

# Nitrous oxide emissions from a fertilized and grazed grassland in the South East of Ireland

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**Abstract** Agriculture currently accounts for 28% of national greenhouse gas emissions in Ireland. Nitrous oxide ( $\text{N}_2\text{O}$ ) emissions from agricultural soils account for 38% of this total. A 2-year study was conducted, using the chamber technique on a fertilized and grazed grassland to quantify the effect of fertilizer application rate, soil and meteorological variables on  $\text{N}_2\text{O}$  emissions. Three N fertilizer regimes (0, 225 & 390 kg N  $\text{ha}^{-1}$ ) were imposed with three replicates of each treatment. Nitrogen fertilizer was applied as urea (46% N) in spring with calcium ammonium nitrate (CAN-26% N) applied in the summer (June–September). Rotational grazing was practiced using steers. Nitrous oxide emissions arising from the unfertilized plots (0 N) were consistently low. Emissions from the N-fertilized plots (225 & 390 kg N  $\text{ha}^{-1}$ ) were concen-

trated in relatively short periods (1–2 weeks) following fertilizer applications and grazing, with marked differences between treatments, relative patterns and magnitudes of emissions at different times of the year and between years. Variation in  $\text{N}_2\text{O}$  emissions throughout both years was pronounced with mean coefficients of variation of 116% in year 1 and 101% in year 2. The study encompassed two climatologically contrasting years. As a result the  $\text{N}_2\text{O}$ –N loss, as a percent of the N applied in the cooler and wetter 2002 (0.2–2.0%), were similar to those used for N-fertilized grasslands under the Intergovernmental Panel on Climate Change (IPCC)  $\text{N}_2\text{O}$  emission inventory calculation methodology ( $1.25\% \pm 1$ ). In contrast, the percentage losses in the warmer and drier 2003 (3.5–7.2%) were substantially higher.

**Keywords** Nitrogen fertilizer · Grassland · Grazed pasture · Nitrous oxide emissions

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## Introduction

The main greenhouse gas (GHG) emitted in Ireland is carbon dioxide, which arises from the burning of fossil fuel in transport, heating and electricity generation (DEHLG 2000). Irish emissions of other GHGs, including methane and  $\text{N}_2\text{O}$ , are proportionally higher than other countries due to a large agriculture sector. The

emission of nitrous oxide is of concern because of its important role in the chemistry of the atmosphere on both regional and global scales (Smith 1997). The emission of  $\text{N}_2\text{O}$  from soil generally represents a small fraction of the annual input of nitrogen (N) within managed or farmed soils. However typical seasonal patterns of  $\text{N}_2\text{O}$  emission from agricultural soils show low levels during the winter which then increase to maximum levels in spring/summer, when soil temperature rises and N fertilizers are applied with a second peak often occurring in autumn, due to N mineralization. During the summer period, low soil moisture contents and limited substrate availability lead to only small emissions of  $\text{N}_2\text{O}$ . Peak  $\text{N}_2\text{O}$  emissions are generally found after N fertilizer application and grazing, except during dry weather. The observed increases in  $\text{N}_2\text{O}$  emission due to N application and the effects of grazing generally last for a period of 1–3 weeks (Velthof and Oenema 1995; Kelliher et al. 2002; van Groenigen et al. 2005).

In terms of estimating  $\text{N}_2\text{O}$  emissions, Bouwman (1996) derived the following relationship from his data compilation:

$$E = 1 + 0.0125 \times F$$

Where  $E$  is the emission rate ( $\text{kg N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$ ), the value of  $1 \text{ kg N ha}^{-1}$  represents the background emission rate and  $F$  is the fertilizer application rate ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ). However, this may not be appropriate for estimating emissions under local conditions or for specific crops, although it may suffice for global analysis. Thus, Fowler et al. (1997) suggested that the fractional loss of  $\text{N}_2\text{O}$  from fertilizer and excreta inputs is substantially larger than the  $1.25 \pm 1\%$  figure used for the global emission inventory of the IPCC derived by Bouwman (1996), and further suggested a range of 0.3–3.7% (median 1.8%) for mowed grassland and a range of 1.2–6.7% (median 2.1%) for grazed grassland. Similar results were found by Clayton et al. (1994) and Merino et al. (2001). The results from both studies (Clayton et al. 1994; Merino et al. 2001) suggest that grazing increases the magnitude of  $\text{N}_2\text{O}$  emissions. In addition Denmead et al. (2000) suggest that approximately 30% of anthropogenic

$\text{N}_2\text{O}$  emissions are produced in grazed pastures, however there is an uncertainty factor of 2 associated with this estimate.

The aim of this study was to quantify the effects of N fertilizer application rates, soil and weather conditions on  $\text{N}_2\text{O}$  emissions from a fertilized and grazed grassland.

## Materials and methods

### Experimental site

The experiment was laid out in the “Tower Field” at the Teagasc (The Irish Agriculture and Food Research Authority) Environmental Research Centre, Johnstown Castle, Co. Wexford, Ireland; latitude  $52^\circ \text{N}$ , longitude  $6^\circ \text{W}$ ; mean annual rainfall and temperature, 1014 mm and  $10^\circ \text{C}$ , respectively. The soil in the “Tower Field” is predominantly an imperfectly drained gley of loam over clay loam texture (FAO classification: gleyic cambisol) derived from Irish Sea Till (Gardiner and Ryan 1964) with 18% coarse sand, 26% fine sand, 38% silt, 18% clay, 5.8 pH ( $1:2 \text{ ml}^{-1}$ ), 3.2% C, 0.28% N and bulk density  $1.23 \text{ g cm}^3$ . The pasture consisted of an established ( $>10$  years) uniform ryegrass (*Lolium perenne*) sward. The experimental area was divided into experimental paddocks/plots varying in size, dependent on N treatment, stocking rate and associated infrastructural and animal husbandry requirements. Three N fertilizer treatments were imposed with three replicates of each treatment. The levels applied were 0, 225 and  $390 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . These correspond to a basal or control level, with the other two treatments representing an intermediate and a maximum N fertilizer application rate as advised by Teagasc for grazed grassland (Coulter 2001). Rotational grazing was practiced using steers (300–350 kg liveweight at turnout in spring). Nitrogen fertilizer was applied to the grazed pasture as urea (46% N) for the first two applications in spring and as calcium ammonium nitrate (CAN-26% N) applied in the remaining applications (May–September).

Fertilizer N applications followed guidelines in relation to the timing of fertilizer N applications (Coulter 2001) and were designed so that

treatments received similar proportions of their annual total simultaneously, thus providing a range of soil mineral N levels and a range of grass production levels across treatments during the year. In 2002 there were seven split fertilizer applications and in 2003 there were nine applications. Nitrogen fertilizer application and grazing rotations followed a pre-determined schedule based on the management of replicate 1 in each treatment. Phosphorus and potassium fertilizer was applied in each of the 2 years at rates which ensured that these nutrients would not limit herbage production and were in line with guidelines on the application of each of these elements to grazed pasture (Coulter 2001). Nitrogen fertilizer was applied using a tractor-mounted fertilizer spreader which was calibrated for the type and rate of fertilizer that was to be applied at each application.

#### Animal husbandry and grazing rotations

A 21-day rotational grazing pattern was practiced. Out of a total of 42 animals over the experimental area, 14 animals were assigned to each treatment in both years, thus creating three separate herds. As herbage supply declined through the grazing season, stock numbers were reduced. These animals were then held in “holding paddocks” receiving the same level of N fertilizer application. In addition, during the summer months any excess herbage was removed by topping to allow uniform re-growth of the grazing sward.

Each treatment can be conceptualized as a separate farmlet in which there were three plots (treatment replicates) which were rotationally grazed in a 3-week grazing rotation. Throughout the grazing season all plots were strip-grazed. For each grazing interval, the N deposited by grazing animals as dung and urine, was calculated based on reported annual nutrient production levels (DAFRD 2000), stock numbers and the grazing plan imposed on the experimental plots (data not shown).

#### Nitrous oxide emission measurement

Nitrous oxide emission measurements were conducted using the closed chamber technique (Smith et al. 1995). This involved placing a col-

lection chamber over the soil surface for a measurement period, thereby enclosing an area of the atmosphere adjacent to the soil surface. The chambers, 11.3 cm in diameter and 15 cm high, were inserted to a depth of 2 cm into the soil to provide a seal from the atmosphere and an effective volume of air above the soil within the chamber headspace of 1303 cm<sup>3</sup>. Eight chambers were used per replicate plot (i.e., a total of 72 chambers per measurement day) placed at random in two lines of four. On each sampling occasion the chambers were in place for 1 h between 10.30 a.m. and 11.30 a.m. to minimise any effects of diurnal variation. A sample of the air within the chamber was withdrawn through a rubber septum (Becton Dickinson, Spain) using a 10 ml polypropylene syringe (BD Plastipak, Becton Dickinson, Spain) which was opened fully (volume~11 ml) and fitted with a hypodermic needle (BD Microlance 3, Becton Dickinson, Spain). The atmosphere in the chamber was mixed by pumping the syringe plunger sharply a number of times prior to the withdrawal of a sample from the chamber. The sample was then immediately transferred into a pre-evacuated 7 ml (nominal) screw-cap septum vial (Perbio Science, UK) fitted with a Tuf-Bond (Teflon-Silicone) septum (Perbio Science, UK) for analysis within 6 h. The injection of ~11 ml over-pressurized the sample vials, thus preventing any back-diffusion of ambient air. Ambient air samples were also taken at the time of chamber sampling. The N<sub>2</sub>O concentration in each sample was analysed using a gas chromatograph (GC) (Varian CP 3800 GC, Varian, USA) using an electron capture detector (E.C.D) at 300°C. From each vial a 0.7 ml sub-sample was automatically injected into the chromatograph using a Combi-Pal automatic sampler (CTC Analysis, Switzerland) under computer control. The system allowed for the unattended analysis of 64 samples. Additional instrument details include: 3 m long, 3 mm outside diameter stainless steel column packed with Poropak Q (80/100 mesh) preceded with a magnesium perchlorite (Mg(ClO<sub>4</sub>)<sub>2</sub> + aq) (14–22 mesh) packed pre-column to remove any moisture in the samples. The column oven and injector temperature were both 60°C, while argon (Ar) (BOC Gases) flowing at 35 ml min<sup>-1</sup> was

used as a carrier gas. Analysis time was of the order of 2.1 min. Areas under  $\text{N}_2\text{O}$  peaks were integrated using Star Chromatography Workstation version 5.51 (Varian, USA). The analytical variability of GC analysis was determined periodically by analysing  $\text{N}_2\text{O}$  standards (ambient i.e., 0.31, 1, 5.2, 10.5, 51.5, and 100.3 ppm) (Scott Speciality Gases, USA) and was found in all cases to be almost linear (average  $r^2 = 0.99$ , data not shown).

#### Soil, environmental and meteorological variables

Throughout the 2-year experimental period, the mineral N content of the soil in experimental plots was measured before and for a number of days after N fertilizer application so as to encompass the peak in  $\text{N}_2\text{O}$  emission found after the application of N fertilizer to the pasture but not on all days that  $\text{N}_2\text{O}$  emission measurements were undertaken. Intact soil cores were taken for nitrate-N ( $\text{NO}_3^-$ -N) and ammonium-N ( $\text{NH}_4^+$ -N) content determination. Immediately after  $\text{N}_2\text{O}$  samples were collected, the chambers were removed and intact soil cores (2.95 cm in diameter by 10 cm deep) were taken within the area that had been covered by the chamber, using a stainless steel soil corer. In total, eight sets of cores were taken from each plot on each sampling day. The 8 sets of soil cores were then bulked into 4 groups of two successive chamber sites giving four bulked samples per plot. Nitrate-N and  $\text{NH}_4^+$ -N concentrations were then determined by extracting 20 g of field-moist soil with 100 ml of 2 M potassium chloride (KCl) (Ellis et al. 1998; Mosier et al. 1997). Extraction was facilitated by intermittent hand-shaking for 2 h or using an automated shaker for 1 h (New Brunswick Scientific Model G-10 Gyrotory shaker). The extract was then filtered through Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, UK) and stored at 4°C prior to colorimetric analysis on a Chemlab System 4 (3 channel) auto analyser (Chemlab Instruments, Essex, England).

Soil moisture content to 15 cm depth and soil temperature at 5 cm depth were measured using frequency domain reflectometry and thermistors, respectively by instrumentation of the experi-

mental site with soil moisture (CS 615) and temperature (T107) probes (Campbell Scientific, UK) connected to dataloggers (CR10X, Campbell Scientific, UK). The probes were programmed using PC 208 software (Campbell Scientific, UK) to take hourly measurements of soil moisture and temperature and to produce daily averages and minimum and maximum temperatures for each day throughout the experimental period. Soil temperature at 10 cm and 30 cm depth and rainfall data were obtained from a meteorological station 1 km distant from the experimental site.

#### Calculation of $\text{N}_2\text{O}$ emission rate

Measurement-day  $\text{N}_2\text{O}$  emission values as  $\text{g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$  were calculated from the concentrations measured in the chambers for the measurement period of 1-h ( $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ) converted to those over a 24-h period. Measurement-day emission values were subjected to statistical analysis. Mean daily emissions rates were calculated for each replicate of each treatment. Cumulative emission totals were calculated by multiplication of the mean value of eight chambers per replicate plot for two consecutive sampling days by the intervening time interval. Summing of all such values yielded a cumulative total  $\text{N}_2\text{O-N}$  evolved.

#### Statistical analysis

Upon inspection of histograms of the distribution of  $\text{N}_2\text{O}$  emission data throughout the study, the data were shown to be positively skewed. As a consequence  $\text{N}_2\text{O}$  emission data were transformed to their natural logarithms prior to statistical analysis. As emission measurements were conducted on the same areas or plots over time, the emission data set is by definition a longitudinal data set (data with repeated measurements on the same units over time). The natural logarithms of daily arithmetic mean emission values were analysed as a split-plot in time design. Statistical differences between treatments were assessed, with the time of N fertilizer application as the “blocking” factor, treatment levels as the “whole plot” factor and time as the sub-plot factor within each whole plot with average daily temperature

and soil moisture content as measured covariates with time.

Pearson correlation coefficients were calculated and linear regression analysis conducted on measurement-day arithmetic means of both soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N contents to identify linear relationships between soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N contents and  $\text{N}_2\text{O}$  emission levels using Microsoft Excel data analysis toolpak (Microsoft Office XP). The relationship between emissions and soil mineral N contents and soil moisture status was further investigated using the REML directive in Genstat 6.2 (Genstat Committee 2003).

## Results

Nitrous oxide emission measurements were conducted on a total of 134 occasions over the 2-year period (9/11/2001 to 26/11/2003). Emissions arising from the unfertilized plots (0 N) were consistently low, with values, on average, less than  $75 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$  recorded. Emissions from the N-fertilized plots ( $225$  &  $390 \text{ kg N ha}^{-1}$ ) were concentrated in relatively short periods (1–2 weeks) following fertilizer N applications and grazing, with marked differences between treatments and the relative patterns and magnitude of emission at different times of the year and between years (Figs. 1 and 2). The higher emission values were associated with N fertilizer applications in the summer months in conjunction with rainfall events and increasing soil temperatures. The lower emission values were found over the winter and early spring periods of both years before the initiation of fertilizer applications and the onset of the grazing season. Variation in  $\text{N}_2\text{O}$  emissions throughout both years was pronounced with mean coefficients of variation of 116% (7–524%) in year 1 and 101% (4–357%) in year 2.

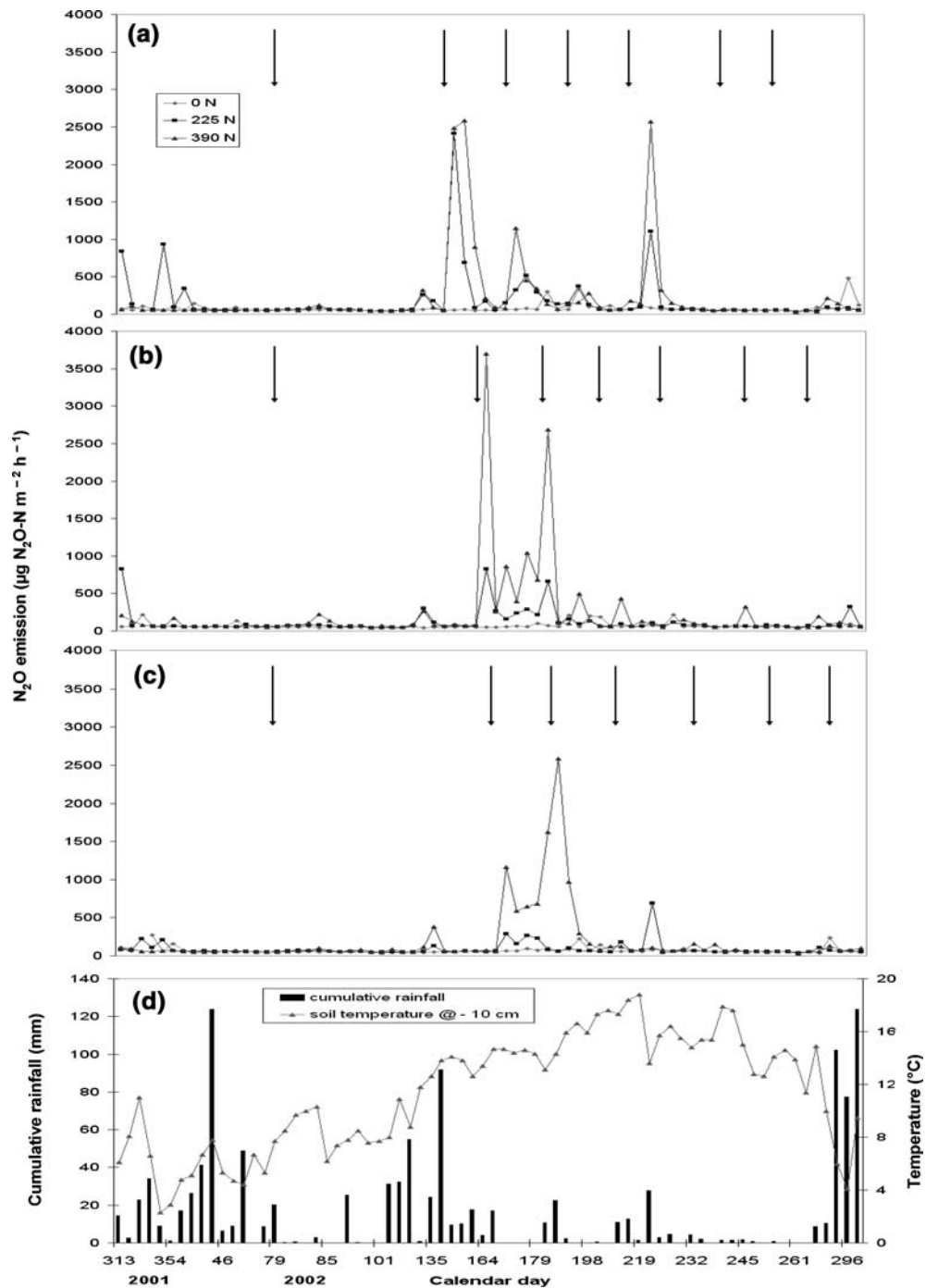
In year 1 the mean cumulative  $\text{N}_2\text{O}$  release from the control plots ( $0 \text{ kg N ha}^{-1}$  treatment) was  $4.21 \pm 0.79 \text{ kg N}_2\text{O-N ha}^{-1}$  compared with  $6.45 \pm 1.95$  and  $12.55 \pm 3.61 \text{ kg N}_2\text{O-N ha}^{-1}$ , in the  $225$  and  $390 \text{ kg N ha}^{-1}$  treatments, respectively. In year 2 the mean cumulative  $\text{N}_2\text{O}$  release from the control plots was  $4.66 \pm 0.55 \text{ kg N}_2\text{O-N ha}^{-1}$  compared with  $18.51 \pm 2.91$  and

$28.93 \pm 4.86 \text{ kg N}_2\text{O-N ha}^{-1}$  in the  $225$  and  $390 \text{ kg N ha}^{-1}$  treatments, respectively. Cumulative annual totals were also calculated for each fertilized treatment minus that of the 0 N treatment by summing cumulative emission values for each fertilizer application and calculating the percentage  $\text{N}_2\text{O}$  loss as a percentage of total N applied in each year in the form of N fertilizer and the N deposited by the grazing animals in the form of dung and urine (Table 1).

Derived variables were created to simplify analysis by calculating mean daily emission values, and mean daily covariate values for each fertilizer N application/grazing rotation for each replicate plot over the 2 years of the study. Using split-plot ANOVA in Genstat 6.2 (Genstat Committee 2003) an initial model was fitted to examine the effect of N treatment on measurement-day  $\text{N}_2\text{O}$  emission values. Nitrogen treatment significantly ( $P < 0.001$ ) affected mean daily  $\text{N}_2\text{O}$  emission in both the whole plot (Block  $\times$  Treatment) and sub-plot (Block  $\times$  Treatment  $\times$  Time) stratum.

Subsequently the effects of covariates and treatment covariate interactions on mean daily  $\text{N}_2\text{O}$  emissions within cycles were tested. The covariates of interest were those directly measured in the experimental plots. In this study soil temperature at 5 cm below the soil surface and the volumetric soil moisture content of the top 15 cm of the soil profile were measured. The effect of soil moisture and soil temperature and the nature of their interaction were examined in the sub-plot stratum of the analysis as inference of these effects in this stratum is more precise. There are generally insufficient degrees of freedom to estimate the error mean square appropriate to between-block totals with the accuracy needed to estimate treatment effects (Mead et al. 1998) in the upper strata.

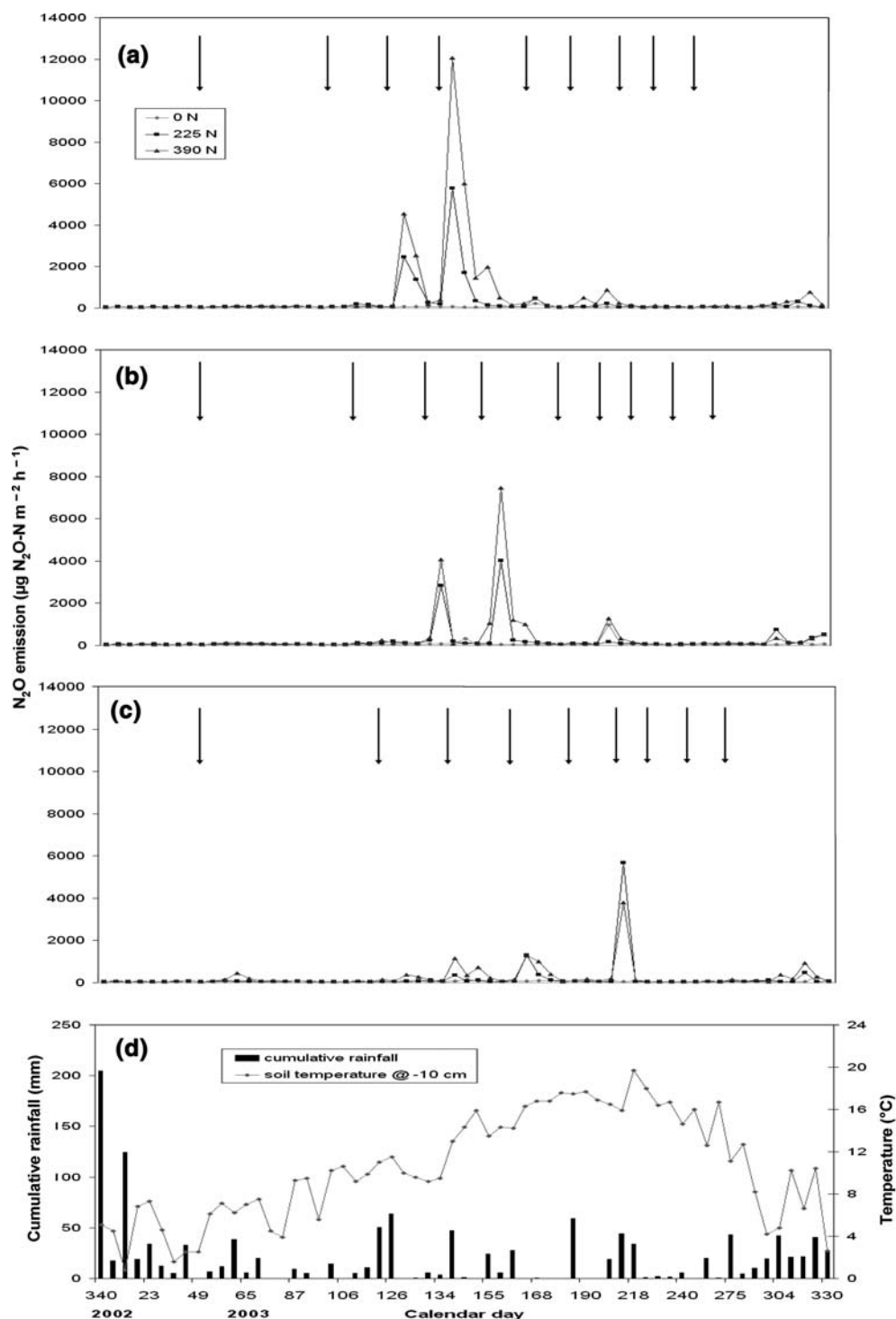
Various model structures were examined by investigating the effects of covariates, the effect of time and treatment covariate interactions by fitting terms sequentially and adopting the principles of hierarchy and marginality (i.e., a model with interacting terms should also include the main effects of the variables involved in the interaction). Although statistically significant ( $P < 0.001$ ), the effect of time was confounded with the effect of soil moisture status, indicating



**Fig. 1** Time course of  $\text{N}_2\text{O}$  emissions in year 1 (Nov 01–Nov 02) as affected by fertilizer N applications indicated by arrows (Replicate 1: (a); Replicate 2: (b); Replicate 3: (c)). Data points are the arithmetic mean of 8 chambers

per plot. Associated soil temperature at 10 cm below the surface and cumulative rainfall between measurement days is also shown (d)





**Fig. 2** Time course of  $N_2O$  emissions in year 2 (Nov 02–Nov 03) as affected by fertilizer N applications indicated by arrows (Replicate 1: (a); Replicate 2: (b); Replicate 3: (c)). Data points are the arithmetic mean of 8 chambers.

Associated soil temperature at 10 cm below the surface and cumulative rainfall between measurement days is also shown (d). (Please note difference in Y-axis scales between Fig. 1 and Fig. 2.)

**Table 1** Cumulative annual N<sub>2</sub>O–N loss (kg N<sub>2</sub>O–N ha<sup>-1</sup>) and percentage loss ((kg N<sub>2</sub>O–N emitted/kg N applied) × 100) for both years

Treatment	Replicate	Total N input* kg N ha <sup>-1</sup>	N <sub>2</sub> O–N evolved (kg)**	Emission factor*** (% N evolved)
Year 1				
225	1	279.7	2.7	1.0
	2	321.2	3.2	1.0
	3	329.6	0.8	0.2
390	1	429.5	6.2	1.4
	2	468.4	8.9	1.9
	3	489.2	10.0	2.0
Year 2				
225	1	287.2	14.0	4.9
	2	312.0	11.0	3.5
	3	311.1	16.6	5.3
390	1	475.7	34.4	7.2
	2	464.7	16.7	3.9
	3	538.0	21.8	4.1

\*Total N input = N fertilizer + N from dung and urine deposition

\*\*N<sub>2</sub>O–N evolved (kg) = Total N<sub>2</sub>O–N evolved (kg) minus that evolved in 0 N control

\*\*\*% loss = (kg N<sub>2</sub>O–N loss per kg N applied) × 100

that the effect of time should be included in the model. Interestingly, a non-significant relationship was found with soil temperature. Based on the latter finding, the effects of soil moisture status and temperature were re-examined by re-analysing the data while excluding the effect of time in the analysis. While soil moisture status effects were identical to those in the previous analysis, soil temperature also showed a highly significant ( $P < 0.001$ ) effect on emission levels when added sequentially as a model term. This observation indicated an interaction between soil moisture status and soil temperature, which was subsequently included in the analysis.

Variability in N<sub>2</sub>O emission over time can thus be explained by N treatment and variations in soil moisture status and temperature. However the effect of cycle as a measure of time must be included in this analysis as the purpose of adopting this statistical method is to account for the longitudinal nature of the data set. Highly significant ( $P < 0.001$ ) interactions, that were established between both soil temperature and soil moisture status and N treatment, suggest that the strength of the N treatment effect depends on both soil moisture status and soil temperature. In addition, where two factors interact independently with a third factor, an interaction between the three factors must exist. Therefore an interaction between treatment, temperature and soil moisture status exists ( $P < 0.05$ ) (Table 2).

**Table 2** Analysis of variance of covariate effects, covariate interactions and treatment covariate interactions

Source of variation	d.f. <sup>a</sup>	P
Cycle	15	< 0.001
Covariates	6	< 0.001
V (Volumetric moisture content)	1	< 0.1
T (Temperature @– 5 cm)	1	ns
T × V	1	ns
Treatment (Tr) × V	1	< 0.001
Tr × T	1	< 0.001
Tr × T × V	1	< 0.05
Residual	114	
Total	143	

<sup>a</sup>Degrees of freedom

Based on the results presented, a model of the data in the  $i$ th block from the  $j$ th treatment in the  $k$ th cycle can be described as follows:

$$y_{ijk} = b_i + t_j + e_{ij} + c_k + tc_{jk} + \beta_{ijk} + f_{ijk}$$

where  $y_{ijk}$  is the response (g N<sub>2</sub>O–N ha<sup>-1</sup> d<sup>-1</sup>);  $b_i$  is the  $i$ th block effect, ( $i = 1, 2, 3$ );  $t_j$  is the  $j$ th treatment effect ( $j = 1, 2, 3$ );  $e_{ij}$  is the main-plot residual;  $c_k$  is the  $k$ th cycle effect ( $k = 1, \dots, 16$ );  $tc_{jk}$  is the interaction effect between the  $j$ th treatment and the  $k$ th cycle;  $\beta_{ijk}$  is a vector of covariates;  $f_{ijk}$  is the subplot residual. We assume that  $e_{ij} \sim \text{NID}(0, \sigma^2)$  and  $f_{ijk} \sim \text{NID}(0, \sigma^2)$ . This implies a constant correlation ( $\sigma^2 / \sigma^2 + \sigma_w^2$ ) between any two observations on the same plot and independence otherwise. Predicted N<sub>2</sub>O emission levels for a selection of



soil moisture and temperature values are presented in Table 3.

### Soil mineral N

Nitrate-N and  $\text{NH}_4^+$ -N contents remained consistently low in the 0 N control treatment, whereas they increased and decreased with time at 225 and 390 kg N  $\text{ha}^{-1}$  as a result of N fertilizer applications (Figs. 3 and 4). The patterns of soil  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N depicted are representative of fertilized and grazed grassland where fertilizer N is applied in several applications throughout

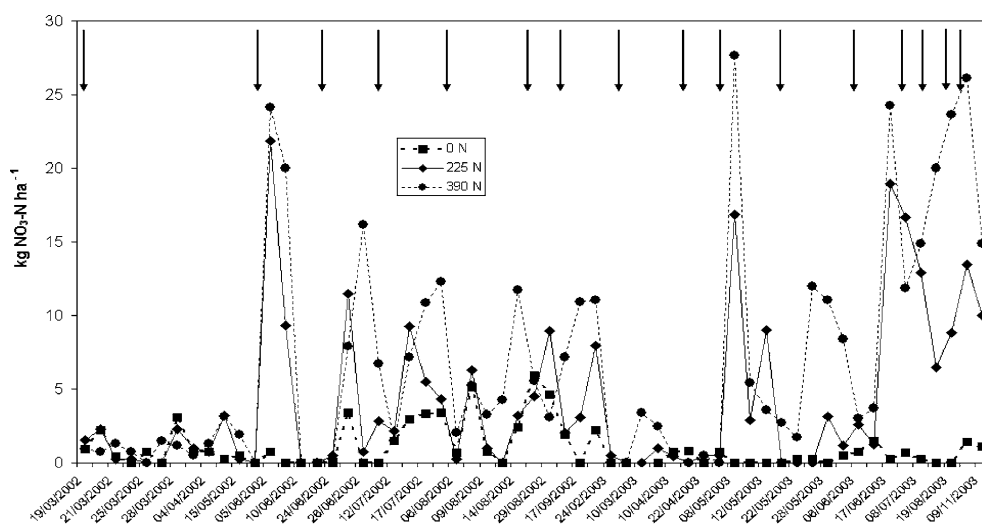
the year. This pattern is caused by fertilizer N applications, subsequent grazing and rapid N uptake by the grass sward during the growing season, as well as gaseous losses of N to the atmosphere as  $\text{N}_2\text{O}$  through denitrification and ammonia through volatilization.

Correlation analysis of soil mineral N data over both years showed that correlations exist between soil  $\text{NO}_3^-$ -N content ( $r = 0.34$ ,  $P < 0.001$ ;  $0.17$ ,  $P < 0.05$ ; and  $0.27$ ,  $P < 0.001$ , for the 0, 225 and 390 kg N  $\text{ha}^{-1}$  treatments, respectively), soil  $\text{NH}_4^+$ -N content ( $r = 0.45$ ,  $P < 0.001$  for the 0 kg N  $\text{ha}^{-1}$  treatment;  $r = 0.16$  and  $0.19$ ,  $P < 0.05$  for the 225 and 390 kg N  $\text{ha}^{-1}$  treatments, respectively) and total mineral N content ( $\text{NO}_3^-$ -N +  $\text{NH}_4^+$ -N) ( $r = 0.47$ ,  $P < 0.001$ ;  $r = 0.18$ ,  $P < 0.05$ ;  $r = 0.25$ ,  $P < 0.01$ , for the 0, 225 and 390 kg N  $\text{ha}^{-1}$  treatments, respectively) and  $\text{N}_2\text{O}$  emission (transformed to its natural logarithm). Correlations also existed between soil  $\text{NO}_3^-$ -N content and soil  $\text{NH}_4^+$ -N content ( $r = 0.48$ ,  $0.59$  and  $0.41$ ,  $P < 0.001$  for the 0, 225 and 390 kg N  $\text{ha}^{-1}$  treatments, respectively).

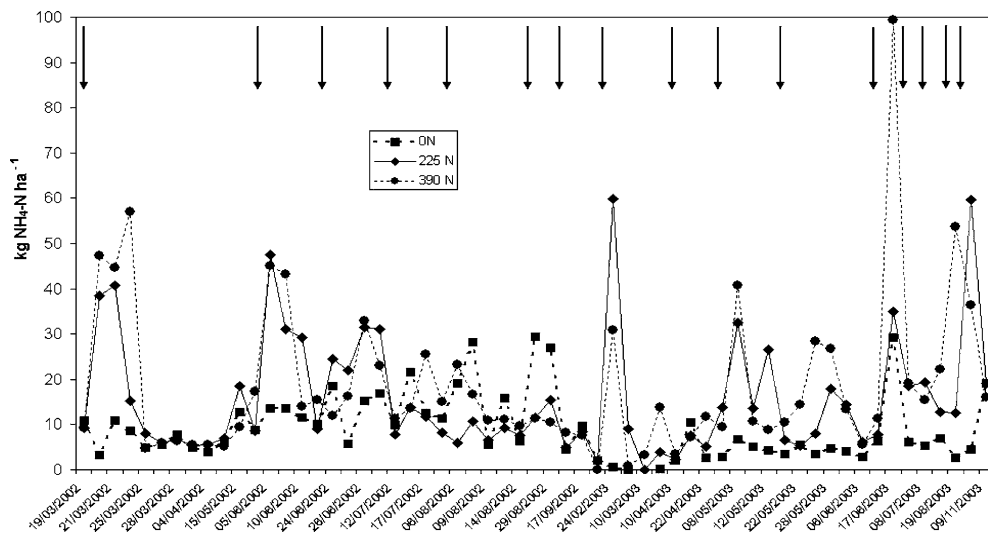
Effects of soil mineral N content, soil temperature and soil moisture status (as gravimetric soil moisture content) were tested using the REML directive in Genstat 6.2 (Genstat Committee 2003). Various models relating treatment, soil  $\text{NO}_3^-$ -N content, soil  $\text{NH}_4^+$ -N content, time after N

**Table 3** Predicted mean  $\text{N}_2\text{O}$  emissions (g  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$   $\text{d}^{-1}$ ) within a cycle for the range of soil temperature and soil moisture contents measured over the course of the study

Temperature (°C)	Volumetric soil moisture content (%)	$\text{N}_2\text{O}$ emission (g $\text{N}_2\text{O}$ -N $\text{ha}^{-1}$ $\text{d}^{-1}$ )		
		0 N	225 N	390 N
8	25	38	47.59	63.22
8	40	31.23	41.84	59.44
8	50	25.67	36.78	55.9
12	25	30.94	25.84	22.89
12	40	21.89	31.03	46.67
12	50	15.48	37.27	95.17
16	25	25.19	14.03	8.29
16	40	15.34	23.02	36.64
16	50	9.34	37.77	162.02



**Fig. 3** Soil  $\text{NO}_3^-$ -N (kg  $\text{NO}_3^-$ -N  $\text{ha}^{-1}$ ) contents throughout the two year period of the study. Arrows represent fertilizer N applications



**Fig. 4** Soil  $\text{NH}_4\text{-N}$  ( $\text{kg NH}_4\text{-N ha}^{-1}$ ) contents throughout the 2 year period of the study. Arrows represent fertilizer N applications

application, soil moisture status and treatment covariate interactions were investigated by fitting and eliminating terms sequentially to arrive at a final model structure which only includes the terms whose elimination would significantly decrease the explanatory power of the model (Table 4). Time after N application was transformed to its natural logarithm for model development to reflect decreases in soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  contents with time.

The model for the response on the  $i$ th plot on the  $j$ th measurement day can be described as follows:

$$y_{ij} = N_i + \beta_1(\text{Ln}T)_{ij} + \beta_2(\text{NO}_3)_{ij} + \beta_3(\text{Grav})_{ij} + \beta_4(\text{NO}_3 \times \text{Grav})_{ij} + \beta_5(N \times \text{Grav})_{ij} + e_{ij}$$

**Table 4** REML variance component analysis

Model terms	Wald statistic ( $\chi^2$ )	d.f.	Wald/d.f.	$\chi^2$ prob
Treatment (Tr)	10.74	2	5.37	0.005
Ln Time	18.20	1	18.20	<0.001
$\text{NO}_3\text{-N kg ha}^{-1}$	42.38	1	42.38	<0.001
Gravimetric soil moisture content (Grav)	9.45	1	9.45	0.002
Tr $\times$ Grav	8.49	2	4.25	0.014
$\text{NO}_3\text{-N} \times \text{Grav}$	18.90	1	18.90	<0.001

where  $y_{ijk}$  is the response ( $\text{g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ );  $N$  is the effect of applied N;  $\text{Ln}T$  is the natural logarithm of the number of days after fertilizer N application;  $\text{NO}_3$  is the soil  $\text{NO}_3\text{-N}$  content ( $\text{kg NO}_3\text{-N}$ );  $\text{Grav}$  is the gravimetric soil moisture content (%);  $\text{NO}_3 \times \text{Grav}$  is the model interaction between soil  $\text{NO}_3$  content and gravimetric soil moisture content;  $N \times \text{Grav}$  is the model interaction between N applied and gravimetric soil moisture content; and  $e_{ij}$  is the residual. It is assumed that observations on different plots are uncorrelated, conditional on the explanatory variables, and that observations on the same plot are correlated. The nature of this intra-correlation was modelled as auto-regressive of order 1 (AR1). Predicted  $\text{N}_2\text{O}$  emission levels at various soil  $\text{NO}_3\text{-N}$  contents, soil moisture levels and number of days after N application are presented in Table 5.

## Discussion

Most  $\text{N}_2\text{O}$  emission studies are of short duration and therefore may not accurately measure the total  $\text{N}_2\text{O}$  evolved from either fertilizer N, N deposited by grazing animals or soils accurately. Thus the use of longer term studies such as that presented herein, provide a more representative estimate of annual emission totals and allow for

**Table 5** Predicted  $\text{N}_2\text{O}$  emissions ( $\text{g N}_2\text{O ha}^{-1} \text{d}^{-1}$ ) for a range of soil  $\text{NO}_3\text{-N}$  contents, soil moisture status values and days after fertilizer N application

$\text{NO}_3\text{-N}$ ( $\text{kg N ha}^{-1}$ )	Soil moisture % <sup>a</sup>	Day after application	Response ( $\text{g N}_2\text{O ha}^{-1} \text{d}^{-1}$ )		
			0 N	225 N	390 N
2	30	2	15.43	19.50	19.19
2	30	10	14.42	18.22	17.93
2	40	2	16.70	25.58	31.29
2	40	10	15.60	23.90	29.23
2	50	2	18.08	33.57	51.03
2	50	10	16.89	31.36	47.67
7	30	2	17.39	21.97	21.62
7	30	10	16.25	20.53	20.20
7	40	2	24.12	36.94	45.18
7	40	10	22.53	34.51	42.21
7	50	2	33.45	62.10	94.40
7	50	10	31.25	58.02	88.20
12	30	2	19.60	24.76	24.36
12	30	10	18.31	23.13	22.76
12	40	2	34.82	53.34	65.23
12	40	10	32.53	49.83	60.94
12	50	2	61.88	114.90	174.66
12	50	10	57.81	107.34	163.17

<sup>a</sup>Soil moisture content = gravimetric soil moisture content (%)

more in-depth analysis of treatment and covariate effects.

Nitrous oxide emissions were measured with static-closed chambers which were removed between measurements thus allowing for greater ground coverage over the course of the 2-year period and integrating more of the spatial variability in  $\text{N}_2\text{O}$  emission that is found as a result of dung and urine deposition on the grazed pasture.

Nitrous oxide emission followed a similar pattern to rainfall. Highest emissions occurred following rainfall soon after or immediately before fertilizer N application and grazing and its consequent effects on soil moisture status. Similar rainfall-driven peaks in  $\text{N}_2\text{O}$  emission coincident with N fertilizer applications and grazing have been shown by a number of authors (Dobbie et al. 1999; Egginton and Smith 1986; Estavillo et al. 1994; Clayton et al. 1994; Anger et al. 2003; Merino et al. 2001). However, a relationship with rainfall and its effect on soil moisture status is unlikely to be straightforward as outlined by Jarvis et al. (1991).

Velthof et al. (1996a) suggest that even though seasonal and inter-annual variations in  $\text{N}_2\text{O}$

emissions from agricultural soils hamper accurate quantification of fluxes, measurements carried out in 1 year nevertheless were reasonably predictive of  $\text{N}_2\text{O}$  losses in other years. However, the sharp contrasts found in this study, between year 1 and year 2, do not agree with this observation. The cooler and wetter conditions in year 1 gave rise to considerably lower emissions than the warmer and drier year 2. Indeed the higher than average rainfall in the first half of 2002 compared with the coincidence of rainfall events and fertilizer applications in 2003 is evidence that short-term vagaries in weather can have a dominating influence on total annual emissions as suggested by Dobbie et al. (1999). Differences in cumulative emission totals are large i.e., up to a 3-fold difference in annual totals between the two years. However, although rainfall increased the potential for higher  $\text{N}_2\text{O}$  emissions at the experimental site, the higher soil moisture contents caused by prolonged rainfall would possibly have decreased the aerobic status of the soil, resulting in lower  $\text{N}_2\text{O}:\text{N}_2$  ratios as gaseous products of the denitrification process (de Klein and van Logtestijn 1996).

Treatment-induced  $\text{N}_2\text{O}$ -N losses ranged from 0.23% to 2.04% of N (fertilizer and dung and urine) applied in year 1. This is within the range of values used for inventory reporting purposes for N-fertilized grasslands under IPCC calculation procedures (IPCC 1997). In contrast, calculated emission factors for year 2 are substantially higher; values ranged from 3.17% to 7.22%. This is in agreement with Fowler et al. (1997) who, in a compilation of some studies of  $\text{N}_2\text{O}$  emissions from grassland soils observed that the fractional loss of  $\text{N}_2\text{O}$  from fertilizer and excretal N inputs to grasslands is substantially higher than the 1.25% used by the IPCC (IPCC 1997). In addition, the values recorded herein agree with those found by Velthof and Oenema (1995, 1997) and McTaggart et al. (1994) who observed that total annual  $\text{N}_2\text{O}$  emissions from grazed and fertilized grasslands were generally in the range of 10–20  $\text{kg N ha}^{-1}$ .

The variation encountered in cumulative emission totals between treatment replicates arises as a consequence of the rotational grazing structure of the experimental study as described.

Each treatment replicate was grazed for a 7-day period within the 21-day grazing rotation imposed. Thus each replicate within a treatment was subjected to differing climatic and environmental conditions relative to the other replicates. In addition, more of the variation caused by short-term vagaries in weather conditions would be likely to have been accounted for using such an approach and furthermore, the effect of interactions between soil temperature, soil moisture and fertilizer-N are more easily explored.

Laboratory research has conclusively established that soil parameters such as moisture status, temperature,  $\text{NO}_3^-$  content and organic carbon are primary factors affecting  $\text{N}_2\text{O}$  losses (Folorunso and Rolston 1984). These variables combine in the control of  $\text{N}_2\text{O}$  emissions from denitrification, and interact in a multiplicative manner. The theoretical consequence of multiplicative interactions is a lognormal distribution in response data (Aitchinson and Brown 1957). This is essential for time series analysis as it is a basic assumption that all variables are normally distributed. However, it does not change any conclusions made from the data. The complexities associated with the analysis of longitudinal data (repeated measurements) in rotationally grazed pasture were overcome with the use of the method of derived variables (Diggle et al. 2003). It offers a simple and easily interpretable analysis with a strong focus on particular aspects of the mean response profiles (Diggle et al. 2003). Indeed, it can be seen from the statistical analysis conducted that the key driving variables, soil moisture status and temperature, explained much of the variation of the data observed over time. The confounding of environmental variables with time is as a direct consequence of soil wetting and drying cycles, due to seasonal fluctuations in soil moisture and soil temperature. Response profiles of modelled  $\text{N}_2\text{O}$  emission levels further explain the multiplicative interaction of N application rate (N fertilizer and urine and dung deposition), soil moisture status and soil temperature which has been reported in other studies (Carran et al. 1995; Hénault et al. 1998; Dobbie et al. 1999; Velthof et al. 1996b; van Groenigen et al. 2005; Merino et al. 2001).

Based on modelled data in this study the decrease in  $\text{N}_2\text{O}$  emission levels with increasing temperature and N treatment (Table 4) is indicative of uptake of N by the grass sward with increasing N treatment level under dry soil conditions such as those found in the summer months. Furthermore, the increase in modelled data shown for the lower temperature value of  $8^\circ\text{C}$  at the 25% volumetric soil moisture content may indeed be as a result of the nitrification of  $\text{NH}_4^+$  in urinary N returns by the grazing animals with higher stocking rates found in the fertilized treatments. The interactions between N treatment, temperature and soil volumetric soil moisture content are especially marked at the 55% level. The larger emission values observed, are as a result of high volumetric soil moisture status, a soil temperature of  $16^\circ\text{C}$  and the application of N to the  $390 \text{ kg N ha}^{-1}$  treatment, which suggest the importance of denitrification as the source of  $\text{N}_2\text{O}$  at this site. In addition, it is shown that emissions from the  $225 \text{ kg N ha}^{-1}$  treatment are unaffected by soil temperature level at this level of soil moisture content. This would suggest that for this site at least that there is a threshold level of N treatment above which large differences in emission levels are seen for different temperatures.

The strong influence of soil  $\text{NO}_3^-$ -N contents on  $\text{N}_2\text{O}$  losses can be seen by comparing  $\text{N}_2\text{O}$  emission levels with soil  $\text{NO}_3^-$ -N contents after fertilizer N applications. Throughout the 2 years of measurement, peaks in  $\text{N}_2\text{O}$  emission levels coincided with peaks in soil  $\text{NO}_3^-$ -N contents, which in turn occurred after application of N fertilizer after which both soil  $\text{NO}_3^-$ -N contents and  $\text{N}_2\text{O}$  emissions returned to background levels within a 3-week period. When compared with  $\text{NH}_4^+$ -N, soil  $\text{NO}_3^-$ -N contents generally remained low throughout the period of the study, indicating uptake of the plant-available  $\text{NO}_3^-$ -N by the grass sward and probably as a result of the transformation of urinary N after grazing (Carran et al. 1995; Merino et al. 2001).

Even though correlations between natural logarithm-transformed  $\text{N}_2\text{O}$  emission values and soil mineral N contents were significant, the correlation coefficients observed were weak both for  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N contents which suggests that much of the variation in  $\text{N}_2\text{O}$  emission remains

unexplained by the values measured. Both spatial and temporal variation may partially account for this. In addition the occurrence of low  $\text{NO}_3^-$ -N concentrations when conditions appeared to favour denitrification and the bulking of soil samples prior to analysis may also offer an explanation.

## Conclusions

The emission of  $\text{N}_2\text{O}$  from grazed fertilized grassland follows an irregular pattern during the year which is dependent on N application rate, grazing management practices, seasonal variations in soil temperature and fluctuations in soil moisture status created by rainfall events, which interact in a multiplicative manner. Prolonged studies are therefore necessary to elucidate individual treatment or covariate effects. Peak emissions were generally associated with fertilizer N applications during the summer months which were coincident with rainfall events which suggest that denitrification was the dominant process leading to  $\text{N}_2\text{O}$  emissions at this site under warm, wet conditions in the topsoil.

Further work is required to identify the contribution of grazing and fertilizer N applications to grassland on a range of soil types. This would lead to more reliable estimates of  $\text{N}_2\text{O}$  emission for inclusion in regional, national and global inventories. The relative contributions of dung and urine on grazed pastures and their interaction with fertilizer N application regimes and soil and environmental variables needs to be examined for distinct periods during the grazing season to provide more robust estimates of  $\text{N}_2\text{O}$  emissions from grazed fertilized grasslands.

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