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## Rapid Methane Oxidation in a Landfill Cover Soil†

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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  $\text{CH}_4$  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  $31^\circ\text{C}$ , respectively, the apparent half-saturation constant was 2.5 to  $9.3 \mu\text{M}$ , and 19 to 69% of oxidized  $\text{CH}_4$  was assimilated into biomass. The  $\text{CH}_4$  oxidation rate of this soil under waterlogged (41% [wt/vol]  $\text{H}_2\text{O}$ ) 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%  $\text{H}_2\text{O}$ ) conditions. Since there are no large physiological differences between this microbial community and other  $\text{CH}_4$  oxidizers, we attribute the high  $\text{CH}_4$  oxidation rate in moist soil to enhanced  $\text{CH}_4$  transport to the microorganisms; gas-phase molecular diffusion is  $10^4$ -fold faster than aqueous diffusion. These high  $\text{CH}_4$  oxidation rates in moist soil have implications that are important in global climate change. Soil  $\text{CH}_4$  oxidation could become a negative feedback to atmospheric  $\text{CH}_4$  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  $\text{CH}_4$  over the next  $\sim 50$  years is expected to be about 25% that of  $\text{CO}_2$  at current rates of atmospheric concentration increases (9, 28), so it is important to understand sources, sinks, and processes modulating atmospheric  $\text{CH}_4$ .

Methane oxidation must be an important modulator of atmospheric  $\text{CH}_4$  flux; roughly half of the organic carbon degraded anaerobically is converted to  $\text{CH}_4$ , yet  $\text{CH}_4$  release to the atmosphere represents only 0.5% of the total carbon turnover (14). However,  $\text{CH}_4$  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  $\text{CH}_4$  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  $\text{CH}_4$  oxidation by a topsoil microbial community from the Berkeley North Waterfront Park near San Francisco, Calif. This  $\sim 40$ -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  $\text{CH}_4$  concentrations were found in air immediately above the topsoil (D. Blake, personal communication), and our initial observations showed rapid rates of  $\text{CH}_4$  consumption by this soil at the atmospheric concentration of  $1.7 \text{ ppm}$  (microliters per liter) (5). These findings suggested active  $\text{CH}_4$  oxidation and led to experiments examining the  $\text{CH}_4$ -oxidizing poten-

tial, the end products of  $\text{CH}_4$  oxidation, and the depth, moisture, and temperature dependence of  $\text{CH}_4$  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  $\text{CH}_4$  oxidation in soils. Methane oxidation in soils is a poorly understood term in the global  $\text{CH}_4$  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  $1.88 \text{ g cm}^{-3}$ , a moisture content of 11% (wt/wt), and an organic content of 4% (wt/wt; loss on ignition at  $550^\circ\text{C}$  of oven dried samples). Freshly collected bulk soils and cores (enclosed in a coring tube) were placed in  $\sim 1$ -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  $4^\circ\text{C}$ .

Experiments were performed at  $25^\circ\text{C}$  (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  $\text{CH}_4$  concentration.

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

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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<sup>-1</sup>) 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 CH<sub>4</sub> 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 <sup>14</sup>CH<sub>4</sub> (8) tracer (200 to 383 kBq; specific activity, 2,005 kBq mol<sup>-1</sup>) were added with milliliter quantities of unlabeled CH<sub>4</sub> to jar or bottle headspaces to determine the products of CH<sub>4</sub> oxidation and their distribution with time. Headspace samples (10 cm<sup>3</sup>) 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 <sup>14</sup>CO<sub>2</sub> was trapped in 1 N NaOH before combustion, and the <sup>14</sup>CH<sub>4</sub> was collected in a phenethylamine-based fluor (1) as <sup>14</sup>CO<sub>2</sub> after combustion; radiocarbon recovery in the stripping-oxidation line was quantitative. The core was frozen to terminate the experiment, and the remaining headspace <sup>14</sup>CH<sub>4</sub> and <sup>14</sup>CO<sub>2</sub> were recovered with a series of He flushes. The <sup>14</sup>C 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 <sup>14</sup>CH<sub>4</sub> 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 <sup>14</sup>CH<sub>4</sub> oxidation. Six serum vials containing the culture were amended with <sup>14</sup>CH<sub>4</sub>, 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 <sup>14</sup>CH<sub>4</sub> addition; zero time), which was used in correcting subsequent samples for abiotic labeling of metabolic pools. The end products of <sup>14</sup>CH<sub>4</sub> 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- $\mu$ 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 <sup>14</sup>C 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 H<sub>2</sub>SO<sub>4</sub>, and <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>CH<sub>4</sub>

were stripped with He. Radioactivity remaining in the filtrate plus rinse was dissolved organic <sup>14</sup>C. The remainder of the culture was acidified with 0.25 ml of 1 N H<sub>2</sub>SO<sub>4</sub> to terminate CH<sub>4</sub> oxidation. Radiolabeled CH<sub>4</sub> and CO<sub>2</sub> were recovered from the culture with the stripping-oxidation line described previously. A 3-ml sample of the culture was then assayed directly for <sup>14</sup>C 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 CH<sub>4</sub>.** 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 CH<sub>4</sub> oxidation with C<sub>2</sub>H<sub>2</sub> (3) and monitoring equilibration of added CH<sub>4</sub> (61 ppm, final concentration) until headspace concentration changes could not be resolved (6 min). The first-order relaxation (equilibration) constant for added CH<sub>4</sub> in this experiment was  $-0.70$  min<sup>-1</sup>. The highest first-order CH<sub>4</sub> oxidation rate constant in a natural core was  $-0.04$  min<sup>-1</sup>, indicating that equilibration of added CH<sub>4</sub> was much faster than consumption. Zero time gas samples were taken >15 min after CH<sub>4</sub> addition in all experiments to ensure complete equilibration of added CH<sub>4</sub>.

We took the following measures to minimize mass transfer resistance during experiments and to enhance CH<sub>4</sub> 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 CH<sub>4</sub> addition. Slurries of composite soil samples were small (<80 cm<sup>3</sup>) to maximize contact between the liquid and gas phases in Mason jars. Slurries were hand shaken after CH<sub>4</sub> 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 CH<sub>4</sub> during incubation.

**Statistical analysis and calculations.** The apparent half-saturation constant for CH<sub>4</sub> oxidation ( $K_s$ ) and the maximum rate of CH<sub>4</sub> oxidation ( $V_{max}$ ) were estimated by directly fitting a rectangular hyperbola to data for oxidation rate versus CH<sub>4</sub> 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 CH<sub>4</sub> 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 CH<sub>4</sub> oxidation was limited to the subsurface soil zones (Fig. 1). The 0- to 3-cm horizon of sectioned 12-cm cores showed no CH<sub>4</sub> oxidation, whereas deeper soil zones showed vigorous CH<sub>4</sub> oxidation. Beyond the consistent absence of activity in the surface layer, there was no correlation between soil depth and rate of CH<sub>4</sub> oxidation for several cores.

The substrate dependence of CH<sub>4</sub> oxidation was deter-

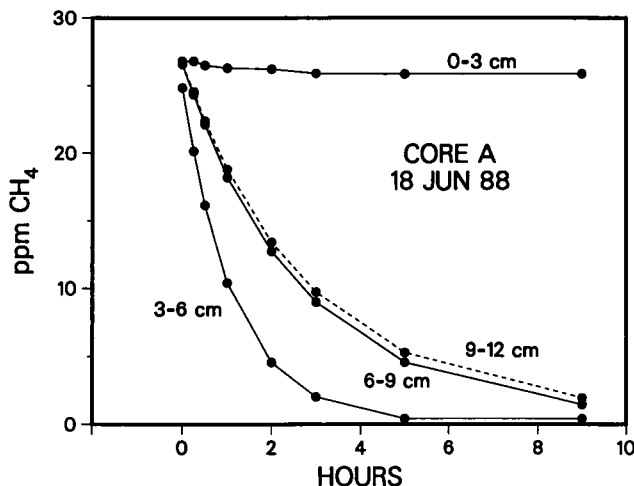


FIG. 1. Time course of CH<sub>4</sub> oxidation for 3-cm sections of a soil core in Mason jars; the headspace was adjusted to an initial CH<sub>4</sub> concentration of about 27 ppm.

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 CH<sub>4</sub> concentrations, and the decrease in headspace CH<sub>4</sub> was determined in single endpoint experiments. One natural core was incubated with 15 different headspace CH<sub>4</sub> concentrations in single endpoint experiments. Residual CH<sub>4</sub> 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 CH<sub>4</sub> and good resolution of the CH<sub>4</sub> concentration decrease. Methane oxidation rates increased with increasing headspace CH<sub>4</sub> in all three experiments over the concentration range of 1.7 to >10<sup>4</sup> ppm. Values for K<sub>s</sub> ranged from 1,800 to 7,000 ppm of CH<sub>4</sub> (gas phase) or from 2.5 to 9.3 μM CH<sub>4</sub> (liquid phase) (Table 1). The V<sub>max</sub> for CH<sub>4</sub> oxidation by a 12-cm core was 61 g m<sup>-2</sup> day<sup>-1</sup>.

Soil cores oxidized CH<sub>4</sub> over a >10<sup>4</sup>-fold concentration range with no lag in response to added CH<sub>4</sub>; core D oxidized CH<sub>4</sub> at headspace concentrations of 1.7 ppm (atmospheric level) and 7.7 × 10<sup>4</sup> ppm in experiments separated by only 20 min (Fig. 2). The corresponding first-order rate constants for CH<sub>4</sub> oxidation were -0.54 and -2.37 h<sup>-1</sup>. Methane oxidation by core D reduced the headspace CH<sub>4</sub> concentration to 0.5 ppm when the zero-time CH<sub>4</sub> concentration was 1.7 ppm. The headspace CH<sub>4</sub> 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 CH<sub>4</sub> oxidation by this microbial community. Substrate-saturated

TABLE 1. Kinetics of CH<sub>4</sub> oxidation by a fresh landfill soil core, a landfill soil composite, and an isolated methanotroph<sup>a</sup>

Sample	K <sub>s</sub> (ppm)	K <sub>s</sub> (μM)	V <sub>max</sub>
Core E	4,600 ± 1,600	6.2 ± 2.1	61 ± 10 g m <sup>-2</sup> day <sup>-1</sup>
Composite	1,800 ± 500	2.5 ± 0.7	60 ± 7 μg g of soil <sup>-1</sup> day <sup>-1</sup>
Culture	7,000 ± 2,500	9.3 ± 3.4	96 ± 18 fg cell <sup>-1</sup>

<sup>a</sup> ±95% confidence intervals are shown.

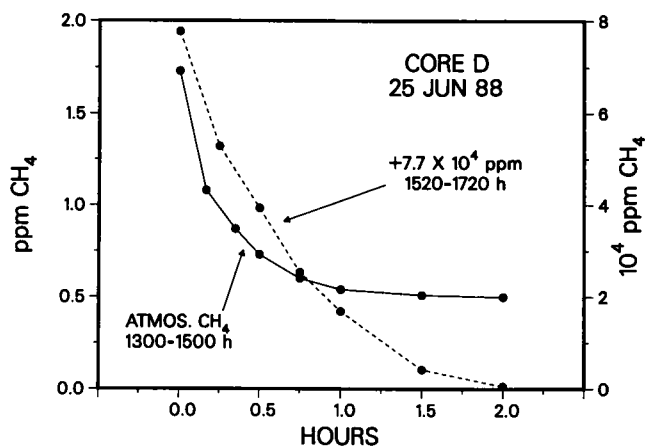


FIG. 2. Time courses for CH<sub>4</sub> oxidation by soil core D at zero-time headspace CH<sub>4</sub> concentrations of 1.7 and 7.7 × 10<sup>4</sup> ppm.

CH<sub>4</sub> oxidation (zero-order consumption kinetics; indicated by linear time course for decrease in headspace CH<sub>4</sub>) was not observed for any core; the highest headspace CH<sub>4</sub> concentration tested was 7.7 × 10<sup>4</sup> ppm. The highest observed rate of CH<sub>4</sub> oxidation (unbiased by equilibration of added CH<sub>4</sub>) was 45 g m<sup>-2</sup> day<sup>-1</sup> (75% of the V<sub>max</sub> for core E; Table 1), between time = 10 min and time = 20 min when the core D headspace was amended with 7.7 × 10<sup>4</sup> ppm of CH<sub>4</sub> (Fig. 2).

The products of CH<sub>4</sub> oxidation in core D (Fig. 2) were determined in a tracer experiment involving <sup>14</sup>CH<sub>4</sub>. The headspace of the jar containing the core was amended with CH<sub>4</sub> to give a zero-time concentration of 3.9 × 10<sup>4</sup> ppm (6,700 μl of <sup>12</sup>CH<sub>4</sub> plus 4.4 μl of <sup>14</sup>CH<sub>4</sub>) at the start of the 2-h time course. The experiment was conducted in the dark to prevent photosynthetic assimilation of <sup>14</sup>CO<sub>2</sub>. Figure 3 shows a decrease in headspace <sup>14</sup>CH<sub>4</sub> accompanied by a steady increase in <sup>14</sup>CO<sub>2</sub>. The apparent increase in <sup>14</sup>CH<sub>4</sub> between zero time and 15 min is due to a leak in the stripping line between the CO<sub>2</sub> and CH<sub>4</sub> traps when the zero-time sample was processed; collection of <sup>14</sup>CO<sub>2</sub> was unaffected, but <sup>14</sup>CH<sub>4</sub> was lost. The leak was sealed before analysis of the next sample. Nearly all (99.6%) of the <sup>14</sup>CH<sub>4</sub> was

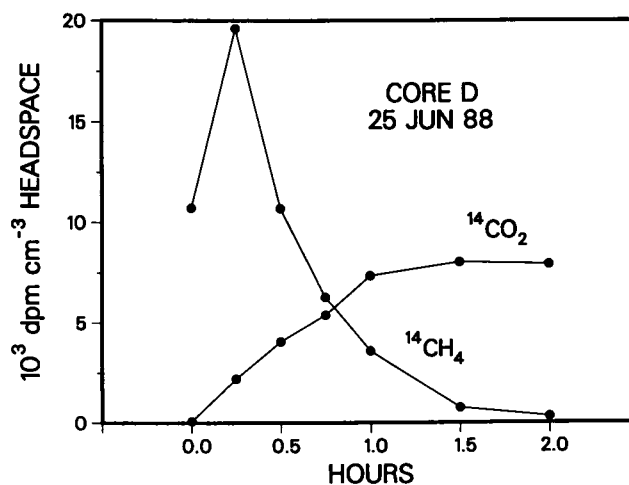


FIG. 3. Time course for <sup>14</sup>CH<sub>4</sub> consumption and <sup>14</sup>CO<sub>2</sub> evolution in the <sup>14</sup>CH<sub>4</sub>-amended headspace of a jar containing soil core D.

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 $\text{CO}_2$	Cell biomass	Cell fractions			
				Polysaccharide	Protein	Lipid	LMW <sup>a</sup>
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

<sup>a</sup> 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  $\text{CH}_4$  oxidation were further fractionated in a culture experiment. Samples of the pure culture were amended to give a zero-time  $\text{CH}_4$  concentration of  $1.5 \times 10^4$  ppm (1,700  $\mu\text{l}$  of  $^{12}\text{CH}_4$  plus 2.2  $\mu\text{l}$  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  $\text{CO}_2$ , and lost as dissolved organic carbon. The relative proportion of  $^{14}\text{C}$  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  $^{14}\text{C}$  (data not shown). The relative  $^{14}\text{C}$  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  $\text{CH}_4$  oxidation was determined by adjusting soil moisture content and measuring the substrate-saturated  $\text{CH}_4$  oxidation rate. The moisture content of 70-g composite soil samples (initially at 11%  $\text{H}_2\text{O}$ ) 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  $\text{CH}_4$  was adjusted to a nominal concentration of  $10.5 \times 10^3$  ppm.  $\text{CH}_4$  oxidation was maximum at the soil moisture content of freshly collected samples, 11% (Fig. 4). All treatments gave linear decreases in headspace  $\text{CH}_4$  concentration over 12 h. Zero-order  $\text{CH}_4$  consumption (linearity) indicates that oxidation rates were moisture dependent rather than  $\text{CH}_4$  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  $\text{CH}_4$  oxidation rates were not significantly different at 5, 41, 56, and 71% moisture content. However, these rates were significantly lower than the  $\text{CH}_4$  oxidation rate at 26% soil moisture, which was significantly lower than the rate at 11%.

The effect of soil temperature on  $\text{CH}_4$  oxidation was determined by adjusting soil temperature and measuring the substrate-saturated  $\text{CH}_4$  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  $\text{CH}_4$  was adjusted to a nominal concentration of

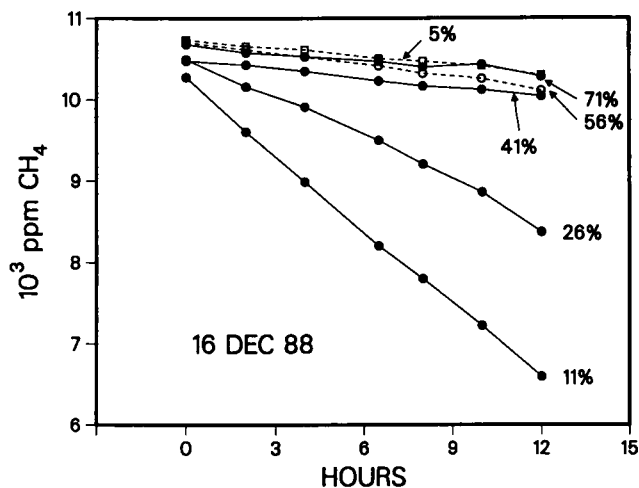


FIG. 4. Time course for substrate-saturated  $\text{CH}_4$  oxidation by 70-g composite soil samples with different moisture contents (percentage by weight). Fresh soil was 11%  $\text{H}_2\text{O}$ .

$9.5 \times 10^3$  ppm. The 12-h time course for headspace  $\text{CH}_4$  concentration decrease was linear (zero order) for all treatments (Fig. 5A), indicating that  $\text{CH}_4$  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  $\text{CH}_4$  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  $\text{CH}_4$  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 ( $T_{\text{opt}}$ ) and  $Q_{10}$  for  $\text{CH}_4$  oxidation (Fig. 5B). A third-order polynomial provided the best fit ( $r^2 = 0.96$ ) to the data and gave a  $T_{\text{opt}}$  of 31°C for  $\text{CH}_4$  oxidation. An exponential model was fitted ( $r^2 = 0.89$ ) to the data, where  $\text{CH}_4$  oxidation increased monotonically with increasing temperature (5 to 26°C). An average  $Q_{10}$  of 1.9 was calculated for  $\text{CH}_4$  oxidation over this temperature range.

## DISCUSSION

Physiological characteristics of natural and cultured methanotrophs are summarized in Table 3. The soil  $\text{CH}_4$ -oxidizing community and isolated bacterium from this study are similar physiologically to communities and isolates from other environments. Our values of  $K_s$  for  $\text{CH}_4$  oxidation are within the range of most values reported for field and laboratory experiments. The  $T_{\text{opt}}$  for  $\text{CH}_4$  oxidation by the soil composite falls midway between values reported for lake waters and is similar to the  $T_{\text{opt}}$  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  $\text{CH}_4$  oxidation. The core D cell production efficiency from  $\text{CH}_4$  oxidation (69%) compares best with the 73% efficiency reported in the only other soil study (27). This high carbon assimilation capacity from  $\text{CH}_4$  oxidation in landfill topsoil agrees with the observation that cell synthesis from oxidized  $\text{CH}_4$  is more efficient under  $\text{CH}_4$ -replete conditions than under  $\text{CH}_4$ -limiting conditions in lake sediment (25). The

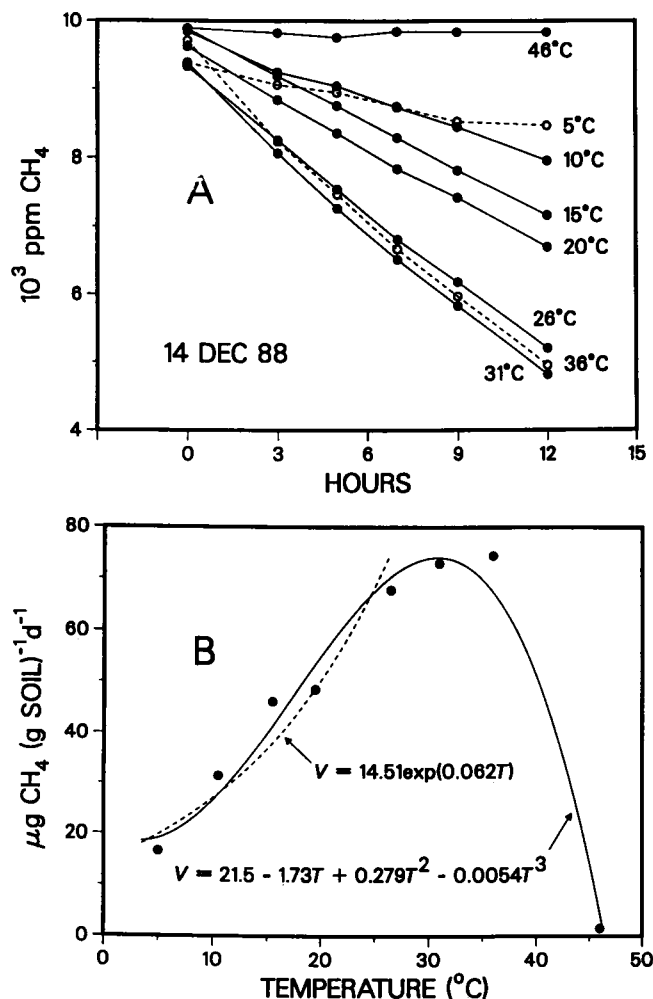


FIG. 5. (A) Time course for substrate-saturated  $\text{CH}_4$  oxidation by 75-g composite soil samples at different temperatures. (B) Methane oxidation rate plotted versus temperature. Third-order polynomial ( $n = 8$ ) and exponential functions ( $n = 5$ ) are fitted to data from panel A.

fraction of oxidized  $\text{CH}_4$  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  $\text{CH}_4$  to biomass by cultured methanotrophs has been linked to the influence of growth conditions on methane monooxygenase (23).

Results of the  $^{14}\text{C}$  culture experiment showed a constant proportion of label in each cell fraction (Table 2), pointing to an equilibrium time of  $<2$  h for  $^{14}\text{C}$  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  $\text{CH}_4$ -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	$K_s$ ( $\mu\text{M}$ )	% of $\text{CH}_4$ in cellular biomass	$T_{\text{opt}}$ ( $^{\circ}\text{C}$ )	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 <sup>a</sup>	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
$\text{CH}_4$ -oxidizing coccus OU-4-1	0.8			18
$\text{CH}_4$ -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

<sup>a</sup>  $T_{\text{opt}}$  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 ( $\sim 11\%$   $\text{H}_2\text{O}$ ) for  $\text{CH}_4$  oxidation in this soil result in markedly reduced  $\text{CH}_4$  oxidation rates. A decrease in soil moisture content from 11% to 5% significantly reduced the  $\text{CH}_4$  oxidation rate in composite soil samples (Fig. 4). Figure 1 shows no  $\text{CH}_4$  oxidation in the 0- to 3-cm horizon of a natural core (3%  $\text{H}_2\text{O}$ ) and active oxidation in deeper soil zones ( $\sim 7$  to 13%  $\text{H}_2\text{O}$ ). An increase in soil moisture above 11% also decreased the  $\text{CH}_4$  oxidation rate in composite soil samples (Fig. 4) until soils were visibly waterlogged (41%  $\text{H}_2\text{O}$ ). When soils were waterlogged (41 to 76%  $\text{H}_2\text{O}$ ),  $\text{CH}_4$  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  $\text{CH}_4$  to a maximum area of cell surface while preventing desiccation. The decrease in  $\text{CH}_4$  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  $\text{CH}_4$  transport to cells. No further decrease in the  $\text{CH}_4$  oxidation rate was observed with increasing moisture content once soils were saturated, because  $\text{CH}_4$  transport to  $\text{CH}_4$  oxidizers was dominated by aqueous molecular diffusion.

Several lines of evidence suggest a fundamental similarity in the  $\text{CH}_4$ -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 ( $2 \mu\text{M CH}_4$

[25]; 1-cm active zone [20];  $5 \times 10^7$  cells  $\text{cm}^{-3}$  [15]), we calculated for these soil microbes a  $\text{CH}_4$  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  $V_{\text{max}}$  for our pure culture (Table 1) is in reasonable agreement with  $\text{CH}_4$  oxidation rates of 2,000 and  $60 \text{ fg cell}^{-1} \text{ h}^{-1}$ , which we calculated from data for population estimates and  $\text{CH}_4$  oxidation rates for bog sediment (13) and lake water (17). Third, although rates are dependent on cell numbers, the substrate-saturated  $\text{CH}_4$  oxidation rate for waterlogged soil (Fig. 4; >26% moisture content) averaged  $6.1 \text{ mg liter}^{-1} \text{ day}^{-1}$ , comparable to the  $V_{\text{max}}$ 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  $\text{CH}_4$  oxidation at 11% soil moisture. The agreement between  $\text{CH}_4$  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  $\text{CH}_4$  oxidation rates in lake sediments suggests that rapid rates are possible for nonwaterlogged lake sediments. Finally, the two end members of  $\text{CH}_4$ -oxidizing communities in well-drained soils have a similar potential for  $\text{CH}_4$  consumption. Moist tundra soils consuming atmospheric  $\text{CH}_4$  (~1.7 ppm) as the sole  $\text{CH}_4$  source had a threshold for  $\text{CH}_4$  oxidation equal to that found here and showed first-order consumption kinetics over the  $10^3$ -fold range of  $\text{CH}_4$  concentrations tested with no lag in response to  $\text{CH}_4$  increases (40).

Enhanced  $\text{CH}_4$  transport through air-filled soil pores increases  $\text{CH}_4$  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  $V_{\text{max}}$  ( $61 \text{ g of CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ ) and observed rate ( $45 \text{ g m}^{-2} \text{ day}^{-1}$ ) of  $\text{CH}_4$  oxidation for 12-cm cores is  $10^4$ -fold higher than the in situ  $\text{CH}_4$  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  $\text{CH}_4$  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  $\text{CH}_4$  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  $\text{CH}_4$  year<sup>-1</sup> (per capita  $\text{CH}_4$  generation rates in municipal and industrial waste [4]). We estimate  $\text{CH}_4$  consumption in U.S. landfills to be 18 Tg of  $\text{CH}_4$  year<sup>-1</sup> (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,  $\text{CH}_4$  oxidation can be 50% of  $\text{CH}_4$  production in U.S. landfills.

Our observations that gas-phase molecular diffusion can rapidly transport  $\text{CH}_4$  through moist, porous soils, that microbes can rapidly consume  $\text{CH}_4$  over a > $10^4$ -fold concentration range, and that methanotrophs capable of this  $\text{CH}_4$  consumption rate may be widely distributed have important implications for the global  $\text{CH}_4$  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  $\text{CH}_4$  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  $\text{CH}_4$  and  $\text{CO}_2$  through increased methanogenesis and permafrost thawing and to provide a positive feedback to global warming (19, 22). However,  $\text{CH}_4$  oxidation has not been considered in these predictions. Most tundra soils are presently waterlogged, so  $\text{CH}_4$  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  $\text{CH}_4$  transport is by gas-phase molecular diffusion. We suggest that  $\text{CH}_4$  oxidizers not only could consume  $\text{CH}_4$  fluxes from tundra soils but also could consume atmospheric  $\text{CH}_4$ , providing a negative feedback to  $\text{CH}_4$  increases and warming. Moreover, the  $\text{CO}_2$  produced by oxidizing  $\text{CH}_4$  is 20-fold less effective as a greenhouse gas (5).

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