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Methane oxidation potentials and fluxes in agricultural soil: Effects of fertilisation and soil compaction

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Abstract. We have studied the inhibiting effect of fertilisation and soil compaction on CH_4 oxidation by measuring gas fluxes and soil mineral N dynamics in the field, and CH_4 oxidation rates in laboratory-incubated soil samples. The fertilisation and soil compaction field experiment was established in 1985, and the gas fluxes were measured from 1992 to 1994. Methane oxidation was consistently lower in fertilised than in unfertilised soil, but there apparently was no effect of repeated fertiliser additions on the fertilised plots. The measured mineral N in fertilised and unfertilised soil showed large differences in NH_4^+ concentrations just after fertilisation, but the levels rapidly converged because of plant uptake and nitrification. The CH_4 oxidation rate did not reflect these contrasting mineral N patterns, suggesting that the CH_4 oxidation capacity remaining in the soil that had been fertilised since 1985 was largely insensitive to ammonia in the new fertiliser. Thus, competitive inhibition by ammonia may have been involved in the early stage of the field fertiliser experiment, but the CH_4 oxidation remaining after 7 to 9 years of continued fertilisation seems not to have been affected by ammonia. The substrate affinity of the CH_4-oxidizing microflora appeared to be the same in both the fertilised soil and the unfertilised control, as judged from the response to elevated CH_4 concentrations (52 μl l^{-1}) in laboratory incubations. Soil compaction resulted in a persistent reduction of CH_4 influx, also seen in laboratory incubations with sieved (4-mm mesh) soil samples. Since the sieving presumably removes diffusion barriers created by the soil compaction, the fact that compaction effects persisted through the sieving may indicate that soil compaction has affected the biological potential for CH_4 oxidation in the soil.

Introduction

CH_4 absorbs infrared (IR) radiation in the atmosphere and thus contributes to global warming (Crutzen 1991). An estimated 15% of anticipated atmospheric warming is due to CH_4 (Houghton et al. 1992). CH_4 also influences

the ozone concentration (Crutzen 1991). The atmospheric concentration of CH_4 is increasing by about 1% per year (Denmead 1991). One factor that contributes to the increased atmospheric CH_4 is the reduction in CH_4 oxidation in terrestrial ecosystems. Decreased CH_4 uptake upon addition of N fertilisers is reported by many authors, both in forest (e.g., Steudler et al. 1989; Sitaula et al. 1995) and agricultural ecosystems (e.g., Hansen et al. 1993; Hütsch et al. 1994). The actual mechanism behind this nitrogen effect remains unclear. It is speculated, however, that N affects CH_4 flux through persistent changes in microbial populations and ecological interactions, rather than direct inhibition of the active organism (Dunfield et al. 1995). On the other hand, results from laboratory studies have clearly shown that NH_3 inhibits CH_4 oxidation (Bedard & Knowles 1989; Adamsen & King 1993; Bosse et al. 1993). Therefore, both long-term and short-term effects of fertilisation seem plausible. This requires a systematic investigation to measure CH_4 oxidation rates together with mineral N content over an extended period (before and after fertilisation). We have been able to do this by measuring the dynamics of CH_4 fluxes and mineral N (NH_4^+ and NO_3^-) over a period of three years in an agricultural soil in Norway. We also report the results from an incubation experiment designed to investigate CH_4 oxidation and substrate affinity of methane oxidizers in response to fertilisation and soil depths when exposed to ambient and high CH_4 concentrations.

Soil compaction is a common problem of modern agriculture (e.g., Douglas & Crawford 1993; Hansen 1996). Soil compaction decreases the proportion of coarse pores (O'Sullivan & Ball, 1993, Breland & Hansen 1996), air permeability and gas diffusion (Ball et al. 1997a), and thus potentially increases the abundance of anaerobic microsites. This is likely to be a serious problem in an easily compacted soil in a humid climate, as in western Norway. Compaction reduced average CH_4 uptake rates by about 50% when measured for some months in 1991 (Hansen et al. 1993). In the present work, we conducted more detailed and extended field flux measurement (for three years), measuring other variables that might be important in explaining compaction effects, such as soil moisture, air-filled pore space, and gas diffusion. This allowed us not only to get some insight into the mechanism behind the compaction effects, but also to study the persistence or temporal changes of such effects, by comparing methane uptake rates in 1991 (Hansen et al. 1993) with the rates after some years of soil compaction. We also wanted to examine the persistence of soil compaction effects for reduced methane oxidation beyond the physical effects in the field. We tested this by measuring CH_4 oxidation rates in the laboratory in compacted and uncompacted soils after eliminating the physical compaction by sieving.

Materials and methods

Site description

Gas fluxes were measured in a field experiment in Surnadal, Norway. This was a continuation of earlier work (Hansen et al. 1993), with additional measurements of soil mineral nitrogen and moisture content, simultaneously with gas measurement (CH_4 fluxes). The present measurements were done for three years (1992–1994). Hansen (1996) gives details on the field site. In brief, the experimental field has a slope of less than 2% and is located on a naturally drained sandy loam developed on fluvial deposits. The soil is a Typic Udorthents (USDA System of soil classification). The topsoil contains 2.2% organic carbon and 0.17% organic nitrogen. The subsoil (beneath 30–100 cm), contains with stones, gravel and coarse sand.

In 1992 the field was ploughed and sown with timothy (*Phleum pratense*) and clover (*Trifolium pratense, T. repens* and *T. hybridum*). This ley remained for the rest of the experimental period.

Treatments and experimental design

The experiment had a split-plot factorial design with two replicates, with soil compaction on main plots and fertilisation on subplots (2.8 m $\times$ 8 m, 2 sample areas of 2 m $\times$ 1 m at each plot with 5.5 m between). For each flux measurement, soil cover chambers were placed at random within each sampling plot. For each combination of fertilisation and compaction treatments, there were four parallel flux air measurements taken on each day of measurement. The three years of fertilisation treatments were **NPK** [NPK fertiliser (18% N as NH_4NO_3; in 1992: 83 kg N ha^{-1}; in 1993: 120 kg N ha^{-1} divided into two applications (70+50 kg N); in 1994: 211 kg N ha^{-1} divided in two applications (123+88 kg N)]; **CSH** [cattle slurry, high levels; in 1992: 62 kg N (26 kg NH_4-N) ha^{-1}; in 1993: 80+48 kg ha^{-1}; 1994: 123+88 kg N (74+52 kg NH_4-N) ha^{-1})]; **CSL** [cattle slurry low levels; in 1992: 34 kg N (15 kg NH_4-N) ha^{-1}; in 1993: 48+32 kg N (24+16 kg NH_4-N) ha^{-1}; in 1994:45+30 kg N (27+18 kg NH_4-N) ha^{-1})] and **UNF** (unfertilised) treatment. Cattle slurry was diluted with water to 200% of the original volume and spread by a can with a small spreading plate. NPK fertiliser was spread by hand. The diluted slurry contained 5% dry matter, 0.15% total-N, 0.09% NH_4-N, 1.3 mg/100g NO_3-N, and had a pH of 7.5 (mean of 3 years).

The soil compaction treatment comprised one wheel-by-wheel pass of a four-tonne tractor each spring, and two passes after each harvest. The rear wheels were double-settings with a total tyre width of 140 cm (inflation pres-

sure of 57 kPa). In front there were low-pressure tyres with a total width of 100 cm.

Similar fertiliser and compaction treatments were carried out every year from 1985 except 1990, when no extra compaction or fertilisation was applied.

Tillage treatments (ploughing, two harrowings, rolling) and sowing were carried out across the experimental plots with tractors similar to those used for compaction. Thus, the uncompacted soil was to some extent affected by tractor traffic.

The average bulk density (soil depth 7–11 cm) in September 1991 and 1993 was 1.3 and 1.2 g cm^{-3} in compacted and uncompacted soil, respectively.

Gas measurements

In 1992, we started measuring gas fluxes from shortly after snow melt (18 May) until 26 June. Ten measurements were taken during this period [18, 20, 22, 25, 27 and 30 May; 1, 4, 23 and 26 June]. Similarly, nine measurements were made in 1993 [8, 11, 19, 27 May; 2, 6, 13 June; 25 August; and 7 September]. In 1994, four measurements were made [27 May, 2 and 18 June and 28 July].

Gas fluxes were measured by soil cover chambers as described by Hansen et al. (1993). Soil cover chambers of two different heights (10.5 cm and 21 cm) with the same inner diameter (22.5 cm) were used. The taller chambers were used in the latter part of the crop growth to accommodate the plants. The chambers had a reflecting outer surface that ensured minimal heating by direct sunlight (Hansen et al. 1993). The chambers were pushed 2.5 cm into the soil. Gas samples (20 ml) were extracted with a syringe (20 ml) immediately after closing the holes on the top of the chambers with butyl rubber stoppers (type 20-B3P, Chromacol Ltd, London). Subsequent gas samples were taken after 1.5 h for short chambers and 2 h for tall chambers. The 20 ml gas samples were transferred to 12 ml evacuated (10^{-3} atm.) glass vials (N 20-10 Macherey-Nagel, Dühren). This was done to secure overpressure in the vials, to enable repeated analysis of the gas if necessary. All gas samples were analysed by gas chromatography within 7 days of sampling (Sitaula et al. 1992).

Mineral-N measurements

In 1993 and 1994, the NH_4^+ and NO_3^- content of soil (0–20 cm) was determined in a composite soil sample for each treatment collected with a soil auger. The soil samples were taken on each date of gas measurement, within

5–10 cm from where each gas flux measurement was done. The gravimetric soil moisture content was determined to express NO_3^- and NH_4^+ content of the soil on a dry weight basis. The soil samples were analysed for NO_3^- and NH_4^+ concentrations by standard methods [20 g soil extracted by 50 ml 2M KCl and flow injection analysis by FIA-star 5010 Analyser, TECATOR, Sweden).

Other measurements

The volumetric moisture content of the top 20 cm of soil was measured at each gas sampling date using the TRIME-system digital moisture meter (IMKO GmbH, Ettingen, Germany) connected to TDR (Time-Domain-Reflectrometry) probes. Soil temperature (soil depth = 10 cm and 20 cm) was measured at each date of gas measurement using a hand-held digital thermometer.

To determine the total pore volume in each treatment plot, undisturbed soil samples were taken in 100-cm^3 cylinders (four replicates) from 7–11 cm soil depth. Particle density used to calculate material and pore volume was determined following the method of De Boodt et al. (1967). This measurement was done only once in September 1993 and used for estimating air-filled porosity based on measured volumetric water contents on each date.

In situ diffusion was measured using the method of Ball et al. (1994), modified to use Freon-22 as the diffusing gas instead of Kr-85, as described in Ball et al. (1997a). Two measurements were taken only once, in June 1993, in both compacted and uncompacted plots.

Laboratory incubation experiment

In July 1994, soil samples were collected from the field plots for conducting two laboratory incubation experiments. For the first incubation experiment, soil samples were taken from the NPK-fertilised and unfertilised treatments (uncompacted plots). A bulk (composite) sample of approximately 1 kg was taken from each soil layer (0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm depth). Samples from each layer were mixed separately, sieved (4 mm), and taken to the laboratory for determination of gravimetric water content (de Boodt et al. 1967), organic C (Nelson & Sommers 1982) and total N (Bremner & Mulvaney 1982). The soils had a moisture content of 45% $\pm$ 4%sd (v/v) in the field and were air-dried to 30% (v/v) (controlled by frequent weighing during drying). Methane oxidation rates at 15 °C were measured by incubating 20 g soil samples in 120 ml serum bottles capped with butyl rubber stoppers (type 20-B3P, Chromacol Ltd, London). Two successive incubations were conducted, the first for measuring oxidation rates at ambient concentrations, the second for measuring oxidation rates at high (52 $\mu l\, l^{-1}$) CH_4 concentra-

tion. For oxidation rates at ambient concentrations, the bottles were capped and incubated for 3 days and the atmosphere inside the serum bottles was sampled through a butyl rubber stopper every 12 h (direct transfer from serum bottle to GC, Sitaula et al. 1992). The bottles then were opened and aerated for 24 h in the 15 °C incubator and recapped, and the atmosphere replaced with air containing 52 μl l^{-1} CH_4. During the following 1-day incubation, CH_4 concentration was measured every 6 h.

In the second incubation experiment, compacted and uncompacted soils were compared. Composite sample of approximately 1 kg was taken from both the compacted and uncompacted treatments [unfertilised plots, 0–10 cm soil depth]. The compacted and uncompacted samples were mixed separately, sieved (4 mm), and taken to the laboratory for the same moisture and temperature treatment as that of the first incubation experiment. Methane oxidation rates at ambient concentrations were measured by sampling the atmosphere inside the serum bottles [direct transfer from a serum bottle to GC, Sitaula et al. 1992]. During the following incubation, CH_4 concentration was measured every 12 h for 3 days.

Flux calculations and statistical analyses

CH_4 concentrations in the soil cover chambers (field experiment, Figure 1) and in the atmosphere of serum bottles (laboratory experiment, Figure 4) closely followed an exponential function during the incubation. Thus, the rate constants (k, h^{-1}) can be estimated from a minimum of two concentration measurements C_1 and C_2 at time t_1 and t_2; $k = [\ln(C_2) - \ln(C_1)]/[t_2 - t_1]$, and the flux (ng CH_4 g^{-1} dry soil d^{-1}) at ambient concentration (Table 2 and Figure 4) is thus $^{G}/_{W} \cdot C_{a.}k$, where C_a is the CH_4 concentration in the atmosphere (1.8 μl l^{-1}) normalised to unit gas volume (G) and unit soil mass (W). The oxidation rate at elevated concentration (52 μl l^{-1}, Table 2) was calculated as the net reduction in CH_4 during the first 6-h incubation, thus assuming zero order kinetics. The half-saturation constant was estimated using the Michaelis-Menten function (Segel 1976).

Field CH_4 uptake rates (μg CH_4 m^{-2} h^{-1}, Table 1 and Figure 3) were calculated accordingly ($^{G}/_{A} \cdot C_{a.}k$), normalised to unit gas volume (G) and unit soil area (A), and assuming ambient CH_4 concentration (C_a = 1.8 μl l^{-1}).

The main effects and interactions of soil compaction, fertilisation and date were tested with analysis of a variance (ANOVA) and the Newman–Keuls test. The interaction between replicate and compaction was used as an error term to test the effect of compaction. Residual plots with studentized residuals were used to test the normality of the distributions (SAS Institute 1988). CH_4 fluxes were normally distributed.

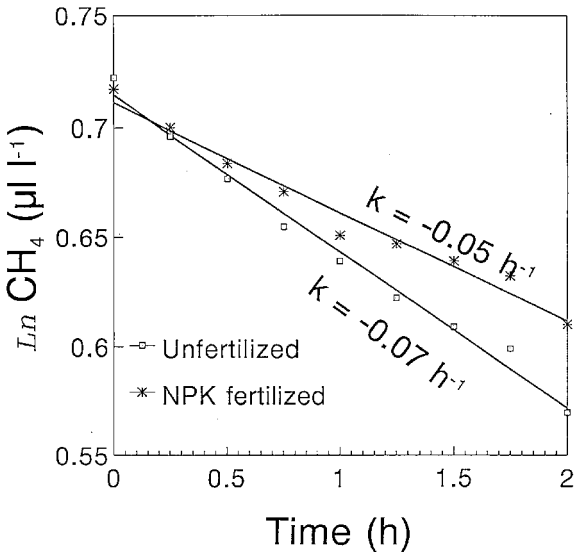

Figure 1. Methane concentrations (ln values) in headspace of field soil cover chamber in Surnadal field experiments. Decay rate constants (k, h^{-1}) were calculated by linear regression.

Correlations among CH_4 fluxes, soil moisture and air-filled porosity were tested with the Pearson correlation coefficient (SAS Institute 1988).

Results and discussion

Kinetics and range of field CH_4 fluxes

Figure 1 shows the results of the soil cover experiment designed to investigate the kinetics of the CH_4 flux during prolonged periods with the soil covered. The CH_4 concentration closely followed an exponential function of time, demonstrating that CH_4 consumption should be estimated as a first-order process. Such determinations of the kinetics of CH_4 consumption have previously been conducted in laboratory and lysimeter experiments (Sitaula et al. 1995; Hütsch et al. 1993; Yavitt et al. 1990). Our experiment, done in field conditions, confirms that in the field the CH_4 oxidation rate is a linear function of the concentration, as shown in Figure 1. The concentration of CH_4 at 0 h (1.9 to 2 μl l^{-1}) was slightly higher than that normally reported for ambient (1.8 μl l^{-1}). We believe the reason for this is that our site is near a heavily farmed area.

The average CH_4 uptake rate (for all dates) in the unfertilised treatment was 6 to 9 μg CH_4 $m^{-2}h^{-1}$ in 1992, 4 to 6 μg CH_4 $m^{-2}h^{-1}$ in 1993, and

Table 1. CH_4 uptake rates as influenced by fertilisation and soil compaction for the period 1992–1994 (average values for each treatment ± standard error).

	CH_4 flux (μg CH_4 m^{-2} h^{-1})**	
Fertilisation*	Uncompacted	Compacted
	1992	
NPK, 83 kg N ha^{-1}	5.5 ± 1bA	2.1 ± 0.7bB
CSH, 62 kg N ha^{-1}	4.1 ± 0.9bA	3.1 ± 0.7bA
CSL, 34 kg N ha^{-1}	5.1 ± 0.9bA	2.7 ± 0.8bB
Unfertilised	9.3 ± 1.2aA	6.1 ± 0.8aB
	1993	
NPK, 120 kg N ha^{-1}	4.2 ± 0.4bA	2.7 ± 0.3bB
CSH,128 kg N ha^{-1}	4.9 ± 0.5abA	3.0 ± 0.4bB
CSL, 82 kg N ha^{-1}	4.5 ± 0.4abA	2.2 ± 0.4bB
Unfertilised	6.1 ± 0.5aA	4.1 ± 0.8aB
	1994	
NPK, 209 kg N ha^{-1}	6.2 ± 0.8abA	2.2 ± 0.6abB
CSH, 211 kg N ha-1	3.6 ± 1.4bA	0.9 ± 0.8bA
CSL, 75 kg N ha^{-1}	8.5 ± 1.1aA	1.3 ± 0.9bB
Unfertilised	9.0 ± 0.9aA	4.2 ± 1.0aB

*NPK = NPK-fertiliser, CSH & CSL = cattle slurry High & Low.
**Within each compaction treatment (columns), the fertilisation treatments are compared with lower case letters (abc) and within each fertilisation treatment (rows), the compaction treatments are compared with upper case letters (*AB*). Values followed by different letters in the same column in lower case or rows in upper case are significantly different (Newman–Keuls test, $\propto$ = 0.05).

4–9 μg CH_4 $m^{-2}h^{-1}$ in 1994. The low fluxes recorded for several dates in 1993 occurred when the soil was nearly water saturated (soil moisture = 40 to 54% v/v, Figure 3). The low fluxes during the wet conditions were probably due to restricted CH_4 diffusion from atmosphere to soil, possibly in combination with CH_4 production in anaerobic microsites induced by soil wetness. Air-filled pore space was generally lower in 1993 than in 1992 and 1994 (Figure 3).

Fertilisation effects

Relative to unfertilised soil, fertilisation reduced CH_4 uptake by 30% to 41% in uncompacted soil and by 35% to 65% in compacted soil (Table 1). There was no significant difference between fertilisation with NH_4NO_3 (NPK) and

with cattle slurries. Neither did the amount of cattle slurry (CSH & CSL) affect CH_4 uptake significantly (Table 1). These effects were in agreement with the previous finding (Hansen et al. 1993) regarding the negative effects of both fertilisation and soil compaction treatments on CH_4 uptake.

One possible explanation for decreased CH_4 uptake caused by input of inorganic N is that NH_3 and CH_4 compete for the same active site of the monooxygenases, the enzymes catalysing the first oxidation step of CH_4 and NH_4^+ in CH_4-oxidising and ammonium-oxidising bacteria (Bedard & Knowles 1989). If this is the only mechanism involved, the inhibitory effect of fertilisation should depend on the comparative NH_4^+ concentration in the fertilised and unfertilised soil. The concomitant measurements of CH_4 fluxes and mineral N contents in soil allow an examination of this relationship between inhibition by fertiliser and NH_4^+ concentration. Figure 2 shows the variation in mineral N (NH_4^+ and NO_3^-) content and corresponding CH_4 fluxes in relation to fertilisation treatment. The fertilised and control treatments did not differ in NH_4^+ or NO_3^- content before fertilisation, i.e., at the start of the experiment. In response to fertilisation, NH_4^+ and NO_3^- concentrations increased, but converged towards the levels in the control plots throughout the growth season. The convergence was particularly rapid for NH_4^+ concentration, which already was similar for the two treatments 1–2 weeks after the fertilisation (Figure 2). In contrast to this transient effect of fertilisation on NH_4^+ and NO_3^- levels in soil, the fertiliser effect on CH_4 oxidation largely persisted throughout the growth season. This persistence of the fertiliser effect on CH_4 oxidation can also be seen at the beginning of the growing season (i.e., prior to fertilisation), as a result of the previous year's fertilisation. In fact, there is no visible direct effect of the fertiliser at all in Figure 2; the whole fertiliser effect is most likely a residual effect of previous fertiliser additions. Thus we are seeing two phenomena here. First, the fertiliser has affected the CH_4 oxidation rate (probably at a very early stage of the field experiment), and this fertiliser effect is not reversed by removing the fertiliser's effect on the ammonium and nitrate level in the soil. Second, adding more fertilisers to the same soil does not seem to have the same inhibiting effect on the CH_4 oxidation rate remaining after the first fertiliser additions.

The first phenomenon, i.e., the long persistence of fertiliser effects, has been seen by others (Mosier et al. 1991), and is possibly explained by irreversible damage to some CH_4-oxidizers or replacement of methanotrophs by nitrifiers in the soil (Castro et al. 1994). The damage is hypothesised to be exerted through inhibition of CH_4 oxidation by NH_3 (Bedard & Knowles 1989), resulting in starvation and death of CH_4-oxidizing bacteria. The slow recovery of this particular functional group is not difficult to explain,

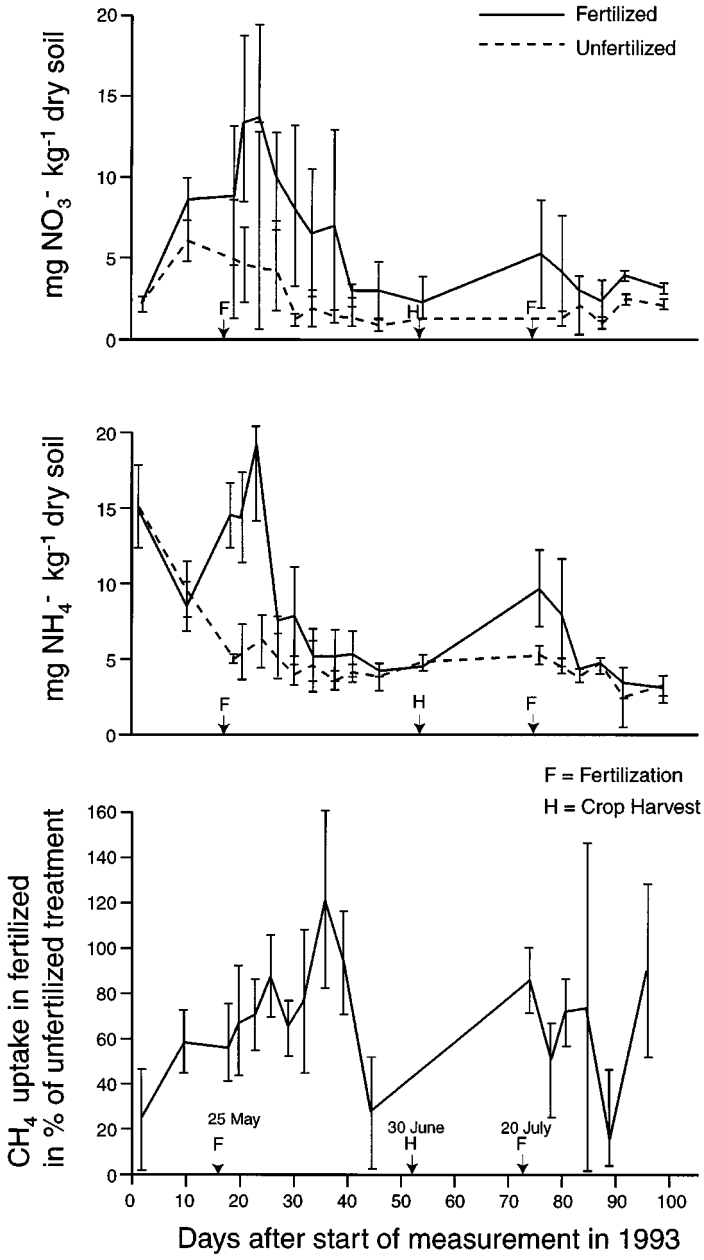

Figure 2. Variation in soil NH_4^+, NO_3^- content and CH_4 uptake rates in unfertilised and NPK fertilised treatment (average value for each treatment ± standard error).

considering the low energy flux through the oxidation of CH_4 at ambient concentrations.

The second phenomenon (a lack of an additional effect of adding more N) suggests that a more "N-tolerant" CH_4-oxidizing microflora remains after the first additions of fertilisers. Explanations of this apparently increased tolerance are not readily at hand. A survival of certain tolerant species may be involved, but a selective advantage depending on their position in the soil matrix may also be involved.

Persistent effects of N fertilisation but the lack of a correlation between mineral N level and CH_4 oxidation rate in a system with a history of N fertilisation may be significant for developing biogeochemical models. If early inhibition of CH_4 oxidation (discussed above) is almost permanent, then the latter N availability would have minor influence on the CH_4 oxidation rates. This is particularly important because ammonium supply is outlined as an important proximal control for CH_4 oxidation (Schimel et al. 1993). This relation did not hold true, particularly in an agricultural soil with a history of N fertilisation. Therefore, one should be cautious in including soil ammonium as a proximal controller of CH_4 oxidation in biogeochemical models.

The inhibiting effect of cattle slurry treatments (in the same amount as that of NPK) was an interesting observation. Neither Willison et al. (1996) nor Hütsch et al. (1993) found inhibition of methane oxidation after long-term addition of farmyard manure (FYM), although abundant NH_4-N was released slowly from FYM. The reason for this discrepancy might be that at the time of application, half of the total N was in the form of NH_4 because of the urine content of cattle slurries. In FYM, most of the N is organic N and the release of NH4-N is a result of mineralization. The inhibitory effect on CH_4 oxidation of NH_4 derived from the mineralization of the FYM is likely to be buffered by increased microbial biomass (Willison et al. 1996). Thus, the observed reduction in CH_4 uptake due to cattle slurry application in our work was probably due to the higher initial content of the NH_4-N at the time of application.

CH_4 oxidation at different soil depths

The CH_4 oxidation rates in the first laboratory incubations of fertilised and unfertilised soil are shown in Table 2. At ambient concentrations, the oxidation rates were 1.5–1.8 ng CH_4 g^{-1} dry soil d^{-1} in the unfertilised soil and 1–1.4 ng CH_4 g^{-1} d^{-1} in the fertilised soil. The difference between fertilised and unfertilised soil was statistically significant ($p < 0.05$); within each treatment there were no significant differences among the soil depths. This lack of vertical zonation is perhaps not surprising, considering that all samples

Table 2. CH_4-consumption rate (ng CH_4 g^{-1} dry soil d^{-1}*) in soil from different depth, average values (n = 24) for each treatment ± standard error.

Soil depth	Unfertilised	NPK, 140 kg N ha^{-1}
*At ambient (1.8 μl l^{-1}) CH_4 concentration***		
0–5 cm	1.8 ± 0.2aA	1.4 ± 0.1aB
5–10 cm	1.7 ± 0.2aA	1.4 ± 0.1aB
10–15 cm	1.7 ± 0.1aA	1.1 ± 0.2bB
15–20 cm	1.5 ± 0.2aA	1.0 ± 0.2bB
*At high (52 μl l^{-1}) CH_4 concentration***		
0–5 cm	25 ± 2aA	16 ± 2aB
5–10 cm	23 ± 3aA	13 ± 3aB
10–15 cm	21 ± 2aA	13 ± 2aB
15–20 cm	20 ± 2aA	13 ± 2aB

*Within each fertilisation treatments (columns) under each starting CH_4 concentration (ambient and 52 μl l^{-1}), the soil depths are compared with lower case letters (ab) and within each soil depth (rows), the fertilisation treatments are compared with upper case letters (ABC). Values followed by different letters in the same column in lower case or rows in upper case are significantly different (Newman–Keuls test, α = 0.05).

**The rates at ambient concentrations were calculated by linear regression of ln [CH_4] verses time assuming first order decay over the entire period. The rates at 52 μl l^{-1} starting concentrations are estimated by net decrease during the first 6 h of incubation.

were taken above the plough layer, which means that there is almost complete vertical mixing at each ploughing event.

This result was consistent both for ambient and high (52 μl l^{-1}) CH_4 concentration (Table 2). The oxidation rate at 52 μl l^{-1} was 20–25 and 13–16 ng CH_4 g^{-1} d^{-1} in the unfertilised and the fertilised soil, respectively (Table 2), which is 9–14 times the measured oxidation rates at ambient concentrations. Because of the short term measurement period (6 h) and the moderate CH_4 concentrations compared with observed lag times and threshold values for the growth of methanotrophs in soil (Bender & Conrad 1995), the measured oxidation rate at 52 μl l^{-1} is probably not influenced by growth. Hence the ratio between oxidation rate at the two concentrations, i.e., V_{52} and $V_{1.8}$ can be used to approximate the apparent substrate affinity (Ks, in μl l^{-1}), since $V_{52}/V_{1.8}$ = 52(1.8 + Ks)/[1.8(52 + Ks)] according to the Michaelis–Menton kinetic equation. The average $V_{52}/V_{1.8}$ ratios for the fertilised and unfertilised soils were 13 ± 0.6 and 11 ± 1.5, respectively (thus not significantly different), giving estimated substrate affinities of 29 (22–36)

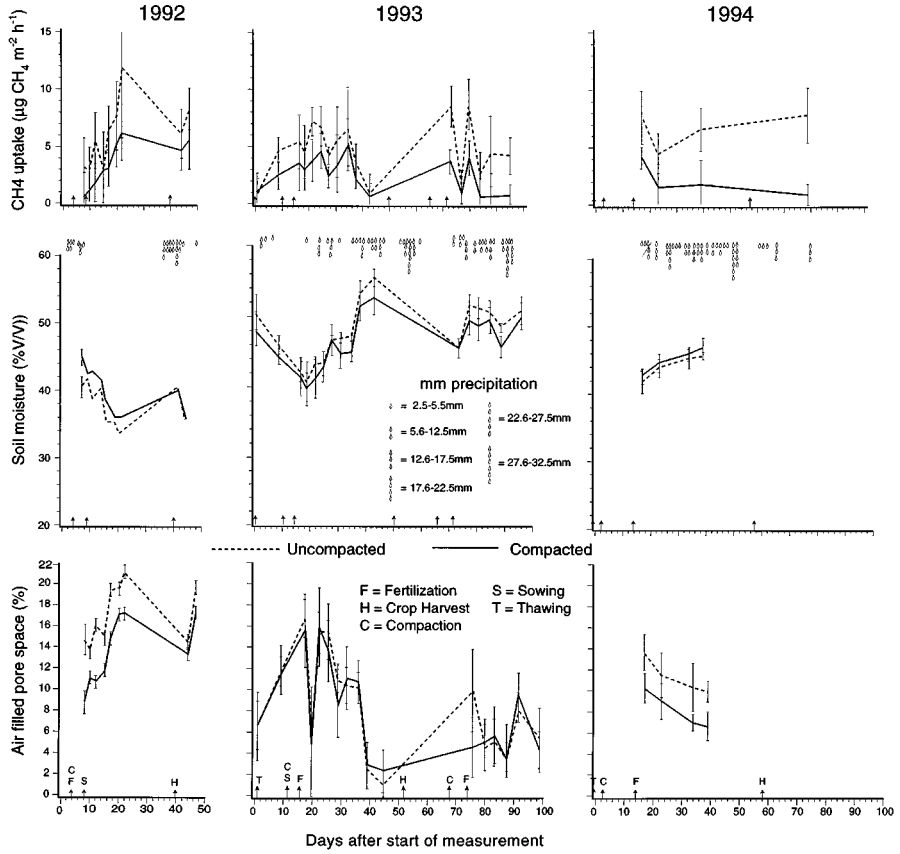

Figure 3. Compaction effects on soil moisture content, air-filled pore space and methane uptake (average value for each treatment ± standard error).

μl l^{-1} and 39 (37–42) μl l^{-1}, respectively (the range in parentheses is calculated from the standard deviation range). These concentrations are equivalent to 31 and 58 nM in the water phase (equilibrium with the gas phase at 20 °C), if calculated according to Wilhelm et al. (1977).

Compaction effects

The reduction in CH_4 oxidation rate (35% to 75%) from soil compaction found in the present work (Table 1, Figure 3) was of the same order of magnitude as found in 1991 (Hansen et al. 1993). This indicates that there were no additional inhibiting effects from repeated soil compaction in the following years.

An important reason for the inhibited CH_4 oxidation as a result of soil compaction is probably a reduction of the coarse pores and thus reduced air-filled pore space (Figure 3) and gas diffusion. Gas diffusion seemed to be decreased by compaction, although treatment differences were not statistically significant (Ball et al. 1997b). *In situ* diffusion of Freon-22 measured at 5–10 cm for uncompacted soil was about 1.9 mm^2s^{-1}, decreasing to 1.5 mm^2s^{-1} in compacted soil. Similarly, *in situ* diffusion at 10–18 cm soil depth for uncompacted soil was about 1.7 mm^2s^{-1}, decreasing to 0.6 mm^2s^{-1} in compacted soil. This decrease in gas diffusion in compacted soils was in agreement to other findings in the same sites where lower soil O_2 concentration, and higher soil CO_2 and N_2O concentrations were found in compacted soil than in uncompacted soil (Hansen & Bakken 1993; Hansen et al. 1993). The restricted soil aeration or decrease in gas diffusivities due to compaction likely to increase the frequency of anaerobic micro sites. This compaction effect would be even more severe in humid climate with higher amount of rain such as those in Western Norway, and in a easily compacted (fine textured) soils (Hansen et al. 1993). The possible CH_4 production in anaerobic soil microsites would reduced the overall net methane uptake (by shifting balance toward CH_4 production) from a given area. Therefore net change in CH_4 uptake rates in the compacted soil is not entirely caused entirely by a change in CH_4 methane oxidation rates. Other phenomena, such as localised methane production in the anaerobic sites and long-term change in methanotrophic activities/population, all will influence the net CH_4 fluxes due to soil compaction (discussed latter).

High soil moisture persisted for longer period in our sites (soil moisture $>$40% v/v, Figure 3), and a negative relationship between CH_4 uptakes rates and moisture moisture (r = –0.3, $p < 0.01$) was observed. In agreement to this, a positive relationship between methane uptake rates and air filled pore space (r = 0.3, $p < 0.01$) (Figure 3) was found.

Persistence of compaction effects

The results of the second soil incubation experiment (Figure 4) show a persistence of reduced CH_4 oxidation rates beyond the physical effect of compaction due to retarded diffusion. The sieving presumably removes any diffusion restriction due to soil compaction, although persistent compaction effects at the microscale level cannot be ruled out. Thus, the fact that the CH_4 oxidation rate in the previously compacted soil (0.7 ± 0.001^{SE}ng CH_4 d^{-1} g^{-1} soil dry weight) was significantly lower than in the uncompacted soil (0.9 ± 0.01^{SE} ng CH_4 d^{-1} g^{-1} soil dry weight) strongly indicates that the soil compaction effects seen in the field flux data (Figure 3 and Table 1) are not due only

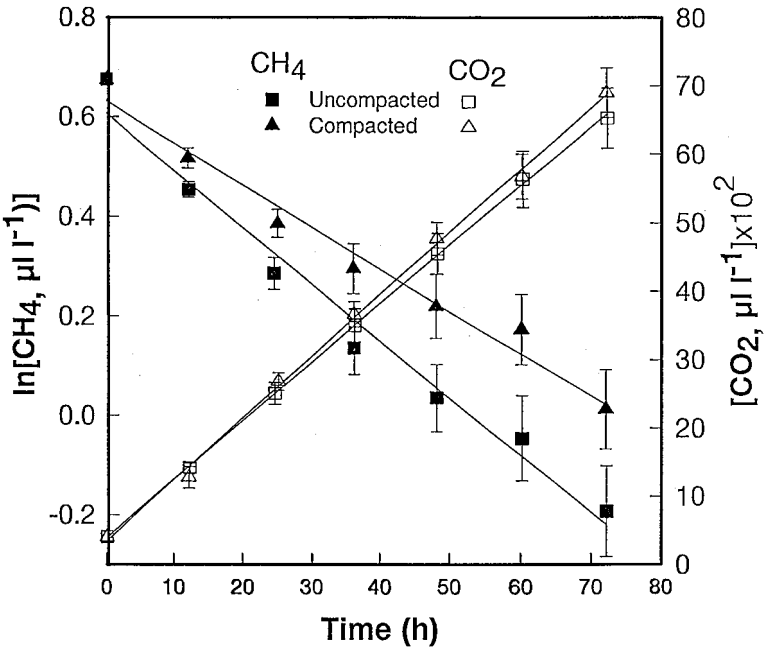

Figure 4. Time-dependent decrease of CH_4 (ln values) and increase of CO_2 in the head space of serum bottle (n = 16) in the sieved samples showing persistence of compaction effects on CH_4 oxidation.

to restriction of diffusion. There also seems to be a significant reduction in the number or activity of CH_4-oxidizing bacteria. In contrast, soil respiration in the laboratory experiment was almost identical for the compacted and the uncompacted soil (Figure 4). One may speculate that the change in physical condition of the soil would modify methanotrophic activities almost permanently in various ways. Soil compaction reduces the total pore volume and may increase the probability of anaerobic conditions (O'Sullivan & Ball 1993). Significant reduction in air-filled pore space due to soil compaction was observed in the present study (Figure 3). A long-term effect of this situation would be a reduction in sites where methanotrophic activities take place, thus reducing the methanotrophic population.

Conclusion

N fertilisation inhibited CH_4 uptake, but the mineral N (NH_4^+, NO_3^-) content of the soil could not explain the fertiliser effect on CH_4 uptake rates, at least after years of N application. Inhibition in methane oxidation by NH_4^+ availa-

bility is plausible only in the early stage of N input. The reduction in methane oxidation rates persisted beyond the physical effect of compaction caused by diffusion. Thus, both soil compaction and fertilisation seems to have long lasting inhibitory effect on soil methane oxidising potentials.

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