Nutrient Cycling in Agroecosystems (2005) 72:189–199
DOI 10.1007/s10705-005-1269-4

Relationship between gross nitrogen cycling and nitrous oxide emission in grass-clover pasture

Per Ambus
[1]*Biosystems Department, Risø National Laboratory, DK-4000, Denmark; e-mail: per.ambus@risoe.dk; fax: +45-4677-4160*

Received 19 April 2004; accepted in revised form 19 January 2005

Key words: Grass-clover pasture, Gross mineralization, Gross nitrification, Nitrogen cycling, Nitrous oxide emission

Abstract

Replacement of high-input N fertilized pastures with low-input grass-legume pastures may provide a mitigation option to reduce agricultural N_2O emissions. This study examined the relationship between N-cycling rates and N_2O production and evolution from the root zone of grass-clover pastures of various ages (production year 1, 2 and 8). The experimental approach included cross-labelling pasture monoliths with ^{15}N-enriched substrates to identify sources of N_2O, in combination with assessment of gross N mineralization and nitrification. Nitrous oxide emissions were generally low, fluctuating between 82 and 136μg N_2O–N m^{-2} d^{-1}, independent of pasture age. The ^{15}N labelling indicated that at least 50% of the N_2O was derived from the soil NH_4^+ pool, approaching 100% in June. In the two year old pasture the NH_4^+ pool contributions to N_2O emissions varied significantly with sampling time. Emission rates of N_2O correlated positively with soil NH_4^+ concentrations and the NH_4^+ supply as expressed by gross mineralization. The N_2O emissions showed a significant inverse relationship with soil NO_3^-, but was not correlated with the supply of NO_3^- as expressed by gross nitrification. The ratio N_2O vs. nitrification averaged 0.05% (range 0.004 to 0.29%) and varied with sampling time showing the lowest value in wet soil conditions.

Introduction

It is well documented that emissions of N_2O can be substantial from intensively managed grasslands. Introduction of less intensively managed systems with a high proportion of grass-legume pastures, such as organic dairy farms, may lead to smaller N_2O losses (Velthof et al. 1998; Flessa et al. 2002). The reduction in N_2O emissions achieved by introducing grass-legume pastures, depends partly on the magnitude by which N_2 fixed by the legumes is recycled in the pasture root zone.

Mitigation options to reduce N_2O emissions from agriculture have received much attention (Mosier et al. 1996; Skiba et al. 1997; Dalal et al. 2003), but quite often the available data for N_2O emissions are rather limited. The ideal dataset for N_2O emissions is based on year-round continuous measurements collected from multiple combinations of land-use and soil conditions. Since this is not practically achievable, models need to be developed to predict N_2O emissions from different land management regimes and soil conditions. Simple empirical modelling based on inputs from

few soil parameters has been proven successful in predicting N_2O fluxes from grassland sites (Velthof et al. 1996). However, the applicability of such models are limited and restricted to sites with specific soil physical and chemical characteristics, similar to those used to parameterize the model (Velthof et al. 1996). Development of widely applicable models to predict N_2O formation and emission requires a more mechanistic approach that incorporates functions accounting for soil physical and climatic characteristics on, for example, soil C and N cycling and plant primary production. An intercomparison of four mechanistic trace gas flux models (Frolking et al. 1998) showed that simulated N cycling in temperate agricultural sites was in close agreement with observed values, but the simulated nitrogen trace gas fluxes were not consistent. From this it can be concluded that our understanding of soil N cycling is quite comprehensive, but our ability to understand and predict the link between N cycling and trace gas emissions still remains unsatisfactory. Formation of N_2O is not a constant fraction of the substrate turned over but is regulated by various other soil parameters as indicated in the hole-in-the-pipe model proposed by Firestone and Davidson (1989). A determination of the fraction of N_2O produced from the flow of N through nitrification and denitrification requires information on the gross cycling rates that actually expresses the amount of N flowing through the various pathways.

Rotations of grass-clover pastures constitute an important element of organic farming systems. Although such rotations are susceptible to significant nitrogen losses (Eriksen 2001), information on loss of nitrogenous gases is sparse. Consequently, the IPCC guidelines for inventories on greenhouse gas emissions do not explicitly address N_2O emissions from nitrogen fixing forage crops (Penman et al. 2000).

In this study relationships between N-cycling rates and N_2O emission from the root zone of grass-clover pastures of various ages and at different times during the growing season were investigated in grass-clover pasture in Denmark. The experimental approach included cross labelling with ^{15}N-enriched NO_3^- and NH_4^+ in order to trace sources of N_2O, in combination with an assessment of gross mineralization and nitrification by the isotope pool dilution technique.

Materials and methods

Site description and soil collection

Soil columns for this experiment were collected during 2001 in an experimental field trial of the Danish Institute of Agricultural Sciences in Foulum, Denmark (9°34′ E, 56°29′ N). The soil is classified as a Typic Hapludult with 8.4% clay, 28.9% silt, 56.5% sand with a pH (H_2O) of 5.6. The field trial included grass-clover pasture swards (perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) of three different ages. In 2001, the swards were entering production years 1, 2 and 8, respectively. The swards were managed according to organic farming practice and no fertilizer was added (Eriksen 2001). Each treatment was replicated in four plots. In March 2001, eight 30-cm diameter × 30-cm long PVC-cylinders were inserted 25 cm into the soil in a 2 × 4 regular grid in each of the replicate field trials giving a total of 96 cylinders, and left for subsequent collection. In addition, two 12.5-cm diameter cylinders were also installed to 25 cm depth in each plot. Areas with the PVC cylinders installed were fenced to avoid damage by grazing animals and the sward was cut by band at approximately 2-week intervals. Four times during the growing season (1 May, 11 June, 20 August, and 8 October) two of the larger cylinders were carefully excavated from each plot. The undisturbed soil column was then placed on sheets of polyethylene foam (15 mm) glued to plywood to create a gas tight seal at the bottom. The two 12.5-cm cylinders were also excavated and two new cylinders of similar size were pushed down for collection at the next sampling event. All samples were transported to Risø National Laboratory within the same day as collection and placed outdoors under a shelter to avoid precipitation and excessive heating by sunlight. The first day after collection, emission of N_2O was determined from all the individual 30-cm soil columns (*T0*). On the second day, half of the columns were labelled with ^{15}N in the form of $^{15}NH_4NO_3$ and N_2O evolution as well as ^{15}N enrichment of the evolved N_2O was determined 24 h (*T24*) and 48 h (*T48*) after labelling. This treatment group will hereafter be described as *AL* (ammonium labelling). Three days after collection the *NL* (nitrate labelling) treatment was initiated by adding ^{15}N label in the form of $NH_4{}^{15}NO_3$ to the remaining

columns, which then was subject to gas sampling as in the *AL* treatment group.

Gas sampling and analysis

For determination of N_2O emission, the cylinders were sealed at the top by Perspex lids. A rubber septum through the lid allowed the headspace gas to be sampled by syringe and needle. A 5 cm extension collar was glued to the lid to increase headspace height to about 10 cm. A rubber draught excluder provided a gas tight seal between the cylinder top and extension collar. At each gas-sampling event the headspace was sealed for 90 min and four 5-ml gas samples were removed at 30 min intervals. Gas samples were stored in pre-evacuated 3-ml Venojects® (Terumo, Leuven, Belgium) until analysis, which took place within a week after collection. Nitrous oxide emission rates were then assessed by the linear increase in headspace concentration over the closure period. At the end of the 90 min chamber closure, a 120 ml headspace sample was collected and transferred to a pre-evacuated serum bottle crimp sealed with a butyl stopper. This sample was subsequently used for analysis of the ^{15}N content in the N_2O. In order to assess the ^{15}N enrichment of the N_2O emitted into the headspace, it was assumed that N_2O confined in the headspace at the time of chamber closure contained no excess ^{15}N, which was verified from random determinations of ^{15}N in the N_2O over the soil columns just prior to mounting the lids. Furthermore, it was also assumed that the N_2O produced was isotopically in equilibrium with the N_2O emitted into the headspace.

Soil sampling and ^{15}N labelling

At each field visit, soil auger samples (2 cm diameter) were taken from 0 to 16 cm depth for initial determinations of soil moisture and N content. Four auger samples were collected in the vicinity of the two excavated columns, bulked and kept in a cool box until processing the next day.

At the onset of treatments *AL* and *NL* the top 0–14 cm of soil in each column was labelled uniformly by adding ^{15}N-enriched (10 atom% excess) solutions of $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$, respectively. Each soil column received 74 mg of NH_4^+-N and 74 mg of NO_3^--N, equivalent to a total of 2.2 g N m^{-2}. The N was added in 188 ml portions to each column, which was distributed in 47 4-ml doses given by syringe. To ensure even distribution by depth each 4-ml dose was added slowly (Hamilton Microlab 500 precision liquid processor) in synchrony with the travel of a 15-cm side-hole needle through the soil profile. Equivalent amounts of N were added in a similar way also to the 12.5-cm diameter, soil cores. These were removed from the PVC sleeves 2 h after additions in order to determine start values of soil moisture, inorganic nitrogen concentrations and the ^{15}N enrichments, Likewise, after the final gas sampling at 48 h, the 30 cm soil columns were removed from the PVC sleeves in order to collect subsamples for soil moisture and nitrogen determinations. All soil in the 0–16 cm top layer was collected from each soil column and coarse roots, stones etc. removed. The soil was then mixed thoroughly by hand, sieved to pass through a 10-mm mesh and then separated into minor portions, which were rapidly cooled to −24 °C. For practical reasons it was not possible to extract the soil samples immediately after sieving, however, cooling of the small soil samples to below freezing occurred within 1–2 h. For subsequent N-extractions, 10-g samples of frozen soil were transferred to 2 M KCl (1:10 w:vol) and extracted for 1 h.

Gross N turnover rates were calculated based. on the ^{15}N-isotope pool dilution principle using the analytical equations of Kirkham and Bartholomew (1954). In treatment *AL*, gross rates of N mineralization were obtained from the isotopic dilution of the NH_4^+ pool. In treatment *NL*, gross rates of nitrification were obtained from the isotopic dilution of the NO_3^- pool.

Analysis

Concentrations of N_2O were measured by gas chromatography (Shimadzu GC 14B, Kyoto, Japan) with electron capture detection. The ^{15}N contents of the N_2O were analyzed following chemical removal of CO_2 and dual cryogenic focusing of the N_2O using a Finnigan MAT Pre-Con unit (ThermoFinnigan, Bremen, Germany) interfaced with a GC coupled in continuous flow-mode to a Finnigan MAT Delta PLUS isotope

ratio mass spectrometer. Soil extracts were analyzed for concentrations of NH_4^+ and NO_3^- on a Bran + Luebbe AutoAnalyzer 3 system (Bran + Luebbe, Norderstedt, Germany). For determinations of the ^{15}N content in the NH_4^+ and NO_3^-, the content of each component in 70-ml portions of the KCl extracts was concentrated on acidified filter paper (Sørensen and Jensen 1991). The two components were concentrated sequentially in order to undertake separate ^{15}N determinations in the two forms of N. Soil total N and ^{15}N was determined in finely ground 40-mg portions of air-dried soil samples. Dried filter papers and soil samples were wrapped in tin cups followed by analysis on a CE 1110 elemental analyser (ThermoFinnigan, Milan, Italy) coupled in continuous flow mode to the isotope ratio mass spectrometer.

Statistical analysis

Statistical evaluation of the data was undertaken using S-PLUS® (Insightful Corporation, Seattle, USA). Two-sample population means were compared using *t*-tests and multiple-means comparisons were made by using the MULTICOMP function. Data were assumed normally distributed except for N_2O fluxes, which were log-transformed before evaluation. All tests were performed at the 5% probability level.

Results

Total N and recovery of ^{15}N

Nitrogen and moisture contents in the 0- to16-cm soil layer generally was not affected by pasture age, except for a few observations on total N and NH_4^+ in October, and NO_3^- in May (Table 1). The NH_4^+ content increased 2-fold from 9.5 mg N kg^{-1} in May to 23 mg N kg^{-1} in October. The NO_3^- content remained at a steady level of about 6.5 mg N kg^- through the season (Table 1).

The amount of ^{15}N-label recovered from the soil columns after 48 h of incubation ranged between 0.33 and 0.52 mg kg^{-1} soil in the $^{15}NH_4^+$-labelled soils and between 0.32 and 0.48 mg ^{15}N kg^{-1} soil in the $^{15}NO_3^-$ labelled soils. Compared with the initial addition 0.55 mg ^{15}N, this kg^{-1} corresponds to

Table 1. Soil contents of total N, ammonium (NH_4^+), nitrate (NO_3^-) and moisture at the different combinations of grass-clover pasture age and sampling times. Numbers are means of n = 4 replicates.

	Total N g kg^{-1}	NH_4^+ mg N kg^{-1} dry soil	NO_3^- mg N kg^{-1} dry soil	H_2O% WFPS[A]
May				
1-year-old	2.9[a,B]	8.7[a]	11.6[a]	69[a]
2-year-old	2.9[a]	9.5[a]	5.3[b]	68[a]
8-year-old	3.1[a]	11.3[a]	7.1[ab]	72[a]
June				
1-year-old	3.0[a]	17.9[a]	7.4[a]	61[a]
2-year-old	2.9[a]	18.3[a]	6.4[a]	57[a]
8-year-old	2.8[a]	26.9[a]	4.9[a]	59[a]
August				
1-year-old	3.1[a]	26.0[a]	6.8[a]	59[a]
2-year-old	2.6[a]	20.4[a]	5.8[a]	53[a]
8-year-old	3.1[a]	24.0[a]	5.0[a]	56[a]
October				
1-year-old	2.9[a]	20.7[b]	7.0[a]	60[a]
2-year-old	2.7[b]	23.2[ab]	6.0[a]	61[a]
8-year-old	3.0[a]	26.2[a]	5.8[a]	65[a]

[A]% water filled pore space.
[B]Lower case letters denotes significant differences ($P < 0.05$) between pasture age for each sampling time.

recovery efficiencies of between 58 and 95%. The recovery of ^{15}N was independent of pasture age but varied between sampling times within several combinations of pasture age and treatment. Generally, the amounts of unrecovered ^{15}N peaked in June and August when up to 40% of the added label was unaccounted for in the soil N pool. This excessive loss of ^{15}N-label in June and August was very likely due to plant uptake, which was not considered in this study. For comparison, the recovery of ^{15}N label 2 h after the additions of $^{15}NH_4^+$ and $^{15}NO_3^-$ averaged 98.8 and 98.6%, respectively. Recovery of residual ^{15}N in the inorganic N fraction (NH_4^+ + NO_3^-) was relatively high at the first incubation in May; 92% in the 1- and 2-year-old pastures, and 76% in the 8-year-old pasture. At the subsequent incubations, the recoveries of ^{15}N in the inorganic N fractions dropped significantly ($P < 0.001$) in the 1- and 2-year-old grass-clover, ranging from 55 to 78%, indicating increased assimilatory activity. A drop was observed in the 8-year-old grass-clover, ranging from 57 to 69%, but this was not statistically significant. It also was observed that between 61 and 92% of the residual inorganic ^{15}N in the *AL* treatment group was present as NO_3^-, which

indicates rapid nitrification in these soils. However, the increase in NH_4^+ over the season (Table 1) suggests that mineralization rates exceeded nitrification rates.

Nitrous oxide emissions

Emissions of N_2O from untreated soil columns (*T0*) ranged between 0 and 200 μg N_2O-N m^{-2} d^{-1} and were not influenced by sampling time for any of the pasture ages (Figure 1). The N_2O emissions over the course of the four sampling events was independent of pasture age and averaged 136, 112 and 82 μg N_2O-N m^{-2} d^{-1} for the 1-, 2- and 8-year-old grass-clover, respectively. Twenty-four hours after N applications (*T24*), the N_2O emissions generally had increased to between 70 and 400 μg N_2O-Nm^{-2} d^{-1}, but a significant effect of the N application was observed only in a few cases; the 2- and 8-year-old pastures in June and in the 8-year-old pasture in October (Figure 1). The effect of N application on N_2O emission was short-lived, and two days after the N application (*T48*) the N_2O emission rates had dropped to between 40 and 215 μg N_2O-N m^{-2} d^{-1} and were not different from the N_2O emissions at *T0*.

Nitrogen-15 enrichment of the N_2O emitted at *T24* fluctuated between 0.465 and 1.735 atom% excess (APE) across all combinations of pasture age and sampling times in the *AL* treatment and between 0.825 and 1.424 APE in the *NL* treatment (Figure 2 left hand plots). At *T48*, ^{15}N enrichment of the N_2O had decreased to between 0.220 and 1.247 APE in the *AL* treatment and between 0.266 and 1.052 APE in the *NL* treatment (Figure 2 right hand plots).

The initial enrichments of the soil inorganic N pools fluctuated between 1.241 and 3.143 APE (NH_4^+ in the *AL* treatment group) and between 3.477 and 5.879 APE (NO_3^- in the *NL* treatment group), depending on soil N concentrations (Table 2). After 48 hours of incubation, APE values of the soil inorganic N pools had dropped significantly (Table 2) indicating mineralization (*AL*) as well as nitrification (*NL*) activity. APE of NH_4^+ in the *NL* treatment group also increased at some events (Table 2), which suggests that remineralization of assimilated NO_3^--^{15}N may have occurred.

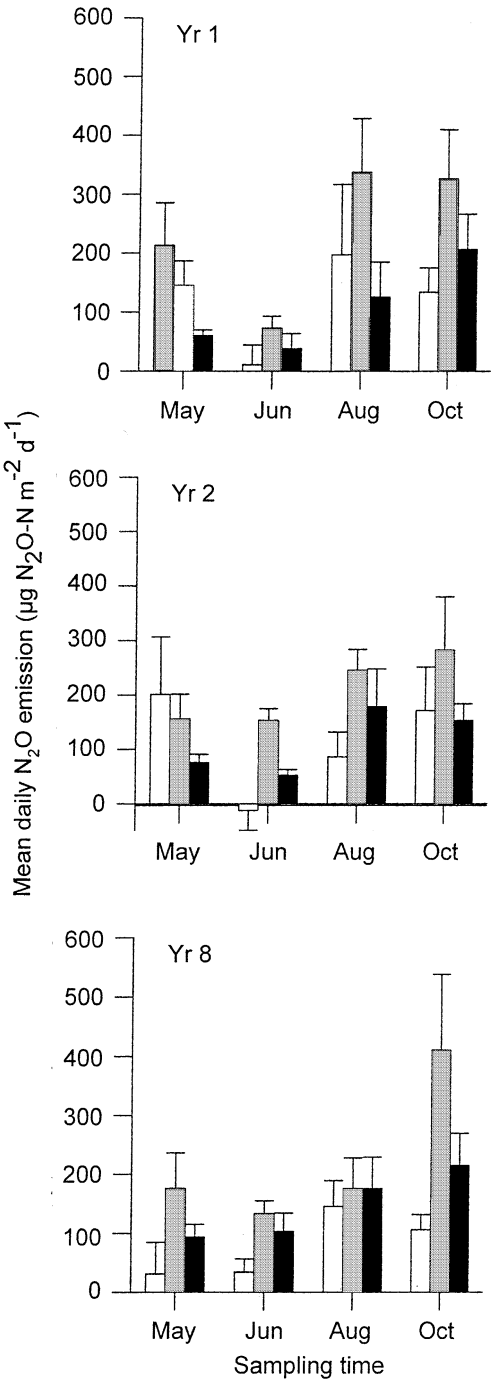

Figure 1. Emissions of N_2O from three different ages of grass-clover. Emissions were recorded in 30-cm diameter soil columns prior to additions of NH_4NO_3 (T0: □), 24 h after the additions (*T24*: ■) and 48 h after the additions (T48: ■), and at different sampling times through the growing season. Data are means of eight replicates + 1 SE.

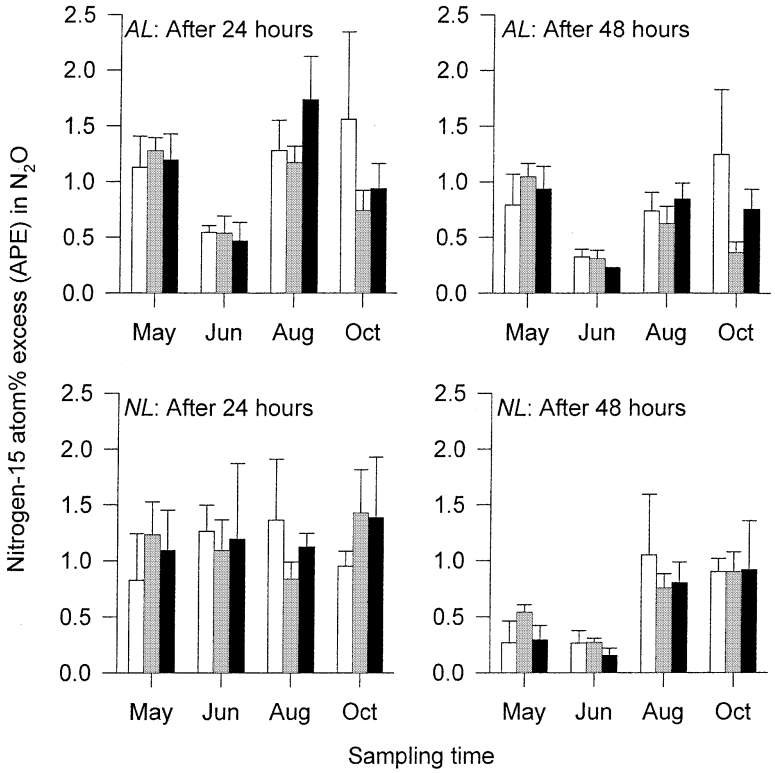

Figure 2. Nitrogen-15 enrichments of N_2O emitted from $^{15}NH_4NO_3$ labelled (*AL* – top plots) and $NH_4{}^{15}NO_3$ labelled (*NL* – bottom plots) soil columns. Emissions were recorded (24 h left hand plots) and 48 h (right hand plots) after labelling and at different sampling times through the growing season. Different bar shadings indicate different grass-clover pastrure ages; 1 year (□), 2 year (▩), and 8 year (■). Data are means of four replicates +1SE.

Isotopic enrichment of N_2O observed at *T48* was intermediate to the enrichment of NH_4^+- and NO_3^--pools in both treatment groups (Figure 2 and Table 2), suggesting that N_2O was derived from both the NH_4^+ and NO_3^- pools at the same time. In the *AL* treatment group in June, however, the enrichment of N_2O closely matched that of the NH_4^+ pool, indicating that NH_4^+ was the sole source for N_2O. Assuming that ^{15}N was distributed homogeneously within each of the inorganic N pools, their relative contribution to N_2O production was assessed by the distribution of N among NH_4^+, NO_3^- and N_2O, respectively, at *T48* (Figure 3). Generally it was found that at least 50% of the N_2O was derived from the NH_4^+ pool, approaching 100% in June. The sampling time variability was significant ($P < 0.05$) for the 2-year-old pasture. Pasture age had no influence on the relative contribution of the NH_4^+-pool for N_2O production (Figure 3). Emissions of ^{15}N as N_2O did not vary significantly between the two labelled treatment groups (not shown), which also indicate that both processes were important for N_2O production.

The relationship between N_2O evolution and soil substrate availability and turnover was examined further from the evolution rates of ^{15}N in N_2O in combination with the available ^{15}N in the soil inorganic N-pools. The concentration of soil inorganic N and ^{15}N at *T24* was estimated as the average between the concentrations observed at *T0* and *T48* assuming a linear change over the 48-h period. Data from the *AL* treatment group was used for this test. Evolution of $^{15}N_2O$ was positively correlated with soil $^{15}NH_4^+$ availability ($R^2 = 0.22$; $P < 0.001$) showing the relationship $F_{15N_2O} = 0.9 \times e^{6.92 \times A}$ where A denotes the concentration of $^{15}NH_4^+$ (Figure 4). In contrast, $^{15}N_2O$ evolution was inversely related to soil $^{15}NO_3^-$ availability ($R^2 = 0.19$; $P < 0.001$) with the relationship $F_{15N_2O} = 1.60 \times e^{-5.95 \times N}$, where N denotes the concentration of $^{15}NO_3^-$ (Figure 4). The relationships illustrated in Figure 4 were

Table 2. Nitrogen-15 enrichments (atom% excess – APE) of NH_4^+-and NO_3-pools immediately after (*T0*) and 48 h after (*T48*) labelling with $^{15}NH_4NO_3$ (*AL*) and $NH_4{}^{15}N_3^-$ (*NL*), respectively.

Label	$^{15}NH_4NO_3$(*AL*)				$NH_4{}^{15}NO_3$(*NL*)			
Compound:	NH_4^+		NO_3^-		NH_4^+		NO_3^-	
Time:	T0	T48	T0	T48	T0	T48	T0	T48
1-year-old grass-clover								
May	2.911	0.246	0.011	1.842	0.001	0.092**	3.697	2.116*
June	2.055	0.268	~0	2.089	0.003	na	3.477	3.442^{ns}
Aug	1.311	0.082	~0	1.741	0.012	0.034	3.645	2.083
Oct	1.504	0.148	~0	1.685	0.012	0.065^{ns}	3.600	2.132
2-year-old grass-cloves								
May	3.143	0.409**	0.018	1.670	< 0.001	0.121	5.879	2.647
June	1.675	0.229	0.002	1.857	0.003	na	3.755	3.539^{ns}
Aug	1.522	0.093	~0	1.402	0.014	0.039*	3.927	2.222
Oct	1.380	0.189	~0	1.877	0.011	0.037^{ns}	3.875	1.545**
8-year-old grass-clover								
May	3.067	0.427	0.018	1.723	0.001	0.104**	5.191	1.511**
June	1.243	0.241	0.001	2.144	0.001	na	4.358	1.598**
Aug	1.346	0.173	0.001	1.419	0.012	0.055^{ns}	4.266	1.907
Oct	1.241	0.220	~0	1.781	0.010	0.062	4.042	2.547**

Changes in atom% excess (APE) between *T0* and *T48* for each combination of pasture age, month and treatment are significant at the 0.1% level unless indicated otherwise. ns, not significant; *, significant at 5% level; **, significant at 1% level; na, indicates data not available due to experimental error.

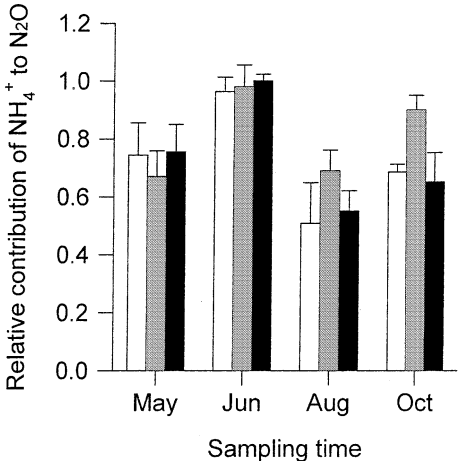

Figure 3. Relative contribution of soil NH_4^+ to N_2O emission in 1 year (□), 2 year (▩) and 8 year (■) old grass-clover pastures, respectively, at different sampling times through the growing season. Data are means of eight replicates + 1 SE.

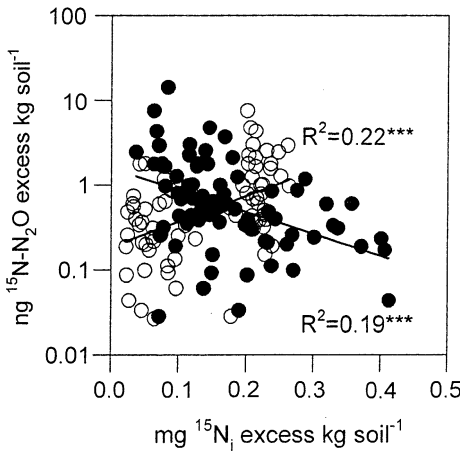

Figure 4. Relationship between Nitrogen-15 emitted in N_2O (Y-axis) and nitrogen-l5 abundance in the soil NH_4^+ (○) and NO_3^- (●) pools (X-axis) recorded in grass-clover columns labelled with $^{15}NH_4NO_3$. Datapoints indicate observations from single columns. *** indicates $P < 0.001$.

validated against data for soil inorganic N and N_2O emissions in the independent *NL* treatment group. This test indicated that 13% ($P < 0.01$) of the variability of the N_2O evolution in the *NL* treatment group could be predicted from the isotopic analysis in the *AL* treatment group.

Gross N cycling

Gross mineralization was calculated from dilution of the $^{15}NH_4^+$-pool corrected for NH_4^+-consumption in the *AL* treatment group. Likewise, gross nitrification rates were calculated from the dilution

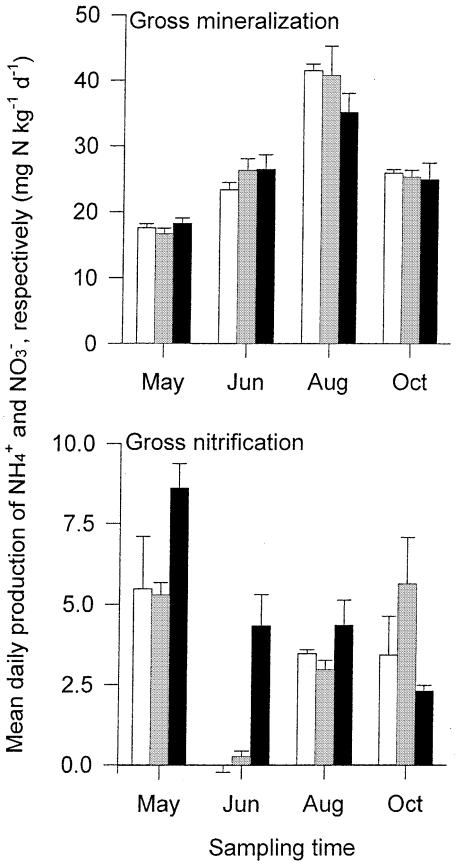

Figure 5. Rates of gross mineralization (top) and gross nitrification (bottom) in 1-year (□), 2-year (▒). and 8-year (■)-old grass-clover pastures, respectively, at different sampling times through the growing season. Data are means of four replicates +1SE.

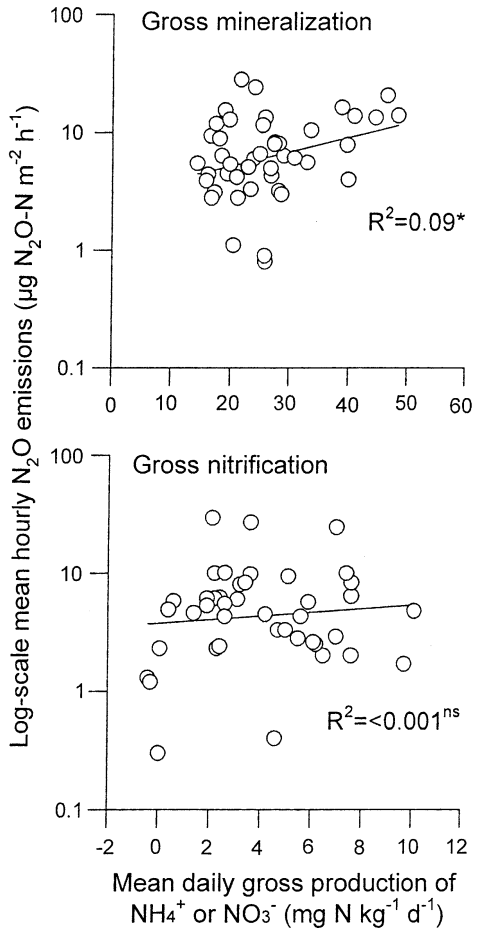

Figure 6. Relationship between N_2O emissions and gross mineralization (top) and gross nitrification (bottom). Data points are observations in single soil columns labelled with $^{15}NH_4NO_3$ for mineralization and $NH_4{}^{15}NO_3$ for nitrification. *indicates $P < 0.05$ ns, not significant.

of the $^{15}NO_3^-$-pool corrected for NO_3^- -consumption in the *NL* treatment group. Gross mineralization activity showed distinct variation among sampling times, with a significant ($P < 0.05$) peak in activity in August for all three pasture ages (Figure 5 top). Pasture age had no effect on the mineralization of N. Unlike gross mineralization, gross nitrification did not fluctuate with sampling time, overall mean of 3.8 mg N $kg^{-1}d^{-1}$ apart from an almost lack of activity in the 1- and 2-year-old pastures in June (Figure 5 bottom). Among all sampling times, gross nitrification was not affected by pasture age.

The relationships between gross N cycling rates and N_2O evolution are illustrated in Figure 6, Gross mineralization and gross nitrification rates were obtained from the distinct *AL* and *NL* treatment groups, and this analysis is based on coherent data for N_2O emissions and gross transformations observed in single soil column. Emissions of N_2O were positively correlated ($P < 0.05$) with gross mineralization rates (Figure 6 top), but not with gross nitrification activity (Figure 6 bottom). Assuming that all N_2O emitted was produced in the 0- to 16-cm soil depth, where turnover 9 rates were measured, it was calculated that 0.05% (range 0.004–0.29%) of the nitrified NH_4^+ was recovered as emitted N_2O within all combinations of pasture age and sampling time. The N_2O:nitrification proportion was not related to pasture age, but changed significantly with sampling time ($P < 0.05$) Thus, for the sequential

sampling times of May, June, August and October the N_2O:nitrification proportion was calculated as 0.01, 0.13, 0.03 and 0.04%, respectively.

Discussion

Nitrous oxide emission from grass-clover

The N_2O emissions of this study compare well with those of other studies on unfertilized pastures (e.g., Kaiser et al. 1998; Poggemann et al. 1999). In order to label the soil with ^{15}N for the examination of gross N turnover it was necessary to apply additional nitrogen to the soil columns. The applied amounts of NH_4^+ and NO_3^- corresponded to approximately 5 mg N kg^{-1} of each form. Although this was only between one and five times less than the amount of inorganic N already in the soil (Table 1), the N_2O emission increased temporarily. Thus, it can be speculated that N_2O production in these pasture soils was limited by the spatial distribution of N already present in the root zone rather than by the bulk concentration. However, the response in N_2O emission to the N additions was relatively low and short-lived in comparison to N_2O emissions after conventional N applications (Clayton et al. 1997). Changes in other N-cycling processes such as nitrification, denitrification and immobilization may also have occurred upon the N addition, but given the modest influence on N_2O emissions these processes also were probably affected only to a minimal extent.

Pasture age significantly influenced above- as well as below-ground plant biomass production (Eriksen et al. 2004), but did not unambiguously influence N_2O emissions nor did it affect magnitudes of soil N pools and N-cycling rates. This is in line with observations in tropical grasslands following forest clear cutting (Keller et al. 1993 and Veldkamp et al. 1999) showing little or no effect of grass pasture age on soil N-cycling and N_2O emissions until >10 years after deforestation. Systematic measurements of N_2O emissions from various ages of temperate pastures are not available.

Although sampling was intended to be representative of the entire growing season, any interpretation from only four time points should be done with extreme care. As pointed out by Veldkamp et al. (1999), sampling may inadvertently take place under certain soil or climatic conditions not reflecting true interannual variability, which then may be insufficient to make a good estimate of trace gas fluxes. In the present study, it is noteworthy for example, that soil moisture showed very little variability among sampling times (Table 1), which emphasizes that the results likely do not represent proper boundaries in seasonal dynamics of the N-cycling processes.

Relationship with soil N concentrations and cycling

Biological nitrification and denitrification are the two dominant sources of nitrous oxide in soil plant systems. In 1984, Linn and Doran proposed a general model describing the relationship between soil water filled pores space (WFPS) and the occurrence of various soil microbial processes (Linn and Doran 1984). This model predicts that denitrification may not occur until WFPS increases above 60%. In my study, %WFPS fluctuated around the anticipated cut off value for denitrification, i.e., 60% (Table 1) and only in the May sampling time would a contribution from denitrification be expected in all three different pasture ages. In other words, given these relatively dry soil conditions a significant contribution of denitrification to the N_2O emission was not anticipated. This consideration agrees well with the empirical relationship illustrated in Figure 4, emphasizing the importance of NH_4^+ turnover for N_2O production in these pastures. On the other hand, it was also observed that significant amounts of $^{15}N_2O$ were produced from the ^{15}N-labelled NO_3^- pool, implying that denitrification was a source for the N_2O at the same time. This was particularly obvious in the *NL* treatment where a dilution of the $^{15}NO_3^-$ pool by a supply from the natural abundance NH_4^+ pool led to a concomitant reduction in the APE of emitted N_2O (Figure 2).

Thus, a lack of, or even an inverse, relationship between N_2O emission and soil NO_3^- concentrations (Figure 4) does not necessarily indicate that NO_3^- is unimportant as a source of N_2O. In the majority of the experiments, the N_2O enrichments were intermediate to the enrichments of the NH_4^+ and NO_3^- pools; i.e., lower than the NH_4^+ enrichments in the $^{15}NH_4^+$-labelled treatment

group and lower than the NO_3^- enrichments in the $^{15}NO_3^-$-labelled treatment group. The relative contribution of NH_4^+ to N_2O was estimated to vary between 50 and 100% (Figure 3). This range is in good agreement with the 70% contribution observed by Stevens et al. (1997) in a laboratory study with water unsaturated soils. It must be emphasized, however, that this result does not necessarily imply that 50–100% of the N_2O was formed directly from the activity of nitrifying organisms as N_2O might be produced by reduction of NO_2^- supplied both from oxidation of NH_4^+ and reduction of the NO_3^- (Watson and Mills 1998; Wrage et al. 2001). Simultaneous nitrification and denitrification also may take place even in well aerated soils (Kuenen and Robertson 1994). However, Russow et al. (2000) state that mixing of NO_2^- from nitrification and denitrification is very unlikely since in most cases rates of NO_2^- consumption exceed the diffusion rates. Under such conditions, the results from this study suggest that nitrification contributed directly to the N_2O production.

Gross nitrification in the grass-clover pastures is in the upper range of values reported for unmanaged grassland in the North-eastern US (Corre et al. 2002). In a Northern Irish grassland with different N-inputs, Watson and Mills (1998) found gross nitrification to vary between 2.7 and 7.8 kg N ha^{-1} d^{-1}, which compares well with the gross nitrification in this study, where the rates can be extrapolated to between 0 and 16.5 kg N ha^{-1} d^{-1} assuming an active depth of 16 cm. The reason for lack of nitrification activity in the 1- and 2-year-old grass-clover in June is not obvious, and is in contrast to the high activity in the 8-year-old pasture. Corre et al. (2002) found that gross nitrification was depressed when soil moisture increased during summer months, but this seems not to be the case (Table 1).

A positive relationship was observed between N_2O emission and gross N mineralization. However, it has never been reported that formation of N_2O occurs during mineralization of soil organic N. This positive relationship may be due to the fact that the gross N mineralization in these grass-clover soils was an important driving parameter for the formation and accumulation of NH_4^+, which proved to be related to N_2O emissions.

It was observed that between 0.004 and 0.29% of the N turned over via gross nitrification was released as N_2O, which compares well with the results from Low et al. (1997) who found that 0.002–0.01% of gross nitrification was released as N_2O in a flask incubation study. In contrast, Watson and Mill (1998), also using flask incubations, suggested that $\gg$1% of gross nitrification was released as N_2O. As already discussed the four sampling events did likely not represent boundaries in seasonality, but nevertheless there was a significant relationship between sampling time and the ratio of N_2O release vs. gross nitrification. The N_2O:gross nitrification ratio would be expected to vary under changing environmental conditions, but the control of this ratio is not well understood (Firestone and Davidson 1989). For example, because N_2O apparently results from a reductive process its importance as a product of nitrification should increase as oxygen availability decreases, but at the same time the overall nitrification rate is reduced by limited oxygen availability making it difficult to predict the total N_2O from nitrification (Hutchinson and Davidson 1993). In the grass-clover the ratio of N_2O from nitrification was lowest under the wet soil conditions in May when oxygen-availability supposedly was low.

Conclusion

Nitrous oxide emissions from grass-clover at various ages were found to be relatively small. The low N_2O emission, however, was not due to a general low N-cycling activity as the gross N flow was several orders of magnitude greater than the N_2O release. Nitrification appeared to be the main source of N_2O. The ratio of nitrified N emitted as N_2O fluctuated significantly between different sampling times. Process-oriented model predictions of N_2O emissions thus require knowledge not only of the main sources for N_2O production, but also detailed knowledge on the controls over N_2O formation.

Acknowledgements

The project received financial support from the Danish Research Center for Organic Farming (DARCOF). Merete Brink Jensen provided technical assistance with the laboratory analysis. Drs.

J. Eriksen and F.P. Vinther at the Danish Institute for Agricultural Sciences are acknowledged for allowing access to the experimental site and help maintaining the experimental plots. John Clifton Brown and Peter Curtis are acknowledged for comments on draft versions of the manuscript.

References

Clayton H., McTaggart I.P., Parker J., Swan L. and Smith K.A. 1997. Nitrous oxide emissions from fertilised grassland: a 2-year study of the effects of N fertiliser form and environmental conditions. Biol. Fert. Soils. 25: 252–260.

Corre M.D., Schnabel R.R. and Stout W.L. 2002. Spatial and seasonal variation in gross nitrogen transformations and microbial biomass in a Northeastern US grassland. Soil Biol. Biochem. 30: 445–457.

Dalal R.C., Wang W.J., Robertson G.P. and Parton W.J. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Aust. J. Soil Res. 41: 165–195.

Eriksen J. 2001. Nitrate leaching and growth of cereal crops following cultivation of contrasting temporary grasslands. J. Agr. Sci. 136: 271–281.

Eriksen J., Vinther F.P. and Søegaard K. 2004. Nitrate leaching and N_2 fixation in grasslands of different composition, age and management. J. Agr. Sci. 142: 141–151.

Firestone M.K. and Davidson E.A. 1989. Microbiological basis of NO and N_2O production and consumption in soil. In: Andreae M.O. and Schimel D.S. (eds), Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere. John Wiley and Sons Ltd, pp. 7–21.

Flessa H., Ruser R., Dörsch P., Kamp T., Jimenez M.A., Munch J.C. and Beese F. 2002. Integrated evaluation of greenhouse gas emissions (CO_2, CH_4, N_2O) from two farming systems in southern Germany. Agr. Ecosyst. Environ. 91: 175–189.

Frolking S.E., Mosier A.R., Ojima D.S., Li C., Parton W.J., Potter C.S., Priesack E., Stenger R., Haberbosch C., Dörsch P., Flessa H. and Smith K.A. 1998. Comparison of N_2O emissions from soils at three temperate agricultural sites: simulations of year-round measurements by four models. Nutr. Cycl. Agroecosyst. 52: 77–105.

Hutchinson G.L. and Davidson E.A. 1993. Processes for production and consumption of gaseous nitrogen oxides in soil. In: Harper L.A., Mosier A.R., Duxbury J.M. and Rolston D.E. (eds), Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. ASA Special publication number 55, Madison, WI, USA, pp. 79–93.

Kaiser E.A., Kohrs K., Kücke M., Schnug E., Munch J.C. and Heinemeyer O. 1998. Nitrous oxide release from arable soil: importance of perennial forage crops. Biol. Fertil. Soils 28: 36–43.

Keller M., Veldkamp E., Weitz A. and Reiners W. 1993. Effect of pasture age on soil trace-gas emissions from a deforested area of Costa-Rica. Nature 365: 244–246.

Kirkham D. and Bartholomew W.V. 1954. Equations for following nutrient transformations in soil utilizing tracer data. Soil Sci. Soc. Am. Proc. 18: 33–34.

Kuenen J.G. and Robertson L.A. 1994. Combined nitrification–denitrification processes. FEMS Microbiol. Rev. 15: 109–117.

Low A.P., Stark J.M. and Dudley L.M. 1997. Effects of soil osmotic potential on nitrification, ammonification, N-assimilation, and nitrous oxide production. Soil Sci. 162: 16–27.

Linn D.M. and Doran J.W. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48: 1267–1272.

Mosier A.R., Duxbury J.M., Freney J.R., Heinemeyer O. and Minami K. 1996. Nitrous oxide emissions from agricultural fields: assessment, measurement and mitigation. Plant Soil 181: 95–108.

Penman J., Kruger D., Galbally I., Hiraishi T., Nyenzi B., Emmanul S., Buendia L., Hoppaus R., Martinsen T., Meijer J., Miwa K. and Tanabe K. (eds) 2000. Good practice guidance and uncertainty management in national greenhouse gas inventories. IPCC National Greenhouse Gas Inventories Programme.

Poggemann S., Weissbach F. and Küntzel U. 1999. Reduction of N surpluses and release of N_2O from pasture. Ber Landwirtsch 77: 21–34.

Russow R., Sich I. and Neue H.-U. 2000. The formation of the trace gases NO and N_2O in soils by the coupled processes of nitrification and denitrification: results of kinetic ^{15}N tracer investigations. Chemos. Global Change Sec. 2: 359–366.

Skiba U., Fowler D. and Smith K.A. 1997. Nitric oxide emissions from agricultural soils in temperate and tropical climates: sources, controls and mitigation options. Nutr. Cycl. Agroecosyst. 48: 139–153.

Stevens R.J., Laughlin R.J., Burns L.C., Arah J.R.M. and Hood R.C. 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biol. Biochem. 29: 139–151.

Sørensen P. and Jensen E.S. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoproethylene trap for ^{15}N determination. Anal. Chim. Acta 252: 201–203.

Veldkamp E., Davidson E., Erickson H., Keller M. and Weitz A. 1999. Soil nitrogen cycling and nitrogen oxide emissions along a pasture chronosequence in the humid tropics of Costa Rica. Soil Biol. Biochem. 31: 387–394.

Velthof G.L., Koops J.G., Duyzer J.H. and Oenema O. 1996. Prediction of nitrous oxide fluxes from managed grassland on peat soil using a simple empirical model. Neth. J. Agr. Sci. 44: 339–356.

Velthof G.L., van Beusichem M.L. and Oenema O. 1998. Mitigation of nitrous oxide emission from dairy farming systems. Environ. Pollut. 102: 173–178.

Watson C.J. and Mills C.L. 1998. Gross nitrogen transformations in grassland soils as affected by previous management intensity. Soil Biol. Biochem. 30: 743–753.

Waage N., Velthof G.L., van Beusichem M.L. and Oenema O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33: 1723–1732.