Capacity for Methane Oxidation in Landfill Cover Soils Measured in Laboratory-Scale Soil Microcosms

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Laboratory-scale soil microcosms containing different soils were permeated with CH_4 for up to 6 months to investigate their capacity to develop a methanotrophic community. Methane emissions were monitored continuously until steady states were established. The porous, coarse sand soil developed the greatest methanotrophic capacity ($10.4 \text{ mol of CH}_4 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$), the greatest yet reported in the literature. Vertical profiles of O_2 , CH_4 , and methanotrophic potential in the soils were determined at steady state. Methane oxidation potentials were greatest where the vertical profiles of O_2 and CH_4 overlapped. A significant increase in the organic matter content of the soil, presumably derived from methanotroph biomass, occurred where CH_4 oxidation was greatest. Methane oxidation kinetics showed that a soil community with a low methanotrophic capacity (V_{max} of $258 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) but relatively high affinity (k_{app} of $1.6 \text{ } \mu\text{M}$) remained in N_2 -purged control microcosms, even after 6 months without CH_4 . We attribute this to a facultative, possibly mixotrophic, methanotrophic microbial community. When purged with CH_4 , a different methanotrophic community developed which had a lower affinity (k_{app} of $31.7 \text{ } \mu\text{M}$) for CH_4 but a greater capacity (V_{max} of $998 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) for CH_4 oxidation, reflecting the enrichment of an active high-capacity methanotrophic community. Compared with the unamended control soil, amendment of the coarse sand with sewage sludge enhanced CH_4 oxidation capacity by 26%; K_2HPO_4 amendment had no significant effect, while amendment with NH_4NO_3 reduced the CH_4 oxidation capacity by 64%. In vitro experiments suggested that NH_4NO_3 additions (10 and $71 \text{ } \mu\text{mol} \cdot \text{g of soil}^{-1}$) inhibited CH_4 oxidation by a nonspecific ionic effect rather than by specific inhibition by NH_4^+ .

Anaerobic decomposition of landfilled solid waste generates significant amounts of landfill gas comprising CH_4 and CO_2 in the approximate proportions of 60%:40% (vol/vol), together with numerous trace gases (35). Methane is a radiatively active trace gas that has been shown to be increasing at a rate of approximately 1%/year over the past 15 years (6, 17). The contribution of landfill CH_4 emissions to the increase in atmospheric CH_4 is uncertain, but estimates have put global emissions from landfills at between 20 and 70 Tg of $\text{CH}_4 \cdot \text{year}^{-1}$ (17, 32). The landfills within the United Kingdom are thought to contribute 1 to 2 Tg of $\text{CH}_4 \cdot \text{year}^{-1}$, which may amount to 25 to 33% of the CH_4 emitted to the atmosphere by the United Kingdom (38).

Microbially mediated CH_4 oxidation is recognized as being globally important and accounts for about 80% of global CH_4 consumption (32). Biological CH_4 oxidation must, therefore, play an important role in reducing emission of CH_4 to the atmosphere. Microbial CH_4 oxidation has been reported to occur at significant rates in many natural environments (1, 19, 40), and soils can act as sinks for CH_4 from the atmosphere (1, 39). Soils exposed to elevated CH_4 concentrations can develop a high capacity for CH_4 oxidation, presumably by selection of methanotrophic bacteria. Large methanotrophic populations, and high rates of CH_4 oxidation, have been reported in landfill cover soils exposed to a CH_4 flux (16, 40). Oxidation of CH_4 by methanotrophic bacteria in landfill cover soil clearly reduces the amount of CH_4 emitted by some landfills, although the quantitative significance of this remains to be determined.

The present research was designed to examine the extent to which methanotrophic microbial communities developed in a variety of soils in response to the presence of CH_4 and hence contributed to CH_4 attenuation. At most large, modern landfill sites, biogas is collected and burnt for safety and environmental reasons. Manipulation of landfill cover soils to maximize their CH_4 oxidation potential might provide a complementary strategy for control of CH_4 emissions, particularly at older sites where the CH_4 concentration in landfill gas is too low for flaring or energy recovery. The capacity of different soils to oxidize CH_4 was examined in experimental microcosms.

MATERIALS AND METHODS

Soil microcosms. Soil core tubes (1 m by 15-cm diameter) were constructed from polyvinyl chloride (PVC) pipe (Fig. 1). Gas sampling ports (2-mm diameter) were drilled at 10-cm intervals down the core, plugged with silicone rubber, and sealed on the inside with PVC tape. A perforated plate was located in the base of the core tube, and soil was packed above it to a depth of 90 cm. The soil was agitated during the filling process to settle the substrate. Core tubes were closed at either end with PVC end caps fitted with two rubber O-rings to ensure a gastight fit. The cores were housed in a room heated by means of a thermostatically controlled electric convection heater, and the temperature was maintained at $19 \pm 3^\circ\text{C}$. After being packed into the cores, the soil was left with an airflow across the top until the output of CO_2 declined to a constant level. At this point, CH_4 (>99% purity; British Oxygen Co., London, United Kingdom) was applied to the base of each core, and gas flow was controlled with needle valves and flow meters at $5 \text{ ml} \cdot \text{min}^{-1}$. This rate was selected as typical of the CH_4 production rate derived from a 20-m-deep layer of landfill (18). Control cores of the coarse sand soil were purged with oxygen-free N_2 (British Oxygen Co.), also at $5 \text{ ml} \cdot \text{min}^{-1}$.

During the experiment, an airflow ($300 \text{ ml} \cdot \text{min}^{-1}$) was passed across the top of each core through ports in the head cap. This maintained the natural vertical redox gradients in the soil and permitted measurement of the flux of CH_4 from the soil surface. The effluent airflow was passed to a 12-line channel changer (Analytical Development Co., Hoddesdon, United Kingdom). The CH_4 in the airflows from the cores was sampled sequentially, each time after equilibration for 5 min, in a cycle with a 1-h interval between sampling cycles; i.e., each core was analyzed every 2 h. Methane concentration was measured in an infrared CH_4

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FIG. 1. Diagram of the soil cores used to study methane oxidation in the laboratory.

analyzer (Analytical Development Co.), and the data were recorded on a data logger (Campbell Scientific, Sharnbrook, United Kingdom). Logged data were downloaded onto a personal computer for analysis.

Measurement of the CH_4 concentration in the effluent airstream and the known flow rate of the air across each core permitted calculation of a CH_4 flux from the surface of each soil core. The discrepancy between the CH_4 applied to the base of each core and the flux of CH_4 from the soil surface was calculated, and this was attributed to CH_4 oxidation. Mean values and standard errors of CH_4 oxidation rates were calculated for the triplicate cores of each soil type.

Application of CH_4 to the base of the soil cores was continuous for 6 months, except for a period after the cores had been purged for 2 months, when the CH_4 supply was interrupted for a period of 8 days.

Soil characteristics. Soils of three types were obtained from landfill sites within Essex, United Kingdom. All were obtained from the upper 15-cm layer of soil following removal of any turf and were sieved through a 20-mm mesh. Any remaining plant material and large stones were removed by hand.

A coarse sand (pH 6.6; 40% water-holding capacity; coarse sand, 70%; fine sand, 18%; silt/clay, 12%; porosity, 0.29) was obtained from Martin's Farm landfill site, St. Osyth (National Grid Reference TM 117175), from an area of the site that had previously been shown to be emitting CH_4 from the surface (15).

A clay top soil (pH 6.4; 55% water-holding capacity; coarse sand, 57%; fine sand, 16%; silt/clay, 27%; porosity, 0.19) was obtained from a section of Martin's Farm landfill site that was restored with clay top cover in 1976 and left undisturbed since. During this time, a lush plant growth had developed, and topsoil was present to a depth of 15 cm. On occasion, CH_4 has been found to be emitted to the atmosphere from this region of the landfill site (18a).

A fine sand (pH 5.3; 45% water-holding capacity; coarse sand, 16%; fine sand, 68%; silt/clay, 16%; porosity, 0.37) was obtained from Buckingham Hill landfill site, Stanford Le Hope (National Grid Reference TQ 671814). This site was capped with a 10-cm-deep clay layer to prevent water penetration and was covered above the clay to a depth of 35 cm with a fine sand.

Triplicate cores of each soil type were purged with CH_4 . A further three cores of the coarse sand were purged with N_2 as a control without CH_4 exposure.

Steady-state gas profiles. After the emission measurements had reached steady state (see Results), profiles of gases within the cores were measured by directly withdrawing duplicate samples (250 μl) of the soil atmosphere through the gas sampling ports into gas syringes (Pressure-lok; Precision Sampling Corporation, Baton Rouge, La.). The samples were analyzed for CH_4 , O_2 , and N_2 by using a thermal conductivity detector on a gas chromatograph (model 4300; Carlo Erba, Milan, Italy) fitted with a stainless steel column (3 m by 2-mm inside diameter) packed with 100/200-mesh Molecular Sieve 5A (Jones Chromatography, Hengoed, United Kingdom). The temperatures used were as follows: injector, 60°C; column oven, 100°C; detector, 250°C; and bridge, 350°C. Carrier gas was helium at 20 $\text{ml} \cdot \text{min}^{-1}$. Peak areas were determined with an integrator (Trivector, Sandy, United Kingdom). Under these conditions, the minimum quantifiable concentrations for the gases were 40 nmol of $\text{CH}_4 \cdot \text{ml}^{-1}$, 20 nmol of $\text{O}_2 \cdot \text{ml}^{-1}$, and 20 nmol of $\text{N}_2 \cdot \text{ml}^{-1}$. Since O_2 and Ar elute together from this column, and Ar comprises 0.93% (vol/vol) of air, this gas interferes with O_2 analysis. To allow for this, the contribution of Ar to the O_2 /Ar peak was subtracted, assuming that both N_2 and Ar would be present at a constant atmospheric ratio throughout the soil cores.

Carbon dioxide was analyzed on the same gas chromatograph with a glass column (2 m by 4-mm inside diameter) packed with 50/100-mesh silica gel.

Samples were injected by using a 1-ml sample loop. Other conditions were the same as described above. The limit of detection was 5 nmol of $\text{CO}_2 \cdot \text{ml}^{-1}$.

Analysis of soil carbon and nitrogen. Samples of soil taken at intervals down the soil cores were dried in a warm (60°C) oven to constant weight and ground in a mortar. Subsamples (~20 mg) were assayed for carbon and nitrogen content in a CHN analyzer (model 2400; Perkin-Elmer, Beaconsfield, United Kingdom).

Soil water content. Samples of soil taken at intervals down the soil cores were dried to a constant weight in an oven (60°C) for 24 h. The water content of the soil was calculated as the percent weight lost on drying.

Methane oxidation kinetics in the coarse sand. Since CH_4 oxidation was most rapid in the coarse sand (see Results), the CH_4 -oxidizing capacity of this soil was further investigated. At the end of the permeation experiment, the soil from one core of coarse sand was mixed, and samples were stored at 4°C. Subsamples of the stored soil (10 g) were placed in 55-ml serum bottles which were sealed with butyl rubber stoppers. Methane was injected into the bottles to a concentration of 5% (vol/vol) and left overnight to equilibrate to the analysis temperature. The bottles were flushed with air, and then fresh CH_4 was injected to give initial concentrations in the headspaces ranging from 500 to 50,000 CH_4 ppm (vol/vol). Bottles were incubated at 25°C. Headspace samples (150 μl) were withdrawn at intervals over a period of 30 min and analyzed in a gas chromatograph (model 4300; Carlo Erba) with a flame ionization detector fitted with a glass column (2 m by 4-mm inside diameter) packed with 50/100-mesh silica gel. Conditions used were as follows: injector, 60°C; column oven, 75°C; and detector, 250°C. Carrier gas was N_2 at 25 $\text{ml} \cdot \text{min}^{-1}$. The detection limit was 20 pmol of $\text{CH}_4 \cdot \text{ml}^{-1}$. The removal of CH_4 from the headspace was rapid, and the loss of CH_4 measured over the first 15 min was used to calculate the rate of CH_4 oxidation. The CH_4 removed during this period was <5% of the initial CH_4 concentration. Estimates of the kinetic parameters, V_{max} and apparent k_m (k_{app}), were obtained by using a computing package (SigmaPlot for Windows; Jandel Scientific, Erkrath, Germany) to fit by a least-squares method the Michaelis-Menten equation to the data.

Effect of nutrient amendment on methane oxidation. Fresh coarse sand obtained from Martin's Farm landfill site was seeded with the contents of one CH_4 -purged core of the same soil from the first experiment. Quantities of this composite soil were amended with either NH_4NO_3 (equivalent to 2 g of N $\cdot$ kg of soil $^{-1}$), K_2HPO_4 (100 mg of P $\cdot$ kg of soil $^{-1}$), or anaerobically digested sewage sludge (2.5 g $\cdot$ kg of soil $^{-1}$; equivalent to ~100 mg of N plus ~100 mg of P $\cdot$ kg of soil $^{-1}$). The nitrogen loading is typical of agricultural application (2 g of N $\cdot$ kg of soil $^{-1}$ = 100 kg of N $\cdot$ ha $^{-1}$) and is equivalent to 71 μmol of N $\cdot$ g of soil $^{-1}$, assuming soil bulk density of 1 g $\cdot$ ml $^{-1}$ and that the application remains within the top 10 cm of soil. The sewage sludge was obtained from Chelmsford Sewage Treatment Works, Essex, United Kingdom, as a dewatered cake containing ~20% solids. The sludge was dried in a fan oven at 35°C and ground to a powder in a blender (Waring Products, New Hartford, Conn.). All amendments were added to the soil as a dried powder and mixed in by repeated turning. The amended soils then were packed into triplicate cores as before, along with a control set of three cores of the unamended soil. All cores were purged with CH_4 (5 $\text{ml} \cdot \text{min}^{-1}$) as described previously.

At the end of the experiment, analysis of CH_4 oxidation kinetics was conducted by using soil from the most actively methanotrophic layers within the cores. This soil was first dried to a constant moisture level to negate any variation in CH_4 oxidation rate due to the varying water content of the soil from different depths within the cores (see Results). The coarse sand from a depth of 20 to 30 cm within the soil cores was removed, homogenized, and dried in an airstream until a moisture content of 10% had been reached. Triplicate soil samples (5 g [dry weight]) were placed into stoppered 25-ml serum tubes, and CH_4 was injected to 5% and equilibrated overnight at room temperature. Before each experimental run, the tubes were flushed with air and resealed, and fresh CH_4 was injected to give initial concentrations in the headspace of the tubes ranging from 50 to 50,000 CH_4 ppm (vol/vol). Tubes were incubated on a tube roller at $20 \pm 1^\circ\text{C}$. Headspace samples (150 μl) were withdrawn at intervals over a period of 30 min and analyzed in a gas chromatograph with flame ionization detector (conditions as described above). Methane removal over the first 15 min was used to calculate rates at that particular CH_4 concentration. Kinetic parameters, V_{max} and k_{app} , were computed as before.

Methane oxidation potential. Methane oxidation potential was assessed in vitro by applying a high concentration of CH_4 so that the soil microflora were saturated with CH_4 , and oxidation of CH_4 followed zero-order kinetics, i.e., the maximum possible rate. This provides an estimate of the relative biomass of the methanotrophic population in the soil, as well as an indication of the CH_4 -oxidizing capacity of each soil. Soil samples (5 g [wet weight]), taken at 10-cm intervals down the cores from the second microcosm experiment, were each placed in a 25-ml serum tube, which was closed with a septum stopper, and CH_4 was injected to give a final concentration of 5% (vol/vol). Preliminary experiments had shown that this concentration of CH_4 was saturating. Bottles were incubated at room temperature ($20 \pm 1^\circ\text{C}$) on a tube roller to avoid gas transfer limitations between headspace and soil. Gas headspace samples (150 μl) were withdrawn at intervals and analyzed for residual CH_4 in a gas chromatograph with a flame ionization detector (conditions as described previously).

Effect of added ions on methane oxidation. Soil samples (5 g [wet weight]) were each placed in a 25-ml serum tube, which was closed with a septum stopper, and CH_4 was injected to give a final concentration of 5% (vol/vol). Bottles were

FIG. 2. Methane oxidation rate for a coarse sand soil purged from the base with methane. Points represent mean daily rates.

incubated at room temperature ($20 \pm 1^\circ\text{C}$) on a tube roller to avoid gas transfer limitations between headspace and soil. Gas headspace samples ($50\ \mu\text{l}$) were withdrawn at intervals and analyzed for residual CH_4 in a gas chromatograph with a flame ionization detector (conditions as described above). After 90 min, NH_4Cl , KNO_3 , or KCl (10 or $71\ \mu\text{mol} \cdot \text{g}^{-1}$ of soil $^{-1}$) was added in $100\ \mu\text{l}$ of ultra-high-purity water. The higher concentration used was equivalent to the loading in the NH_4NO_3 -amended microcosms. Control samples received $100\ \mu\text{l}$ of ultra-high-purity water only. Rates of CH_4 oxidation before and after amendment were calculated by passing a linear regression line through the data.

RESULTS

Soil core microcosms. In all soil cores purged with CH_4 , the flux of CH_4 from the surface of the soil declined with time, demonstrating a developing capacity for CH_4 oxidation. For the first 13 days of incubation with the coarse sand, there was an initial steady-state CH_4 efflux that indicated CH_4 oxidation at a low initial rate of around $4\ \text{mmol}$ of $\text{CH}_4 \cdot \text{core}^{-1} \cdot \text{h}^{-1}$, probably due to the original methanotrophic community (Fig. 2). After 13 days, the CH_4 oxidation rate within the cores of coarse sand rapidly increased, and it subsequently levelled off at $10.4 \pm 0.1\ \text{mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ (Table 1). At this point, a second steady state had developed between CH_4 input and oxidation which presumably reflected the selection of a larger and more active methanotrophic community.

Over the same period, the clay topsoil and the fine sand also showed a developing methanotrophic potential, although to a

TABLE 1. Steady-state CH_4 oxidation rates measured in soil cores of landfill cover soil and in cores of coarse sand amended with various nutrients, after application of CH_4 for 6 months

Soil type	Methane oxidation rate (mol of $\text{CH}_4 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) $\pm$ SE	% Oxidation
Expt 1		
Coarse sand	10.4 ± 0.1	61
Clay topsoil	6.8 ± 0.3	40
Fine sand	6.9 ± 0.3	41
Expt 2		
Control (no amendment)	5.75 ± 0.17	34
NH_4NO_3	2.06 ± 0.12	12
K_2HPO_4	5.75 ± 0.15	34
Sewage sludge	7.25 ± 0.16	43

FIG. 3. Depth profiles of methane (Δ), oxygen ($\circ$), nitrogen ($\bullet$), and carbon dioxide ($\blacktriangle$) in coarse-sand soil cores, at the end of purging from the base for 6 months with methane (A) or nitrogen (B).

lesser extent than in the coarse sand. After 2 and 4.5 months, respectively, the CH_4 oxidation rates in these soils had increased to steady-state rates of 65 and 66%, respectively, of that for the coarse sand (Table 1). Over the course of the experiment, the N_2 -purged control showed no significant CH_4 efflux, confirming that CH_4 was not being produced in the soil cores.

Fifty-one days from the start of CH_4 application to the soil cores, the cylinder supplying the gas emptied and there was an interruption of the CH_4 purge until a replacement could be fitted. During the interruption, air was able to diffuse throughout the whole of the soil core. After 8 days, when the CH_4 supply was reestablished, the CH_4 oxidation rates recovered rapidly (48 h) to regain the same steady-state CH_4 oxidation rates as were present prior to the interruption of supply.

Steady-state gas profiles. Vertical profiles of gases in the soils demonstrated broadly similar patterns in all of the CH_4 -purged soils (Fig. 3). In the coarse sand, O_2 concentrations declined with depth and the soil became anaerobic at 30 cm. The CH_4 concentration profile showed a sharp decrease upward toward the surface, although the depth of maximum decline was around 30 cm. At the same depth, a peak of CO_2 was measured also. By contrast, the cores of the same coarse sand purged with N_2 showed only a slow decline in O_2 concentration with depth, and the whole soil profile was aerobic. Thus, the removal of O_2 in the presence of CH_4 was not due merely to outgassing of the O_2 by the CH_4 purge but also to its utilization by the methanotrophic microflora.

Similar patterns for O_2 and CH_4 were measured in the other two CH_4 -purged soils. However, in each core, the soil became anaerobic at deeper depths than in the cores of coarse sand (around 45 cm for the clay and 55 cm for the fine sand).

Soil organic matter. The total carbon and nitrogen content

FIG. 4. Depth profiles of total carbon (hatched bars) and total nitrogen (black bars) in cores of various landfill cover soils after purging for 6 months. Soil type (purge gas): A, coarse sand (CH_4); B, coarse sand (N_2); C, clay topsoil (CH_4); D, fine sand (CH_4). Bars represent means of duplicate analyses.

of the soils was not uniformly distributed (Fig. 4). In the unamended coarse sand, there was a marked peak in carbon content at 25 cm that was three times the values present in the rest of the core. No such elevation in soil carbon content was present in the N_2 -purged coarse sand. A similar peak in carbon content was present in the clay soil at 25 to 45 cm, although there was overall a higher level of carbon and nitrogen in this soil. The fine sand soil had a lower carbon and nitrogen content than the other soils, but a slight elevation in the carbon content was measured at a depth of 45 cm.

Soil water content. The moisture content of the soils established that the passage of air across the top had desiccated the surface of each core (<3% water lost on drying) to a depth of 10 to 15 cm (Fig. 5). The soils were wetter toward the bottom of the cores, and a small amount of water had collected in the base caps.

Methane oxidation kinetics in the coarse sand. Kinetic parameters for CH_4 oxidation in the CH_4 - and N_2 -purged coarse sand (Table 2) showed that there was a distinct difference in the responses of the two soils. The CH_4 -purged soil showed a pattern of oxidation characterized by a high activity ($V_{\max}$, $998 \pm 58 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) but low affinity for CH_4 (k_{app} , $31.7 \mu\text{M}$). In contrast, the N_2 -purged soil displayed a lower maximum activity for CH_4 ($V_{\max}$, $258 \pm 12 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) but had a higher affinity (k_{app} , $1.6 \mu\text{M}$), and relatively faster CH_4 oxidation rates were measured at lower CH_4 concentrations.

Effect of nutrient amendment on methane oxidation. In the second microcosm experiment, the steady-state CH_4 oxidation rate in the cores of sewage sludge-amended coarse sand ($7.25 \pm 0.16 \text{ mol} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) was significantly enhanced by 26% ($P < 0.001$) compared with the unamended control (Table 1). Initially, the cores of NH_4NO_3 -amended coarse sand showed no CH_4 oxidation at all, but after 2 months, the rate began to increase such that 5 months from the start of the

experiment, a steady-state for CH_4 oxidation had been reached that was significantly lower ($P < 0.01$) than in the control soil. Amendment of the coarse sand with K_2HPO_4 had no significant effect on the CH_4 oxidation rate.

Profiles of the steady-state gas concentrations within the cores showed that the NH_4NO_3 -amended cores were oxidized to the base (Fig. 6). The cores of K_2HPO_4 - and sewage sludge-amended coarse sand and the control soil showed a pattern of gas concentration similar to that in the coarse sand, with the soil becoming anaerobic below around 30 cm.

Kinetic parameters for CH_4 oxidation in the unamended and NH_4NO_3 -amended soils (Table 2) were distinctly different. The unamended soil showed CH_4 oxidation that possessed a high activity ($V_{\max}$, $2,342 \pm 165 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) but low affinity for CH_4 (k_{app} , $8.1 \mu\text{M}$). The NH_4NO_3 -amended soil displayed a lower maximum activity for CH_4 ($V_{\max}$, $390 \pm 146 \text{ nmol} \cdot \text{g of soil}^{-1} \cdot \text{h}^{-1}$) but had a similar affinity (k_{app} , $14.4 \mu\text{M}$). The discrepancy between the low $V_{\max}$ measurement in the sewage sludge-amended coarse sand (Table 2) and the relatively high activity in these amended microcosms (Table 1) was probably a result of the higher water content of this soil. During the measurement of CH_4 oxidation kinetics of this treatment, the soil clumped inside the rolled tubes, with the result that it became CH_4 transport rate limited.

Analysis of the carbon content within the cores of coarse sand (Fig. 7) showed a peak of organic matter in the soil at a depth of 20 to 30 cm. A similar peak in carbon content at the same depth was present in the K_2HPO_4 - and sewage sludge-amended soils. No such elevation in soil carbon content was present in the NH_4NO_3 -amended soil.

Methane oxidation potential. Variations of headspace CH_4 concentration with time were linear ($P < 0.05$), suggesting that CH_4 oxidation was indeed CH_4 saturated and conformed to zero-order kinetics. The potential CH_4 oxidation capacities on a dry weight basis were calculated from the data by linear

FIG. 5. Depth profiles of soil water content in cores of various landfill cover soils after purging for 6 months. Soil type (purge gas): A, coarse sand (CH_4); B, coarse sand (N_2); C, clay topsoil (CH_4); D, fine sand (CH_4).

regression. Measurement of the CH_4 oxidation potentials demonstrated that each of the soils had a capacity for methanotrophy at most depths (Fig. 8), even extending into the anoxic layer. The cores of K_2HPO_4 -amended and sewage sludge-amended coarse sand and the unamended control soils showed peak CH_4 oxidation potential at a depth of 20 to 30 cm below the surface of the core. At this depth, the maximum potential CH_4 oxidation rate was around three times higher than in the remainder of the core. The NH_4NO_3 -amended coarse sand showed no such peak in activity at any depth, and rates were uniformly low throughout the core.

Effect of added ions on methane oxidation. Before amendment of the soils, all treatments showed rapid CH_4 oxidation (Fig. 9; Table 3). In the controls, to which only water was added, there was a small but statistically significant ($P < 0.05$) decrease of CH_4 oxidation rate. Addition of any of the solutes (at both 10 and 71 $\mu\text{mol} \cdot \text{g}^{-1}$ [wet weight] of soil $^{-1}$) resulted in significant inhibition of CH_4 oxidation by the soil, while at the higher concentration inhibition was complete (slopes not significantly different from zero at $P = 0.05$).

DISCUSSION

The immediate establishment of a steady-state rate of CH_4 oxidation in the coarse sand cores after initiation of the CH_4 purge demonstrated the presence of a microbial community with at least some methanotrophs (Fig. 2). After 13 days of exposure to CH_4 , a marked increase in CH_4 oxidation indicated further enhancement of the methanotrophic community. This rapid increase in CH_4 oxidation suggested that methanotrophs in the coarse sand were initially carbon limited but that application of an available carbon substrate, CH_4 , resulted in the selective enrichment of the methanotrophic community. The CH_4 oxidation rate in the coarse sand attained a second steady state after 30 days, at which point further development of the methanotrophic microflora became limited by some factor other than CH_4 availability. The steady-state methanotrophic activity which developed was, therefore, the maximum capacity for CH_4 oxidation which could develop in the particular soil and set of environmental conditions. Enrich-

TABLE 2. Kinetic parameters for methane oxidation in a coarse sand landfill soil after a steady-state for CH_4 oxidation had been reached

Soil type	$V_{\max} \pm \text{SE}$ ($\text{nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$)	$k_{\text{app}} \pm \text{SE}$ ($\text{nmol} \cdot \text{ml}^{-1}$)	Methane concn (μM) ^a
Expt 1			
CH_4 purged	998 ± 58	$1,005 \pm 113$	31.7
N_2 purged	258 ± 12	50 ± 12	1.6
Expt 2			
Unamended	$2,342 \pm 165$	233 ± 36	8.1
NH_4NO_3 amended	390 ± 146	416 ± 288	14.4
K_2HPO_4 amended	470 ± 160	535 ± 313	18.6
Sewage sludge amended	719 ± 222	693 ± 340	24.0

^a Dissolved concentration of methane equivalent to k_{app} .

FIG. 6. Depth profiles of methane (Δ), oxygen (○), nitrogen (●), and carbon dioxide (▲) in cores of coarse sand amended with various nutrients, after purging from the base with methane for 6 months. (A) Control; (B) NH₄NO₃; (C) K₂HPO₄; (D) sewage sludge. Dotted lines indicate the depths of the light-colored oxic surface layer.

ment of methanotrophs in response to high CH₄ has been reported previously (4, 40).

In both the clay soil and fine sand, there was no initial CH₄ oxidation, presumably because of the absence of active methanotrophs. Their methanotrophic capacities developed more

slowly than in the coarse sand, and the steady-state methanotrophic activities were lower. These lower oxidation rates in clay and fine sand were not attributable solely to slower rates of gas transport in these two soils, as the surface oxic layers at steady state penetrated deeper than in the coarse sand. This, in

FIG. 7. Depth profiles of total carbon (hatched bars) and total nitrogen (black bars) in cores of coarse sand amended with various nutrients, after purging from the base with methane for 6 months. (A) Control; (B) NH₄NO₃; (C) K₂HPO₄; (D) sewage sludge. Bars represent means of duplicate analyses.

FIG. 8. Rates of methane oxidation potential recorded *in vitro* in subsamples of coarse sand, taken from various depths within the cores. Soils contained no addition (A) or were amended with NH_4NO_3 (B), K_2HPO_4 (C) or sewage sludge (D) and purged with methane for 6 months.

turn, suggests that it was the development of a very active methanotroph community in the coarse sand which rapidly removed O_2 and limited its penetration into this soil. In contrast, the development of equally active communities in the other two soils was prevented by other factors such as nutrient limitation.

Following the unplanned interruption of the CH_4 supply to the soil cores, the CH_4 oxidation activity returned to previous steady-state rates almost immediately after the CH_4 supply was reestablished (Fig. 2). This finding emphasizes the ability of the methanotrophic community to survive periods of CH_4 starvation, which is particularly important in landfill cover soils, where the upward flux of biogas into the cover soil may vary in response to changes in atmospheric pressure (45, 46). Anticyclonic weather patterns cause atmospheric pressure to rise and interrupt the supply of CH_4 to the microorganisms in the cover soil, while a fall in atmospheric pressure may result in an upward surge of CH_4 . The retention of methanotrophic capability in the cover soil, despite a temporarily interrupted CH_4 supply, will tend then to reduce the probability of emission of CH_4 from the soil surface.

Roslev and King (33) showed that methanotrophs could survive extended periods in the absence of CH_4 , particularly under anoxic conditions when anaerobic endogenous metabolism apparently provided maintenance energy. In our experiments, methanotrophic potential was detectable even in soil strata which were anoxic (Fig. 8). The persistence of methanotrophic potential under conditions of anoxia has been demonstrated previously in other environments (19, 20, 22) and has been attributed to two functional types of methanotrophs. One type is an obligate aerobe rapidly inactivated under anoxia, while the second type can withstand extended periods of an-

FIG. 9. Methane concentration measured in tubes containing a coarse sand soil. The soil was amended after 90 min (arrowed) with ultra-high-purity water (○), NH_4Cl (■), KNO_3 (●), or KCl (□).

TABLE 3. Methane oxidation rates in landfill soil before and after addition of inorganic nutrients

Amendment	CH ₄ oxidation rate ($\mu\text{mol} \cdot \text{g}$ [wet wt] of soil ⁻¹ · h ⁻¹) $\pm$ SE	
	Before addition	After addition
71 $\mu\text{mol} \cdot \text{g}$ (wet wt) of soil ⁻¹		
Control	2.58 $\pm$ 0.21	1.50 $\pm$ 0.08
NH ₄ Cl	2.29 $\pm$ 0.17	0.08 $\pm$ 0.11 ^a
KNO ₃	3.06 $\pm$ 0.51	-0.03 $\pm$ 0.46 ^a
KCl	2.16 $\pm$ 0.16	0.06 $\pm$ 0.12 ^a
10 $\mu\text{mol} \cdot \text{g}$ (wet wt) of soil ⁻¹		
Control	2.43 $\pm$ 0.29	1.58 $\pm$ 0.15
NH ₄ Cl	2.64 $\pm$ 0.20	0.44 $\pm$ 0.10 ^b
KNO ₃	2.16 $\pm$ 0.32	0.60 $\pm$ 0.21 ^b
KCl	2.07 $\pm$ 0.17	0.46 $\pm$ 0.11 ^b

^{a,b} Analysis of variance not significantly different at $P = 0.05$.

oxia and rapidly recover its CH₄ oxidation activity upon the return of oxic conditions.

In our work, the methanotrophic community survived for >180 days in the N₂-purged control cores in the absence of CH₄ (Table 2). These cores were essentially oxic throughout their lengths (Fig. 3) and therefore not optimal for starvation or survival of methanotrophs, which is favored under anoxia (33). Roslev and King (33) showed that all oxic methanotroph cultures lost CH₄ oxidation activity within 42 days, and even anoxic cultures did so by 70 days (although a small proportion [4 to 10%] of the population could be subsequently recovered on agar plates after prolonged incubation). Significant activity from survival of obligate methanotrophs after >180 days of CH₄ starvation in the control cores must, therefore, be doubted. An alternative hypothesis is that this surviving population consists of facultative methanotrophs which, in the absence of CH₄, metabolize other substrates. The low specificity of methane monooxygenase (10, 31) may be of survival advantage in the absence of CH₄, as other hydrocarbon substrates may be scavenged, or the bacteria may survive by mixotrophic growth with other organic substrates. Facultative methanotrophs, including yeasts (42, 43) and bacteria (30), have been reported (9), and the reported presence of methanotrophic populations in soils apparently unexposed to elevated CH₄ concentrations (4, 5, 16) may reflect the presence of an autochthonous, mixotrophic, facultative methanotrophic population with a high affinity for CH₄. King (21) has recently pointed out that because of the selection techniques that have been used to isolate them initially, the methanotrophs currently available in pure culture may not be representative of the species abundant in natural environments. Jones and Nedwell (16) reported that methanotrophs isolated in CH₄ from landfill soil subsequently grew better when subcultured onto organic medium in the absence of CH₄. Exposure to abundant CH₄ appears, however, to select a specialized high-capacity/low-affinity methanotrophic community in soil, which may be obligate methanotrophs.

Analysis of the soils within the cores after a steady state for CH₄ oxidation had been reached showed vertical concentration gradients of gases (Fig. 6), which are similar to those found in the cover soil of a CH₄-producing landfill site (15). In the coarse sand cores, the soil became anaerobic below 30 cm (Fig. 3), and aerobic CH₄ oxidation could not have occurred below this depth. Anaerobic CH₄ oxidation has been reported in marine sediments linked to sulfate reduction (e.g., reference 2), but the decline in the CH₄ concentration in the anaerobic

region of the core was constant with depth, consistent with passive dilution of the gas by N₂ from the air and indicating an absence of anaerobic CH₄ oxidation. The soil became desiccated in the top 10 cm, and most (if not all) of the aerobic CH₄ oxidation within the coarse sand cores must have occurred at a depth of between 10 and 30 cm. In contrast, aerobic CH₄ oxidation occurred in the clay and fine sand soils to depths of 35 and 45 cm, respectively. Even though CH₄ oxidation was occurring to greater depths in these soils than in the coarse sand, the oxidation rates measured in these cores were lower. This finding emphasizes the much higher rate of CH₄ oxidation that was occurring in the narrow active band of the coarse sand.

Potential CH₄ oxidation rates in the cores at steady state showed a peak in activity that corresponded to the depth at which the decline in CH₄ and O₂ was most rapid (as reported elsewhere [25]). This depth was 20 cm in the coarse sand and coincided with the peaks of carbon and nitrogen in the soil organic matter at the end of the experiment (Fig. 7). Soil carbon increased above that initially present, and the peaks did not occur in the N₂-purged controls, indicating significant carbon assimilation and organic matter synthesis by the methanotrophic community during the course of the experiment. Experiments using ¹⁴CH₄ showed that 25 to 38% of carbon was assimilated during CH₄ oxidation (data not shown); others have reported that up to 70% of the carbon from CH₄ assimilated into microbial cell material during CH₄ oxidation (29, 40). The increase in soil nitrogen content at the same depth may indicate some N₂ fixation in the zone of active CH₄ oxidation. In the other two soils, there were no such obvious peaks of soil carbon or nitrogen, although the higher background organic content of the clay would make such accumulation less easily detected.

The results of the kinetic study show that there were apparently distinct populations of methanotrophs in the coarse sand soil that was CH₄ purged and in the control coarse sand cores purged with N₂. In soil that was exposed to high concentrations of CH₄, at steady state there was a population of methanotrophs with a high capacity for CH₄ oxidation but relatively low affinity (k_{app} , 31.7 μM) for CH₄. Bender and Conrad (4) reported k_{app} s of 100 μM for CH₄-purged forest soil and 28 μM for forest luvisol. The control soil that was N₂ purged had not been exposed to CH₄ for a period of 6 months and yet still showed some CH₄ oxidation activity, characterized by a lower capacity (V_{max}) for CH₄ oxidation but a higher affinity (k_{app} , 1.6 μM). Bender and Conrad (4) detected two populations of methanotrophs in soils: low-capacity/high-affinity populations in soil exposed to only ambient atmospheric CH₄, and an additional high-capacity/low-affinity methanotrophic population in soil exposed to elevated CH₄. However, the k_{app} s that they reported for the high-affinity populations (0.013 to 0.47 μM) were much lower than in our N₂-purged control. This difference may reflect the origin of the soil in our N₂-purged control, which was from a landfill site and may have been exposed to CH₄ before the experiment. The only previous report of V_{max} and k_m in landfill soil (40) showed values which were broadly in accordance with those for our N₂-purged soil.

Amendment with NH₄NO₃ of the cores of coarse sand significantly reduced the capacity of the soil to oxidize CH₄. Ammonium addition has been suggested to diminish the capacity for uptake of atmospheric CH₄ by forest soil (36, 37), agricultural soil (14), and sediment (7). The mode of action of NH₄⁺ has been proposed to be through competition with CH₄ at the active site of methane monooxygenase (3, 41), although the mechanism is controversial (8, 12, 28). Recent work (23, 24, 34) has suggested that there may be an interaction between the

ambient CH_4 concentration and the degree of inhibition of CH_4 oxidation induced by NH_4^+ . Nitrite derived from NH_4^+ may also play an inhibitory role (24).

While our core microcosm experiments indicated inhibition of CH_4 oxidation by addition of NH_4NO_3 (but not by K_2HPO_4 addition), the in vitro experiments (Fig. 9) showed that inhibition of CH_4 oxidation was not specifically by NH_4^+ . The supposition that NH_4^+ specifically inhibits methane monooxygenase is based upon a small number of papers reporting in vitro experiments (8, 13, 28, 41, 44) in which ionic concentrations were not controlled. The comparison with NH_4^+ addition was usually a distilled water blank. Reports that CH_4 oxidation is diminished by NH_4^+ in fertilized field sites (14, 26, 36, 37) could equally well be explained by oxidation of the applied NH_4^+ to NO_2^- , or even by an increased ionic concentrations in fertilized soils. While attributing specific effects to NH_4^+ addition, Nesbit and Breitenbeck (27) also suggested that methanotrophs in soil were sensitive to increases in osmotic potential. They pointed out that the inhibitory effect of added NH_4^+ persisted in soil long after it had disappeared through nitrification, which is not consistent with a direct inhibitory action by NH_4^+ . Adamsen and King (1) reported that NH_4Cl decreased CH_4 uptake rate constants by 93%, while an equivalent concentration of NaNO_3 decreased uptake by 76%. Therefore, in their work, while NH_4^+ addition had the greater inhibitory effect on CH_4 oxidation, the change in ionic concentration probably accounted for 80% of the total inhibition. Recently, Crill et al. (11) reported that CH_4 oxidation in fertilized peat plots was subjected to an inhibitory salt effect which was not the result of NH_4^+ addition, and they concluded that the mechanism of inhibition of CH_4 oxidation remained unclear. Our additions in the in vitro experiments were, in molar concentrations, similar to those used in the other works (11, 14, 27, 37) and typical of agricultural application, and the results support the concept of an inhibitory effect linked to ionic concentration. We do not suggest that inhibition of CH_4 oxidation is solely due to the change in ionic strength; NH_4^+ may also impose a specific inhibition. Clearly, the inhibitory mechanism is complex (11, 34), and the major inhibitor may change in response to changing concentrations of CH_4 , NH_4^+ , and ionic strength.

The significant enhancement of CH_4 oxidation after amendment of the soil with sewage sludge (Table 1) demonstrated that full development of the methanotrophic community in the unamended soil must have been limited by a lack of an available nutrient or nutrients in the soil. Thus, augmentation of the soil with a mixed source of elements, such as anaerobically digested sewage sludge, removed the nutrient limitation and permitted further development of the methanotrophic community in the soil, thus stimulating the capacity to oxidize CH_4 . Amendment with K_2HPO_4 had no effect on CH_4 oxidation, which indicated that PO_4^{3-} was not limiting methanotrophy in the coarse sand. The lack of ionic inhibition of CH_4 oxidation in the sand cores by K_2HPO_4 addition probably reflected the much smaller molar concentration added compared with the NH_4NO_3 addition.

In conclusion, our data show that the soil microbial community adapts rapidly to the presence of elevated CH_4 concentrations and can effectively reduce emissions of CH_4 from the surface of landfill soil. The capacity for CH_4 oxidation in soil is related to the depth of O_2 penetration and to the nutrient status of the soil, which may regulate the extent to which the methanotrophic community can develop. The capacity of a soil to respond to the challenge of a CH_4 purge may be enhanced by amendment with a combined nutrient source, such as sewage sludge, which may be acting through removal of limitations

to development of the methanotrophic community. We are carrying out further experiments to determine the degree to which the types and amounts of organic soil amendments can regulate the capacity of a soil to oxidize CH_4 .

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