

RESEARCH ARTICLE

Methane turnover and methanotrophic communities in arctic aquatic ecosystems of the Lena Delta, Northeast Siberia

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One sentence summary: Lake systems on Samoylov Island (Lena Delta) in contrast to the Lena River showed high variation in the methane concentration, the abundance and composition of MOB communities and consequently methane oxidation rates.

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ABSTRACT

Large amounts of organic carbon are stored in Arctic permafrost environments, and microbial activity can potentially mineralize this carbon into methane, a potent greenhouse gas. In this study, we assessed the methane budget, the bacterial methane oxidation (MOX) and the underlying environmental controls of arctic lake systems, which represent substantial sources of methane. Five lake systems located on Samoylov Island (Lena Delta, Siberia) and the connected river sites were analyzed using radiotracers to estimate the MOX rates, and molecular biology methods to characterize the abundance and the community composition of methane-oxidizing bacteria (MOB). In contrast to the river, the lake systems had high variation in the methane concentrations, the abundance and composition of the MOB communities, and consequently, the MOX rates. The highest methane concentrations and the highest MOX rates were detected in the lake outlets and in a lake complex in a flood plain area. Though, in all aquatic systems, we detected both, Type I and II MOB, in lake systems, we observed a higher diversity including MOB, typical of the soil environments. The inoculation of soil MOB into the aquatic systems, resulting from permafrost thawing, might be an additional factor controlling the MOB community composition and potentially methanotrophic capacity.

Keywords: bacterial methane oxidation; Samoylov Island; global climate change; methane emission; active layer soil; pyrosequencing; pmoA

INTRODUCTION

Methane is an important radiatively active trace gas that is responsible for ~20% of the greenhouse effect (Cicerone and Oremland 1988; IPCC 2014). Large amounts of organic carbon, which can be mineralized to methane and carbon dioxide, are stored in the Arctic permafrost environments (Zimov, Schuur and Chapin 2006; Tarnocai et al. 2009; Knoblauch et al. 2013; Hugelius et al. 2014). With the predicted global climate warming and the resultant thawing of the permafrost, more methane is expected to escape into the atmosphere, contributing positive feedback for climate warming (Wagner, Kobabe and Liebner 2009; Graham et al. 2012; IPCC 2014). Water bodies, which are abundant in the Arctic Lena Delta (8900 km²; Schneider, Grosse and Wagner 2009), represent a transition zone between the soil and the atmosphere and play a key role in methane emission and in the carbon cycle in general (Walter, Smith and Chapin 2007; Bastviken et al. 2011; Boike et al. 2012; Knoblauch et al. 2015). Additionally, water bodies act as transportation systems. The methane dissolved in the water can be carried away by rivers until it reaches the open ocean (Shakhova, Semiletov and Bel'Cheva 2007; Busmann 2013; Crawford et al. 2014).

The process of microbial methane formation, termed methanogenesis, is mediated by methanogenic archaea and occurs under strictly anoxic conditions. There is also evidence of methanogenesis in the oxic zones of the water column, which is linked to algal dynamics and driven by acetoclastic production (Bogard et al. 2014); however, this process needs further investigation. In water bodies, methanogenesis takes place mostly in anoxic sediments (Bartlett et al. 1988) and depends mainly on the supply of organic matter, the availability of electron acceptors and the temperature (Duc, Crill and Bastviken 2010; Lofton, Whalen and Hershey 2014). Methane from the sediments and the water column reaches the atmosphere either by ebullition or by diffusion from a water surface. Additionally, methane flux can be associated with plant-mediated transport in the littoral zones (Knoblauch et al. 2015). The contribution of each pathway to the methane emission rates is uncertain due to their spatial and temporal variations. Ebullition, which acts as a methane transport pathway, was previously underestimated and is now considered to drive methane emissions from the Arctic lakes (Bastviken et al. 2004; Walter, Smith and Chapin 2007). Ebullition can also contribute to the budget of methane dissolved in the water column. According to the estimations of Greene et al. (2014), up to 80% of the methane in bubbles trapped under the ice during the winter can dissolve into the lake water. Thus, each pathway of methane transport, as well as the methane-related processes that affect this transport, is important for understanding the methane dynamics in the Arctic permafrost ecosystems.

The fate of the methane dissolved in water is largely dependent on bacterial methane oxidation (MOX). The main factors controlling the MOX rates are methane and oxygen availability (Hanson and Hanson 1996). In the low- and mid-latitude freshwater, up to 90% of the methane can be oxidized before reaching the atmosphere (Guérin and Abril 2007; Bastviken et al. 2008). At the same time, a very few MOX rate measurements have been conducted in the Arctic freshwater environments, especially in the water column (He et al. 2012; Busmann 2013; Lofton, Whalen and Hershey 2014; Martinez-Cruz et al. 2015). These studies reveal high spatial variability, which makes the assessment of the role of MOX as a methane sink difficult. Additionally, very little is known about the abundance and the diversity of aerobic methane-oxidizing (methanotrophic) bacteria (MOB), which are responsible for MOX in oxic freshwater environments at high latitudes.

Methanotrophic bacteria are a subset of methylotrophic bacteria that are specialized to utilize methane or methanol as a sole source of carbon and energy (Hanson and Hanson 1996). The ability to utilize methane is ensured by the activity of the key enzyme methane monooxygenase (MMO), which can exist in soluble and particulate forms. The particulate form is almost ubiquitous in MOB, while the soluble form is rare. Currently, all known cultivated MOB belong to two subphyla: Alphaproteobacteria (Type II MOB), Gammaproteobacteria (Type I MOB) and the recently discovered phylum Verrucomicrobia (Dunfield et al. 2007; Pol et al. 2007; Islam et al. 2008). Apart from their phylogeny, the differences between Type I and II MOB include the internal membrane arrangement and the carbon assimilation pathway used. Type I methanotrophs have disc-shaped membrane bundles distributed throughout the cytoplasm and assimilate carbon as formaldehyde via the RuMP pathway. Type II methanotrophs have paired internal membrane structures aligned with the periphery of the cell and assimilate formaldehyde via the serine pathway (Hanson and Hanson 1996). Other traits that were previously thought to be exclusive to one type or the other, such as nitrogen fixation, the phospholipid fatty acid profile or the formation of resting stages, have been found to be more widespread throughout methanotrophic Proteobacteria (Knief 2015). Types I and II are further divided into the following subtypes: Ia, Ib, Ic, IIa and IIb. This categorization, however, is not consistent among different publications. In the current study, we will follow the classification proposed by Dumont et al. (2014). According to this classification, Type Ia has *pmoA* sequences affiliated with the classic Type I methanotrophs (i.e. not Type X). Type Ib, also referred to elsewhere as Type X are those methanotrophs that belong to *Methylococcus* and closely related genera. Type Ic are all other Type I-related sequences with a more ambiguous affiliation. Type IIa was used to group the primary *pmoA* sequences of *Methylocystaceae*. Type IIb was used to group all other Type II-related (i.e. *Alphaproteobacteria*) sequences, including those from *Beijerinckiacae* and the alternative pMMO2 identified in some *Methylocystis* species.

Large uncertainties are connected to the factors that determine the community structure of MOB in lake environments. Apart from the various environmental variables that favor the proliferation of certain groups of microorganisms, the microbial community composition can be largely affected by the physical transfer of microorganisms from neighboring terrestrial and aquatic environments (Crump, Amaral-Zettler and Kling 2012).

In this study, we investigated the methane fluxes in the water bodies of Samoylov Island (Lena Delta, Northeast Siberia) and the surrounding Lena River. The main foci of this study were two questions: (1) How variable are the methane concentrations and MOX rate in different types of Arctic water bodies, and what are the controlling environmental factors? And (2) are the methane concentrations and MOX rates related to the diversity and abundance of MOB in Arctic freshwater environments? To better understand the specific importance of the different types of water bodies as methane sources and to assess the methane transport along lake–river transects, our study included five lakes and their outlet streams, which connect these lakes with the surrounding Lena River and the river itself. We assumed that the land–water/water–water transfers of substances and MOB determine the characteristics of the methane flux. Furthermore, these results should help us understand to what extent bacterial MOX contributes as a methane sink in these systems. In addition to the methane concentrations, we measured the MOX rates using radiotracer technique and performed quantitative and qualitative analyses of the methane-oxidizing communities by analyzing the *pmoA* gene, using qPCR and

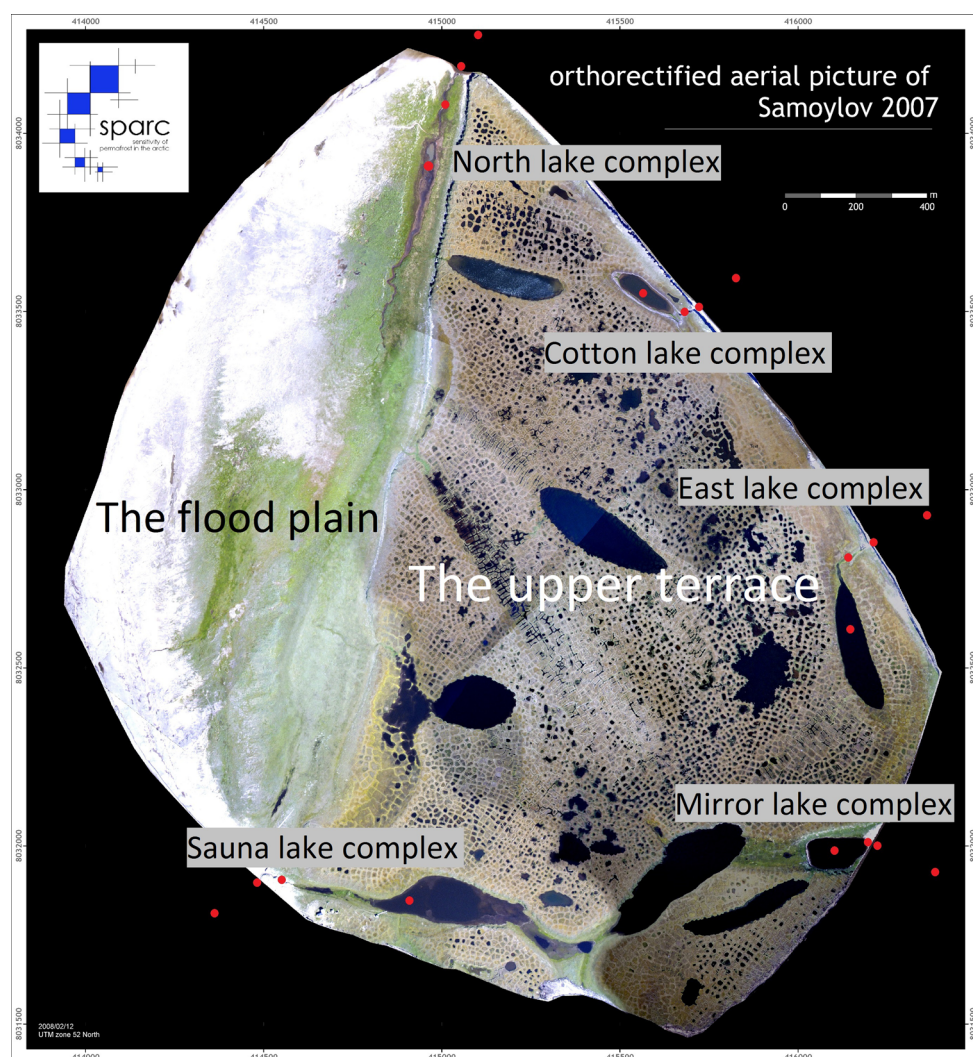


Figure 1. Location of the studied lake complexes on Samoylov Island. Lake names are unofficial and were invented by the authors. The aerial image is provided by Boike, Wille and Abnizova (2008).

pyrosequencing. Environmental factors, such as temperature, salinity, oxygen and suspended particulate matter (SPM), were included in the investigation to study their influence on methane turnover and the methane-oxidizing community.

MATERIALS AND METHODS

Study area and water sampling

With an area of 29 000 km², the Lena Delta is one of the largest deltas in the world (Schneider, Grosse and Wagner 2009). It extends 100 km into the Laptev Sea and is ~400 km wide. Field work was conducted on Samoylov Island, which is representative of the currently active portion of the Lena Delta. The island is located in the central part of the Lena Delta (72.37N, 126.47E). The island hosts a research station that has been operated since the late 20th century, which made it a center for various research campaigns. Samoylov Island consists of a flood plain in the west and an elevated river terrace in the east that is characterized by a polygonal tundra, which is a typical peatland of the Arctic zone (Minke et al. 2007). More details about Samoylov Island are given by Boike et al. 2012. Samples were collected from five lake–river

transects (hereafter called lake complexes), and each included four sampling sites: a lake, its outlet, a river close to the outlet and a river in the middle (Fig. 1). All the lakes were located at the coastline of the island and were of thermokarst origin, with a surface area up to 0.03 km² and a depth up to 6 m. The North Lake complex was located at the flood plain area. The remaining lake complexes were located on the upper terrace. Outlets represented shallow streams (2–10 cm deep) varying from several meters to several tens of meters in length. The Lena River was approximately half a meter deep near the mouth of an outlet and up to 6 m deep in the middle. The geomorphological data of the water bodies investigated are summarized in Table 1.

The sampling campaign took place from 13 July 2012 to 26 July 2012. Water samples from the lakes and the middle of the river were collected from aboard a small rubber boat using a Niskin bottle (KC Denmark, Silkeborg, DK). Due to the shallow depths, water samples from the other locations were collected using 60-mL plastic syringes.

Samples used for the methane concentration and MOX rate measurements, as well as for the molecular studies of MOB, were collected from the surface waters of all the lake complexes (Mirror, Cotton, Sauna, East, and North Lake complexes). The

Table 1. Freshwater characteristics (temperature, salinity, oxygen content and SPM), methane concentrations, MOX rates and MOB abundance in the studied lake complexes. In cases of replicate measurements (technical replicates), the average value with standard deviation is given.

Lake complex	Complex constituents	Coordinates (north latitude, east longitude)	Water surface area (lakes, m ²) and length (outlets, m)	Depth (m)	Methane concentration (nmol L ⁻¹)	MOX rate (nmol L ⁻¹ h ⁻¹)	Abundance of MOB (cells L ⁻¹ *10 ³)	Temperature (°C)	Salinity	Oxygen (mg L ⁻¹)	SPM (mg L ⁻¹)
North lake complex	Lake surf.	72.3867, 126.4826	9000	n.d.	22 974 ± 1442	357 ± 24	41 ± 13	15	0.04	9.5	11.5
	Lake bot.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Outlet	72.3887, 126.4837	200	0.1	5409 ± 110	151 ± 31	18 ± 3	14	0.04	8.96	8.8
	River near the shore	72.3893, 126.4844	n.d.	0.5	6406 ± 626	109 ± 0.4	130 ± 30	14	0.04	9.77	43.72
Mirror lake complex	River middle surf.	72.3916, 126.4870	n.d.	n.d.	153 ± 1	1 ± 0.2	1.6 ± 0.4	17	0	9.68	27.85
	River middle bot.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Lake surf.	72.3701, 126.5185	11500	6	258 ± 7	8 ± 0.3	0.7 ± 0.2	11.3	0	9.82	7.02
	Lake bot.		n.d.	n.d.	240 ± 16	9 ± 1	n.d.	n.d.	0	9.92	29.55
Sauna lake complex	Outlet	72.3706, 126.5224	10	0.1	24 343 ± 176	480 ± 19	24 ± 3	11.3	0.2	9.51	56.81
	River near the shore	72.3705, 126.5233	n.d.	0.5	140 ± 3	0.4 ± 0.03	11 ± 2	17	0	9.52	91.8
	River middle surf.	72.3700, 126.5265	n.d.	n.d.	146 ± 3	0.3 ± 0.01	6 ± 2	n.d.	0	9.54	22.05
	River middle bot.		n.d.	n.d.	146	0.4 ± 0.05	n.d.	n.d.	0	9.39	27.1
East lake complex	Lake surf.	72.3684, 126.4832	27 000	4	747	8.5 ± 0.7	1.8 ± 0.3	n.d.	0	10.02	9.42
	Lake bot.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.
	Outlet	72.3688, 126.4736	100	0.1	912 ± 8	13 ± 0.4	4.8 ± 0.1	n.d.	0	9.02	229.2
	River near the shore	72.3681, 126.4722	n.d.	0.5	150 ± 0.03	0.7 ± 0.03	2.8 ± 1	n.d.	0	8.64	12.27
East lake complex	River middle surf.	72.3668, 126.4697	n.d.	n.d.	197	1 ± 0.1	1.2 ± 1.3	n.d.	0	8.82	8.37
	River middle bot.		n.d.	n.d.	171 ± 10	0.9 ± 0.01	n.d.	n.d.	n.d.	8.44	26.65
	Lake surf.	72.3752, 126.5197	22 000	n.d.	205 ± 6	0.2 ± 0.02	n.d.	15.7	0	9.16	3.55
	Lake bot.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.
Cotton lake complex	Outlet	72.3778, 126.5178	100	0.1	166 ± 0.5	0.6 ± 0.03	n.d.	n.d.	0	9.4	2.52
	River near the shore	72.3790, 126.5193	n.d.	0.5	193 ± 15	1.4 ± 0.2	8.5 ± 2.2	n.d.	0	9.61	32.75
	River middle surf.	72.3798, 126.5232	n.d.	n.d.	146 ± 2	0.7 ± 0.1	8	17	0	9.07	29.97
	River middle bot.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.
Cotton lake complex	Lake surf.	72.3839, 126.5010	8000	6	249 ± 7	0.3 ± 0.03	n.d.	13.4	0	8.3	1.1
	Lake bot.		n.d.	n.d.	308 ± 16	9.1 ± 1.9	n.d.	n.d.	0	8.3	n.d.
	Outlet	72.3835, 126.5045	100	0.1	12 490 ± 293	151 ± 10	1 ± 0.1	13.4	0	8.13	9.95
	River near the shore	72.3837, 126.5061	n.d.	0.5	256 ± 2	1.1 ± 0.1	n.d.	16.5	0	8.11	29.55
n.d.: not determined.	River middle surf.	72.3843, 126.5087	n.d.	n.d.	216 ± 7	1.2 ± 0.1	6 ± 4.7	16.5	0	7.96	25.05
	River middle bot.		n.d.	n.d.	216 ± 3	1.4 ± 0.2	n.d.	n.d.	0	7.95	50.5

n.d.: not determined.

bottom water samples were taken for only the methane concentration and MOX rate measurements from the Mirror and Cotton Lakes and from the mid-river sites of the Mirror, Cotton and Sauna Lake complexes. For the methane concentration and MOX rate measurements, the collected water was transferred bubble-free into 120 mL glass serum bottles that were flushed out with the sampled water several times, capped with black rubber stoppers and sealed with an aluminum crimp according to Bussmann et al. (2015). To eliminate agents that inhibit MOX (such as soap), the glass bottles and the stoppers received extensive chemical cleaning before being used, as suggested by Osudar et al. (2015). Samples used for the methane concentration and MOX measurements were collected in duplicate and triplicate, respectively. Samples for the molecular studies of MOB were collected without replicates.

Temperature, salinity, oxygen and SPM measurements

The temperature, salinity and oxygen content of the water column were measured from the surface of all the sampling sites. In most of the lakes and the mid-river sites, the salinity, temperature and SPM were also measured at the bottom of the water column. The measurements were performed immediately after sampling using a Universal Pocket Meter (Multi 340i, WTW GmbH, Weilheim, Germany) with a precision of 1% for salinity, 0.1°C for temperature and 0.5% for oxygen content. The salinity was measured in $\mu\text{S cm}^{-1}$ and converted according to the Practical Salinity Scale. To measure the SPM, the sampled water was filtered using pre-washed and pre-weighed GFC filters (Whatman, Sigma-Aldrich Chemie, Munich, Germany). After drying the filters for 48 h at room temperature, they were weighed again. The SPM concentration was calculated as the difference between the filter weights before and after filtering divided by the water volume. The water volumes varied from 250 to 400 mL, depending on the turbidity. The measurements of pH at the sampling sites were not performed in the current study. However, it was previously shown that the pH values of the lakes on Samoylov Island vary over a relatively narrow range from 6.8 to 7.5 (Abnizova et al. 2012). The pH of the Lena River also does not substantially vary (7.8–7.9) (Semiletov et al. 2011).

Methane concentration measurements

Immediately after filling, capping and sealing the bottles, 0.3 mL of 5 N NaOH was added to the samples to prevent methane oxidation. NaOH was added using a syringe with a second needle to allow for the displacement of water. The samples were stored in the dark at $\sim +10^\circ\text{C}$ for 1–2 weeks before further processing. Methane concentrations were measured with the headspace technique by adding 10 mL of N_2 (McAuliffe 1971). Three 1 mL headspace aliquots from each sample were analyzed using gas chromatography (Chromatec-Crystal 5000.1, Chromatec, Yoshkar-Ola, Russia). Gas standards (Air Liquide, Kornwestheim, Germany) with concentrations of 10 and 100 ppm methane were used for calibration. The atmospheric equilibrium solubility of the methane in the water (the equilibrium concentrations of the methane in the water column with respect to atmospheric concentrations) was calculated, according to the formula proposed by Wiesenburg and Guinasso (1979). The data measuring the methane concentration of the atmosphere were obtained from the Tiksi Hydrometeorological Observatory in Russia (<http://www.esrl.noaa.gov/gmd/dv/iadv/>). The saturation rates given in percent were calculated as the ratio between the observed methane concentration in the water column and the equilibrium concentrations multiplied by 100.

MOX rate measurements

The MOX rates were measured using the radiotracer technique with tritiated methane (20 Ci mmol^{-1} , American Radiolabeled Chemicals, St. Louis, Missouri, USA) according to a modified method from Bussmann et al. (2015). Immediately after filling the bottles, the diluted tracer (0.1 mL) was added to the samples (2 kBq mL^{-1}). The samples were vigorously shaken and incubated for 11–16 h in the dark at near in situ temperatures ($\sim +15^\circ\text{C}$). After incubation, the MOX was stopped by adding 0.3 mL of 5 N NaOH. The MOX of the control samples was stopped before the addition of the tracer. The MOX rate estimation is the comparison of the total amount of radioactivity added to the water sample (C^*H_4) and the radioactive (tritiated) water (H_2O^*) that was produced due to the oxidation of the tritiated methane. The ambient MOX rate is the ratio of these values ($r = \text{H}_2\text{O}^*/\text{C}^*\text{H}_4$) multiplied by the ambient methane concentration ($[\text{CH}_4]$) corrected for the incubation time (t).

$$\text{MOX} = r \times [\text{CH}_4] / t \quad (1)$$

Additionally, we calculated the turnover time, which is the time it would take to oxidize all the methane at a given MOX rate, assuming that MOX is a first-order reaction. A detailed description of the calculation can be found in a publication by Osudar et al. (2015). To determine the total radioactivity of the sample and the radioactivity of the tritiated water, 4-mL aliquots of water were mixed with 10 mL of the scintillation cocktail (Ultima Gold LLT, Perkin Elmer, Waltham, MA, USA) and analyzed using a liquid scintillation counter (Tri-Carb® 2800 TR, Perkin Elmer, Waltham, MA, USA); decays per minute (dpm) were used as unit.

DNA extraction

DNA from water samples was extracted by filtering 150 mL onto 0.2 μm filters, right after sampling. Afterwards, the filters were stored at -20°C before they were brought to the laboratory for further procedures. The DNA extraction was performed using the RapidWater® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The total DNA concentration was calculated using a Qubit®2.0 (Invitrogen, Carlsbad, CA, USA) fluorometer.

Polymerase chain reaction

Due to the generally low concentrations of MOB DNA in the samples collected from the East and Cotton Lake complexes sequencing was performed using only the samples from the North, Mirror and Sauna lake complexes. The detection of aerobic methanotrophs was performed by targeting the *pmoA* gene, which encodes a subunit of the methane monooxygenase particulate and is a functional marker for most of the representatives of this group. Amplification of the *pmoA* gene was performed using the A189f/mb661r primer pair (Holmes et al. 1995; Costello and Lidstrom 1999; McDonald et al. 2008). The total Polymerase chain reaction (PCR) mixture, which had a volume of 25 μL , contained the following ingredients: HotStar Taq Plus DNA polymerase (Qiagen, Hilden, Germany) ($5 \text{ U } \mu\text{L}^{-1}$), 0.25 μL ; forward and reverse primers (10 μM), 0.5 μL of each; dNTP mix (10 mM), 0.5 μL ; BSA ($0.2 \mu\text{g } \mu\text{L}^{-1}$), 1 μL ; CoralLoad PCR buffer (Qiagen, Hilden, Germany), 2.5 μL ; PCR water, 17.75 μL ; and template, 2 μL . The total DNA concentration of the templates varied from 2 to 20 $\text{ng } \mu\text{L}^{-1}$. The PCR conditions were as follows: initial denaturation and polymerase activation at 95°C for 5 min, 33 cycles

of the denaturation temperature of 95°C for 1 min and an annealing temperature of 57°C for 1 min, elongation at 72°C for 1 min and a final elongation at 72°C for 10 min. The PCR products were loaded onto a 1.5% agarose gel containing GelRed stain (Biotium). Amplicons of the expected size were excised from the gel and purified using the HiYield®PCR Clean-up/Gel Extraction Kit (SLG, Gauting, Germany) according to the manufacturer's protocol.

A second round of PCR was performed using the purified amplicons as a template and primers containing the multiplex identifier (MID). Each sample was analyzed in triplicate. The PCR conditions were the same as for the first PCR, but with 20 cycles. The PCR products were purified using the HiYield®PCR Clean-up/Gel Extraction Kit (SLG, Gauting, Germany) according to the manufacturer's protocol for the purification of PCR products. Finally, 454 pyrosequencing was performed by Eurofins MWG Operon (Ebersberg, Germany).

Pyrosequencing data analysis

MOTHUR software (Schloss et al. 2009) was used for most of the sequence processing and the operational taxonomic unit (OTU) assignments. The sequences that did not pass the translation check using FrameBot (Wang et al. 2013) were discarded from the whole dataset. Afterwards, the sequences with a quality score below 25 were considered to be poor quality and were removed from the dataset. In addition, the sequences that did not have the exact primer sequence, sequences that contained an ambiguous base, sequences with a homopolymer stretch longer than eight bases and sequences shorter than 350 bp were also removed from the datasets in MOTHUR. The remaining sequences were aligned against the pre-aligned *pmoA* nucleotide sequences, which were originally retrieved from the FunGene database (<http://fungene.cme.msu.edu/>). After pre-clustering, a chimera check was conducted in MOTHUR using the default settings. The valid sequences were binned into different OTUs at a cutoff of 0.13, which corresponds to a 97% species cutoff value based on the 16S rRNA genes (Degelmann et al. 2010).

Construction of a phylogenetic tree

A phylogenetic tree was constructed to show the relationship of the *pmoA* gene sequences of aerobic methane-oxidizing bacteria from the investigated water bodies of Samoylov Island, Lena Delta, to the most closely branching *pmoA* gene sequences of the known methanotrophic isolates as references. The *pmoA* gene sequences were selected according to their affiliation with the 28 most abundant OTUs detected in this study.

A phylogenetic tree was built using the NJ (neighbor-joining) tree algorithm of the MEGA6 software with 500 bootstrap replications. The closest relatives were obtained by BLASTing the querying sequences against the NCBI database. The representative sequences for each OTU from our *pmoA* library were used to construct the tree.

Data deposition

The *pmoA* gene sequence data were deposited in the NCBI Sequence Read Archive (SRA) under the submission ID SRP062221.

Quantitative PCR

Quantitative PCR (qPCR) of the *pmoA* gene was performed using the same primer pair: A189f/mb661r. The total PCR mixture,

which had a volume of 12.5 mL, contained the following ingredients: SYBR Green Master Mix (Bio-Rad, Munich, Germany), 6.25 µL; forward and reverse primers (10 µM), 0.5 µL of each; PCR-grade water, 0.25 µL; and template, 5 µL. The PCR conditions were as follows: the initial denaturation and polymerase activation at 95°C for 10 min, 37 cycles of the denaturation temperature at 95°C for 30 s and the annealing temperature at 62°C for 30 s, elongation at 72°C for 45 s, and the denaturation of the primer dimers at 80°C for 3 s.

The cell numbers were estimated according to Kolb et al. (2003), assuming that each bacterial cell contains, on average, two copies of the *pmoA* gene. The detection limit, determined by the volume of the water samples collected and the DNA concentration, was 10² cells L⁻¹.

Statistical analysis

The methane concentrations and MOX rates for each sampling site are given as averages (arithmetic means) of the procedural triplicates and duplicates, respectively. MOB abundance and the relative abundance of the OTUs are given as averages of the technical triplicates. When several sampling sites were combined as described, the average of all the replicate measurements performed at the mentioned sampling sites is given with the standard deviation (SD). The SDs of the measurements performed at the individual sampling sites are omitted in the main text but are given in Table 1 and Fig. 2. The SD of the relative abundance of most of the OTUs did not exceed 3% of the total abundance of the OTUs at the sampling site; thus, these SDs are not shown. To investigate if the methane concentration, the MOX rate and the MOB abundance, are correlated with each other and dependent on the temperature or the SPM, we performed simple linear regression analyses. When the linear correlation was not significant or the power of the performed test was less than desired due to the dispersion of the data, we performed a Spearman rank-order correlation analysis, which indicates whether the variables are monotonically related or not, i.e. if an increase in one variable causes an increase/decrease in the other variable.

RESULTS

Most of the measurements (methane concentrations, MOX rates and MOB abundance) revealed that all the lake complexes could be divided into two different groups according to their location. The first group included the lake complexes on the upper terrace, while the second group included the North Lake complex, which was located in the flood plain area. Thus, these sampling sites will be described separately. All the measurements are summarized in Table 1 and are published on www.pangaea.de. The measurements of the methane concentrations, MOX rates and the MOB abundance, are also presented in Fig. 2a, b and c, respectively.

Freshwater characteristics: temperature, salinity, oxygen content and SPM

The water temperature of all the sampling sites varied from 11°C to 17°C and was 2°C–4°C higher in the river than in the lakes and the outlets. The salinity varied from 0 to 0.2. The oxygen content varied relatively little, at a range from 8 to 10 mg L⁻¹. No difference between the surface and bottom water was detected for the oxygen content. The SPM varied from 1 to 230 mg L⁻¹. In the river sites, the SPM was generally higher (32 ± 20 mg L⁻¹) than in the

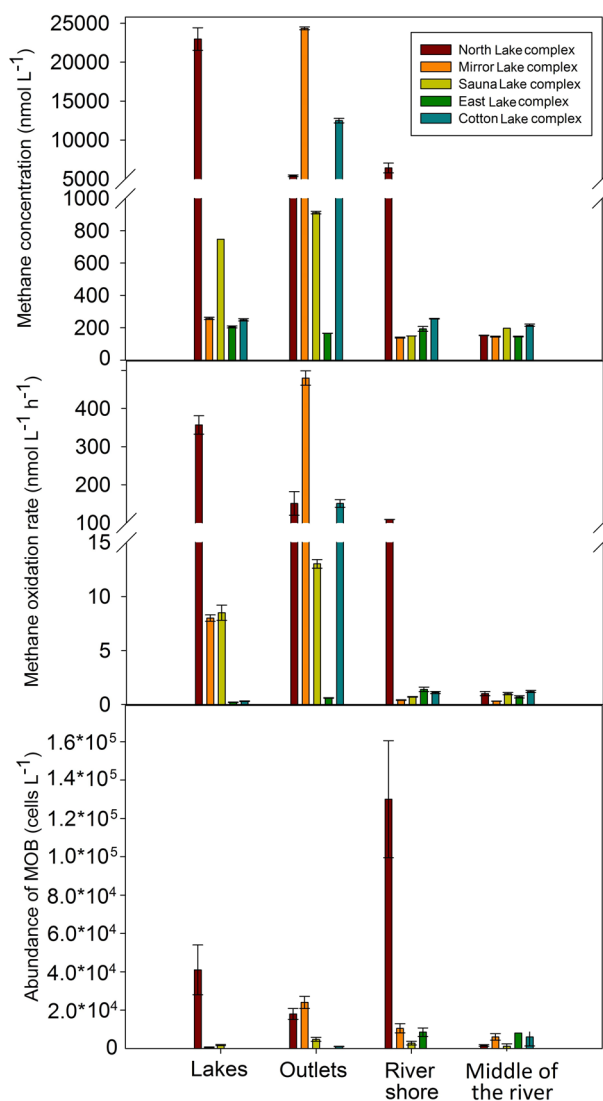


Figure 2. Methane concentrations, MOX rate and the abundance of methane-oxidizing bacteria in the surface water of the different lake complexes. Data represent the mean of technical replicates (\pm SD). Measurements of methane concentrations and abundance of methane-oxidizing bacteria are performed in triplicate, and MOX rates are performed in duplicate.

lakes (10 ± 10 mg L⁻¹). The SPM in the outlets was the highest on average (60 ± 97 mg L⁻¹); however, it varied over a wide range of 3 to 230 mg L⁻¹. We did not find a correlation between any of these parameters and the methane concentration, the MOX rate or the MOB abundance.

Methane concentration in the water column

The calculated equilibrium methane concentration in the water column with respect to the methane concentration in the atmosphere was ~ 4 nmol L⁻¹. The methane concentrations of all the sampling sites varied over two orders of magnitude, from 140 to 24 000 nmol L⁻¹. Thus, all investigated water bodies were supersaturated with methane. The saturation rates varied from $\sim 3500\%$ to 600 000%. The surface and bottom methane concentrations were comparable (the ratio between the average values did not exceed 1.3) for both the lakes (Mirror Lake and Cotton Lake) and the sites in the middle of the river (Mirror, Cotton

and Sauna). Because our data set from the surface was more consistent, we will focus on the surface data in the following discussion.

The distribution of the methane concentrations along the lake complex of the upper terrace generally followed a specific trend: from the lake to the outlet, the concentration rose (up to 100 times—Mirror Lake) or stayed the same but dropped upon reaching the river sites. The methane concentrations of these lakes varied over a relatively narrow range, with an average of 370 ± 260 nmol L⁻¹ and were slightly higher than those of the river sites, which had an average of 180 ± 40 nmol L⁻¹. The outlets varied over a wide range, from 210 (East Lake complex) to 24 000 nmol L⁻¹ (Mirror Lake complex). In comparison with the upper terrace lake complexes, the North Lake complex had substantially higher methane concentrations in the lake and in the river near the shore (23 000 and 6400 nmol L⁻¹, respectively), while in the outlet and the middle of the river (5400 and 150 nmol L⁻¹, respectively), the methane concentrations were comparable.

MOX rates in the water column

The MOX rates of the surface and the bottom of Mirror Lake and of the three river sites were comparable (the ratio between the surface and bottom average values did not exceed 1.4), except in Cotton Lake, which had MOX rates 30 times higher at the bottom than at the surface. The MOX rates were correlated with the methane concentrations (Spearman Rank Order Correlation, $r_s = 0.84$, $n = 20$; Fig. s2, Supporting Information). However, there were some deviations. Despite comparable methane concentrations, the MOX rates of the Mirror and Sauna Lakes (8 and 8.5 nmol L⁻¹ h⁻¹, respectively) were higher than those of the East and Cotton Lakes (0.2 and 0.3 nmol L⁻¹ h⁻¹, respectively) and higher than those of the corresponding river sites. The MOX rates were generally the highest in the outlets, where they varied over a wide range, from 0.6 (East Lake complex) to 480 nmol L⁻¹ h⁻¹ (Mirror Lake complex). The MOX rates of the river sites of the upper terrace were comparable (average of 0.8 ± 0.4 nmol L⁻¹ h⁻¹). In comparison with the upper terrace lake complexes, the North Lake complex had substantially higher MOX rates in the lake and in the river near the shore (360 and 8 nmol L⁻¹ h⁻¹, respectively), while in the outlet and the middle of the river (150 and 1 nmol L⁻¹ h⁻¹, respectively), the MOX rates were comparable with those of the other lake complexes.

Abundance of methane-oxidizing bacteria (MOB)

The total amount of DNA calculated related to the volume of the filtered water varied from 1 to 14 ng mL⁻¹. The highest concentrations (8–14 ng mL⁻¹) were detected at the North Lake complex (except for the river in the middle site) and at the Mirror and Sauna streams. The concentration of the total DNA from most of the other sites did not exceed 4 ng mL⁻¹.

The MOB abundance was positively correlated with the methane concentrations, but only at the lake–outlet complexes (Spearman Rank Order Correlation, $r_s = 0.75$, $n = 7$; Fig. s3, Supporting Information). The deviations included the lakes of the upper terrace. Despite the comparable methane concentrations, the East and Cotton Lakes had a lower MOB abundance ($<10^2$ cells L⁻¹) than the Mirror and Sauna Lakes (0.7×10^3 and 1.8×10^3 cells L⁻¹, respectively). The river sites of the upper terrace had a slightly higher MOB abundance (average of $6.2 (\pm 3.4) \times 10^3$ cells L⁻¹) than the Mirror and Sauna Lakes. The cell

numbers were generally the highest in the outlets, varying from 4.8×10^3 cells L^{-1} (Sauna Lake complex) to 24.0×10^3 cells L^{-1} (Mirror Lake complex). The North Lake complex had substantially higher MOB abundance in the lake and in the river near the shore (41×10^3 and 130×10^3 cells L^{-1} , respectively), while in the outlet and the middle of the river, the MOB abundance was comparable to the rest of the complexes (18×10^3 and 1.6×10^3 cells L^{-1} , respectively).

Diversity of MOB

The number of sequences analyzed per sampling site was ~5000 on average (Fig. S1, Supporting Information). The total number of the OTUs with a cutoff of 0.13 (Degelmann et al. 2010) was 391. A total of 28 OTUs, each of which represented >3% of the total abundance of at least one sampling site, were identified. The other OTUs represented minor groups (<3% each) and together constituted ~13% of the total MOB abundance. The most relatively abundant OTUs (>3%) were clustered into five groups: Types Ia, -b and -c and Types IIa and -b. (Dumont et al. 2014). Their relation to the previously isolated MOB strains is demonstrated using the neighbor-joining tree (Fig. 3). Type Ia was represented by a single OTU (OTU 14) related to *Methylobacter*. Type Ib was represented by 12 OTUs. According to the classification proposed by Dumont et al. (2014), we subclustered these OTUs into the following groups (typical habitats or origin are in brackets): FWs, LWs (freshwater lakes), RPCs, RPC-1, JRC-4 (rice field soil) and OSC-related (organic soil). Type Ic was represented by a single OTU (OTU 65), which belonged to the USC-g (upland soils) cluster (Dumont et al. 2014). Type IIa was represented by 13 OTUs from the genus *Methylocystis*. Type IIb was represented by a single OTU (OTU 31) that belongs to the genus *Methylocapsa*.

The relative abundance of the main clusters is summarized in Table S1 (Supporting Information), and together with the most relatively abundant OTUs is shown in Fig. 4. In all three investigated lakes, Type Ib was prevalent, constituting 60%–70% of the total MOB abundance. At the North Lake complex, Type Ib was also dominant in the outlet (77%) and in the river near the shore (76%). At the outlets and river sites of the East and Sauna Lake complexes, Type IIa MOB (46%–68% of the total MOB abundance) was dominant. The remaining clusters were minor ($\leq 5\%$). The Type Ia (*Methylobacter*) cluster was the only exception, constituting 6% and 14% of the total MOB abundance of the Mirror Lake and its outlet, respectively.

Cluster analysis based on the abundance of the dominant OTUs (Fig. 4) indicated that the sampling sites could be divided into river sites and lake–outlet complexes. The river sites (except for the North river near the shore site) were very similar to each other compared to the lake–outlet complexes, with the *Methylocystis* cluster (OTUs 1, 2, 5) and the FWs cluster (OTUs 3, 16) being dominant. The neighbor-joining tree (Fig. 3) also showed that the OTUs that were abundant in the river sites were related to previously identified MOB strains typical for water rather than for soil environments. The other group contained the lakes and outlets. The North Lake complex (the lake, the outlet and the river near the shore) was characterized by the high abundance of the RPCs cluster (42%–59%), while at the rest of the sampling sites, the relative abundance of this cluster did not exceed 8%. Sauna Lake differed from the rest of the lakes in its high abundance of the RPC-1 cluster (29%). Mirror Lake differed from the other sampling sites because it was the only habitat where the JRC-4 and OSC-related clusters were relatively abundant (38% and 25%, respectively). On the contrary, the abundance of the FW clusters in Mirror Lake was minor in comparison with the other lake–outlet

complexes. The outlets of the Mirror and Sauna Lakes were dominated by the *Methylocystis* cluster, but the OTUs within the cluster differed from those dominant in the river sites.

DISCUSSION

The fresh water bodies in the Arctic permafrost environment are an important ecosystem involved in the global methane cycle. Our results show that the water bodies, which included lakes, their outlets and the Lena River, represent a heterogeneous system with large variations in methane distribution, MOX rates and methane-oxidizing community structure and abundance.

All the water bodies investigated were supersaturated with methane in relation to the atmosphere; thus, they act as a methane source for the atmosphere (Middelburg et al. 2002; Bange 2006). The methane distribution in all the lakes and outlets was highly variable, which is remarkable, given the small size of Samoylov Island (4.34 km^2). The methane concentrations in the lakes of the upper terrace varied over a relatively narrow range ($365 \pm 256 \text{ nmol L}^{-1}$) and were substantially lower than those of the North Lake ($22\,974 \pm 1442 \text{ nmol L}^{-1}$). All these values are in the range of previously reported methane concentrations of high-latitude lakes and ponds (Table 2). The uniqueness of North Lake is likely related to its location in the flood plain area of the island. All of the lakes investigated, except for Eastern Lake, are flooded in the spring at a different periodicity. This flooding, however, has a local, sporadic impact, while the flood plain area, which is flooded annually, forms a unique ecosystem also in regard to the vegetation (Boike et al. 2012). The production of methane, which takes place mainly in lake sediments, is strongly dependent upon carbon loading (Zimov et al. 1997). We expect that the substantial supply of organic matter brought by the river leads to higher methane concentrations. Flooding can also result in the input and transport of nutrients and other substances. Indeed, the concentrations of many ions are higher in the North Lake (Abnizova et al. 2012; Chetverova et al. 2013). Additionally, high proportions of silt and clay material in the flood plain soil support the availability and uptake of substrates (Liebner and Wagner 2007), which may result not only in enhanced MOX but also in methane production.

In most of the outlets of the upper terrace, the methane concentrations were higher (up to 100 times) than in the corresponding lakes. Shallow streams can accumulate organic carbon in sediments, which results in enhanced methane production (Sanders et al. 2007). Additionally, a higher saturation of methane diffusing from the outlet sediments is explained by the small volume of the outlets. Our findings corroborate the hypothesis that streams might be important sources of methane (Crawford et al. 2014).

Upon reaching the river, the methane-rich water from the outlet is diluted with the methane-poor river water. On the upper terrace, the methane concentrations of the river near the shore did not substantially differ from those of the middle of the river. However, at the North Lake complex, the methane concentrations of the river near the shore remained high. This indicates that, at least in some cases, the input of methane from the streams into the river can be significant and can alter the concentrations of methane, at least in the near-shore area.

The MOX rates at all the sampling sites varied from 0.2 to $480 \text{ nmol L}^{-1} \text{ h}^{-1}$, which is in the range previously reported in studies of high-latitude lakes and rivers (Table 2). The turnover time of methane varied from 1 to 43 days, at an average of 10 ± 11 days, indicating that MOX can be an important methane

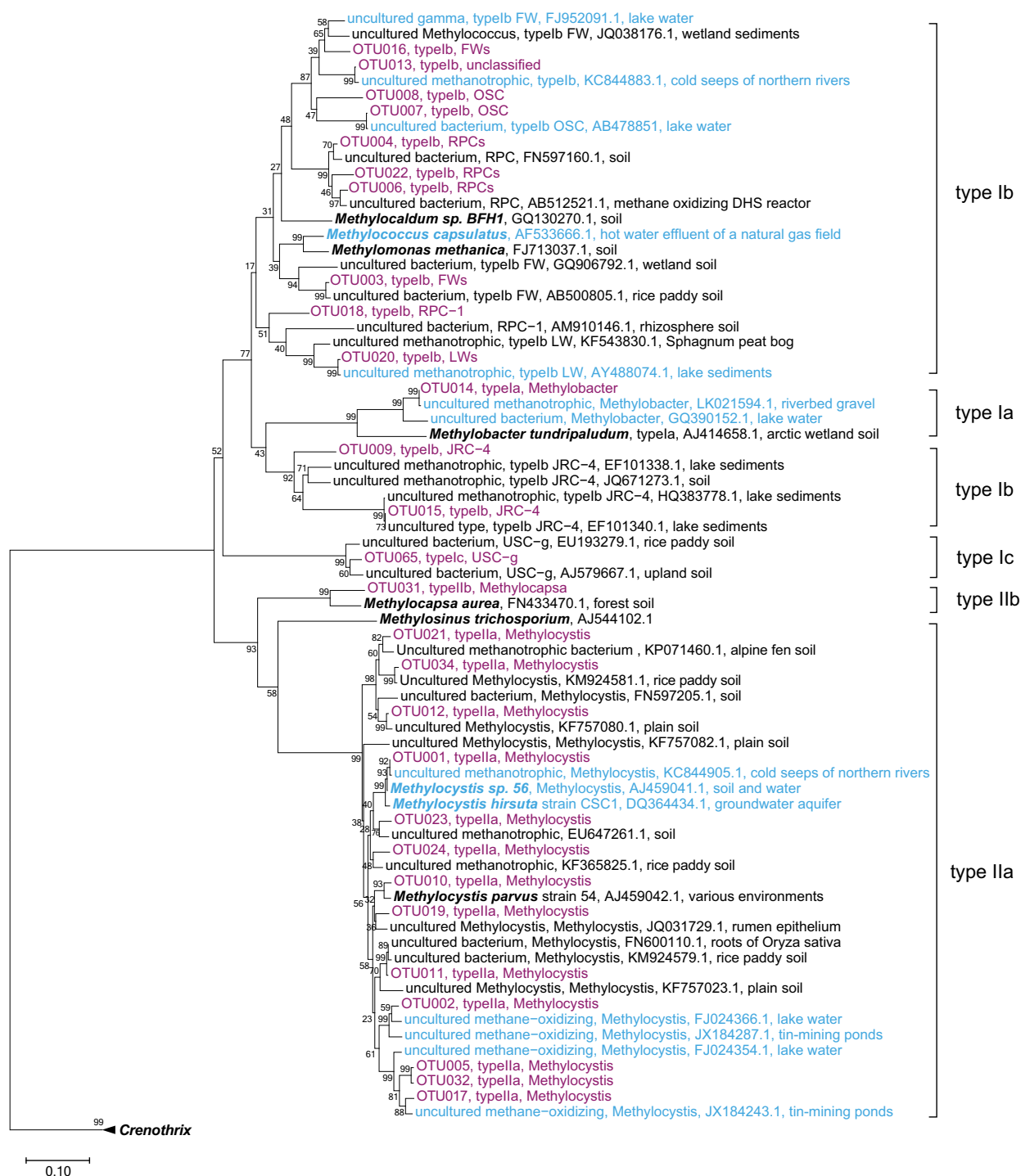


Figure 3. A neighbor-joining tree of *pmoA* gene sequences, showing the relationship between the most abundant OTUs from this study (red color font) and strains from previous studies. Blue or black font indicates the strains obtained from the aquatic and terrestrial environments, respectively. A neighbor-joining tree was built with Mega6 software, NJ (neighbor-joining) tree algorithm, with bootstrap of 500 times. The close relatives were obtained by blasting the querying sequences against the NCBI database.

sink in lakes, streams and rivers. The MOX rates generally coincided with the methane concentrations, especially when we compared the sites with low and high methane concentrations (Fig. s2, Supporting Information). A positive correlation between the MOX rates and the methane concentrations was shown in many other studies (Gentz et al. 2013; Jakobs et al. 2013; Osudar et al. 2015). The abundance of MOB, in turn, was gen-

erally positively correlated to the methane concentration (Fig. s3, Supporting Information). However, at our study sites, neither the methane concentration nor the MOB abundance could fully explain the observed differences in the MOX rates. According to Mau and colleagues (2013), the size of the MOB community is an important variable for methane flux, but we showed that the community structure also controls the effectiveness of

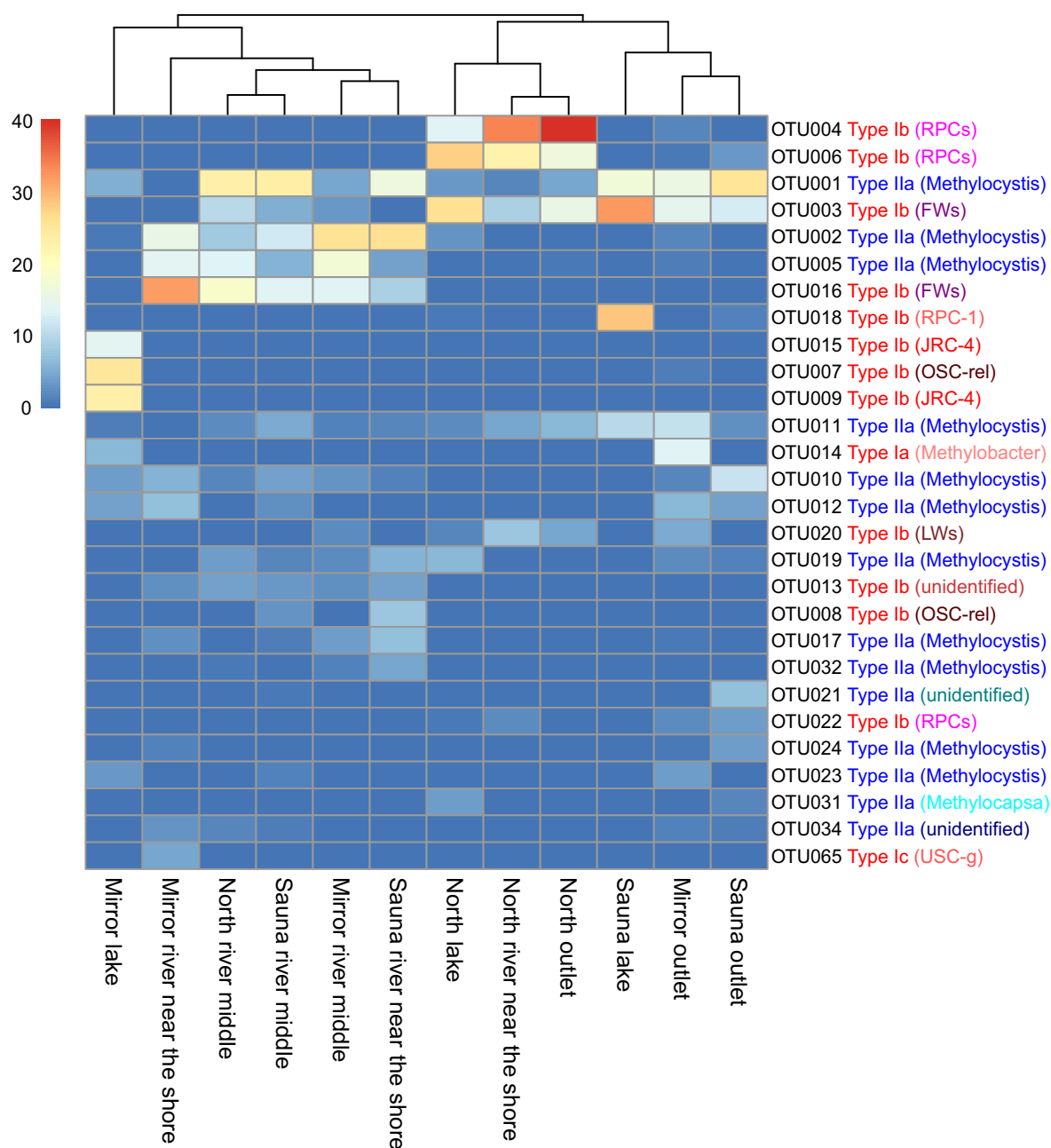


Figure 4. Heat map showing the relative abundance (%) of the most prevalent OTUs demonstrating the relationship between the MOB communities from the different sampling sites.

methane oxidation. For instance, the Mirror and Sauna lakes had methane concentrations comparable with the river sites but had a lower abundance of MOB and higher MOX rates. Therefore, we assume that MOB in these lakes are more efficient in oxidizing methane. Indeed, the analysis of the MOB community structure revealed that the lakes, outlet streams and river showed individual patterns in terms of their MOB community structure. Though we did not find any correlation between the MOX rates and the relative abundance of any MOB cluster, the sites with the highest MOX rates were characterized by a higher diversity of the MOB clusters (Table s1, Supporting Information). Thus, for example, the river sites were characterized by a rather homogeneous MOB community, with the dominance of three OTUs

related to the genus *Methylocystis* and two OTUs related to the FWs cluster. The MOB composition of the lake-outlet complexes was more heterogeneous. Apart from the MOB clusters common for all the sampling sites, each lake and outlet had unique MOB clusters. Additionally, the dominant OTUs of the river sites were related to strains typical of water rather than of soil environments, while in the lakes and outlets, the trend was opposite. Therefore, it is reasonable to assume that the lakes and outlets are more affected by the input of MOB from the soil environment, which occurs due to erosion and flooding. In the outlets, the inoculation of MOB from the sediments additionally occurs due to high water velocity and sediment resuspension. The highest diversity of MOB in this study was observed

Table 2. Methane concentrations and MOX rates in the water columns of high-latitude freshwater lakes and rivers.

Sampling area	Methane concentration (nmol L ⁻¹)	MOX rate (nmol L ⁻¹ h ⁻¹)	Reference
Lakes and ponds			
North Slope of Alaska (9 lakes)	80–16 700, 165 000 ¹	n.d.	Kling, Kipphut and Miller (1992)
Canadian High Arctic (four ponds)	1000–3400	n.d.	Negandhi et al. (2013)
Western Siberia (three lakes)	66–7800	n.d.	Repo et al. (2007)
North Slope of Alaska (two lakes)	780–1520	2–340	Lofton, Whalen and Hershey (2014)
North slope of Alaska and Alaska's interior (two lakes)	n.d.	0–60000	He et al. (2012)
Alaska (30 lakes)	<600–940 000	0–1400	Martinez-Cruz et al. (2015)
Northeast Siberia (five lakes)	200–23 000	0.2–360	This study
Rivers			
North Siberia, Ob estuary	7–41	n.d.	Shakhova, Semiletov and Bel'Cheva (2007)
North Siberia, Yenisei estuary	7–131	n.d.	Shakhova, Semiletov and Bel'Cheva (2007)
Northeast Siberia, Lena estuary	62–651	n.d.	Shakhova, Semiletov and Bel'Cheva (2007)
Northeast Siberia, Lena estuary	30–85	n.d.	Bussmann (2013)
Northeast Siberia, Lena estuary	150–200	0.3–1	This study

n.d.: not determined.

¹The only measurement, which substantially stood out of the rest.

in the outlets, which corroborates the observations of Crump and colleagues (2012). At the same time, presumably terrestrial MOB were not restricted to the Type I or Type II clusters. The lakes were dominated by Type I MOB, while the outlets and river sites were dominated by Type II MOB.

The high abundance of Type II MOB in all the water bodies, however, is remarkable, regardless of its origin. Most of the previous research showed that Type I MOB generally dominate over Type II, both in the freshwaters of the temperate zone (including sediments; Costello et al. 2002; Rahalkar et al. 2009, and the water column; Eller, Känel and Krüger 2005; Sundh, Bastviken and Tranvik 2005; Tsutsumi et al. 2011), as well as in the Arctic wetlands (Wartiainen, Hestnes and Svenning 2003; Graef et al. 2011). Methane and oxygen concentrations as the controlling factors (Hanson and Hanson 1996) could not explain the abundance of Type II MOB in our study. Ho et al. (2013), suggested that according to the universal adaptive strategy theory, Type I MOB should be classified as competitor-ruderal organisms. This could explain the predominance of Type I MOB in the Arctic wetlands, which are exposed to freeze–thaw cycles. Consequently, the presence of the Type II MOB cluster in our study can be explained by the rather stable environmental conditions with lower disturbance. The thermal regimes of the permafrost-affected soil and the lakes and rivers are different. The lakes of this study, which were 4–6 m deep, do not freeze to the bottom in the winter and are most likely underlain with layers of year-round unfrozen ground called taliks (Boike et al. 2015). The same is true for the Lena River (Costard and Gautier 2007). An alternative hypothesis is that Type II methanotrophs become relatively abundant in the summer, due to the warming of the surface water layer. All the samples were collected from the surface during the summer when the water temperature varied between 11°C and 15°C in the lakes and was ~17°C in the river. Type II MOB were shown to successfully compete with Type I MOB in low-latitude soils starting at 15°C (Börjesson, Sundh and Svensson 2004).

CONCLUSION

This study contributes to the understanding of methane fluxes in the Arctic water bodies of the Lena Delta area, including

the lakes, the outlets and the river. Our results suggest that the MOX rates are determined by the methane distribution and the abundance and diversity of MOB. The lake–outlet complexes, in contrast to the Lena River, represent a more heterogeneous ecosystem, which is reflected in the high variation of the MOX rates and the controlling environmental factors. The lake complexes thus appear as more individual systems that have a pronounced interaction with the surrounding soil environment. The flood plain area, for example, facilitates the increased availability of substrates and represents a potential 'hot spot' for methane emissions. The Lena River sites represent a connected and, consequently, more uniform environment with a weaker terrestrial imprint. With the expected climate change and consequent thawing of the permafrost, more terrestrial MOB will be transferred into the aquatic environments, most likely leading to a more pronounced terrestrial methanotrophic fingerprint.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflict of interest. None declared.

REFERENCES

- Abnizova A, Siemens J, Langer M et al. Small ponds with major impact: The relevance of ponds and lakes in permafrost landscapes to carbon dioxide emissions. *Global Biogeochem Cy* 2012;26:GB2041.
- Bange HW. Nitrous oxide and methane in European coastal waters. *Estuarine Coast Shelf S* 2006;70:361–74.
- Bartlett KB, Crill PM, Sebacher DI et al. Methane flux from the central Amazonian floodplain. *J Geophys Res: Atmos* 1988;93:1571–82.
- Bastviken D, Cole J, Pace M et al. Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem Cy* 2004;18:GB4009.
- Bastviken D, Cole JJ, Pace ML et al. Fates of methane from different lake habitats: connecting whole-lake budgets and CH₄ emissions. *J Geophys Res-Bioge* 2008;113:G02024.
- Bastviken D, Tranvik LJ, Downing JA et al. Freshwater methane emissions offset the continental carbon sink. *Science* 2011;331:50.
- Bogard MJ, Del Giorgio PA, Boutet L et al. Oxic water column methanogenesis as a major component of aquatic CH₄ fluxes. *Nat Commun* 2014;5:5350.
- Boike J, Georgi C, Kirilin G et al. Physical processes of thermokarst lakes in the continuous permafrost zone of northern Siberia: observations and modeling (Lena River Delta, Siberia). *Biogeosci Discuss* 2015;12:6637–88.
- Boike J, Kattenstroth B, Abramova K et al. Baseline characteristics of climate, permafrost, and land cover from a new permafrost observatory in the Lena River Delta, Siberia (1998–2011). *Biogeosci Discuss* 2012;9:13627.
- Boike J, Wille C, Abnizova A. Climatology and summer energy and water balance of polygonal tundra in the Lena River Delta, Siberia. *J Geophys Res-Bioge* 2008;113:G03025.
- Börjesson G, Sundh I, Svensson B. Microbial oxidation of CH₄ at different temperatures in landfill cover soils. *FEMS Microbiol Ecol* 2004;48:305–12.
- Bussmann I. Distribution of methane in the Lena Delta and Buor Khaya Bay, Russia. *Biogeosciences* 2013;10:4641–52.
- Bussmann I, Matousu A, Osudar R et al. Assessment of the radio 3H-CH₄ tracer technique to measure aerobic methane oxidation in the water column. *Limnol Oceanogr-Meth* 2015;13:312–27.
- Chetverova A, Fedorova I, Potapova T et al. Hydrological and geochemical features of lakes of Samoylov Island of the Lena River Delta. *Proc AARI*, 2013 2013;1:97–110.
- Cicerone RJ, Oremland RS. Biogeochemical aspects of atmospheric methane. *Global Biogeochem Cy* 1988;2:299–327.
- Costard F, Gautier E. The Lena River: hydromorphodynamic features in a deep permafrost zone. *Large Rivers: Geomorphol Manage* 2007:225–33.
- Costello AM, Auman AJ, Macalady JL et al. Estimation of methanotroph abundance in a freshwater lake sediment. *Environ Microbiol* 2002;4:443–50.
- Costello AM, Lidstrom ME. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microb* 1999;65:5066–74.
- Crawford JT, Lottig NR, Stanley EH et al. CO₂ and CH₄ emissions from streams in a lake-rich landscape: Patterns, controls, and regional significance. *Global Biogeochem Cy* 2014;28:197–210.
- Crump BC, Amaral-Zettler LA, Kling GW. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME J* 2012;6:1629–39.
- Degelmann DM, Borken W, Drake HL et al. Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils. *Appl Environ Microb* 2010;76:3228–35.
- Duc N, Crill P, Bastviken D. Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. *Biogeochemistry* 2010;100:185–96.
- Dumont MG, Lüke C, Deng Y et al. Classification of pmoA amplicon pyrosequences using BLAST and the lowest common ancestor method in MEGAN. *Front Microbiol* 2014;5:34.
- Dunfield PF, Yuryev A, Senin P et al. Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature* 2007;450:879–82.
- Eller G, Känel L, Krüger M. Cooccurrence of aerobic and anaerobic methane oxidation in the water column of Lake Plußsee. *Appl Environ Microb* 2005;71:8925–8.
- Gentz T, Damm E, Schneider von Deimling J et al. A water column study of methane around gas flares located at the West Spitsbergen continental margin. *Cont Shelf Res* 2013;72:107–18.
- Graef C, Hestnes AG, Svenning MM et al. The active methanotrophic community in a wetland from the high Arctic. *Environ Microbiol Rep* 2011;3:466–72.
- Graham DE, Wallenstein MD, Vishnivetskaya TA et al. Microbes in thawing permafrost: the unknown variable in the climate change equation. *ISME J* 2012;6:709–12.
- Greene S, Walter Anthony K, Archer D et al. Modeling the impediment of methane ebullition bubbles by seasonal lake ice. *Biogeosciences* 2014;11:6791–811.
- Guérin F, Abril G. Significance of pelagic aerobic methane oxidation in the methane and carbon budget of a tropical reservoir. *J Geophys Res-Bioge* 2007;112:G03006.
- Hanson RS, Hanson TE. Methanotrophic bacteria. *Microbiol Rev* 1996;60:439–71.
- He R, Wooller MJ, Pohlman JW et al. Diversity of active aerobic methanotrophs along depth profiles of arctic and subarctic lake water column and sediments. *ISME J* 2012;6:1937–48.
- Ho A, Kerckhof FM, Luke C et al. Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environ Microbiol Rep* 2013;5:335–45.
- Holmes AJ, Costello A, Lidstrom ME et al. Evidence that participate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiol Lett* 1995;132:203–8.
- Hugelius G, Strauss J, Zubrzycki S et al. Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps. *Biogeosciences* 2014;11:6573–93.
- IPCC 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri RK, Meyer LA (eds). *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC, 2014, 151.
- Islam T, Jensen S, Reigstad LJ et al. Methane oxidation at 55 C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. *P Natl Acad Sci USA* 2008;105:300–4.
- Jakobs G, Rehder G, Jost G et al. Comparative studies of pelagic microbial methane oxidation within two anoxic basins of the central Baltic Sea (Gotland Deep and Landsort Deep). *Biogeosci Discuss* 2013;10:12251–84.

- Kling GW, Kipphut GW, Miller MC. The flux of CO₂ and CH₄ from lakes and rivers in arctic Alaska. *Hydrobiologia* 1992;**240**:23–36.
- Knief C. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Front Microbiol* 2015; **6**:1346.
- Knoblauch C, Beer C, Sosnin A et al. Predicting long-term carbon mineralization and trace gas production from thawing permafrost of Northeast Siberia. *Global Change Biol* 2013;**19**:1160–72.
- Knoblauch C, Spott O, Evgrafova S et al. Regulation of methane production, oxidation, and emission by vascular plants and bryophytes in ponds of the northeast Siberian polygonal tundra. *J Geophys Res-Biogeophys* 2015;**120**:2525–41.
- Kolb S, Knief C, Stubner S et al. Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays. *Appl Environ Microb* 2003;**69**:2423–9.
- Liebner S, Wagner D. Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. *Environ Microbiol* 2007;**9**:107–17.
- Lofton DD, Whalen SC, Hershey AE. Effect of temperature on methane dynamics and evaluation of methane oxidation kinetics in shallow Arctic Alaskan lakes. *Hydrobiologia* 2014;**721**:209–22.
- McAuliffe C. G.C. determination of solutes by multiple phase equilibrium. *Chem Tech* 1971;**1**:46–51.
- McDonald IR, Bodrossy L, Chen Y et al. Molecular ecology techniques for the study of aerobic methanotrophs. *Appl Environ Microb* 2008;**74**:1305–15.
- Martinez-Cruz K, Sepulveda-Jauregui A, Walter Anthony K et al. Geographic and seasonal variation of dissolved methane and aerobic methane oxidation in Alaskan lakes. *Biogeosci Discuss* 2015;**12**:4213–43.
- Mau S, Blees J, Helmke E et al. Vertical distribution of methane oxidation and methanotrophic response to elevated methane concentrations in stratified waters of the Arctic fjord Storfjorden (Svalbard, Norway). *Biogeosciences* 2013;**10**:6267–78.
- Middelburg J, Nieuwenhuize J, Iversen N et al. Methane distribution in European tidal estuaries. *Biogeochemistry* 2002;**59**:95–119.
- Minke M, Donner N, Karpov NS et al. Distribution, diversity, development and dynamics of polygon mires: examples from Northeast Yakutia (Siberia). *Peatlands Int* 2007;**1**:36–40.
- Negandhi K, Laurion I, Whitticar MJ et al. Small thaw ponds: an unaccounted source of methane in the Canadian High Arctic. *PLoS ONE* 2013;**8**:e78204.
- Osudar R, Matoušů A, Alawi M et al. Environmental factors affecting methane distribution and bacterial methane oxidation in the German Bight (North Sea). *Estuarine Coast Shelf S* 2015;**160**:10–21.
- Pol A, Heijmans K, Harhangi HR et al. Methanotrophy below pH 1 by a new Verrucomicrobia species. *Nature* 2007;**450**:874–8.
- Rahalkar M, Deutzmann J, Schink B et al. Abundance and activity of methanotrophic bacteria in littoral and profundal sediments of Lake Constance (Germany). *Appl Environ Microb* 2009;**75**:119–26.
- Repo ME, Huttunen JT, Naumov AV et al. Release of CO₂ and CH₄ from small wetland lakes in western Siberia. *Tellus B* 2007;**59**:788–96.
- Sanders IA, Heppell CM, Cotton JA et al. Emission of methane from chalk streams has potential implications for agricultural practices. *Freshwater Biol* 2007;**52**:1176–86.
- Schloss PD, Westcott SL, Ryabin T et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microb* 2009;**75**:7537–41.
- Schneider J, Grosse G, Wagner D. Land cover classification of tundra environments in the Arctic Lena Delta based on Landsat 7 ETM+ data and its application for upscaling of methane emissions. *Remote Sens Environ* 2009;**113**:380–91.
- Semiletov I, Pipko I, Shakhova N et al. Carbon transport by the Lena River from its headwaters to the Arctic Ocean, with emphasis on fluvial input of terrestrial particulate organic carbon vs. carbon transport by coastal erosion. *Biogeosciences* 2011;**8**:2407–26.
- Shakhova N, Semiletov I, Bel'Cheva N. *The Great Siberian Rivers as a Source of Methane on the Russian Arctic shelf*. *Doklady Earth Sciences* 2007;**415**(1):734–36.
- Sundh I, Bastviken D, Tranvik LJ. Abundance, activity, and community structure of pelagic methane-oxidizing bacteria in temperate lakes. *Appl Environ Microb* 2005;**71**:6746–52.
- Tarnocai C, Canadell J, Schuur E et al. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem Cy* 2009;**23**:GB2023.
- Tsutsumi M, Iwata T, Kojima H et al. Spatiotemporal variations in an assemblage of closely related planktonic aerobic methanotrophs. *Freshwater Biol* 2011;**56**:342–51.
- Wagner D, Kobabe S, Liebner S. Bacterial community structure and carbon turnover in permafrost-affected soils of the Lena Delta, northeastern Siberia this article is one of a selection of papers in the special issue on polar and alpine microbiology. *Can J Microbiol* 2009;**55**:73–83.
- Walter KM, Smith LC, Chapin FS. Methane bubbling from northern lakes: present and future contributions to the global methane budget. *Philos T Roy Soc A* 2007;**365**:1657–76.
- Wang Q, Quensen JF, Fish JA et al. Ecological patterns of nifH genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. *MBio* 2013;**4**:e00592–13.
- Wartiainen I, Hestnes AG, Svenning MM. Methanotrophic diversity in high arctic wetlands on the islands of Svalbard (Norway)-denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. *Can J Microbiol* 2003;**49**:602–12.
- Wiesenburg DA, Guinasso NL, Jr. Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. *J Chem Eng Data* 1979;**24**:356–60.
- Zimov S, Voropaev YV, Semiletov I et al. North Siberian lakes: a methane source fueled by Pleistocene carbon. *Science* 1997;**277**:800–2.
- Zimov SA, Schuur EA, Chapin FS, III. Permafrost and the global carbon budget. *Science* 2006;**312**:1612–3.