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## Denitrification in intact subsoil cores

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**Abstract** Intact, unamended subsoil cores taken from two contrasting field sites were incubated in the laboratory at 12 °C under aerobic (21% O<sub>2</sub>) and anaerobic (1.1–5.5% O<sub>2</sub>) conditions. Denitrification of <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> diffusing into the cores across a semi-permeable membrane was estimated by: (1) direct observation of <sup>15</sup>N-labelled N<sub>2</sub> and N<sub>2</sub>O, and (2) mass balance following sectioning at the end of the experiment. The in situ denitrification potential (rates where the supply of NO<sub>3</sub><sup>-</sup> is not limited by diffusion) was estimated using a finite-difference approximation to a diffusion reaction equation based on comparison of time and depth profiles of NO<sub>3</sub><sup>-</sup> and Br<sup>-</sup> in the incubated cores. Potentials between 0.2 and 2.5 mg N kg<sup>-1</sup> day<sup>-1</sup> were obtained under low O<sub>2</sub> and natural C concentrations. These results indicate the potential for denitrification in glacial till subsoils to reduce NO<sub>3</sub><sup>-</sup> leaching to ground or surface waters to levels unlikely to result in a pollution hazard. The major product of NO<sub>3</sub><sup>-</sup> reduction in these subsoils was observed to be N<sub>2</sub>, rather than the greenhouse gas and catalyst of stratospheric O<sub>3</sub> removal, N<sub>2</sub>O.

**Key words** Subsoil · Denitrification · Nitrogen-15 · Nitrous oxide · Natural carbon concentrations

### Introduction

Denitrification is commonly thought to be an insignificant mechanism for the reduction of NO<sub>3</sub><sup>-</sup> below a soil depth of about 1.6 m (e.g. Parkin and Meisinger 1989),

although N<sub>2</sub>O production has been observed during anoxic incubation of soil samples from as deep as 20 m (Lind and Eiland 1989; Yeomans et al. 1992). The denitrifying ability of micro-organisms generally decreases with increasing depth in the absence of amendment with a source of oxidisable organic C (e.g. McCarty and Bremner 1992). A source of available organic C is generally considered to be the major factor limiting denitrification in subsoils (Lind and Eiland 1989; Yeomans et al. 1992; Weier et al. 1993a). Where organic C is added, a significant denitrifying potential may be revealed at depths as great as 7 m (McCarty and Bremner 1992; Jarvis and Hatch 1994).

A previously reported attempt to compile N budgets for drained plots of two contrasting soil types (Vinten et al. 1994) showed lower than expected leaching losses with little evidence of denitrification in the topsoil. One possible explanation for this is that some of the NO<sub>3</sub><sup>-</sup> was denitrified during transport through subsoil horizons. Laboratory incubations were set up using a diffusion-cell technique to investigate the extent of denitrification in intact subsoil cores from these sites. No C amendments were made; the focus of interest being the potential for in situ reduction of NO<sub>3</sub><sup>-</sup> without the addition of C.

### Materials and methods

#### Subsoil sampling

Subsoil was sampled from two field sites on the Bush Estate near Edinburgh: Glencorse Mains, a clay loam topsoil overlying glacial till; and No. 3 Field, a sandy loam topsoil overlying partially sorted glacial till. Two cores from each site, from 1 m to 2 m depth, were taken using a Stiboka (88 mm diameter) corer and percussion hammer. A smaller diameter (63 mm) core sampler, with a perspex insert of 1 m length, and percussion hammer was then used to core from 1 m to 2 m depth. The perspex insert within the corer allowed subsoil to be removed as intact cores. The sleeving and cores were sliced into 20 cm sections, sealed in polythene bags to prevent aeration and stored at 4 °C.

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## Slurry Incubations

The potential for denitrification was first assessed using slurries of both subsoils. Grad tubes containing either 20 g of field moist subsoil from Glencorse Mains (129–156 cm) or No. 3 Field (133–166 cm) were slurried with 20 ml of 20 µg N ml<sup>-1</sup> (KNO<sub>3</sub>). The tubes were made airtight with greased seals (Suba Seal 49). One set of duplicate tubes was left aerobic and the second set made anaerobic by flushing with N<sub>2</sub> for 2 min while being agitated. All slurries had 6 ml of headspace (approximately 10% volume gas phase) replaced with C<sub>2</sub>H<sub>2</sub> to prevent the reduction of N<sub>2</sub>O to N<sub>2</sub> and permit the total denitrification rate to be estimated by the rate of N<sub>2</sub>O production (Ryden et al. 1987). The C<sub>2</sub>H<sub>2</sub> gas was first passed through concentrated H<sub>2</sub>SO<sub>4</sub> and distilled H<sub>2</sub>O to remove traces of CH<sub>3</sub>COCH<sub>3</sub> (Walter et al. 1979).

Subsoil slurries were incubated in the dark for 16 days, in a shaking incubator to minimise diffusional limitations. The slurries were incubated at 12 ± 1 °C to replicate the in situ soil temperature at 1–2 m depth during the months when fertiliser NO<sub>3</sub><sup>-</sup> may have leached into the subsoil. Headspace was sampled regularly and analysed for N<sub>2</sub>O and CO<sub>2</sub>. Following the first and second gas samplings the volume of sample removed was replaced with the correct proportion of air/N<sub>2</sub> and C<sub>2</sub>H<sub>2</sub>.

## Intact core incubations

In situ rates and denitrification potentials were investigated using intact cores. Both ends of the 20-cm core sections were cut off to remove soil which may have been aerated during sectioning and storage, leaving cores approximately 12 cm in length. Soil from the discarded core ends was analysed for mineral N, water soluble organic C (WSOC), organic C, Br<sup>-</sup> and pH. These data appear in Table 1.

The bottom end of each core was sealed with a rubber bung. At the top end of the core a thin layer of silica flour and a hydrophilic membrane (Millipore GV filter, 0.22 µm pore size) separated the subsoil from the head chamber. The silica flour allowed good contact between the subsoil and the membrane. The hydrophilic membrane acted as a barrier to mass flow of H<sub>2</sub>O and gas, while allowing diffusion of dissolved gases and ions between the subsoil and the head chamber. The head chamber consisted of a 250-ml plastic bottle, with two three-way taps inserted through the container walls to allow gas and solution sampling. It was attached to the perspex sleeve of the core with araldite, which was allowed to dry for 24 h in a fume cupboard before 200 ml distilled H<sub>2</sub>O was added to the head chamber. The headspace was then left at ambient O<sub>2</sub> or flushed with N<sub>2</sub> and sealed. The headspace

was sampled and analysed for O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub>O over the following 3 weeks. There was no sign of gas leaking into or out of the cores, and there was no increase in the rate of CO<sub>2</sub> respiration which might have been attributable to the use of araldite as a C source.

The distilled water was replaced with 200 ml of stock solution [100 µg Br<sup>-</sup> ml<sup>-1</sup> and 20 µg N ml<sup>-1</sup> (99.6 atom %, <sup>15</sup>N-KNO<sub>3</sub>)]. The cores were incubated in a dark incubator at 12 ± 1 °C. The cores were continuously shaken to minimise diffusive barriers to gas and solute exchange between the head chamber and the soil core surface. The headspace and head solution were analysed weekly. A syringe was used to remove 3 ml of head solution for NO<sub>3</sub><sup>-</sup>-N and Br<sup>-</sup> analysis, while gas samples were taken for analysis of N<sub>2</sub>O, CO<sub>2</sub>, O<sub>2</sub> and <sup>15</sup>N-labelled denitrified gases. The O<sub>2</sub> concentration in the headspace of the anaerobic incubations increased slightly over the course of the incubation, while that of the aerobic incubations generally decreased (Table 1).

After 6 weeks the incubation was stopped. The head solution was removed, and analysed for NO<sub>3</sub><sup>-</sup>-N and Br<sup>-</sup>. Soil cores were sliced into 2-cm sections and each section was analysed for mineral N, Br<sup>-</sup> and WSOC. Soil physical analyses are shown in Table 1.

## Gas sampling and analysis

Gas samples were analysed by gas chromatography, using an electron capture detector for N<sub>2</sub>O and a thermal conductivity detector for O<sub>2</sub> and CO<sub>2</sub>. The <sup>15</sup>N content of headspace gas (N<sub>2</sub>O + N<sub>2</sub>) was analysed as described by Arah et al. (1993), with calculations of pool enrichment and denitrified gas fluxes based on the ion current ratios of the 28, 29 and 30 N<sub>2</sub> isotopes (Arah 1992).

## Soil and head solution analysis

Soil was analysed for mineral N following extraction in 1M KCl to give a 1:5 ratio of fresh soil:extractant. Extractions were shaken for 1 h and filtered (Whatman 42 filter papers). Concentrations of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N in the KCl extractions and head solution samples were determined by continuous flow analysis using the methods of Henriksen and Selmer-Olsen (1970), using CuSO<sub>4</sub> and N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>SO<sub>4</sub> as a reducing reagent in place of Cd, and Crooke and Simpson (1971), respectively. Br<sup>-</sup> was extracted from the soil with a 1:3 ratio of fresh soil:distilled H<sub>2</sub>O. Extractions were shaken and filtered as with the above mineral N extractions. The Br<sup>-</sup> in extractions and head solutions was analysed using an Orion Br<sup>-</sup> electrode with a 90.01 single junction reference elec-

**Table 1** Intact core incubations. Initial pH, total C and water soluble organic C (WSOC) concentrations and headspace O<sub>2</sub> concentration. Final headspace O<sub>2</sub> concentration, WSOC, dry bulk density, total porosity and volumetric moisture content

Subsoil	Glencorse Mains			No. 3 Field		
	100–130	131–165	166–200	100–130	131–165	166–200
<b>Initial values</b>						
pH (H <sub>2</sub> O)	7.5	8.0	8.6	6.5	6.6	6.6
Organic C (%)	0.81	0.60	0.78	0.68	0.50	0.67
WSOC (mg litre <sup>-1</sup> )	87	132	76	92	76	74
Headspace O <sub>2</sub> – aerobic (%)	21.2	20.2	22.8	20.4	19.6	21.6
Headspace O <sub>2</sub> – anaerobic (%)	1.4	1.1	2.6	1.8	1.5	1.3
<b>Final values</b>						
Headspace O <sub>2</sub> – aerobic (%)	20.1	16.1	19.6	21.4	15.2	19.3
Headspace O <sub>2</sub> – anaerobic (%)	4.3	5.5	5.5	3.0	2.3	2.6
WSOC (mg litre <sup>-1</sup> )	95	115	90	109	147	83
<b>Soil physics</b>						
Dry bulk density (g cm <sup>-3</sup> )	1.76	1.79	1.96	1.70	1.73	1.80
Total porosity (%)	34	33	26	36	35	32
Volumetric moisture content (%)	26	27	22	30	30	27

trode. Water-soluble organic C was extracted in a 1:2 ratio of fresh soil:distilled H<sub>2</sub>O. Following shaking for 15 min the extractant was centrifuged for 20 min at 4000 rpm. The supernatant was decanted, and prior to analysis, acidified with H<sub>3</sub>PO<sub>4</sub>, sparged for 5–6 min to remove inorganic C, and filtered through 0.5- $\mu$ m filter units (Millipore Millex-LCR). Filtered samples were analysed on a Dohrman DC-80 TOC analyser. Organic C was determined on dried and milled soils by the Walkley and Black method (Nelson and Sommers 1982). Soil pH was determined in distilled H<sub>2</sub>O.

## Results

Means and SE for slurry incubations are from non-transformed data because of the small sample size ( $n=2$ ). In intact core incubations, N<sub>2</sub>O and denitrified gas concentrations and rates were log-normalised, prior to correlation and one-way analysis of variance.

### Slurry incubations

Mean denitrification rates in slurried subsoils are shown in Table 2. Anaerobic incubations denitrified faster (produced more N<sub>2</sub>O) and more efficiently (more N<sub>2</sub>O per unit of CO<sub>2</sub>) than aerobic incubations. No. 3 Field showed more activity than Glencorse Mains. This

data demonstrates that a potential for denitrification exists in these soils. This was investigated further using intact cores.

### Intact subsoil core incubations

#### Gaseous emissions

Time courses of the evolution of N<sub>2</sub>O and total denitrified gas are shown in Fig. 1. Mean N<sub>2</sub>O emissions and those of CO<sub>2</sub> are given in Table 3. Significantly ( $P<0.01$ ) more N<sub>2</sub>O was emitted from anaerobic than from aerobic cores, the difference being around one order of magnitude. Emissions of denitrified gas measured by mass spectrometry (DEN-MS) were also significantly greater ( $P<0.01$ ) from anaerobic cores. The estimates of mean N<sub>2</sub>O loss and DEN-MS (Table 3) almost certainly underestimated true denitrification losses as N<sub>2</sub>O is only one of the possible products of denitrification, mass spectrometric calculations inevitably underestimate the true flux (Arah 1992), and both methods are vulnerable to leakage.

Denitrified gas emissions from the subsoil cores (Table 3) were generally lower than the potential denitrification rates observed in the slurries (Table 2), with two

**Table 2** Mean rates of denitrification and CO<sub>2</sub> production in aerobic and anaerobic subsoil slurries

Subsoil	Glencorse Mains		No. 3 Field	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Denitrification ( $\mu\text{g N}_2\text{O-N kg}^{-1}$ dry soil day <sup>-1</sup> )	34	87	40	156
CO <sub>2</sub> production ( $\mu\text{g CO}_2\text{-C kg}^{-1}$ dry soil day <sup>-1</sup> )	231	1769	12000	978

**Table 3** Mean rates from anaerobic (N<sub>2</sub>) and aerobic (O<sub>2</sub>) incubations of intact subsoil cores of: observed N<sub>2</sub>O; observed denitrified gas (DEN-MS); denitrification by mass balance of N (DEN-

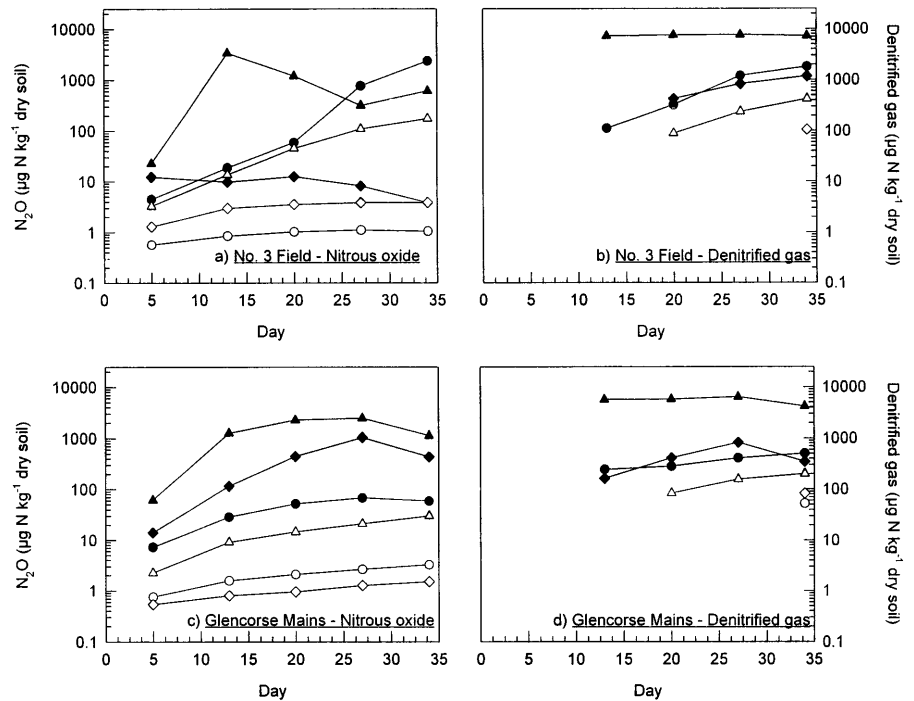
MB); estimated potential NO<sub>3</sub><sup>-</sup> reduction rate (Q); and CO<sub>2</sub> production. ND=Denitrified gas concentration below mass spectrometer detection limit

Subsoil cores	N <sub>2</sub> O ( $\mu\text{g N kg}^{-1}$ day <sup>-1</sup> )	DEN-MS ( $\mu\text{g N kg}^{-1}$ day <sup>-1</sup> )	DEN-MB ( $\mu\text{g N kg}^{-1}$ day <sup>-1</sup> )	Q ( $\mu\text{g N kg}^{-1}$ day <sup>-1</sup> )	CO <sub>2</sub> ( $\mu\text{g C kg}^{-1}$ day <sup>-1</sup> )
No. 3 Field					
N <sub>2</sub> 1a (100–130cm)	69	53	38	733	224
1b (131–165 cm)	108	224	123	>2490 <sup>a</sup>	299
1c (166–200 cm)	0.5	35	32	377	217
O <sub>2</sub> 2a (100–130 cm)	0.02	ND	13	227 <sup>b</sup>	115
2b (131–165 cm)	5.1	12	37	169 <sup>b</sup>	398
2c (166–200 cm)	0.10	3.1	31	142 <sup>b</sup>	155
Glencorse Mains					
N <sub>2</sub> 1a (100–130cm)	2.0	15	36	186	159
1b (131–165 cm)	73	201	115	>2582 <sup>a</sup>	337
1c (166–200 cm)	30	30	21	68	80
O <sub>2</sub> 2a (100–130 cm)	0.08	1.5	7	141 <sup>b</sup>	131
2b (131–165 cm)	0.87	5.9	22	32	267
2c (166–200 cm)	0.03	2.4	25	100 <sup>b</sup>	77

<sup>a</sup> Potential denitrification rate may be underestimated

<sup>b</sup> Potential denitrification rate may be overestimated, rates based on NO<sub>3</sub><sup>-</sup>-N in soil

**Fig. 1a–d** Concentration of  $N_2O$  and denitrified gas emitted from anaerobic and aerobic incubations of intact subsoil cores from No. 3 Field (**a, b**) and Glencorse Mains (**c, d**). Cores for anaerobic (filled symbols) and aerobic (open symbols) incubations were taken from 100–130 cm (●), 131–165 cm (▲) and 166–200 cm (◆) depth. Denitrified gas concentrations in No. 3 Field core 2a were below the detection limit of the mass spectrometer



exceptions (No. 3 Field core 1b and Glencorse Mains core 1b; both anaerobic incubations). Under aerobic conditions, 0.01–8% of the potential denitrification measured in the slurries was expressed, while in anaerobic incubations 8–231% was expressed. Rates of  $CO_2$  emissions from subsoil cores were 4–41% in anaerobic cores, and 1–135% in aerobic cores of those observed in the subsoil slurry incubations. Only Glencorse Mains core 2b expressed a higher mean  $CO_2$  production rate than that observed in the equivalent subsoil slurry. Patterns of  $CO_2$  emission were similar for both subsoils, suggesting that microbial activity was unaffected by soil type or aeration status (Table 3). From Table 1 it can be seen that the cores with the highest WSOC (No. 3 Field core 1b and Glencorse Mains core 1b) also had high denitrification rates and  $CO_2$  emissions. However, there was no significant correlation between either soil WSOC and organic C concentration, or  $CO_2$  production and denitrification.

### Mass balance

An independent estimate of total denitrification was obtained from the N and  $Br^-$  mass balance at the end of the incubation:

$$^{15}N_{den} = \frac{^{15}N_{init} - ^{15}N_{rec} \left( \frac{Br_{init}^-}{Br_{rec}^-} \right)}{t} \quad (1)$$

where  $^{15}N_{init}$  and  $Br_{init}^-$  are equal to the total N and  $Br^-$  present in and applied to the cores before incubation,  $^{15}N_{rec}$  and  $Br_{rec}^-$  are the total  $^{15}N$  and  $Br^-$  recov-

ered at the end of incubation in the headspace, head solution and soil, and  $t$  is the incubation time in days.  $^{15}N_{den}$  is the mass balance estimate of denitrification loss, referred to in Table 3 and throughout the rest of this paper as DEN-MB. Equation 1 assumes that  $Br^-$  is inert, so that at the end of the incubation it has to be present in either the subsoil core or the head solution. Estimates of DEN-MB were significantly greater than observed  $N_2O$  losses ( $P < 0.01$ ) and DEN-MS ( $P < 0.05$ ). There was little difference between aerobic and anaerobic cores ( $P < 0.50$ ). However, DEN-MB rates probably overestimate denitrification due to the basic assumption that all the missing  $NO_3^-$  is denitrified, taking no account of the possibility of microbial immobilisation. The true denitrification rate probably lies somewhere between DEN-MS and DEN-MB.

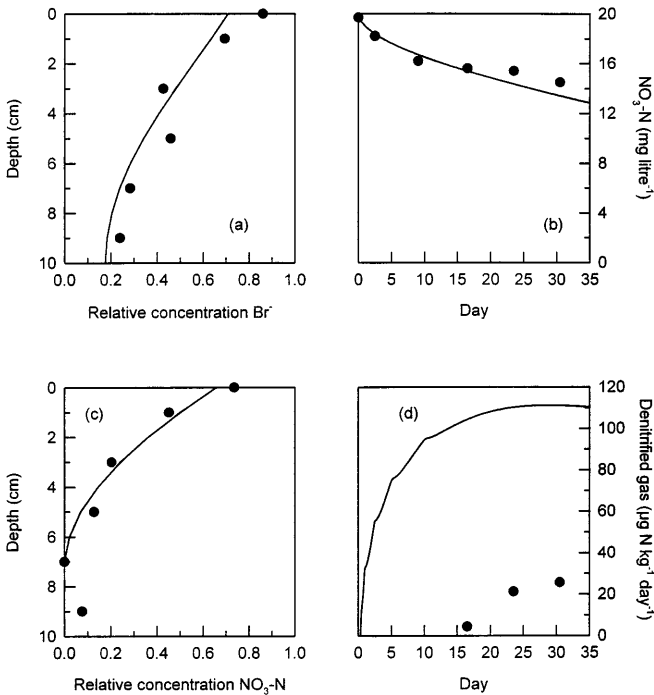
Denitrification accounted for 0–2% of the initial N in No. 3 Field aerobic cores, 7–47% in anaerobic cores, 0.3 to 1.4% in Glencorse Mains aerobic cores, and 3–32% in anaerobic cores. Approximately 4 times as much of the initial N was denitrified during the anaerobic incubation of slurries and intact cores compared to during the aerobic incubation.

### Denitrification potential

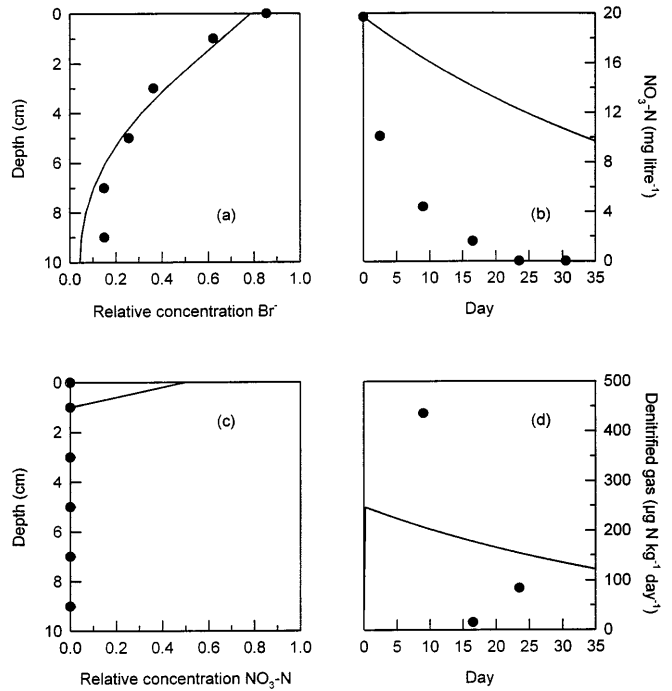
DEN-MS and DEN-MB estimate denitrification rates in the whole core during the incubation period. In practice, denitrification will only occur in the portion of the core into which  $NO_3^-$  has diffused. The rate of in situ denitrification potentials was calculated by parameter fitting, using a finite-difference approximation of Fick's second law, with a zero-order reaction term:

$$\frac{\delta y}{\delta t} = D \frac{\delta^2 y}{\delta z^2} - QB_d \quad (2)$$

where  $y$  is the bulk concentration ( $\mu\text{g m}^{-3} \text{Br}^-$  or  $\text{NO}_3^-$ ),  $t$  the time (day),  $D$  the bulk diffusion constant ( $\text{m}^2 \text{day}^{-1}$ ),  $z$  the depth (m),  $Q$  the consumption rate ( $\mu\text{g kg}^{-1} \text{day}^{-1}$ ) and  $B_d$  the soil dry bulk density ( $\text{kg m}^{-3}$ ).  $Q$  is taken to be zero for  $\text{Br}^-$  and equal to the denitrification potential for  $\text{NO}_3^-$  wherever  $\text{NO}_3^-$  is present. Diffusion of  $\text{Br}^-$  down the soil profile was fitted to the curve of observed  $\text{Br}^-$  concentration, and used to obtain the  $\text{Br}^-$  diffusion coefficient for each subsoil core. The  $\text{NO}_3^-$  diffusion coefficient was then determined using the self-diffusion coefficients in aqueous solution at  $25^\circ\text{C}$  of  $\text{Br}^-$  and  $\text{NO}_3^-$ , assuming a ratio of 1.17 for the  $\text{Br}^-:\text{NO}_3^-$  diffusion coefficient ratio. The denitrification potential  $Q$  was then estimated by curve-fitting to the time course of the  $\text{NO}_3^-$  concentration in the head solution, assuming uniform dispersal of  $\text{NO}_3^-$  down the subsoil core at the calculated diffusion rate (Figs. 2, 3). In some aerobic cores the pattern of  $\text{NO}_3^-$



**Fig. 2a-d** Observed (●) and estimated (—) reaction rate parameters in diffusion limited denitrification in aerobic incubation of an intact subsoil core from No. 3 Field (131–165 cm). **a**  $\text{Br}^-$  diffusion through the core relative to the initial concentration of  $\text{Br}^-$  added to the core ( $D_{Br}$ ). 0 depth is the final concentration of the head solution.  $D_{Br}=0.55 \text{ cm}^2 \text{ day}^{-1}$ , estimated potential  $\text{NO}_3^-$  reduction rate ( $Q$ )=0. **b**  $\text{NO}_3^- \text{N}$  concentration in the head solution of the subsoil core, using optimised parameter values for  $D$  and  $Q$  – see (c). **c**  $\text{NO}_3^- \text{N}$  diffusion through the subsoil core relative to the initial concentration of  $\text{NO}_3^- \text{N}$  added to the core ( $D_{NO_3}$ ). 0 depth is the final concentration of the head solution.  $D_{NO_3}=0.47 \text{ cm}^2 \text{ day}^{-1}$ ,  $Q=169 \mu\text{g N kg}^{-1} \text{ day}^{-1}$ . **d** Denitrification in the subsoil core using optimised parameter values for  $D$  and  $Q$  – see (c)



**Fig. 3a-d** Observed (●) and estimated (—) reaction rate parameters in diffusion limited denitrification in anaerobic incubation of an intact subsoil core from Glencorse Mains (131–165 cm). **a**  $\text{Br}^-$  diffusion through the core relative to the initial concentration of  $\text{Br}^-$  added to the core. 0 depth is the final concentration of the head solution.  $D_{Br}=0.28 \text{ cm}^2 \text{ day}^{-1}$ ,  $Q=0$ . **b**  $\text{NO}_3^- \text{N}$  concentration in the head solution of the subsoil core.  $D_{NO_3}=0.24 \text{ cm}^2 \text{ day}^{-1}$ ,  $Q=2582 \mu\text{g N kg}^{-1} \text{ day}^{-1}$ . **c**  $\text{NO}_3^- \text{N}$  diffusion through the subsoil core relative to the initial concentration of  $\text{NO}_3^- \text{N}$  added to the core, using optimised parameter values for  $D$  and  $Q$  see (b). 0 depth is the final concentration of the head solution. **d** Denitrification in the subsoil core using optimised parameter values for  $D$  and  $Q$  – see (b). For abbreviations, see Fig. 2

loss from the head solution was too close to that of diffusion only for a reliable estimate of  $Q$  to be made (Fig. 2). In these cases  $Q$  was better estimated by curve-fitting to the final  $\text{NO}_3^-$  concentration profile in the core. In some cores (No. 3 Field core 1b and Glencorse Mains core 1b) nearly all the headspace  $\text{NO}_3^-$  disappeared, so parameter estimates became indeterminate, and only a lower limit to the values of  $Q$  can be given. The high denitrification rates observed in these two cores coincide with high WSOC concentrations (Table 1). Figure 2 and 3 show specimen-measured and simulated data for cores from No. 3 Field and Glencorse Mains, respectively. Simulations of the time-course of gaseous emission are relatively poor since the model takes no account of the time required for gaseous denitrification products to diffuse through the core to the headspace.

Results of this parameter estimation exercise are given in Table 3. Values of  $Q$  were significantly higher than  $\text{N}_2\text{O}$  emission rates ( $P<0.05$ ,  $P<0.001$ ) and DEN-MS ( $P<0.05$ ,  $P<0.001$ ) for anaerobic and aerobic incubations, respectively. Values of  $Q$  corresponded stoi-

chiometrically with the emission rate of CO<sub>2</sub> reasonably well. The high denitrification rates observed in cores 1b from No. 3 Field and 1b from Glencorse Mains again coincided with high WSOC concentrations, indicating increased denitrification due to the presence of higher levels of C than in the other cores (Table 1).

## Discussion

Potential denitrification rates as high as 103 and 672 µg N<sub>2</sub>O-N kg<sup>-1</sup> dry soil day<sup>-1</sup> were observed in slurries of subsoil from Glencorse Mains and No. 3 Field subsoils respectively, with no C additions. These rates are lower than those reported in other studies looking at potential denitrification in subsoils in the absence of C amendment. Jarvis and Hatch (1994) reported potential denitrification rates of 1000 µg N kg<sup>-1</sup> dry soil day<sup>-1</sup> in chalk subsoil samples, taken from a depth of 1–2 m, under a long-term arable treatment, while rates of 1400–5100 µg N kg<sup>-1</sup> dry soil day<sup>-1</sup> have been reported in Iowa agricultural subsoils (Yeomans et al. 1992). The higher potential denitrification observed in these studies may be due to the presence of soluble C leaching down the soil profile from the surface crops, and the shorter incubation period – 4 days compared to the 16 days employed here.

The ratio of readily available C:N (WSOC:mineral N ratio) is likely to control microbial function in the subsoil, since most of the C present will have been leached down through the soil. Previous studies have indicated that denitrification is significantly correlated with WSOC and organic C (Burford and Bremner 1975; Beauchamp et al. 1980; Yeomans et al. 1992). Although the cores with the highest WSOC concentration at the end of incubation were also those with high denitrified gas and CO<sub>2</sub> concentrations, there was no statistical correlation between either WSOC and organic C, or CO<sub>2</sub> production and denitrification. Weier et al. (1993a, b) reported a strong relationship between emissions of CO<sub>2</sub> and N<sub>2</sub>O in cores of clay subsoil. While rates of CO<sub>2</sub> emission in subsoil slurries were not correlated with denitrification rates, there was good correlation between the total amount of CO<sub>2</sub> emitted and total denitrified N gas emissions in anaerobic cores ( $r^2=0.714$ ,  $P>0.05$ ) and aerobic cores ( $r^2=0.766$ ,  $P>0.05$ ). Emissions of CO<sub>2</sub> from denitrifying micro-organisms accounted for a larger percentage of total CO<sub>2</sub> emissions in anaerobic incubations (4–47%) than aerobic incubations (0.2–6%). This was expected due to the increased importance of microbial denitrification at low O<sub>2</sub> concentrations.

When denitrification occurs in subsoil, denitrified gas has to diffuse back up the soil profile before detection at the soil surface. During this slow diffusion process there is an increased likelihood of N<sub>2</sub>O undergoing further microbial reduction to N<sub>2</sub>. The slow diffusion rate through the subsoil also results in longer periods of

time before denitrified gas is measurable at the soil surface compared to denitrified gas resulting from topsoil denitrification. When nitrogenous gas from subsoil denitrification does appear at the soil surface it is in the form of N<sub>2</sub> which is more difficult to analyse than N<sub>2</sub>O. Subsoil denitrification may therefore be occurring in the field, without necessarily being measured as a surface flux.

Anaerobic incubations of subsoil slurry and intact cores denitrified approximately 4 times as much as those incubated under high O<sub>2</sub> concentrations. This agrees with findings by Parkin and Tiedje (1984), i.e. that when the O<sub>2</sub> concentration in soil cores decreased from 20–5%, the denitrification rate increased by a factor of 1.2–5.

The diffusion-mediated transport observed in the intact subsoil core incubations has been proposed as a mechanism for NO<sub>3</sub><sup>-</sup> transport in the glacial till subsoil at both Glencorse Mains and No. 3 Field (Vinten et al. 1992). It has been estimated that the annual amount of drainage water lost to deep percolation through the glacial till at Glencorse Mains and No. 3 Field is 25% and 44% of drainflow, respectively, accounting for substantial losses of NO<sub>3</sub><sup>-</sup>. Moreover, deep percolation may be more significant during periods of slow flow when NO<sub>3</sub><sup>-</sup> concentrations may be higher (Vinten et al. 1992).

Gambrell et al. (1975) suggested that in poorly drained subsoil, a soluble C concentration of 12–15 mg Cl<sup>-1</sup> was sufficient to act as an effective potential energy source for denitrification. The initial WSOC concentration in the intact subsoil cores ranged from 73 to 132 mg l<sup>-1</sup> pore water, suggesting there was adequate soluble C to act as a C source for denitrification. Assuming: a low O<sub>2</sub> concentration; sufficient soluble, assimilable C percolating down with the NO<sub>3</sub><sup>-</sup>; uniform NO<sub>3</sub><sup>-</sup> and denitrifying microsite dispersal down the soil profile, the subsoil at a depth of 1–2 m could denitrify up to 1.8 kg N ha<sup>-1</sup> day<sup>-1</sup> at No. 3 Field and 1.5 kg N ha<sup>-1</sup> day<sup>-1</sup> at Glencorse Mains. This suggests that subsoil denitrification could reduce most, if not all, the NO<sub>3</sub><sup>-</sup> that was transported below the water table at both field sites. Recent calculations (Vinten 1999) showed that including subsoil respiration rates in keeping with those presented in this paper led to much better simulation of observed NO<sub>3</sub><sup>-</sup> leaching over several years at the Glencorse Mains and No. 3 Field sites.

The results presented here suggest that in subsoils at low, and ambient O<sub>2</sub> concentrations, without additional C amendment, denitrifier activity may lead to significant levels of subsoil denitrification, which may result in the removal of some NO<sub>3</sub><sup>-</sup> from the leachate before it reaches ground and surface waters and becomes a potential pollutant. This denitrification is unlikely to add to concerns over global atmospheric N<sub>2</sub>O concentrations due to the further reduction of N<sub>2</sub>O during diffusion up the soil profile. Subsoil denitrification may also go some way to explain the unexpectedly low concentrations of NO<sub>3</sub><sup>-</sup> in drainage water, particularly at the No. 3 Field site.

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