



Dynamics of the denitrification process in soil from the Brimstone Farm experiment, UK

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Abstract

The effect of restricted drainage during winter on the dynamics of denitrification was investigated in clayey soil from the Brimstone Experimental Farm, Oxfordshire, UK. Soil taken in autumn 1995 and spring 1996 from the 0–20 and 20–50 cm layers of plots with restricted and unrestricted drainage were amended with or without C_2H_2 to inhibit reduction of N_2O to N_2 and with or without chloramphenicol to inhibit de novo synthesis of reduction enzymes. Samples were incubated anaerobically for 72 h at 25°C and the production of CO_2 and N_2O and the concentrations of NO_3^- and NO_2^- were monitored. Restricting drainage had no significant effect on the concentrations of NO_3^- and NO_2^- nor on the production of N_2O . The production of N_2O was very variable and less than 1.5 mg $N_2O-N\ kg^{-1}$ after 72 h in both plots. The N_2O -to- CO_2 ratio on both plots was only 0.02 and no significant amounts of N_2 were produced. However, conditioning soil from the 0–20 cm of the plot with restricted drainage by keeping it under waterlogged conditions for 28 d at 25°C in the laboratory changed the dynamics of the denitrification process substantially: the N_2O -to- CO_2 ratio increased to 0.56, 54% of the gaseous product of denitrification was N_2 and the resulting N_2O -to- N_2 ratio was 1.84. The application of either 50 mg $NO_3^- -N$ or 50 mg $NO_3^- -N$ plus 100 mg glucose C decreased the production of N_2 ; consequently the N_2O -to- N_2 ratio increased to 2.66 and 3.37, respectively. With a clay content of 55–60% and very slowly permeable subsoil, the Brimstone Farm soil was assumed to be well adapted to anaerobic conditions, but its capacity in 1995/1996 to reduce NO_3^- to N_2O and N_2 was very limited, even when it was incubated under strict anaerobic conditions and supplied with NO_3^- . However, waterlogging the soil in the laboratory caused the microbial community to change its functional characteristics by adapting to the anaerobic conditions. The inability to replicate this effect in the field by restricting winter drainage can be attributed to dry antecedent soil conditions resulting from the very low rainfall in summer 1995. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The antecedent water regime of a soil affects the denitrification process and its gaseous products (Dendooven et al., 1996b). In the Brimstone Farm leaching experiment (Oxfordshire, UK) the drainage of a clay soil is restricted experimentally by closing a U-bend in the drain in an attempt to decrease the amount of nitrate and other contaminants leaching to the surface water (Catt et al., 1996). Drainage restriction may decrease NO_3^- leaching by increasing denitri-

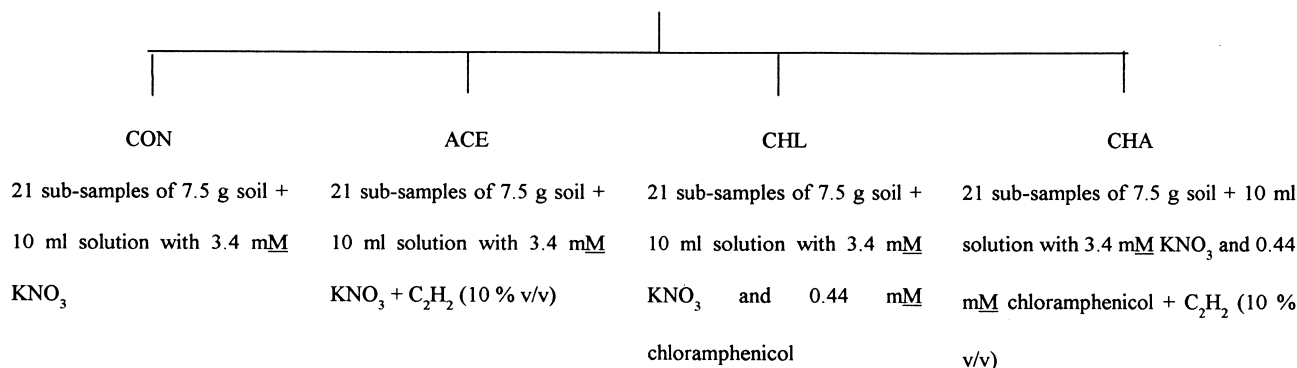
fication, so that more N is lost as N_2O or N_2 to the atmosphere, or it may decrease mineralization of soil organic matter (a major source of NO_3^-) or it may simply decrease NO_3^- losses by limiting the flow of water.

Our aim was to investigate the effect of the restricted drainage on the dynamics of the denitrification process. Soil was sampled at the beginning and end of the 1995/1996 winter drainage season from the 0–20 and 20–50 cm layers of two plots, one (plot 1) with restricted and the other (plot 15) with unrestricted drainage. Denitrification dynamics were studied by incubating the soil samples anaerobically for 72 h with or without C_2H_2 , which inhibits the reduction of N_2O to N_2 (Balderstone et al., 1976), and with or without chloramphenicol which inhibits de novo synthesis of reduction enzymes (Smith and Tiedje, 1979).

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(a)

Assays for enzyme activity carried out on soil from the 0–20 cm and 20–50 cm layers of Plots 1 and 15



(b)

soil sampled from the 0–20 cm layer of Plot 1

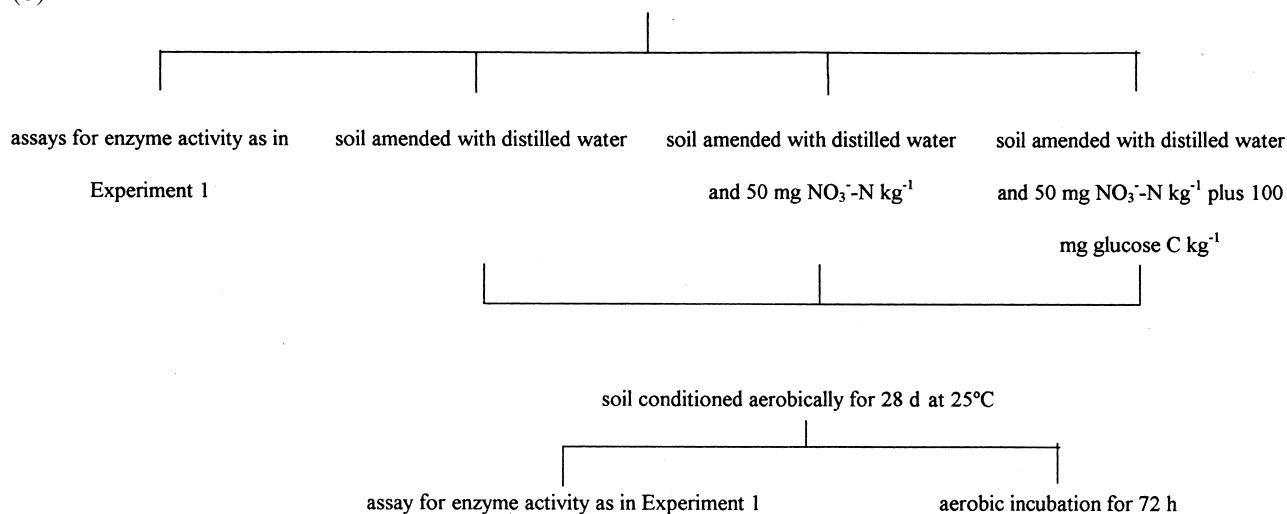


Fig. 1. Experimental procedure to investigate the effect of a restricted or unrestricted drainage on the denitrification process in soil from the Brimstone Farm and the effect of waterlogging the soil with or without the addition of 50 mg NO₃⁻ kg⁻¹ or 50 mg NO₃⁻ kg⁻¹ and 100 mg glucose C kg⁻¹.

Production of CO₂ and N₂O and the concentrations of NO₂⁻ and NO₃⁻ were monitored.

2. Materials and methods

2.1. Experimental site

Brimstone Farm near Faringdon, Oxfordshire (UK) is operated jointly by ADAS and IACR–Rothamsted as an experimental site for leaching studies. Twenty hydrologically isolated plots, which are large enough for normal agricultural operations, are used to study water movement and the transport of NO₃⁻, phosphorus and pesticide residues in an artificially drained clay soil. Detailed descriptions of the original site facilities were given by Cannell et al. (1984) and Harris et al. (1984). The soil is a uniform heavy clay of the

Denchworth series (Jarvis, 1973) derived from Upper Jurassic Oxford clay. In the top 20 cm of the profile the soil contains 54% clay (< 2 μm), 39% silt (2–60 μm) and 7% sand (> 60 μm). The altitude is 100–106 m O.D. and the slope is approximately 2%. The mean annual rainfall is 680 mm. Both plots sampled (plots 1 and 15) have 3.2% organic C, less than 0.1% inorganic C and 0.6% total N in the 0–20 cm horizon. The pH_(H₂O) of the 0–20 cm horizon is approximately 7.0 as a result of recent liming.

The work involved two experiments (Fig. 1). The first investigated the dynamics of the denitrification process in the two layers of the two plots, and the second investigated the effect of conditioning a soil by waterlogging it for 28 d at 25°C with or without 50 mg NO₃⁻-N kg⁻¹ or 50 mg NO₃⁻-N kg⁻¹ plus 100 mg glucose C kg⁻¹.

2.2. Experiment 1: anaerobic incubation to assess potential denitrifying activity

Soil was sampled from the 0–20 and the 20–50 cm layers of plots 1 and 15 in January 1995, broken up by hand and sieved to pass 6.5 mm while still moist. Eighty-four 7.5 g subsamples from each plot and layer were added to 250-ml Erlenmeyer flasks giving a total of 336 flasks. Ten ml of a solution containing 3.4 mM KNO_3 , equivalent to approximately 50 mg NO_3^- -N kg^{-1} , were added to 42 flasks from each plot and layer, and the atmosphere inside was isolated by glass adapters fitted with rubber bungs and sampling tubes. The flasks were evacuated for a total of 5 min and the head-space replaced with O_2 -free N_2 to create an anaerobic atmosphere. From half of the flasks from each plot and layer, 30 ml of the head-space gas was removed and replaced with C_2H_2 (ACE treatment) to give an approximate head-space concentration of 10%, which was sufficient to prevent the reduction of N_2O to N_2 (Yoshinari and Knowles, 1976). The other 21 flasks were considered to be the control (CON) treatment.

Ten ml of a solution containing 34 mM KNO_3 and 0.44 mM chloramphenicol were added to the remaining 42 flasks from each plot and layer, and the atmosphere inside was isolated by glass adapters fitted with rubber bungs and sampling tubes. The flasks were evacuated for a total of 5 min and the head-space replaced with O_2 -free N_2 to create an anaerobic atmosphere. Half of the samples were amended with C_2H_2 (CHA treatments) while the unamended samples were considered the CHL treatment. The concentration of chloramphenicol was approximately 300 mg kg^{-1} , sufficient to inhibit protein synthesis without adversely affecting the activity of the indigenous N-reductase enzymes (Dendooven and Anderson, 1994), although Brooks et al. (1992) and Wu and Knowles (1995) provided evidence for a possible small inhibitory effect on these enzymes. It has also been shown that chloramphenicol additions can increase NO_2^- formation in soils; Dendooven et al. (1994) give a full discussion of the possible mechanisms.

The samples were well mixed to create a slurry, thus minimizing the influence of irregular NO_3^- distribution, and were incubated at 25°C ($\pm 1^\circ\text{C}$) in an orbital incubator. At the onset of the experiment and after approximately 2, 6, 12, 24, 48 and 72 h, three flasks were chosen at random from each of the CON, ACE, CHL and CHA treatments for each of the two plots and layers and the head-space was analyzed for N_2O , CO_2 and C_2H_2 by gas chromatography (GC) (Ai 93 chromatogram, Ai Cambridge). N_2O was determined on an electron capture detector and CO_2 and C_2H_2 on a thermal conductivity detector, using the method of Hall and Dowdell (1981). The concentrations of CO_2

and N_2O were corrected for dissolution in water (Weast, 1968; Moraghan and Buresh, 1977, respectively). The flasks were then opened and inorganic-N was extracted by shaking with 40 ml of distilled water for 30 min and filtering through Whatman No. 42 paper. The NO_3^- , NO_2^- and NH_4^+ concentrations were determined colorimetrically on a Tecator 5010 flow injection analyzer (UK).

2.3. Experiment 2: conditioning of soil under waterlogged conditions

Soil sampled from the 0–20 cm layer on January 1996 was broken up and sieved to <6.5 mm, then divided into four equal amounts. Enzyme activity was assayed on one portion of the soil as described in experiment 1 (Fig. 1). One hundred and fifty ml of three different liquids were added to the three remaining portions of soil: (a) distilled H_2O (b) a 10 mM solution of KNO_3 and (c) a solution of 10 mM KNO_3 and 4 mM of glucose. This procedure resulted in a water content of approximately twice the water holding capacity of 39% and concentrations of ca. 50 mg NO_3^- -N kg^{-1} dry soil in (b) or 50 mg NO_3^- -N kg^{-1} dry soil and 100 mg glucose C kg^{-1} dry soil in (c). The soil was then conditioned aerobically for 28 d at 25°C in drums containing two separate vessels with 100 ml of H_2O to avoid desiccation and 100 ml of a 2 M NaOH solution to trap CO_2 evolved. Afterwards enzyme activity was assayed on the three treatments as described in experiment 1.

Additionally, 18 subsamples of 7.5 g of soil from each plot and layer were added to 100 ml conical flasks, sealed and incubated aerobically on an orbital incubator at 25°C ($\pm 1^\circ\text{C}$) for 72 h. The flask head-space was sampled for CO_2 and N_2O after approximately 2, 6, 12, 24, 48 and 72 h.

2.4. Statistical analysis

CO_2 production was regressed on elapsed time using a linear regression model, which was forced to pass through the origin but allowed different slopes (production rates) for each plot. This approach assumes that no CO_2 is produced at time zero. Production of N_2O under anaerobic conditions was analyzed in the same way. Production of NO_2^- and NO_3^- were analyzed in similar ways, but the regression lines were not forced to pass through the origin and were allowed different intercepts in different plots and layers.

Inorganic N concentrations (NO_2^- and NO_3^-) and CO_2 and N_2O production were subjected to a one-way analysis of variance to test for significant differences between the plots, layers and treatments. All analysis were performed using the statistical package from SAS Institute (1988).

Table 1

Rates of CO_2 ($\mu\text{g C kg}^{-1} \text{ h}^{-1}$) and N_2O production ($\mu\text{g N kg}^{-1} \text{ h}^{-1}$) in soil from the 0–20 and 20–50 cm layers of plot 1 and 15 from the Brimstone Farm experiment amended with 50 mg $\text{NO}_3^- \text{N kg}^{-1}$, with or without C_2H_2 and with or without chloramphenicol and incubated anaerobically for 72 h at 25°C. The rates shown are estimated slopes (and associated standard errors) from the linear regression analysis of production over time

Treatment	Plot 1		Plot 15	
	0–20 cm	20–50 cm	0–20 cm	20–50 cm
<i>CO₂ production ($\mu\text{g C kg}^{-1} \text{ h}^{-1}$)</i>				
Control (CON)	709(39)	788(148)	1048(165)	749(85)
10 vol% C_2H_2 (ACE)	725(59)	795(121)	1131(175)	849(85)
300 mg kg^{-1} chloramphenicol (CHL)	798(37)	826(134)	1092(172)	842(81)
10 vol% C_2H_2 + 300 mg chloramphenicol kg^{-1} (CHA)	809(52)	781(119)	1066(174)	814(115)
<i>N₂O production ($\mu\text{g N kg}^{-1} \text{ h}^{-1}$)</i>				
Control (CON)	7.6(3.4)	14.4(12.8)	7.4(4.4)	42.6(10.1)
10 vol% C_2H_2 (ACE)	91.1(41.2)	4.8(0.8)	2.8(1.2)	24.7(6.9)
300 mg kg^{-1} chloramphenicol (CHL)	1.8(0.8)	0.5(0.1)	21.1(18.4)	10.6(3.4)
10 vol% C_2H_2 + 300 mg chloramphenicol kg^{-1} (CHA)	22.4(5.6)	18.7(9.4)	15.9(11.3)	22.1(5.7)

Numbers in parentheses are standard errors of the estimates.

3. Results

3.1. Experiment 1: anaerobic incubation to assess potential denitrifying activity

There was a significantly greater CO_2 production in the 0–20 than in the 20–50 cm layer ($P < 0.05$) and significantly greater CO_2 production in plot 15 (unrestricted drainage) than in plot 1 (restricted drainage) ($P < 0.01$) (Table 1). The addition of C_2H_2 or chloramphenicol had no significant effects on production of CO_2 .

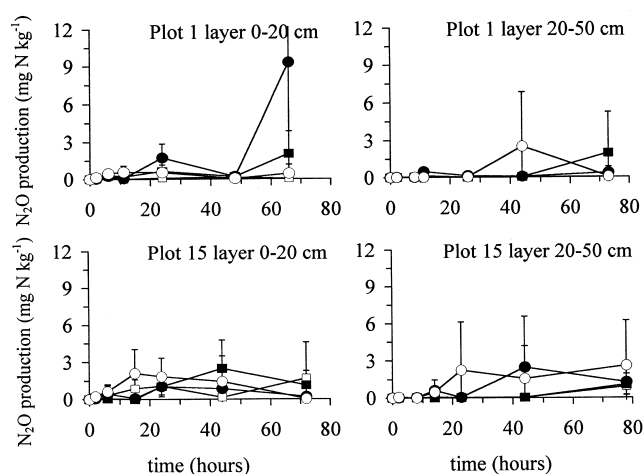


Fig. 2. The N_2O production ($\text{mg N}_2\text{O-N kg}^{-1}$) in soil from the 0–20 and 20–50 cm layer of plots 1 and 15 of the Brimstone Farm experiment anaerobically incubated for ca. 72 h at 25°C following the application of 50 mg $\text{NO}_3^- \text{N kg}^{-1}$ soil. Control (—○—) CON, C_2H_2 (10 vol%) (—●—) ACE, 300 mg chloramphenicol kg^{-1} (—□—) CHL, and 300 mg chloramphenicol kg^{-1} plus C_2H_2 (10 vol%) (—■—) CRA. Bars indicate plus and minus S.D.

Concentrations of NO_2^- were very variable (data not shown). There was no significant difference in NO_2^- concentrations between plots, but there was a significantly greater mean concentration in the 0–20 cm than in the 20–50 cm layers ($P < 0.01$). The addition of C_2H_2 significantly decreased ($P < 0.001$) and chloramphenicol significantly increased ($P < 0.05$) the concentration of NO_2^- .

The concentration of NO_3^- significantly decreased over time in plot 15 ($P < 0.0001$) but not in plot 1. The decreases in NO_3^- concentrations over time were similar in both soil layers. The additions of both C_2H_2 and chloramphenicol had no significant effects on the decrease in the NO_3^- concentrations.

Production of N_2O was always less than 10 mg $\text{N}_2\text{O-N kg}^{-1}$ but was very variable (Fig. 2). There was no significant difference in N_2O production between the two plots nor between the two soil layers (Table 1). The addition of C_2H_2 and chloramphenicol had no significant effect on the production of N_2O .

3.2. Experiment 2: conditioning of soil under waterlogged conditions

The assay for enzyme activity on the soil sampled in spring gave similar results to that in the first experiment. The addition of C_2H_2 or chloramphenicol had no significant effects on production of CO_2 ; the concentrations of NO_2^- were very variable; the concentration of NO_3^- did not significantly decrease over time and the N_2O production was very variable over time, with an average value of less than 2 mg $\text{N}_2\text{O-N kg}^{-1}$ for all treatments (data not shown).

Conditioning the same soil for 28 d after it was amended with distilled H_2O , a 10 mM solution of

Table 2
Rates of CO₂ ($\mu\text{g C kg}^{-1} \text{ h}^{-1}$) and N₂O production ($\mu\text{g N kg}^{-1} \text{ h}^{-1}$) in soil from 0–20 cm layer of plot 1 from the Brimstone Farm experiment amended with distilled water, a solution of 10 mM KNO₃ or a solution of 10 mM KNO₃ and 4 mM glucose conditioned aerobically for 28 d at 25°C. Soil was incubated aerobically or amended with 50 mg NO₃[−]-N kg^{−1}, with or without C₂H₂ and with or without chloramphenicol and incubated anaerobically for 72 h at 25°C

Treatment	Distilled H ₂ O	Distilled H ₂ O and 50 mg NO ₃ [−] -N kg ^{−1}	Distilled H ₂ O, 50 mg NO ₃ [−] -N kg ^{−1} and 100 mg glucose C kg ^{−1}
<i>CO₂ production ($\mu\text{g C kg}^{-1} \text{ h}^{-1}$)</i>			
Aerobic			
Control (CON)	1450(175) ^a	1291(173)	1742(144)
10 vol% C ₂ H ₂ (ACE)	1191(56)	1044(55)	1409(58)
300 mg kg ^{−1} chloramphenicol (CHL)	1276(73)	1104(125)	1339(41)
10 vol% C ₂ H ₂ + 300 mg chloramphenicol kg ^{−1} (CHA)	1459(161)	1032(83)	1377(54)
	1149(61)	1054(82)	1161(67)
<i>N₂O production ($\mu\text{g N kg}^{-1} \text{ h}^{-1}$)</i>			
Aerobic			
Control (CON)	75(14)	67(13)	76(11)
10 vol% C ₂ H ₂ (ACE)	229(70)	480(78)	557(54)
300 mg kg ^{−1} chloramphenicol (CHL)	711(88)	907(67)	885(65)
10 vol% C ₂ H ₂ + 300 mg chloramphenicol kg ^{−1} (CHA)	521(73)	743(59)	675(59)
	898(78)	908(82)	972(73)

^a Numbers in parentheses are standard errors of the estimates.

KNO₃ or a solution of 10 mM KNO₃ and 4 mM of glucose changed the characteristics of the denitrification process. The CO₂ production (average of all treatments) was significantly less in the soil amended with 50 mg NO₃[−]-N kg^{−1} than in the soil amended with distilled H₂O or 50 mg NO₃[−]-N kg^{−1} plus 100 mg glucose C kg^{−1} ($P < 0.001$) (Table 2). The application of C₂H₂ or chloramphenicol had no significant effect on the production of CO₂.

There were no significant differences in the production of N₂O between soil samples amended with distilled H₂O, a 10 mM solution of KNO₃ and a solution of 10 mM KNO₃ and 4 mM of glucose for the ACE, CHA and CHL treatments (Table 2). The N₂O production in the CON treatment of the soil amended with distilled H₂O was significantly less than in the soil amended with either a 10 mM solution of KNO₃ or a solution of 10 mM KNO₃ and 4 mM of glucose ($P < 0.05$).

4. Discussion

Some of the results for the soil from Brimstone Farm do not correspond with those obtained for enzyme activity elsewhere. The CO₂ production under anaerobic conditions was approximately half of that evolved from a pasture soil (Dendooven and Anderson, 1995) and twice as much as from soil under a Norway spruce plantation (Dendooven et al., 1996a) but was similar to that expected for an arable soil under continuous wheat (unpublished data). However, the pattern and amount of N₂O evolved and the N₂O-to-CO₂ ratio in the assays for enzyme activity were less than those observed previously. The average amount of N₂O produced at the end of the incubation was only 1.46 mg N₂O-N kg^{−1} compared with 12 mg N₂O-N kg^{−1} in the ACE treatment of the soil under Norway spruce, even though the CO₂ production of the soil under spruce (Dendooven et al., 1996a) was less than half of that from Brimstone soil. As there was no lag in the production of N₂O, no de novo synthesis of reduction enzymes occurred. The mean N₂O-to-CO₂ ratio was very small and only 0.02. It therefore seems that the activity of the denitrifiers in the soil of Brimstone Farm is very low, and that they are not well adapted to anaerobiosis, despite its high clay percentage and very slowly permeable subsoil (Youngs and Goss, 1988). However, in study of soil from the same site Colbourn et al. (1984) stated that the soil had potential to denitrify large quantities of added nitrate rapidly. In an incubation under anaerobic conditions for 72 h at 20°C, they found that the production of N₂O was on average 38 mg N₂O-N kg^{−1} in the 0–20 cm layer and of 8 mg N₂O-N kg^{−1} in the 20–60 cm layer.

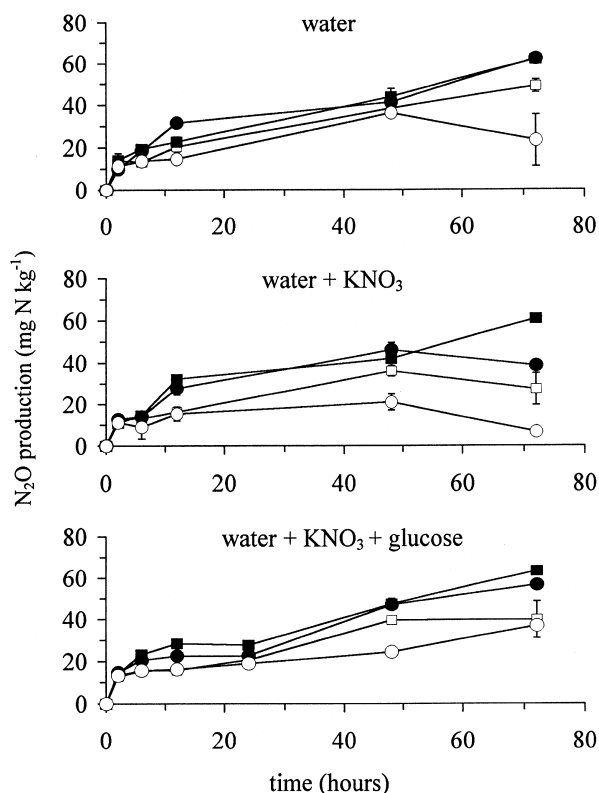


Fig. 3. The N_2O production ($\text{mg N}_2\text{O-N kg}^{-1}$) in soil from the 0–20 layer of plot 1 of the Brimstone Farm experiment waterlogged for 28 d with or without $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ or $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ plus $100 \text{ mg glucose C kg}^{-1}$ and then anaerobically incubated for ca. 72 h at 25°C . Soil was amended with $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ soil and treatments were Control (—○—) CON, C_2H_2 (10 vol%) (—●—) ACE, $300 \text{ mg chloramphenicol kg}^{-1}$ (—□—) CHL and $300 \text{ mg chloramphenicol kg}^{-1}$ plus C_2H_2 (10 vol%) (—■—) CHA. Bars indicate plus and minus S.D.

We had expected similar values to those reported by Colbourn et al. (1984). For instance, in an experiment investigating the effect of long-term application of inorganic N fertilizer on denitrification in an arable soil with a clay content of ca. 22%, a comparable annual rainfall of 725 mm and a comparable microbial biomass activity, the N_2O production after 72 h was ca. $25 \text{ mg N}_2\text{O-N kg}^{-1}$ (unpublished data). As it was possible that an error occurred during the experiment, we repeated the assay for enzyme activity with soil from plot 1 sampled in January 1996. The results were comparable with those obtained in the first experiment. The CO_2 production rate was on average $1.18 \text{ mg CO}_2\text{-C kg}^{-1} \text{ h}^{-1}$, the average N_2O production at the end of the incubation was only $0.4 \text{ mg N}_2\text{O-N kg}^{-1}$ and the N_2O -to- CO_2 ratio was only 0.034. Therefore it seems that there had been no error in the first experiment.

The low amount of enzymes for reduction of NO_3^- can be explained by: (i) a soil specific inhibitory effect on the denitrification process (ii) the microbial biomass was not adapted to anaerobiosis and the small amount

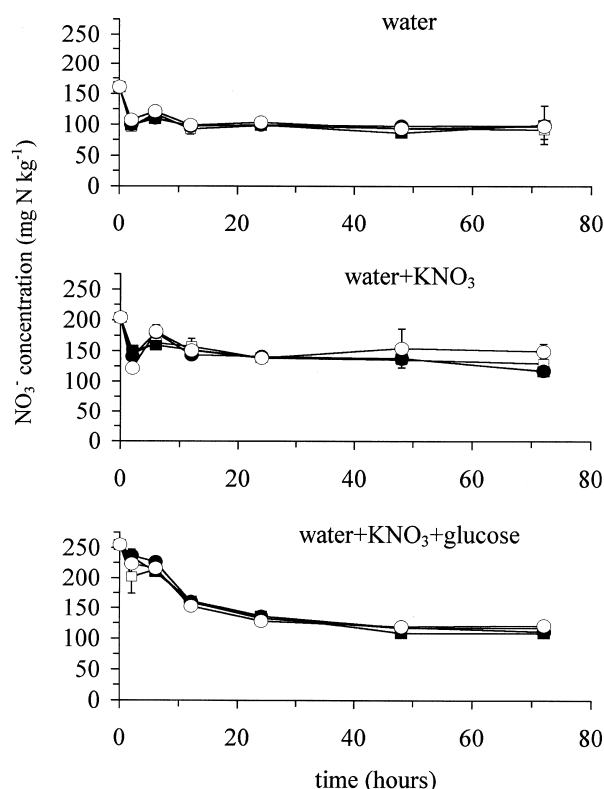


Fig. 4. The NO_3^- concentration ($\text{mg NO}_3^- \text{ kg}^{-1}$) in soil from the 0–20 layer of plot 1 of the Brimstone Farm experiment waterlogged for 28 d with or without $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ or $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ plus $100 \text{ mg glucose C kg}^{-1}$ and then anaerobically incubated for ca. 72 h at 25°C . Soil was amended with $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ soil and treatments were Control (—○—) CON, C_2H_2 (10 vol%) (—●—) ACE, $300 \text{ mg chloramphenicol kg}^{-1}$ (—□—) CHL and $300 \text{ mg chloramphenicol kg}^{-1}$ plus C_2H_2 (10 vol%) (—■—) CHA. Bars indicate plus and minus S.D.

of N_2O produced was related to the absence of previous anaerobic events (Dendooven et al., 1996b) (iii) there was insufficient NO_3^- in the soil prior to the experiment as NO_3^- is sometimes a prerequisite for de novo synthesis of NO_3^- reductase or (iv) there was a limited availability of C substrate for denitrification activity.

Experiment 2 tested these hypotheses by conditioning soil from plot 1 for 28 d at 25°C under waterlogged conditions with or without $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ or $50 \text{ mg NO}_3^- \text{ kg}^{-1}$ plus $100 \text{ mg glucose C kg}^{-1}$. It was clear from the production of N_2O in the soil waterlogged for 28 d that there was no soil specific inhibitory effect on the denitrification process (Fig. 3). The production of N_2O increased over time, reaching $40 \text{ mg N}_2\text{O-N kg}^{-1}$ in the C_2H_2 -amended soil after 72 h. The N_2O -to- CO_2 ratio was 0.56, a value similar to that (0.6) for a soil under permanent pasture (Dendooven and Anderson, 1994). Waterlogging a forest soil for 6 months in the field also increased the N_2O -to- CO_2 ratio but only from 0.08 to 0.31 (unpub-

lished data), probably because less C was available (Beauchamp et al., 1989).

The addition of either 50 mg NO_3^- -N kg^{-1} or 50 mg NO_3^- -N kg^{-1} plus 100 mg glucose C kg^{-1} before conditioning increased the N_2O -to- N_2 ratio, as indicated by the rates of N_2O production (Table 2) and by the fact that more electrons would be required to reduce NO_3^- to N_2 , from 1.84 in the unamended soil to 2.66 and 3.37, respectively. These changes in ratios are due to changes in N_2 production rather than in N_2O production, which was similar (Table 2). Addition of NO_3^- or glucose seems to have influenced the denitrifiers in such a way that it affected the reduction of N_2O (Blackmer and Bremner, 1978).

The patterns of decrease of NO_3^- and production of N_2O were similar for the soil samples amended with distilled water and 50 mg NO_3^- -N kg^{-1} . The concentration of NO_3^- decreased by ca. 60 mg NO_3^- -N kg^{-1} within 2 h (Fig. 4) but N_2O production was only 10 mg of N_2O -N. The NO_3^- concentration increased again after 6 h and then decreased and remained constant for the remainder of the incubation but the production of N_2O continued. However, the amount of N_2O + N_2 produced after 72 h was similar to the decrease in NO_3^- -N concentration. These changes in NO_3^- concentrations as well as an excess in uptake of NO_3^- in relation to the N_2O produced within the first few hours of anaerobicity also occurred in both pasture and forest soil (Dendooven et al., 1997). The patterns of decrease of NO_3^- and production of N_2O were not similar for the soil amended with 50 mg NO_3^- -N kg^{-1} plus 100 mg glucose C kg^{-1} , and the decrease of NO_3^- -N exceeded the N_2O produced even after 72 h. Immobilization of N could not account for this phenomenon as concentrations of NH_4^+ -N increased (data not shown). Dissimilatory reduction could neither account for the phenomenon as the increase in NH_4^+ was too small. This points at a capacity of denitrifiers to take-up NO_3^- (electron acceptor) in excess to the amount required by the oxidation of organic material (electron donor) (Ellis et al., 1996).

With a clay content of 55–60% and a very slowly permeable subsoil, the microorganisms in soil from the Brimstone Farm were assumed to be well adapted to anaerobic conditions, but its capacity in 1995/1996 to reduce NO_3^- to N_2O and N_2 was very limited, even when it was incubated under strict anaerobic conditions and supplied with NO_3^- . However, waterlogging the soil in the laboratory for 28 d caused the microbial community to change its functional characteristics by adapting to the anaerobic conditions. This adaptation did not occur in the field by the restricting winter drainage of the plots probably because of dry antecedent soil conditions resulting from the very low rainfall in summer 1995.

5. Conclusions

Our results show that in the clay-rich and slowly permeable soil at Brimstone Farm the rate of denitrification is likely to vary from almost negligible values to those typical of other clayey arable soils, as a result of antecedent moisture conditions. This probably explains the very different results obtained by Colbourn et al. (1984) and our similar experiment 1. It implies that it will be difficult to quantify the denitrification component of the N budget at this site unless autumn and winter rainfall are taken into account. Also it suggests that the effect of drainage restriction on winter leaching of NO_3^- is likely to vary from year to year, unless the effects of decreased mineralization and decreased drainflow greatly exceed that of increased denitrification.

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