Biogeochemistry **47:** 1–14, 1999.

Release of fossil methane from mineral soil particles, and its implication for estimation of methane oxidation in a mineral subsoil

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Accepted 20 October 1998

Key words: ammonia oxidizers, isotopic composition, methane oxidation, thermogenic methane

Abstract. In a preliminary experiment we found that methane evolved from a sandy subsoil during aerobic incubation of shaken soil slurries. In the study presented here the methane was found to be released from the sand particles by mechanical weathering, caused by the grinding effect of the shaking. Large amounts of gas (about 0.5 ml gas g^{-1} soil) were extracted by intense grinding of the soil in gas tight serum vials. Methane was the main hydrocarbon in the emitted gas, but also a considerable amount of ethane was present, as well as minor amounts of heavier hydrocarbons (up to C_6). The δ^{13}C-values of the emitted methane and ethane were –33 ‰ and –29 ‰, respectively. Together these results demonstrate a thermogenic origin of the gas. This paper also reports the results of an incubation experiment where possible methane oxidation was looked for. If a possible release of methane is not accounted for, methane oxidation may be overlooked, as illustrated in this paper. Methane consumption was detected only in soil from 40 cm, in contrast to soil sampled at 100 cm and deeper where a slight production was measured. When methane oxidation was inhibited by dimethyl-ether, a significant release of methane was seen. The release was probably caused by chemical weathering. When this methane release was taken into account, methane oxidation was found to be present at all measured depths (40 to 200 cm). Fertilization with urea inhibited the methane oxidation at 40 cm but not at deeper layers. It is hypothesized that ammonia oxidizing bacteria were the main methane oxidizers in this mineral subsoil (deeper than 1 m), and that oxidation of methane might be a survival mechanism for ammonia oxidizers in ammonia limited environments.

Introduction

Methane is a major greenhouse gas, accounting for a fifth to a quarter of short-term anthropogenic warming impact. Bacterial methane oxidation is known to occur in most aerobic environments (Coleman et al. 1981; Born et al. 1990; Schipper & Reddy 1996), and aerobic soil acts as a sink for atmospheric methane and methane produced in deeper anaerobic layers (Levin et al. 1993). The terrestrial methane oxidation may significantly affect the global methane budget. It is estimated that 6 to 60 Tg CH_4 is oxidized per year in terrestrial ecosystems, contributing 1 to 15% of the total annual methane destruction (Born et al. 1990). The oxidation of methane has been found to decrease in soil fertilized with nitrogen, specially ammonium (Hansen et al. 1993; Bédard & Knowles 1989). Increased N input to soils may therefore be a contributor to increased atmospheric methane concentrations and global warming.

During a field experiment aiming to follow transport and transformations of urea-N through the unsaturated zone of a sandy, glaciofluvial deposit (Swensen 1997; Swensen & Singh 1997), soil gas was sampled from a soil profile (from 40 to 230 cm depth) and analysed for CH_4, CO_2, and N_2O at different times of the year. The temporal and spatial variations in composition of the soil gas indicated a presence of methane oxidation as well as ureolytic and nitrifying activity at all investigated depths of the mineral subsoil. Soil was then sampled at the field site for further investigations in the laboratory. One purpose of the laboratory experiments was to estimate the methane oxidation rate in this subsoil and to investigate a possible inhibition by N-fertilization. However, during a preliminary experiment, where soil slurries were shaken to ensure aerobic conditions, a surprising increase in methane concentration was detected. Biological methane production is known to occur only under anaerobic conditions, and it was very unlikely that anaerobic conditions could develop in this coarse sand with less than 0.7% (w/w) organic material. Reported in this paper are the efforts made to find the cause of this methane, and the results leading us to conclude that thermogenic methane was released from some of the sand particles.

Also reported in this paper is how the knowledge gained about the methane release from this subsoil turned out to be crucial for the interpretation of methane data obtained during an incubation experiment aiming to quantify the methane oxidation rate in this subsoil.

Materials and methods

The soil

The soil used in this investigation was sampled from a lysimeter trench located at the Hauerseter glacial-contact delta approximately 40 km north of Oslo in south-eastern Norway. The glaciofluvial deposit has a heterogeneous, layered structure, and consists mainly of coarse/medium sand with some gravel and fine sand/silt lenses. A typical sample of the deposit contains about

Table 1. Overview of replicates and treatments used in the soil-slurry experiment

	Number of replicates				
Sampling depth	Total	"Hot-spots" (great CH_4 emissions)	BESA	Nitrate and $HgCl_2$(*)	Incubated on bench
40 cm	6	0	1	1(*)	1
40 cm	6	1	1	1	0
105 cm	6	2	1	1(*)	1
230 cm	8	1	2	2(1*)	1

50% quartz, 25% feldspars and 25% shale fragments (Teveldal et al. 1990). The organic matter content is less than 0.7% (w/w).

The four soil samples used in the methane release experiments (shaking of soil slurries and collection of gas) were sampled at 40 cm (2x), 105 cm and 230 cm depth during the excavation of a lysimeter trench (French et al. 1994). The trench (2.5 m deep, 3 m wide, 7 m long) is part of Moreppen research station (French et al. 1994) and has been used in a field study of transport processes and transformations of urea-N in the unsaturated zone (Swensen 1997; Swensen & Singh 1997).

Soil collected later through one of the lysimeter trench walls was used in the incubation experiment where methane oxidation potential was examined. (This experiment was mainly designed to investigate ureolytic and nitrifying activity, Swensen & Bakken, 1998). Results reported in this paper are from seven samples, three collected at 40 cm and four from 100 to 200 cm depth. A more detailed description of the lysimeter trench and the soil sampling is given in Swensen and Singh (1997) and Swensen and Bakken (1998).

The background respiration is low in this subsoil, only 5 to 10 nmol CO_2 g^{-1} soil h^{-1} in soil from 40 cm and less than 1 nmol CO_2 g^{-1} soil h^{-1} in soil from deeper layers (Swensen & Bakken 1998).

Experiment to reveal the origin of emitted methane

The four soil samples collected during the trench excavation were used in this experiment. Each sample was divided into six (one into eight) portions of about 4 g soil (a total of 26 replications, Table 1), and each portion was filled into autoclaved glass bottles (38 ml). Five millilitre of a liquid mineral medium was added. This medium was buffered at pH 7.5 with 15 mM HEPES-buffer (sodium salt of 2-[4-2-hydroxyethyl-1-piperazinyl]-ethanesulfonic acid) and sodium bicarbonate, and containing 3.8 mM urea (a medium optimized for cultivation of ammonia oxidizing bacteria, Jiang 1996). This medium was used to make the conditions similar to the preliminary experiment where methane emission first was observed. The bottles were closed with Teflon coated rubber septa and placed on a shaker (160 strokes min^{-1}). To be sure that all samples produced methane, all bottles were shaken for three days before the first measurement. An increase in methane concentration was found in all bottles. Three of the bottles were then placed outside the shaker and incubated further on the bench to see whether the shaking had any effect. For the bottles incubated further on the shaker the shaking intensity was increased two times during the incubation period (to 170 strokes min^{-1} after 168 h and to 185 strokes min^{-1} after 408 h).

The headspace of the serum bottles was analysed for both CH_4 and CO_2 at subsequent times (after 72, 168, 288, 408, 480, 624 and 648 h). Both gases were analysed in the same sample injection on a Carlo-Erba gas chromatograph equipped with a wide bore capillary column and three detectors (flame ionization, thermal conductivity and electron capture detectors) (Sitaula et al. 1992). The CO_2-concentration served as an indication of aerobic conditions in the bottles throughout the experiment. Anaerobic conditions could not exist as long as the CO_2 concentration was below 100 ml l^{-1} (100 000 ppm). In our experiment, the CO_2 concentration did not exceed 7 ml l^{-1} in any of the bottles, in fact it was less than 2 ml l^{-1} in those bottles where the methane concentration increased most.

Because the methane production ceased in the three bench incubated bottles and new increases in methane concentrations were found after the first increase in shaking intensity, we suspected that the methane was not produced by biological activity in the bottles. To get better proof for this, different compounds known to inhibit biological methanogenesis in soil were added to some of the bottles after 408 h of shaking. Nitrate, which acts as an electron acceptor in an anaerobic environment, was added to five bottles (Table 1) to a final concentration of 2 mM. Five other bottles were added 2-bromoethanesulfonic acid (BESA) to a final concentration of 0.5 mM. BESA is a specific inhibitor in the last step of microbial methane formation. In pure cultures of methanogenic bacteria 1 μM BESA has been found to inhibit the methane production by 50% (Brock et al. 1994). No decrease in methane production was found in the first measurement (at 480 h) after these additions. Therefore, more BESA was added to the five BESA-bottles (final concentration of 15 mM). To three of the five nitrate-bottles (Table 1) $HgCl_2$ was added to a final concentration of 300 mg l^{-1}. This should be sufficient to stop biological activity in the soil. However, the best way of eliminating biological activity would have been to heat-sterilise the soil, but because possibly occluded methane could be stripped off by heating, this method was not chosen. The three bench-incubated bottles were finally put on the shaker (after the 480 h measurement). Three control bottles were also included in the experiment (5 ml liquid medium without soil in sealed 38 ml bottles).

Gas sampling for isotope measurements

Because shaking intensity was the only factor having an influence upon the methane production in the soil slurries, it was strongly indicated that formerly produced methane, entrapped in some of the sand particles, was released by mechanical weathering caused by the shaking. To get more information about the released gas, effort was made to collect enough gas for isotopic determinations ($^{13}C/^{12}C$ and $^{2}H/^{1}H$ ratios).

About 1 mg CH_4 was needed for this and enough gas was obtained by grinding the soil in gas tight serum bottles and collecting the emitted gas. Soil sampled during excavation of the trench was used also in this experiment. Portions of ~25 g soil were transferred to 38 ml glass bottles and added 10 ml de-ionized water, 25 mg $HgCl_2$ (to prevent any biological activity), and 0.2 ml 3 N NaOH (to entrap all CO_2 which otherwise may interfere in the isotopic analysis). To create a mill inside the bottles, three steel bars (length 50 mm, diameter 10 mm) were added. The bottles were then placed on a roller and the mineral particles were effectively ground between the steel bars. The methane concentration in the headspace levelled off at about 20 ml l^{-1}, and the gas from all bottles (about 20) was then transferred to two sterile, evacuated serum bottles (120 ml).

The collected gas was analysed for total composition of hydrocarbon gases on a gas chromatograph with a flame ionization detector. The isotopic signature of those gases being in sufficient amount ($\delta^{13}C$ in methane and ethane, and δD ($\delta^{2}H$) in methane) was determined. The carbon and hydrogen isotope concentrations are expressed in the usual δ-notation:

$$\delta(‰) = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000,$$

where R is the isotope ratios $^{13}C/^{12}C$ ($\delta\ ^{13}C$) and $^{2}H/^{1}H$ (δD), respectively (e.g., Fritz & Fontes 1980).

For the isotopic determinations of hydrocarbon gases, a preparative gas chromatograph system and an isotope mass spectrometer were used (Andresen & Throndsen 1989). After separation on the gas chromatograph (equipped with a Porapak QS 12′ × 14″ stainless steel column, hot wire and flame ionization detectors), the gas components were oxidized in separate CuO-ovens at 850 °C. The combustion products (CO_2 and H_2O) were collected in special vessels by cryogenic separation. Water was reduced to H_2 with zinc metal in sealed quartz tubes at 900 °C. The isotope measurements were done with a Finnigan Mat 251 and a Finnigan Delta mass spectrometer. The uncertainty in the $\delta^{13}C$ values is ± 0.3 ‰ and includes all analytical steps. The $\delta^{13}C$ value on NBS 22 (lube oil from National Bureau of Standards) is –29.77 ± 0.06 ‰. The uncertainty in the δD values is estimated to ± 10 ‰ (Andresen, pers. comm.).

Methane oxidation potential in the subsoil

Because methane oxidation activity was indicated during an earlier, associated field experiment (soil gas analyses), measurements of the methane concentration was included in an incubation experiment primarily set up to investigate the ureolytic and nitrifying potential of the subsoil (Swensen & Bakken 1998). Soil sampled from one of the lysimeter trench walls was used in this experiment. Reported in this paper are the results from three samples collected at 40 cm and four samples collected at 100 to 200 cm depth.

Each sample was leached with sterile, de-ionized water and drained at a suction of 10 kPa. Ten grams were then put in a gas tight 120 ml serum bottle. The remaining part of the soil sample was flooded with a 3.8 mM urea solution, drained at a suction of 10 kPa, and 10 g transferred to another 120 ml serum bottle. The draining standardized the soil moisture tension at a value known to ensure aerobic conditions (Bakken 1988). The bottles were incubated still-standing in the dark at room temperature (20 to 22 °C). The gas composition of the headspace was monitored for 50 days. Methane and CO_2 (plus N_2O) were analysed in a single injection on the Carlo Erba gas chromatograph described above. Both unfertilized and urea fertilized soil was monitored for possible methane oxidation. Low CO_2 concentrations (< 7 ml l^{-1}) showed that aerobic conditions were achieved in all bottles throughout the incubation period. A more detailed description of the experimental set-up is found in Swensen and Bakken (1998).

After incubation for 730 h, dimethyl ether (DME) was added (final concentration of 2%) to all of the unfertilized samples. This is known to inhibit methane oxidation (Larsen 1994). The DME did not interfere with the CH_4 measurements.

Results and discussion

Methane release from shaken soil slurries and its origin

Methane had been produced in all 26 soil slurries after 72 h of shaking. However, at the next measurements (168 h), the production seemed to have

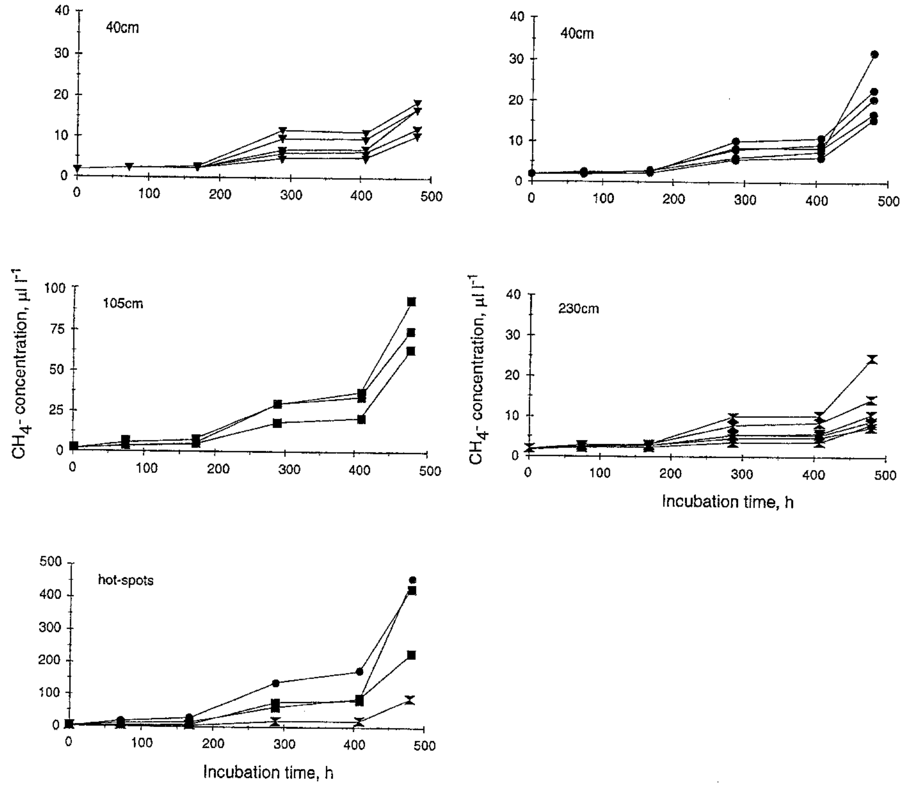

Figure 1. Methane concentration (μl l^{-1}) measured in the headspace of all the 23 replicates incubated on the shaker. The shaking intensity was increased from 160 to 170 strokes min^{-1} after 168 h and to 185 strokes min^{-1} after 408 h. (Note the different scales.)

levelled off in the 23 bottles on the shaker and ceased completely in the three bottles placed on the bench. The shaking intensity was then increased from 160 to 170 strokes min^{-1}. This resulted in a new increase in methane concentration in the 23 shaken bottles (Figure 1). After this increase, the methane concentration again levelled off. The pattern was similar in all samples despite the great variation in methane concentrations both between and within samples (Figure 1). At this point we suspected the methane emission to have a nonmicrobial cause. Once more the shaking intensity was increased (to 185 strokes min^{-1} after the 408 h measurements). At the same time, five bottles were added nitrate and five others were added BESA (Table 1). None of these potential inhibitors of methanogenesis seemed to have any effect (Figure 2(a–c)). Neither had the second addition of BESA nor the $HgCl_2$-addition. When the three bottles incubated on the bench were placed on the shaker (after 480 h), the methane release started immediately

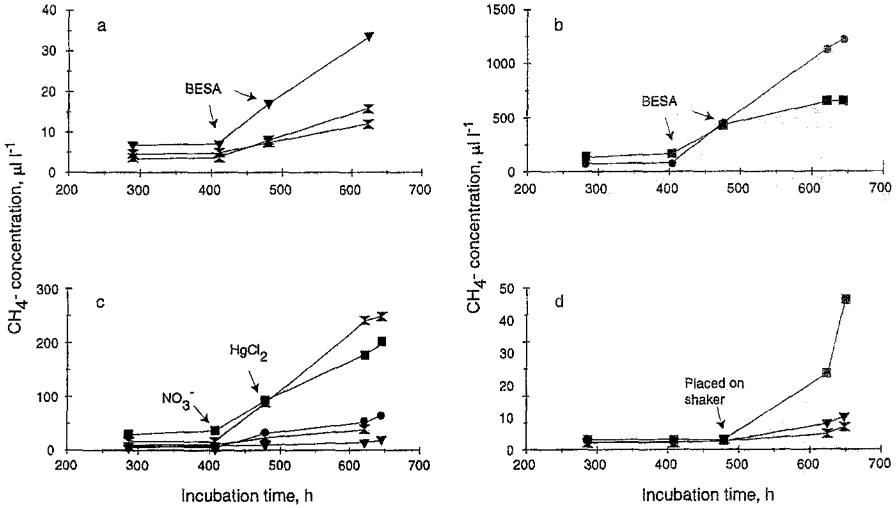

Figure 2. Methane concentration (μl l^{-1}) measured in the headspace after addition of BESA (a and b), nitrate and HgCl$_2$ (c) and in the bench incubated bottles (d). (Note the different scales.)

Table 2. The hydrocarbons (C$_1$–C$_4$) and their concentrations (μl l^{-1}) in the gas emitted from the sub-soil

Methane	Ethane	Ethene	Propane	i-Butane	n-Butane
22700	830	40	180	30	20

(Figure 2(d)). No methane was detected in the three control bottles without soil (only shaking of liquid mineral medium). This excludes the possibility of methane coming from other sources than the soil (e.g. the medium, bottles or capsules). When taking into account both the aerobic incubation conditions, the effect of grinding and the lack of response to any of the potential inhibitors, we were quite sure that mechanical weathering released earlier formed methane from the soil particles. However, some methanogenic activity could hypothetically exist in very small, occluded pores in some of the sand particles. In such pores anaerobic conditions could have been created and inhibitors could be restricted from reaching the methanogenes. Also, if not being active at present, methanogenic activity could have existed in the deposit during some part of the last postglacial period of about 10,000 years. To get more information about the emitted gas, as much gas as possible was collected by grinding of soil in closed bottles.

Table 3. Isotopic composition of carbon and hydrogen, given as δ (‰)-values ($\delta^{13}C$ and δD, respectively) in methane and ethane collected from the sub-soil, and typical values found in thermogenic and biogenic gas

Values found in the gas from this subsoil			Values from literature[a]				
			Thermogenic			Biogenic	
Methane		Ethane	Methane		Ethane	Methane	
$\delta^{13}C$	δD	$\delta^{13}C$	$\delta^{13}C$	δD	$\delta^{13}C$	$\delta^{13}C$	δD
-33.0	-178	-29.3	-60/-30	-300/-150	$\delta^{13}C_2 \approx \delta^{13}C_1 + 8$	-90/-60	-280/-350

[a] (Levin et al. 1993; Schoell 1988).

Methane was the dominating hydrocarbon in the collected gas, but considerable amounts of ethane and propane were also measured, together with smaller amounts of ethene, i-butane and n-butane (Table 2). Even trace amounts of pentanes and hexanes (1 to 5 $\mu l\ l^{-1}$) were detected (not shown). The total concentration of these higher weight hydrocarbons (C_{2+}) was 4.6% relative to methane. These gases are not expected to be found together with biologically produced methane. Some ethane may form during early diagenesis of bacterial methane, but will constitute less than 0.5% of the methane (Whiticar et al. 1986). The C_{2+} content in our sample strongly argues for a thermogenic origin of the gas (Schoell 1988).

This view was reinforced by the isotopic signatures found. Only methane and ethane were present in sufficient amounts for isotopic determinations. The δ-values found are listed in Table 3, together with values normally found for bacterial methane and thermogenic gases (Levin et al. 1993; Schoell 1988). The methane was found to have a $\delta^{13}C$-value of –33 ‰ and a δD-value of –178 ‰. These values are very much higher than what is normally found in bacterial methane. The $\delta^{13}C$-value was high even compared to those commonly found for thermogenic methane (–60 to –30 ‰) (Lilley et al. 1993; Schoell 1988).

Bacterial methane oxidation can change isotopic composition of the remaining gas (Coleman et al. 1981) by fractionation. The light isotope is oxidized more rapidly than the heavy isotope, leading to an enrichment of the heavy isotope in the residual gas (higher δ-values). However, Coleman et al. (1981) found that during oxidation the δD value in the residual methane changed about 10 times the change in $\delta^{13}C$ value. This implies that if the high $\delta^{13}C$-value found was caused by fractionation of an originally bacterial methane (e.g with an initial $\delta^{13}C$-value of –75 ‰), the initial δD should have been around –450 ‰. This is a very unlikely δD-value according to data referred in literature (Schoell 1988).

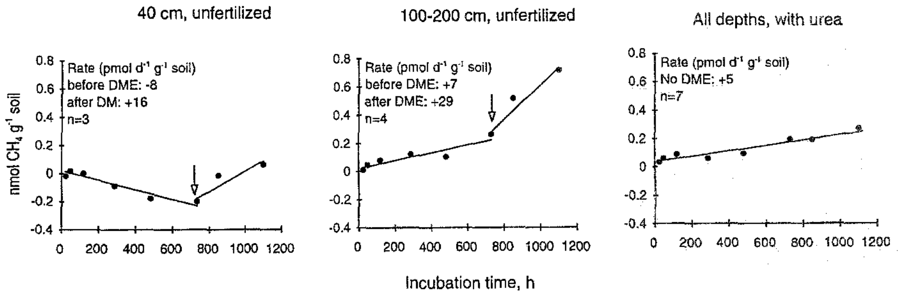

Figure 3. Means of the methane production (+) and consumption (–) rates estimated from linear regression (Table 4) in the sub-soil during the final incubation experiment. The dots represent means at subsequent times (three samples from 40 cm and four from 100 to 200 cm). To inhibit methane oxidation dimethylether (DME) was added (2 vol%) after 730 h incubation (arrow) to the unfertilized bottles.

Because both the gas composition and all the isotopic values found were consistent with a thermogenic gas, and because shaking intensity was the only factor influencing on the methane production in the soil slurries, it seems justifiable to conclude that thermogenic methane was emitted from the sand because of mechanical weathering.

When and how the methane was produced is however still a question. We can only conclude that gas, being produced during some earlier geological time in one or more of the source rocks constituting the glacial-contact deposit, still is entrapped within some of the sand particles.

Methane oxidation in the sub-soil

The finding that methane could be released from the sand particles turned out to be important also for interpreting the results of the methane oxidation study (incubation of drained soil). Methane oxidation was observed only in unfertilized samples from 40 cm depth. The unfertilized samples from deeper layers and the samples fertilized with urea (from all depths), showed either no changes or a slight increase in methane concentrations (Figure 3, before addition of DME). Methane release/consumption rate (pmol $d^{-1}g^{-1}$ soil) was calculated for each of the seven samples by linear regression of the measured values ($r^2 > 0.8$ for all) (Table 4). From these results we could have concluded that a small methane oxidation activity existed in the upper layer of the soil profile only and that it was inhibited by addition of urea. However, if methane could be released from the sand particles and then oxidized at almost the same rate, methane oxidation could be present without being detected. Therefore, dimethyl ether (DME, a selective inhibitor of methane oxidation) was added to all unfertilized bottles after 730 h incubation. A significant increase (p <

Table 4. Rates of oxidation (–) or release (+) of methane (pmol d^{-1} g^{-1} soil) determined by linear regression of measured methane concentrations at subsequent times for each of the seven samples ($r^2 > 0.8$ for all samples)

Soil depth (cm)	Measured rates			Estimated oxidation rates	
	Unfertilized		With urea	Unfertilized[c]	With urea[d]
	Before DME[a]	After DME[b]	No DME		
	pmol CH_4 d^{-1} g^{-1} soil				
	–9.4	+17	+3.3	–26	–14
40	–2.3	+11	+4.5	–13	–6.5
	–13	+21	+5.6	–34	–15
Mean(±SE)	**–8.2**(±5)	**+16**(±5)	**+4.5**(±1)	**–24**(±10)	**–12**(±5)
	+22	+25	+8.4	–3	–16
	+6.0	+11	+4.4	–5	–6.6
100–200	–1.6	+39	+3.2	–41	–36
	+0.5	+13	+4.8	–12	–8.2
Mean(±SE)	**+7**(±10)	**+22**(±13)	**+5.2**(±2)	**–15**(±17)	**–17**(±13)

[a] Based on values before addition of dimethylether (DME).
[b] Based on values after addition of DME (2 vol%) at 730 h incubation.
[c] Estimated from: (Unfertilized before DME)-(Unfertilized after DME).
[d] Estimated from: (With urea)-(Unfertilized after DME).

0.005 at 40 cm, $p < 0.10$ at 100–200 cm) in methane concentration was then detected (Figure 3). This implies that the actual methane consumption must have been higher than actually measured. When the methane release was accounted for, it appeared that methane oxidation occurred at all depths (40–200 cm) without any significant difference (Table 4).

Both measured and estimated oxidation rates demonstrated that the methane oxidation was inhibited by ammonia in samples from 40 cm depth, but no such inhibitory effect was found in soil from deeper layers (Table 4 and Figure 3). This indicates that parts of the methane oxidation at 40 cm may have been carried out by some other mechanism than further down in the soil profile. Methane is oxidized by both methanotrophs and ammonia oxidizing bacteria (Bédard & Knowles 1989). It is reported that methane oxidation by methanothrophs may be more inhibited by NH_4^+ than methane oxidation by ammonia oxidizing bacteria (Hyman & Wood 1983; Hyman & Wood 1984; Jones & Morita 1983). The latter is in fact found to be stimulated in the presence of small amounts (10 ppm) of ammonium, possibly due to production of reducing power when ammonia is oxidized. From this, and our results from the present and associated study, it is suggested that ammonia oxidizers are the main methane oxidizers in this subsoil. The methane oxidation rate of *Nitrosomonas eoropaea* has been found to be in the same range (2 μmol CH_4 h^{-1} mg^{-1} dw cells) as is reported for methane oxidizers (Jones & Morita 1983). Assuming the number of ammonia oxidizers to be $1 \cdot 10^4$ to $5 \cdot 10^5$ (as found in this subsoil, Swensen & Bakken 1998) and a cell dry-weight of $0.1 \cdot 10^{-12}$ g $cell^{-1}$ (Bakken & Olsen 1983; Jiang 1996) a methane oxidation rate of 2 to 100 pmol g^{-1} soil h^{-1} can be calculated. This is surprisingly equal to the total methane oxidation rates estimated in this study (Table 4) and supports the hypothesis that ammonia oxidizers may be responsible for some, if not most, of the methane oxidation in the deeper layers of this subsoil. We can only speculate about why methanotrophs seem to be restricted to the upper soil layer, but one reason might be a better access to other substrates (e.g., methanol and formate, or even multicarbon substrates if they are facultative methanotrophs, Bédard & Knowles 1989) which may be used for growth in addition to methane. It has been hypothesized that methane oxidation might be a survival mechanism for ammonia oxidizers in ammonia limited habitats, but attempts to grow them on methane as the sole energy source has failed (Jones & Morita 1983). However, several studies using nanomolar concentrations of $^{14}CH_4$, reviewed by Bédard and Knowles (1989), have found $^{14}CH_4$-C to be incorporated into cellular material and $^{14}CO_2$ to be produced by ammonia oxidizers. This has also been found when $^{14}CH_3OH$ was used as substrate, indicating that ammonia oxidizers in fact are able to metabolize both CH_4 and CH_3OH produced during methane oxidation. This, and the finding of ammonia oxidizers in this very ammonia limited subsoil (Swensen & Bakken 1998), where methane seems to be abundant, supports the idea that oxidation of methane might be a survival mechanism for ammonia oxidizers. Even though methane is insufficient for growth, it may support an endogenous metabolism for survival.

Conclusions

Mechanical weathering (shaking) released nonbacterial methane from this mineral subsoil. This is concluded because none of the inhibitors used to prevent biological methane production stopped the methane emission and because shaking intensity was the only factor influencing the methane production.

From the isotopic signatures of the released methane and the content of hydrocarbons in the emitted gas, it is concluded that the gas entrapped in the mineral particles had a thermogenic, fossil origin.

It was also indicated that entrapped methane was released by chemical weathering, because methane was also emitted from unshaken soil (incubation of drained soil). To be aware of, and quantify, this methane release was very important for the determination of methane oxidation rates in the subsoil. Without taking this methane release into account, methane oxidation would have been strongly underestimated in samples taken at 40 cm and completely overlooked in samples from deeper layers. When accounted for, methane oxidation was found to be present in soil from all investigated depths (40 to 230 cm).

Fertilization with urea inhibited methane oxidation in samples from 40 cm but not in samples from deeper layers. It is hypothesized that ammonia oxidizing bacteria were the main methane oxidizers in this mineral subsoil (deeper than 1 m), and that oxidation of methane might be a survival mechanism for ammonia oxidizers in ammonia limited environments.

Acknowledgements

This work was partly funded by the Norwegian Research Council (NFR). Special thanks to B. Andresen (IFE, Kjeller) for the isotopic analyses.

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