



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

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Accepted 9 October 1998

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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.

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### 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.

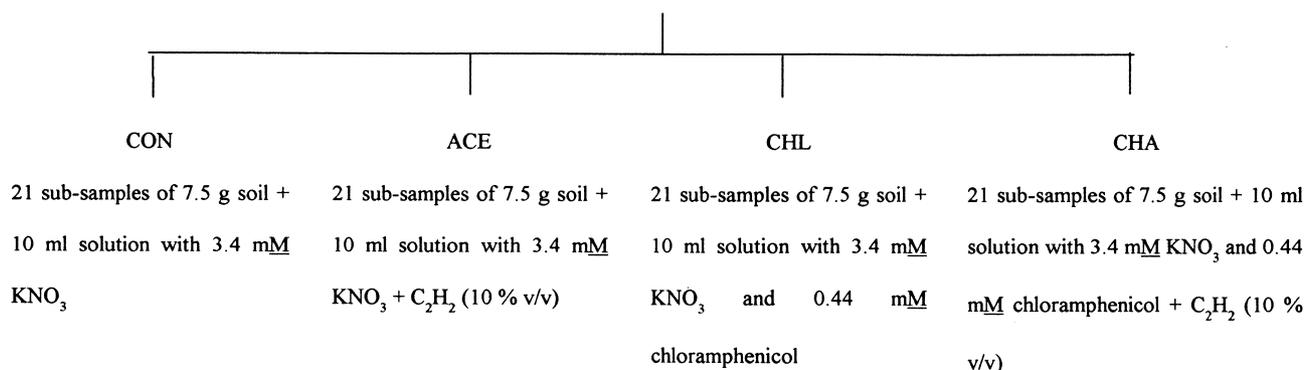
Our aim was to investigate the effect of the restricted drainage on the dynamics of the denitrification process. Soil was sampled at the beginning and end of the 1995/1996 winter drainage season from the 0–20 and 20–50 cm layers of two plots, one (plot 1) with restricted and the other (plot 15) with 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

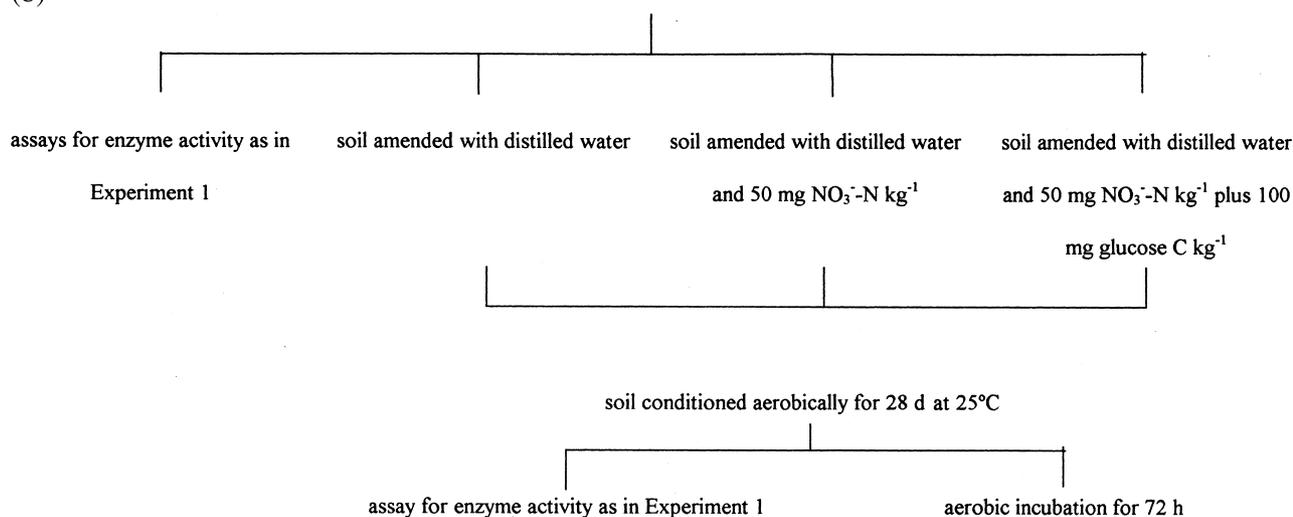


Fig. 1. Experimental procedure to investigate the effect of a restricted or unrestricted drainage on the denitrification process in soil from the Brimstone Farm and the effect of waterlogging the soil with or without the addition of 50 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> or 50 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> and 100 mg glucose C kg<sup>-1</sup>.

Production of CO<sub>2</sub> and N<sub>2</sub>O and the concentrations of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup>, 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<sub>(H<sub>2</sub>O)</sub> 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 NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> or 50 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> plus 100 mg glucose C kg<sup>-1</sup>.

## 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  $\text{KNO}_3$ , equivalent to approximately 50 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$ , 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  $\text{O}_2$ -free  $\text{N}_2$  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  $\text{C}_2\text{H}_2$  (ACE treatment) to give an approximate head-space concentration of 10%, which was sufficient to prevent the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Yoshinari and Knowles, 1976). The other 21 flasks were considered to be the control (CON) treatment.

Ten ml of a solution containing 34 mM  $\text{KNO}_3$  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  $\text{O}_2$ -free  $\text{N}_2$  to create an anaerobic atmosphere. Half of the samples were amended with  $\text{C}_2\text{H}_2$  (CHA treatments) while the unamended samples were considered the CHL treatment. The concentration of chloramphenicol was approximately 300 mg  $\text{kg}^{-1}$ , 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  $\text{NO}_2^-$  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  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{C}_2\text{H}_2$  by gas chromatography (GC) (Ai 93 chromatogram, Ai Cambridge).  $\text{N}_2\text{O}$  was determined on an electron capture detector and  $\text{CO}_2$  and  $\text{C}_2\text{H}_2$  on a thermal conductivity detector, using the method of Hall and Dowdell (1981). The concentrations of  $\text{CO}_2$

and  $\text{N}_2\text{O}$  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  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  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  $\text{H}_2\text{O}$  (b) a 10 mM solution of  $\text{KNO}_3$  and (c) a solution of 10 mM  $\text{KNO}_3$  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  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  dry soil in (b) or 50 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  dry soil and 100 mg glucose C  $\text{kg}^{-1}$  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  $\text{H}_2\text{O}$  to avoid desiccation and 100 ml of a 2 M NaOH solution to trap  $\text{CO}_2$  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  $\text{CO}_2$  and  $\text{N}_2\text{O}$  after approximately 2, 6, 12, 24, 48 and 72 h.

## 2.4. Statistical analysis

$\text{CO}_2$  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  $\text{CO}_2$  is produced at time zero. Production of  $\text{N}_2\text{O}$  under anaerobic conditions was analyzed in the same way. Production of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  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 ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) and  $\text{CO}_2$  and  $\text{N}_2\text{O}$  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  $\text{CO}_2$  ( $\mu\text{g C kg}^{-1} \text{h}^{-1}$ ) and  $\text{N}_2\text{O}$  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  $50 \text{ mg NO}_3^- \text{N kg}^{-1}$ , with or without  $\text{C}_2\text{H}_2$  and with or without chloramphenicol and incubated anaerobically for 72 h at  $25^\circ\text{C}$ . 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>CO<sub>2</sub> production (<math>\mu\text{g C kg}^{-1} \text{h}^{-1}</math>)</i>				
Control (CON)	709(39)	788(148)	1048(165)	749(85)
10 vol% $\text{C}_2\text{H}_2$ (ACE)	725(59)	795(121)	1131(175)	849(85)
300 mg $\text{kg}^{-1}$ chloramphenicol (CHL)	798(37)	826(134)	1092(172)	842(81)
10 vol% $\text{C}_2\text{H}_2$ + 300 mg chloramphenicol $\text{kg}^{-1}$ (CHA)	809(52)	781(119)	1066(174)	814(115)
<i>N<sub>2</sub>O production (<math>\mu\text{g N kg}^{-1} \text{h}^{-1}</math>)</i>				
Control (CON)	7.6(3.4)	14.4(12.8)	7.4(4.4)	42.6(10.1)
10 vol% $\text{C}_2\text{H}_2$ (ACE)	91.1(41.2)	4.8(0.8)	2.8(1.2)	24.7(6.9)
300 mg $\text{kg}^{-1}$ chloramphenicol (CHL)	1.8(0.8)	0.5(0.1)	21.1(18.4)	10.6(3.4)
10 vol% $\text{C}_2\text{H}_2$ + 300 mg chloramphenicol $\text{kg}^{-1}$ (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  $\text{CO}_2$  production in the 0–20 than in the 20–50 cm layer ( $P < 0.05$ ) and significantly greater  $\text{CO}_2$  production in plot 15 (unrestricted drainage) than in plot 1 (restricted drainage) ( $P < 0.01$ ) (Table 1). The addition of  $\text{C}_2\text{H}_2$  or chloramphenicol had no significant effects on production of  $\text{CO}_2$ .

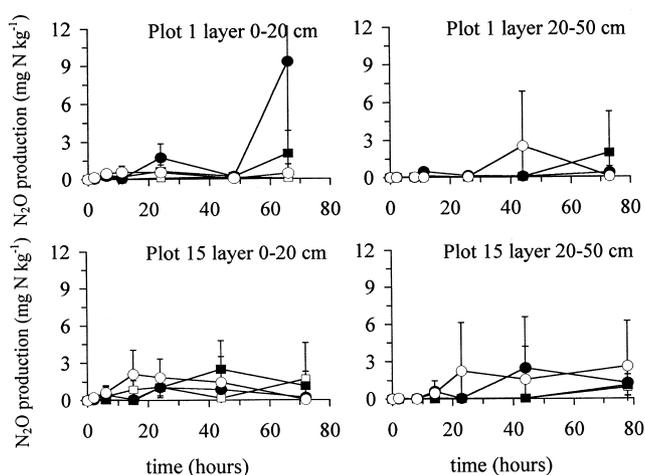


Fig. 2. The  $\text{N}_2\text{O}$  production ( $\text{mg N}_2\text{O-N kg}^{-1}$ ) in soil from the 0–20 and 20–50 cm layer of plots 1 and 15 of the Brimstone Farm experiment anaerobically incubated for ca. 72 h at  $25^\circ\text{C}$  following the application of  $50 \text{ mg NO}_3^- \text{N kg}^{-1}$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

Concentrations of  $\text{NO}_2^-$  were very variable (data not shown). There was no significant difference in  $\text{NO}_2^-$  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  $\text{C}_2\text{H}_2$  significantly decreased ( $P < 0.001$ ) and chloramphenicol significantly increased ( $P < 0.05$ ) the concentration of  $\text{NO}_2^-$ .

The concentration of  $\text{NO}_3^-$  significantly decreased over time in plot 15 ( $P < 0.0001$ ) but not in plot 1. The decreases in  $\text{NO}_3^-$  concentrations over time were similar in both soil layers. The additions of both  $\text{C}_2\text{H}_2$  and chloramphenicol had no significant effects on the decrease in the  $\text{NO}_3^-$  concentrations.

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

#### 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  $\text{C}_2\text{H}_2$  or chloramphenicol had no significant effects on production of  $\text{CO}_2$ ; the concentrations of  $\text{NO}_2^-$  were very variable; the concentration of  $\text{NO}_3^-$  did not significantly decrease over time and the  $\text{N}_2\text{O}$  production was very variable over time, with an average value of less than  $2 \text{ mg N}_2\text{O-N kg}^{-1}$  for all treatments (data not shown).

Conditioning the same soil for 28 d after it was amended with distilled  $\text{H}_2\text{O}$ , a 10 mM solution of

Table 2  
Rates of CO<sub>2</sub> ( $\mu\text{g C kg}^{-1} \text{ h}^{-1}$ ) and N<sub>2</sub>O 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 KNO<sub>3</sub> or a solution of 10 mM KNO<sub>3</sub> and 4 mM glucose conditioned aerobically for 28 d at 25°C. Soil was incubated aerobically or amended with 50 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup>, with or without C<sub>2</sub>H<sub>2</sub> and with or without chloramphenicol and incubated anaerobically for 72 h at 25°C

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

<sup>a</sup> Numbers in parentheses are standard errors of the estimates.

KNO<sub>3</sub> or a solution of 10 mM KNO<sub>3</sub> and 4 mM of glucose changed the characteristics of the denitrification process. The CO<sub>2</sub> production (average of all treatments) was significantly less in the soil amended with 50 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> than in the soil amended with distilled H<sub>2</sub>O or 50 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup> plus 100 mg glucose C kg<sup>-1</sup> ( $P < 0.001$ ) (Table 2). The application of C<sub>2</sub>H<sub>2</sub> or chloramphenicol had no significant effect on the production of CO<sub>2</sub>.

There were no significant differences in the production of N<sub>2</sub>O between soil samples amended with distilled H<sub>2</sub>O, a 10 mM solution of KNO<sub>3</sub> and a solution of 10 mM KNO<sub>3</sub> and 4 mM of glucose for the ACE, CHA and CHL treatments (Table 2). The N<sub>2</sub>O production in the CON treatment of the soil amended with distilled H<sub>2</sub>O was significantly less than in the soil amended with either a 10 mM solution of KNO<sub>3</sub> or a solution of 10 mM KNO<sub>3</sub> 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 CO<sub>2</sub> 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 N<sub>2</sub>O evolved and the N<sub>2</sub>O-to-CO<sub>2</sub> ratio in the assays for enzyme activity were less than those observed previously. The average amount of N<sub>2</sub>O produced at the end of the incubation was only 1.46 mg N<sub>2</sub>O-N kg<sup>-1</sup> compared with 12 mg N<sub>2</sub>O-N kg<sup>-1</sup> in the ACE treatment of the soil under Norway spruce, even though the CO<sub>2</sub> 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 N<sub>2</sub>O, no de novo synthesis of reduction enzymes occurred. The mean N<sub>2</sub>O-to-CO<sub>2</sub> 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 N<sub>2</sub>O was on average 38 mg N<sub>2</sub>O-N kg<sup>-1</sup> in the 0–20 cm layer and of 8 mg N<sub>2</sub>O-N kg<sup>-1</sup> in the 20–60 cm layer.

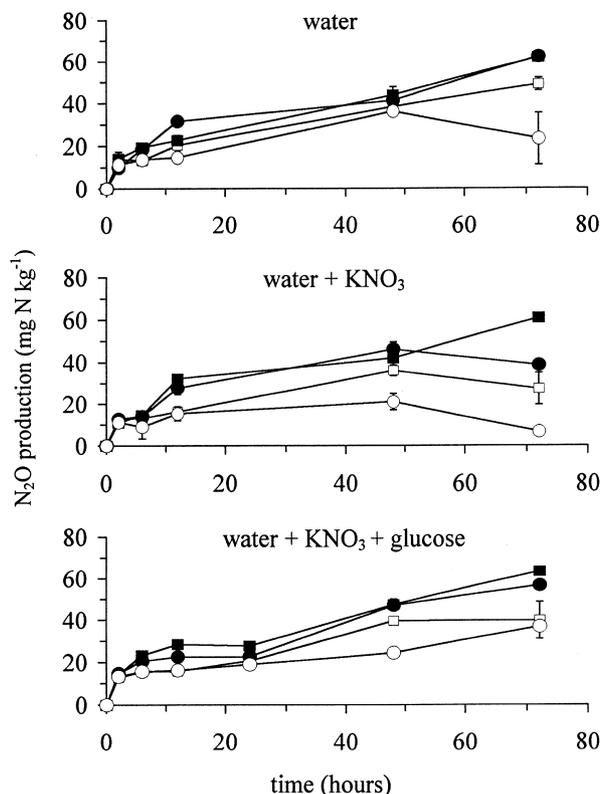


Fig. 3. The  $\text{N}_2\text{O}$  production ( $\text{mg N}_2\text{O-N kg}^{-1}$ ) in soil from the 0–20 layer of plot 1 of the Brimstone Farm experiment waterlogged for 28 d with or without  $50 \text{ mg NO}_3^- \text{ kg}^{-1}$  or  $50 \text{ mg NO}_3^- \text{ kg}^{-1}$  plus  $100 \text{ mg glucose C kg}^{-1}$  and then anaerobically incubated for ca. 72 h at  $25^\circ\text{C}$ . Soil was amended with  $50 \text{ mg NO}_3^- \text{ N kg}^{-1}$  soil and treatments were Control (—○—) CON,  $\text{C}_2\text{H}_2$  (10 vol%) (—●—) ACE,  $300 \text{ mg chloramphenicol kg}^{-1}$  (—□—) CHL and  $300 \text{ mg chloramphenicol kg}^{-1}$  plus  $\text{C}_2\text{H}_2$  (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  $\text{N}_2\text{O}$  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  $\text{CO}_2$  production rate was on average  $1.18 \text{ mg CO}_2\text{-C kg}^{-1} \text{ h}^{-1}$ , the average  $\text{N}_2\text{O}$  production at the end of the incubation was only  $0.4 \text{ mg N}_2\text{O-N kg}^{-1}$  and the  $\text{N}_2\text{O}$ -to- $\text{CO}_2$  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  $\text{NO}_3^-$  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

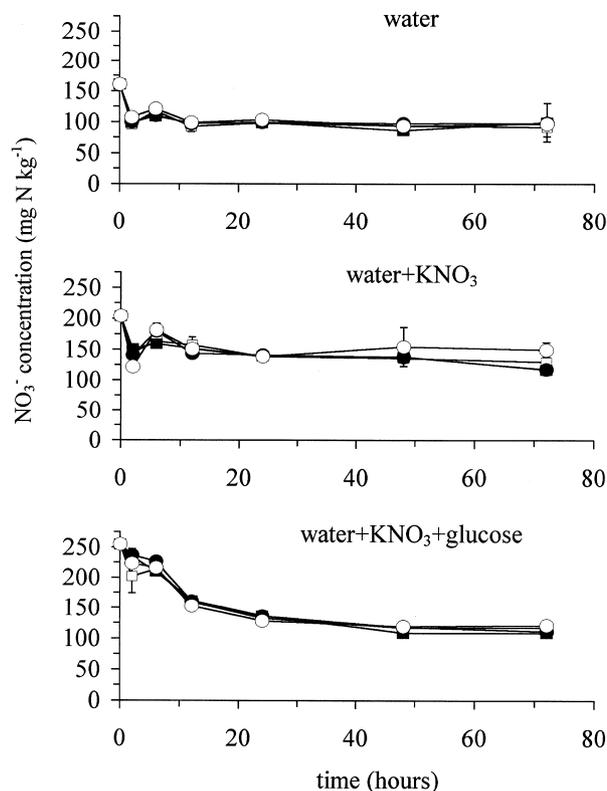


Fig. 4. The  $\text{NO}_3^-$  concentration ( $\text{mg NO}_3^- \text{ N kg}^{-1}$ ) in soil from the 0–20 layer of plot 1 of the Brimstone Farm experiment waterlogged for 28 d with or without  $50 \text{ mg NO}_3^- \text{ kg}^{-1}$  or  $50 \text{ mg NO}_3^- \text{ kg}^{-1}$  plus  $100 \text{ mg glucose C kg}^{-1}$  and then anaerobically incubated for ca. 72 h at  $25^\circ\text{C}$ . Soil was amended with  $50 \text{ mg NO}_3^- \text{ N kg}^{-1}$  soil and treatments were Control (—○—) CON,  $\text{C}_2\text{H}_2$  (10 vol%) (—●—) ACE,  $300 \text{ mg chloramphenicol kg}^{-1}$  (—□—) CHL and  $300 \text{ mg chloramphenicol kg}^{-1}$  plus  $\text{C}_2\text{H}_2$  (10 vol%) (—■—) CHA. Bars indicate plus and minus S.D.

of  $\text{N}_2\text{O}$  produced was related to the absence of previous anaerobic events (Dendooven et al., 1996b) (iii) there was insufficient  $\text{NO}_3^-$  in the soil prior to the experiment as  $\text{NO}_3^-$  is sometimes a prerequisite for de novo synthesis of  $\text{NO}_3^-$  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  $25^\circ\text{C}$  under waterlogged conditions with or without  $50 \text{ mg NO}_3^- \text{ N kg}^{-1}$  or  $50 \text{ mg NO}_3^- \text{ N kg}^{-1}$  plus  $100 \text{ mg glucose C kg}^{-1}$ . It was clear from the production of  $\text{N}_2\text{O}$  in the soil waterlogged for 28 d that there was no soil specific inhibitory effect on the denitrification process (Fig. 3). The production of  $\text{N}_2\text{O}$  increased over time, reaching  $40 \text{ mg N}_2\text{O-N kg}^{-1}$  in the  $\text{C}_2\text{H}_2$ -amended soil after 72 h. The  $\text{N}_2\text{O}$ -to- $\text{CO}_2$  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  $\text{N}_2\text{O}$ -to- $\text{CO}_2$  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  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  or 50 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  plus 100 mg glucose C  $\text{kg}^{-1}$  before conditioning increased the  $\text{N}_2\text{O}$ -to- $\text{N}_2$  ratio, as indicated by the rates of  $\text{N}_2\text{O}$  production (Table 2) and by the fact that more electrons would be required to reduce  $\text{NO}_3^-$  to  $\text{N}_2$ , from 1.84 in the unamended soil to 2.66 and 3.37, respectively. These changes in ratios are due to changes in  $\text{N}_2$  production rather than in  $\text{N}_2\text{O}$  production, which was similar (Table 2). Addition of  $\text{NO}_3^-$  or glucose seems to have influenced the denitrifiers in such a way that it affected the reduction of  $\text{N}_2\text{O}$  (Blackmer and Bremner, 1978).

The patterns of decrease of  $\text{NO}_3^-$  and production of  $\text{N}_2\text{O}$  were similar for the soil samples amended with distilled water and 50 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$ . The concentration of  $\text{NO}_3^-$  decreased by ca. 60 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  within 2 h (Fig. 4) but  $\text{N}_2\text{O}$  production was only 10 mg of  $\text{N}_2\text{O}$ -N. The  $\text{NO}_3^-$  concentration increased again after 6 h and then decreased and remained constant for the remainder of the incubation but the production of  $\text{N}_2\text{O}$  continued. However, the amount of  $\text{N}_2\text{O}$  +  $\text{N}_2$  produced after 72 h was similar to the decrease in  $\text{NO}_3^-$ -N concentration. These changes in  $\text{NO}_3^-$  concentrations as well as an excess in uptake of  $\text{NO}_3^-$  in relation to the  $\text{N}_2\text{O}$  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  $\text{NO}_3^-$  and production of  $\text{N}_2\text{O}$  were not similar for the soil amended with 50 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  plus 100 mg glucose C  $\text{kg}^{-1}$ , and the decrease of  $\text{NO}_3^-$ -N exceeded the  $\text{N}_2\text{O}$  produced even after 72 h. Immobilization of N could not account for this phenomenon as concentrations of  $\text{NH}_4^+$ -N increased (data not shown). Dissimilatory reduction could neither account for the phenomenon as the increase in  $\text{NH}_4^+$  was too small. This points at a capacity of denitrifiers to take-up  $\text{NO}_3^-$  (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  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  and  $\text{N}_2$  was very limited, even when it was incubated under strict anaerobic conditions and supplied with  $\text{NO}_3^-$ . 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  $\text{NO}_3^-$  is likely to vary from year to year, unless the effects of decreased mineralization and decreased drainflow greatly exceed that of increased denitrification.

## Acknowledgements

We thank Professor D.S. Powlson for comments on an early draft of this paper, K.R. Howse for technical assistance, Dr. L. Duchateau for statistical advice, Mrs. J. Day for total C and N analysis and M. Howe for analytical analysis of inorganic N. The research of LD was funded by the EC–Copernicus Project IACR receives grant-aided support from BBSRC, UK.

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