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Alex De Visscher · Michael Schippers
Oswald Van Cleemput

Short-term kinetic response of enhanced methane oxidation in landfill cover soils to environmental factors

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Abstract This paper aims at a better understanding of methane oxidation under conditions that are representative of landfill cover soils. The kinetics of methane oxidation were studied in landfill cover soils that had been exposed to high methane mixing ratios. This was done in batch experiments, under various environmental conditions. V_{max} increased exponentially with temperature in the range 5–35 °C, with a Q_{10} value of 2.8. K_m increased approximately linearly in this range from 1.2 μM to 7 μM. Consequently, the influence of temperature on methane consumption was more pronounced at high concentrations than at low concentrations. The inhibition by ammonium of methane consumption was much stronger after 6–7 months of exposure to high methane mixing ratios than after 5–7 weeks of exposure, indicating that there was a shift of dominating methanotrophic species in soils after long exposure times. Additions of nitrifying sludge or compost to soils initially inhibited methane oxidation, followed by a stimulation after a few days.

Keywords Methane oxidation · Landfills · Kinetics

Introduction

Methane (CH_4) is an important greenhouse gas, contributing around 15–20% to the greenhouse effect (IPCC 1996). Soils can oxidize atmospheric CH_4 microbially, with a global sink strength of about 6% of the global CH_4 emission (Crutzen 1991). In some environments, such as landfill cover soils, CH_4 is oxidized efficiently, because high CH_4 mixing ratios lead to enhanced CH_4 oxidation (Kightley et al. 1995; Schnell and King 1995; Bogner et al. 1997). Methane oxidation rates were found to depend on moisture and temperature (Whalen et al. 1990; Boeckx et al. 1996), nitrogen input (Mosier et al. 1991; Hütsch et al. 1993), soil amendments (Boeckx and Van Cleemput 1996; De Visscher et al. 1999), soil tillage (Hütsch 1998) and the presence of copper (Leak and Dalton 1986; Graham et al. 1993).

In past research, two methods were generally used to study the response of methanotrophs to environmental factors. The first method, generally adopted by soil scientists, is to incubate soil samples under different environmental conditions, and to compare the CH_4 uptake rates (Bender and Conrad 1994). However, this method allows a different microbial community to develop at each incubation, so it is not clear whether the influence observed is a result of physiology or population dynamics. The second method, usually adopted by microbiologists, is to expose microbial cultures to different environmental conditions (Leak and Dalton 1986). In this case, the observed influence is certainly physiological, but the microbial strains are not necessarily the same ones dominant in situ. Furthermore, some environmental factors, such as mineral nitrogen concentration, may not be representative of in situ conditions. A compromise between the two approaches has been the use of soil slurries (Megraw and Knowles 1987).

The aim of this study was to investigate the physiological response to environmental factors of a soil ecosystem that is representative of a landfill cover soil. The procedure was as follows: soil was incubated in the presence of CH_4 in order to obtain an active methanotrophic community, samples of this soil were then transferred to bottles, and CH_4 was added. The kinetics of the CH_4 uptake were determined in the bottles under different environmental conditions. Thus, the influence of the environmental factor was isolated from possible changes in the composition of soil microbiota, while preserving the representativeness of the sample. The influence of temperature, moisture, mineral nitro-

A. De Visscher (✉) · M. Schippers · O. Van Cleemput
Laboratory of Applied Physical Chemistry,
Faculty of Agricultural and Applied Biological Sciences,
Ghent University, Coupure Links 653, 9000 Ghent, Belgium
e-mail: alex.devisscher@rug.ac.be
Fax: +32-9-2646242

gen, copper, and sources of microorganisms (compost and nitrifying sludge) were thus determined.

Materials and methods

Soil characteristics and soil handling

A sandy loamy cover soil was taken from a landfill in Schoten (Belgium), on the site described by De Visscher et al. (1999). The soil properties were: 60% sand; 31.6% silt; 8.4% clay; 1.7% carbon; particle density 2532 kg m^{-3}; soil pH_{H_2O} 6.6. The soil was air-dried, ground, and rewetted up to 140 g of water/kg air-dried soil unless indicated otherwise. A week after rewetting, the soil was incubated in a flow-through system with cylinders, a modification of the setup described by De Visscher et al. (1999). In the present study, a mixture of 5–6% CH_4 in air was fed at the bottom of the cylinders, and no air was fed at the top. The flow rate was sufficiently high to ensure that the CH_4 concentration was almost constant throughout the column. This was checked using GC analysis.

Soil analyses

Soil moisture contents were determined gravimetrically, by drying 10 g samples for 48 h at 105 °C. Soil pH was determined in a 1:2.5 soil–water mixture.

Inorganic N was extracted from 30 g soil samples with 60 ml 2 M KCl under shaking for 1 h and then the soil slurry was filtered. The ammonium (NH_4^+) content of the filtrate was determined acidimetrically after steam distillation. Nitrate (NO_3^-) was determined as NH_4^+ after reduction with Devarda alloy (Keeney and Nelson 1982).

Methane oxidation kinetics

Soil samples were taken from the incubation column, the environmental conditions were adjusted to the desired level, and the samples were transferred to 215-ml incubation bottles. The batch incubation technique described by De Visscher et al. (1999) was used for the determination of the CH_4 oxidation kinetics, but instead of 10–30 g of fresh soil, 80–90 g of fresh soil was incubated in the bottles. The bottles were sealed with rubber septa and filled with 20,000 ppm CH_4. Methane degradation curves (i.e., concentration versus time curves) were obtained by repeatedly analysing the headspace with GC-FID until the CH_4 concentration was below 50 ppm. In order to assess microbial growth, the procedure was followed on three consecutive days unless indicated otherwise. Kinetic parameters were obtained by fitting a computer-simulated time course of the CH_4 concentration in the bottles to the measured concentrations. Michaelis-Menten kinetics were fitted to the data, according to the following relationship:

$$r_{CH_4} = -\frac{V_{max}[CH_4]}{K_m + [CH_4]} \quad (1)$$

with $[CH_4]$ representing the CH_4 concentration of soil water (μM). These CH_4 concentrations were calculated from the gas-phase concentrations using the solubility data of Sander (1999). The difference between the CH_4 uptake rates on consecutive days indicated bacterial growth. This growth was accounted for in the simulation by means of a growth model.

Experiments were performed in duplicate or triplicate. The standard deviation was always <10% for V_{max} and <5% for K_m, except at low temperatures, when K_m was about 1,000 ppm in the gas phase. In this case the standard deviation for K_m was around 10%.

In some cases, a first-order term ($-k_1$ $[CH_4]$) was added to the kinetic Eq. 1 in order to allow for deviations from Michaelis-Menten kinetics (De Visscher et al. 1999).

Influence of temperature

Soil samples were taken from the flow-through incubation after 6 days of exposure to 5–6% CH_4, and transferred to eight bottles. Two bottles were incubated at each of 5, 15, 25 or 30 °C. After 18 h, a degradation curve was determined at an initial concentration of 20,000 ppm CH_4. The following day, a degradation curve was determined at an initial concentration of 2,500 ppm CH_4. Subsequently, the incubation temperature of the samples kept at 5, 15 or 30 °C was increased by 5 °C and 18 h later the degradation curves were determined again.

Moisture content

Soil samples were taken from a flow-through incubation after 7 days, and transferred to bottles after adjustment of the moisture content. Experiments were performed at moisture contents of 9, 14 and 20% (90, 140 and 200 g water/$kg_{soil\ DW}$). The lowest moisture content was attained by drying soil in an airstream at room temperature. The highest moisture content was attained by adding deionized water. Two degradation curves were determined at initial CH_4 concentrations of 20,000 ppm, followed by a degradation curve at 2,500 ppm.

Influence of mineral nitrogen

Soil samples were taken from the flow-through incubation after 34 days. Ammonium was added as NH_4Cl (1–5 g N l^{-1} in aqueous solution), blanks were prepared by adding equal amounts of deionized water. The soil samples were transferred to bottles and degradation curves were determined on three consecutive days, each time at an initial concentration of 20,000 ppm. A similar experiment was performed with soil taken after 48 days of flow-through incubation. In this case, however, the initial CH_4 concentration in the bottles was 50,000 ppm on three consecutive days. Similar experiments were performed with soils taken after 176 and 210 days (initial CH_4 concentration 30,000 ppm).

In some preliminary experiments, the influence of NO_3^- (added as $NaNO_3$) was also determined.

Influence of nitrifying sludge and compost

Soil samples were taken from the flow-through incubation after 28 days. Nitrifying sludge (0.5 ml, 1 ml or 2 ml) was added to 90 g of moist soil, and transferred to bottles. Degradation curves were obtained on four consecutive days at initial concentrations of 20,000 ppm. The nitrifying culture (ABIL, commercially produced by Avecom, Beernem, Belgium) was a specific enrichment of *Nitrosomonas* species, containing about 1 g of nitrifier biomass dry weight per liter on a total of 10 g of total solids per liter. It also contained 100 mg/l NO_3^--N. The specific activity of the biomass was 1 g NH_4^+-N nitrified per liter of ABIL per day.

The influence of adding compost from an aerobic fruit, vegetable and garden waste composting plant on methane oxidation was investigated. Soil samples were taken from the flow-through incubation after 20 days. Samples (2, 5 or 10 g) of moist compost were added to 90 g of moist soil and transferred to bottles. Degradation curves were obtained on five consecutive days at initial concentrations of 20,000 ppm. The compost, obtained from ILVA, Schendelbeke, Belgium, had the following properties: 420 g moisture/$kg_{compost\ DW}$, 40% organic matter, 2% total N (C:N 10.7), 480 mg NH_4^+-N/$kg_{compost\ DW}$, 130 mg NO_3^--N/$kg_{compost\ DW}$, pH_{H_2O} 8.8. The compost was routinely analysed for the content of heavy metals, which was necessary for certification

as a soil amendment. The heavy metal contents were: 0.71 mg Cd $kg^{-1}_{compost\ DW}$, 16 mg Cr $kg^{-1}_{compost\ DW}$, 39 mg Cu $kg^{-1}_{compost\ DW}$, 0.14 mg Hg $kg^{-1}_{compost\ DW}$, 84 mg Pb $kg^{-1}_{compost\ DW}$, 13.6 mg Ni $kg^{-1}_{compost\ DW}$, and 220 mg Zn $kg^{-1}_{compost\ DW}$.

Influence of copper content

The copper content of the soil (26 μg Cu^{2+} $kg^{-1}_{soil\ DW}$) was determined by atomic absorption spectrometry, after extraction with 0.01 M $CaCl_2$. The CH_4 oxidation kinetics of this soil (taken after 41 days of flow-through incubation) was compared with the CH_4 oxidation kinetics of soil samples to which 26 or 52 μg Cu^{2+} $kg^{-1}_{soil\ DW}$ were added as $CuSO_4$. Degradation curves were determined on four consecutive days (initial concentration 20,000 ppm).

Influence of incubation time

All experiments were performed with samples taken from the same flow-through incubation, with the exception of some preliminary experiments on the influence of mineral N, and the experiment on the influence of the moisture content. An unamended sample was used on each occasion as a control. Consequently, the CH_4 oxidation kinetics of the unamended soil were determined after 6, 20, 28, 34, 41, 48, 176, and 210 days of flow-through incubation. One additional experiment was performed, after 217 days of flow-through incubation (initial CH_4 concentration 30,000 ppm; two consecutive degradation curves).

Results and discussion

Influence of incubation time

Kinetic experiments with soil samples were performed after 6, 20, 28, 34, 41, 48, 176, 210, and 217 days of incubation at 5–6% CH_4. The values of V_{max} were 0.200, 0.213, 0.725, 0.820, 0.552, 0.508, 0.496, 0.288, and 0.445 μmol $kg^{-1}_{soil\ DW}$ s^{-1}, respectively. The value of K_m increased from 6 to 10 μM in the liquid phase during these 217 days (4,000–7,000 ppm in the gas phase). Apparently, after an initial steady state (activity around 0.2 μmol $kg^{-1}_{soil\ DW}$ s^{-1}), the soil evolved to a more active phase between day 20 and day 28 of the incubation. A similar phenomenon was found by Kightley et al. (1995) and De Visscher et al. (1999), and no reasonable hypotheses to interpret it have been proposed. After about 5 weeks, the activity started to decrease again. Hilger et al. (2000) found a similar decrease, and attributed it to the production of exopolymers that clog the soil pores, or hinder the gas diffusion to the microorganisms.

Influence of temperature

The concentration versus time profiles of all experiments are shown in Fig. 1. The highest activity was found at 35 °C, the highest temperature tested. This is somewhat at variance with earlier studies indicating that the optimum temperature for CH_4 oxidation is near 30 °C or below (Boeckx et al. 1996; Whalen and Reeburgh 1996). However, the short-term (< 48 h) temperature response was investigated in our study. Exposure to temperatures exceeding 30 °C for longer periods can lead to a loss of activity due to enhanced cell decay. Consequently, the temperature optimum depends on the timescale of the experiment.

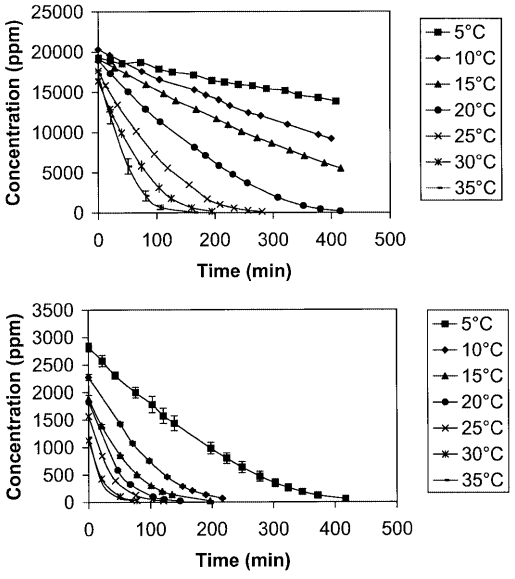

Fig. 1 Concentration versus time profiles of batch methane oxidation experiments at different temperatures, and initial methane concentrations of 20,000 (*top*) or 2,500 ppm (*bottom*). *Error bars* are standard deviations from experiments on duplicate soil samples

It is clear from Fig. 1 that the influence of temperature was stronger at 10,000–20,000 ppm than at 0–3,000 ppm. This can also be seen from the kinetic parameters. Table 1 shows the values of V_{max} and K_m that best fitted the kinetic data (average of measurements on duplicate soil samples). The kinetic parameters were within the same range of those reported by Czepiel et al. (1996) and De Visscher et al. (1999). Both V_{max} and K_m increased with temperature. The increase of V_{max} was exponential over the whole temperature range

Table 1 Kinetic parameters of methane consumption in soils at different temperatures ± standard deviations based on measurements on duplicate soil samples. Parameter values were based on fits to degradation curves in Fig. 1, at 20,000 ppm and 2,500 ppm initial concentrations. Values of K_m are liquid concentrations, calculated from gas concentrations using the solubility data of Sander (1999)

T (°C)	V_{max} (μmol $kg^{-1}_{soil\ DW}$ s^{-1})	K_m (μM)
5	0.0233 ± 0.0002	1.18 ± 0.12
10	0.0478 ± 0.0001	2.10 ± 0.24
15	0.0546 ± 0.0001	2.20 ± 0.14
20	0.1069 ± 0.0001	4.20 ± 0.02
25	0.200 ± 0.003	5.60 ± 0.01
30	0.3026 ± 0.0006	6.71 ± 0.10
35	0.503 ± 0.015	7.01 ± 0.31

($R^2 = 0.989$; $n = 7$), with a Q_{10} value of 2.8. This roughly coincides with an activation energy of 72 kJ mol^{-1}. The increase in K_m was approximately linear. Ignoring the value at 15 °C, which was below the trend of the other values, the relationship best fitting the experimental values of K_m was:

$$K_m(\mu M) = 0.143 + 0.207\ T \quad (2)$$

where T represents the temperature in °C ($R^2 = 0.987$; $n = 6$).

If $[CH_4] < K_m$, Eq. 1 becomes:

$$r_{CH_4} = -\frac{V_{max}}{K_m}[CH_4] \quad (3)$$

a first-order equation with a constant rate (V_{max}/K_m). At these low concentrations, the reaction rate had a Q_{10} of only 1.4 between 10 and 20 °C, and 1.9 between 20 and 30 °C in our experiments. Usually, the $[CH_4]$ of Eq. 3 refers to the gas-phase CH_4 concentration. For instance, when $[CH_4] = 1.7$ ppm, Eq. 3 yields the reaction rate of CH_4 at its natural concentration in the atmosphere (Lessard et al. 1994; Whalen and Reeburgh 1996). In that case, the Q_{10} values we found at low CH_4 concentrations were even lower (1–1.4). This is due to the temperature dependency of the CH_4 solubility in water.

A concentration-dependent influence of temperature on CH_4 consumption has also been observed by King and Adamsen (1992) in a pure-culture study with *Methylomonas rubra*. They attributed the difference to mass transfer limitations. If mass transfer resistance is the cause of the change in K_m, then K_m should also decrease with decreasing moisture level. To check this, batch experiments at 9% moisture content were compared with batch experiments at 14% moisture content. The kinetic parameters were as follows: $V_{max} = 0.0239$ μmol $kg^{-1}_{soil\ DW}$ s^{-1}, $K_m = 2.24$ μM, $k_1 = 0.00143$ μmol μM^{-1} $kg^{-1}_{soil\ DW}$ s^{-1} for the experiment at 9% moisture content, and $V_{max} = 0.0289$ μmol $kg^{-1}_{soil\ DW}$ s^{-1}, $K_m = 2.33$ μM, $k_1 = 0.00188$ μmol μM^{-1} $kg^{-1}_{soil\ DW}$ s^{-1} for the experiment at 14% moisture content. The activity was lower at 9% moisture content than at 14% moisture content, likely due to water stress, but K_m was practically the same in both cases, and the ratio between the first-order term and V_{max} was nearly constant. By substitution of the values of V_{max}, K_m and k_1 found at 9% moisture content and at 14% moisture content into:

$$r_{CH_4} = -\frac{V_{max}[CH_4]}{K_m + [CH_4]} - k_1[CH_4] \quad (4)$$

the CH_4 consumption can be calculated as a function of the CH_4 concentration. This is shown in Fig. 2. The curves are plotted on different scales to eliminate the effect of water stress, and to allow comparison of the shapes of the curves. If there was a considerable limitation to mass transfer, the kinetic curve would have resembled a straight line more closely as the soil became wetter and the mass transfer resistance increased. The shapes of the curves were nearly identical. It can be concluded that mass transfer resistances were negligible in these experiments, and that the K_m shift shown in Table 1 was a physiological response of soil microbiota to changes in temperature. In natural CH_4-rich environments, CH_4 production decreases with decreasing temperature (Arah and Stephen 1998; van Hulzen et al. 1999). The decrease in K_m may be a survival strategy of the methanotrophs under such conditions. Similarly, methanotrophs adapted to long-term incubation with CH_4 (< 275 ppm) showed a decrease in K_m value (Dunfield et al. 1999).

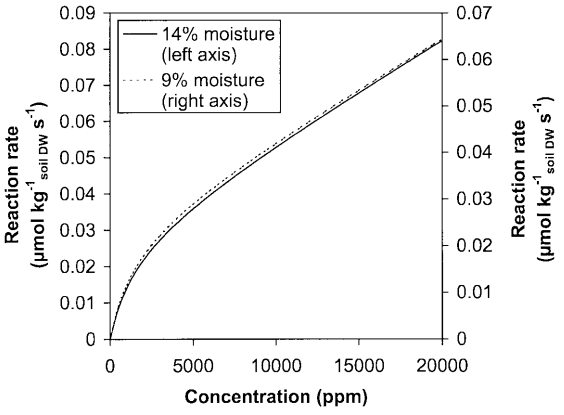

Fig. 2 Methane consumption rate as a function of methane concentration, at two moisture levels. Calculations based on parameter values estimated from fits to two degradation curves at initial methane concentrations of 20,000 ppm and one degradation curve at initial methane concentration 2,500 ppm

Mass transfer limitations were not negligible at a moisture content of 20%. The CH_4 uptake activity was less than half the activity at 14% moisture content, whereas the K_m value was higher, and the first-order term was more dominant ($V_{max} = 0.00732$ μmol $kg^{-1}_{soil\ DW}$ s^{-1}, $K_m = 4.02$ μM, $k_1 = 0.00095$ μmol μM^{-1} $kg^{-1}_{soil\ DW}$ s^{-1}) than at 14% moisture.

Influence of mineral nitrogen

Some preliminary experiments were performed to study the effect of adding NH_4^+ and NO_3^- to the soil on CH_4 consumption kinetics. These experiments were performed after 7–14 days of soil exposure to high CH_4 mixing ratios, in different flow-through systems. The length of time between wetting of the soil and incubation in the flow-through systems was different for each experiment (between 5 days and several weeks). The addition of ammonium caused a slight inhibition or a strong increase in the CH_4 oxidation rate (data not shown). The influence of NO_3^- was negligible when the NH_4^+ addition inhibited CH_4 oxidation, whereas it stimulated the oxidation when this was also increased by the NH_4^+ addition (data not shown).

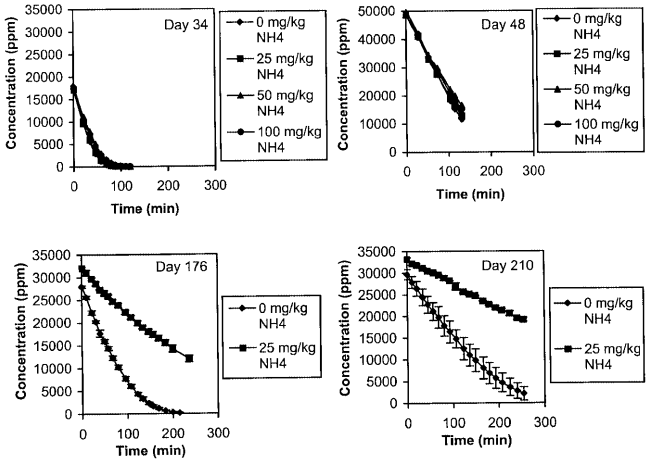

Fig. 3 Effect of different ammonium contents on batch methane oxidation by soil after four different times of exposure to high methane mixing ratios

In order to eliminate this variability, the influence of NH_4^+ on CH_4 oxidation was studied with samples from the same flow-through system, after 34, 48, 176, and 210 days of exposure to 5–6% CH_4. Figure 3 shows the first CH_4 degradation curve of each experiment. The initial CH_4 oxidation rates are shown in Table 2. After 34 days of exposure, the inhibition was 7% at 25 mg NH_4^+-N $kg^{-1}_{soil\ DW}$, and 15% at 50 and 100 mg NH_4^+-N $kg^{-1}_{soil\ DW}$. Similar results were found after 48 days. After long incubation times, the inhibition by NH_4^+ was much stronger. Indeed, the inhibition was 65% at 25 mg NH_4^+-N $kg^{-1}_{soil\ DW}$ after 176 days of exposure. The inhibition was nearly the same after 210 days. Thus, the inhibitory effect of NH_4^+ increased with incubation time, in spite of the fact that the moisture content increased as well (from a value of 15–18% after 34 and 48 days to a value of 30–33% after 176 and 210 days). Boeckx et al. (1996) found that the inhibition of CH_4 consumption by NH_4^+ ions decreased with increasing moisture content. Therefore, it can be assumed that the inhibition would have been worse if the water content had not changed.

The different effects of NH_4^+ addition on CH_4 oxidation were probably due to changes in the composition of the microbial community with CH_4 exposure. Shortly after exposing the soil to high CH_4 concentration, the methanotrophic strains that grow fast (or hatch rapidly out of cysts) dominated. As the methanotrophic community developed, the mineral N content of the soil decreased (De Visscher et al. 1999). After depletion of the mineral N, only the methanotrophs able to fix N were still able to grow, thus suppressing strains that thrived when mineral N was still present.

Table 2 Initial reaction rates of the degradation curves shown in Fig. 3. The influence of added NH_4^+ on the methane oxidation rates was monitored after four different exposure times at 5–6% CH_4

High-CH_4 exposure period (days)	Added ammonium content (mg $kg^{-1}_{soil\ DW}$)	Initial CH_4 oxidation rate (μmol CH_4 $kg^{-1}_{soil\ DW}$ s^{-1})
34	0	0.61
	25	0.56
	50	0.50
	100	0.52
48	0	0.46
	25	0.42
	50	0.38
	100	0.40
176	0	0.44
	25	0.16
210	0	0.26
	25	0.10

Influence of nitrifying sludge additions

Figure 4 shows the results of batch CH_4 consumption experiments with added nitrifying sludge, on four consecutive days. The initial CH_4 oxidation rates are shown in Table 3. Initially, adding the sludge inhibited the CH_4 consumption. The inhibition increased with increasing amounts of sludge. As time proceeded, the inhibition became less pronounced. On day 4, 0.5 ml of nitrifying sludge added to 90 g of soil dry weight even stimulated the CH_4 consumption slightly.

Nitrifying bacteria are known to oxidize CH_4, but they do not derive energy from it for growth (Bédard and Knowles 1989). Therefore, it was expected that adding nitrifying sludge would stimulate CH_4 oxidation. This stimulation did occur, but an inhibitory effect was apparent as well, especially on the first few days. Ammonium may well have been the cause of the inhibition.

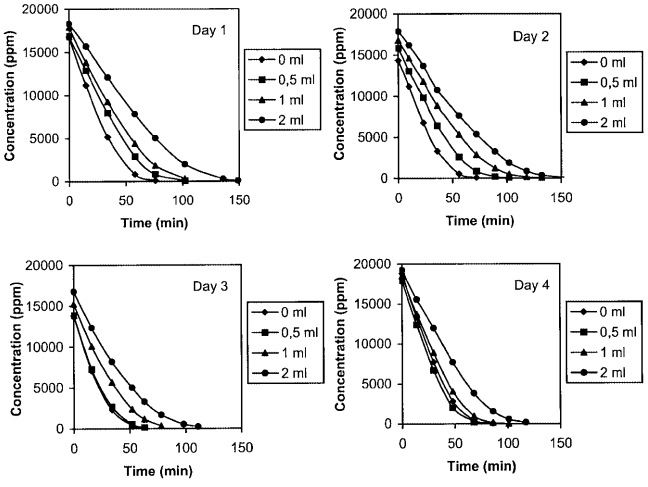

Fig. 4 Effect of different amounts of nitrifying sludge on batch methane oxidation by soil. Day 1 was the day on which the sludge was added; Days 2–4 were the three following days

Table 3 Initial reaction rates of the degradation curves shown in Fig. 4. The influence of the amounts of nitrifying sludge (added to 90 g of soil moist weight) on the methane oxidation rate. Day 1 was the day on which the sludge was added; days 2–4 were the three following days

Time	Amount of nitrifying sludge added (g)	Initial CH_4 oxidation rate (μmol CH_4 $kg^{-1}_{soil\ DW}$ s^{-1})
Day 1	0	0.56
	0.5	0.39
	1	0.40
	2	0.26
Day 2	0	0.51
	0.5	0.41
	1	0.34
	2	0.29
Day 3	0	0.63
	0.5	0.61
	1	0.48
	2	0.40
Day 4	0	0.59
	0.5	0.59
	1	0.50
	2	0.39

After the experiments, the soil with 2 ml nitrifying sludge added contained 15.7 mg NH_4^+-N $kg^{-1}_{soil\ DW}$. This was the only soil still showing a pronounced inhibition on the fourth day. Indeed, the other soils contained 3–5 mg NH_4^+-N $kg^{-1}_{soil\ DW}$.

Influence of compost additions

Similar results were obtained with compost and nitrifying sludge amendments, although the inhibition was less pronounced with compost (data not shown). In the first batch experiment, the unamended soil had the highest CH_4 consumption activity. After 5 days, all amended soils had at least the same CH_4 consumption activity as the unamended soil. The highest activity was found in the soil amended with 2 g compost fresh weight (added to 90 g of soil fresh weight). After 5 days, the NH_4^+ content decreased with increasing amounts of added compost (10.3 mg NH_4^+-N $kg^{-1}_{soil\ DW}$ without compost, 2.2 mg NH_4^+–N $kg^{-1}_{soil\ DW}$ with 10 g compost), whereas the pH increased with increasing amounts of added compost (6.6 without compost, 7.1 with 10 g added compost). Increasing the pH led to an increasing sensitivity to NH_4^+ inhibition, because free NH_3 is a stronger inhibitor of CH_4 oxidation than the NH_4^+ ion (Carlsen et al. 1991). Apparently, under our experimental conditions, the NH_4^+ content was decreased sufficiently for a reduced inhibition when 2 g of compost was added to the soil, while the pH increase was not yet sufficient to increase the sensitivity to NH_4^+ inhibition. Since the compost was certified for use as a fertilizer, it is unlikely that there was any inhibition due to metal toxicity.

Table 4 Kinetic parameters of methane consumption in soils at different copper concentrations. Parameter values based on fits to three degradation curves with initial concentrations 20,000 ppm

Cu^{2+} content (μg $kg^{-1}_{soil\ DW}$)	V_{max} (μmol $kg^{-1}_{soil\ DW}$ s^{-1})	K_m (μM)
26	0.57	8.1
52	0.58	8.3
78	0.54	8.1

Influence of copper

Table 4 shows the kinetic parameters calculated from batch experiments performed with three copper contents. Clearly, copper is not the limiting element in our experiments. When copper is not limiting, CH_4 is oxidized to CH_3OH by the particulate methane monooxygenase (pMMO), whereas in the absence of copper, the soluble methane monooxygenase (sMMO) is the active enzyme. The absence of any influence of copper on CH_4 oxidation kinetics in our experiments indicates that the pMMO was already active at the lowest copper content, i.e., at 26 μg Cu^{2+} $kg^{-1}_{soil\ DW}$.

Conclusions

In batch experiments, the affinity constant of microbial methane consumption by soils adapted to high mixing ratios of methane increased with increasing temperature. Consequently, methane consumption was more sensitive to temperature at high mixing ratios than at low mixing ratios. The effect was a physiological response to the temperature change, and did not reflect mass transfer limitations. The inhibition by ammonium was hard to assess because it depended on the time of exposure to high methane mixing ratios. Long exposure times increased the sensitivity to ammonium inhibition. Adding nitrifying sludge or compost enhanced the methane consumption after a brief inhibition effect. Adding copper did not affect the methane consumption kinetics, indicating that the particulate methane monooxygenase was the active enzyme in the system.

References

Arah JRM, Stephen KD (1998) A model of the processes leading to methane emission from peatland. Atmos Environ 32:3257–3264

Bédard C, Knowles R (1989) Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers. Microbiol Rev 53:68–84

Bender M, Conrad R (1994) Microbial oxidation of methane, ammonium and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils. Biogeochemistry 27:97–112

Boeckx P, Van Cleemput O (1996) Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature and nitrogen-turnover. J Environ Qual 25:178–183

Boeckx P, Van Cleemput O, Villaralvo I (1996) Methane emission from a landfill and the methane oxidising capacity of its covering soil. Soil Biol Biochem 28:1397–1405

Bogner J, Meadows M, Czepiel P (1997) Fluxes of methane between landfills and the atmosphere: natural and engineered controls. Soil Use Manage 13:268–277

Carlsen HN, Joergensen L, Degn H (1991) Inhibition by ammonia of methane utilization in *Methylococcus capsulatus* (Bath). Appl Microbiol Biotechnol 35:124–127

Crutzen PJ (1991) Methane's sinks and sources. Nature 350:380–381

Czepiel PM, Mosher B, Crill PM, Harriss RC (1996) Quantifying the effect of oxidation on landfill methane emissions. J Geophys Res 101D:16721–16729

De Visscher A, Thomas D, Boeckx P, Van Cleemput O (1999) Methane oxidation in simulated landfill cover soil environments. Environ Sci Technol 33:1854–1859

Dunfield PF, Liesack W, Henckel T, Knowles R, Conrad R (1999) High-affinity methane oxidation by a soil enrichment culture containing a Type II methanotroph. Appl Environ Microbiol 65:1009–1014

Graham DW, Chaudhary JA, Hanson RS, Arnold RG (1993) Factors affecting competition between Type I and Type II methanotrophs in two-organism, continuous-flow reactors. Microb Ecol 25:1–17

Hilger HA, Cranford DF, Barlaz MA (2000) Methane oxidation and microbial exopolymer production in landfill cover soil. Soil Biol Biochem 32:457–467

Hulzen JB van, Segers R, Bodegom PM van, Leffelaar PA (1999) Temperature effects on soil methane production: an explanation for observed variability. Soil Biol Biochem 31:1919–1929

Hütsch BW (1998) Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. Biol Fertil Soils 27:284–292

Hütsch BW, Webster CP, Powlson DS (1993) Long-term effects of nitrogen fertilization on methane oxidation in soil of the Broadbalk wheat experiment. Soil Biol Biochem 25:1307–1315

IPCC (1996) Climate change 1995: the science of climate change. Cambridge University Press, Cambridge

Keeney DR, Nelson DW (1982) Nitrogen – inorganic forms. In: Page AL, Miller RH, Keeney DW (eds) Methods of soil analysis: part 2, chemical and microbiological properties. 2nd edn. American Society of Agronomy, Soil Science Society of America, Madison, Wis., pp 643–698

Kightley D, Nedwell DB, Cooper M (1995) Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms. Appl Environ Microbiol 61:592–601

King GM, Adamsen APS (1992) Effects of temperature on methane consumption in a forest soil and in pure cultures of the methanotroph *Methylomonas rubra*. Appl Environ Microbiol 58:2758–2763

Leak DJ, Dalton H (1986) Growth yields of methanotrophs. 1. Effect of copper on the energetics of methane oxidation. Appl Microbiol Biotechnol 23:470–476

Lessard R, Rochette P, Topp E, Pattey E, Desjardins RL, Beaumont G (1994) Methane and carbon dioxide fluxes from poorly drained adjacent cultivated and forest sites. Can J Soil Sci 74:139–146

Megraw SR, Knowles R (1987) Active methanotrophs suppress nitrification in a humisol. Biol Fertil Soils 4:205–212

Mosier A, Schimel D, Valentine D, Bronson K, Parton W (1991) Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature 350:330–332

Sander R (1999) Compilation of Henry's law constants for inorganic and organic species of potential importance in environmental chemistry (Version 3). http://www.mpch-mainz.mpg.de/~sander/res/henry.html

Schnell S, King GM (1995) Stability of methane oxidation capacity to variations in methane and nutrient concentrations. FEMS Microbiol Ecol 17:285–294

Whalen SC, Reeburgh WS (1996) Moisture and temperature sensitivity of CH_4 oxidation in boreal soils. Soil Biol Biochem 28:1271–1281

Whalen SC, Reeburgh WS, Sandbeck KA (1990) Rapid methane oxidation in a landfill cover soil. Appl Environ Microbiol 56:3405–3411