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Field method to determine N_2O emission from nitrification and denitrification

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Abstract Nitrous oxide (N_2O) emissions via the nitrification (I_{nit}) and denitrification (I_{den}) pathways were successfully measured with in-field incubation of soil cores in preserving jars at 0 Pa and 5–10 Pa acetylene. From the incubations, fractions of nitrification – N_2O over total N_2O (I_{nit}/I_{tot}) – and denitrification – N_2O over total N_2O (I_{den}/I_{tot}) – were obtained. Actual field emissions of N_2O via nitrification (F_{nit}) and denitrification (F_{den}) were calculated by multiplying the fractions from the incubation technique with the daily N_2O emission (F_{day}) determined with a direct soil cover method. The approach presented here was successful for a whole range of soil moisture conditions in intensive grassland. F_{nit} and F_{den} followed the trends of soil ammonium and soil nitrate.

Key words Nitrous oxide · Nitrification · Denitrification · Soil cores · Acetylene

Introduction

Denitrification and nitrification are believed to be the two main nitrous oxide (N_2O) producing processes in soils (Davidson 1991). Therefore, an easy-to-apply field-based method would be very valuable in order to track the relative contributions of the two processes under various soil and environmental conditions.

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Currently, there are two methods under development to distinguish nitrification and denitrification N_2O production in soil. The first method involves a ^{15}N technique (Stevens and Laughlin 1996) and seems to provide good results. However, so far it has been tested only in laboratory incubations and it appears labor intensive, which may preclude routine measurements in the field. Moreover, the addition of ^{15}N fertilizers to the soil may preclude observations in natural ecosystems where changes in the N pool may alter the nitrification and denitrification rates.

The second approach distinguishes the two processes by comparing N_2O emissions from soil previously treated with and without nitrification inhibitors. Research has shown that acetylene (C_2H_2) applied to soil at low concentrations (5–10 Pa C_2H_2) is an effective nitrification inhibitor without affecting the last reduction step in the denitrification sequence (Klemedtsson et al. 1998). In comparison to other nitrification inhibitors such as nitrapyrin, C_2H_2 appears to have the least impact on soil conditions and other microbiological processes and can be readily applied in short-term incubations (5–10 h) (Klemedtsson et al. 1988).

The C_2H_2 method to distinguish N_2O from nitrification and denitrification is based on the inhibition of nitrification in the presence 5–10 Pa C_2H_2. The resulting flux from this treatment is therefore due only to denitrification. The contribution due to nitrification is then estimated by the difference between emissions from soils with and without the C_2H_2 block.

In this paper, an approach is presented where C_2H_2 incubations are performed in the field alongside a range of other accompanying measurements. This has the advantage that incubations can be directly related to the concomitant observations. The main advantage of the field incubation is that possible changes in the fractions of nitrification and denitrification N_2O due to soil temperature changes can be precluded. The relative contributions of nitrification N_2O and denitrification N_2O from the incubations are converted to actual emissions of nitrification N_2O (F_{nit}) and denitrification

N_2O (F_{den}) by multiplying them with the integrated N_2O emission measurement (F_{day}) over the incubation period using a direct soil cover method. These measurements were performed on a gas sampling plot adjacent to the plots from where the soil cores originated.

Nomenclature

A_{arr} = Arrhenius constant (g N_2O-N ha^{-1} h^{-1}) (direct cover method 2000 to 0800 hours estimate)
E = Apparent activation energy, Arrhenius equation (J mol^{-1})
F_{day} = Integrated daily N_2O flux (g N_2O-N ha^{-1} day^{-1})
F_{arr} = N_2O emission calculated with an Arrhenius relationship (g N_2O-N ha^{-1} h^{-1})
F_{meas} = N_2O emission measured between 800 and 2000 hours (g N_2O-N ha^{-1} day^{-1}) (soil cover method)
F_{nit} = N_2O emission due to nitrification (g N_2O-N ha^{-1} day^{-1}) (soil cover method)
F_{den} = N_2O emission due to denitrification (g N_2O-N ha^{-1} day^{-1}) (soil cover method)
I_{tot} = Total N_2O emission (g N_2O-N ha^{-1} day^{-1}) (jar incubation)
I_{den} = N_2O emission due to denitrification (g N_2O-N ha^{-1} day^{-1}) (jar incubation)
I_{nit} = N_2O emission due to nitrification (g N_2O-N ha^{-1} day^{-1}) (jar incubation)
I_{0Pa} = Mean N_2O emission from incubations with 0 Pa C_2H_2 (control) (g N_2O-N ha^{-1} day^{-1})
I_{5Pa} = Mean N_2O emission from incubations with 5 Pa C_2H_2 (g N_2O-N ha^{-1} day^{-1})
N_{NO3} = Soil nitrate concentration (kg N ha^{-1})
N_{NH4} = Soil ammonium concentration (kg N ha^{-1})
R = Gas constant (8.314) (J mol^{-1} K^{-1})
T_t = average soil temperature, 0–5 cm (K)

Materials and methods

Experimental design

Investigations were carried out during April 1994, in a field trial near Lincoln University, New Zealand. The site was sown in ryegrass (*Lolium perenne*) and white clover (*Tifolium repens*) on Templeton silt loam (Udic Ustochrept; USDA Soil Taxonomy). Texture, bulk density and soil moisture characteristics from the top 5 cm of the soil are presented in Table 1.

The experimental plots were arranged in a randomized block design with three replications. Separate adjacent areas were designated for gas sampling (each plot: width 40 cm, length 40 cm) and soil sampling (each plot: width 60 cm, length 60 cm). Three moisture treatments were maintained throughout the experiment: maximum (S_1) (application of 5 mm rain day^{-1}; approximate suction: 70 cm H_2O); intermediate (S_2) (application of 2.5 mm rain day^{-1}; approximate suction: 130 cm H_2O) and minimum (S_3) (no irrigation; approximate suction: >16000 cm H_2O).

Synthetic sheep urine was applied at a rate of 4.073 l m^{-2} to all plots according to the recipe given in Fraser et al. (1994). This resulted in applications of N: 500 kg ha^{-1}, K: 400 kg ha^{-1}, Cl: 100 kg ha^{-1} and S: 15 kg ha^{-1}, respectively.

Table 1 Particle size analysis, soil bulk density and soil moisture characteristic from Templeton site loam under intensive pasture (0–5 cm) (mean with standard error in parentheses)

Particle size analysis		Soil moisture characteristic	
Size fraction (μm)	% by wt. (±SE)	Volumetric water content (cm^3 cm^{-3})	Soil water suction (cm H_2O)
>63	19.0 (1.3)	0.433	10
63–20	39.8 (3.8)	0.425	30
20–2	20.6 (1.3)	0.384	50
<2	19.4 (1.0)	0.373	100
		0.277	1019
		0.240	3058
Dry bulk density (g cm^{-3})	1.10	0.200	15290

The following measurements were carried out on days 1, 5, 12 and 19 after urine application: total N_2O emission with the soil cover technique (F_{day}); N_2O emission from nitrification (I_{nit}) and denitrification (I_{den}) with the field C_2H_2 incubation technique; soil NO_3-N; soil NH_4-N; water soluble C; soil temperature and soil water content. All soil analyses were performed at the 0–5 cm depth.

Gas collection and analysis of N_2O

A modified version of the soil cover technique described by Hutchinson and Mosier (1981) was used to determine N_2O emissions from synthetic urine affected soil. Emission measurements were carried out on each sampling day at 0800, 1200, 1400, 1600 and 2000 hours to give a range of times in order to make a better prediction of the daily N_2O flux. At each sampling time, three samples at 0, 10 and 20 min after coverage were taken from the enclosed head space.

N_2O was analyzed on a gas chromatography system equipped with ECD similar to the one described in Mosier and Mack (1980). The detector, switching valves and column temperatures were 350 °C, 20 °C and 20 °C, respectively. The carrier gas was oxygen-free nitrogen (NZIG) at a flow rate of 45 ml min^{-1}.

N_2O fluxes were calculated using the nonlinear equation given in Hutchinson and Mosier (1981) and expressed in g N_2O-N ha^{-1} day^{-1}. For a cover period of 20 min, the method was capable of resolving N_2O fluxes to a precision of ±0.2 g N_2O-N ha^{-1} day^{-1}. The F_{day} was calculated according to Eq. (1):

$$F_{day} = \sum_{t=800}^{2000\,h} F_{means} + \sum_{t=2000}^{0800\,h} F_{arr} \quad (1)$$

with,

$F_{arr} = A_{arr} \cdot e^{(-E/RT_t)}$ (Conrad et al. 1983; Müller 1996)

The Arrhenius parameters A_{arr} and E were determined from the five measurements throughout the day correlated with the average soil temperature from the top 5 cm (T_t).

Field C_2H_2 incubation

Incubations of soil cores under the two C_2H_2 concentrations (0 Pa and 5–10 Pa) were performed in glass jars (Agee; volume approx. 1100 ml) inserted into the ground and covered with a thin wooden plate to track field temperature conditions as closely as possible [modified according to IAEA (1992)] (Fig. 1). The maximum soil temperature difference on a sunny day between soil cores in the

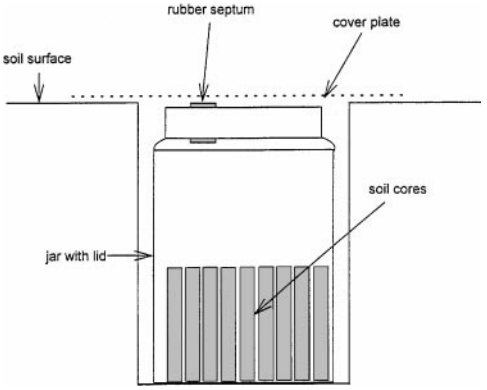

Fig. 1 Positioning of jar during the field incubation

jar and soil outside was 2.5 °C. Each jar contained nine soil cores (soil core diameter = 2.5 cm, depth = 5 cm) taken 1 day prior to the measurements from the soil sampling plots. The headspace volume of each jar was determined according to the total jar volume minus the volume of the soil cores (soil atmosphere volume in the cores was added to the headspace). The 5–10 Pa C_2H_2 concentration in the jar atmosphere was adjusted by exchanging, for each jar, an exact calculated headspace volume (approximately 5 ml) with a 834 Pa C_2H_2 standard (freshly prepared). The C_2H_2 was previously purified by passing it through three gas wash bottles filled with distilled water (Gross and Bremner 1992). Incubations were carried out for 5 h (1130 to 1630 hours); the optimum incubation time was determined in a preliminary experiment (data not presented). After the incubation, gas samples were taken with syringes and analyzed on the gas chromatograph system as described above.

I_{nit} and I_{den} were expressed as fractions of total N_2O (I_{tot}) (Eqs. 2, 3), using geometrically calculated mean emissions of 0 Pa C_2H_2 (I_{0Pa}) and 5 Pa C_2H_2 (I_{5Pa}) from jar incubations.

$$I_{den}/I_{tot} = \frac{I_{5Pa}}{I_{0Pa}} \quad (2)$$

$$I_{nit}/I_{tot} = \frac{I_{0Pa} - I_{5Pa}}{I_{0Pa}} \quad (3)$$

F_{nit} and F_{den} were calculated by multiplying the two fractions (I_{den}/I_{tot}, I_{nit}/I_{tot}) by F_{day} (Eq. 1) and making the assumption that the fractions determined from the jar incubation were equal to the average daily fractions in the plots where N_2O was measured with the soil cover technique, i.e. Eq. 4.

$$I_{den}/I_{tot} = F_{den}/F_{day},\ I_{nit}/I_{tot} = F_{nit}/F_{day} \quad (4)$$

Soil NH_4, soil NO_3

Soil NH_4 and NO_3 were extracted immediately after sampling in 2 M KCl (10 g moist soil in 50 ml KCl) (Maynard and Kalra 1993) and analyzed colorimetrically by flow-injection analysis (Tector Flow Injection Analyzer). Results were adjusted to the water content and the bulk density of the soil (Table 1).

Soil and air temperature

Soil and air temperature were measured with thermistors (Campbell Scientific) and logged half hourly with a datalogger (21X, Campbell Scientific).

Statistical analysis

The analysis of variance and the test for differences between means with Duncan's multiple range test was done in Quattro Pro version 4 (Borland) and Minitab (Minitab Inc. Release 8.2, 1991). For gas fluxes, the analysis was done on lognormal transformed values (geometric mean). All other values were assumed to be normally distributed (arithmetic mean).

Results and discussion

N_2O emissions from incubation at the two C_2H_2 concentrations followed, for all treatments, the trend $I_{5Pa} < I_{0Pa}$, allowing the separation into F_{nit} and F_{den} (Fig. 2). This provides a clear indication that nitrification-induced N_2O emissions were inhibited with 5–10 Pa C_2H_2 without blocking the N_2O reductase in the denitrification pathway (Klemedtsson et al. 1988). Simultaneous N_2O emissions via nitrification and denitrification are expected under conditions where both processes are active, such as in a urine patch (Monaghan and Barraclough 1993; Stevens and Laughlin 1996).

Not all results between the two C_2H_2 concentrations were statistically different on all sampling occasions (data not presented), which can be explained by the high spatial variability among cores distributed in the different jars. However, the definite trend in N_2O emission described above was considered to be a more important indicator of the potential application of this

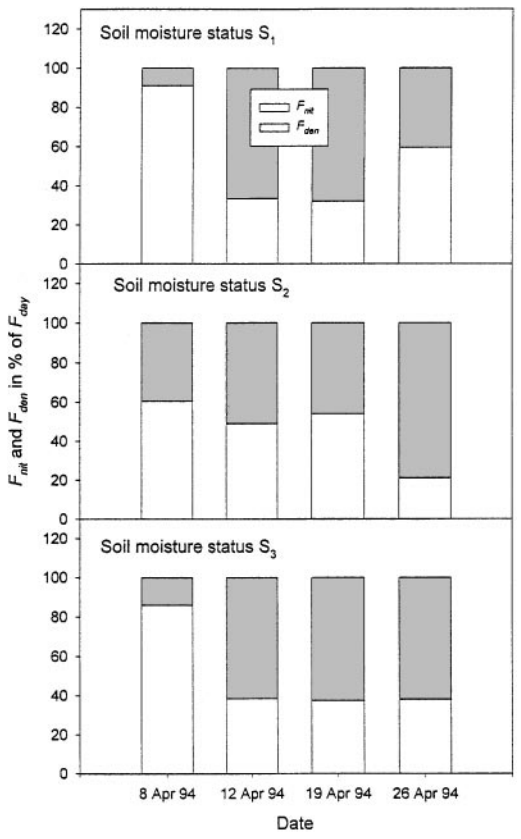

Fig. 2 Results of the C_2H_2 method for N_2O via nitrification (F_{nit}) and N_2O via denitrification (F_{den}) in % of total N_2O (F_{day}) from urine-affected intensive grassland soil at different soil moisture contents (S_1 to S_3)

method than the actual statistical differences as influenced by differing C_2H_2 concentrations.

Fractions F_{tot}/F_{day} and F_{den}/F_{day} generally followed the trends of soil NH_4: $N_{NH4}/N_{NH4}+N_{NO3}$ and soil NO_3: $N_{NO3}/N_{NH4}+N_{NO3}$, respectively (Fig. 3). This observation is expected since NH_4^+ and NO_3^- are the key substrates for nitrification and denitrification, respectively (Granli and Bøckman 1994). The rate of nitrification N_2O seems to be related to the soil water status (Fig. 3). While in the moist treatments (S_1 and S_2) NH_4 is present in the water and most likely available at the sites where nitrification occurs, there appears to be a much stronger limitation for the actual NH_4 present at the reaction sites in the dry treatment. This behavior is indicated by the much steeper slope of F_{nit} versus N_{NH4} for the dry treatment compared to the wetter treatments. Additional evidence for this observation was provided by the much lower nitrification rate of the dry treatment as indicated by a lower build-up of soil NO_3, compared to the nitrification rates in the other two treatments.

F_{den} is also positively related to the soil NO_3 fractions (Fig. 3). This behavior is not surprising since water-soluble C was at no time during the experiment stoichometrically limiting denitrification (data not presented) (Burford and Bremner 1975).

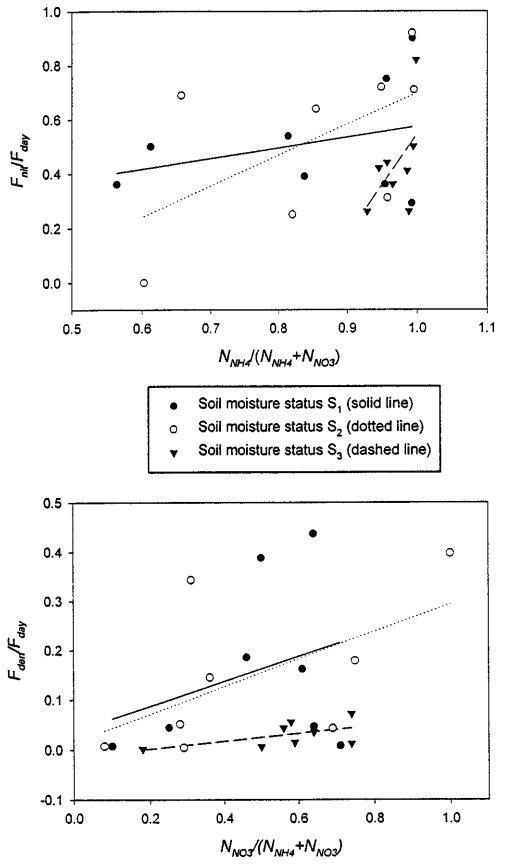

Fig. 3 Relationships between fractions of F_{nit}/F_{day} and $N_{NH4}/(N_{NH4}+N_{NO3})$ and F_{den}/F_{day} vs $N_{NO3}/(N_{NO3}+N_{NH4})$ for intensive grassland at three different soil moisture conditions (S_1 to S_3)

The in-field incubation method provides a way of distinguishing N_2O emitted by nitrification and denitrification and is in agreement with the laboratory-based observations of Klemedtsson et al. (1988). The N_2O emission via nitrification and denitrification, as calculated here, is one of the first attempts to quantify the mechanisms for N_2O emissions from urine-affected intensive grassland. Therefore, it is difficult to comment on the validity of the relationships found, since no independent figures are available. Other researchers have reported that N_2O emission via nitrification occurs but they were unable to provide accurate figures (Colbourn 1992; Monaghan and Barraclough 1993; de Klein and van Logtestijn 1994).

Many studies have used C_2H_2 inhibition to quantify denitrification losses from soil under field conditions by using a 0.1–10 kPa C_2H_2 concentration to block the reduction step from N_2O to N_2 (IAEA 1992). However, if nitrification is an important mechanism for N_2O production, this approach may seriously underestimate the total N_2O+N_2 production from soil since the N_2O from nitrification cannot be accounted for due to the blockage of nitrification under high C_2H_2 concentrations. Large differences in total N_2O+N_2 from this error are reported by de Klein and van Logtestijn (1994) who found, depending on whether nitrification or denitrification is the source of N_2O production, that the total N_2O+N_2 could change by a factor of as much as 2.

It is difficult to comment on the validity of the approach described in this paper because it seems that no in-field incubations of this kind have been carried out previously. Other methods to distinguish N_2O emissions from the two pathways include mathematical modeling such as the models developed by Mosier and colleagues. These models combine climate data with mineral N data to simulate total N_2O production by nitrification and denitrification (Mosier et al. 1983; Mosier and Parton 1985; Parton et. al. 1988; Parton et al. 1996). However, their approach did not use actual measurements of N_2O via nitrification and denitrification but correlations of total N_2O with soil NH_4 and soil NO_3 to develop emission relationships. Validations of their approach as well as improvement of the accuracy of their relationships would certainly be achieved by actually measuring the F_{nit} and F_{den} fractions.

An extension of the method described here for field N_2 emissions and an application of the relationships obtained with soil and environmental factors to a data set where F_{nit} and F_{den} have not been measured are presented in Müller (1996).

Clearly, more work has to be done to validate this in-field approach to distinguish nitrification and denitrification N_2O. The method presented here is being further developed and applied in a long-term field experiment in Giessen, Germany, on an old grassland site under a wide range of soil and environmental conditions.

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