represented <1% of the fertilizer addition; the comparable figures for Drayton and North Wyke were <4 and <2%, respectively.

Rates of denitrification: daily variation

The activity of denitrifying bacteria is favoured by high NO_3^-–N concentrations, restricted aeration, high soil temperatures and an adequate carbon supply from readily mineralized organic matter. Ryden (1986) suggested that for grassland soils, the potential for loss by denitrification is at its greatest when soil NO_3^-–N is >5 μg g^{-1}; soil air-filled porosity is <15–40% and soil temperature exceeds 5°C. The extent of the interactions between these factors has been the subject of extensive discussion (see *e.g.* Fillery, 1983; Haynes and Sherlock, 1986). The present rates of loss were relatively low at most times of the year, comparable to other grazed swards (Ryden, 1986). However, in all treatments the patterns of loss were characterized by spikes of very high rates: this has also been demonstrated in other studies (Scholefield *et al.*, 1990). Over the range of fertilizer additions which is representative of most grassland fertilizer managements, denitrification rates ranged from <0.008 (in all treatments at both sites) to 0.324, 0.866 and 0.268 kg N ha^{-1} d^{-1} at Hurley, and 1.25, 1.545 and 1.33 kg N ha^{-1} d^{-1} at Jealotts Hill in 100, 250 and 450 kg N ha^{-1} treatments, respectively. These very high rates of loss, which were similar at all rates of input within each site, were almost always associated with fertilizer additions either when the soil was already wet or immediately preceding rainfall. The similarity in maximum rates of loss can be explained by the fact that, at all levels of addition, the supply of substrate NO_3^-–N is likely to be considerably greater than the immediate potential for denitrification.

Variation between the site means for soil temperature, soil-water/aeration and NO_3^-–N accounted for only 6% of the overall variance in denitrification in the top 10 cm in pooled data from Hurley and Jealotts Hill. There were, however, significant interactions between the site and the effects of the other variables. Simple and multiple linear and quadratic regressions were therefore carried out on a within site basis. It was always possible to explain more of the variance at Jealotts Hill than at Hurley using the same independent variates.

Because of the apparent association of high denitrification with fertilizer dressings or rainfall events, the relationships between individual daily rates and soil water, soil temperature and NO_3^-–N content were poor and respectively accounted for 0.3, 3.1 and 14.3% of the variance for Hurley and 2.8, 3.0 and 20.0% of that at Jealotts Hill using simple quadratic regressions. Calculated values for air-filled porosity were also used as an independent variate but these generally decreased the proportion of the variance accounted for when compared with simple gravimetric water contents. This is surprising since the availability of anaerobic sites should have been more accurately described by an assessment of the degree to which pores were filled by water. However, these estimates were based on published bulk density values and not on densities of the individual samples which may have varied considerably both spatially and temporally.

The best multiple regression models, as fitted by backward elimination of variables were:

for Hurley:

$$\text{denitrification} = -0.1644\,(0.0503) + 0.01536\,(0.00388)\,\text{TEMP} - 0.000734\,(0.000230)\,\text{TEMP}^2 + 0.00569\,(0.000196)\,H_2O + 0.00899\,(0.00119)\,NO_3 - 0.0001123\,(0.0000192)\,NO_3^2\ (R^2 = 0.191)$$

for Jealotts Hill:

$$\text{denitrification} = -0.367\,(0.130) + 0.00825\,(0.00445)\,\text{TEMP} + 0.01294\,(0.00354)\,H_2O - 0.002247\,(0.00963)\,NO_3 - 0.0003082\,(0.0000572)\,NO_3^2 + 0.001414\,(0.000336)\,\text{TEMP} \times NO_3 + 0.001269\,(0.000267)\,H_2O \times NO_3\ (R^2 = 0.334)$$

where denitrification = kg N ha^{-1} d^{-1}, TEMP = soil temperature (°C), H_2O = soil water (% dry soil) and NO_3 = soil NO_3^--N ($\mu g\ g^{-1}$, air dry soil) (figures in parentheses = s.e.) [data for the top 10 cm of the soil profile].

In both of these models a quadratic term in NO_3^- was required; the data suggested that the curve of denitrification rate against NO_3^- was rising to an asymptote. An exponential form for the effect of NO_3^- was therefore used, but did not improve the relationships. The fitted curve showed that there was little effect of NO_3^--N concentration above 5 $\mu g\ g^{-1}$ air dried soil, *i.e.* the supply of substrate NO_3^- was considerably greater than the immediate potential for denitrification. A number of other curvilinear forms was also used, but none provided an improvement in fit compared to the quadratic model. Improvements in the relationships thus appear to depend on the inclusion of further variables rather than on improvements in the functional forms of those already available. Possibilities include time elapsed since rainfall events and water soluble carbon contents (as discussed later). Any relationship with rainfall is, however, not likely to be straightforward since it will be mediated by the advance and retreat of a front of anaerobic conditions especially in the top few cm of the soil profile which will also be affected by drainage and evapotranspiration.

An attempt was also made to use days since the last fertilizer application as an independent variable, but the relationship was, however, very poor. Models were also fitted to data excluding the high rates assocated with rainfall: this improved the relationships slightly, but the functional form and the relative size and sign of the coefficients were not affected.

Separate regressions were fitted within each site × year × fertilizer level subset. This analysis accounted for 37.7% of the overall variation in denitrification rate at Hurley and 51.0% of that at Jealotts Hill. At Hurley, the effect of water content was never significant and there was also a significant negative effect of temperature. This is surprising as denitrifying activity might have been expected to be directly proportional to temperature. However, it seems likely that this apparent effect reflects the low water content of this soil over the summer period (when there was a correlation of −0.457 between temperature and water content). This observation was not apparent from the Jealotts Hill data. Although the regressions were fitted within fertilizer level, thus removing gross differences in NO_3^- levels, the relationship with NO_3^- content was still generally significant. This probably results from the decline in NO_3^- contents with time after fertilizer application, although, as indicated above, the modelling of time explicitly in this context was not useful.

Provision of good overall correlations would have enabled predictive models to be constructed or tested. The lack of correlations in the present studies with a restricted intensity of sampling is therefore disappointing although not surprising: other studies (Christensen and Tiedje, 1988; Scholefield *et al.*, 1990) have shown that good relationships are obscured by the considerable temporal and spatial variation that occurs with denitrification.

Available carbon supply

Another major environmental regulator of denitrifying losses is the supply of a readily available energy source for the denitrifying organisms. Most grassland soils are thought to contain large quantities of mobile forms of C which can be utilized. Examination of three of the soils on two occasions during the period of measurement indicated that there were generally high concentrations of water soluble C present in these soils during winter periods. It is of interest to note that high levels of soluble C were present in treatments which received the lowest N addition (Table 3). On average, over all treatments and all sites, soils with 100 kg N ha^{-1} contained 31% more soluble C than the other treatments. Similarly, organic C contents at 750 kg N ha^{-1} were, on average, 22% lower than under the 250, 350 and 450 treatments. At Hurley and Jealotts Hill greater amounts of soluble C were present in February than in the preceding October: the reverse was true at Ravenscroft. Despite the differences between treatments there was little indication that shortage of available C was limiting denitrification in any of the present soils.

Denitrification at depth

Ryden (1985) has suggested that because of the sensitivity of denitrification to changes in environmental conditions in the upper 20-cm of the soil profile, it is from within this part of the

Table 3. Denitrification rates under grazed swards at Hurley, Jealott's Hill and Ravenscroft swards in relation to water soluble carbon contents

Site/date	Fertilizer treatment (kg N ha^{-1})	NO_3^--N ($\mu g\ g^{-1}$)	Soil moisture % (w/w)	Water soluble carbon ($\mu g\ g^{-1}$)	Denitrification rate (kg N ha^{-1} d^{-1})
Hurley					
8/10/87	100	<0.1	14.9	194	<0.01
	250	0.2		177	<0.01
	450	19.9		188	0.01
	750	79.5		91	0.04
4/2/88	100	0.7	24.6	383	0.13
	250	0.5		286	0.03
	450	0.5		229	0.40
	750	3.0		250	0.05
Jealotts Hill					
8/10/87	100	<0.1	18.7	355	<0.01
	250	<0.1		152	<0.01
	450	2.7		251	<0.01
	750	63.8		231	0.19
4/12/88	100	<0.1	32.6	369	0.03
	250	<0.1		327	0.06
	450	7.5		357	0.15
	750	1.7		297	0.27
Ravenscroft					
28/10/87	100	<0.1	32.9	460	<0.01
	250	<0.1		432	0.02
	350	3.2		467	0.03
	450	1.3		474	0.06
	750	2.6		341	<0.01
22/2/88	100	1.9	34.8	313	<0.01
	250	1.5		319	<0.01
	350	1.3		256	0.01
	450	1.1		319	0.01
	750	1.3		159	0.02

profile that the major proportion of denitrification losses occur. This is in agreement with the rapid decrease in denitrification potential that is found with increasing depth (Germon and Couton, 1981) and also with the pattern of organic matter distribution in most grassland soils. Our data (Table 4) also indicate the extent of the differences at the three different sampling depths and that the top 10 cm of the soil profile provides the largest source of denitrification. Nevertheless, it is important to note that significant rates of loss took place from both 10–20 and 20–30 cm depths. For example, for the 450 kg N ha^{-1} treatment at Jealotts Hill in January the estimated daily denitrification loss from 10–30 cm was equivalent to 19% of the total loss. Whilst denitrification rates in the deeper parts of the soil profile (both absolute and relative to that at 10 cm) were variable, the extent of this deeper activity is likely to result in significant underestimation of loss by the present coring/incubation technique on some occasions. However, it is also important to note that this bias is likely to be small during periods of maximum activity, *i.e.* following fertilizer application when the bulk of the NO_3^- substrate will be residing within a very shallow band at the top of the soil profile. Rates of loss will then reflect a balance between the effects of rainfall on (i) restricting the O_2 supply to the surface layer (*i.e.* by decreasing air filled porosity even over a relatively short period) and therefore increasing denitrification rate, (ii) moving NO_3^--N further down the profile (iii) influencing the possibility of competition for NO_3^--N either by denitrification or by uptake and removal into microbial biomass or plant roots and (iv) the possible obstruction of the escape of N_2O and N_2 and also removal of N_2O in solution down the profile. The net result of these interactions and the consequent effects of pulses of rainfall and NO_3^- on measured rates of loss at different depths are therefore difficult to predict and further work is required to quantify their impact.

Acknowledgements

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Table 4. Denitrification losses at different depths under grazed swards at Hurley and Jealotts Hill

Site/date	Sampling depth (cm)	Soil temperature (°C*)	Soil moisture (%)	Soil NO_3^--N ($\mu g\ g^{-1}$) N treatment kg N ha^{-1}				Denitrification loss (kg N ha^{-1} d^{-1}) N treatment (kg N ha^{-1})			
				100	250	450	750	100	250	450	750
Hurley											
15/10/87	0–10	11.0	24.6	<0.1	0.2	12.9	17.1	<0.01	0.03	0.25	0.39
	10–20		15.0	0.2	0.6	8.1	6.8	<0.01	<0.01	0.01	0.44
	20–30		14.0	1.5	1.8	8.3	2.9	<0.01	<0.01	<0.01	0.03
25/1/88	0–10	6.0	27.1	1.0	0.2	0.6	1.5	0.09	0.04	0.75	0.07
	10–20		17.2	1.2	0.3	0.6	2.8	0.01	0.01	<0.01	0.01
	20–30		16.3	0.3	0.3	1.0	5.3	<0.01	<0.01	<0.01	<0.01
4/4/88	0–10	10.7	20.8	<0.1	<0.1	0.8	0.5	<0.01	<0.01	0.01	<0.01
	10–20		15.1	<0.1	0.3	0.9	1.9	<0.01	<0.01	0.01	<0.01
	20–30		14.4	0.7	1.1	2.1	n.d.	<0.01	<0.01	<0.01	<0.01
Jealotts Hill											
28/1/88	0–10	5.5	35.8	<0.1	<0.1	<0.1	3.9	0.01	0.06	0.21	0.23
	10–20		25.3	<0.1	<0.1	0.8	2.1	0.03	0.01	0.03	0.06
	20–30		20.2	<0.1	<0.1	0.9	13.4	<0.01	<0.01	0.02	0.07
14/4/88	0–10	8.3	29.0	5.0	11.1	6.1	8.2	0.06	0.29	<0.01	<0.01
	10–20		20.7	2.1	2.3	3.5	4.4	0.02	<0.01	0.02	<0.01
	20–30		16.2	0.5	2.0	1.3	11.6	0.02	0.02	<0.01	<0.01

* Measured at 10 cm.

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