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Short communication

Biogenic gas emissions from soils measured using a new automated laboratory incubation system

L.M. Cárdenas*, J.M.B. Hawkins, D. Chadwick, D. Scholefield

Institute of Grassland and Environmental Research, North Wyke, Okehampton EX20 2SB, UK

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Laboratory studies of nitrous oxide (N_2O) and nitrogen (N_2) emissions due to denitrification in soils normally require the use of enzymatic inhibitors such as acetylene (C_2H_2) (Parkin et al., 1984) and oxygen (O_2) (Fazzolari et al., 1998). These may disturb other processes and C_2H_2 can serve as an additional source of carbon (Swerts et al., 1996a). Data interpretation is difficult as diffusion of the C_2H_2 gas throughout the soil core cannot be guaranteed (Smith and Arah, 1992). Studies involving direct measurement of both N_2O and N_2 have been limited by low N_2 sensitivities (Scholefield et al., 1997a). Here we describe a new laboratory system based on the replacement of the soil atmosphere by an inert gas allowing the measurement of the evolution of both N_2O and N_2 (Stefanson and Greenland, 1970). In an early study, Scholefield et al. (1997a) modified the technique by using intact cores and added an irrigation assembly for fertiliser application.

Soil was collected by a cylindrical corer (143 mm diameter, 120 mm height) pushed into the ground to a depth of 100 mm. The corer and soil were then placed inside a cylindrical incubation vessel to an exact fit once the base of each core had been pared level with the corer edge and vegetation trimmed off. A mixture of Helium (He) + O_2 was passed through the soil core (via the bottom of the vessel) in order to purge (flow-through mode) the soil atmosphere, headspace and all gas lines of N_2. Flow rates of He and O_2 (>100 ml min^{-1}) were regulated using mass flow controllers to provide an O_2 concentration of c. 20% (Scholefield et al., 1997a). The He + O_2 mixture was then directed to the vessel via the lid (flow-over mode) after reducing the flow rate to approximately 30 ml min^{-1} and N_2 measured until N_2 levels reached baseline. We observed that after the removal of 99.996% of the soil and vessel atmosphere, the variability of each blank varied between 30 and 38% for a flow rate of 30 ml min^{-1}.

Effluent gases from each of the 12 vessels passed through an outlet in the lid of the incubation vessel to an actuated 16-port selection valve for either analysis or venting to the atmosphere. After replacement of the atmosphere within the soil cores, amendments were added via a secondary vessel fitted to the centre of each lid after being flushed with He (to avoid any atmospheric N_2 contamination). The amendment vessel was cylindrical (147 ml volume) with a funnel shaped bottom.

The effluent gas sample was split to analyse N_2O and N_2. Nitrous oxide was analysed by Electron Capture Detection (ECD) and separation achieved by a stainless steel packed column (2 m long, 4 mm bore) filled with 'Porapak Q' (80–100 mesh) and using N_2 as the carrier gas. The detection limit for N_2O was equivalent to 2.3 g N ha^{-1} d^{-1}. Linearity of the detector was observed between 1 and 3000 ppmv. Nitrogen was quantified by He Ionisation Detection (HID) and separation achieved by a PLOT column (30 m long 0.53 mm i.d.), with He as the carrier gas. The detection limit was 9.6 g N ha^{-1} d^{-1} and the detector was linear from 1 to 1000 ppmv N_2. The new denitrification system has a series of improvements compared to the system developed by Scholefield et al. (1997a) with which the main limitation was the sensitivity to atmospheric N_2. The new system shows a detection limit five times better than its precursor (Scholefield et al., 1997a) achieved not only by means of a better analytical system, but also by using better quality materials and seals.

Incubations were carried out in a 1.3 m^3 temperature controlled cabinet containing the incubation chambers,

* Corresponding author. Tel.: +44-1837-883-500; fax: +44-1837-82139.
E-mail address: laura.cardenas@bbsrc.ac.uk (L.M. Cárdenas).

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amendment vessels and gas lines capable of operating between 3 and 30 °C (±1 °C). Headspace temperatures inside the vessels were logged hourly.

Soil was sampled from an experimental site at the Institute of Grassland and Environmental Research, North Wyke Research Station in Devon, UK (January–June, 2001). The soil is a clayey pelostagnogley of the Hallsworth series (Claysden and Hollis, 1984), a FAO dystric gleysol (FAO, 1990). The top 10 cm is characterised by 36.6% clay, 47.7% silt and 13.9% fine sand and 1.8% coarse sand in the inorganic fraction. Organic carbon (C) was 5.3% and pH 5.7 (Harrod, 1981; Armstrong and Garwood, 1991; Scholefield et al., 1997a).

The effect of the application of N and C on N_2O and N_2 fluxes was evaluated and the results compared to those of Scholefield et al. (1997a,b) from similar experiments. The soil cores were watered twice with 100 ml and left to drain for 2 h before being placed in the incubation vessels to insure they were at maximum soil water holding capacity (~90% water filled pore space, WFPS). Flushing with $He + O_2$ was carried out at about 200 ml min^{-1} in flow-through mode for about 16 h and then the flow rate was reduced to 38 ml min^{-1} with 16% O_2 in flow-over mode until background levels of N_2 were achieved. Nitrate was applied as KNO_3 at a rate of 50 kg N ha^{-1} and C as glucose at 363 kg C ha^{-1}. The samples were kept at 15 °C throughout the experiment. Emissions of N_2 and N_2O were measured simultaneously. The treatments applied were 70 ml H_2O (vessels 1–12), 49.7 kg N ha^{-1} (KNO_3) (vessels 5–12) and 363 kg C ha^{-1} (glucose) (vessels 9–12).

Extra soil cores were analysed (square cores of 5 cm × 5 cm and 10 cm depth) for mineral N as nitrate (NO_3^-) and ammonium (NH_4^+) before incubation. After incubation, subsamples were extracted from the cylindrical cores for similar analysis. The analysis was carried out after extraction with 2 M KCl by means of a segmented flow analyser using a colorimetric technique (Kamphake et al., 1967; Searle, 1984). Total C and N in the soil were analysed using a TCD Carlo Erba instrument. Soil moisture was determined by gravimetric analysis after drying at 100 °C before and after incubation.

Results showed that no N_2O or N_2 was emitted in the water only treatment. This could be the result of denitrification occurring during the pre-incubation and flushing of the soil cores, causing exhaustion of the original NO_3^- content in the soil. Nitrous oxide was observed in the NO_3^- only treatment with an average maximum for four vessels of 2.7 ± 1.23 kg N ha^{-1} d^{-1}, 26 h after amendment application (Fig. 1). Nitrous oxide emissions decreased slowly for the remainder of the experiment. The cumulative emission (calculated from the area under the curve) during the 5 days of the experiment was 8.25 kg N ha^{-1} (16.6% of the added N) and the coefficient of variation was 40%. No N_2 evolution was observed in the NO_3^- only treatment unlike the results of Scholefield et al. (1997b).

The N_2O fluxes measured from the NO_3^- + glucose treatment (Fig. 1) gave a maximum rate for three vessels of 9.8 ± 2.43 kg N ha^{-1} d^{-1}, 33.2 h after the application of the amendment. Calculation of the area under the curve gave a total of 25.0 kg N ha^{-1} during the 5 days of the experiment (50.4% of the total N added) with a coefficient of variation of 37%.

Statistical analysis of the fluxes illustrated in Fig. 1 is not simple, as different treatments produce varied temporal responses. We therefore compared the rates of increase and decrease of the fluxes. A 2-pool model was applied to fit a mathematical function to the experimental data (Dhanoa et al., 1985; Gill et al., 1985). The model calculates the rate of increase, k_2, and rate of decrease, k_1, of the fluxes after the application of the amendment, the lag time TT, which is the time it takes for the system to respond to the amendment application, and the mean retention time, MRT, which is the mean of the total time it takes for the process to occur. The inputs required by the model are the date and fluxes in kg N ha^{-1} d^{-1}. The model reproduced the data for the NO_3^-

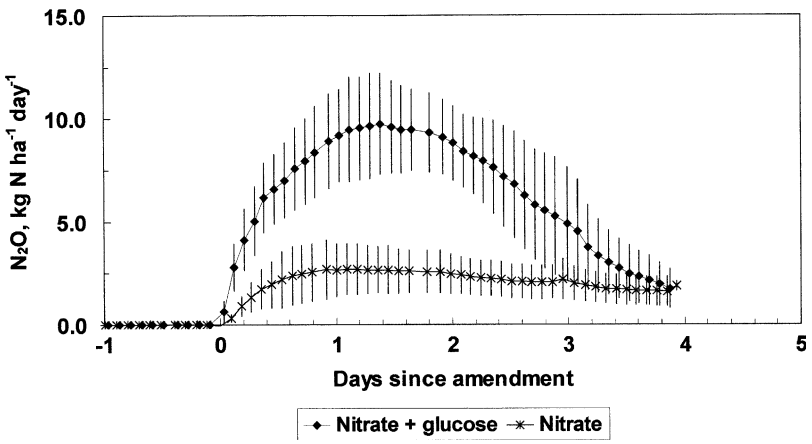

Fig. 1. N_2O fluxes from the NO_3^- only and NO_3^- + glucose treatments (each data point is an average of four vessels for NO_3^- only and three vessels for NO_3^- and glucose treatments, the vertical bars correspond to the standard deviation of these averages). Day 0 corresponds to the date and time of the application of the amendment.

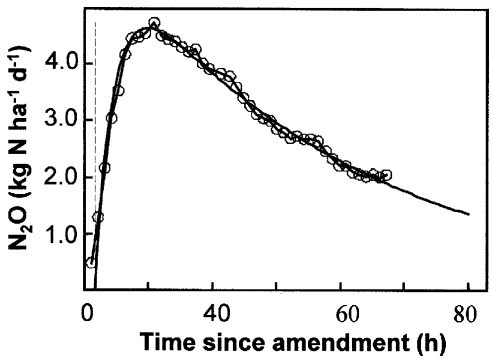

Fig. 2. Application of a 2-pool model to the N_2O fluxes from one vessel. Day 0 corresponds to the date and time of the application of the amendment. The circles correspond to experimental data, the line is the modelled results. The corresponding equation for the model is: $Y = Be^{-k_1(X-TT)} + Ce^{-k_2(X-TT)}$, where rate constants $k_1 = -0.013/h$, $k_2 = -0.1683/h$ and lag TT = 3.2*h*. *B* and *C* are scaling constants.

Table 1
Parameters obtained from the 2-pool model.

Vessel	Treatment	k_1	k_2	TT	MRT
5	NO_3^-	−0.0077	−0.0913	5.5	146.4
6	NO_3^-	−0.0130	−0.1683	3.2	86.1
7	NO_3^-	−0.0167	−0.0984	3.0	73.1
8	NO_3^-	−0.0021	−0.0085	−278	310.0
9	NO_3^- + glucose	−0.0331	−0.0672	4.0	49.0
10	NO_3^- + glucose	−0.0512	−0.0665	12.3	46.8
11	NO_3^- + glucose	−0.0495	−0.0760	1.7	35.1

treatments better than for NO_3^- + glucose treatments. Fig. 2 shows an example of the application of the model to the experimental data of the average N_2O fluxes for vessel six (NO_3^- only).

The results were analysed using the statistical package Genstat fifth edition (Table 1). *P* values calculated for k_2 and TT were 0.117 and 0.555, respectively, higher than 0.05 showing that these variables were the same for both treatments (NO_3^- and NO_3^- + glucose). *P* values for k_1 and MRT were 0.007 and 0.048, respectively, significantly different for the two treatments. The similar rates of increase and lag time for the NO_3^- only and NO_3^- + glucose treatments, seem to suggest that the initiation of the processes responsible for the emissions from the soil after fertilisation, depends on the N added but not the C, that is the original C content of the soil is sufficient to initialise the process. However, the rate of decrease in emissions and, in consequence, the mean retention time, are different for both treatments suggesting that the effect of C is important after the process is initialised and throughout the whole period until the effect of the application expires. The k_1 value was larger for the NO_3^- + glucose treatment than the NO_3^- only, in agreement with a shorter MRT for the former.

The results of the N_2 fluxes observed are shown in Fig. 3. Only the NO_3^- + glucose treatment showed emission of N_2. The corresponding blanks were subtracted from each vessel in order to calculate the net flux of N_2. The average maximum flux observed was 2.6 ± 0.69 kg N ha^{-1} d^{-1}, 71 h after amendment application and coefficient of variation was 45%. The fluxes of N_2O for the NO_3^- + glucose treatment are also shown in Fig. 3. The total N_2 produced during the period of measurements was 4.97 kg N ha^{-1} or 9.95% of the total N added. The lag period between the appearance of N_2O and N_2 and especially the maxima, may be due to the removal of NO_3^- from the soil. Nitrate has preferential acceptance for electrons compared with N_2O, allowing N_2O to accumulate and be further reduced to N_2 once NO_3^- is depleted (Swerts et al., 1996b). Although we did not measure NO_3^- content in the soil throughout the experiment, the observed trend for both gases suggests that this was possible. Another possible explanation is the sequential synthesis of denitrification enzymes (Firestone et al., 1980).

The timing of the N_2O maxima for both, NO_3^- and NO_3^- + glucose treatments (26 and 33.2 h, respectively), were in agreement with Scholefield et al. (1997a) (30 h).

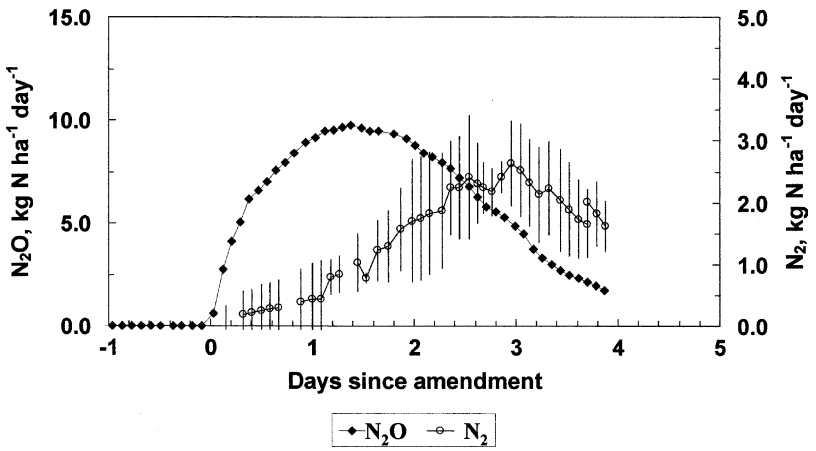

Fig. 3. Fluxes of N_2O and N_2 from the NO_3^- + glucose treatment (each data point is an average of three vessels, the N_2O data are the same as Fig. 1). Day 0 corresponds to the date and time of the application of the amendment.

However, the N_2 peak in Scholefield et al. (1997a) appeared 1 d after the N_2O peak, earlier than our results at 71 h after applying the amendment.

The total N evolved during the 5 days of the experiment in the NO_3^- + glucose treatment as $N_2O + N_2$ (30 kg N ha^{-1}) accounted for 60% of the N applied, higher than the 50% that Scholefield et al. (1997a) could account for. We found that soil samples contained NH_4^+-N and NO_3^--N after the experiment: 2.67 and 12.24 kg N ha^{-1} (respectively) for NO_3^- only, and 3.49 and 3.94 kg N ha^{-1} (respectively) for NO_3^- + glucose. Taking into account all the measured N species we can account for a total of 23.2 kg N ha^{-1} in the NO_3^- only treatment (46% of the N applied) and 37.4 kg N ha^{-1} (75% of the N applied) in the NO_3^- + glucose treatment. These total values are low compared with the results of Scholefield et al. (1997a). This was mainly due to the fact that we did not find a large amount of NH_4^+–N in the soil after the experiment in the NO_3^- + glucose treatment whereas Scholefield et al. (1997a) found 37.4% of the N applied was in the form of NH_4^+.

The results of the soil analysis could have also been affected by the method of collection of the subcore from the sample after the experiment, since it was taken from the middle and might not have represented the whole core. Total C and N did not show a great difference with the original C and N content of the soil. Other forms of N not accounted for, such as biomass assimilation and N_2 fixation, could have affected our balance.

Another factor that could explain some of the differences between these results and those of Scholefield et al. (1997a) is the shape and size of the core. The collection of 25 small soil cores to fit a large chamber in Scholefield et al. (1997a) was replaced by one larger circular core in the present work. It is possible that the ratio of flow rate:surface area of the core affects the proportion of N_2O to N_2. In the previous system a flow rate of 20 ml min^{-1} was used for a surface area of 506 cm^2. The new system has a flow rate of 38 ml min^{-1} for a surface area of 154 cm^2. This results in a 5.5 times faster flow rate relative to surface area in the new system, which could help in the transport of the gases emitted in the soil to the headspace. Further studies will help in the understanding of the effect of some of these parameters (such as flow rate) on the emissions.

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