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

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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 floodplain 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.

Introduction

Methane is an important radiatively active trace gas that is responsible for approximately 20% of the greenhouse effect (Cicerone & 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 *et al*., 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 *et al*., 2009, Graham *et al*., 2012, IPCC, 2014). Water bodies, which are abundant in the Arctic Lena Delta (8900 km^2, Schneider *et al*. (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 *et al*., 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 *et al*., 2007, Bussmann, 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 *et al*., 2010, Lofton *et al*., 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 *et al*., 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, are 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 & Hanson, 1996). In the low- and mid-latitude fresh waters, up to 90% of the methane can be oxidized before reaching the atmosphere (Guérin & Abril, 2007, Bastviken *et al*., 2008). At the same time, very few MOX rate measurements have been conducted in the Arctic freshwater environments, especially in the water column (He *et al*., 2012, Bussmann, 2013, Lofton *et al*., 2014, Martinez-Cruz *et al*., 2015). These studies reveal high spatial variability, which makes the assessment of the role of methane oxidation as a methane sink difficult. Additionally, very little is known about the abundance and the diversity of aerobic 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 & 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 three phyla: *Alphaproteobacteria* (Type II MOB), *Gammaproteobacteria* (Type I MOB) and the recently discovered *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 & 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 *Beijerinckiaceae* and the alternate 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 *et al*., 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 methane

oxidation contributes as a methane sink in these systems. In addition to the methane concentrations, we measured the MOX rates using the radiotracer technique and performed quantitative and qualitative analyses of the methanotrophic bacterial communities by analyzing the *pmoA* gene using qPCR and 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 methanotrophic community.

Materials and methods

Study area and water sampling

With an area of 29,000 km^2, the Lena Delta is one of the largest deltas in the world (Schneider *et al*., 2009). It extends 100 km into the Laptev Sea and is approximately 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 20^{th} 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 km^2 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 13-07-2012 to 26-07-2012. Water samples from the lakes and the middle of the river were collected from aboard a small rubber boat using a Niskin bottle. 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 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 methane oxidation (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) with a precision of 1% for salinity, 0.1 °C for temperature, and 0.5% for oxygen content. The salinity was measured in µS 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™). After drying the filters for 48 hours 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 approximately +10 °C for 1-2 weeks

before further processing. The methane concentrations were measured using the headspace technique; 10 mL of N_2 was added (McAuliffe, 1971). Three 1 mL headspace aliquots from each sample were analyzed using gas chromatography (Chromatec-Crystal 5000.1). Gas standards (Air Liquide) 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) were calculated according to the formula proposed by Wiesenburg & Guinasso Jr. (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 were calculated as the ratio between the observed methane concentration in the water column and the equilibrium concentrations multiplied by 100%.

Methane oxidation (MOX) rate measurements

The MOX rates were measured using the radiotracer technique with tritiated methane (American Radiolabeled Chemicals, 20 Ci $mmol^{-1}$) 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 hours in the dark at near *in situ* temperatures (approximately +15 °C). After incubation, the methane oxidation was stopped by adding 0.3 mL of 5 N NaOH. The methane oxidation 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 between these values ($r = {}^*H_2O / C^*H_4$) multiplied by the ambient methane concentration ($[CH_4]$) corrected for the incubation time (t).

$$MOX = r \times [CH_4] / t \qquad (1)$$

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 methane oxidation 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) and analyzed using a liquid scintillation counter (Tri-Carb® 2800 TR, Perkin Elmer); decays per minute (dpm) were used as the units.

DNA extraction

The recovery of the DNA from water samples was conducted using 0.2 µm filters to filter 150 mL of water right after the sample was taken. 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) according to the manufacturer's protocol. The total DNA concentration was calculated using a Qubit®2.0 fluorometer.

Polymerase chain reaction (PCR)

Due to the generally low concentrations of MOB DNA in the samples collected from the East and Cotton Lake complexes (section 3.4), 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 & Lidstrom, 1999, McDonald *et al.*, 2008). The total PCR mixture, which had a volume of 25 µL, contained the following ingredients: HotStar Taq Plus DNA polymerase (Qiagen) (5 U μL^{-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 µg μL^{-1}), 1 µL; CoralLoad PCR buffer (Qiagen), 2.5 µL; PCR water, 17.75 µL; and template, 2 µL. The total DNA concentration of the templates varied from 2 to 20 ng μL^{-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) 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 the first PCR but with 20 cycles. The PCR products were purified using the HiYield®PCR Clean-up/Gel Extraction Kit (SLG) according to the manufacturer's protocol for the purification of PCR products. Finally, 454 pyrosequencing was performed by Eurofins MWG Operon in 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 8 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 preclustering, 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 methanotrophic 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 shown 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 (qPCR)

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), 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, 2 copies of the *pmoA* gene. The detection limit, determined by the volume of the water samples collected and the DNA concentration, was 10^2 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. The concentration of the MOB cells (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. The standard deviations 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 standard deviation 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 standard deviations 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. Pangea.de. The measurements of the methane concentrations, MOX rates and the MOB abundance are also presented in Figs. 2a, b and c, respectively.

Freshwater characteristics: temperature, salinity, oxygen content and SPM

The water temperature of all the sampling sites varied from 11 to 17 °C and was 2-4 °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 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 approximately 4 nmol L^{-1}. The methane concentrations of all the sampling sites varied over two orders of magnitude, from 140 nmol L^{-1} to 24,000 nmol L^{-1}. Thus, all the water bodies that were investigated were supersaturated with methane. The saturation rates varied from approximately 3,500 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 nmol L^{-1} (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 6,400 nmol L^{-1}, respectively), while in the outlet and the middle of the river (5,400 and 150 nmol L^{-1}, respectively), the methane concentrations were comparable.

Methane oxidation (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, supplementary materials). 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 nmol L^{-1} h^{-1} (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, supplementary materials). 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 (± 3.4) × 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 approximately 5,000 on average (Fig. s1, supplementary materials). 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 approximately 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, 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 (≤ 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 methanotrophic bacterial 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 ± 256 nmol L^{-1}) and were substantially lower than those of the North Lake (22,974 ± 1,442 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 floodplain 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 floodplain 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 floodplain soil support the availability and uptake of substrates (Liebner & Wagner, 2007), which may result not only in enhanced methane oxidation 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 nmol L^{-1} 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 methane oxidation can be an important methane 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). 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 generally positively correlated to the methane concentration (Fig. s3). 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 also community structure controls the effectiveness of 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). 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 in the outlets, which corroborates the observations of Crump *et al*. (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 fresh waters of the temperate zone (including sediments (Costello *et al*., 2002, Rahalkar *et al*., 2009) and the water column (Eller *et al*., 2005, Sundh *et al*., 2005, Tsutsumi *et al*., 2011)), as well as in the Arctic wetlands (Wartiainen *et al*., 2003, Graef *et al*., 2011). Methane and oxygen concentrations as the controlling factors (Hanson & 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 to 6 meters 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 & 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 and 15 °C in the lakes and was approximately 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 *et al*., 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 floodplain 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.

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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^2)	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.	22974 ± 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
	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.
Mirror lake complex	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
	Outlet	72.3706, 126.5224	10	0.1	24343 ± 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
Sauna lake complex	Lake surf.	72.3684, 126.4832	27000	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
	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
East lake complex	Lake surf.	72.3752, 126.5197	22000	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.
	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	12490 ± 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
	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

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 – 16700, 165000*	n.d.	Kling *et al.*,1992
Canadian High Arctic (4 ponds)	1000 – 3400	n.d.	Negandhi *et al.*, 2013
Western Siberia (3 lakes)	66 – 7800	n.d.	Repo *et al.*, 2007
North Slope of Alaska (2 lakes)	780 – 1520	2 – 340	Lofton *et al.*, 2014
North slope of Alaska and Alaska's interior (2 lakes)	n.d.	0 – 60000	He *et al.*, 2012
Alaska (30 lakes)	<600 – 940000	0 – 1400	Martinez-Cruz *et al.*, 2015
Northeast Siberia (5 lakes)	200 – 23000	0.2 – 360	This study
Rivers			
North Siberia, Ob estuary	7 – 41	n.d.	Shakhova *et al.*, 2007
North Siberia, Yenisei estuary	7 – 131	n.d.	Shakhova *et al.*, 2007
Northeast Siberia, Lena estuary	62 – 651	n.d.	Shakhova *et al.*, 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

*the only measurement, which substantially stood out of the rest

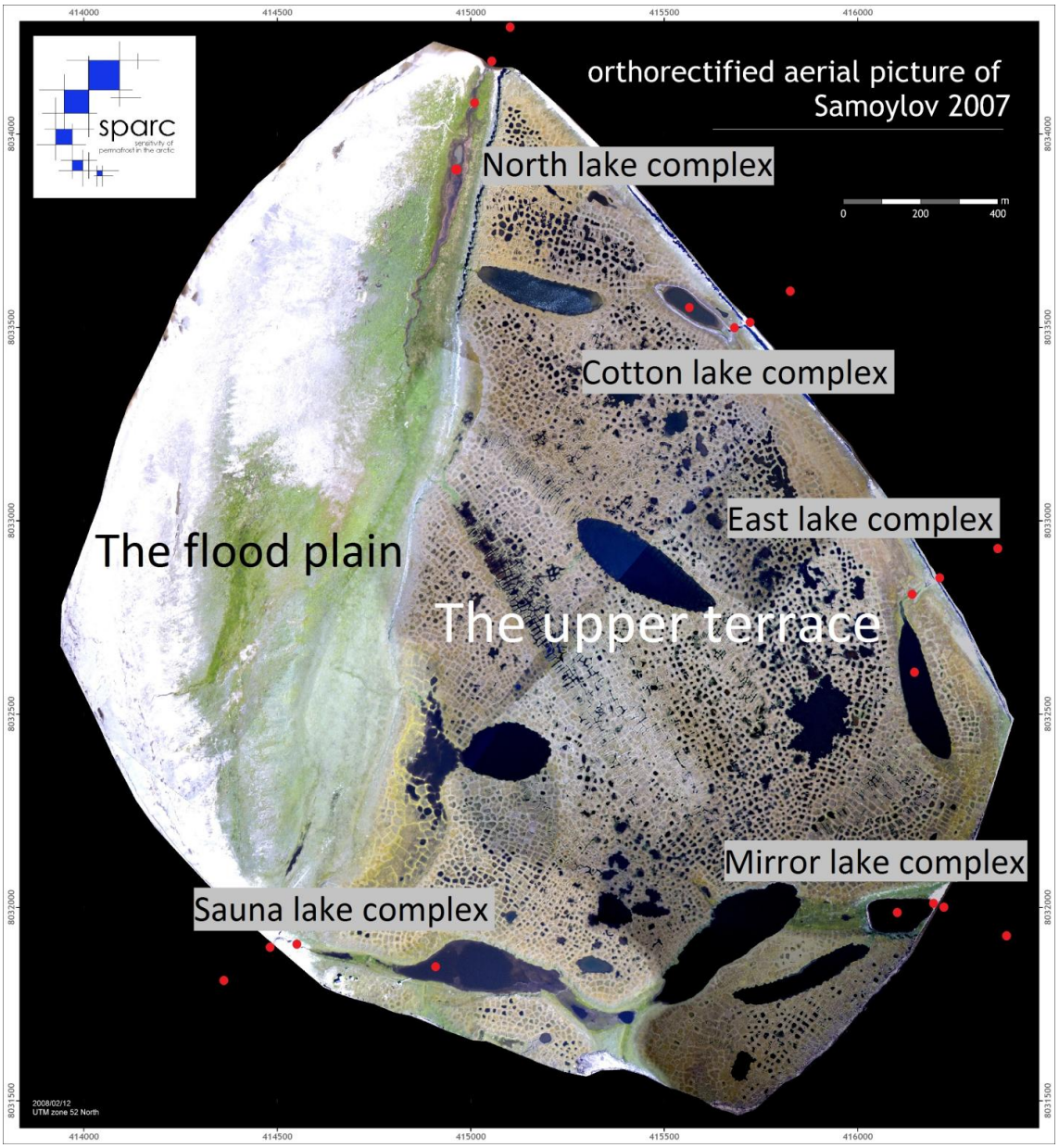

Fig. 1. Location of the studied lake complexes on Samoylov Island. Lake names are unofficial and were invented by the authors. The aerial image is provided by Boike *et al*. (2008).

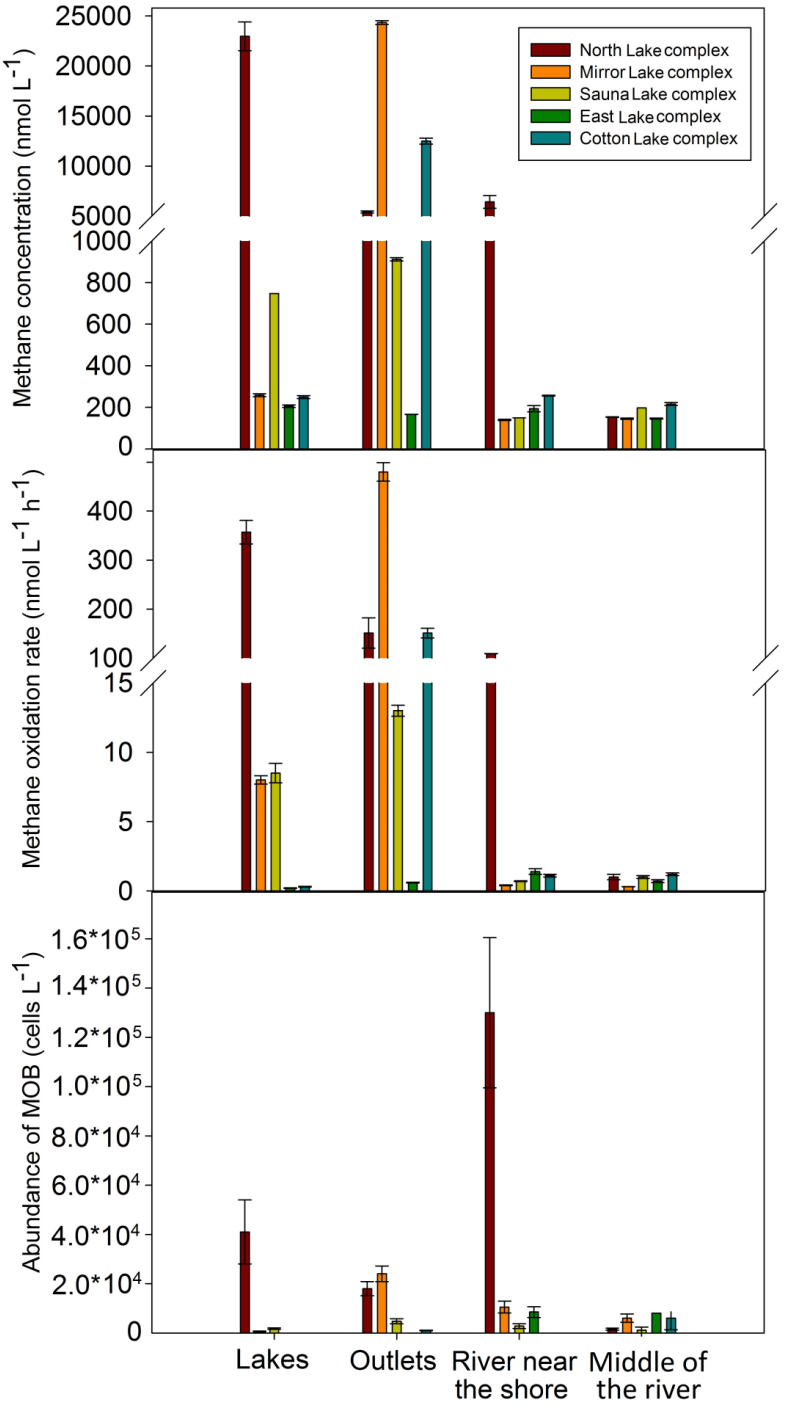

Fig. 2. Methane concentrations, methane oxidation rate and the abundance of methanotrophic bacteria in the surface water of the different lake complexes. Data represent the mean of technical replicates (± standard deviation, SD). Measurements of methane concentrations and abundance of methanotrophic bacteria are performed in triplicate, methane oxidation rates are performed in duplicate.

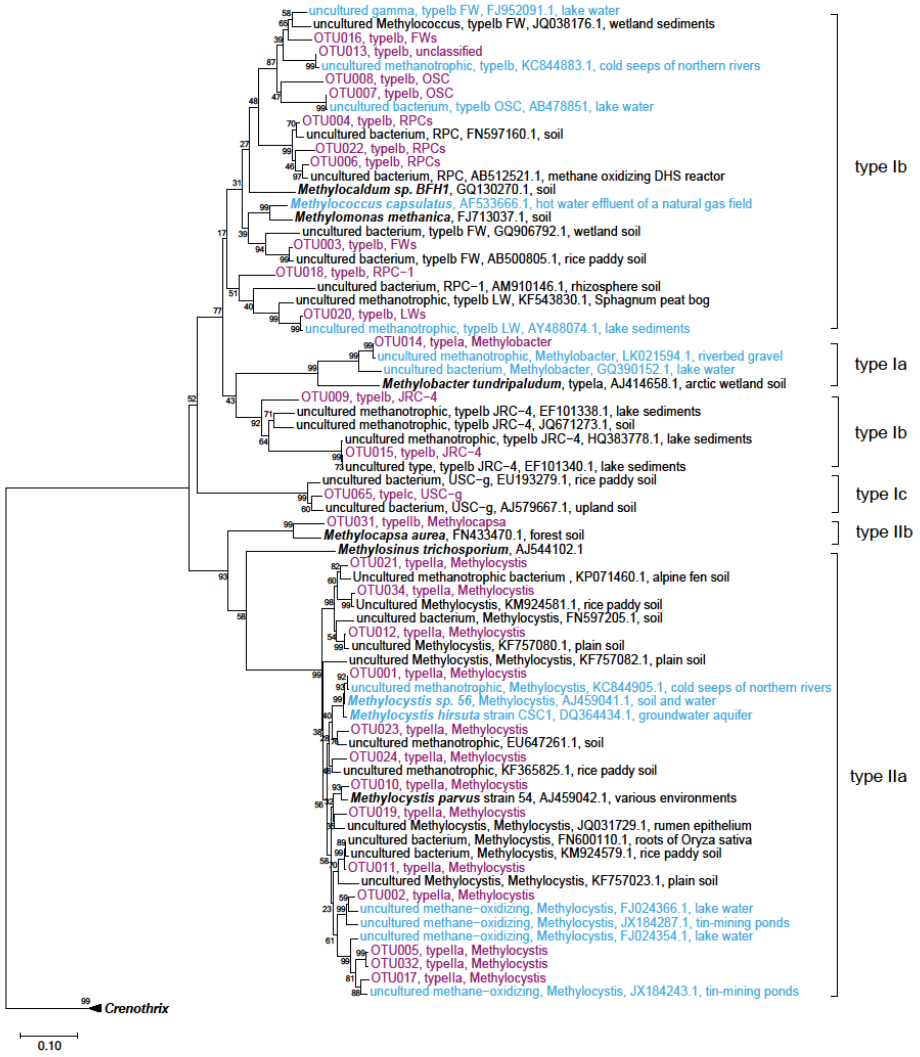

Fig. 3. A neighbor-joining tree of *pmoA* gene sequences, showing the relationship between the most abundant OTUs from this study (red color font) and strains from previous studies. Blue or black font indicates the strains isolated from the aquatic and terrestrial environments, respectively. A neighbor-joining 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.

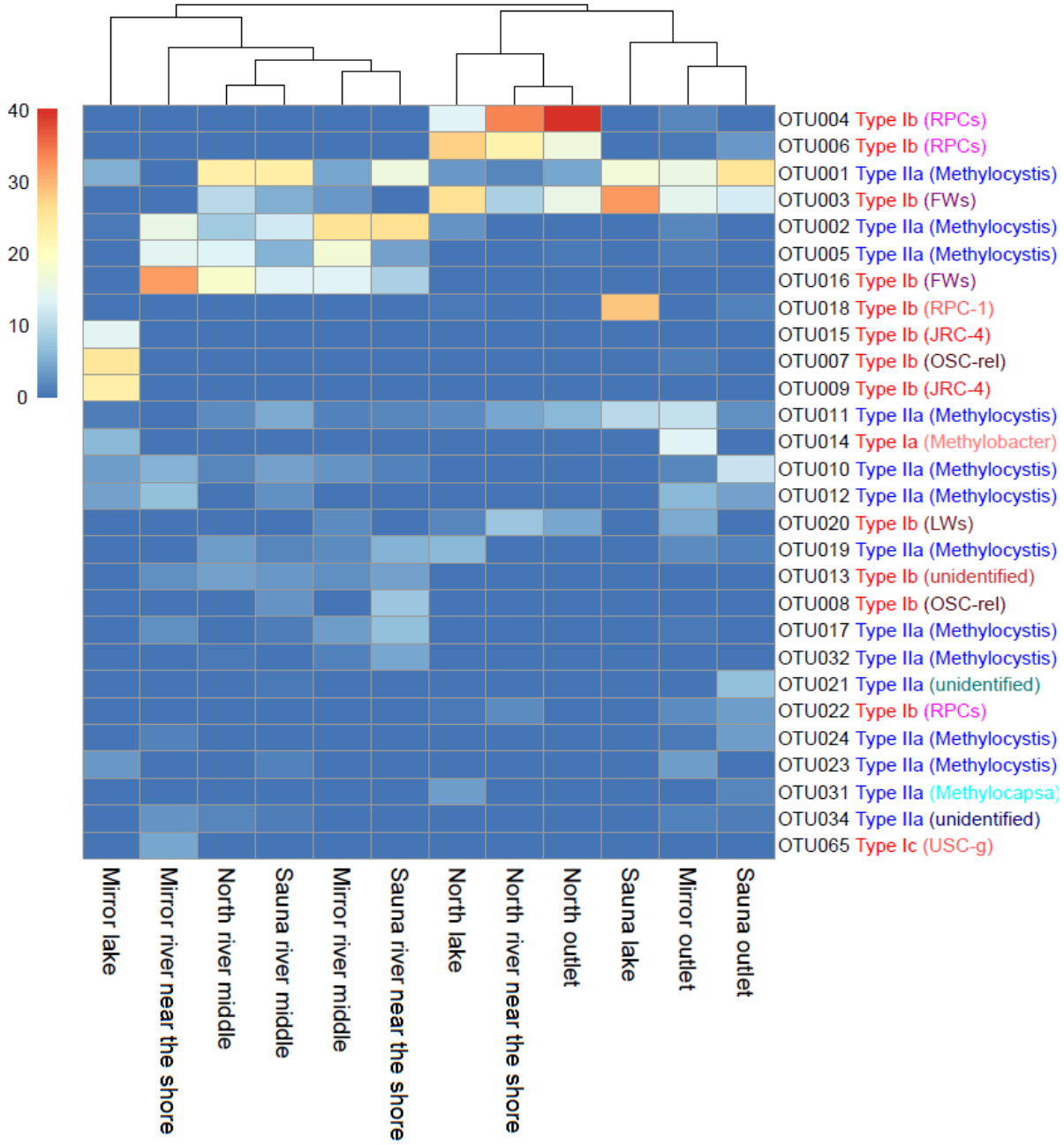

Fig. 4. Heat map showing the relative abundance (%) of the most prevalent OTUs with the dendrogram demonstrating the relationship between the MOB communities from the different sampling sites.