Shifts in Identity and Activity of Methanotrophs in Arctic Lake Sediments in Response to Temperature Changes

Ruo He,^{a,b} Matthew J. Wooller,^{c,d} John W. Pohlman,^e John Quensen,^f James M. Tiedje,^f and Mary Beth Leigh^b

Department of Environmental Engineering, Zhejiang University, Hangzhou, China^a; Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska, USA^b; Alaska Stable Isotope Facility, Water and Environmental Research Center, University of Alaska Fairbanks, Fairbanks, Alaska, USA^c; School of Fisheries and Ocean Sciences, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, Alaska, USA^d; U.S. Geological Survey, Woods Hole Coastal and Marine Science Center, Woods Hole, Massachusetts, USA^e; and Center for Microbial Ecology, Michigan State University, East Lansing, Michigan, USA^f

Methane (CH₄) flux to the atmosphere is mitigated via microbial CH₄ oxidation in sediments and water. As arctic temperatures increase, understanding the effects of temperature on the activity and identity of methanotrophs in arctic lake sediments is important to predicting future CH₄ emissions. We used DNA-based stable-isotope probing (SIP), quantitative PCR (Q-PCR), and pyrosequencing analyses to identify and characterize methanotrophic communities active at a range of temperatures (4°C, 10°C, and 21°C) in sediments (to a depth of 25 cm) sampled from Lake Qalluuraq on the North Slope of Alaska. CH₄ oxidation activity was measured in microcosm incubations containing sediments at all temperatures, with the highest CH₄ oxidation potential of 37.5 μmol g⁻¹ day⁻¹ in the uppermost (depth, 0 to 1 cm) sediment at 21°C after 2 to 5 days of incubation. Q-PCR of *pmoA* and of the 16S rRNA genes of type I and type II methanotrophs, and pyrosequencing of 16S rRNA genes in ¹³C-labeled DNA obtained by SIP demonstrated that the type I methanotrophs *Methylobacter*, *Methylomonas*, and *Methylosoma* dominated carbon acquisition from CH₄ in the sediments. The identity and relative abundance of active methanotrophs differed with the incubation temperature. Methylobacter were also abundant in the microbial community that derived carbon from CH₄, especially in the deeper sediments (depth, 15 to 20 cm) at low temperatures (4°C and 10°C), and showed a good linear relationship ($R = 0.82$) with the relative abundances of methanotrophs in pyrosequencing reads. This study describes for the first time how methanotrophic communities in arctic lake sediments respond to temperature variations.

Climate change is an important issue, with some of the most marked changes occurring in the Arctic (63). The mean annual temperature of permafrost in some areas of the Arctic has recently increased (59), and continued warming and thawing are predicted (36). Freshwater thermokarst lakes can form in topographic depressions as permafrost melts and are widely distributed in the Arctic, contributing significantly to the atmospheric methane (CH₄) budget (70, 71). CH₄ is an important greenhouse gas that is about 23 times more potent than carbon dioxide over a 100-year period (31). Increased thawing of permafrost as a result of warming in the Arctic could act as a positive feedback to climate warming through the release of carbon previously sequestered in permafrost (1, 60) as CH₄ emissions from arctic lakes (1, 70). If the frozen Pleistocene-age carbon in the Arctic were to be released during the next 500 to 1,000 years, the average CH₄ emission rate from lakes would be approximately 50 to 100 Tg year⁻¹ (70), which is 8 to 17% of the contemporary atmospheric CH₄ budget.

The net flux of CH₄ to the atmosphere is mitigated to some extent by CH₄ oxidation within the water column and sediments (41, 42, 67). Microbial CH₄ oxidation occurs aerobically and anaerobically. At the sediment-water interface in freshwater ecosystems, where CH₄ and oxygen gradients overlap, aerobic CH₄ oxidation is an important process in reducing CH₄ release to the atmosphere (4, 10, 21, 27).

Methanotrophic community structure and activity differ with temperature (12, 41, 49) as well as with other environmental conditions, such as the concentrations of CH₄ and O₂, pH, and nitrogen stress (27, 61). Temperature also influences the enzymatic activity of methanotrophs, including hydroxypyruvate reductase, hexulose phosphate synthase, formate dehydrogenase, and ribulose biphosphate carboxylase activities (45). Most metha-

notrophs available in pure culture are mesophiles, although psychrophilic methylobacter have been described (7, 51). Although active type I and type II methanotrophs have been found over a wide range of temperatures (67), in general, type II methanotrophs are favored at temperatures of > 15°C (47, 68) while type I methanotrophs prevail at low temperatures (0 to 10°C) (6, 26, 41, 69, 72, 73). However, type II methanotrophs—*Methylocella* species—have been found to predominate in acidic arctic tundra soils (5, 20). Some mesophilic methanotrophs can maintain viability with exposure to subzero temperatures, while psychrophilic and psychrotolerant methanotrophs can maintain growth and/or activity at low temperatures (67).

In the Arctic, ice on shallow ponds and lakes generally begins to melt in June and is absent between June and September (46). The average temperatures at the surfaces of the sediments in some shallow ponds and lakes in the Arctic have been shown to be very close to the average water temperatures. The mean daily water temperature is usually below 4°C but rises rapidly in June, with a seasonal peak at about 16 to 20°C in the first week of July (9, 18, 46). Moreover, most shallow arctic lakes are thermokarst lakes (70) and would be expected to undergo complete drainage once the underlying permafrost has thawed, enabling permeation of

Received 14 March 2012 Accepted 9 April 2012

Published ahead of print 20 April 2012

Address correspondence to Mary Beth Leigh, mbleigh@alaska.edu.

Supplemental material for this article may be found at <http://aem.asm.org/>.

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doi:10.1128/AEM.00853-12

water downward through the thaw bulbs (34). At that time, methanotrophs in the shallow, drained sediments in a drained landscape would have direct exposure to climate perturbations and greater depth of O₂ penetration. Because of the crucial role of methanotrophic activity in controlling CH₄ emissions (27, 61), describing how the activity and structure of methanotrophic communities respond to temperature fluctuations in Arctic lake sediments is important for predicting how future climate warming and permafrost thaw will influence CH₄ emissions from Arctic lakes. However, the response of active methanotrophs to temperature changes in arctic lakes has not been well studied, although these lakes are significant contributors to atmospheric CH₄ (70, 71).

We employed DNA-based stable-isotope probing (SIP), quantitative PCR (Q-PCR), and pyrosequencing analyses to identify and characterize the metabolically active methanotrophs in arctic lake sediments along a 25-cm sediment core sectioned at intervals of 0 to 1, 1 to 3, 3 to 5, 5 to 10, 10 to 15, 15 to 20, and 20 to 25 cm. We also tracked the activity and community structure of the active methanotrophs in sediments in response to three incubation temperatures (4°C, 10°C, and 21°C), representing a range above and below the peak annual temperatures recorded for some arctic lake sediments (about 16 to 20°C) (46).

MATERIALS AND METHODS

Study site and sampling. The study site, Lake Qalluuraq, is located on the North Slope of the Brooks Range in Alaska, about 136 m away from a site with continuous CH₄ ebullition (70°22.669'N, 157°20.925'W) (75). The depth, temperature, and dissolved oxygen (DO) concentration of water were about 170 cm, 16 to 17°C, and 10 mg liter⁻¹, respectively, when sampling was conducted in July 2009. Core tubes (length, 2.5 m; diameter, 7 cm) were used for the collection of sediment samples in depth increments of 1 to 2 cm within the top 5 cm of sediment and then every 5 cm for the remaining length of the sediment core. The sediment subsamples were immediately placed in plastic zipper freezer bags and were homogenized; then subsamples were frozen at -80°C for later molecular studies. Sediment samples in the plastic bags were kept at 4°C for CH₄ oxidation incubations and SIP experiments (described below). The particle composition of the experimental sediment mixture (depth, 0 to 25 cm) was as follows: at >2 mm, 0.6%; at 0.05 to 0.1 mm, 67.2%; at 0.05 to 0.002 mm, 22.2%; at <0.002 mm, 10%. The total carbon and total nitrogen contents of the sediments, measured every 5 cm, were 14.8% ± 3.1% and 0.9% ± 0.2%, respectively (means ± standard deviations for 5 samples).

For measurement of dissolved CH₄ concentrations, sediments were collected as 3-ml plugs at 2-cm intervals from a 20-cm core. The samples were transferred to 20-ml serum vials, sealed with 1-cm-thick septa, and stored at -20°C. The headspace hydrocarbon concentrations were in the vials was analyzed by gas chromatography-flame ionization detection (GC-FID) (52). Headspace concentrations were converted to aqueous concentrations by using the method of Hoehler et al. (29).

SIP microcosms and measurement of CH₄ oxidation potential. Incubations for SIP and for measurement of CH₄ oxidation potential were conducted in microcosms. Sediment microcosms were constructed by adding approximately 5 g (wet weight) of each subsample of the sediments to a sterile 60-ml glass serum vial that was crimp-sealed with a butyl rubber stopper. Each microcosm was injected with ¹³CH₄ (99 atom% ¹³C; Sigma-Aldrich) or CH₄ (99.5% pure, as a control) to 10% (vol/vol). Calculated by Henry's law and the van't Hoff equation to correct for the effect of temperature on CH₄ solubility (39), the theoretical saturated CH₄ concentration in the pore water of microcosm sediments was 151 μmol liter⁻¹ at the initial CH₄ concentration of 10% (vol/vol). In the shallow ponds and lakes in the Arctic, the mean daily water temperature is usually below 4°C but rises rapidly in June and experiences a seasonal peak at

about 16 to 20°C in the first week of July (9, 18, 46). Considering the seasonal temperatures of the arctic lakes, microcosms were incubated at 4°C, 10°C, or 21°C on a shaker (100 rpm). All treatments were performed in triplicate. The disappearance of CH₄ from the headspace was monitored using gas chromatography. Gas headspace samples (50 μl) were withdrawn from microcosms at intervals and were analyzed for residual CH₄ by using GC-FID during incubation.

CH₄ oxidation potential was measured at incubation times of 2 days, 5 days, and 10 days at 21°C, 4 days, 10 days, and 20 days at 10°C, and 5 days, 15 days, and 30 days at 4°C; assessed from the zero-order decrease in CH₄ concentration in the headspace of the serum vials within 12 to 72 h (35); and expressed as micromoles of CH₄ per gram (dry weight) per day. For SIP, after >90% of the initial CH₄ concentration had been oxidized, the serum vials were flushed with air, and the initial CH₄ concentration (10% [vol/vol]) was reestablished. The incubation was continued in the same manner until approximately 0.2 mmol ¹³CH₄ or CH₄ g (wet weight)⁻¹ was consumed (55 to 74 days at 21°C, 144 to 212 days at 10°C, and 212 to 248 days at 4°C), quantities reportedly required to obtain a visible ¹³C-labeled DNA band following ultracentrifugation of DNA from 1 g of soil (56). Sediment samples were then harvested and were frozen immediately at -80°C.

DNA extraction and separation of ¹³C-labeled DNA. DNA was extracted from about 0.5 g of the sediment subsamples subjected to SIP by using the Bio 101 Fast DNA Spin kit for soil (MP Biomedicals, Solon, OH). The equilibrium (isopycnic) density gradient centrifugation and fractionation procedures were modified from the method described by Leigh et al. (38) and used a cesium trifluoroacetate (CsTFA; GE Healthcare, United Kingdom) solution with a starting buoyant density (BD) of 1.60 g ml⁻¹. Samples of 3 μg DNA were combined with the CsTFA solution and were loaded into Beckman polyallomer Quick-Seal centrifuge tubes (13 by 51 mm), which were then sealed and spun in an NVT 100 rotor in an Optima L-100 XP ultracentrifuge (Beckman Coulter) at 134,260 × g and 25°C for about 70 h. Gradients were fractionated into 250-μl fractions by displacement with nuclease-free water by using a syringe pump at a flow rate of 500 μl min⁻¹. The BDs of fractions were measured gravimetrically by weighing aliquots of each fraction from a blank gradient, containing water in place of DNA, run in parallel. Sample DNA was precipitated from fractions with isopropanol overnight at -20°C; then pellets were washed twice with isopropanol and were resuspended in 20 μl nuclease-free water (Sigma-Aldrich).

The relative abundances of bacterial DNA in gradient fractions ranging in BD from 1.558 to 1.663 g ml⁻¹ were determined by Q-PCR as described by Leigh et al. (38). After the range of fractions containing ¹³C-labeled DNA or unlabeled DNA was identified, they were combined to compose compiled "heavy" fractions from each sample for subsequent molecular analyses.

Q-PCR analysis. Q-PCR of 16S rRNA genes of type I and type II methanotrophs and of the *pmoA* gene in the heavy fractions from ¹³C-labeled DNA and unlabeled control DNA was performed using primer sets U785F (3)/MethT1bR (74) and U785F/MethT2R (74) for 16S rRNA genes of type I and type II methanotrophs, respectively, and primer set A189F (30)/mb661R (17) for the *pmoA* gene. Q-PCR was conducted in 4 replicates of 15-μl reaction mixtures containing SYBR green master mix (Applied Biosystems, Foster City, CA), 4.5 pmol each primer, and 1 μl template. Thermal cycler conditions were as follows: an initial stage at 50°C for 2 min; denaturation at 95°C for 5 min; and 60 cycles of 95°C for 30 s, 58°C for 30 s, and either 72°C for 45 s for the *pmoA* gene or 72°C for 30 s for the 16S rRNA genes of type I and type II methanotrophs. Standards were made from 10-fold dilutions of linearized plasmids containing the same fragments of the 16S rRNA genes of type I and type II methanotrophs and the *pmoA* gene as the Q-PCR product that was cloned from amplified pure-culture DNA. The limit of detection by Q-PCR was about 10² copies per reaction for the *pmoA* gene and about 10³ copies per reaction for the 16S rRNA genes of type I and type II methanotrophs.

FIG 1 Sediment depth profile for dissolved CH₄ concentrations at the sampling site.

16S rRNA gene sequence analysis. Sequence analyses were performed on bar-coded 16S rRNA gene amplicons from ¹³C-labeled DNA by pyrosequencing. The primer set used consisted of 577F (5'-CGTATCGCCTCCTCGCGCCATCAG [bar code] AYTGGGYDTAAAGNG-3') and 926R (5'-CTATGCGCCTTGCCAGCCCGCTCAGCCGTCATTCMTTTRAGT-3') (with the sequencing adaptors in boldface). PCR was run using the same conditions as for terminal restriction fragment length polymorphism (T-RFLP) but with different primers. For each sample, 12 replicate 25-μl PCR mixtures were created to obtain abundant PCR products and to span variations in template and amplification bias. PCR products were then purified and concentrated using the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA). Products were gel purified using the QIAquick gel extraction kit (Qiagen Inc., Valencia, CA), cleaned again with the QIAquick PCR purification kit, and eluted with 20 μl of EB buffer (10 mM Tris-Cl, pH 8.5). Clean products were quantified using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and were mixed in equal amounts (20 ng each). Pyrosequencing was performed using Roche 454 GS FLX Titanium sequencing (454 Life Sciences, Branford, CT) at the Research Technology Support Facility at Michigan State University (East Lansing, MI).

Sequences were first trimmed of the primer region, and low-quality sequences were removed by the pyrosequencing pipeline of the Ribosomal Database Project (RDP). In our libraries, 41% to 66% of the sequences in the libraries (sequence libraries ranged from 2,961 to 4,474 reads in size) passed this filter (average length, 330 to 331 bp) and were assigned to taxonomic groups by RDP's Naïve Bayesian Classifier (confidence threshold, 80%). The only exception was the library obtained from ¹³C-labeled DNA (sediment depth, 0 to 1 cm) at 10°C, in which 23% of the 3,102 total library sequences passed the filter.

Nucleotide sequence accession number. The nucleotide sequences determined in this study have been deposited in the NCBI Short Read Archive under accession number [SRP005485](#).

RESULTS

In situ CH₄ concentrations. Dissolved CH₄ concentrations increase gradually from 1.5 μmol liter⁻¹ at the sediment-water interface to 861.3 μmol liter⁻¹ at a 19-cm sediment depth (Fig. 1). At the continuous CH₄ ebullition site in the lake reported here, dissolved CH₄ concentrations were less than 15 μmol liter⁻¹ from a sediment depth of 0 to 10 cm and increased to 1,334 μmol liter⁻¹ at a 25-cm sediment depth. In other parts of this lake, as well as in other lakes in Alaska in our study, dissolved CH₄ concentrations at a sediment depth of 1.5 cm were 109 to 505 μmol liter⁻¹, and the maximum measured dissolved CH₄ concentration was 2,062

μmol liter⁻¹ at a 25.5-cm sediment depth (28). The saturated CH₄ concentration, calculated by Henry's law and the van't Hoff equation, in the pore water in SIP microcosms was 151 μmol liter⁻¹ at the initial CH₄ concentration of 10% (vol/vol). In comparison, the CH₄ concentration in the pore water in the microcosms is within the range of ambient conditions.

CH₄ oxidation potential. CH₄ oxidation potential measurement compares the relative activities of the methanotrophic populations among samples from different environmental conditions (35). CH₄ oxidation potential was measured during SIP incubations conducted at 4°C, 10°C, and 21°C, with a headspace of CH₄ at an initial concentration of 10% (vol/vol) in air. Measurable CH₄ consumption was first detected after 2 days of incubation in the microcosms from a sediment depth of 0 to 10 cm incubated at 21°C. The highest CH₄ oxidation potential, 37.5 μmol g⁻¹ day⁻¹, was recorded in sediments collected from a depth of 0 to 1 cm (Fig. 2). Although CH₄ consumption was initially slow or undetectable in some microcosms (for example, in those from depths of 10 to 15 cm, 15 to 20 cm, and 20 to 25 cm), CH₄ consumption was observed in all the sediment microcosms at 21°C after incubation for 5 days. After incubation at 21°C and 10°C for 10 days and 20 days, respectively, the sediments from the depth of 10 to 25 cm showed higher CH₄ oxidation potentials, 47.2 to 53.6 μmol g⁻¹ day⁻¹ and 27.3 to 29.5 μmol g⁻¹ day⁻¹, than the upper sediments. The CH₄ oxidation potentials observed increased as the incubation temperature increased from 4°C to 21°C. In the 0- to 5-cm sediment depth range, the CH₄ oxidation potentials at 21°C (on day 5) were 0.6 to 1.6 times and 37.0 to 160.5 times greater than those at 10°C (on day 4) and 4°C (on day 5), respectively.

Identification of the CH₄-utilizing bacterial community. Q-PCR analyses of gradient fractions, indicating the relative abundance of bacterial DNA throughout the gradient, demonstrated that significant quantities of ¹³C-labeled DNA were present in the sediments incubated with ¹³CH₄ (see Fig. S1 in the supplemental material). Unlabeled control DNA from the sediment at 21°C had a peak indicating abundant DNA at a BD of 1.600 g ml⁻¹ (see Fig. S1d), and the DNA distributions in unlabeled controls at 4°C and 10°C were similar to those at 21°C (data not shown). Little or no bacterial DNA was detected in fractions with BDs higher than 1.619 g ml⁻¹ for unlabeled control DNA, while a second peak was present in labeled DNA, indicating the presence of ¹³C-labeled DNA (see Fig. S1a, b, and c). Based on the Q-PCR results, we combined the fractions with BDs of 1.619 to 1.643 g ml⁻¹ as compiled heavy fractions for each sample prior to downstream molecular analyses.

To characterize bacterial communities active in assimilating carbon from CH₄, we selected ¹³C-labeled DNA samples from the uppermost sediment (depth, 0 to 1 cm) and from deeper sediments (15 to 20 cm) incubated at the three temperatures (i.e., 4°C, 10°C, and 21°C) for pyrosequencing analyses. Because the *pmoA* genes (encoding a subunit of particulate methane monooxygenase) of some novel aerobic methanotrophs, including *Crenothrix polyspora*, and of members of the phylum *Verrucomicrobia* are not detectable by use of standard primers in PCR amplification (22, 33, 53, 66) and no *pmoA* gene is present in *Methylocella* species (19), we relied on bacterial 16S rRNA gene sequencing instead of *pmoA* genes for ¹³C-labeled DNA pyrosequencing analysis to ensure that we captured the diversity of active methanotrophs. Although the numbers of pyrosequencing reads ranged from 699 to 2,440 in the uppermost sediment and from 1,782 to 2,934 in the

FIG 2 CH₄ oxidation potentials of sediments at incubation temperatures of 4°C (a), 10°C (b), and 21°C (c).

deep sediment (Table 1), rarefaction curves showed a higher diversity in the uppermost sediment than in the deep sediment (Fig. 3). The relative abundances of different phylogenetic groups present in ¹³C-labeled DNA were determined based on the frequency of pyrosequencing reads associated with different taxa as determined using the RDP classifier (Table 1). The phylum most frequently detected in ¹³C-labeled DNA was *Proteobacteria*. However, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes* were also found within the bacterial community that derived carbon from CH₄. In addition, some pyrosequencing reads could not be assigned by the RDP classifier; 12.3 to 22.2% of pyrosequencing reads from the uppermost sediment were assigned to unclassified bacteria, while only about 1.4 to 1.7% of those from the deep sediment were unclassified.

Variation in metabolically active methanotrophs and methylophils with temperature. Methanotrophs are divided into two main taxonomic groups, type I and type II, based on their cell morphology, ultrastructure, phylogeny, and metabolic pathways (27, 44, 61). In our study, the 16S rRNA genes of type I methanotrophs and the *pmoA* gene were both detected by Q-PCR in ranges of 1.1×10^6 to 1.8×10^7 and 1.2×10^6 to 2.0×10^7 copies μl⁻¹, respectively, in all ¹³C-labeled DNA samples (Fig. 4). The 16S rRNA genes of type II methanotrophs were detected at 1.8×10^4 and 6.4×10^4 copies μl⁻¹ in the ¹³C-labeled DNA from sediments at depths of 1 to 3 cm at 10°C and 5 to 10 cm at 21°C, respectively, while they were below the limit of detection of 10^3 copies reaction⁻¹ in other ¹³C-labeled DNA. For all the heavy fractions from unlabeled control DNA, the 16S rRNA genes of type I and type II methanotrophs and the *pmoA* gene were below detection limits.

Pyrosequencing showed that the type I methanotrophs *Methylobacter*, *Methylomonas*, *Methylosoma*, and unclassified *Methylococcaceae* and the type II methanotroph *Methylocystis* were all

present in the ¹³C-labeled DNA fractions (Fig. 5). Compared with those of type II methanotrophs, type I methanotroph sequences were more frequently detected in the ¹³C-labeled DNA pyrosequencing libraries. *Methylocystis*, the dominant type II methanotroph, was found mainly in the uppermost sediment (depth, 0 to 1 cm), with a relative abundance of 0.5% of pyrosequencing reads for ¹³C-labeled DNA from the sediment subjected to SIP that was incubated at 4°C, and with higher relative abundances at higher temperatures: 1.4% at 10°C and 2.5% at 21°C. In the deep sediment (depth, 15 to 20 cm), *Methylocystis* constituted a smaller proportion (0 to 1.1%) of the active bacterial community involved in CH₄ oxidation.

Type I methanotrophs were more abundant in the deep sediments (depth, 15 to 20 cm). *Methylomonas* was dominant in the uppermost sediment incubated at 10°C and 21°C. *Methylosoma* was found mainly in the sediment incubated at 10°C, while *Methylobacter* was dominant in the deep sediment and the sediment incubated at 4°C, especially in the deep sediment at 4°C, where it accounted for 39.7% of the total. In addition, we also detected *Verrucomicrobia* in the ¹³C-labeled DNA. However, our sequences belonging to the phylum *Verrucomicrobia* did not have high similarity to the three new *Verrucomicrobia* isolates identified as CH₄ oxidizers (22, 33, 53); rather, they were classified as members of the genus *Opitut*, subdivision 3 genera *incertae sedis* and the genus *Prostheobacter* and as unclassified *Verrucomicrobiaceae* and unclassified *Opitutaceae* by RDP's Naïve Bayesian Classifier (confidence threshold, 80%).

In addition to methanotrophs, *Methylophilus*, a known obligate methylophilus that uses methanol as the sole source of carbon and energy, was found to be abundant in ¹³C-labeled DNA from the deep sediment, particularly at low temperatures. The relative abundance of *Methylophilus* in the ¹³C-labeled DNA from the uppermost sediment at 4°C was about 13.2%, but it dropped to 4.6% and 0.4% at 10°C and 21°C, respectively. In the deep sediment, the

TABLE 1 Relative abundances of pyrosequencing reads from ^{13}C -labeled DNA assigned to different taxa by using the Ribosomal Database Project classifier

Phylogenetic affiliation ^a	% (no.) of pyrosequencing reads ^b from ^{13}C -labeled DNA at:					
	4°C		10°C		21°C	
	0-1 cm	15-20 cm	0-1 cm	15-20 cm	0-1 cm	15-20 cm
<i>Proteobacteria</i>						
<i>Alphaproteobacteria</i>						
<i>Methylocystaceae</i>						
<i>Methylocystis</i> (type II)	0.5 (12)	0 (1)	1.4 (10)	1.1 (19)	2.5 (30)	0.2 (4)
Unclassified <i>Methylocystaceae</i>	— ^c	—	0.3 (2)	0.1 (2)	0.2 (3)	—
<i>Hyphomicrobiaceae</i> , <i>Hyphomicrobium</i> ^d	—	—	2.0 (14)	0.3 (5)	4.2 (51)	0.3 (8)
Other <i>Alphaproteobacteria</i>	6.1 (149)	11.1 (325)	6.6 (46)	10.8 (193)	13.5 (164)	15.2 (359)
<i>Betaproteobacteria</i>						
<i>Methylophilales</i>						
<i>Methylophilus</i> ^d	13.2 (322)	20.0 (586)	4.6 (32)	26.2 (467)	0.4 (5)	9.8 (230)
Unclassified <i>Methylophilaceae</i> ^d	0.3 (7)	0.2 (5)	0.1 (1)	1.7 (31)	—	6.6 (156)
<i>Burkholderiales</i> , <i>Methylibium</i> ^d	0.8 (19)	0 (1)	—	—	0.2 (3)	0.1 (2)
Other <i>Betaproteobacteria</i>	7.7 (188)	16.2 (474)	31.8 (222)	7.5 (133)	17.9 (217)	19.1 (449)
<i>Gamma</i> <i>proteobacteria</i>						
<i>Methylococcaceae</i>						
<i>Methylobacter</i> (type I)	8.7 (212)	39.7 (1166)	1.7 (12)	5.9 (106)	0.4 (5)	21.6 (509)
<i>Methylomonas</i> (type I)	0.4 (10)	—	4.0 (28)	0.1 (1)	5.2 (63)	0.3 (6)
<i>Methylsoma</i> (type I)	0.2 (4)	—	5.4 (38)	25.3 (450)	—	—
Unclassified <i>Methylococcaceae</i> (type I)	0.4 (9)	0.1 (2)	0.4 (3)	0.7 (12)	0.6 (7)	—
Other <i>Gamma</i> <i>proteobacteria</i>	6.6 (161)	1.0 (29)	6.4 (45)	1.7 (31)	7.6 (92)	2.7 (64)
<i>Deltaproteobacteria</i>	9.6 (234)	1.8 (52)	7.7 (54)	0.3 (5)	10.8 (131)	0.8 (18)
Unclassified <i>Proteobacteria</i>	0.8 (19)	0.7 (22)	1.1 (8)	0.8 (15)	2.9 (35)	0.6 (13)
<i>Actinobacteria</i>	1.4 (35)	3.3 (96)	1.6 (11)	4.9 (88)	1.2 (14)	6.4 (150)
<i>Bacteroidetes</i>	4.5 (109)	0.6 (19)	3.4 (24)	3.0 (53)	3.1 (37)	5.2 (122)
<i>Acidobacteria</i>	3.5 (86)	0.1 (4)	2.9 (20)	0.4 (8)	9.1 (110)	0.5 (11)
<i>Verrucomicrobia</i>	5.7 (138)	0.1 (4)	2.3 (16)	0.5 (9)	0.8 (10)	1.7 (41)
<i>Planctomycetes</i>	3.5 (85)	1.5 (43)	1.3 (9)	4.7 (84)	1.6 (19)	4.8 (113)
Unclassified bacteria	22.2 (542)	1.4 (40)	12.3 (86)	1.8 (32)	14.1 (171)	1.7 (40)
Other	4.1 (99)	2.2 (65)	2.6 (18)	2.1 (38)	3.6 (44)	2.5 (60)
Total	100 (2,440)	100 (2,934)	100 (699)	100 (1,782)	100 (1,211)	100 (2,355)

^a "Type I" and "type II" stand for type I and type II methanotrophs.^b The numbers of pyrosequencing reads were assigned using the RDP classifier (80% confidence threshold).^c —, no pyrosequencing reads assigned.^d Methylophilus. **FIG 3** Rarefaction curves for the numbers of pyrosequencing reads and operational taxonomic units (OTUs) (at 3% sequencing dissimilarity).

relative abundance of *Methylophilus* reached 20 to 26.2% at low temperatures (4°C and 10°C). In addition to *Methylophilus*, unclassified *Methylophilaceae*, *Methylibium*, and *Hyphomicrobium* (14, 32) were also present in the ^{13}C -labeled DNA. At lower incubation temperatures (4°C), the relative abundance of methylo-trophs in the ^{13}C -labeled DNA from the uppermost sediment increased slightly over that at 21°C. We observed a strong correlation ($R = 0.82$) between the relative abundances of methanotrophs and methylo-trophs in pyrosequencing reads of ^{13}C -labeled DNA (Fig. 6).

DISCUSSION

In freshwater sediments, the activity of methanotrophs is largely restricted to a narrow layer at the oxic-anoxic interface (4, 10, 21, 27). In our study, which used a 10% (vol/vol) CH_4 concentration in the headspace during incubations, CH_4 consumption was observed at all depth intervals tested (down to a sediment depth of 25 cm) and at all three incubation temperatures (4°C, 10°C, and

FIG 4 Q-PCR of the 16S rRNA genes of type II (open bars) and type I (hatched bars) methanotrophs and *pmoA* (filled bars) in ^{13}C -labeled DNA from sediments subjected to SIP. The limit of detection by Q-PCR was about 10^2 copies per reaction for the *pmoA* gene and about 10^3 copies per reaction for the 16S rRNA genes of type I and type II methanotrophs. (a) 4°C; (b) 10°C; (c) 21°C.

21°C) (Fig. 2), indicating that the potential for methanotrophy exists down to a sediment depth of at least 25 cm in arctic lakes. Since aerobic methanotrophs cannot be metabolically active in the absence of O_2 , the methanotrophs from the deeper anoxic sediment layers might be dormant as cysts or exospores *in situ* (8) and could have become active during the aerobic incubations (57).

The CH_4 oxidation potentials in the sediment samples increased with increasing incubation temperature from 4°C to 21°C. A greater increase in the CH_4 oxidation potential occurred from 10°C to 21°C than from 4°C to 10°C, indicating that many of the active methanotrophs in the arctic lake sediments might be psychrotolerant (growing at <0 to $\leq 35^\circ\text{C}$, with an optimal temperature of $\leq 25^\circ\text{C}$) rather than psychrophilic (growing at <0 to $\leq 20^\circ\text{C}$, with an optimal temperature of $\leq 15^\circ\text{C}$) (50, 58). Similar results were obtained from analyses of the active-layer soils below the surface (top 10 cm) from Ellesmere Island in the Canadian high Arctic, where the active methanotrophs were psychrotolerant rather than psychrophilic (43). In permafrost from Lena Delta, Siberia, the methanotrophic communities in the upper layers were also dominated by psychrotolerant organisms or a mixed community of mesophiles and psychrotolerant organisms, while those

FIG 5 Relative abundances of known methanotrophs and methylotrophs within sediment microbial populations that derived carbon from CH_4 during SIP, based on the abundances of 16S rRNA gene sequences detected in ^{13}C -labeled DNA by use of pyrosequencing. Taxonomic assignments were made using the classifier of the Ribosomal Database Project (confidence threshold, 80%). Sediments subjected to SIP were analyzed after about $0.2 \text{ mmol } ^{13}\text{CH}_4 \text{ g}^{-1}$ (wet weight) was consumed at incubation temperatures of 4°C, 10°C, and 21°C. (a) Methanotrophs; (b) methylotrophs.

close to the permanently frozen ground (30 to 54 cm) were dominated by psychrophiles (41).

Q-PCR of 16S rRNA genes of type I and type II methanotrophs and of *pmoA* genes, and pyrosequencing of 16S rRNA genes in the heavy fractions (^{13}C -labeled DNA), demonstrated that type I methanotrophs were more abundant and active than type II methanotrophs in the sediments (Table 1; Fig. 4 and 5). Approximately 95.1 to 100% of methanotrophs active at 4°C were assigned to type I methanotrophs. In Lena Delta, Siberia, type I methanotrophs were also more abundant than type II methanotrophs throughout the permafrost active layer of 45 cm or 49 cm according to phospholipid fatty acid (PLFA) analysis (69). In

FIG 6 Correlation between relative abundances of methanotrophs and methylotrophs within the ^{13}C -labeled DNA from sediments (depths, 0 to 1 and 15 to 20 cm) subjected to SIP, based on pyrosequencing reads classified with the classifier of the Ribosomal Database Project (confidence threshold, 80%).

the active-layer soils from Eureka Island in the Canadian high Arctic, Q-PCR of 16S rRNA genes also showed the dominance of type I methanotrophs over type II methanotrophs in the native soil samples, and all methanotrophic 16S rRNA and *pmoA* gene sequences found in the heavy fractions were related to the type I methanotrophs *Methylosarcina* and *Methylobacter*, while no sequence related to type II methanotrophs was identified (43). Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA and *pmoA* genes showed that the type I methanotrophs *Methylobacter* and *Methylosarcina* were dominant in an arctic permafrost active layer of the Lena Delta, Siberia (40). DGGE of the 16S rRNA gene and RNA-SIP also indicated that *Methylobacter tundripaludum* dominated in soil samples and enrichments from Svalbard (26, 72, 73).

Methylocystis was the only type II methanotroph detected in our sediment samples. The increase in the relative abundance of *Methylocystis* with an increase in temperature from 4°C to 21°C indicated that high temperatures favored the growth of these type II methanotrophs. Similar results have been obtained for landfill cover soils, where it was found that type I methanotrophs were more dominant at 10°C than at 20°C, while levels of type II methanotrophs were highly elevated only at 20°C (6). In our study, not only did the relative abundances of type I and type II methanotrophs change with temperature, but the composition of the type I methanotrophic community was influenced by temperature. This was most likely due to differences in relative optimum temperatures (2, 27, 54, 62, 65). Microorganisms in permafrost are primarily cold adapted, including psychrophilic and psychrotolerant organisms; very few mesophilic or thermophilic isolates have been identified (25, 41, 64, 67). Based on the relative abundance of methanotrophs in our pyrosequencing reads, we hypothesize that *Methylosoma* in the sediment was psychrophilic, with an optimum temperature of about 10°C. *Methylomonas* was likely psychrotolerant or mesophilic, with a decrease in activity and growth at 4°C relative to 21°C. *Methylobacter* was likely psychrotolerant, as evidenced by substantial growth at 21°C and 4°C.

In addition to methanotrophs, we found that methylotrophs, especially *Methylophilus*, were active in the acquisition of carbon originally derived from CH₄. The relative abundances of methanotrophs, including both type I and type II methanotrophs, correlated significantly with those of methylotrophs, including *Methylophilus*, unclassified *Methylophilaceae*, *Methylibium*, and *Hyphomicrobium* (Fig. 6) ($R = 0.82$). Methanol is the first compound produced by methanotrophs during aerobic CH₄ oxidation (27). With a high concentration of CH₄ and extended incubation times, SIP techniques can lead to significant cross-feeding of ¹³C into secondary populations (44, 55). Methylotrophs have been widely detected in SIP studies targeting methanotrophic bacteria (11, 26, 42, 43). Cébron et al. (11) suggested that an abundance of methylotrophs such as *Methylophilus*, *Methylovorus*, *Aminomonas*, and *Hyphomicrobium* in 16S rRNA gene libraries from SIP heavy fractions might result from the incorporation of methanol produced by ¹³CH₄ oxidation. Martineau et al. (43) considered that it was possible that methylotrophs might have acquired carbon by direct utilization of ¹³CH₄ through some previously unknown pathway, because the diversity of methanotrophs is broader than what has generally been reported (15). In our study, methylotrophs were more dominant in ¹³C-labeled DNA libraries from the deep (15- to 20-cm) sediment, accounting for 20.1 to 27.9% of pyrosequencing reads at low temperatures of

10°C and 4°C, than in those from the uppermost (0- to 1-cm) sediment at 21°C. There may be characteristics of deep sediments or their communities that are associated with the interruption of methanotrophic enzymatic reactions, due to the presence of mutations or inhibitors, such as NaCl, EDTA, and HCOONa, or due to gas composition, leading to an excess of methanol that accumulates extracellularly (16, 23, 24, 37). *Methylosoma* has also been reported to produce formaldehyde that accumulates extracellularly, which might provide ¹³C for methylotrophs (48). In addition, because of the *in vitro* SIP incubations, these methylotrophs could have derived carbon through cross-feeding, in which non-methanotrophs incorporate ¹³C into their DNA through the metabolism of by-products, such as ¹³CO₂ or organic matter, derived from methanotrophs (44, 55). It is challenging to determine whether these methylotrophs in ¹³C-labeled DNA incorporated ¹³C directly or indirectly from ¹³CH₄. Additional studies are required for understanding of the role of methylotrophs in CH₄ cycling in arctic lakes, especially during the period when the lakes are covered with ice.

Despite the enormous potential of SIP techniques for the investigation of microbial community function without the need for cultivation, SIP may distort the original microbial community structure due to *in vitro* incubation, which can only partially reflect conditions *in situ*; thus, the data should be interpreted with some caution (13, 44, 55, 56). However, most of the results of this study are consistent with those of the studies in permafrost regions, such as the dominance of type I methanotrophs, including *Methylobacter*, *Methylomonas*, and *Methylosoma*, rather than type II methanotrophs and of psychrotolerant rather than psychrophilic organisms in the arctic lake sediment (40, 41, 43, 69).

Taken together, our findings indicate that CH₄ oxidation can occur at low temperatures characteristic of arctic lake sediments, down to at least 4°C. As temperatures increase, the CH₄ oxidation potential also increases and is associated with shifts in the composition of bacteria actively growing at the expense of CH₄. These shifts are evident within temperature ranges that already occur seasonally in arctic lakes, suggesting that active methanotrophic populations may also change seasonally. As temperatures continue to rise in the Arctic, predominant oxidation rates and active methanotrophic populations will shift. Whether these changes can offset predicted increases in the activity of methanogens is a critical question underlying models of future CH₄ flux and resultant climate change.

ACKNOWLEDGMENTS

We thank Ben Gaglioti, Nathan Stewart, Doug Whiteman (Atkasuk, AK), and Monica Heintz for field assistance; Catharine Catranis, John Guido Cable, Heather Slater, Catherine Glover, Robert Burgess, and Mary-Catherine Leewis for laboratory assistance; and the Alaska Stable Isotope Facility staff, including Tim Howe and Norma Haubenstock. We thank the community in Atkasuk for hosting us. Notably, we thank the Mayor of Atkasuk and the members of the City Council. We thank Thomas Itta, Wanda Kippi, Doug Whiteman, and Kimberly Brent for their input and for allowing us to share our work with them and with high school students at the Meade River School in Atkasuk.

This work was conducted under a Bureau of Land Management permit (FF095556) and North Slope Borough permits (NSB 09-0478 and NSB 10-018). Any use of trade names is only for descriptive purposes and does not imply endorsement by the U.S. Government.

This work was supported by funding from the U.S. Department of

Energy National Energy Technology Laboratory (grant DE-NT000565), awarded to Matthew J. Wooller and Mary Beth Leigh.

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