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COMBINING THE ^{15}N AND ACETYLENE INHIBITION TECHNIQUES TO EXAMINE THE EFFECT OF ACETYLENE ON DENITRIFICATION

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Summary—Using acetylene to inhibit the reduction of N_2O to N_2 is the simplest and most commonly used technique to quantify denitrification. The acetylene treatment may not always inhibit N_2O-reductase completely when soils are wet, low in NO_3^- or with a high C-to-NO_3^- ratio. Acetylene blocks nitrification as well as N_2O-reductase, so if nitrification is the source of NO_2^- or NO_3^- for denitrification, then the denitrification rate would be underestimated by the C_2H_2 inhibition technique. Acetylene can also be metabolised in soil and so enhance denitrification. We show how the ^{15}N method for measuring denitrification can be combined with the C_2H_2 inhibition technique to check the effect of acetylene on N_2O-reductase, respiration and nitrification. No method exists, however, for the analysis of ^{15}N in the N_2 and N_2O of headspace samples which also contain 10% v/v C_2H_2. We describe a technique to remove C_2H_2 from headspace samples with alkaline $KMnO_4$ prior to analysis by isotope-ratio mass spectrometry. The C_2H_2 removal procedure had no effect on the determination of N_2O concentration, or on the determination of the ^{15}N content of N_2O and N_2. Then using data for fluxes of N_2O, N_2, (N_2O + N_2), CO_2 and the size and ^{15}N content of the soil NO_2^- pool for a soil incubation experiment, we show how the effects of C_2H_2 can be quantitatively assessed. The data also allowed the amount of denitrification, as estimated by the C_2H_2 inhibition technique, to be compared with that measured using the ^{15}N technique. For the soil conditions during this incubation, 10% v/v C_2H_2 completely blocked N_2O-reductase, slightly inhibited respiration and completely blocked nitrification. The average total flux of N gases as measured by ^{15}N (4.3 nmol N g^{-1} h^{-1}) was not significantly different ($P > 0.05$) from the average flux measured by the C_2H_2 inhibition method (3.9 nmol N g^{-1} h^{-1}). Including C_2H_2 as a treatment in the ^{15}N method provides a direct way of confirming that some of the assumptions in the C_2H_2 inhibition technique are valid. © 1997 Elsevier Science Ltd

INTRODUCTION

Denitrification can be quantified directly by the C_2H_2 inhibition technique or by using ^{15}N (Mosier and Klemedtsson, 1994). The C_2H_2 inhibition technique is simpler and more commonly used. Procedures for its use are well established (Tiedje *et al.*, 1989). The technique relies on C_2H_2 selectively inhibiting N_2O reduction to N_2, allowing the total denitrification flux to be measured as N_2O. The proportion of the N-gas flux occurring as N_2O can be measured by excluding C_2H_2 during the assay. There are problems, however, with the C_2H_2 inhibition technique (Knowles, 1990). The assumption that C_2H_2 inhibits N_2O-reductase completely may not always be true. When soils are wet, clayey or compacted, C_2H_2 may not diffuse to all sites of denitrifying microbial activity. The blocking of N_2O-reductase activity by C_2H_2 is incomplete when NO_3^- concentrations are low, especially if there is decomposable organic C readily available and the C-to-NO_3^- ratio is high (Yeomans and Beauchamp, 1978; Simarmata *et al.*, 1993). An C_2H_2 concentration of 5–10% v/v is needed to block N_2O-reductase (Tiedje *et al.*, 1989), but a concentration of 0.01% v/v is sufficient to block nitrification (Berg *et al.*, 1982). If nitrification is the source of NO_2^- or NO_3^- for denitrification, then the denitrification rate could be underestimated by the C_2H_2 inhibition technique. Acetylene can be metabolised in soil and so enhance denitrification. Several laboratory experiments have shown that C_2H_2 increased NO_3^- reduction, and the production of N gases and CO_2 (Haider *et al.*, 1983; Topp and Germon, 1986).

Ideally when using the C_2H_2 inhibition technique with a particular soil under specific conditions, the completeness of the inhibition of N_2O-reductase should be checked and the contribution of nitrification to denitrification measured. Including C_2H_2 as a treatment in the ^{15}N gas-flux method would be one way to check whether the inhibition of N_2O-reductase is complete. The ^{15}N gas-flux method involves the application of $^{15}NO_3^-$ to soil, with the

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subsequent quantification of the flux of N_2 and N_2O into a headspace (Mosier and Klemedtsson, 1994). In the presence of C_2H_2 and $^{15}NO_3^-$, no labelled N_2 should be formed if the inhibition of N_2O-reductase is complete.

In the ^{15}N gas-flux method, the flux of N_2 is quantified by comparing the isotopic composition of the enriched atmosphere with normal atmosphere. The flux of N_2O is quantified by measuring the change in N_2O concentration with time. The determination of ^{15}N in the N_2 and quantification of N_2O are easily achieved by automated continuous-flow isotope-ratio mass spectroscopy (CF-IRMS) as described by Stevens *et al.* (1993). If the headspace samples contain C_2H_2, an C_2H_2-removal step is required to prevent C_2H_2 being introduced into the mass spectrometer source along with N_2. In the comparison of ^{15}N and C_2H_2-inhibition techniques by Mosier *et al.* (1986b), liquid N_2 was used as a trap before determining ^{15}N in N_2 with a manual inlet mass spectrometer (Mosier *et al.*, 1986a). As liquid N_2 also traps N_2O, we developed a technique which selectively removes C_2H_2 from headspace samples prior to automated analysis for ^{15}N in N_2 and N_2O by CF-IRMS analysis. The technique uses alkaline $KMnO_4$ to oxidise C_2H_2 to potassium formate and CO_2 (Lee and Chang, 1979; Haines, 1985). We determined the optimum amounts of $KMnO_4$ needed to oxidise the C_2H_2 and of KOH needed to absorb the CO_2 produced by the oxidation. Then we measured the effect of the C_2H_2-removal procedure on the determination of N_2O concentration, and on the ^{15}N content of N_2O and N_2. By presenting additional data on fluxes of N_2O, N_2, (N_2O + N_2), CO_2 and on the size and ^{15}N content of the soil NO_2^- pool for an experiment on N_2O (Stevens *et al.*, 1997) we show quantitatively under different NO_3^- and C concentrations how the effects of C_2H_2 on N_2O-reductase, respiration and nitrification can be measured. These data also allowed us to compare the rate of denitrification measured by the C_2H_2 inhibition technique with that measured using the ^{15}N gas-flux technique.

METHODS AND MATERIALS

Removing acetylene from gas samples with potassium permanganate

Potassium permanganate can oxidise C_2H_2 to oxalic acid, formic acid and CO_2. Acidic or alkaline $KMnO_4$ provide harsher conditions for the oxidation and result in a more complete oxidation than neutral $KMnO_4$. With acidic or alkaline $KMnO_4$ the main end-products are formic acid and CO_2. Strongly alkaline $KMnO_4$ has the advantage over acidic or neutral $KMnO_4$ because the CO_2 produced by the oxidation is absorbed by the alkali (KOH in this study), and hence is not available to overload the existing CO_2 scrubber on the CF-IRMS system.

Aqueous solutions containing 0, 60, 120, 180, 240, 300 and 360 mM $KMnO_4$ were prepared. A square (40 × 40 mm) of glass fibre paper (GF/D, Whatman Ltd, U.K.) was rolled into a cylinder and placed in each of 21 10-ml vials (Chromacol Ltd, U.K.). The vials were then crimp-capped with septa (20 mm chlorobutyl septa, Chromacol Ltd, U.K.) and evacuated to < 100 Pa. Using a gas-tight syringe, 13.5 ml of 300 μl N_2O l^{-1} in N_2 (Bedfont Scientific Ltd, U.K.) and 1.5 ml of C_2H_2 (to make up 10% v/v C_2H_2) were injected into each vial. One ml of each $KMnO_4$ solution was then injected into each of three gas-filled vials. The solution was absorbed onto the filter paper, which provided a large surface area on which the C_2H_2 could react. The vials were left for 1 h at 20°C before analysis by gas chromatography. A Varian Genesis headspace auto-sampler was used to transfer 0.5 ml aliquots to a Perkin Elmer 8500 GC. The GC was fitted with a 5 m × 2 mm column packed with Porapak QS (80–100 mesh) operated at 40°C and a hot wire detector operated with filament current of 240 mA. The carrier gas was He at a flow rate of 20 ml min^{-1}. A drying column (4 × 100 mm) containing $Mg(ClO_4)_2$ was fitted between the auto-sampler and the GC to remove water vapour. Concentrations of the C_2H_2, CO_2 and N_2O were quantified by comparing the peak heights with those of calibration mixtures.

Aqueous solutions containing 300 mM $KMnO_4$ were prepared in 0, 25, 50, 75, 100 and 125 mM KOH. Vials were filled with 300 μl N_2O l^{-1} + 10% v/v C_2H_2 as before and 1 ml of each $KMnO_4$-KOH solution was injected into each of three gas-filled vials. The vials were left for 1 h at 20°C and the contents analysed by gas chromatography.

Effect of acetylene removal on the determination of nitrous oxide concentration

A square (40 × 40 mm) of glass fibre paper was rolled into a cylinder and placed in each of 54 12-ml cylindrical screw-top vials (15 × 100 mm). The vials were capped with septa and evacuated to < 100 Pa. Gas mixtures containing 10, 100, 300, 500, 750 and 1000 μl N_2O l^{-1} with or without 10% v/v C_2H_2 were made up in gas sampling bags. Samples of gas mixtures were transferred in triplicate to the vials for comparison between three treatments: (i) 12 ml of each gas mixture without C_2H_2; (ii) 12 ml of each gas mixture without C_2H_2 and with 1 ml the C_2H_2-removal solution added; (iii) 13.3 ml of each gas mixture with C_2H_2 and with 1 ml of the C_2H_2-removal solution added (12 ml of gas should remain after removal of the C_2H_2). The C_2H_2-removal solution (300 mM $KMnO_4$ and 100 mM KOH) was purged with He for 10 min to remove atmospheric N_2 and N_2O before the 1-ml

aliquots were injected into each vial. After 1 h at 20°C the concentration of N_2O in each vial was determined by automated CF-IRMS (Stevens *et al.*, 1993) using an Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser interfaced to an Europa Scientific Trace Gas Preparation System with Gilson auto-sampler. A segment of I_2O_5 activated by H_2SO_4 (Gastec Analyser Tube, catalogue no. 1HH), which oxidises small quantities of C_2H_2 to CO_2, was included in the scrubber train between the $Mg(ClO_4)_2$ and the ascarite to ensure complete removal of C_2H_2 before the gas stream entered the mass spectrometer.

Effect of acetylene removal on the determination of ^{15}N in nitrous oxide and di-nitrogen

Mixtures of N_2O (150 $\mu l\,l^{-1}$) at natural abundance, 10 and 20 atom % ^{15}N with or without 10% v/v C_2H_2 were made up in gas sampling bags. The ^{15}N-labelled N_2O was generated from ^{15}N-enriched $(NH_4)_2SO_4$ reacted with alkaline NaOBr (Hauck, 1982). Di-nitrogen is the main product of the reaction, but 1–3% of the gas produced is N_2O. The effect of C_2H_2 removal on the determination of the ^{15}N content of each enrichment of N_2O was tested using the same three treatments as were used to test the effect of removal of C_2H_2 on the determination of N_2O concentration. The ^{15}N content of the N_2O was determined by CF-IRMS. The system was as described by Stevens *et al.* (1993), but with automation of the valve switching and source setting so that the ^{15}N content of N_2 and N_2O could be determined in the same sample. The ion currents (I) at m/z 44 45, and 46 enabled ^{45}R ($^{45}I/^{44}I$) and ^{46}R ($^{46}I/^{44}I$) to be calculated for N_2O. The ^{15}N content of the N_2O was calculated from either the ^{45}R, using equations (5) and (7) or ^{46}R, using equations (6) and (7) of Stevens *et al.* (1993). The concentration of N_2O was calculated as described by Stevens *et al.* (1993) from measurements of ^{44}I, ^{45}I and ^{46}I.

Samples of N_2 at natural abundance, 0.4, 0.6 and 0.8 atom % ^{15}N with or without 10% v/v C_2H_2 were made up in gas sampling bags. The ^{15}N-labelled N_2 was generated by reacting ^{15}N-enriched $(NH_4)_2SO_4$ with alkaline NaOBr prepared as described previously. The effect of C_2H_2 removal on the determination of the ^{15}N content of the N_2 was tested using the same three treatments as were used to test the effect of C_2H_2 on the determination of ^{15}N in the N_2O. For N_2, the ion currents at m/z 28, 29 and 30 enabled ^{29}R ($^{29}I/^{28}I$) and ^{30}R ($^{30}I/^{28}I$) to be measured by CF-IRMS (Stevens *et al.*, 1993).

Testing the effect of acetylene on soil processes using a soil incubation experiment

In a soil incubation experiment, a grassland soil was treated in factorial combination with differentially labelled NH_4NO_3 at one N rate (1.46 μmol N g^{-1} oven dry soil), two rates of glucose (42 and 83 μmol C g^{-1} oven dry soil), with or without 10% v/v C_2H_2 in the headspace, and three incubation times (6, 12 and 24 h). Each treatment was replicated six times, replicates being arranged randomly during incubation at 20°C. Control treatments without N or C addition were included for time zero and each incubation time. Fresh 5-mm sieved soil (200 g containing 458 mg H_2O g^{-1} oven-dry soil) was placed in 500-ml Kilner jars. The required amounts of N and C were dissolved in 20 ml of water and dispensed uniformly over the soil surface using a syringe. Control treatments received 20 ml of water, bringing all soil moisture contents to 60% (oven-dry basis). Immediately after liquid addition, a nylon lid with a gas-sampling septum was fitted to each jar using an O-ring to form a gas-tight seal. The volume of headspace in each jar containing treated soil was 363 ml. For the treatments with C_2H_2, 36 ml of C_2H_2 at atmospheric pressure were added to the headspace after removing an equal amount of air. The C_2H_2 had been scrubbed through water to remove acetone and other impurities (Gross and Bremner, 1992). For the treatments without C_2H_2, He was used instead of C_2H_2 to maintain the same mass of N_2 (306 mg) and O_2 in the headspace of each jar.

At the end of each incubation period the headspace of each jar was analysed for ^{15}N in N_2 and N_2O concentration by CF-IRMS and for CO_2 by gas chromatography. The flux of N_2 was calculated according to Mulvaney and Boast (1986). The soil in each jar was then extracted with 2 M KCl solution according to the procedure of Stevens and Laughlin (1995). Nitrite concentration was determined colorimetrically by the Griess–Ilosvay procedure (Keeney and Nelson, 1982). The ^{15}N content of the NO_2^- was determined by producing N_2O for CF-IRMS (Stevens and Laughlin, 1994). Data for fluxes of N_2O, N_2, (N_2O + N_2) and CO_2 into the headspace are only shown for the $NH_4^{15}NO_3$ treatment and data for the size and enrichment of the NO_2^- pool are only shown for the $^{15}NH_4NO_3$ treatment. Some of the data for this test have already been published in our paper on measuring the contribution of nitrification and denitrification to the flux of nitrous oxide from soil (Stevens *et al.*, 1997). The data used come from the second experiment described in that paper which was designed to test the uniformity of the NO_3^--labelled pool using C_2H_2 as a nitrification inhibitor.

Statistical analyses

Analysis of variance was used to determine the effect of the C_2H_2 removal procedure on the measurement of N_2O concentration and on the measurement of ^{15}N in N_2 and in N_2O, and to determine the effect of treatments on the flux of N_2O, N_2, (N_2O + N_2), CO_2, and on the size and enrichment of the soil NO_2^- pool.

RESULTS AND DISCUSSION

Removal of acetylene from gas samples

The effects of $KMnO_4$ on the concentrations of C_2H_2 and N_2O are shown in Fig. 1. The use of 1 ml of 300 mM $KMnO_4$ solution totally removed 10% v/v C_2H_2 to below detectable limits (10 μl C_2H_2 l^{-1}). The equation for the reaction is:

$$C_2H_2 + 4[O] \longrightarrow CHOOH + CO_2$$

The concentration of N_2O was unaffected by neutral $KMnO_4$. The effect of KOH concentration in the 300 mM $KMnO_4$ absorbent on the CO_2 concentration is shown in Fig. 2. A concentration of 100 mM KOH absorbed all the CO_2 produced from the oxidisation of the C_2H_2 by the $KMnO_4$. A solution of 300 mM $KMnO_4$ and 100 mM KOH is, therefore, suitable for use as a C_2H_2 absorbent. Theoretically, 1 ml of this mixture is capable of removing 3.4 ml of pure C_2H_2. The large excess of $KMnO_4$ drove the reaction to completion within 1 h.

Effect of acetylene removal on gas analyses

The C_2H_2 removal procedure had no effect on the determination of N_2O concentration in the gas samples (Table 1). It also had no effect on the determination of the ^{15}N content in N_2O as calculated from either ^{45}R or ^{46}R (Table 2) or on the measured values of ^{29}R and ^{30}R in N_2 (Table 3). The values for ratios in Table 3 are calculated from measured ion currents and have not been corrected to true values using background correction or instrument performance factors. The success of this C_2H_2 removal procedure allows the isotopic analysis of gases to proceed in ^{15}N studies where C_2H_2 is included as a treatment.

The efficiency of inhibition of nitrous oxide reductase

The effects of C_2H_2 on the fluxes of N_2 and N_2O during the soil incubation with different stresses of NO_3^- and C are shown in Table 4. In the presence of C_2H_2 the isotopic composition of the N_2 was not significantly different from normal atmosphere, so no N_2 flux was detectable. Therefore, for the soil conditions during this incubation, 10% v/v C_2H_2 completely blocked N_2O-reductase. In all treatments without C_2H_2, the N_2 flux was greater than the N_2O flux. The higher concentrations of C caused an increase in the total amount of denitrification. In these high C treatments, the NO_3^- concentrations after 24 h of incubation were less than the native soil NO_3^- concentrations (Stevens *et al.*, 1997). This NO_3^- limitation, however, did not affect the inhibition efficiency of the C_2H_2.

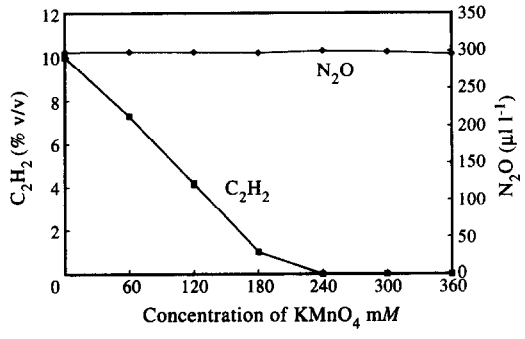

Fig. 1. The effects of $KMnO_4$ in neutral aqueous solution on the concentrations of C_2H_2 and N_2O in gas samples. Error bars indicate SE of the mean (n = 3) or are smaller than the symbols.

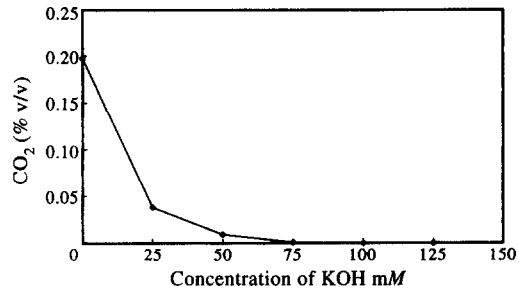

Fig. 2. The effect of KOH concentration in 300 mM $KMnO_4$ on the removal of CO_2 produced from the oxidation of C_2H_2 in gas samples. Error bars indicate SE of the mean (n = 3) or are smaller than the symbols.

The effect of acetylene on respiration and nitrification

Comparison of treatments with or without C_2H_2 within sampling times showed that C_2H_2 had no significant effect ($P > 0.05$) on CO_2 flux (Table 4). When averaged overall the times, the CO_2 flux from the treatments without C_2H_2 (535 nmol C g^{-1} h^{-1}) was significantly higher ($P < 0.012$) than the flux from those with C_2H_2 (510 nmol C g^{-1} h^{-1}). This shows that C_2H_2 slightly inhibited respiration. Flather and Beauchamp (1992) showed that C_2H_2 could inhibit fermentative bacteria. In our experiment, there was no evidence for the microbial metabolism of C_2H_2. The soil microbial community probably needs more than 24 h to adapt to the metabolism of C_2H_2.

The size and enrichment of the NH_4^+ and NO_3^- pools in this experiment were described in detail by Stevens *et al.* (1997). In the $^{15}NH_4^+$-labelled treatment, the NO_3^- pool was not enriched in the presence of C_2H_2, but was enriched in the absence of C_2H_2. Nitrification was, therefore, blocked by C_2H_2 but occurred without C_2H_2. Further evidence of the effect of C_2H_2 on nitrification can be obtained by examination of the size and enrichment of the NO_2^- pool (Table 5). The NO_2^- pool was significantly ($P < 0.001$) larger in the absence of C_2H_2. In the $^{15}NH_4^+$-labelled treatment, the NO_2^- pool was not enriched in the presence of C_2H_2, but was enriched in the absence of C_2H_2. Nitrite exists in a small and dynamic pool compared with NH_4^+ and NO_3^-. Any

Table 1. The effect of $KMnO_4$ solution and C_2H_2 removal with $KMnO_4$ solution on the concentration of N_2O in gas samples

Theoretical N_2O concentration ($\mu l\ l^{-1}$)	Measured N_2O concentration ($\mu l\ l^{-1}$)			
	N_2O	N_2O + $KMnO_4$	N_2O + C_2H_2 + $KMnO_4$	SEM (n = 3)
10	9.9	9.6	10.1	0.11
100	99.4	99.1	99.3	0.29
300	298.7	298.9	296.5	0.47
500	500.4	499.0	497.8	1.41
750	751.3	749.2	748.5	1.52
1000	999.2	998.2	997.6	1.13

Table 2. The effect of $KMnO_4$ solution and acetylene removal with $KMnO_4$ solution on the ^{15}N content of N_2O in gas samples

Theoretical enrichment atom % ^{15}N	Measured atom % ^{15}N from ^{45}R				Measured atom % ^{15}N from ^{46}R			
	N_2O	N_2O + $KMnO_4$	N_2O + C_2H_2 + $KMnO_4$	SEM (n = 3)	N_2O	N_2O + $KMnO_4$	N_2O + C_2H_2 + $KMnO_4$	SEM (n = 3)
0.37	0.37	0.37	0.37	0.001	ND	ND	ND	ND
10.00	10.14	10.13	10.15	0.009	9.97	9.96	9.97	0.074
20.00	20.25	20.27	20.25	0.024	20.00	20.02	20.01	0.012

Table 3. The effect of $KMnO_4$ solution and acetylene removal with $KMnO_4$ solution on the measured values of ^{29}R and ^{30}R in N_2

Theoretical enrichment atom % ^{15}N	$^{29}R \times 10^3$				$^{30}R \times 10^5$			
	N_2	N_2 + $KMnO_4$	N_2 + C_2H_2 + $KMnO_4$	SEM (n = 3)	N_2	N_2 + $KMnO_4$	N_2 + C_2H_2 + $KMnO_4$	SEM (n = 3)
0.366	7.19	7.19	7.19	0.001	2.42	2.40	2.43	0.020
0.400	7.96	7.97	7.96	0.014	2.85	2.82	2.85	0.018
0.600	11.77	11.51	11.84	0.068	4.94	4.78	4.95	0.032
0.800	15.37	15.48	15.61	0.127	7.63	7.67	7.77	0.086

change in its size or enrichment is a sensitive indicator of nitrification. The first step of the autotrophic oxidation of NH_4^+ is inhibited by 0.01% v/v of acetylene (Berg *et al.*, 1982).

Comparison of acetylene inhibition and ^{15}N gas-flux methods

If nitrification is contributing to the NO_3^- pool, potentially N-gas flux measured by the ^{15}N method could be greater than the N-gas flux measured by the C_2H_2 inhibition method. Although the N-gas flux from the ^{15}N method was usually greater than that from the C_2H_2 inhibition method within incubation times (Table 4), the average flux over all incubation times for the ^{15}N method (4.3 nmol N g^{-1} h^{-1}) was not significantly different ($P > 0.05$) from that for the C_2H_2 inhibition method (3.9 nmol N $g^{-1}h^{-1}$). Variability has also made it difficult to detect small differences between the ^{15}N method and the C_2H_2 inhibition technique in other studies (Klemedtsson *et al.*, 1990).

In this experiment the native plus added NO_3^- at the start of the incubation was 1.16 μmol N g^{-1} oven-dry soil, so NO_3^- was not limiting for denitrifi-

Table 4. The effect of C_2H_2 on the fluxes of CO_2, N_2O and N_2 from soil treated with $NH_4^{15}NO_3$ and two concentrations of glucose for different times

C_2H_2 (%v/v)	Glucose (μmol C g^{-1} h^{-1})	Time (h)	CO_2 flux (nmol C g^{-1} h^{-1})	N_2O flux	N_2 flux	(N_2 + N_2O) flux
				(nmol N g^{-1} h^{-1})		
0	42	0–6	450	0.78	1.32	2.11
0	42	0–12	532	1.65	2.03	3.68
0	42	0–24	406	1.46	2.25	3.71
0	83	0–6	521	0.93	2.49	3.41
0	83	0–12	630	2.69	3.72	6.41
0	83	0–24	670	1.13	5.26	6.39
10	42	0–6	405	1.78	0.00	1.78
10	42	0–12	483	4.64	0.00	4.64
10	42	0–24	399	3.31	0.00	3.31
10	83	0–6	499	2.24	0.00	2.24
10	83	0–12	642	5.71	0.00	5.71
10	83	0–24	629	5.98	0.00	5.98
SEM (df = 60, n = 6)			16.9	0.180	0.235	0.355

Table 5. The effect of C_2H_2 on the size and enrichment of the NO_2^- pool in soil treated with $^{15}NH_4NO_3$ and two concentrations of glucose for different times

C_2H_2 (%v/v)	Glucose (μmol C g^{-1})	Time (h)	NO_2^-	
			(nmol N g^{-1})	(Atom % excess ^{15}N)
0	42	0	1.9	0.0
0	42	0–6	6.9	9.4
0	42	0–12	4.0	6.5
0	42	0–24	4.1	2.1
0	83	0	1.9	0.0
0	83	0–6	7.0	8.9
0	83	0–12	7.1	7.6
0	83	0 24	3.8	1.5
10	42	0	1.9	0.0
10	42	0–6	2.7	0.6
10	42	0–12	2.8	0.7
10	42	0–24	3.2	0.5
10	83	0	1.9	0.0
10	83	0–6	3.9	0.5
10	83	0–12	5.5	0.3
10	83	0–24	3.3	0.2
SEM (df = 60, n = 6)			0.36	0.29

cation. Nitrification occurred in the treatments without C_2H_2 and increased the size of the NO_3^- pool, but did not increase significantly the total losses of N gases. Inhibition of nitrification which is coupled to denitrification can upset estimations of denitrification if NO_3^- concentrations are limiting. Care should, thus, be taken when applying the C_2H_2 inhibition technique to soils which have been fertilised with artificial NH_4^+ fertilisers or manures where there is a large NH_4^+ pool available for nitrification. In these circumstances the C_2H_2 inhibition technique could underestimate denitrification significantly. Including C_2H_2 as a treatment in conjunction with the ^{15}N gas-flux method provides a direct method of determining whether the assumptions in the C_2H_2 technique are valid for a particular soil and conditions.

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