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ACCELERATED PAPER

LONG-TERM EFFECTS OF NITROGEN FERTILIZATION ON METHANE OXIDATION IN SOIL OF THE BROADBALK WHEAT EXPERIMENT

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Summary—Methane uptake by a temperate arable soil was investigated in incubation experiments with intact soil cores. The measurements were carried out with a soil moisture content of 16–17% (w/w) and at 25°C. The decrease of the CH_4 concentrations in an amended atmosphere (10 μl CH_4 l^{-1}) was measured during a 212 h period. There was no decrease of CH_4 if the soil was autoclaved showing that the disappearance of methane was entirely mediated by microbial activity. The long-term application (140 yr) of mineral nitrogen fertilizer caused significant differences in the ability of the soil to oxidize CH_4: the larger the amount of fertilizer applied the lower the rate of CH_4 oxidation. No significant short-term effect of mineral-N fertilization could be observed whether applied as $(NH_4)_2SO_4$ or KNO_3. An organic manure treatment, which has received nearly 240 kg N ha^{-1} each year as farmyard manure, showed almost the same ability to oxidize CH_4 as an unfertilized plot and had a significantly higher CH_4 oxidation rate after an application of 144 kg N ha^{-1} as nitrate fertilizer. For the mineral-N treatments the inhibition of the CH_4 oxidation increased with increasing N turnover rate but was independent of the mineral nitrogen content of the soil at the time of measurement. Therefore, the continued application of mineral-N fertilizer for an extended period (at least 7 yr) caused a depletion of the bacterial methane sink in soil and may have contributed to the continuous increase in atmospheric CH_4 over the past decades.

INTRODUCTION

Methane is a radiatively active trace gas, which is estimated to contribute between 10% (Bouwman, 1989) and 15% (Rohde, 1990) to global warming. Its concentration in the atmosphere has increased by 1% per year over the last decade (Blake and Rowland, 1988). A positive correlation exists between the atmospheric CH_4 mixing ratios and the world's human population, which indicates a strong influence of anthropogenic activity on the atmospheric CH_4 cycle (Schütz *et al.*, 1990). The imbalance between CH_4 production and consumption is most likely caused by an increase of emissions (e.g. by ruminants, rice paddies), but a decrease in consumption may also be involved.

The major tropospheric methane sinks are a reaction with OH radicals, transport to the stratosphere (Bolle *et al.*, 1986) and absorption in soils, which can contribute up to 15% to the total methane destruction (Born *et al.*, 1990). Since Seiler *et al.* (1984) first demonstrated the uptake of CH_4 by soils in the tropics, methane consumption has been measured in a Humisol (Megraw and Knowles, 1987), moss-derived peat soils (Yavitt *et al.*, 1990), tundra soils (Whalen and Reeburgh, 1990), temperate forest soils (Steudler *et al.*, 1989; Born *et al.*, 1990), grasslands (Mosier *et al.*, 1991) and desert soils (Striegl *et al.*, 1992).

Investigations on arable land have been reported by Mosier *et al.* (1991) and Born *et al.* (1990). Mosier *et al.* detected a decrease in CH_4 uptake rate after applications of nitrogen fertilizer to a pasture; an annual fertilizer application equivalent to 22 kg N ha^{-1} as NH_4NO_3 reduced the CH_4 uptake by an average of 41% compared to an adjoining unfertilized pasture. However, N fertilizer application to a regularly-fertilized wheat field had no effect on CH_4 uptake (Mosier and Schimel, 1991). In addition to these investigations in agroecosystems, Steudler *et al.* (1989) found nitrogen fertilization (37 or 120 kg N ha^{-1} yr^{-1}) resulted in an inhibition of the methane uptake in temperate forest soils.

We report laboratory measurements which investigated CH_4 oxidation in the topsoil from the Broadbalk Wheat Experiment at Rothamsted as affected by short- and long-term inorganic N fertilizer addition and by long-term farmyard manure (FYM) application.

MATERIALS AND METHODS

Experimental site

The investigations were carried out with soil samples from Section 1 of the Broadbalk Wheat

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Table 1. Properties of the topsoil (0–23 cm) at Broadbalk Wheat Experiment

Classification (FAO)	Texture	Particle size distribution (%) 2000–60	60–2	<2 μm	pH (in H_2O)	Total N (%) N0–N288	FYM	Organic C (%) N0–N288	FYM
Chromic luvisol	Silty clay loam	20	51	28	7.0–8.0	0.12*	0.28	1.1*	2.8

*Details of small differences in total C and N between plots receiving different rates of inorganic N are given by Glendining *et al.* (1992).

Experiment at Rothamsted Experimental Farm on soil classified as Chromic Luvisol. The topsoil (about 0–23 cm) is a flinty clay loam to silty clay loam containing about 28% clay, 51% silt, 14% fine sand and 6% coarse sand (Avery and Bullock, 1969; Johnston, 1969).

The Broadbalk experiment was started in 1843 on a field which had been in cultivation for at least two centuries, and probably much longer. Wheat has been grown every year on Section 1, except for the period 1925–1966 when it was bare fallowed 1 yr in 5 to control weeds. The plots used for these CH_4 uptake measurements receive 0, 48, 96, 144, 192, 240 or 288 kg N ha^{-1} yr^{-1} as a single dressing of 'Nitro-Chalk' (ammonium nitrate–calcium carbonate) in April. These treatments are termed N0, N48 etc. An organic manure treatment, which receives 35 t ha^{-1} farmyard manure (FYM) each year, applied before ploughing, was also investigated.

The N0, N48, N96, N144 and FYM treatments have been given the same annual fertilizer application since 1852 (between 1843 and 1852 the N rates varied); the N192 treatment was begun in 1968 and N240 and N288 in 1985 (see Johnston and Garner, 1969, for full details of past treatments). These different treatments caused a variation in pH, total N and organic carbon, as can be seen in Table 1. The total N and organic carbon contents of soil in the FYM treatment are more than twice those in the mineral-N plots.

Soil sampling

Measurements of methane oxidation were made on undisturbed soil cores, which were collected in plastic tubes, 6.4 cm i.d. and 12 cm deep. Cores were collected on 6 April 1992, before the mineral-N fertilizer was applied, and on 4 September 1992, after harvest of winter wheat. At each sampling date, additional soil for measurement of water content, inorganic N and pH was collected to a depth of 12 cm around each core, using a 2 cm dia auger, and bulked for each plot. Cores were immediately wrapped in plastic film (to prevent evaporation) and, together with bulk soils, stored at 4°C. Soil loss from cores was prevented by covering the base of each core with nylon voile secured with rubber bands.

Incubation procedure

Incubation experiments to measure methane uptake were performed at 25°C in the dark in 1 litre Kilner jars with lids fitted with two septa which allowed the headspace to be sampled with syringe and sideport needle. To ensure hermetic seals new septa were used in each incubation and rubber gaskets were given a thin film of vacuum grease. All cores were conditioned at 25°C for 42 h to allow microbial activity to adapt to this temperature. This procedure caused a drying of the soil to about 16–17% H_2O (w/w). Tests had shown that with this moisture content CH_4 uptake was higher than with the original content of 19–20% H_2O (w/w). After conditioning, the cores contained in plastic tubes were transferred to the jars, sealed and CH_4 added to increase the headspace concentration by 8 μl l^{-1}. Headspace samples (800 μl) were collected and analysed after 0, 3, 6, 24, 48, 72, 120, 168 and 212 h. Zero time gas samples were actually taken about 15 min after CH_4 addition in all experiments to ensure complete equilibration of added CH_4 (compare Whalen *et al.*, 1990). At the end of each experiment (after 212 h) CO_2 concentration was also measured.

Between 16 and 24 cores were incubated at the same time with four replicates per treatment. With each series there were two additional control jars, which received an identical CH_4 addition but which contained no soil. For the period of a normal incubation the CH_4 concentration in these control jars stayed nearly constant (±1.5%). Between the measurements the septa of all jars were covered with distilled water to prevent leakage.

In experiments where inorganic N was applied to the soil cores, it was dissolved in 2 ml of solution and injected into the soil (1–2 cm deep) with a syringe, just before closing the jars. It was added either as $(NH_4)_2SO_4$ (30–170 mM) or as KNO_3 (60–330 mM), equivalent to the amount which is added annually to the field plots. For these experiments the CH_4 uptake ability of the unamended soil cores was measured first and, after the 212 h experiment, the jars were changed and the inorganic N applied to the same set of cores. Thus the effect of unamended vs recently-fertilized soil could be compared directly in the same cores. However, in the second incubation the soil had already been exposed to a CH_4-amended atmosphere; it was therefore necessary to test whether or not the conditioning in an atmosphere containing 10 μl CH_4 l^{-1}, compared to 2 μl CH_4 l^{-1} (ambient concentration), altered the CH_4 oxidation rate. This was tested using the N48, N144 and FYM treatments. In these experiments the CH_4 concentration was measured at $t = 0$ and $t = 212$ h, then inorganic N

was applied (144 kg N ha^{-1} to the FYM treatment) and the main incubation started.

Soil samples from the N0, N144 and FYM treatments, with or without autoclaving, were used to confirm that CH_4 disappearance was biologically mediated. Soil cores were kept at 25°C for 42 h, then half were wrapped loosely in aluminium foil and autoclaved at 123°C (30 min sterilizing time, 10 min purge time). The weight of the soil cores did not change (±0.6%) so it was assumed that no major alteration of the water content took place. There was no visible change in the physical structure of the soil. Immediately the soil cores had cooled to room temperature, the incubation of autoclaved and non-autoclaved cores in the Kilner jars was started. In this experiment CO_2 concentration in the headspace was measured after 24, 72, 120, 168 and 212 h.

Gas analysis

Headspace CH_4 and CO_2 concentrations were determined by gas chromatography. For CH_4 a flame ionization detector (125°C) was used with a Porapak Q column (oven temperature: 50°C) and N_2 as carrier gas (flow rate: 25 ml min^{-1}). The mean of duplicate samples is reported in most cases. CO_2 was measured using a thermal conductivity detector (100°C) and a Poropak Q column, maintained at 90°C with He as carrier gas (40 ml min^{-1}).

Soil preparation and analysis

For mineral N measurements, analysis on bulk samples represented conditions at the start of incubations and the individual cores at the end. All soils were sieved (≤5 mm), and 50 g (fresh weight) extracted by shaking with 200 ml 2 M KCl for 1 h and filtered through Whatman No. 1 filter paper. The aqueous extracts were stored frozen until measurement of the NO_3^- and NH_4^+ concentrations with an ALPKEM rapid flow analyser. The concentrations are expressed as kg N ha^{-1}, taking into account the different soil weights (≤6.25 mm) of the mineral-N and FYM treatments.

Soil moisture content was measured gravimetrically by drying the soil samples at 105°C. For determination of pH, dried samples were ground (<2 mm), mixed with distilled water (soil:water ratio 1:2.5) and pH measured in the supernatant liquid with a glass combination electrode.

Statistical analysis

The decrease in the CH_4 concentration in the headspace, measured in four replicate cores of each field treatment, followed first-order-kinetics and an exponential function ($y = ae^{bt}$) could be fitted ($r^2 > 0.99$). A log-transformation, $\ln y = a + bt$, resulted in straight lines with one individual slope for each treatment. 'Analysis of parallelism' (Ross, 1984) was used to test whether slopes were significantly different. If this was the case, the least significant difference for 5, 1 and 0.1% was calculated and the slopes compared. These slopes, or b-values, can be interpreted as methane oxidation rates and are, therefore, characteristic values for the methane uptake ability of soil in a given treatment. All these calculations were carried out using the computer programme 'Genstat'.

RESULTS

Effect of autoclaving on disappearance of CH_4

Figure 1(a)–(c) shows changes in concentration of CH_4 in the jars during 212 h at 25°C and the effect of

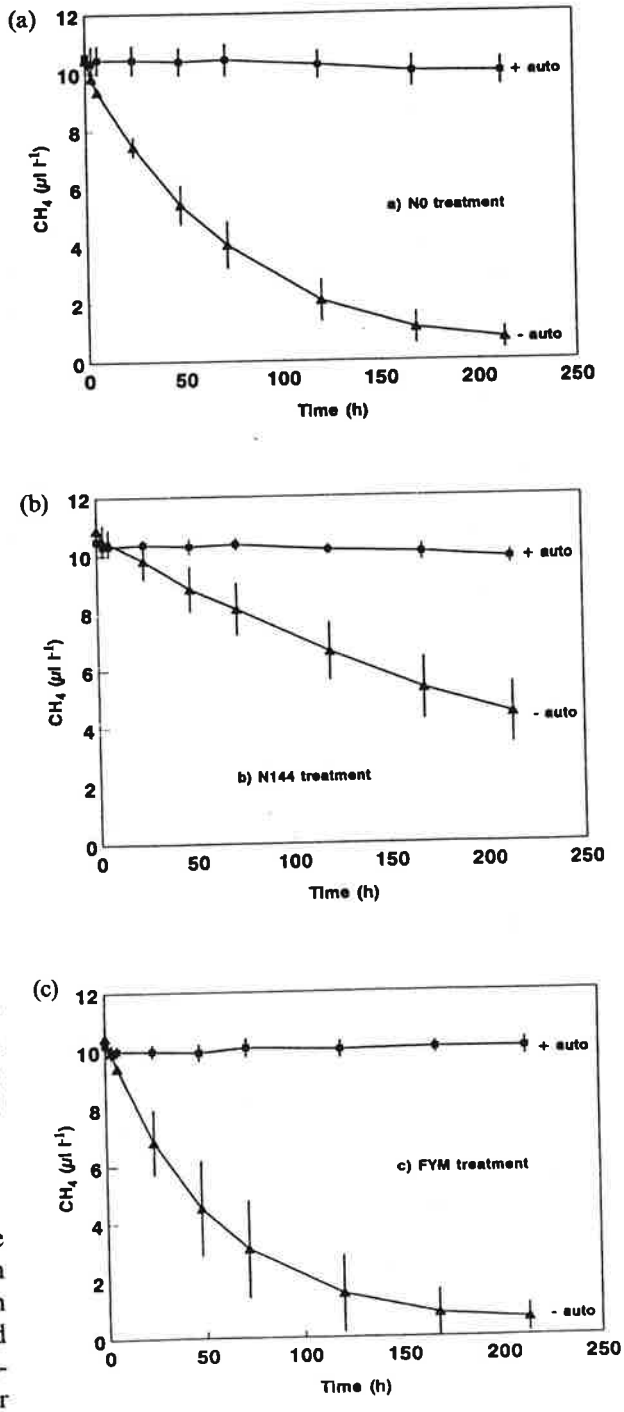
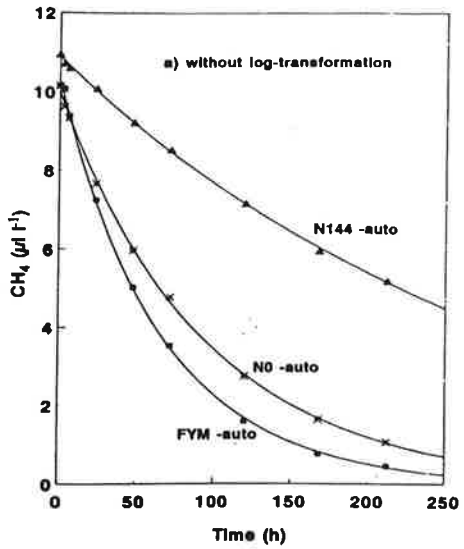


Fig. 1. Effect of autoclaving on the CH_4 uptake of (a) N0 (b) N144 and (c) FYM treatment ($n = 4$), vertical lines = standard deviations, sampling date: 4 September 1992.

(a)



(b)

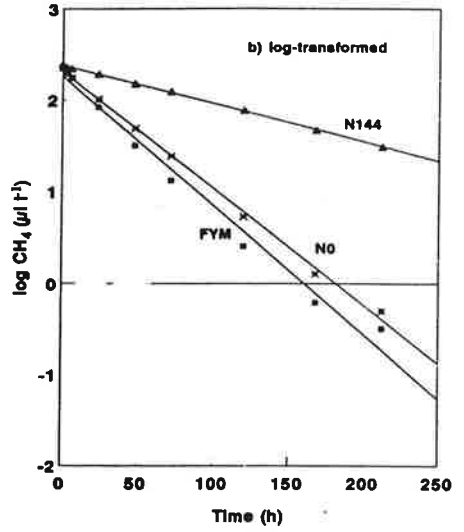


Fig. 2. Model simulation (curves) and observed concentrations (data points) for the non-autoclaved N0, N144 and FYM treatments, (a) before ($y = a\ e^{bt}$) and (b) after log-transformation ($\ln y = a + bt$).

autoclaving. Where soils were autoclaved there was virtually no decrease in the CH_4 concentration, showing that the process responsible was biological. We presume that the process is microbial oxidation, although we cannot distinguish between microbial assimilation and conversion to CO_2. With non-autoclaved soil cores CH_4 concentration decreased greatly and there were significant treatment differences. Disappearance of methane was much faster in the N0 and the FYM treatments than the N144 treatment. After 212 h CH_4 concentration in the N0 and FYM treatments reached values of 0.7 and 0.6 μl l^{-1}, respectively, whereas in the N144 treatment the concentration only decreased to 4.4 μl l^{-1}. The relatively large differences in CH_4 oxidation rate between replicate cores, shown by the large standard deviations in Fig. 1, were not unexpected as undisturbed cores were used rather than homogenized soil.

The decrease in CH_4 concentrations shows typical first-order-kinetics and was fitted to the exponential function $y = a\ e^{bt}$ where:

$y = CH_4$ concentration in headspace, μl l^{-1}
t = time of incubation, h

and a and b are constants.

Figure 2(a) shows the good fit between the data points and the predicted curves. A log-transformation of the form ($\ln y = a + bt$) was calculated which resulted in straight lines [Fig. 2(b)], for which the slopes (i.e. values of b) can be interpreted as methane oxidation rates. The rate for the N144 treatment was significantly lower ($P < 0.001$) than the rates for N0 and FYM, but the difference between N0 and FYM was not significant (Table 2). At the start of the autoclaving experiment the mineral-N contents were small and similar in all three treatments (Table 2). At the end of incubation nitrate had accumulated in non-autoclaved soils. In autoclaved soils large amounts of ammonium were measured, with most in the FYM treatment; this was expected as organic matter and microbial biomass are destroyed during heating (Sonneveld, 1979; Powlson and Jenkinson, 1976). The pH values showed only a small variation (pH 7.1–8.0) with the lowest value in N144 and the highest in N0. The moisture contents of the soil cores lay between 16 and 17% (w/w).

Long- and short-term effects of N fertilizer and FYM application on CH_4 oxidation

Figure 3(a) shows the CH_4 consumption rates for the whole range of N treatments for soil cores collected in April. The cores were incubated for 212 h without any addition of inorganic N and then for a

Table 2. Methane decrease rates (b values, μl CH_4 l^{-1} h^{-1}, × 1000), mineral nitrogen content, pH and moisture content at start and end of incubation in the autoclaving experiment ($n = 4$; sampling date: 4 September 1992)

				End of incubation					
	Start			Autoclaved			Non-autoclaved		
N treatment	N0	N144	FYM	N0	N144	FYM	N0	N144	FYM
b values (× 1000)	—	—	—	−0.2	−0.2	0.0	−13.2	−4.3***	−15.6
NO_3-N [kg ha^{-1}]	4.9	4.9	7.2	3.2	4.3	9.3	9.7	9.2	11.8
NH_4-N [kg ha^{-1}]	1.6	0.8	1.5	14.1	16.8	49.4	0.7	0.7	1.3
pH (in H_2O)	7.7	7.1	7.4	8.0	7.2	7.5	8.0	7.2	7.7
% H_2O (by weight)	ND	ND	ND	16.1	17.4	17.4	15.7	17.1	17.6

ND = not determined.
***Significant difference at the 0.1% level.

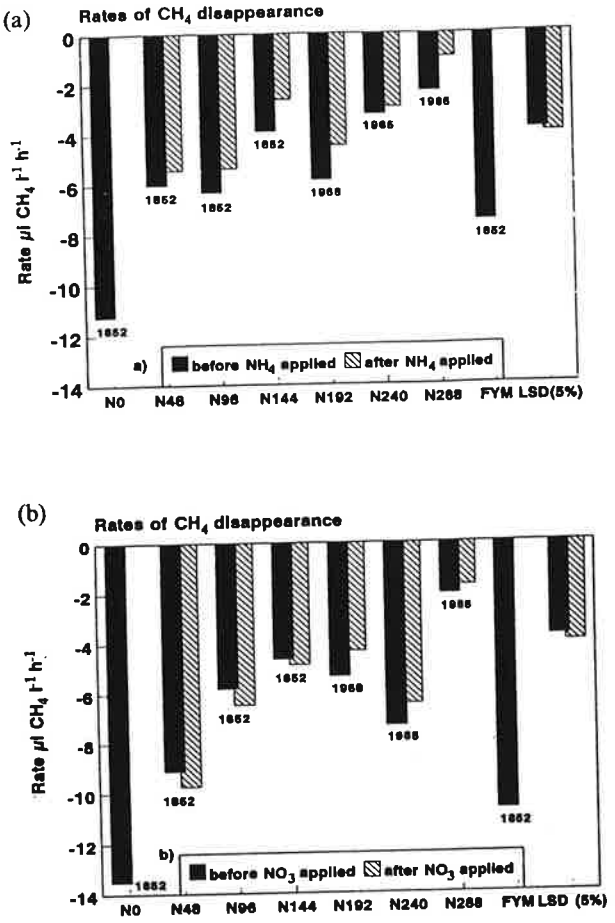


Fig. 3. Methane decrease rates (b values, μl CH_4 l^{-1} h^{-1}, $\times 1000$) for the different N treatments ($n = 4$, sampling date: 6 April 1992); (a) before and after NH_4^+ application, (b) before and after NO_3^- application, no N applied to N0 and FYM; 1852, 1968 and 1985 indicate dates when treatments began, LSD = least significant difference.

second 212 h period after addition of NH_4-N at rates equivalent to the annual field application. The NO and FYM treatments were amended with 2 ml of water only; this had no effect on CH_4 oxidation rate (data not shown). At the date of sampling (in April) the mineral nitrogen content of all soils was <5 kg N ha^{-1} to 12 cm depth (Table 3) so the rates of CH_4 oxidation measured before addition of NH_4^+ represent the long-term effect of nitrogen fertilization, not the effect of residual inorganic N from fertilizer application. Methane oxidation rate in the N0 treatment was significantly (5% level) greater than in all the mineral-N treated plots, and the FYM treatment was intermediate. Adding NH_4^+ to the cores caused only small decreases in the CH_4 oxidation rate which were not statistically significant [Fig. 3(a)].

Results from a repeat set of soil cores, sampled at the same time, and incubated without adding inorganic N are shown in Fig. 3(b). Again, the highest CH_4 oxidation rate was observed in the N0 treatment and rates declined progressively for increases in N applications up to N144. The FYM treatment was intermediate between N0 and N48. Adding nitrate to the soil cores had no significant effect.

Table 3. Mineral nitrogen content, pH and moisture content in the different N treatments of the NH_4 experiment and the NO_3 experiment (sampling date: 6 April 1992); no N applied to N0 and FYM

	Start of incubation (adjacent samples)			End of incubation (after NH_4 treatment)				End of incubation (after NO_3 treatment)			
Treatments	NO_3-N [kg ha^{-1}]	NH_4-N [kg ha^{-1}]	pH (in H_2O)	NO_3-N [kg ha^{-1}]	NH_4-N [kg ha^{-1}]	pH (in H_2O)	H_2O (% w/w)	NO_3-N [kg ha^{-1}]	NH_4-N [kg ha^{-1}]	pH (in H_2O)	H_2O (% w/w)
N0	2.3	0.2	7.9	10.1	0.8	7.7	15.3	12.7	0.9	7.7	15.5
N48	3.4	0.3	7.8	70.6	2.0	7.3	15.9	80.4	0.8	7.4	17.5
N96	3.4	0.3	7.7	97.3	22.2	7.0	15.6	137.8	0.9	7.1	16.8
N144	3.2	0.4	7.6	141.8	46.1	6.7	17.3	224.8	1.1	7.0	16.4
N192	3.2	0.6	8.0	225.1	4.1	7.5	16.6	264.2	1.2	7.6	16.2
N240	3.8	0.5	7.6	167.4	182.2	6.7	16.0	338.7	0.9	7.0	16.1
N288	3.2	0.2	8.0	267.5	53.5	7.3	16.3	392.6	0.8	7.4	17.0
FYM	3.3	0.4	8.0	19.6	0.6	7.7	16.7	22.1	0.6	7.7	16.3

Table 4. Methane decrease rates (b values, μl CH_4 l^{-1} h^{-1}, $\times$ 1000) after either 2 μl CH_4 l^{-1} (ambient concentration, $-CH_4$) or 10 μl CH_4 l^{-1} ($+CH_4$) preincubation ($n = 8$; sampling date: 4 September 1992), LSD (5%) = 2.5

N treatment	$-CH_4$	$+CH_4$
FYM	−19.7	−17.3
N_{48}	−8.9	−8.5
N_{144}	−3.9	−4.1

These experiments clearly point to a long-term effect of nitrogen fertilization on CH_4 uptake, which is much more pronounced than any short-term effect of added inorganic N. As with the soils used in the autoclaving experiment, mineral-N content was small (<4 kg N ha^{-1} to 12 cm depth) and very similar in all treatments at the time of sampling (Table 3). There was also very little variation in pH between treatments (range 7.6–8.0 before incubation; Table 3).

Soil cores taken in September were used to test whether previous exposure of soil to atmospheres containing 10 μl CH_4 l^{-1} influenced oxidation rates during a subsequent incubation. Table 4 shows that there was no effect compared to cores incubated at the ambient atmospheric CH_4 concentration of 2 μl l^{-1} so the comparison of unamended soil and that amended with NH_4^+ or NO_3^- (Fig. 3) should not be influenced by previous CH_4 exposure. The same cores were also used to compare the effects of NH_4^+ and NO_3^- which was applied to N48 and N144 at their historic rates and to FYM at 144 kg N ha^{-1}. Rates of CH_4 uptake in the N48 and N144 treatment (Table 5) were similar to those measured from the same treatments collected from the field in April [Fig. 3(a),(b)]. Furthermore the values for N144 corresponded with those found in the autoclaving experiment, which was conducted with a separate set of cores, but also taken in September (Table 2). For the N48 and N144 treatments addition of inorganic N, whether as NH_4^+ or NO_3^-, did not alter the rate of methane oxidation (Table 5; compare rates with those in Table 2). By contrast, in the FYM treatment the nitrate amended soil cores showed a much higher CH_4 oxidation rate compared to the ammonium-treated cores ($P < 0.001$). Ammonium addition made little difference to the methane oxidizing ability of FYM-treated soil, but NO_3^- application enhanced it (Table 5; compare rates with those in Table 2).

CO_2 evolution

The CO_2 concentrations in the headspace of the incubation jars did not exceed 6.5% indicating that anaerobic conditions never developed and that O_2 content had not decreased to <13.5% by the end of an incubation. In the autoclaving experiment, CO_2 concentrations were measured more frequently, and increased steadily from 24 to 212 h with final values in the non-autoclaved cores from the N0, N144 and FYM plots of 2.9, 3.9 and 6.4% CO_2, respectively. The autoclaved cores showed some CO_2 evolution with final values of 0.2, 0.5 and 1.3% CO_2, respectively. There could have been some microbial activity in the autoclaved cores due to contamination during the transfer into the incubation jars. Nevertheless, autoclaving entirely inhibited CH_4 consumption [Fig. 1(a)–(c)]. In the other experiments there was a general trend for greater CO_2 evolution after NH_4^+ than NO_3^- application (data not shown).

DISCUSSION

There is evidence that addition of inorganic N fertilizer decreases the rate at which CH_4 is oxidized in acid forest soils (Steudler *et al.*, 1989) and in grassland soils (Mosier *et al.*, 1991) but only few data have been reported on the effects in arable soil. The Broadbalk experiment at Rothamsted offers an ideal site for investigation as winter wheat has been grown continuously for 149 yr and a regime of constant annual application of inorganic N at rates up to 144 kg N ha^{-1} and of farmyard manure has been maintained for 140 yr since 1852; between the start of the experiment in 1843 and 1852 the fertilizer N rates varied somewhat between years.

In our work intact soil cores were used so measurements of CH_4 fluxes were comparable to *in situ* rates (King *et al.*, 1990). Conditions in slurries cannot mimic the CH_4 and O_2 gradients that occur *in situ* (Conrad and Rothfuss, 1991) so an undisturbed soil structure is of importance. Methane oxidizers and ammonium oxidizers both tend to favour similar habitats in soil, namely aerobic–anaerobic interfaces (Bedard and Knowles, 1989). As alterations in soil moisture can have a considerable effect on gas diffusion, we tried to keep within a narrow range (16–17% w/w). Investigations by Whalen *et al.* (1990) with soil

Table 5. Methane decrease rates (b values, μl CH_4 l^{-1} h^{-1}, $\times$ 1000), mineral nitrogen content, pH and moisture content at start and end of incubation with either NH_4 or NO_3 application (144 kg N ha^{-1} to FYM), sampling date: 4 September 1992

				End of incubation ($n = 8$)					
	Start			$+NH_4$			$+NO_3$		
N treatment	N48	N144	FYM	N48	N144	FYM	N48	N144	FYM
b values ($\times$ 1000)	—	—	—	−8.0	−2.9	−13.2	−9.3	−5.1	−23.8***
NO_3-N [kg ha^{-1}]	4.6	4.7	5.9	54.8	81.7	85.2	69.6	192.4	171.1
NH_4-N [kg ha^{-1}]	1.1	1.0	1.0	9.8	100.8	78.7	0.6	0.9	1.2
pH (in H_2O)	7.4	7.1	7.5	7.3	6.9	7.5	7.4	7.0	7.5
% H_2O (by weight)	ND	ND	ND	17.4	16.3	17.2	17.4	16.7	17.0

ND = not determined.
***Significant difference at the 0.1% level.

covering a landfill site showed the highest methane oxidation rate at a moisture content of 11% (w/w) so our data may underestimate the potential for CH_4 oxidation on Broadbalk. As the solubility of CH_4 in water is low (*ca.* 24 mg l^{-1} at 20°C and ambient pressure; Schütz and Seiler, 1989) dissolved CH_4 can be neglected under these conditions (Born *et al.*, 1990). By measuring changes in CH_4 concentrations in headspace, as in our work, only a net CH_4 flux can be measured and CH_4 could be produced in soil as well as oxidized. However, a very low redox potential (−200 mV) is required for CH_4 production which is most unlikely under the conditions of incubation, even within microsites. Nor can this method distinguish between CH_4 that is assimilated into microbial biomass and that used as an energy source and oxidized to CO_2; such measurement would require isotopic labelling. But, whatever the fate of CH_4, its removal from the atmosphere and partial replacement by CO_2 is beneficial as CO_2 is about 20 times less effective as a greenhouse gas than CH_4 (Blake and Rowland, 1988).

Our incubations involved exposure of soils to 10 μl CH_4 l^{-1} rather than the ambient concentration at about 2 μl l^{-1}; this was to improve analytical precision so that differences in the rate of CH_4 disappearance between soils could be detected more readily. According to Schütz *et al.* (1990) the rate of CH_4 uptake by soils may increase with an increase in atmospheric CH_4 mixing ratios, and Whalen *et al.* (1990) observed higher CH_4 oxidation rates with increasing CH_4 concentrations in the headspace over the range of 1.7 to >10,000 μl l^{-1}. This phenomenon was also observed in our investigations. Soil cores were conditioned by exposure to either 10 μl CH_4 l^{-1} or 2 μl l^{-1} and gas measurements made at 0 and 212 h. Methane oxidation in the amended atmosphere increased by the same factor as the increase in CH_4 concentration (Table 6). Hence the *differences* in the CH_4 uptake rate between the different treatments investigated was independent of the initial CH_4 concentration. Ideally measurements should be made in the field rather than in a closed incubation vessel where CH_4 concentration can decrease well below the natural atmospheric concentration [e.g. Fig. 1(a)]. However, laboratory studies permit greater precision in detecting differences.

Most of the CH_4 uptake rates reported in the literature were measured under field conditions and resulted from short-term flux measurements (<30 min) so it is difficult to compare them with our results. The rates measured in the field will be higher than those measured in jars because the CH_4 concentration at the soil surface will be buffered. It is therefore not surprising that the uptake rates we measured (0.8–1.3 μg C m^{-2} h^{-1}, Table 6) are rather lower than those reported by Mosier and Schimel (1991) for a fertilized wheat field (2.5–3.75 μg C m^{-2} h^{-1}) and by Born *et al.* (1990) on cultivated arable land (0.71–17.12 μg C m^{-2} h^{-1}).

Mosier *et al.* (1991) and Mosier and Schimel (1991) observed that N-fertilizer applied to a fertilized wheat field did not affect CH_4 uptake. A reduction of CH_4 uptake after N fertilization was found in hitherto unfertilized forest and grassland soils (Steudler *et al.*, 1989; Mosier *et al.*, 1991). Mosier *et al.* concluded that N-turnover, rather than the actual mineral-N content of soil, influenced CH_4 uptake: our results support this suggestion. Fresh application of mineral-N to a soil which had received mineral-N for many years did not alter the methane-consuming ability; i.e. no short-term effect of mineral-N application could be observed [Fig. 3(a),(b); Table 5]. By contrast, a large long-term effect of the different fertilizer treatments was observed. This was especially true of the oldest treatments—N0-N48-N96-N144, which were established 140 yr ago [Fig. 3(b)]. In these treatments, soil total N contents have been virtually constant since 1881 (Jenkinson, 1977), thus steady-state conditions can be assumed, with the nitrogen immobilized into soil organic N equal to the amount mineralized each year. There is evidence that the higher rates of N fertilizer application on Broadbalk have led to both increased immobilization of N and also increased mineralization (Powlson *et al.*, 1986; Shen *et al.*, 1989) so our findings are consistent with the suggestion that decreased CH_4 oxidation is associated with increased N turnover, though not necessarily with mineral N content at the time of measurement. However, the FYM treatment is an exception to this. It receives *ca* 240 kg N ha^{-1} yr^{-1} as organic manure, its N-turnover is 2–3 times greater than in any other treatment, yet it has a methane-oxidizing ability comparable to that of the N0 plot [Fig. 1(a),(c)]. In addition, the FYM treatment reacted differently to an addition of mineral N. All the other fertilized plots showed no response to either fresh NH_4^+ or NO_3^-, whereas FYM had

Table 6. Methane decrease rates for either ~2 μl CH_4 l^{-1} ($-CH_4$) or 10 μl CH_4 l^{-1} ($+CH_4$) at $t = 0$, calculated with the concentrations at start (t_0) and end of incubation (t_{end}: 208.5 h for N48, 213 h for N144, 188 h for FYM)

N treatment	CH_4 preincubation	Decrease t_0–t_{end} (μl CH_4 l^{-1})	Factor $+CH_4/-CH_4$ at t_0	Decrease rates (μl CH_4 l^{-1} h^{-1})	Factor $+CH_4/-CH_4$ of decrease rates	Decrease rates (μg C m^{-2} h^{-1})
N48	$-CH_4$	2.5–0.6		0.00911		0.83
	$+CH_4$	10.7–1.8	4.4	0.0422	4.6	
N144	$-CH_4$	2.4–0.8		0.00756		0.69
	$+CH_4$	10.6–2.9	4.4	0.0365	4.8	
FYM	$-CH_4$	2.8–0.2		0.0140		1.28
	$+CH_4$	10.1–0.3	3.6	0.0521	3.7	

a much higher CH_4 uptake rate after NO_3^- was applied.

There is uncertainty regarding the relative importance of methanotrophs and ammonia oxidizers for the oxidation of CH_4 in soil. The key enzymes of these bacteria are similar (methane monooxygenase and ammonia monooxygenase) and both are able to oxidize either CH_4 or NH_3 (O'Neill and Wilkinson, 1977; Hyman and Wood, 1983; Jones and Morita, 1983; Bedard and Knowles, 1989). Opinion is divided about the rate at which methanotrophs oxidize NH_3 and ammonia-oxidizers oxidize CH_4. Bedard and Knowles (1989) showed that the maximum CH_4 oxidation rate for an ammonia oxidizer was about one-fifth that of the slowest methanotroph. On the other hand, Jones and Morita (1983) postulated that the rate of CH_4 oxidation by NH_3 oxidizers is significant and may actually exceed that of the classical methane oxidizers. Furthermore, from an energetics viewpoint, the oxidation of 1 mol CH_4 provides more energy than the oxidation of 1 mol NH_3 and is therefore a more advantageous option. In our work there is one point suggesting that the methane uptake was more likely mediated by methanotrophs. The CH_4 oxidation pattern for the N0 and FYM treatments were similar [Fig. 1(a),(c)]. This is despite the FYM treatment having a 2–3 times greater N-turnover and 8–9 times more ammonia-oxidizing bacteria than the N0 plot (Ziemiecka, 1932).

A possible explanation of our results and those of Mosier *et al.* (1991) and Mosier and Schimel (1991) is that the continued application of inorganic N fertilizer increases the numbers of ammonia-oxidizers at the expense of methane-oxidizers. This is possible if they occupy a similar niche in soil (Bedard and Knowles, 1989), and if the total size of the two populations is limited by the availability of suitable sites: this possibility requires testing. If this explanation is correct a similar change in the balance between ammonia-oxidizers and methane-oxidizers would be expected in the FYM-treated soil, yet CH_4 oxidation rate in FYM-treated soil was not decreased compared to the unfertilized treatment. Two factors might tend to reverse the effect in the FYM-treated soil. First, the total microbial population is much greater in the FYM treatment than the untreated soil: total soil microbial biomass is more than twice that in the unfertilized soil (Jenkinson and Powlson, 1976). Thus the size of the methane-oxidizing population is likely to be greater, even if it comprises a smaller proportion of the total. Second, the annual application of FYM is likely to have increased the temporary occurrence of anaerobic zones in this soil as aerobic decomposition of the large organic matter additions will have depleted oxygen concentration in the soil air, at least for some periods of the year. The opportunity for CH_4 formation in this soil is thus greater than in the other treatments. This could have increased the numbers of methanotrophs, as occurs in landfill cover soils which are exposed to enhanced CH_4 concentration (Whalen *et al.*, 1990).

In our investigations, and those of Mosier *et al.* (1991), soils treated with mineral-N fertilizer all had a slightly lower pH than unfertilized soil. Although it is possible that this might affect CH_4 uptake, it seems unlikely. Such an effect was not observed by Bedard and Knowles (1989) who investigated effects of pH changes on methanotrophs. Indeed, they noted an inhibition of CH_4 oxidation at higher pH because of the increase in NH_3 concentration in relation to NH_4^+, NH_3 being more inhibitory. Jones and Morita (1983) investigated CH_4 oxidation by *Nitrosomonas europaea*; between pH 7 and 8 activity changed little but decreased substantially below pH 6.5 and above 8.0–8.5. In our present study all soils were between pH 7 and 8 (Tables 2, 3 and 5) so the CH_4-oxidizing activity of nitrifiers was unlikely to have been affected. However, in the study of Mosier *et al.* (1991) the unfertilized soils were in the range pH 6.0–6.5 whilst the fertilized ones were at pH 5.6, so this change could have had an effect.

In conclusion, our results are the first to show that prolonged and continuous applications of inorganic N fertilizer can have a negative effect on the methane-oxidizing capacity of an aerobic arable soil. They are consistent with earlier results showing such an effect in forest and grassland soils. Because of the importance of N fertilizer in agricultural production worldwide it is essential that this effect is examined in a wide range of soil types, climates and agricultural systems to see whether it is reproduced elsewhere. It would be premature to conclude that there is a direct relationship between the increasing atmospheric concentration of methane and a decreased sink strength of aerobic arable soil resulting from N fertilizer use. Sources of methane have increased and it is possible that other sinks, such as photochemical reactions in the troposphere, may have changed. However, the results do show that agricultural practices, including nitrogen fertilizer use, may have significant and unforeseen effects at a global scale and therefore warrent close scrutiny. The observation that organic manure application had a much smaller effect than inorganic N fertilizer on the methane-oxidizing ability of arable soil is intriguing. Ruminants are thought to be a major source of methane; this observation emphasizes the importance of viewing the environmental effects of agricultural systems as a whole. If the effect of organic manure is by increasing soil organic matter content, and thus maintaining a larger soil microbial population, this result may indicate one previously unrecognized benefit of maintaining soil organic matter at high levels or of attempting to increase the organic matter contents of humus-depleted soils.

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