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Authors

Whalen, SC
Reeburgh, WS
Sandbeck, KA

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Rapid Methane Oxidation in a Landfill Cover Soil†

S. C. WHALEN, W. S. REEBURGH,* AND K. A. SANDBECK‡

Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99775-1080

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Methane oxidation rates observed in a topsoil covering a retired landfill are the highest reported ($45 \text{ g m}^{-2} \text{ day}^{-1}$) for any environment. This microbial community had the capacity to rapidly oxidize CH_4 at concentrations ranging from $<1 \text{ ppm}$ (microliters per liter) (first-order rate constant $[k] = -0.54 \text{ h}^{-1}$) to $>10^4 \text{ ppm}$ ($k = -2.37 \text{ h}^{-1}$). The physiological characteristics of a methanotroph isolated from the soil (characteristics determined in aqueous medium) and the natural population, however, were similar to those of other natural populations and cultures: the Q_{10} and optimum temperature were 1.9 and 31°C , respectively, the apparent half-saturation constant was 2.5 to $9.3 \mu\text{M}$, and 19 to 69% of oxidized CH_4 was assimilated into biomass. The CH_4 oxidation rate of this soil under waterlogged (41% [wt/vol] H_2O) conditions, $6.1 \text{ mg liter}^{-1} \text{ day}^{-1}$, was near rates reported for lake sediment and much lower than the rate of $116 \text{ mg liter}^{-1} \text{ day}^{-1}$ in the same soil under moist (11% H_2O) conditions. Since there are no large physiological differences between this microbial community and other CH_4 oxidizers, we attribute the high CH_4 oxidation rate in moist soil to enhanced CH_4 transport to the microorganisms; gas-phase molecular diffusion is 10^4 -fold faster than aqueous diffusion. These high CH_4 oxidation rates in moist soil have implications that are important in global climate change. Soil CH_4 oxidation could become a negative feedback to atmospheric CH_4 increases (and warming) in areas that are presently waterlogged but are projected to undergo a reduction in summer soil moisture.

Methane is a radiatively active trace gas that has shown an atmospheric concentration increase of $1\% \text{ year}^{-1}$ over the last decade (5). Greenhouse heating from CH_4 over the next ~ 50 years is expected to be about 25% that of CO_2 at current rates of atmospheric concentration increases (9, 28), so it is important to understand sources, sinks, and processes modulating atmospheric CH_4 .

Methane oxidation must be an important modulator of atmospheric CH_4 flux; roughly half of the organic carbon degraded anaerobically is converted to CH_4 , yet CH_4 release to the atmosphere represents only 0.5% of the total carbon turnover (14). However, CH_4 oxidation is generally not considered in climate change models (28). Methanotrophic bacteria have been isolated from diverse aerobic environments and are well characterized biochemically (2), but most of the information on their ecology derives from investigations in aquatic environments. Studies of rates and controls of CH_4 oxidation in natural and landfill soils are surprisingly few, despite fundamental physical differences between aquatic and terrestrial ecosystems.

We report results from a study of CH_4 oxidation by a topsoil microbial community from the Berkeley North Waterfront Park near San Francisco, Calif. This ~ 40 -ha park was constructed over a municipal landfill that has been retired since 1983. The landfill is presently covered with 1.5 to 3 m of soil in the area of sample collection. Low CH_4 concentrations were found in air immediately above the topsoil (D. Blake, personal communication), and our initial observations showed rapid rates of CH_4 consumption by this soil at the atmospheric concentration of 1.7 ppm (microliters per liter) (5). These findings suggested active CH_4 oxidation and led to experiments examining the CH_4 -oxidizing poten-

tial, the end products of CH_4 oxidation, and the depth, moisture, and temperature dependence of CH_4 oxidation for this microbial community. Further, we isolated a methanotroph from this soil and compared its physiological characteristics with published values for other pure cultures. Results reported here are typical for repeated experiments. Although landfills can be considered an end-member environment, the results from this study provide general information concerning controls on CH_4 oxidation in soils. Methane oxidation in soils is a poorly understood term in the global CH_4 budget that could become more important under projected future conditions of a warmer, dryer climate.

MATERIALS AND METHODS

Soil samples. Soil cores 7 to 12 cm in length were collected in plastic tubes (6.7-cm inner diameter, 2-mm wall) by cutting around the tube perimeter with a serrated knife as the tubes were inserted. Bulk samples were obtained from the 3- to 12-cm zone with a garden trowel after removal of the surface soil. Soils were sparsely covered with grass and consisted of sand mixed with brown and gray clays. A typical core had a pH of 5.4, a bulk density of 1.88 g cm^{-3} , a moisture content of 11% (wt/wt), and an organic content of 4% (wt/wt; loss on ignition at 550°C of oven dried samples). Freshly collected bulk soils and cores (enclosed in a coring tube) were placed in ~ 1 -liter Mason jars and shipped in coolers with frozen gel packs to our Fairbanks laboratory. Bulk samples of the 3- to 12-cm zone were combined after removal of stones and twigs and were stirred to form a homogeneous soil composite with a 9 to 11% moisture content. All samples were stored at 4°C .

Experiments were performed at 25°C (unless noted) in 1-liter Mason jars with lids fitted with a septum for syringe sampling of headspace gas. Soil cores were capped at the bottom, and the capped, tubed core was placed in the Mason jar. All experiments involved time-course measurement of the headspace CH_4 concentration.

Bacterial cultures. Topsoil slurries were made from $\sim 1 \text{ g}$ of soil in 10 ml of liquid mineral medium (41). A methan-

* Corresponding author.

† Contribution no. 836 from the Institute of Marine Science, University of Alaska, Fairbanks.

‡ Present address: Department of Microbiology, SC-42, University of Washington, Seattle, WA 98195.

otrophic bacterium was isolated by incubating the slurries for 1 month in a methane-air (1:1) atmosphere and streaking the supernatant onto medium solidified with 7.5% Gelrite agar. The pure culture was maintained at room temperature in the liquid mineral medium with a methane-air (3:1) headspace at 1 atm (101.29 kPa). Experiments on cultures were conducted at room temperature. Samples (8 to 9 ml) of exponential-phase culture ($\sim 7 \times 10^7$ cells ml⁻¹) were dispensed into 125-ml serum bottles and sealed with black butyl stoppers (Bellco Glass, Vineland, N.J.) and an aluminum crimp. Methane addition and headspace gas sampling were done with a syringe through the stopper. Cells in each bottle were enumerated with an electronic particle counter (Coulter Corp., Hialeah, Fla.).

Gas analysis. Headspace CH₄ concentrations in all experiments were determined by gas chromatography with a flame ionization detector (precision of <1% [39]). The mean of duplicate syringe samples is reported in most cases.

Radioactive methane and analysis of radioactivity. Microliter quantities of biogenically produced ¹⁴CH₄ (8) tracer (200 to 383 kBq; specific activity, 2,005 kBq mol⁻¹) were added with milliliter quantities of unlabeled CH₄ to jar or bottle headspaces to determine the products of CH₄ oxidation and their distribution with time. Headspace samples (10 cm³) from an experiment involving a core in a Mason jar were injected into an aluminum stripping-oxidation line (1) and carried in an He stream through Harvey traps (Harvey Instrument Corp., Hillsdale, N.J.) and a stainless steel combustion tube packed with CuO heated to 800°C. The ¹⁴CO₂ was trapped in 1 N NaOH before combustion, and the ¹⁴CH₄ was collected in a phenethylamine-based fluor (1) as ¹⁴CO₂ after combustion; radiocarbon recovery in the stripping-oxidation line was quantitative. The core was frozen to terminate the experiment, and the remaining headspace ¹⁴CH₄ and ¹⁴CO₂ were recovered with a series of He flushes. The ¹⁴C incorporated in the core was assayed by dry combustion of freeze-dried, homogenized subsamples in a Harvey Biological Material Oxidizer. Label recovered by dry combustion represents ¹⁴CH₄ assimilated into microbial biomass and inorganic matter. The inorganic fraction was probably small and was not assayed; the low soil pH (5.4) suggests that these soils are essentially carbonate free.

A pure culture of the isolated methanotroph was also used to assess the time course of product formation from ¹⁴CH₄ oxidation. Six serum vials containing the culture were amended with ¹⁴CH₄, and one randomly chosen bottle was analyzed at each of six selected time intervals to determine label distribution over time. The six observations included a procedural control (killed immediately after ¹⁴CH₄ addition; zero time), which was used in correcting subsequent samples for abiotic labeling of metabolic pools. The end products of ¹⁴CH₄ oxidation were determined as follows. A 2-ml sample of the culture was withdrawn with a syringe and filtered through a 25-mm (0.2- μ m pore diameter) Gelman PTFE filter. Particle counts indicated that this filter retained 99% of the cells. The filter was rinsed with 1 ml of distilled water, and ¹⁴C incorporated by bacterial cells was fractionated by a serial solvent extraction technique (24) into methanol-water-soluble, chloroform-soluble, hot trichloroacetic acid-soluble, and trichloroacetic acid-insoluble fractions. Following definitions published previously (24), we operationally define these fractions as low-molecular-weight metabolites, lipids, polysaccharides, and proteins, respectively, recognizing that no sequential extraction method perfectly fractionates each class of compounds. The 3 ml of filtrate plus rinse was acidified with 0.1 ml of 1 N H₂SO₄, and ¹⁴CO₂ and ¹⁴CH₄

were stripped with He. Radioactivity remaining in the filtrate plus rinse was dissolved organic ¹⁴C. The remainder of the culture was acidified with 0.25 ml of 1 N H₂SO₄ to terminate CH₄ oxidation. Radiolabeled CH₄ and CO₂ were recovered from the culture with the stripping-oxidation line described previously. A 3-ml sample of the culture was then assayed directly for ¹⁴C as an independent check of label recovery in the cellular fractions; radioactivity in dissolved organic carbon was subtracted from that of the 3-ml sample to estimate whole-cell incorporation. The sum of the metabolite fractions was 100.1% \pm 8.9% (\bar{x} \pm standard deviation) of the whole-cell assimilation after adjusting for differences in sample size, indicating full recovery of assimilated label during fractionation. The radioactivity of all samples was determined with a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.).

Equilibration and mass transfer of added CH₄. Interpretation of experiments involving amended atmospheres requires an understanding of the time scale of equilibration between soil and headspace for added gas. The equilibration time was evaluated for a natural core with a relaxation technique (34) that involved inhibiting CH₄ oxidation with C₂H₂ (3) and monitoring equilibration of added CH₄ (61 ppm, final concentration) until headspace concentration changes could not be resolved (6 min). The first-order relaxation (equilibration) constant for added CH₄ in this experiment was -0.70 min⁻¹. The highest first-order CH₄ oxidation rate constant in a natural core was -0.04 min⁻¹, indicating that equilibration of added CH₄ was much faster than consumption. Zero time gas samples were taken >15 min after CH₄ addition in all experiments to ensure complete equilibration of added CH₄.

We took the following measures to minimize mass transfer resistance during experiments and to enhance CH₄ equilibration before zero time headspace gas sampling. Experiments involving liquid cultures were conducted on a rotary shaker at 250 rpm, and zero time samples were taken >1 h after CH₄ addition. Slurries of composite soil samples were small (<80 cm³) to maximize contact between the liquid and gas phases in Mason jars. Slurries were hand shaken after CH₄ addition, and zero time samples were taken 1 h later. A volume ratio of >12:1 for headspace to liquid was maintained in all experiments to prevent significant depletion of headspace CH₄ during incubation.

Statistical analysis and calculations. The apparent half-saturation constant for CH₄ oxidation (K_s) and the maximum rate of CH₄ oxidation (V_{max}) were estimated by directly fitting a rectangular hyperbola to data for oxidation rate versus CH₄ concentration (7). Values of K_s are expressed as gas-phase concentrations (parts per million) and also as aqueous-phase concentrations (micromolar) by using Bunsen solubility coefficients (42). The Q_{10} value for CH₄ oxidation was calculated from the van't Hoff equation (35). Statistical analyses were as described by Zar (44). A significance level of $\alpha = 0.05$ was used for all tests.

RESULTS

Analysis of core segments showed that CH₄ oxidation was limited to the subsurface soil zones (Fig. 1). The 0- to 3-cm horizon of sectioned 12-cm cores showed no CH₄ oxidation, whereas deeper soil zones showed vigorous CH₄ oxidation. Beyond the consistent absence of activity in the surface layer, there was no correlation between soil depth and rate of CH₄ oxidation for several cores.

The substrate dependence of CH₄ oxidation was deter-

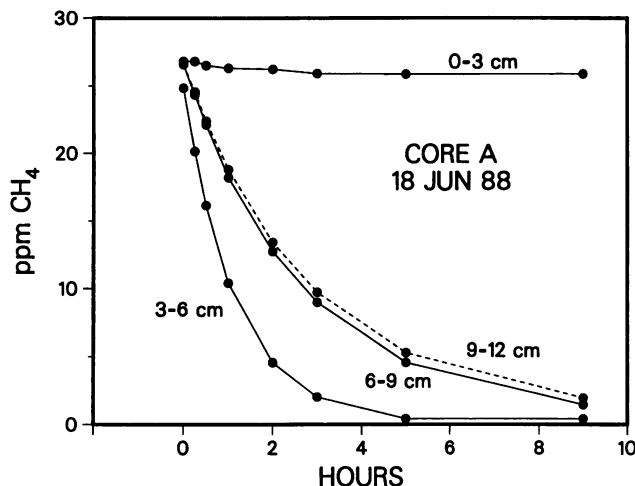


FIG. 1. Time course of CH₄ oxidation for 3-cm sections of a soil core in Mason jars; the headspace was adjusted to an initial CH₄ concentration of about 27 ppm.

mined in initial velocity experiments on a natural core, 75-g soil composites, and a culture. Ten or 11 samples of pure culture and composite soil were incubated with different initial headspace CH₄ concentrations, and the decrease in headspace CH₄ was determined in single endpoint experiments. One natural core was incubated with 15 different headspace CH₄ concentrations in single endpoint experiments. Residual CH₄ was purged from the jar headspace between experiments by equilibration with ambient air. Based on preliminary experiments, an incubation period of several minutes (core and soil composites) to 1 h (culture) was used to ensure a linear decrease in headspace CH₄ and good resolution of the CH₄ concentration decrease. Methane oxidation rates increased with increasing headspace CH₄ in all three experiments over the concentration range of 1.7 to >10⁴ ppm. Values for K_s ranged from 1,800 to 7,000 ppm of CH₄ (gas phase) or from 2.5 to 9.3 μM CH₄ (liquid phase) (Table 1). The V_{max} for CH₄ oxidation by a 12-cm core was 61 g m⁻² day⁻¹.

Soil cores oxidized CH₄ over a >10⁴-fold concentration range with no lag in response to added CH₄; core D oxidized CH₄ at headspace concentrations of 1.7 ppm (atmospheric level) and 7.7 × 10⁴ ppm in experiments separated by only 20 min (Fig. 2). The corresponding first-order rate constants for CH₄ oxidation were -0.54 and -2.37 h⁻¹. Methane oxidation by core D reduced the headspace CH₄ concentration to 0.5 ppm when the zero-time CH₄ concentration was 1.7 ppm. The headspace CH₄ concentration decreased to 0.2 ppm in similar 2-h experiments with other cores. Methane concentrations reached the analytical detection limit (0.1 ppm) in the headspace of cores allowed to run down for several hours. This suggests a threshold of <0.1 ppm for CH₄ oxidation by this microbial community. Substrate-saturated

TABLE 1. Kinetics of CH₄ oxidation by a fresh landfill soil core, a landfill soil composite, and an isolated methanotroph^a

Sample	K _s (ppm)	K _s (μM)	V _{max}
Core E	4,600 ± 1,600	6.2 ± 2.1	61 ± 10 g m ⁻² day ⁻¹
Composite	1,800 ± 500	2.5 ± 0.7	60 ± 7 μg g of soil ⁻¹ day ⁻¹
Culture	7,000 ± 2,500	9.3 ± 3.4	96 ± 18 fg cell ⁻¹

^a ±95% confidence intervals are shown.

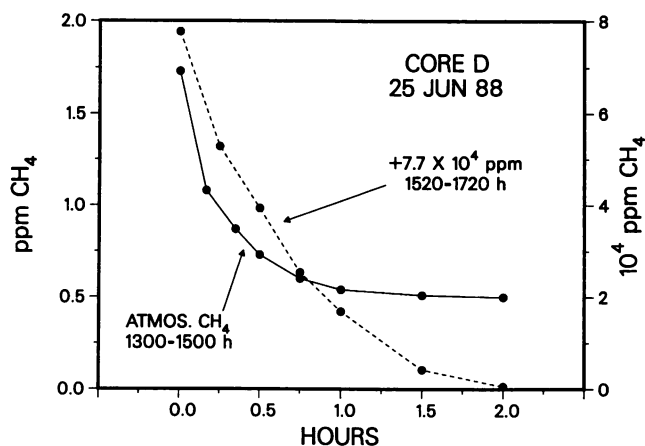


FIG. 2. Time courses for CH₄ oxidation by soil core D at zero-time headspace CH₄ concentrations of 1.7 and 7.7 × 10⁴ ppm.

CH₄ oxidation (zero-order consumption kinetics; indicated by linear time course for decrease in headspace CH₄) was not observed for any core; the highest headspace CH₄ concentration tested was 7.7 × 10⁴ ppm. The highest observed rate of CH₄ oxidation (unbiased by equilibration of added CH₄) was 45 g m⁻² day⁻¹ (75% of the V_{max} for core E; Table 1), between time = 10 min and time = 20 min when the core D headspace was amended with 7.7 × 10⁴ ppm of CH₄ (Fig. 2).

The products of CH₄ oxidation in core D (Fig. 2) were determined in a tracer experiment involving ¹⁴CH₄. The headspace of the jar containing the core was amended with CH₄ to give a zero-time concentration of 3.9 × 10⁴ ppm (6,700 μl of ¹²CH₄ plus 4.4 μl of ¹⁴CH₄) at the start of the 2-h time course. The experiment was conducted in the dark to prevent photosynthetic assimilation of ¹⁴CO₂. Figure 3 shows a decrease in headspace ¹⁴CH₄ accompanied by a steady increase in ¹⁴CO₂. The apparent increase in ¹⁴CH₄ between zero time and 15 min is due to a leak in the stripping line between the CO₂ and CH₄ traps when the zero-time sample was processed; collection of ¹⁴CO₂ was unaffected, but ¹⁴CH₄ was lost. The leak was sealed before analysis of the next sample. Nearly all (99.6%) of the ¹⁴CH₄ was

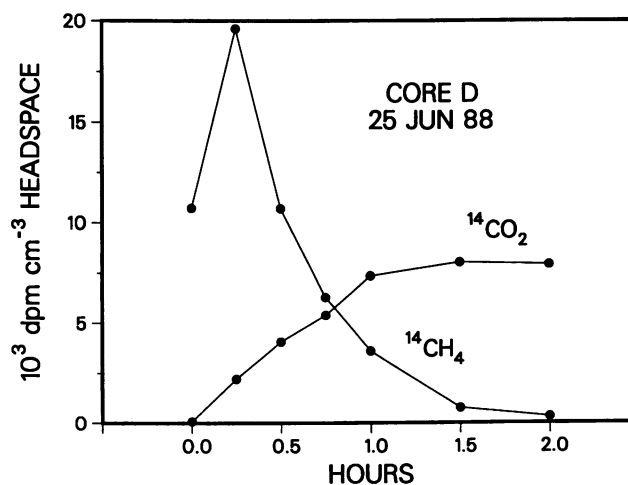


FIG. 3. Time course for ¹⁴CH₄ consumption and ¹⁴CO₂ evolution in the ¹⁴CH₄-amended headspace of a jar containing soil core D.

TABLE 2. Distribution $^{14}\text{CH}_4$ utilized by a landfill methanotroph isolate

Time (h)	% of $^{14}\text{CH}_4$ in:						
	Dissolved organic C	Respired CO_2	Cell biomass	Cell fractions			
				Polysaccharide	Protein	Lipid	LMW ^a
2.0	2.9	80.5	16.6	7	53	10	30
3.5	2.0	80.1	17.9	7	57	10	26
5.0	1.7	80.7	17.6	6	61	7	26
6.5	1.5	76.9	21.6	6	60	8	26
8.0	1.4	77.0	21.6	7	58	9	26
Mean	1.9	79.0	19.1	7	58	9	27

^a LMW, Low-molecular-weight compounds.

consumed within 2 h. We accounted for 86% of the added label; 31% of the recovered label was respired as $^{14}\text{CO}_2$, and 69% was incorporated into microbial biomass. The leak in the stripping line introduced a negligible error in the overall isotope budget.

The products of CH_4 oxidation were further fractionated in a culture experiment. Samples of the pure culture were amended to give a zero-time CH_4 concentration of 1.5×10^4 ppm (1,700 μl of $^{12}\text{CH}_4$ plus 2.2 μl of $^{14}\text{CH}_4$). We recovered $101.9\% \pm 1.5\%$ ($\bar{x} \pm$ standard deviation) of the added label. The data (not shown) indicate a continuous increase in $^{14}\text{CH}_4$ incorporated into cells, respired as CO_2 , and lost as dissolved organic carbon. The relative proportion of ^{14}C in each pool remained essentially constant throughout the time course (Table 2); $1.9\% \pm 0.5\%$ ($\bar{x} \pm$ standard deviation) was excreted as dissolved organic carbon, $79.0\% \pm 1.9\%$ was respired, and $19.1\% \pm 2.3\%$ was assimilated into cellular biomass. All intracellular pools showed a continuous increase in ^{14}C (data not shown). The relative ^{14}C content for each pool showed no time dependence (Table 2). Most of the cellular label was incorporated into protein ($58\% \pm 3\%$), followed by low-molecular-weight compounds ($27\% \pm 2\%$), lipids ($9\% \pm 1\%$), and polysaccharides ($7\% \pm 1\%$).

The effect of soil moisture on CH_4 oxidation was determined by adjusting soil moisture content and measuring the substrate-saturated CH_4 oxidation rate. The moisture content of 70-g composite soil samples (initially at 11% H_2O) was increased in 15% steps to 71% with a mist of distilled water or decreased to 5% by air drying with intermittent mixing. Samples were acclimated overnight to the changed moisture content, and headspace CH_4 was adjusted to a nominal concentration of 10.5×10^3 ppm. CH_4 oxidation was maximum at the soil moisture content of freshly collected samples, 11% (Fig. 4). All treatments gave linear decreases in headspace CH_4 concentration over 12 h. Zero-order CH_4 consumption (linearity) indicates that oxidation rates were moisture dependent rather than CH_4 dependent. Slopes were significant for all treatments (linear regression) but were not homogeneous (analysis of covariance) when pooled. Tukey's test for multiple comparisons indicated that CH_4 oxidation rates were not significantly different at 5, 41, 56, and 71% moisture content. However, these rates were significantly lower than the CH_4 oxidation rate at 26% soil moisture, which was significantly lower than the rate at 11%.

The effect of soil temperature on CH_4 oxidation was determined by adjusting soil temperature and measuring the substrate-saturated CH_4 oxidation rate. Composite soil samples (75 g, 11% moisture content) were acclimated for 2 h to a range of temperatures from 5 to 46°C (5°C increments), and headspace CH_4 was adjusted to a nominal concentration of

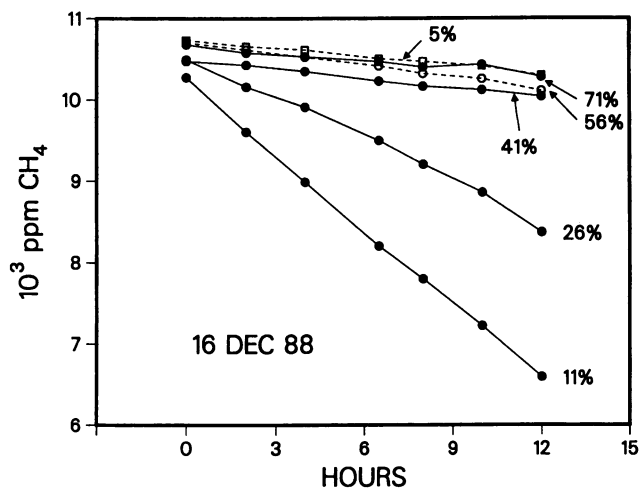


FIG. 4. Time course for substrate-saturated CH_4 oxidation by 70-g composite soil samples with different moisture contents (percentage by weight). Fresh soil was 11% H_2O .

9.5×10^3 ppm. The 12-h time course for headspace CH_4 concentration decrease was linear (zero order) for all treatments (Fig. 5A), indicating that CH_4 oxidation rates were temperature dependent. Linear regression showed significant, negative slopes for all temperatures except 46°C, where the slope was not statistically different from zero. Pooled slopes for data from 5 to 36°C were not homogeneous (analysis of covariance). Further analysis (Tukey's test) showed a significant, stepwise increase in the rate of CH_4 oxidation from 5 to 15°C. Methane oxidation rates at 15 and 20°C were similar and significantly lower than rates at 26°C. Rates of CH_4 oxidation were equal at 31 and 36°C and were significantly higher than rates at all other temperatures.

Methane oxidation rates were plotted against temperature to estimate the optimum temperature (T_{opt}) and Q_{10} for CH_4 oxidation (Fig. 5B). A third-order polynomial provided the best fit ($r^2 = 0.96$) to the data and gave a T_{opt} of 31°C for CH_4 oxidation. An exponential model was fitted ($r^2 = 0.89$) to the data, where CH_4 oxidation increased monotonically with increasing temperature (5 to 26°C). An average Q_{10} of 1.9 was calculated for CH_4 oxidation over this temperature range.

DISCUSSION

Physiological characteristics of natural and cultured methanotrophs are summarized in Table 3. The soil CH_4 -oxidizing community and isolated bacterium from this study are similar physiologically to communities and isolates from other environments. Our values of K_s for CH_4 oxidation are within the range of most values reported for field and laboratory experiments. The T_{opt} for CH_4 oxidation by the soil composite falls midway between values reported for lake waters and is similar to the T_{opt} for growth of *Methylococcus* spp. The average Q_{10} of 1.9 given here for the temperature range of 5 to 26°C has not been reported previously for CH_4 oxidation. The core D cell production efficiency from CH_4 oxidation (69%) compares best with the 73% efficiency reported in the only other soil study (27). This high carbon assimilation capacity from CH_4 oxidation in landfill topsoil agrees with the observation that cell synthesis from oxidized CH_4 is more efficient under CH_4 -replete conditions than under CH_4 -limiting conditions in lake sediment (25). The

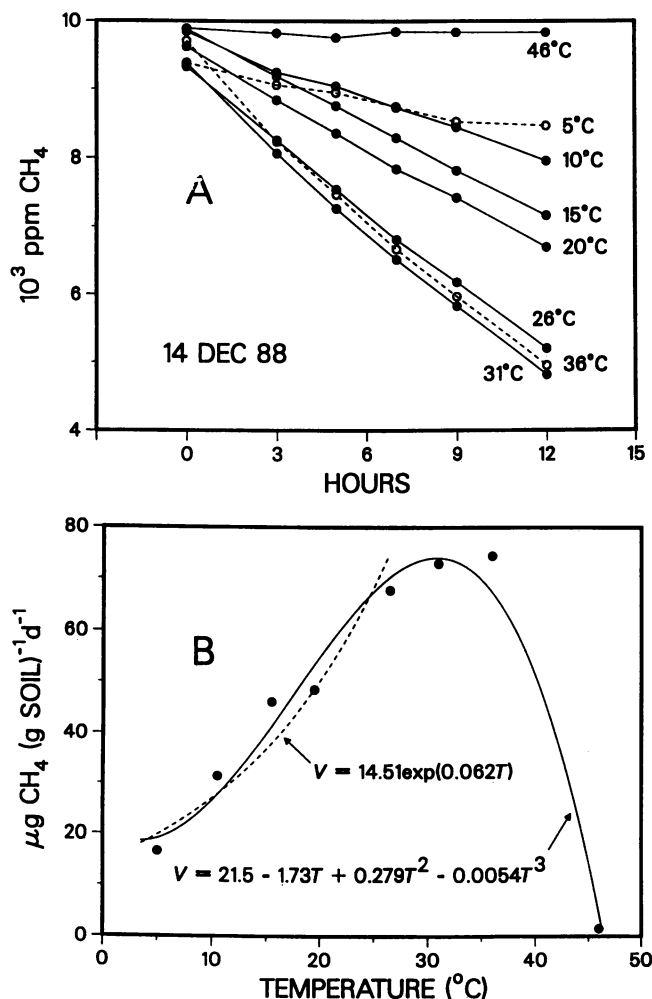


FIG. 5. (A) Time course for substrate-saturated CH_4 oxidation by 75-g composite soil samples at different temperatures. (B) Methane oxidation rate plotted versus temperature. Third-order polynomial ($n = 8$) and exponential functions ($n = 5$) are fitted to data from panel A.

fraction of oxidized CH_4 used for cell production in the culture experiment (19%) is only about a third of that assimilated by the soil core. Both experiments were reproducible, indicating that the difference was real. Growth conditions in the culture may be responsible; variability in conversion efficiency of CH_4 to biomass by cultured methanotrophs has been linked to the influence of growth conditions on methane monooxygenase (23).

Results of the ^{14}C culture experiment showed a constant proportion of label in each cell fraction (Table 2), pointing to an equilibrium time of <2 h for ^{14}C distribution among cell pools. The pattern of photosynthate allocation has been reported to differ in the log and stationary phases for cultured autotrophs (16), suggesting that relative label distribution here may be dependent on the culture growth stage.

What accounts for the high CH_4 -oxidizing capacity of this soil microbial community? Since the physiological properties of this natural population and isolated bacterium do not lie outside the envelope of values exhibited by other methanotrophs, we suggest a nonbiological explanation. Results from our moisture dependence experiment imply a physical

TABLE 3. Physiological characteristics of methane-oxidizing bacteria

Organism or environment	K_s (μM)	% of CH_4 in cellular biomass	T_{opt} ($^{\circ}\text{C}$)	Source or reference
Landfill soil, Berkeley, Calif.	2.5–6.2	69	31	This study
Landfill soil, isolated bacterium	9.3	19		This study
<i>Methylococcus</i> sp.			30–55 ^a	2
Lake 227, E.L.A., Ontario	4.7	30–60	25	31
Lake 227, E.L.A., Ontario		50		32
Lake Washington surface sediment	8.3–10.7	2–61		25
Lake Washington surface sediment	5.1–10.0			20
<i>Methylosinus trichosporium</i> OB3b	2			18
CH_4 -oxidizing coccus OU-4-1	0.8			18
CH_4 -oxidizing mixed culture	1.73			21
<i>Methylococcus</i> sp.	32			26
Mixed culture	44			26
Surface peat, West Virginia	45	15		43
Cultivated humisol	66.2	73		27
Water, Lake Mendota, Wis.		22	35	12
Lake Saqvaqujac, Canadian Arctic		34		38
Lake Tanganyika, Africa		26		30
Surface water, Cariaco Basin		9		37
Bering Sea water		2		11

^a T_{opt} values for growth of five species of *Methylococcus*; four of five values were in the range of 30 to 37°C.

explanation; they indicate that deviations from the optimum moisture content ($\sim 11\%$ H_2O) for CH_4 oxidation in this soil result in markedly reduced CH_4 oxidation rates. A decrease in soil moisture content from 11% to 5% significantly reduced the CH_4 oxidation rate in composite soil samples (Fig. 4). Figure 1 shows no CH_4 oxidation in the 0- to 3-cm horizon of a natural core (3% H_2O) and active oxidation in deeper soil zones (~ 7 to 13% H_2O). An increase in soil moisture above 11% also decreased the CH_4 oxidation rate in composite soil samples (Fig. 4) until soils were visibly waterlogged (41% H_2O). When soils were waterlogged (41 to 76% H_2O), CH_4 oxidation rates were independent of soil moisture and not significantly different. These observations suggest that a soil moisture content of 11% supports rapid gas-phase molecular diffusion of CH_4 to a maximum area of cell surface while preventing desiccation. The decrease in CH_4 oxidation rates as soil moisture is increased above 11% results from a change from the gas phase to aqueous molecular diffusion (10^4 -fold less rapid) for CH_4 transport to cells. No further decrease in the CH_4 oxidation rate was observed with increasing moisture content once soils were saturated, because CH_4 transport to CH_4 oxidizers was dominated by aqueous molecular diffusion.

Several lines of evidence suggest a fundamental similarity in the CH_4 -oxidizing capability of diverse communities. Some of this evidence relies on manipulation of the kinetic data for our pure culture (Table 1), and we caution that a microbe isolated under test conditions may not be representative of the entire community of methanotrophs. First, using kinetic parameters for the pure culture (Table 1) and representative data for surficial lake sediment ($2 \mu\text{M } \text{CH}_4$

[25]; 1-cm active zone [20]; 5×10^7 cells cm^{-3} [15]), we calculated for these soil microbes a CH_4 oxidation rate of about $0.2 \text{ g m}^{-2} \text{ day}^{-1}$, which falls between rates reported for sediment (0.004 to $0.007 \text{ g m}^{-2} \text{ day}^{-1}$ [10, 20]) and potential rates given for a peat bog (0.08 to $1.27 \text{ g m}^{-2} \text{ day}^{-1}$ [43]). Second, the V_{max} for our pure culture (Table 1) is in reasonable agreement with CH_4 oxidation rates of 2,000 and $60 \text{ fg cell}^{-1} \text{ h}^{-1}$, which we calculated from data for population estimates and CH_4 oxidation rates for bog sediment (13) and lake water (17). Third, although rates are dependent on cell numbers, the substrate-saturated CH_4 oxidation rate for waterlogged soil (Fig. 4; >26% moisture content) averaged $6.1 \text{ mg liter}^{-1} \text{ day}^{-1}$, comparable to the V_{max} s of 10.1 to $16.5 \text{ mg liter}^{-1} \text{ day}^{-1}$ reported for lake sediment (20, 25). Fourth, the 95% confidence intervals about the K_s for the pure culture (5.9 to $12.7 \mu\text{M CH}_4$) and core E (4.1 to $8.3 \mu\text{M CH}_4$) overlap (Table 1), indicating that they are not significantly different. Our K_s data suggest that the physiological characteristics of the isolated bacterium were, in fact, representative of the whole soil community responsible for rapid CH_4 oxidation at 11% soil moisture. The agreement between CH_4 oxidation rates observed (composite soil samples) and calculated (using K_s and V_m from the aqueous pure culture) here for waterlogged soil compared with CH_4 oxidation rates in lake sediments suggests that rapid rates are possible for nonwaterlogged lake sediments. Finally, the two end members of CH_4 -oxidizing communities in well-drained soils have a similar potential for CH_4 consumption. Moist tundra soils consuming atmospheric CH_4 (~ 1.7 ppm) as the sole CH_4 source had a threshold for CH_4 oxidation equal to that found here and showed first-order consumption kinetics over the 10^3 -fold range of CH_4 concentrations tested with no lag in response to CH_4 increases (40).

Enhanced CH_4 transport through air-filled soil pores increases CH_4 oxidation rates on a volume basis and an areal basis. The areal rate increase results from an increase in the vertical extent of the oxidized zone. The calculated V_{max} ($61 \text{ g of CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) and observed rate ($45 \text{ g m}^{-2} \text{ day}^{-1}$) of CH_4 oxidation for 12-cm cores is 10^4 -fold higher than the in situ CH_4 oxidation rates reported above for lake sediment with a <1-cm oxidized zone (10, 20) and >35-fold higher than potential oxidation rates given above for a peat bog with the water table at or near the soil surface (43).

Methane oxidation can be an important modulator of CH_4 flux from moist landfill topsoil to the atmosphere. Methane concentrations as high as 47% by volume in subsurface landfill soil (33) can easily support a CH_4 oxidation rate as high as the $45 \text{ g m}^{-2} \text{ day}^{-1}$ observed here, and higher rates can be attained with a >12-cm oxidized zone. Methane production in U.S. landfills is estimated to be 35 Tg of CH_4 year $^{-1}$ (per capita CH_4 generation rates in municipal and industrial waste [4]). We estimate CH_4 consumption in U.S. landfills to be 18 Tg of CH_4 year $^{-1}$ (landfill area, $2 \times 10^9 \text{ m}^2$ [36]; 200-day active period; V , $45 \text{ g of CH}_4 \text{ m}^{-2} \text{ day}^{-1}$). Thus, CH_4 oxidation can be 50% of CH_4 production in U.S. landfills.

Our observations that gas-phase molecular diffusion can rapidly transport CH_4 through moist, porous soils, that microbes can rapidly consume CH_4 over a > 10^4 -fold concentration range, and that methanotrophs capable of this CH_4 consumption rate may be widely distributed have important implications for the global CH_4 budget. Some computer climate models predict warmer, dryer summer conditions at high latitudes (reviewed in reference 28). High-latitude tundra accounts for 10% of the annual CH_4 release to the atmosphere from all sources (6) and stores 15%

of the global soil carbon (29). The summer temperature increase is projected to mobilize the large tundra carbon reservoir as CH_4 and CO_2 through increased methanogenesis and permafrost thawing and to provide a positive feedback to global warming (19, 22). However, CH_4 oxidation has not been considered in these predictions. Most tundra soils are presently waterlogged, so CH_4 transport to the consumption zone (the oxic-anoxic boundary near the water table) is by aqueous molecular diffusion. A lowered water table resulting from permafrost melting or decreased precipitation will increase the oxic, aerated soil zone, where CH_4 transport is by gas-phase molecular diffusion. We suggest that CH_4 oxidizers not only could consume CH_4 fluxes from tundra soils but also could consume atmospheric CH_4 , providing a negative feedback to CH_4 increases and warming. Moreover, the CO_2 produced by oxidizing CH_4 is 20-fold less effective as a greenhouse gas (5).

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