Denitrification in the subsoil of the Broadbalk Continuous Wheat Experiment

John E. Richards[a], Colin P. Webster[b],*

[a]*Agriculture and Agri-Food Canada, Atlantic Cool Climate Crop Research Centre, 30 Brookfield Road, P.O. Box 39088. St John's, Nfld, Canada AIE 577*
[b]*I.A.C.R., Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK*

Abstract

Denitrification in subsoils may be a natural process able to decrease NO_3 contamination of groundwater. We studied this process and how it was affected by long-term fertilization practices. We collected soil to a depth of 2 m from four treatments in the Broadbalk Continuous Wheat Experiment. One treatment (FYM) had received manure applications annually since 1843, while the other treatments were N0 (0 kg N ha^{-1} since 1843), N4 (196 kg N ha^{-1} since 1968) and N6 (288 kg N ha^{-1} since 1984). Using the acetylene inhibition method, we measured N_2O production from anaerobic slurries of either unamended or amended (plus C and NO_3) samples of surface soil and three subsoil depth layers. These results will not equate to actual field denitrification rates, but the amended treatments (i.e. plus C and NO_3) will indicate the relative denitrification capacities of the soils. The unamended treatment will more accurately indicate the relative field denitrification rates. In the surface soils, denitrification rates were approximately proportional to total organic C (TOC) and they increased in the following order: N0, N4, N6, FYM. In the subsoil, denitrification declined with depth, and at 1.2 m was only a small fraction of that at the surface. There was no relationship between surface soil fertilization practices and either subsoil denitrification capacity, TOC, dissolved organic carbon (DOC) or total N. Indeed subsoil (60–200 cm) denitrification capacity was not significantly different between treatments. Denitrification capacity in unamended subsoils was not related to DOC. However, there was a significant relationship ($R^2 = 0.41$) between TOC and denitrification rate. When amended soils were conditioned aerobically for 5 days, N_2O production increased, indicating the presence of a small community of denitrifiers which multiplied when given a C source. We conclude that subsoil denitrification has the potential to decrease NO_3 concentrations of percolating waters but is in reality limited by biologically available C. On our Broadbalk site, surface soil management had no effect upon the amount of 'available' C in the subsoil. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In Britain, as in many other parts of the world, there is concern about the environmental damage caused by nitrate (NO_3) leached from agricultural land to surface or ground water. Aside from tangible ill-effects, such as eutrophication, in Europe, an EU directive prohibits NO_3 concentrations in excess of 11.3 mg N l^{-1} in potable water and surface waters judged to be at risk of eutrophication. There is, there-fore, concerted effort to identify all factors affecting NO_3 concentration in water as it passes from the soil surface to its point of abstraction or outflow.

Denitrification in subsoil or the underlying porous strata is a natural process which may decrease NO_3 concentrations in percolating waters. Denitrifying organisms have been widely observed in subsoil (Parkin and Meisinger, 1989; Yeomans et al., 1992) and throughout the vadose zone to the water table (Whitelaw and Rees, 1980; Lind and Eiland, 1989). They have also been found in chalk and sandstone aquifers (Foster et al., 1985; Trudell et al., 1986).

The size of a microbial community is largely deter-mined by the amount of 'available' C. Denitrifying

microorganisms can compete with other heterotrophs for added C, hence the denitrification capacity of a soil is often highly correlated with its C content (Myrold and Tiedje, 1985; Drury et al., 1991). For example, Katz et al. (1985) reported that denitrification in unamended soils was proportional to the soils' soluble C content. With the exception of C deposited at the time of strata formation (geological carbon) and that produced by autotrophic bacteria, any C in the subsoil will have originated in the top-soil. This movement of soluble C (dissolved organic C or DOC) is, therefore, a critical factor controlling subsoil denitrification. It is not unrealistic to suppose that management practices which increase surface soil total C may increase DOC both in the surface soil and in the subsoil and lead to increased subsoil microbial communities. Myers and McGarity (1971) found that soluble C, added as glucose, cowpea straw or wheat straw leached from the zone of application. They concluded that high denitrification activities observed in some B horizons of Australian solonetz soils were due to soluble C leached from the A horizon. In contrast, McCarty and Bremner (1992) found the increase in soluble C concentrations of surface soil following addition of plant residues was short lived, and concluded that this C was so rapidly decomposed in surface soils that it could not leach and hence promote subsoil denitrification.

The conditions under which DOC may be exported from the topsoil to the subsoil are unclear. Furthermore there is little information on the influence of surface soil management on DOC leaching. At the time of our investigation, the Broadbalk Continuous Wheat Experiment at Rothamsted contained plots which had received farmyard manure (FYM) annually for 149 years and others which received various sustained annual applications of inorganic fertilizer N. As a result, there was a wide range of total C and total N concentrations in the surface soil of the various fertility treatments. This experiment was conducted to test the hypothesis that the various long term fertility treatments also markedly influenced soil total C, DOC, total N and therefore denitrification capacity to a depth of 2 m.

2. Materials and methods

The study was conducted on Batcombe Series silty-clay loam soil (Chromic luvisol — FAO classification) collected from the Broadbalk Continuous Wheat Experiment at the Rothamsted Experimental Farm, Harpenden, Herts, UK. The soils were described in detail by Avery and Bullock (1969).

Soil was collected from four plots that had histories of different N and C inputs. They were 1: FYM —

plot 2.2, to which 35 t ha^{-1} (dry weight) of farmyard manure was applied in each autumn since 1843; the manure supplied about 240 kg N ha^{-1} and 3 t C ha^{-1} annually. 2: N0 — plot 3 which had received no fertilizer N or manure since 1843. 3: N4 — plot 9 which had received 196 kg N ha^{-1} y^{-1}, as NH_4NO_3 since 1968 and before that 48 kg N ha^{-1} y^{-1} since 1852. 4: N6 — plot 16 which had received 288 kg N ha^{-1} y^{-1}, as NH_4NO_3, since 1985 and 96 kg N ha^{-1} y^{-1} between 1884 and 1984. Plots 9 and 16 received their N as a single top dressing in spring and also P and K fertilizer annually and Mg once every 3 years.

Soil samples were collected between sections 8 and 9, an area which had grown continuous winter wheat (with occasional fallow breaks) annually since 1843; see Johnston and Garner (1969) and Dyke et al. (1982) for details of cropping history. Currently, the total above-ground plant material, apart from stubble, is removed prior to ploughing. Soils were collected on 6 and 7 October 1992, 6 weeks after wheat was harvested, but before FYM was applied and the site was ploughed and sown for the 1993 crop. Using a 4-cm diameter Dutch auger, four profile samples were collected from each plot to a 2 m depth in the following increments: 0–23 cm (this being the depth of ploughing at this site), 23–40 and 20 cm increments thereafter. For each plot, soil from the same horizons were combined and sieved to pass a 5-mm mesh and mixed. A subsample was taken for air-drying, the remainder was stored in plastic bags in a field moist condition at 4°C.

2.1. Analysis of denitrification

Ideally denitrification should have been measured on intact subsoil cores maintained in conditions which mirrored those in the field. However equipment to collect such cores and to suitably incubate was not available, so we used a laboratory method, based on that described by Smith and Tiedje (1979). It was not intended to measure actual field denitrification rates, but rather to give an index of each soil's denitrification capacity and potential.

Two experiments were done. The first experiment used soils from four depth layers (0–23; 60–80; 120–140 and 180–200 cm). Nitrous oxide production was measured from slurries created by mixing 25 g field moist soil and 15 ml of an appropriate solution in a 250-ml flask. There were two treatments, one in which the solution contained only distilled water and chloramphenicol (500 μg g^{-1} soil) hereafter referred to as unamended. In the second treatment the solution contained sucrose (1000 μg C g^{-1} soil) and NO_3 (100 μg N g^{-1} soil) in addition to the chloramphenicol, hereafter referred to as amended soil. Each combination was replicated three times. The flasks were sealed with ground glass lids and made anoxic by alternately evac-

uating and flushing with N_2 three times. Acetylene was then added to give a 10% by volume concentration and the flasks were put on an orbital incubation shaker (100 rev min^{-1}) at 25°C. Ten ml headspace samples were collected after 4 and 24 h and analyzed for N_2O and CO_2 by gas chromatography as described by Webster and Goulding (1989). The amount of N_2O dissolved in the water was accounted for when total N_2O production was calculated (Tiedje, 1982).

Chloramphenicol was added to prevent cell division without altering microbial metabolism and the anoxic conditions ensured that only anaerobic respiration could occur. With this approach, in the amended treatment, microbial metabolism is unconstrained by substrate and each unit of denitrifying biomass will produce N_2O at the same rate in each soil sample. Therefore these denitrification rates will be a measure of the relative sizes of denitrifier communities, and therefore of the intrinsic *denitrifying capacity* of the soils. This measurement has been described as denitrifying enzyme activity (DEA) by Smith and Tiedje (1979). However in the context of this research the term in italic above is a more informative description and so will be used. Both amended and unamended treatments had the same denitrifier communities but the latter had only the available C and N substrate that was in the soil when it was collected. Values from the unamended treatment therefore gave a better indication of the relative denitrification rates which actually exist in the field or potentially exist when the soil becomes anaerobic.

The second experiment involved only the three subsoil depth layers (60–80, 120–140 and 180–200 cm). Soil samples (25 g) were incubated aerobically for 5 days at 25°C after addition of 7.5 ml of aqueous solution amended or unamended with sucrose and NO_3 as before but now with *no* chloramphenicol added. After 5 days, another 7.5 ml of aqueous solution (which now contained chloramphenicol) was added to each flask, and the denitrification rate was immediately measured from anoxic slurries as above. We refer to these rates as *denitrification potential*. During the 5-day aerobic incubation, the original microflora was able to multiply at near their optimum temperature and, in the unamended treatment, using just the C and N substrate originally in the soil. In the amended treatment, microbial growth was unconstrained by available C and N. The ratio between the denitrification capacity and potential (of amended treatments) gives an indication of the relative increase in subsoil denitrification which could occur if carbon and nitrate substrates were made available.

2.2. Chemical analyses

Soil mineral N was extracted from duplicate samples of field moist soil by shaking for 1 h with 200 ml 2 M KCl (Maynard and Kalra, 1993). The NH_4 and NO_3 concentrations in the filtered extracts were analyzed colorimetrically using the method of Henriksen and Selmer-Olsen (1970). Total soil N was measured by a modified Kjeldahl method on air-dried soil ground to pass a 0.45-mm sieve (Crooke and Simpson, 1971), the NH_4 produced by the digestion was measured colorimetrically as above. Dissolved organic carbon (DOC) was extracted from three samples of each soil; 5 g of field moist soil was placed into a 50-ml centrifuge tube, followed by 20 ml distilled water. The mixture was shaken on a reciprocating shaker for 30 min, centrifuged at $40,000 \times g$ and the supernatant filtered through a 0.45 μm cellulose membrane filter. The DOC was analyzed on a Dohrmann DC-80 analyzer (Wu et al., 1990) which used an acidified potassium persulfate–ultra violet digestion; the CO_2 produced was measured by infrared detection. Total organic carbon (TOC) was determined (on all the depth increments, not just the four used for denitrification measurements) by combustion on a LECO C–N–S analyzer. Soil pH was measured in 10 mM $CaCl_2$ (1:1 soil:solution ratio).

3. Results

3.1. Soil properties

Biological and chemical properties of the surface soil (0–23 cm) were markedly affected by long-term soil fertility practices (Table 1). The annual application of FYM for 149 years resulted in a soil with greater concentrations of total soil N and total organic carbon (TOC) relative to soils which received inorganic N fertilizers. The dissolved organic carbon (DOC) content of the FYM treatment was significantly ($P < 0.05$) greater than N0 and N6, but not the N4. Within the inorganic N treatments surface soil total N, TOC and DOC of the N0 treatment were significantly ($P < 0.05$) smaller than in the two fertilized treatments (N4 and N6).

In contrast, subsoils showed no treatment differences in total N, DOC or TOC at each depth layer, with the exception of DOC in the 60–80 cm section from the N0 which was significantly ($P < 0.05$) lower than the FYM and N4 treatments. The inorganic N concentrations were affected by the different long-term fertility treatments (Table 1). In the top soil (0–23 cm), they were greatest in soils from the FYM treatment and, in the inorganic N addition sequence, increased progressively with larger annual N inputs. Subsoil

Table 1
Selected chemical and biological properties of soils

Plot	Sample depth (cm)	2 M KCl extractable (µg^{-1} O.D. soil)		Total N (%)	Organic C		pH
		NH$_4$	NO$_3$		Total (TOC) (%)	Dissolved (DOC) (µg C g^{-1} soil)	
FYM	0–23	0.5	10.5	0.27	3.01	63	6.9
N0		0.5	2.8	0.11	0.91	24	7.4
N4		1.1	4.8	0.14	1.24	54	7.3
N6		2.4	7.3	0.13	1.16	37	7.4
SEM		0.3	0.4		0.06	9	
Error d.f		4	4		8	8	
FYM	60–80	0.6	4.1	0.08	0.55	37	7.5
N0		0.6	1.4	0.08	0.47	18	7.3
N4		0.4	2.5	0.07	0.40	34	7.3
N6		0.4	8.0	0.07	0.39	27	7.4
SEM		0.2	0.3		0.03	4	
Error df		4	4		8	8	
FYM	120–140	0.4	1.8	0.07	0.33	26	7.3
N0		0.3	0.4	0.07	0.34	20	7.1
N4		0.3	0.9	0.05	0.19	30	7.2
N6		0.4	5.4	0.05	0.24	21	7.2
SEM		0.1	0.2		0.03	5	
Error df		4	4		8	8	
FYM	180–200	0.3	2.5	0.05	0.24	38	6.5
N0		0.5	0.3	0.07	0.26	22	7.9
N4		0.4	1.6	0.06	0.18	35	6.7
N6		0.3	4.9	0.07	0.28	26	7.0
SEM		0.1	0.2		0.03	7	
Error df		4	4		8	8	
SEM between depths		0.2	0.2		0.08	8	
Error df		9	9		25	25	

inorganic N concentrations generally increased with increasing annual fertilizer N application rates, with soils from the FYM treated plot being intermediate between those from the N4 and N6 plots. The soil for these measurements were collected on either 6 or 7 October 1992 and so their values represent one point in the annual cycle.

3.2. Denitrification capacities of surface soils

Surface soil denitrification (both with or without amendment) was significantly ($P < 0.05$) greater in the FYM treatment than from the inorganic N treatments (Table 2). Within the inorganic N sequence, N$_2$O emission from the N0 treatment was significantly ($P < 0.05$) lower than from the N4 and N6. The relative denitrification capacities (i.e. the with amendment) will indicate the relative population of denitrifier biomass in these surface soils. Means of the 4 and 24 h measurements give an approximate biomass ratio of 1:2:2:4 for the N0, N4, N6 and FYM treatments. The relative increase in N$_2$O emission between without and with amendments was similar from all treatments

(Table 2) indicating that their biomass responded in a similar way to the added substrate. For a particular treatment, denitrification rates between 0–4 and 0–24 h were approximately the same and showed no consistent trend to increase or decrease.

3.3. Denitrification capacity of subsoil

The denitrification capacity for the 60–80 depth increment was, for all treatments, only a small fraction of the rate in surface soil, less than 3% (Table 3). It further declined with increasing depth to 120–140 cm, although there were no significant differences between the two deepest depth layers (Table 3), with the exception of the N4 soil. Rates were again similar between 0–4 and 0–24 h, and hence only the latter are shown. In the 60–80 cm depth layer, subsoil denitrification capacity was significantly ($P < 0.05$) greater in the N4 soil than in the others and the value in the FYM soil was significantly greater than in N0 and N6. In the 120–140 cm depth layer the largest value was again in the N4 treatment. There were no significant differences between treatments for the 180–200 cm depth layer. Some denitrification

Table 2
The influence of different long-term soil fertility practices on the denitrification capacity of the Ap horizon (0–23 cm) of soil from the Broadbalk Continuous Wheat Experiment

| Plot[a] | Incubation time (h) | | | |
| | 4 h (μg N_2O–N g^{-1} soil) | | 24 h (μg N_2O–N g^{-1} soil) | |
	Unamended[b]	Amended[b]	Unamended[b]	Amended[b]
FYM	2.9	7.2	16.6	36.1
N0	0.6	2.1	3.0	7.4
N4	1.0	3.6	7.6	23.0
N6	1.8	3.8	8.1	18.3
SEM	0.2		1.4	
Error df	11		11	

[a] Where FYM is farmyard manure (240 kg N ha^{-1} y^{-1}) and N0, N4 and N6 are 0, 196 and 288 kg N ha^{-1} y^{-1}, respectively.

[b] Where unamended indicates that no additional C or NO_3 were added to the slurry and amended indicates that 1000 μg C g^{-1} soil and 100 μg NO_3–N g^{-1} soil were added with the slurry.

occurred in all unamended (Table 3) soils, indicating they had at least some substrate in their natural state.

3.4. Denitrification potential of subsoil

The denitrification potentials of all subsoils were higher than their denitrification capacities (Table 4). In all treatments, the 60–80 cm depth layer had the greatest denitrification potential. Values for the 120–140 and the 180–200 cm depth layers were not significantly different with the exception of the N4 treatment. The ratio of denitrification potential to denitrification capacity (Table 4) increased with soil profile depth. Hence after the five-day aerobic incubation, the 180–

Table 3
The influence of different long-term soil fertility practices on the amount of N_2O released during 24 h from slurries which had not been conditioned. Note that denitrification capacity was taken as the N_2O evolved from amended slurries

| Plot[a] | Sample depth (cm) | Soil treatment[b] (μg N_2O–N kg^{-1}) | |
		Unamended	Amended (soil denit. capacity)
FYM	60–80	270.3	541.9
N0		97.0	185.4
N4		419.1	750.3
N6		141.5	289.6
SEM		37.7	
Error df		16	
FYM	120–140	21.0	47.3
N0		19.6	29.1
N4		73.2	140.9
N6		3.3	75.8
SEM		8.9	
Error df		16	
FYM	180–200	19.9	13.5
N0		18.2	66.5
N4		1.9	5.3
N6		8.5	22.7
SEM		3.5	
Error df		16	
SEM between depths		43.3	
Error df		32	

[a] Where FYM is farmyard manure (240 kg N ha^{-1} y^{-1}) and N0, N4 and N6 are 0, 196 and 288 kg N ha^{-1} y^{-1}, respectively.

[b] Where in the unamended soil no additional C or NO_3 were added to the slurry while in the amended soil treatment 1000 μg C g^{-1} soil and 100 μg NO_3–N g^{-1} soil were added with the slurry.

Table 4
The influence of different long-term soil fertility practices on the amount of N_2O released during 24 h from slurries which were conditioned for 5 days. Note that denitrification potential was taken as the N_2O evolved from amended slurries

| Plot[a] | Sample depth (cm) | Soil treatment[b] (μg N_2O–N kg^{-1}) | | Ratio |
		Unamended	Amended (soil denit. potential)	denit. potential/denit. capacity
FYM	60–80	130.5	789.6	1.46
N0		68.1	229.2	1.23
N4		148.8	1212.2	1.62
N6		56.9	866.0	2.99
SEM		173		
Error df		16		
FYM	120–140	33.3	109.0	2.32
N0		15.8	153.0	5.26
N4		29.6	724.4	5.14
N6		21.8	620.2	8.18
SEM		38		
Error df		16		
FYM	180–200	6.5	112.3	8.32
N0		28.4	227.5	3.42
N4		4.2	304.7	57.5
N6		7.4	498.5	22.0
SEM		72		
Error df		16		
SEM between depths		161.3		
Error df		32		

[a] Where FYM is farmyard manure (240 kg N ha^{-1} y^{-1}) and N0, N4 and N6 are 0, 196 and 288 kg N ha^{-1} y^{-1}, respectively.
[b] Where in the unamended soil no additional C or NO_3 were added to the slurry while in the amended soil treatment, 1000 μg C g^{-1} soil and 100 μg NO_3–N g^{-1} soil were added with the slurry.

200 cm depth layer showed the greatest relative increase in N_2O emission. In unamended soils, the 5-day aerobic incubation actually decreased N_2O emissions compared to unincubated soils (Table 4). Presumably this was due to consumption of available C and NO_3 during the 5-day incubation.

3.5. N_2O emission and TOC

The denitrification rates of the unamended, unincubated subsoils (which were the best indicator of actual field denitrification rate in this experiment) were not significantly ($P < 0.05$) related to DOC. There was, however, a significant relationship between TOC and N_2O production given by:

$$Y = 0.0119 \times e^{(5.8 \times TOC)} \quad R_2 = 0.41, \tag{1}$$

Where Y is the nitrous oxide production from unamended soils which were not aerobically incubated for 5 d (mg N_2O kg^{-1} soil) and TOC the total organic carbon content of subsoil (%C).

In this study N_2O production was only measured in the top soil and three other depth layers but TOC was measured in all layers. On the assumption that Eq. (1) holds for all depths, the TOC values were used to esti-mate N_2O released from soil in each layer between 60–200 cm depth. These values were multiplied by the appropriate bulk density and summed to provide the estimated cumulative N_2O loss from this zone. This value will be subject to considerable error which inevitably follows such mass balance calculations and there was in fact, no significant difference between plots. All returned a value of about 2 kg N ha^{-1}. This value has no relation to actual field denitrification but allows the denitrification activity of the subsoil relative to the top soil to be gauged. Subsoil activity (60–200 cm) was equivalent to 5.5, 35, 11 and 8% of that in the top soil in the FYM, N0, N4 and N6 plots, respectively.

4. Discussion

Our work addressed two questions. First, was there the potential for denitrification in the subsoil? Second, does management practice at the soil surface affect subsoil denitrification? In particular does a practice such as the long and continued application of FYM increase subsoil denitrification through the leaching of readily decomposable C to depth?

4.1. Subsoil denitrification

Some denitrification was measured at all depths in all treatments even without C or NO_3 amendment. Even though our measurements were made in artificially created anaerobic environments and at 25°C, it shows there was some potential for the NO_3 concentration in drainage water to be decreased by denitrification while percolating from surface soil to the saturated zone. As this potential may operate over a long time throughout a long drainage path, subsoil denitrification may significantly reduce NO_3 concentration in drainage water.

Use of the model in Eq. (1) indicates that, in the inorganic N-treated soil (N4 and N6), denitrification activity of the subsoil (60–200 cm) was only about 10% of that of the surface soil. Other workers have also shown that subsoil denitrification capacity is considerably lower than that of the surface soil and declines sharply with increasing soil depth (Gambrel et al., 1975; Cho et al., 1979; Yeomans et al., 1992). The occurrence of some denitrification at all depths supports observations that denitrifiers are ubiquitous in subsoils and their greater denitrification potential to denitrification capacity ratio (Table 4) indicate their ability to respond to added substrate. We recognize this is a simplistic approach and the proportion of denitrification in the subsoil (60–200 cm) relative to the topsoil will vary with seasonal fluctuations in soil available carbon and nitrogen, temperature and oxygen status. However we cannot be more quantitative on the basis of this work.

Soil processes other than denitrification will lead to the production of gaseous N compounds (Robertson and Tiedje, 1987). For example, in some soil conditions the emission of nitrous oxide as a by-product of nitrification exceeds that from denitrification (Myrold and Tiedje, 1985). Generally these processes will not be competing with denitrification for available N and will be active under different conditions (i.e. nitrification in aerobic conditions). In natural field conditions they will, of course, make a contribution to the total gaseous N losses, however they will not in this laboratory study, the anoxic treatment and presence of acetylene precluding the nitrification process.

4.2. The influence of fertility treatment

Perhaps the most significant finding of our work is that long-term manure application had little influence on some subsoil chemical and biological properties. This result is similar to that of Parkin and Meisinger (1989) who reported that surface soil straw management had no effect upon subsoil denitrification.

When this study was started, we hypothesized that long-term FYM applications would increase subsoil DOC concentrations and hence denitrification capacity. This did not happen. Dissolved solutes are mobile in the soils at Rothamsted, demonstrated by the leaching of organic forms of P (Johnston, 1976). Also in our work, NO_3 concentrations were elevated in the 180–200 cm depth layer in the N6 treatment. Hence, there was no physical barrier preventing DOC from entering the subsoil.

McCarty and Bremner (1993) found that no DOC leached from the surface into the subsoil during the decomposition of freshly added plant tissue. They concluded the DOC was rapidly metabolized by the microbial community in the surface soil. Perhaps a similar situation occurred on Broadbalk, that is, the increase of surface soil DOC concentration which followed manure application, so stimulated microbial activity that all DOC was assimilated before it could be leached. This situation will not apply to all soil types, indeed it may not even be the general rule. The subsoil of cracking clays soils of Australia exhibit rapid denitrification rates; this has been attributed to their accumulation of substrate C and N (Myers and McGarity, 1971; Weier et al., 1993).

Straw has always been removed from these plots so the increase in TOC and total N in the N4 and N6 compared to the N0 must result from enhanced organic matter deposition from wheat roots, root exudates and stubble. Decay of roots is clearly a means by which DOC can be introduced to a subsoil. Previous work at Rothamsted on cores collected to a depth of 1 m found that root mass declined exponentially with depth, with over 50% in the top 20 cm of soil (Barraclough and Leigh, 1984). Fertilizer application was found to increase root mass, but the increase was small. From these results we could deduce there would be only small differences between plots in the amount of DOC introduced into the subsoil by decomposition of roots; a deduction supported by the results (Table 1). The rate of DOC release from root decomposition will vary during the year, being greatest soon after crop harvest. However rapid utilization of DOC by microbes (McCarty and Bremner, 1993) may result in the amount that accumulates in the soil varying little during the year.

The capacity of microbes to intercept and assimilate DOC before it leaves the top soil increases the importance of any C deposited at the time of strata formation (geological C) or that transported as colloidal material. Trudell et al. (1986) and Whitelaw and Edwards (1980) suggested these sources of C provided the energy source for denitrification within an unconfined shallow sand and chalk aquifers. In the light of our finding we cannot automatically assume that the small quantities of available C in the Broadbalk subsoil originated in the topsoil and were leached down.

The relationship between TOC and N_2O production from unamended subsoil confirms the findings of Yeomans et al. (1992) and McCarty and Bremner (1992, 1993) who postulated that denitrification in Iowa subsoils is limited by a shortage of available C. However it is not immediately clear why DOC was unrelated to N_2O production from unamended soils in our work. Beauchamp et al. (1980) reported that, in poorly drained soils, denitrification through the soil profile was related better to TOC than to other measurements of microbially-available C. Perhaps, in a single soil sampling, TOC is a better indication than DOC of the long-term C available to support microbial growth.

All the Broadbalk subsoils we used had some denitrification activity and therefore held at least a small microbial community. The increasing ratio of denitrification potential: capacity with increase in depth (Table 4) indicates the deep soil microflora will readily expand if provided with substrate. Stimulation of subsoil denitrification by added C has been reported for laboratory studies (Lind and Eiland, 1989; Yeomans et al., 1992; McCarty and Bremner, 1992), and for field studies (Weier et al., 1993). However it is difficult to envisage a rational agricultural practice which could so increase subsoil C in field soil. Coupled with the findings of Foster et al. (1985) and of Whitelaw and Rees (1980), it would appear that NO_3 in waters leaching from the rooting zone are still susceptible to denitrification during percolation to and containment in limestone aquifers of southern England. Where the process can be sustained for many years it may be able to consume significant quantities of NO_3. More work is required to determine the extent and under what conditions C may be transported to subsoil. Work such as ours can provide indications of actual soil processes, but is no substitute to the measurement of actual in situ losses nor of identifying the composition of the gaseous products.

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