ORIGINAL ARTICLE

Diversity of active aerobic methanotrophs along depth profiles of arctic and subarctic lake water column and sediments

Ruo He^{1,2}, Matthew J Wooller^{3,4}, John W Pohlman⁵, John Quensen⁶, James M Tiedje⁶ and Mary Beth Leigh²

¹Department of Environmental Engineering, Zhejiang University, Hangzhou, China; ²Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, USA; ³Alaska Stable Isotope Facility, Water and Environmental Research Center, University of Alaska Fairbanks, Fairbanks, AK, USA; ⁴School of Fisheries and Ocean Sciences, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK, USA;

⁵US Geological Survey, Woods Hole Coastal and Marine Science Center, Woods Hole, MA, USA and

⁶Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA

Methane (CH₄) emitted from high-latitude lakes accounts for 2–6% of the global atmospheric CH₄ budget. Methanotrophs in lake sediments and water columns mitigate the amount of CH₄ that enters the atmosphere, yet their identity and activity in arctic and subarctic lakes are poorly understood. We used stable isotope probing (SIP), quantitative PCR (Q-PCR), pyrosequencing and enrichment cultures to determine the identity and diversity of active aerobic methanotrophs in the water columns and sediments (0–25 cm) from an arctic tundra lake (Lake Qalluuraq) on the north slope of Alaska and a subarctic taiga lake (Lake Killarney) in Alaska's interior. The water column CH₄ oxidation potential for these shallow (~2 m deep) lakes was greatest in hypoxic bottom water from the subarctic lake. The type II methanotroph, *Methylocystis*, was prevalent in enrichment cultures of planktonic methanotrophs from the water columns. In the sediments, type I methanotrophs (*Methylobacter*, *Methylosoma* and *Methylomonas*) at the sediment-water interface (0–1 cm) were most active in assimilating CH₄, whereas the type I methanotroph *Methylobacter* and/or type II methanotroph *Methylocystis* contributed substantially to carbon acquisition in the deeper (15–20 cm) sediments. In addition to methanotrophs, an unexpectedly high abundance of methylotrophs also actively utilized CH₄-derived carbon. This study provides new insight into the identity and activity of methanotrophs in the sediments and water from high-latitude lakes.

The ISME Journal (2012) 6, 1937–1948; doi:10.1038/ismej.2012.34; published online 17 May 2012

Subject Category: microbial ecology and functional diversity of natural habitats

Keywords: methanotrophs; CH₄ oxidation potential; arctic lakes; stable isotope probing

Introduction

Methane (CH₄) is a potent greenhouse gas responsible for about 20% of the direct radiative forcing from all long-lived greenhouse gases (IPCC, 2001). The concentration of CH₄ in the atmosphere has increased from 0.7 to 1.8 p.p.m.v. since the industrial revolution because of increased anthropogenic inputs (Etheridge *et al.*, 1998). Natural aquatic systems, in particular wetlands and lakes, also contribute substantial quantities of CH₄ to the atmosphere and are an important component of the continental greenhouse gas budget (IPCC, 2007;

Reeburgh, 2007; Bastviken *et al.*, 2011). Presently, lakes north of 45°N emit an estimated 24.2 ± 10.5 Tg CH₄ per year directly to the atmosphere (Walter *et al.*, 2007), which is more than twice that from the oceans (Reeburgh, 2007). Moreover, with additional climate warming, CH₄ emissions from high-latitude lakes are expected to increase during this century, particularly in arctic regions with continuous, organic-rich permafrost (Zimov *et al.*, 2006).

Aerobic CH₄ oxidation in the sediment and water column has an important role in mitigating CH₄ release from mid and low latitude freshwater ecosystems to the atmosphere (Frenzel *et al.*, 1990; Bender and Conrad, 1994; Hanson and Hanson, 1996; Carini *et al.*, 2005; Duc *et al.*, 2010). The availability of CH₄ and oxygen (O₂), the substrates for aerobic methanotrophy, are the most significant factors determining the location and rates of CH₄ oxidation in sediments (Hanson and Hanson, 1996).

Correspondence: MB Leigh, Institute of Arctic Biology, University of Alaska Fairbanks, PO Box 757000, Fairbanks, AK 99775-7000, USA.

E-mail: mbleigh@alaska.edu

Received 17 November 2011; revised 21 February 2012; accepted 22 February 2012; published online 17 May 2012

CH₄ oxidation is generally most active at the oxic-anoxic transition in sediments near the sediment-water interface (Kuivila *et al.*, 1988; Frenzel *et al.*, 1990; Bender and Conrad, 1994). In addition, CH₄ is also consumed in oxygenated portions of the water column in lakes (Carini *et al.*, 2005; Bastviken *et al.*, 2008).

Aerobic methanotrophic diversity in lakes is greater than that of peat or marine environments (Costello and Lidstrom, 1999). Diverse methanotrophs have been reported in low and mid-latitude freshwater ecosystems and varied with environmental conditions (Auman, 2001; Jugnia *et al.*, 2006; Rahalkar and Schink, 2007). Arctic lakes differ substantially from low and mid-latitude lakes in terms of the seasonal shifts in temperature, dissolved oxygen (DO) and CH₄ concentration associated with the dynamics of ice cover and thaw (Miller *et al.*, 1980; Phelps *et al.*, 1998; Clilverd *et al.*, 2009). These environmental conditions could favor an aerobic methanotrophic community distinct from warmer climates. However, to date, no study has addressed the identity or ecological ramifications of arctic lake CH₄ oxidation. In this study, we characterize the microbial community associated with aerobic CH₄ oxidation in the water column and sediments (0–25 cm) from two thermokarst lakes in Alaska, an arctic tundra lake on the North Slope of the Brooks Range and a subarctic taiga lake in Alaska's interior. In addition to the cultivation of aerobic methanotrophs from the water column, we applied DNA-based stable isotope probing (DNA-SIP) coupled with terminal restriction fragment length polymorphism (T-RFLP), quantitative PCR (Q-PCR) and pyrosequencing to determine the identity and diversity of active aerobic CH₄-oxidizing microorganisms along sediment depth profiles.

Materials and methods

Study site and sampling

Water and sediment samples were collected in July 2009 from two lakes: (a) Lake Qalluuraq, a tundra lake with a persistent CH₄ seep located near Atkasuk on the north slope of the Brooks Range in Alaska; and (b) Lake Killarney, a subarctic taiga lake with moderate CH₄ ebullition in Fairbanks, Alaska (Brosius, 2010) (Figure 1 and Table 1). Two sites, LQ-BGC and LQ-WEST, were sampled at Lake Qalluuraq. Site LQ-BGC is the location with high CH₄ ebullition (Wooller *et al.*, 2009), while CH₄ ebullition was not observed in the LQ-WEST site. A single site (LK-NW) was sampled in the northwest portion of Lake Killarney.

Water samples were collected from 20 cm under the water surface (top), 1 m below the water surface (middle) and 10 cm above the sediment-water interface (bottom), respectively, at each sampling location. Water temperature, DO and pH at the different sampling depths at each sampling location were measured using a YSI 556 MPS meter (YSI Environmental Inc., Yellow Springs, OH, USA). Cores of sediment 25 cm long were collected at each site using polycarbonate tubes (7 cm o.d.). The sediment sub-samples were immediately placed into plastic zipper freezer bags, homogenized and then sub-samples were kept at 4 °C for CH₄ oxidation incubations and SIP experiments. Additional sub-samples were freeze-dried and analyzed for their organic carbon and nitrogen content as described by Wang and Wooller (2006). Sediment particle size was analyzed by the Bouyoucos hydrometer method (Gee and Bauder, 1986).

Sediments for determination of dissolved CH₄ concentrations were collected as 3 ml plugs at 4–5 cm intervals from the sediment cores. The

Figure 1 Sampling sites in the study site. Lake Qalluuraq, an arctic tundra lake with a persistent CH₄ seep located near Atkasuk on the North Slope of the Brooks Range in Alaska; and Lake Killarney, a subarctic taiga lake with moderate CH₄ ebullition in Fairbanks, Alaska.

Table 1 Physical and chemical characteristics of sediment and water column in the study sites

Sampling site	GPS position	Sediment				Water column			
		Granular composition (%)				Water depth (cm)	Layer	Temperature (°C)	pH
		> 2 mm	0.1–2 mm	0.05–0.1 mm	0.002–0.05 mm				
LQ-BGC	70°22.669'N, 157°20.925'W	2.0	85.5	12.5	—	190	Top Middle Bottom	17.0 17.0 16.9	6.37 6.45 6.43
LQ-WEST	70°22.675'N, 157°21.856'W	—	—	68.2	20.4	170	Top Middle Bottom	17.0 16.9 16.8	6.03 5.94 6.02
LK-NW	64°52.204'N, 147°54.144'W	—	—	45.0	41.5	177	Top Middle Bottom	18.7 13.7 9.4	7.32 7.19 7.23
									DO ^a (mg l ⁻¹)
									10.10 9.91 9.89 10.11 9.94 9.94 7.15 1.43 0.27

Abbreviation: GPS, global positioning system.

^aDissolved oxygen.^bMean ± s.d. in the experimental sediment.

samples were transferred to 20 ml serum vials, sealed with 1 cm thick septa and stored at -20°C . Headspace CH_4 concentration in the vials was analyzed using a gas chromatograph equipped with a flame ionization detector and converted to aqueous concentrations using the method of Hoehler *et al.* (2000).

SIP microcosms and CH_4 oxidation potential

Incubations for SIP and CH_4 oxidation potential were conducted in microcosms in sterile 60 ml glass serum vials, containing either 5 g (wet weight) of sediment or 10 ml of water, sealed with butyl rubber stoppers. Microcosms were injected with $^{13}\text{CH}_4$ (99 atom % ^{13}C , Sigma-Aldrich, Saint Louis, MO, USA) or CH_4 (99.5% pure, as control) to achieve a headspace concentration of 10% CH_4 (v/v) and incubated on a rotary platform shaker (100 r.p.m.) at 4, 10 or 21°C . Calculated by Henry's Law and the van't Hoff equation to correct for the effect of temperature on CH_4 solubility (Lide and Frederikse, 1995), the theoretical saturated CH_4 concentration in the pore water is $151\ \mu\text{M}$ at the initial CH_4 concentration of 10% (v/v). CH_4 consumptions at 4 and 10°C were both very slow in the sediments from the LQ-BGC site (Supplementary Figure S1). Therefore, we focused on incubations at 21°C , a temperature that is similar to the maximum water temperatures of 20°C (July) detected during a 4-year study of arctic lakes (Miller *et al.*, 1980) and the measured temperature of water at Lake Qalluuraq, to investigate the comparative maximum activity and community structure of methanotrophs between and within the lakes. All samples were incubated in triplicate. Autoclaved water and sediment samples were used as abiotic controls.

Gas headspace samples (50 μl) were periodically withdrawn from the headspace of microcosms and analyzed for residual CH_4 using gas chromatograph-flame ionization detector. CH_4 oxidation potential was assessed from the zero-order decrease in CH_4 concentration in the headspace of the serum vials within 12 h (Kightley *et al.*, 1995), and expressed as $\mu\text{mol CH}_4$ per g dry weight per day ($\mu\text{mol g}^{-1} \text{d}^{-1}$) or $\text{nmol cm}^{-3} \text{d}^{-1}$. CH_4 oxidation potential of water samples was measured during 48 h incubations. SIP incubation was performed as described previously (He *et al.*, 2012) and was continued until $\sim 0.2\ \text{mmol } ^{13}\text{CH}_4/\text{CH}_4 \text{ g}^{-1}$ (wet weight) was oxidized (38–74 days for the LQ-WEST and LK-NW sediments and 108–212 days for the LQ-BGC sediments), which is sufficient to produce a UV visible ^{13}C -DNA band with ethidium bromide after an equilibrium (isopycnic) density gradient centrifugation (Radajewski *et al.*, 2002). At the end of the incubation, the sediment samples were harvested and frozen immediately at -80°C .

SIP DNA extraction and ^{13}C -DNA separation

DNA was extracted from 0.5–1 g (wet weight) of the SIP sediment sub-samples using the Bio101 Fast

DNA Spin Kit for soil (MP Biomedicals, Solon, OH, USA). Equilibrium (isopycnic) density gradient centrifugation and fractionation were performed using cesium trifluoroacetate (CsTFA, GE Healthcare, Chalfont St Giles, UK) solution as described by He *et al.* (2012). Gradients were fractionated into 20 250- μ l fractions, buoyant density (BD) was measured and DNA was precipitated and re-suspended as previously reported (Leigh *et al.*, 2007).

The relative abundance of bacterial DNA in gradient fractions ranging in BD from 1.559 to 1.663 g ml⁻¹ was determined by Q-PCR as described by Leigh *et al.* (2007). 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.

Planktonic methanotroph enrichment cultures

Fifty milliliters each of the triplicate water samples were centrifuged at 5000 r.p.m. for 15 min. The cell pellets were transferred into a sterile 60 ml glass serum vial with 10 ml methanotroph medium prepared as described by Graham *et al.* (1992). The vials were sealed with a butyl rubber stopper, injected with CH₄ to 10% (v/v) and incubated at room temperature ($\sim 21^\circ\text{C}$), 10 or 4 $^\circ\text{C}$, on a shaker (100 r.p.m.). CH₄ consumption was very slow at low temperatures (4 and 10 $^\circ\text{C}$) in the enrichment cultures from Lake Qalluuraq (that is, LQ-BGC and LQ-WEST sites) even if they were incubated for 1 month (Supplementary Figure S2). Considering this, we chose 21 $^\circ\text{C}$ incubations to investigate and compare culturable planktonic methanotrophs between and within the lakes. When the cultures became turbid, 1 ml was transferred into fresh methanotroph medium, and incubated again. After the enrichment, the culture was centrifuged at 14 000 r.p.m. for 10 min and the pellet was harvested and frozen at -80°C . The DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) was used to extract DNA from enrichment cultures.

Methanotroph Q-PCR analyses

Q-PCR of 16S rRNA genes of type I and type II methanotrophs and *pmoA* in the heavy fractions from ¹³C-labeled DNA and unlabeled control DNA was performed using the primer sets described by Martineau *et al.* (2010) and conducted in four replicates of 15 μ l reactions containing SYBR green master mix (Applied Biosystems, Foster City, CA, USA), 4.5 pmol each primer and 1 μ l template. Thermal cycler conditions were as follows: an initial stage at 50 $^\circ\text{C}$ for 2 min; denaturation at 95 $^\circ\text{C}$ for 5 min; 60 cycles of 95 $^\circ\text{C}$ for 30 s, 58 $^\circ\text{C}$ for 30 s and 72 $^\circ\text{C}$ for 45 s for *pmoA* or 72 $^\circ\text{C}$ for 30 s for 16S rRNA genes of type I and type II methanotrophs. Standards were made from 10-fold dilutions of linearized plasmids containing the same fragment of 16S rRNA

genes of type I and type II methanotrophs and *pmoA* as 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 *pmoA* and about 10³ copies per reaction for 16S rRNA genes of type I and type II methanotrophs.

T-RFLP analysis and multivariate analyses

Bacterial community profiling was performed using T-RFLP of 16S rRNA gene amplicons as described previously (He *et al.*, 2012). T-RFLPs were analyzed using GeneMapper software version 3.7 (Applied Biosystems). The peak height threshold for T-RFLPs was set at 300 FU (fluorescence units). Terminal restriction fragments less than 50 and above 1000 bp were eliminated from all data sets, and then normalized such that peak heights represented a percentage of the total peak height in each sample. The different bacterial communities were then subjected to a multivariate cluster analysis using Euclidean distance with the open source statistical application PAST (Hammer *et al.*, 2001).

Sequencing and phylogenetic analyses

Sequence analysis was performed on 16S rRNA gene amplicons from ¹³C-DNA and DNA from the enrichment cultures of water using pyrosequencing as described by He *et al.* (2012). Sequences were first trimmed of the primer region and low-quality sequences were removed by Ribosomal Database Project's (RDP) pyrosequencing pipeline. From 41 to 66% of the total sequences (2631–4794) except for 31% of the total sequences of 2931 for LQ-WEST (0–1 cm) passed this filter (average length of 330–331 bp) and were assigned to taxonomic groups by RDP's Naïve Bayesian Classifier (80% confidence threshold). The nucleotide sequences have been deposited to NCBI Short Read Archive under the accession number of SRP005485.

Results

Physical and chemical characteristics

The water in Lake Qalluuraq (LQ-BGC and LQ-WEST sites) was weakly acidic and DO was between 9.89 mg l⁻¹ and 10.11 mg l⁻¹ at all depths (Table 1). At 17 $^\circ\text{C}$, the temperature of the lake during sampling, the DO concentrations correspond to 102–105% saturation (the theoretically saturated concentration of DO is 9.65 mg l⁻¹). Water in Lake Killarney (LK-NW site) had apparently high dissolved organic carbon (dark in color) and a sharp decrease of DO and temperature with increasing depth. The sediment from the seep site (LQ-BGC site) was mostly composed of sand-sized particles of 0.1–2 mm in size, accounting for 85.5%. However, the sediments from the LQ-WEST and LK-NW sites were mainly composed of fine-grained particles of 0.05–0.1 mm (45.0–68.2%) and 0.002–0.05 mm

Figure 2 Sediment depth profile for dissolved CH₄ concentration in the sampling sites. (a) LQ-BGC site; (b) LQ-WEST site; and (c) LK-NW site.

(20.4–41.5%), respectively. The sediments from LQ-WEST site had the highest carbon (11.14%) and nitrogen (0.69%) contents, which are about 5 and 70 times higher than those from the LK-NW and LQ-BGC sites, respectively.

At the LQ-BGC site, dissolved CH₄ concentrations were less than 15 μM from 0–10 cm sediment depth, and increased to 1334 μM at 25 cm sediment depth (Figure 2). At the LQ-WEST site, dissolved CH₄ concentrations from near the sediment-water interface to 25.5 cm sediment depth varied from 483 μM to 2062 μM. Lower dissolved CH₄ concentrations (25–68 μM) were found in sandy intervals from 30–36 cm sediment depth. Compared with Lake Qalluuraq (LQ-BGC and LQ-WEST sites), dissolved CH₄ concentration in the pore water from LK-NW site was lower (about 16–390 μM).

CH₄ oxidation potential

The highest water column CH₄ oxidation potential occurred in the subarctic lake site (LK-NW) samples, followed by the arctic lake seep location (LQ-BGC site) (Figure 3). At all locations the CH₄ oxidation potential increased with depth, and the highest rates were obtained from water samples collected from near the bottom of the lakes. The CH₄ oxidation potential in water from the near-bottom of the LK-NW site was 1450 nmol cm⁻³ d⁻¹, which is approximately seven times higher than that from the LQ-BGC site. Near zero CH₄ consumption was observed in water from the LQ-WEST site, which is located 583 m from the Lake Qalluuraq seep (LQ-BGC site).

The CH₄ oxidation potential of surface sediment (0–1 cm) from the LQ-WEST site (77–80 μmol g⁻¹ d⁻¹) is over an order of magnitude higher than the LQ-BGC seep site and 1.8–13.0 times higher than surface sediment from the subarctic lake site, LK-NW (Figure 4). After 2 days of incubation,

Figure 3 CH₄ oxidation potential of water samples from indicated depths: top (20 cm under the water surface); middle (1 m below the water surface); bottom (10 cm above the sediment-water interface). Different letters within the graph refer to significant difference at 5% level based on least significant difference (LSD) method.

measurable CH₄ consumption occurred at the 0–20 cm sediment depths from LQ-BGC and LQ-WEST sites, and at the 0–5 cm depths from the LK-NW site. After 10 days of incubation, CH₄ consumption was measured in sediments from every depth (0–25 cm), with CH₄ oxidation potentials of 0.7–76.8 μmol g⁻¹ d⁻¹.

Enrichment cultures of planktonic methanotrophs

Microorganisms harvested from discrete samples from different water and then incubated in methanotroph medium showed rapid CH₄ consumption (~0.1 mmol CH₄ was first completely consumed within 2 days in the cultures from LK-NW water samples, and ~7 days from LQ-BGC and LQ-WEST water samples). Although near zero CH₄ consumption was measured in water from the LQ-WEST site, the T-RFLP profiles of 16S rRNA gene amplicons from the LQ-WEST enrichments were similar to

Figure 4 CH₄ oxidation potential of sediment from (a) LQ-BGC site; (b) LQ-WEST site; and (c) LK-NW site. □ incubated for 2 days; ▨ incubated for 5 days; ■ incubated for 10 days. Note different scales on y axes (CH₄ oxidation potential).

those from enrichment cultures from the seep site (LQ-BGC) within the same lake (Supplementary Figures S3a, b and S4a, b). Some variation in T-RFs with water depth was visible within the LK-NW site (Supplementary Figures S3c and S4c).

As T-RFLP analyses indicated that the enrichment cultures from various depths were generally similar in community structure (Supplementary Figure S4), we combined DNA extracts obtained from the top, middle and bottom depths together at each sampling location for pyrosequencing analysis of 16S rRNA gene amplicons. RDP classification of pyrosequencing reads showed that the type II methanotroph, *Methylocystis*, was dominant in the water sample enrichment cultures (Table 2). The type I methanotrophs, *Methylomonas*, *Methylobacter* and unclassified *Methylococcaceae*, were detected as a small proportion (1.7%) of the enrichment culture from water from the LK-NW site, while they were not evident in the sites from Lake Qalluuraq.

Interestingly, *Methylophilus*, an obligate methylo-troph that uses methanol as the sole source of carbon and energy (Vries *et al.*, 1990; Bratina *et al.*, 1992), was present in all the enrichment cultures and was especially abundant in the LK-NW water culture, where 25.2% of pyrosequencing reads for the enrichment culture were affiliated with

Table 2 Relative abundance (% of total reads) and phylogenetic affiliations of pyrosequencing reads from DNA of enrichment cultures in methanotroph medium cultivated from lake water samples

Phylogenetic affiliation	Sampling site		
	LQ-BGC	LQ-WEST	LK-NW
<i>Proteobacteria</i>			
<i>Alphaproteobacteria</i>			
<i>Methylocystaceae</i>			
<i>Methylocystis</i>	74.1 (1436) ^a	75.4 (2028)	33.6 (1005)
<i>Methylosinus</i>	0.2 (3)	— ^b	1.9 (58)
Unclassified	0.8 (15)	0.8 (21)	18.9 (564)
<i>Methylocystaceae</i>			
<i>Hyphomicrobiaceae</i>			
<i>Hyphomicrobium</i>	1.4 (27)	1.9 (52)	1.2 (35)
Other	6.6 (127)	6.8 (184)	6.6 (196)
<i>Alphaproteobacteria</i>			
<i>Betaproteobacteria</i>			
<i>Methylophilales</i>			
<i>Methylophilus</i>	7.9 (154)	3.2 (86)	25.2 (754)
Unclassified	0.1 (1)	0.2 (6)	0.2 (7)
<i>Methylophilaceae</i>			
<i>Rhodocyclales</i>	0.1 (2)	—	0.1 (2)
<i>Burkholderiales</i>	4.1 (80)	5.4 (146)	2.6 (77)
Other	1.1 (22)	0.5 (13)	1.4 (43)
<i>Betaproteobacteria</i>			
<i>Gammaproteobacteria</i>			
<i>Methylococcales</i>			
<i>Methylomonas</i>	—	—	0.4 (12)
<i>Methylobacter</i>	—	—	0.5 (14)
Unclassified	—	—	0.8 (25)
<i>Methylococcaceae</i>			
Other	1.5 (30)	2.3 (61)	1.6 (49)
<i>Gammaproteobacteria</i>			
<i>Actinobacteria</i>	1.1 (21)	0.6 (15)	0.1 (3)
<i>Bacteroidetes</i>	0.6 (11)	2.2 (59)	1.7 (51)
<i>Verrucomicrobia</i>	0.2 (3)	0.2 (5)	1.1 (34)
Unclassified Bacteria	0.2 (3)	0.4 (11)	1.1 (34)
Other	0.2 (3)	0.1 (4)	0.9 (27)
Total	100 (1938)	100 (2691)	100 (2990)

Taxonomic assignments were generated using the Ribosomal Database Project's classifier.

^aThe numbers of pyrosequencing reads assigned using the RDP classifier (80% confidence threshold) are shown in parentheses.

^bNo pyrosequencing reads from this group were detected.

Methylophilus. In addition to the *Proteobacteria*, members of the *Actinobacteria*, *Bacteroidetes* and *Verrucomicrobia* (note there are three previously known methanotrophs in the phylum *Verrucomicrobia*; Dunfield *et al.*, 2007; Pol *et al.*, 2007; Islam *et al.*, 2008) were also found in the enrichment cultures from all the study locations.

Active CH₄ oxidizers in sediments

To characterize the active sedimentary methanotrophic community, we employed SIP to track the incorporation of ¹³C-labeled CH₄ carbon into microbial DNA (Radajewski *et al.*, 2003; Dumont and Murrell, 2005). Q-PCR data indicate that significant quantities of ¹³C-DNA were present in SIP sediment

Figure 5 Quantitative real-time PCR (Q-PCR) of *pmoA* (■) and 16S rRNA genes of type I (▨) and type II (□) methanotrophs in ^{13}C -DNA from SIP sediments. The limit of detection by Q-PCR was about 10^2 copies per reaction for *pmoA* and about 10^3 copies per reaction for 16S rRNA genes of type I and type II methanotrophs. (a) LQ-BGC site; (b) LQ-WEST site; and (c) LK-NW site.

incubated with $^{13}\text{CH}_4$ (Supplementary Figure S5). Unlabeled control DNA had a peak of abundant DNA at BD ranging from 1.589 to 1.601 g ml^{-1} . Negligible bacterial DNA was detected when the BD was $>1.620 \text{ g ml}^{-1}$ for unlabeled control DNA fractions, while a second DNA peak in high density fractions was present in the $^{13}\text{CH}_4$ incubated samples. Based on the Q-PCR results, we combined the fractions with BD ranging from 1.620 to 1.644 g ml^{-1} into a compiled heavy fraction for each sample for subsequent molecular analyses.

The 16S rRNA gene of type I methanotrophs and *pmoA* were detected using Q-PCR at 7.0×10^5 – 5.7×10^7 and 1.4×10^6 – 7.1×10^7 copies μl^{-1} , respectively, in all ^{13}C -DNA samples (Figure 5). The 16S rRNA gene of type II methanotrophs was present at levels of 3.0×10^5 – 1.0×10^7 copies μl^{-1} in the ^{13}C -DNA from the LK-NW sediment ranging in depth from 1–25 cm, but was only detected in ^{13}C -DNA from deeper LQ-WEST sediment (20–25 cm) at 1.4×10^7 copies μl^{-1} as well as in LQ-BGC sediments (15–25 cm), which had 2.3×10^6 – 8.1×10^6

Figure 6 Cluster analysis of T-RFLP profiles of 16S rRNA genes amplicons digested with *HhaI* for ^{13}C -DNA from SIP of sediment collected after about $0.2 \text{ mmol } ^{13}\text{CH}_4 \text{ g}^{-1}$ (wet weight) consumed. The hierarchical tree was produced by PAST software (Hammer *et al.*, 2001) based on Euclidean distance. The sign (*) in the figure shows the sediment samples used for pyrosequencing analysis.

copies μl^{-1} . Type II methanotroph sequences were below the limit of Q-PCR detection of 10^3 copies per reaction in all other ^{13}C -DNA samples. For all the heavy fractions from unlabeled control DNA, the 16S rRNA genes of type I and type II methanotrophs and *pmoA* were below detection limits.

T-RFLP profiles of bacterial 16S rRNA gene amplicons for ^{13}C -DNA from sediment showed that a diverse array of bacteria derived carbon from $^{13}\text{CH}_4$ in all sediment incubations, while few obvious peaks were found in the heavy fractions from the control DNA (Supplementary Figures S6, S7). The bacteria active in CH_4 utilization varied in composition among different lakes, sampling sites and sediment depths. A multivariate cluster analysis of T-RFLP profiles using Euclidean distance showed that the composition and structure of active methanotrophic communities clustered by depth and site (Figure 6).

On the basis of the T-RFLP-based multivariate cluster analysis for ^{13}C -DNA from the SIP sediments of different depths and sites, we selected ^{13}C -DNA from the uppermost (0–1 cm) sediment and the deeper (15–20 cm) sediment at each sampling location for comparative pyrosequencing analysis (Figure 6, pyrosequencing samples noted with asterisks). Although numbers of pyrosequencing reads were low in the LQ-WEST 0–1 cm sediment (903) (Table 3), rarefaction curves showed a higher diversity in the sediment than the deep sediments and LK-NW uppermost sediment (Supplementary Figure S8). Phylogenetic affiliations of pyrosequencing reads from ^{13}C -DNA by RDP

Table 3 Relative abundance (% of total reads) and phylogenetic affiliations of pyrosequencing reads from ^{13}C -DNA obtained from SIP of lake sediments with $^{13}\text{CH}_4$

Phylogenetic affiliations	LQ-BGC		LQ-WEST		LK-NW	
	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>	1.7 (43) ^a	25.4 (719)	1.0 (9)	0.2 (6)	0.2 (5)	40.4 (1166)
<i>Methylosinus</i>	— ^b	—	—	—	—	1.5 (43)
Unclassified <i>Methylocystaceae</i>	0.1 (2)	0.5 (13)	0.4 (4)	0.2 (4)	1.6 (45)	7.6 (220)
<i>Beijerinckiacae</i>						
<i>Methylocella</i>	0.1 (2)	—	—	—	0.1 (2)	2.3 (66)
<i>Methylovirgula</i>	—	—	—	—	0.7 (21)	1.1 (32)
Unclassified <i>Beijerinckiacae</i>	2.2 (56)	—	0.3 (3)	0 (1)	0.2 (7)	2.1 (62)
<i>Hyphomicrobiaceae</i>						
<i>Hyphomicrobium</i>	6.8 (173)	4.4 (124)	5.8 (52)	0.8 (22)	—	0.2 (6)
<i>Methylobacteriaceae</i>						
<i>Methylobacterium</i>	—	—	—	—	—	1.6 (45)
Other <i>Alphaproteobacteria</i>	15.6 (396)	22.3 (631)	8.9 (80)	6.8 (178)	1.2 (36)	9.6 (278)
<i>Betaproteobacteria</i>						
<i>Methylophilales</i>						
<i>Methylophilus</i>	2.9 (74)	0.3 (8)	1.7 (15)	29.3 (765)	—	—
Unclassified <i>Methylophilaceae</i>	1.1 (27)	0 (1)	0.7 (6)	1.0 (27)	—	0.2 (2)
<i>Rhodocyclales</i>	0.1 (3)	0 (1)	1.4 (13)	6.7 (174)	6.4 (184)	2.0 (59)
<i>Burkholderiales</i>	1.1 (29)	1.1 (31)	3.1 (28)	5.6 (146)	2.0 (57)	0.6 (17)
Other <i>Betaproteobacteria</i>	15.9 (403)	0.7 (21)	12.2 (110)	2.3 (59)	16.0 (462)	0.9 (25)
<i>Gammaproteobacteria</i>						
<i>Methylococcaceae</i>						
<i>Methylobacter</i>	0 (1)	0 (1)	0.1 (1)	30.3 (790)	60.3 (1742)	20.0 (578)
<i>Methylomonas</i>	—	0 (1)	7.0 (63)	1.2 (30)	—	—
<i>Methylosoma</i>	3.2 (82)	—	0.4 (4)	—	—	—
Unclassified <i>Methylococcaceae</i>	2.4 (60)	0 (1)	1.3 (12)	0.1 (2)	4.7 (136)	0.1 (4)
Other <i>Gammaproteobacteria</i>	4.8 (121)	6.5 (184)	6.1 (55)	0.9 (23)	2.7 (77)	3.5 (101)
<i>Deltaproteobacteria</i>	5.7 (144)	5.2 (146)	13.6 (123)	4.9 (127)	0 (1)	0.5 (14)
Unclassified <i>Proteobacteria</i>	1.7 (42)	5.1 (144)	2.3 (21)	1.2 (31)	0.3 (8)	0.6 (18)
<i>Actinobacteria</i>	0.7 (18)	0 (1)	0.9 (8)	5.4 (140)	0.6 (17)	1.3 (38)
<i>Firmicutes</i>	—	0.1 (3)	0.1 (1)	1.1 (29)	2.1 (60)	0.2 (6)
<i>Bacteroidetes</i>	2.6 (67)	0.7 (20)	5.9 (53)	0 (1)	—	1.4 (39)
<i>Acidobacteria</i>	11.3 (286)	0.7 (19)	3.5 (32)	0.1 (2)	—	0.4 (11)
<i>Verrucomicrobia</i>	0.7 (18)	0.6 (16)	2.7 (24)	—	0 (1)	—
<i>Planctomycetes</i>	4.1 (103)	0.5 (15)	3.4 (31)	0.2 (5)	0.3 (9)	0.5 (13)
<i>Chloroflexi</i>	2.0 (51)	2.4 (67)	0.4 (4)	—	0.1 (2)	0.1 (2)
Unclassified bacteria	12.2 (310)	22.3 (629)	12.7 (115)	1.4 (37)	0.3 (10)	0.9 (25)
Other	1.1 (27)	1.2 (30)	4.0 (36)	0.3 (8)	0.2 (7)	0.5 (14)
Total	100 (2538)	100 (2826)	100 (903)	100 (2607)	100 (2889)	100 (2884)

Taxonomic assignments were generated using the Ribosomal Database Project's classifier.

^aThe numbers of pyrosequencing reads assigned using the RDP classifier (80% confidence threshold) are shown in parentheses.^bNo pyrosequencing reads from this group were detected.

classifier showed a diverse bacterial community was involved directly or indirectly in acquiring CH_4 -derived carbon. Members of the phylum *Proteobacteria* predominated CH_4 carbon assimilation, especially in the sediments from the LK-NW site and the deep (15–20 cm) sediment from the LQ-WEST site, where *Proteobacteria* accounted for

more than 91% of the total pyrosequencing reads. The phyla *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Chloroflexi* were all found in the deep (15–20 cm) sediment from the LQ-BGC site and the uppermost (0–1 cm) sediment from the LQ-WEST site.

Both type I (*Methylobacter*, *Methylobacter*, *Methylosoma* and unclassified *Methylococcaceae*) and type II methanotrophs (*Methylocystis*, *Methylosinus* and *Methylocella*) were detected in the ^{13}C -DNA from sediments. Compared with type II methanotrophs, type I methanotrophs were more dominant in ^{13}C -DNA from the uppermost (0–1 cm) sediment, especially at the LK-NW site, where *Methylobacter* accounted for 60.3% of the pyrosequencing reads (Table 3). Type I (*Methylobacter*) and type II methanotrophs (*Methylocystis*, *Methylosinus* and *Methylocella*) were all detected in the deeper (15–20 cm) sediment from the LK-NW site. The type II methanotroph, *Methylocystis*, was the most abundant methanotroph in the deeper sediment from the seep site (LQ-BGC) at Lake Qalluuraq, while the type I methanotroph, *Methylobacter*, was the most abundant type in deeper sediments from LQ-WEST site in the same lake.

Discussion

Differences in CH_4 oxidation potential in the water column were observed both between and within our study lakes, Lake Qalluuraq (a tundra arctic lake) and Lake Killarney (a subarctic taiga lake) (Figure 1). A 15 to 60-fold greater CH_4 oxidation potential was observed in the water column above the active CH_4 seep (LQ-BGC) site compared with the non-seep (LQ-WEST) site within the same arctic lake, which was likely due to the seep providing a constant input of carbon and energy sources that caused increased microbial biomass in the water column (Reay *et al.*, 1993; Treude and Ziebis, 2010) (Figure 3). Compared with the arctic lake CH_4 seep, a considerably (4 to 7-fold) higher CH_4 oxidation potential was exhibited by the subarctic lake (LK-NW site) water. The highest CH_4 oxidation potential was present in the deep water from this subarctic lake (LK-NW site), which was a hypoxic zone *in situ* (Table 1).

Culturable methanotrophic communities varied with water depth in the subarctic lake (Supplementary Figures S3c and S4c), but not in the arctic lake (Supplementary Figures S3a, b and S4a, b). The vertical variation of the culturable methanotrophic communities is consistent with stratification of the water column in the subarctic Lake Killarney. Cultivation in a methanotroph medium in our study appeared to favor *Methylocystis*, especially in the enrichment cultures of water from arctic Lake Qalluuraq, whose representatives accounted for 74–75% of the total pyrosequencing reads (Table 2). The prevalence of *Methylocystis* in the enrichment cultures of water may be an indication of a predominance of these organisms in water and/or their more rapid growth in cultures than other dominant organisms, which has been reported previously in a mid-latitude freshwater system (Busmann *et al.*, 2004).

In sediments, the communities of active CH_4 utilizers varied with depth as well as sampling site (Supplementary Figures S6 and S7), and were likely governed by depth of O_2 penetration. The metabolically active community of aerobic methanotrophs in sediment is limited by the *in situ* availability of O_2 and CH_4 (Hanson and Hanson, 1996). The O_2 penetration depth depends largely on the input of degradable organic matter to the sediment (Brune *et al.*, 2000). In our three study locations, the highest sediment organic matter content was observed in the LQ-WEST site, but the DO was still oversaturated in the water column due to physical mixing of the shallow (<2 m depth) lake (Miller *et al.*, 1980; Burn, 2002; Crump *et al.*, 2003). Compared to the LQ-WEST and LK-NW sediment, the LQ-BGC (active CH_4 seep site) sediment was much sandier, potentially allowing a deeper penetration of O_2 into the sediment due to higher permeability and lower sediment microbial biomass and metabolic activity, as well as in a faster removal of potentially inhibitory reduced end products (Treude and Ziebis, 2010). Based on microbial community shifts revealed by cluster analysis of T-RFLP profiles (Figure 6), we hypothesize that the position of the oxic-anoxic interface relative to the sediment surface was at about 15 cm depth for the LQ-BGC site, but near the sediment-water interface for the LQ-WEST and LK-NW sites. Compared to the sediment depth profile for dissolved CH_4 concentration in the LQ-BGC site (Figure 2a), O_2 penetration was deeper based on the T-RFLP-based multivariate cluster analysis for ^{13}C -DNA, most likely due to CH_4 seep flow which is similar to hydrothermal vent flow entraining lateral inputs of oxygenated water into the seep. The metabolically active communities of bacteria established in the oxic sediment layers were thus markedly different from those in the deeper, anoxic and anaerobic layers, where methanotrophs are likely dormant, existing as cysts or exospores (Bowman *et al.*, 1993), even when they were exposed to the same SIP incubation conditions. A sharp decrease in dissolved CH_4 concentration was observed in the pore water from the LK-NW 15–25 cm sediments (Figure 1), which was likely due to anaerobic CH_4 oxidation. Additional studies such as total methanotroph communities (including *pmoA*) *in situ* and SIP microcosms incubated under anaerobic conditions need to be conducted to better understand the active methanotrophs in arctic lakes.

Both type I and type II methanotrophs were found to derive carbon from CH_4 during the lake sediment microcosm incubations (Table 3), with type I methanotrophs being more active in the uppermost sediment at all sites. In our another study, direct sequencing of 16S rRNA genes of methanotrophs also demonstrated that type I methanotrophs were more abundant than type II methanotrophs in the upper sediment from Lake Qalluuraq (He *et al.*, 2012). However, different genera of

type I methanotrophs were dominant in the uppermost sediment at the three study sites: *Methylosomas* in the LQ-BGC site; *Methylomonas* in the LQ-WEST site; and *Methylobacter* in the LK-NW site. In the deep sediment, the type II methanotroph *Methylocystis* was dominant in the LQ-BGC and LK-NW sites; while type I *Methylobacter* was abundant in the LQ-WEST and LK-NW sites. Type I methanotrophs grow better in low CH₄ and high O₂ concentrations whereas type II methanotrophs dominate under high CH₄ and low O₂ concentrations (Amaral and Knowles, 1995; Henckel *et al.*, 2000). In arctic lakes, the uppermost sediments can be a temporally heterogeneous environment due to the dramatic seasonal variations in the concentrations of O₂, CH₄ and temperature (Miller *et al.*, 1980; Phelps *et al.*, 1998; Clilverd *et al.*, 2009). Type I methanotrophs generally grow more rapidly than type II methanotrophs, and dominate in variable environments (Henckel *et al.*, 2001), perhaps explaining their dominance in the uppermost sediments in our study lakes.

As SIP often requires *in vitro* incubations that only partially reflect conditions *in situ*, this method may distort the relative abundance of organisms active in a particular process (Radajewski *et al.*, 2002, 2003). Nonetheless, SIP is valuable for revealing the identity of active methanotrophs in the environment and enables tracking carbon from a specific substrate through the microbial food web by examining the increase in diversity of ¹³C-labeled organisms over time (Radajewski *et al.*, 2003; McDonald *et al.*, 2005). Methanotrophic community structure and activity varies with temperature (Mor *et al.*, 2006; Liebner and Wagner, 2007; Chanton *et al.*, 2008). Although we conducted SIP microcosms at 21 °C that is similar to the maximum water temperature in arctic lakes (Miller *et al.*, 1980) and harvested SIP samples at the similar CH₄ consumption and different incubation time, our some core samples and depths show type I methanotrophs while others show that type II methanotrophs are dominant, which might be due to factors such as depth and geochemistry known to be associated with varying microbial community structure.

In addition to methanotrophs, we found an unexpectedly high abundance of methylotrophs, especially *Methylophilus*, to be active in utilizing carbon from CH₄ in the deep (15–20 cm) sediment from the LQ-WEST site (Table 3). Methanotrophic bacteria usually metabolize CH₄ completely to CO₂, with methanol, formaldehyde, and formate produced as intermediates (Hanson and Hanson, 1996). This series of reactions reportedly occurs intracellularly and no methanol is released extracellularly (Corder *et al.*, 1986). However, the interruption of the enzymatic reactions by manipulation of the environmental conditions or mutation has been reported to produce excess extracellular methanol (Corder *et al.*, 1986; Lee *et al.*, 2004). Because of the SIP *in vitro* 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 derived from methanotrophs such as ¹³CO₂ or organic matter (Radajewski *et al.*, 2003; McDonald *et al.*, 2005). In addition to using carbon derived from CH₄ during SIP of sediments, surprisingly, *Methylophilus* was also detected by pyrosequencing analyses in our enrichment cultures of water. We isolated several methanotrophic strains from the enrichment cultures of water, but the 16S rRNA sequences of isolates were not affiliated with *Methylophilus* (data not shown). Taken together, our results suggest that methanotrophs and methylotrophs, including *Methylophilus*, play important roles in the microbial food web that processes carbon from CH₄ oxidation in these arctic and subarctic lakes.

In conclusion, SIP and cultivation methods demonstrated that type I methanotrophs, including *Methylomonas*, *Methylobacter* and *Methylosoma*, and type II methanotrophs, including *Methylocystis* and *Methylosinus*, are abundant and actively assimilate CH₄ in these arctic and subarctic lakes. The community structure and activity of methanotrophs varied alongside the physical-chemical properties of the sediments and water, such as sediment composition, oxygen and CH₄ concentrations. Our findings provide new fundamental information regarding the activity and diversity of methanotrophs in cold regions that may aid predicting and modeling future CH₄ flux from warming arctic and subarctic environments.

Acknowledgements

We thank Ben Gaglioti, Nathan Stewart, Doug Whiteman, Monica Heintz, Catharine Catranis for field and laboratory assistance. We also thank Carolyn Ruppel for help with mapping the sampling sites. 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 United States Department of Energy National Energy Technology Laboratory (Grant DE-NT000565).

References

- Amaral JA, Knowles R. (1995). Growth of methanotrophs in methane and oxygen counter gradients. *FEMS Microbiol Lett* **126**: 215–220.
- Auman AJ. (2001). *Molecular analysis of methanotroph ecology in lake Washington sediment*, PhD Thesis. University of Washington: Washington.
- Bastviken D, Cole JJ, Pace ML, Van de Bogert MC. (2008). Fates of methane from different lake habitats: connecting whole-lake budgets and CH₄ emissions. *J Geophys Res* **113**: G02024.
- Bastviken D, Tranvik LJ, Downing JA, Crill PM, Enrich-Prast A. (2011). Freshwater methane emissions offset the continental carbon sink. *Science* **331**: 50.

- Bender M, Conrad R. (1994). Methane oxidation activity in various soils and freshwater sediments: occurrence, characteristics, vertical profiles and distribution on grain size fractions. *J Geophys Res* **99**: 16531–16540.
- Bowman JP, Sly LI, Nichols PD, Hayward AC. (1993). Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov., validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *Int J Syst Bacteriol* **43**: 735–753.
- Bratina BJ, Brusseau GA, Hanson RS. (1992). Use of 16S rRNA analysis to investigate phylogeny of methylobacterial bacteria. *Int J Syst Evol Microbiol* **42**: 645–648.
- Brosius LS. (2010). *Investigation controls over methane production and bubbling from interior Alaskan lakes using stable isotopes and radiocarbon ages*, MS Thesis. University of Alaska Fairbanks: Fairbanks.
- Brune A, Frenzel P, Cypionka H. (2000). Life at the oxic-anoxic interface: microbial activities and adaptations. *FEMS Microbiol Rev* **24**: 691–710.
- Burn CR. (2002). Tundra lakes and permafrost, Richards Island, western Arctic coast, Canada. *Can J Earth Sci* **39**: 1281–1298.
- Bussmann I, Pester M, Brune A, Schink B. (2004). Preferential cultivation of Type II methanotrophic bacteria from littoral sediments (Lake Constance). *FEMS Microbiol Eco* **47**: 179–189.
- Carini S, Bano N, LeClerc G, Joye SB. (2005). Aerobic methane oxidation and methanotroph community composition during seasonal stratification in Mono Lake, California (USA). *Environ Microbiol* **7**: 1127–1138.
- Chanton JP, Powelson DK, Abichou T, Fields D, Green R. (2008). Effect of temperature and oxidation rate on carbon-isotope fractionation during methane oxidation by landfill cover materials. *Environ Sci Technol* **42**: 7818–7823.
- Clilverd H, White D, Lilly M. (2009). Chemical and physical controls on the oxygen regime of ice-covered arctic lakes and reservoirs. *J Am Water Resources Ass* **45**: 500–511.
- Corder RE, Johnson ER, Vega JL, Clausen EC, Gaddy JL. (1986). Biological production of methanol from methane. URL http://www.anl.gov/PCS/acsfuel/preprint%20archive/Files/33_3_LOS%20ANGELES_09-88_0469.pdf.
- Costello AM, Lidstrom ME. (1999). Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol* **65**: 506–5074.
- Crump BC, Kling GW, Bahr M, Hobbie JE. (2003). Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Appl Environ Microbiol* **69**: 2253–2268.
- Duc NT, Crill P, Bastviken D. (2010). Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. *Biogeochemistry* **100**: 185–196.
- Dumont MG, Murrell JC. (2005). Stable isotope probing-linking microbial identity to function. *Nat Rev Microbiol* **3**: 499–504.
- Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou SB et al. (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature* **450**: 879–882.
- Etheridge DM, Steele LP, Francey RJ, Langenfelds RL. (1998). Atmospheric methane between 1000 A.D. and present: Evidence of anthropogenic emissions and climatic variability. *J Geophys Res* **103**: 15979–15993.
- Frenzel P, Thebrath B, Conrad R. (1990). Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). *FEMS Microbiol Lett* **73**: 149–158.
- Gee GW, Bauder JW. (1986). Particle-size analysis. In: Klute A (ed) *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods*. American Society of Agronomy/Soil Science Society of America: Madison, pp 383–411.
- Graham DW, Korich DG, Leblanc RP, Sinclair NA, Arnold RG. (1992). Applications of a colorimetric plate assay for soluble methane monooxygenase activity. *Appl Environ Microbiol* **58**: 2231–2236.
- Hammer Ø, Harper DAT, Ryan PD. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* **4**: 9 pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
- Hanson RS, Hanson TE. (1996). Methanotrophic bacteria. *Microbiol Rev* **60**: 439–471.
- He R, Wooller MJ, Pohlman JW, Catranis C, Quensen J, Tiedje JM, Leigh MB. (2012). Identification of functionally active aerobic methanotrophs in sediments from an arctic lake using stable isotope probing. *Environ Microbiol*; e-pub ahead of print 20 March 2012; doi:10.1111/j.1462-2920.2012.02725.x.
- Henckel T, Jäkel U, Conrad R. (2001). Vertical distribution of the methanotrophic community after drainage of rice field soil. *FEMS Microbiol Ecol* **34**: 279–291.
- Henckel T, Roslev P, Conrad R. (2000). Effects of O₂ and CH₄ on presence and activity of the indigenous methanotrophic community in rice field soil. *Environ Microbiol* **2**: 666–679.
- Hoehler TM, Borowski WS, Alperin MJ, Rodriguez NM, Paull CK. (2000). Model, stable isotope, and radio-tracer characterization of anaerobic methane oxidation in gas hydrate-bearing sediments of the Blake Ridge. *Proc ODP Sci Res* **164**: 79–85.
- Intergovernmental Panel on Climate Change (IPCC) (2001). Climate change 2001: the Scientific Basis. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Xiaos D (eds). *Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge.
- Intergovernmental Panel on Climate Change (IPCC) (2007). Climate Change 2007—The Physical Science Basis. In: Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB, Miller HL, Chen Z (eds). *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Chang*. Cambridge University Press: Cambridge.
- Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK. (2008). Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. *Proc Natl Acad Sci USA* **105**: 300–304.
- Jugnia LB, Roy R, Pacheco-Oliver M, Planas D, Miguez CB, Greer CW. (2006). Potential activity and diversity of methanotrophic bacteria in forest soil, peat, and sediments from a hydroelectric reservoir (Robert-bourassa) and lakes in the Canadian Taiga. *Soil Sci* **171**: 127–137.
- Kightley D, Nedwell DB, Cooper M. (1995). Capacity for methane oxidation in landfill cover soils measured in

- laboratory scale soil microcosms. *Appl Environ Microbiol* **61**: 592–601.
- Kuivila KM, Murray JW, Devol AH, Lidstrom ME, Reimers CE. (1988). Methane cycling in the sediments of Lake Washington. *Limn Oceanogr* **33**: 571–581.
- Lee SG, Goo JH, Kim HG, Oh JI, Kim YM, Kim SW. (2004). Optimization of methanol biosynthesis from methane using *Methylosinus trichosporium* OB3b. *Biotechnol Lett* **26**: 947–950.
- Leigh MB, Pellizari VH, Uhlík O, Sutka R, Rodrigues J, Ostrom NE *et al.* (2007). Biphenyl-utilizing bacteria and their functional genes in a pine root zone contaminated with polychlorinated biphenyls (PCBs). *The ISME J* **1**: 134–148.
- Lide DR, Frederikse HPR. (1995). *CRC Handbook of Chemistry and Physics*, 76th edn. CRC Press, Inc: Boca Raton, FL.
- Liebner S, Wagner D. (2007). Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. *Environ Microbiol* **9**: 107–117.
- Martineau C, Whyte LG, Greer CW. (2010). Stable isotope probing analysis of the diversity and activity of methanotrophic bacteria in soils from the Canadian High Arctic. *Appl Environ Microbiol* **76**: 5773–5784.
- McDonald IR, Radajewski S, Murrell JC. (2005). Stable isotope probing of nucleic acids in methanotrophs and methylotrophs: a review. *Org Geochem* **36**: 779–787.
- Miller MC, Prentki RT, Barsdate RJ. (1980). Physics. In: Hobbie JE (ed) *Limnology of Tundra Ponds: Barrow, Alaska* Hutchinson and Ross/Dowden, pp 76–178.
- Mor S, Visscher AD, Ravindra K, Dahiya RP, Chandra A, Cleemput OV. (2006). Induction of enhanced methane oxidation in compost: Temperature and moisture response. *Waste Manage* **2006**: 381–388.
- Phelps AR, Peterson KM, Jeffries MO. (1998). Methane efflux from high-latitude lakes during spring ice melt. *J Geophys Res* **103**: 29029–29036.
- Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM. (2007). Methanotrophy below pH1 by a new Verrucomicrobia species. *Nature* **450**: 874–878.
- Radajewski S, McDonald IR, Murrell JC. (2003). Stable-isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Curr Opin Biotechnol* **14**: 296–302.
- Radajewski S, Webster G, Reay DS, Morris SA, Ineson P, Nedwell DB *et al.* (2002). Identification of active methylotroph populations in an acidic forest soil by stable-isotope probing. *Microbiology* **148**: 2331–2342.
- Rahalkar M, Schink B. (2007). Comparison of aerobic methanotrophic communities in littoral and profundal sediments of Lake Constance by a molecular approach. *Appl Environ Microbiol* **73**: 4389–4394.
- Reay WG, Gallagher D, Simmons GM. (1993). *Sediment-water column nutrient exchanges in Southern Chesapeake Bay nearshore environments*. Virginia Polytechnic Institute and State University: Virginia.
- Reeburgh WS. (2007). Oceanic methane biogeochemistry. *Chem Rev* **107**: 486–513.
- Treude T, Ziebis W. (2010). Methane oxidation in permeable sediments at hydrocarbon seeps in the Santa Barbara Channel, California. *Biogeosciences* **7**: 3095–3108.
- Vries GE, Kües U, Stahl U. (1990). Physiology and genetics of methylotrophic bacteria. *FEMS Microbiol Lett* **75**: 57–101.
- Walter KM, Smith LC, Chapin FS III. (2007). Methane bubbling from northern lakes: present and future contributions to the global methane budget. *Phil Trans R Soc A* **365**: 1657–1676.
- Wang Y, Wooller MJ. (2006). The stable isotopic (C and N) composition of modern plants and lichens from northern Iceland: with ecological and paleoenvironmental implications. *Jökull* **56**: 27–37.
- Wooller MJ, Ruppel C, Pohlman JW, Leigh MB, Heintz M. (2009). Permafrost gas hydrates and climate change: Lake-based seep studies on the Alaskan north slope. *Fire in the Ice* **4**: 6–9.
- Zimov SA, Schuur EAG, Chapin FS III. (2006). Permafrost global carbon budget. *Science* **312**: 1612–1613.

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