Vol. 82: 1–18, 2018
https://doi.org/10.3354/ame01878

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online August 31

Microbial communities involved in aerobic and anaerobic methane cycling in a meromictic ferruginous subarctic lake

Anna Kallistova[1,*], Vitaly Kadnikov[2,*], Igor Rusanov[1], Natalia Kokryatskaya[3], Alexey Beletsky[2], Andrey Mardanov[2], Alexander Savvichev[1], Nikolai Ravin[2], Nikolai Pimenov[1,]**

[1]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia

[2]Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia

[3]N. Laverov Federal Center for Integrated Arctic Research, Arkhangelsk 163000, Russia

ABSTRACT: Since boreal lakes are now considered an important source of atmospheric methane, new information concerning the activity and diversity of methane cycle microorganisms is critical for understanding the causes of methane emission from these lakes. This study investigated the diversity of microbial communities in the water column of subarctic Lake Svetloe, belonging to a rare type of freshwater sulfate-poor meromictic basins with high concentrations of Fe^{2+} and methane in the permanently anoxic hypolimnion. A combination of physicochemical and radiotracer analysis, high-throughput sequencing of the 16S rRNA genes and incubation experiments was used to link microbial community profile and methane cycle processes. It was shown that methane was produced by aceticlastic *Methanothrix* and hydrogenotrophic *Methanoregula*, which were also detected in the oxygenated epilimnion, together with a small increase in methane concentration. Radiotracer analysis revealed methane oxidation (MO) in oxic and anoxic zones with 2 maxima at the chemocline. The first MO peak was attributed to aerobic *Methylobacter* trophically interacting with cyanobacteria, which was confirmed by obtaining light-dependent MO. The highest MO activity matched the lower chemocline layer where aerobic methanotrophs were less abundant; this suggested that other microorganisms contributed to MO together with *Methylobacter*. Known anaerobic methanotrophs were not detected, and incubations with Fe^{3+} did not reveal methane consumption under anoxic conditions. Thus, further investigations are required to determine the microorganisms and electron acceptors driving anaerobic MO. Although some questions remain open, our study may provide insight into the methane cycle microbial communities in boreal lakes.

KEY WORDS: Meromictic freshwater lakes · Ferruginous lakes · Lake Svetloe · Methane cycle · Methane oxidation · Methanogenesis · 16S rRNA profiling

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

High-latitude boreal freshwater basins located north of 50° N are considered an important source of the greenhouse gas methane (Walter et al. 2007, Bastviken et al. 2011, Saunois et al. 2016, Wik et al. 2016a). Among those, ponds and glacial, post-glacial, and thermokarst lakes are the dominant water body types (Wik et al. 2016a). Thermokarst lakes cause the greatest concern due to their high emission rates and prospective increase in methane production promoted by permafrost thawing (Walter et al. 2007,

*These authors contributed equally to this work
**Corresponding author: npimenov@mail.ru

© Inter-Research 2018 · www.int-res.com

Laurion et al. 2010, Martinez-Cruz et al. 2015, Vonk et al. 2015, Wik et al. 2016a). It was, however, found that while glacial and post-glacial lakes emit less methane per unit area, they are a greater methane source due to their larger total area (Wik et al. 2016a).

Methane emission from boreal surface waters mostly results from diffusion, ebullition and ice-out of methane (see references within Wik et al. 2016a,b). Methane concentration in the surface water is a function of sources and losses and is limited by microbial methane production and oxidation in the sediments and water column (Bastviken et al. 2004). Estimation of methane production and oxidation rates and identifying the microbial communities involved in the methane cycle and factors affecting their activity are therefore important. In contrast to many studies conducted on evaluation of methane emissions (e.g. Bastviken et al. 2004, 2011, Walter et al. 2007, Laurion et al. 2010, Sepulveda-Jauregui et al. 2015, Saunois et al. 2016, Wik et al. 2016a,b, DelSontro et al. 2017, Erkkilä et al. 2018), the diversity of microbial communities involved in the methane cycle processes in boreal lakes is poorly investigated, especially for freshwater meromictic lakes with a permanently anoxic hypolimnion.

Biogenic methane is produced primarily by methanogenic archaea, the terminal agents of anaerobic decomposition of organic matter in various anoxic environments (see references within Conrad 2009, Borrel et al. 2011, Lehours et al. 2016). In a number of different sulfate-poor freshwater lakes, methane production rates are maximal at the water–sediment interface. In meromictic lakes, methanogens are also abundant in the permanently anoxic hypolimnion, where they are responsible for significant methane production (see references within Borrel et al. 2011). Under conditions of no competition with sulfate reducers, acetate becomes available to methanogens and acts as the major substrate for methanogenesis (Liu & Whitman 2008, Borrel et al. 2011, Lofton et al. 2015). However, the concentrations of acetate and hydrogen in the anoxic zones of freshwater lakes are often low, resulting in the limitation of aceticlastic and hydrogenotrophic methanogenesis by substrate availability (see references within Borrel et al. 2011). The effect of temperature and substrate availability on methanogenic potential has been studied in sediments from a number of boreal lakes (e.g. Duc et al. 2010, Lofton et al. 2015), while the information concerning the diversity of methanogenic archaea in boreal lakes is rather scarce. Very few studies have shown the abundance of hydrogenotrophic (*Methanobacteriaceae*, *Methanoregulaceae*) and aceticlastic (*Methanosaetaceae*) methanogens in boreal lakes (Peura et al. 2015, Rissanen et al. 2017).

The amount of methane produced is usually much higher than the amount emitted, since microorganisms consume a high portion of methane before it is released into the atmosphere (see references within Conrad 2009). Methane oxidation (MO) under both oxic and anoxic conditions is carried out by methanotrophic microorganisms, which act as an efficient environmental biofilter decreasing methane emission from various environments (Hanson & Hanson 1996, Knittel & Boetius 2009). High rates of aerobic MO are usually detected at the oxic–anoxic interface of lakes (upper sediment layer if overlaid by oxic water or the oxycline of a stratified water column), where both methane and oxygen concentrations sharply decrease (Lidström & Somers 1984, Sundh et al. 2005, Borrel et al. 2011). Aerobic methanotrophic members of *Alpha*- and *Gammaproteobacteria* are typical inhabitants of freshwater lakes. In most stratified lakes, independent of their trophic status, predominance of methanotrophic *Gammaproteobacteria* (type I methanotrophs) over *Alphaproteobacteria* (type II) has been shown (Costello et al. 2002, Sundh et al. 2005, Pimenov et al. 2010, Oswald et al. 2016a, Savvichev et al. 2017). Aerobic MO in boreal lakes, including determination of the effect of low temperatures on MO potential and diversity of aerobic methanotrophs, is much better studied than methanogenesis (Kankaala et al. 2007, Duc et al. 2010, He et al. 2012, Peura et al. 2012, 2015, Martinez-Cruz et al. 2015, 2017, Denfeld et al. 2016, Crevecoeur et al. 2017). Generally, type I methanotrophic gammaproteobacteria of *Methylococcaceae* family, particularly *Methylobacter* spp., are most frequently detected in boreal lakes, which agrees with the data for other freshwater basins (Borrel et al. 2011).

Methanotrophic gammaproteobacteria are considered strict aerobes capable of activity under microaerobic conditions (Chistoserdova 2015, Danilova et al. 2016). The classical aerobic methanotrophic gammaproteobacteria were recently shown to be involved in MO in anoxic waters of stratified freshwater lakes, probably due to trophic interactions with oxygenic phototrophs (Milucka et al. 2015, Oswald et al. 2015, 2016b) and/or methylotrophs (Chistoserdova 2015), or using alternative electron acceptors (Fe^{3+}, Mn^{4+}, or nitrate) (Oswald et al. 2016a, 2017). As components of microbial communities, aerobic methanotrophic gammaproteobacteria of the genus *Methylobacter* were shown under anoxic conditions to oxidize up to 32% of methane formed in the upper sediments of

shallow ferruginous subarctic Lake Vault (Alaska) (Martinez-Cruz et al. 2017). The recent discoveries of methanotrophic gammaproteobacteria in hypoxic and anoxic conditions cast doubt concerning the strictly aerobic nature of these bacteria (Chistoserdova 2015).

For a number of temporally stratified boreal lakes it was, however, found that irrespective of oxygen availability, MO was generally the highest in the methane-saturated hypolimnion (Bastviken et al. 2002). In the absence of oxygen, other electron acceptors (e.g. SO_4^{2-}, NO_3^-, NO_2^-, Fe^{3+}, Mn^{4+}, or Cr^{6+}) could be used in the process of anaerobic oxidation of methane (AOM). Sulfate- and nitrate-dependent AOM carried out by the anaerobic methane-oxidizing archaea (ANME) via reverse methanogenesis are the better studied processes compared with metal-dependent AOM (Knittel & Boetius 2009, Welte et al. 2016, Timmers et al. 2017). Since most microorganisms of the ANME clusters depend on sulfate reducers for AOM (Knittel & Boetius 2009), sulfate limitation could be a reason for insignificant contribution of sulfate-dependent methanotrophic archaea in a number of freshwater lakes (Oswald et al. 2016b, Martinez-Cruz et al. 2017, Rissanen et al. 2017). In the absence of sulfate, AOM may be carried out by nitrate- and nitrite-dependent methanotrophic archaea and bacteria, '*Candidatus* Methanoperedens nitroreducens' (ANME-2d) and '*Candidatus* Methylomirabilis oxyfera' (NC10), respectively (Ettwig et al. 2010, Haroon et al. 2013, Welte et al. 2016). While metal-dependent AOM coupled to Fe^{3+}, Mn^{4+}, or Cr^{6+} reduction has also been proposed, the underlying mechanisms and taxonomic affiliation of the microorganisms involved in this process still remain unclear. Different bacteria and archaea have been hypothesized to participate in this process (Egger et al. 2015, Fu et al. 2016, Timmers et al. 2017, He et al. 2018), and until now the only proof was obtained for nitrate-dependent ANME-2d archaea related to '*Candidatus* M. nitroreducens' (Ettwig et al. 2016). The effect of different electron acceptors (SO_4^{2-}, NO_3^-, Fe^{3+}, Mn^{4+}, O_2) on the activity and diversity of methanogens and methanotrophs was recently studied for the sediments of 2 shallow boreal lakes enriched with iron. It was, however, shown that the addition of any electron acceptor did not enhance the potential AOM activity (Rissanen et al. 2017). Another process that should be considered when evaluating MO under anoxic conditions is trace methane oxidation (TMO), which is apparently performed by methanogenic archaea due to enzymatic back flux along with general methane production (Timmers et al. 2017).

All boreal lakes referred to above either have oxygenated water column or are temporally stratified. The object of the present study was the subarctic (65° N) meromictic Lake Svetloe (Russia) with a permanently anoxic hypolimnion. This lake is of glacial origin and belongs to a very rare type of freshwater meromictic lakes with high concentrations of ferrous iron (240 µM) and dissolved methane (960 µM) in the hypolimnion; in contrast, the concentrations of sulfate and sulfide are extremely low (~2 µM). Due to its considerable depth (39 m) and protection from wind mixing, the major hydrological parameters of the water column are stable (Savvichev et al. 2017). Iron-enriched and sulfur-poor meromictic lakes are currently considered as modern analogs of the ancient Archean ocean with suitable conditions for photoferrotrophy (Canfield et al. 2006, Crowe et al. 2011, Camacho et al. 2017). There are only few such lakes known from the literature: tropical lakes Matano (Indonesia) and Kabuno Bay (DR Congo) and temperate lakes Pavin (France) and La Cruz (Spain) (Biderre-Petit et al. 2011, Walter et al. 2014, Crowe et al. 2011, Llirós et al. 2015, Lehours et al. 2016, Camacho et al. 2017). Two boreal (56° N) meromictic iron-enriched lakes — Kuznechikha (Russia) and Ørn (Denmark) — have also been described (Gorlenko et al. 1980, Norði et al. 2013), while both have lower maximum depth (20 and 10.5 m, respectively) and higher sulfate concentration in the water column (up to 125 and 250 µM, respectively) compared with Lake Svetloe.

One of the most important features of Lake Svetloe is high methane content in the hypolimnion and bottom sediments. Our radiotracer analysis carried out when the lake surface was covered with ice (April 2014) revealed MO in both oxic and anoxic conditions with the highest rate at the chemocline, where aerobic methanotrophs were detected under oxygen limitation (Savvichev et al. 2017). It was previously shown for another ferruginous lake, Lake La Cruz, that only aerobic methanotrophs were responsible for MO under all (oxic, hypoxic, and anoxic) conditions. Light-dependent MO coupled with oxygenic photosynthesis was shown to be the mechanism for methane removal under anoxic conditions (Oswald et al. 2016b). We thus hypothesized that similar relations may exist in Lake Svetloe, e.g. oxygen production by oxygenic phototrophs concentrated at the chemocline zone provided for MO by aerobic methanotrophs under oxygen limitation. Our data on MO in the anoxic hypolimnion of Lake Svetloe were also of some interest. Since the concentrations of sulfate, nitrate, and nitrite were low in the hypolimnion of the lake (Table 1), and ferric iron ions were present, we

Table 1. Physicochemical parameters of Lake Svetloe. DOC and DIC: dissolved organic and inorganic carbon, respectively. R: conductivity

Parameter	Epilimnion (0–20 m)	Chemocline (20–24 m)	Hypolimnion (24–39 m)	Reference
DOC (µM)	100–150	183	150–230	Chupakov
DIC (mM)	2.44–2.7	3.6	3.9–4.1	et al. (2017)
NO_2–N (µM)	≤0.2	2.0	2.0–2.9	Ershova
NO_3–N (µM)	5.3–11.5	3.6–11.5	8.3–20.2	et al. (2015)
NH_4–N (µM)	0.7–1.4	1.4–143	143–214	
SO_4^{2-} (µM)	45–50	14–50	2–14	Savvichev
Fe (µM)	0.75–2.0	1.2–34	34–240	et al. (2017)
Fe^{3+} (µM)	0–0.1	0.9–11.5	0–7.3	This study
pH	7.66–8.15	7.45–7.9	7.33–7.46	
R (µS cm^{-1})	228–263	286–377	401–419	

hypothesized that AOM might be coupled to Fe^{3+} reduction. AOM coupled to Fe^{3+} or $Mn^{3+/4+}$ reductions was previously proposed for the ferruginous Lake Matano (Crowe et al. 2011, Sturm et al. preprint doi: 10.5194/bg-2015-533). High rates of AOM were also measured in the anoxic, nitrate-free, Fe^{3+}- and sulfate-containing sediments of the ferruginous boreal Lake Ørn. Due to the considerable sulfate concentration in Lake Ørn, it was however difficult to differentiate which of the electron acceptors (Fe^{3+} or SO_4^{2-}) was responsible for the AOM (Norði et al. 2013).

The goal of the present study was to analyze the diversity and distribution of the microbial communities between the oxic epilimnion, oxic–anoxic interface (chemocline), and anoxic hypolimnion of the subarctic freshwater ferruginous meromictic Lake Svetloe in relation to the processes involved in the methane cycle (methane production and oxidation, CO_2 assimilation by oxygenic and anoxygenic phototrophs). The combination of physicochemical analysis of the water column with a radiotracer technique and high-throughput sequencing of the 16S rRNA genes was used to link detailed description of the microbial communities profile to the methane cycle processes. The incubation experiments were focused on light- and Fe^{3+}-dependent MO, which were hypothesized to take place in the hypoxic and anoxic zones of the lake.

MATERIALS AND METHODS

Study site and sample collection

The freshwater meromictic Lake Svetloe (65° 04.98′ N, 41° 06.26′ E) is located in the northern taiga zone with an average annual temperature of 0°C. Duration of the ice-cover period for the lake is about 200 d. The watershed is on a glacial moraine over late Carboniferous limestones (Chupakov et al. 2017). The maximum depth of the lake is 39 m; the chemocline is located at a depth interval of 20–24 m, and the hypolimnion below is anoxic throughout the year (Chupakov et al. 2017, Savvichev et al. 2017). The average annual temperature of the water column below 8 m is about 4°C (Chupakov et al. 2017, Savvichev et al. 2017). Physicochemical parameters of the lake were measured in our previous studies (Zabelina et al. 2013, Ershova et al. 2015, Chupakov et al. 2017, Savvichev et al. 2017); some parameters are summarized in Table 1.

Water samples were collected in May 2016 from the ice-free lake surface to a depth of 35 m. The samples were collected using a 5 l pre-cleaned polycarbonate horizontal water sampler (Aquatic Research).

Analytical techniques

Temperature and dissolved oxygen concentration profiles were measured using a WTW Oxi 330i probe. Water pH was measured with a HANNA HI8314F portable ion meter with temperature compensation and a combined electrode. Specific conductivity was determined with a HANNA HI8733 portable conductometer. Alkalinity was determined immediately after sampling by titration with the standard Aquamerck reagent kit. Dissolved sulfide was determined by the colorimetric method (Cline 1969); samples were preserved with zinc acetate saturated solution. Concentrations of iron were determined by the modified ferrozine method, which makes it possible to measure dissolved Fe^{2+} and the sum of Fe^{2+} and Fe^{3+} after the addition of hydroxylamine hydrochloride as a reducing agent and ammonium acetate buffer; all procedures are described in detail by Viollier et al. (2000). The concentration of Fe^{3+} was calculated as the difference between the concentrations of $\Sigma(Fe^{2+} + Fe^{3+})$ and Fe^{2+}. The samples for iron analysis were collected from the water sampler into 50 ml plastic syringes through the special adapter preventing contact with air. The samples were immediately filtered through 0.45 µm acetate cellulose filters, dispensed to capacity into plastic vials (25–50 ml), preserved

with HNO_3, and sealed avoiding air bubbles. Analysis of dissolved iron species was carried out at the day of sampling. Methane content in the water samples was determined using the headspace method (McAuliffe 1971). For this purpose, 30 ml glass vials were filled with the water sample, and 3.5 ml of the water was replaced immediately by ambient air used as a headspace gas. The vials were then sealed with gas-tight rubber stoppers, and covered with perforated aluminum caps; KOH was used for preservation. The vials were stored upside down in the dark at air temperature (16–18°C) for ≤1 wk. Methane concentration was measured on a Kristall 5000.1 gas chromatograph (Chromatec) equipped with a flame ionization detector and HayeSep N 80/100 as a sorbent; the vials were shaken well prior to gas chromatography analyses. Methane concentrations were corrected for methane content in ambient air similar to that described in Denfeld et al. (2016).

Radiotracer techniques

For measurement of the rates of microbial processes (CH_4 oxidation, hydrogenotrophic H_2/CO_2 methanogenesis, and CO_2 assimilation by oxygenic and anoxygenic phototrophs), water samples were dispensed into 35 ml glass vials, avoiding air bubbles, sealed with gas-tight rubber stoppers, and covered with perforated aluminum caps. The rate of MO was measured using $^{14}CH_4$ (2 µCi per sample, specific activity of 1.16 GBq mmol^{-1}, JSC Isotope). For the purpose, each vial was injected with 0.2 ml of labeled methane dissolved in degassed sterile water. The rates of CO_2 assimilation and H_2/CO_2 methanogenesis were measured using sterile $NaH^{14}CO_3$ (specific activity of 2.04 GBq mmol^{-1}) solutions of 5 and 10 µCi per sample for CO_2 fixation and H_2/CO_2 methanogenesis, respectively. To determine the rates of light and dark CO_2 assimilation, 2 transparent vials and 1 darkened vial were used for each sampling horizon. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) at a final concentration of 10^{-7} mM was used as an inhibitor of oxygenic photosynthesis. No electron donors were added. To determine photosynthetic production and the rates of microbial processes, the vials with water samples and labeled substrates were incubated for 24 h under *in situ* conditions by submerging the vials suspended on nylon lines to the sampling depths. After incubation, the samples were fixed with 0.5 ml 2 N NaOH and transported to the stationary laboratory. The samples were treated as described previously (Pimenov & Bonch-Osmolovskaya 2006). Each experiment was carried out with at least 3 replicates. Photosynthetic production was calculated as the difference between the values for transparent and dark vials. Production of oxygenic photosynthesis was calculated as the difference between total and anoxygenic (transparent vial with DCMU) photosynthetic production.

Light-dependent MO was determined by *in situ* incubation of water samples with ^{14}C-labelled methane. Water samples were taken from the chemocline zone (21, 21.5, and 22 m), and $^{14}CH_4$ was added as described above. Three variants of incubation were used for each sample taken in triplicate: (1) addition of $^{14}CH_4$ into transparent vials, (2) addition of $^{14}CH_4$ into darkened vials, and (3) simultaneous addition of $^{14}CH_4$ and DCMU into transparent vials. The vials were incubated for 24 h directly at the sampling depths, fixed with 0.5 ml 2 N NaOH and further processed as described above.

Molecular genetic techniques

Water samples were collected from depths of 0.5, 5, 10, 17, 21, 21.5, 22, 22.5, 23, 27, 33, and 35 m by overfilling plastic 0.5 l bottles and sealing, avoiding gas bubbles. Microbial cells from a whole volume of each water sample (0.5 l) were concentrated on 0.2 µm filters (GTBP 2500, Millipore) on the day of sampling. The filters were homogenized by grinding with liquid nitrogen, and stored at −70°C prior to further processing. The metagenomic DNA was isolated using a method based on cell lysis and subsequent treatment with 1% N-cetyl N,N,N-trimethylammonium bromide (CTAB) (Wilson 2003). Per water sample, over 1 µg DNA was obtained.

PCR amplification of the 16S rRNA gene fragments containing the V3–V6 variable regions was carried out using the universal primers PRK341F (5′-CCT ACG GGR SGC AGC AG-3′) and PRK806R (5′-GGA CTA CYV GGG TAT CTA AT-3′) (Yu et al. 2005). PCR fragments were then sequenced on a GS FLX genome analyzer (Roche) according to the Titanium protocol using the GS FLX Titanium Sequencing Kit XL+. Creation of the library, its amplification, and sequencing were carried out according to the relevant Roche protocols.

Prior to analysis of the 16S rRNA gene sequences, the reads with both primers at the termini were selected. Potentially chimeric sequences were deleted using the Uchime algorithm implemented in the Usearch package (Edgar et al. 2011). The reads occurring only once in the whole dataset were excluded

from analysis using Mothur (Schloss et al. 2009) as possible pyrosequencing errors. After preliminary filtration, mainly by removal of short sequences, the datasets for the samples consisted of 2486 to 16 862 sequences (Table 2).

Usearch was used for clusterization and determination of the representative sequences for the operational taxonomic units (OTUs); 97% percentage of homology was used for OTUs clustering. Data were normalized to the total number of reads in clusters for each sample. The representative sequences were taxonomically identified using RDP classifier (Cole et al. 2009). For taxonomic identification of the representative sequences, they were also compared with the GenBank database of the 16S rRNA gene sequences using BLASTN. The OTUs with sequences exhibiting over 95% similarity to those of a validly described microorganism were assigned to relevant genera. The obtained sequences were deposited in the Sequence Read Archive (SRA) via the National Center for Biotechnology Information (NCBI) under the accession numbers SRX3205080–SRX3205091.

Hierarchical cluster analysis of microbial community compositional profile was done using R language (hc function) as reported by Gies et al. (2014); Manhattan distance was used for construction of the OTU tree.

Table 2. Number of the 16S rRNA gene fragments sequenced from the samples of Lake Svetloe water column

Depth (m)	Number of 16S rRNA gene reads
0.5	8024
5	9308
10	14 707
17	16 862
21	13 053
21.5	14 323
22	2486
22.5	5993
23	8571
27	16 122
33	11 458
35	11 955

Microscopy

Total microbial number was determined in the samples fixed with glutaraldehyde (2% final concentration). The fixed specimen (1–5 ml) was filtered through 0.2 µm black polycarbonate membranes (Millipore). The filters were stained with acridine orange and examined under an Axio Imager.M2 epifluorescence microscope equipped with an Axiocam 503 mono digital camera and the 16 filter set for acridine orange-stained cells (Carl Zeiss Microscopy). The images were analyzed using the ZEN 2 bundled software package.

Incubation experiments with addition of Fe^{3+}

Incubation experiments with addition of Fe^{3+} as a possible electron acceptor for AOM were performed for the samples from the chemocline zone (22 m), near-bottom water (35.3 m), and upper sediments (0–5 cm). Serum bottles (0.5 l) were filled from the water sampler avoiding air bubbles, sealed with gastight rubber stoppers, and covered with perforated aluminum caps. The sediment samples were dispensed into 50 ml plastic boxes and tightly sealed. All samples were stored at +5°C in the dark within 1 wk until further processing. Manipulations with the samples were carried out in the laboratory under argon atmosphere. The experiment was performed in triplicate for each sample in the following variants: (1) control, headspace of argon + 2.5% methane; (2) headspace of argon + 2.5% methane and 5 mM $Fe_2(SO_4)_3$; and (3) headspace of argon + 2.5% methane and ~5 mM synthetic ferrihydrite ($Fe(OH)_3$) prepared according to Zavarzina et al. (2016). The volumes of water and sediment were 30 and 10 ml, respectively. The incubations lasted 6 mo at 10°C in the dark. Headspace methane concentration was measured once a month on a Kristall 5000.1 gas chromatograph (Chromatec) equipped with a flame ionization detector and HayeSep N 80/100 as a sorbent.

Statistical analysis

All experiments were carried out in triplicate and means ± SD were calculated. It was not possible to proof normality of the data due to small sample size (n = 3). We applied parametric statistical tests assuming that our data had a normal distribution. Correlation tests between OTUs and between OTUs and MO rates were carried out using the Pearson method in R programming language (Hmisc and ggpubr libraries). Student's *t*-test was used to detect significant differences between 2 sets of data in incubation experiments, i.e. differences between MO rates in light and dark incubations, and differences between methane concentrations at the end of the experiment in control and one of each Fe^{3+} additions.

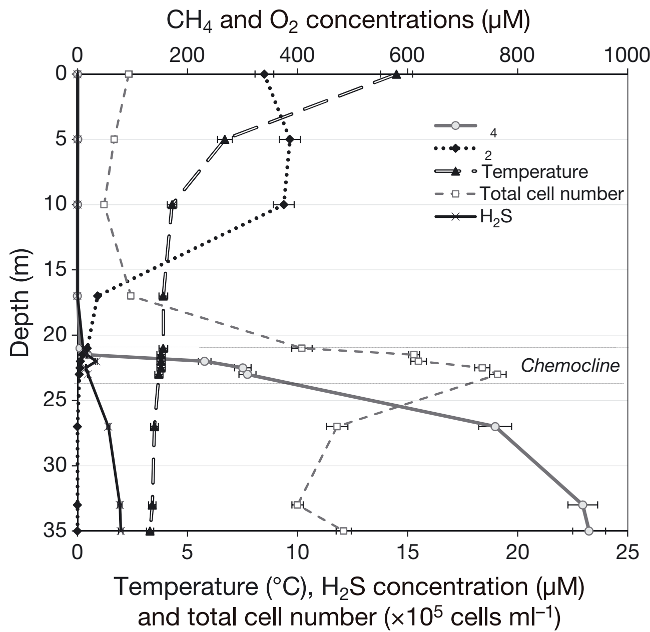

Fig. 1. Physicochemical parameters (temperature, concentrations of oxygen, methane, and sulfide) and total microbial cell numbers in Lake Svetloe water column. Dashed lines denote the chemocline zone. Values are shown as averages ± SD (n = 3)

RESULTS

Hydrochemical parameters of the water column

Our research confirmed stratification of the water column of Lake Svetloe: the chemocline was defined as within the 21–24 m depth interval based on the profile of oxygen concentrations (Figs. 1 & 2A). The upper 10 m of the water column was oxygen saturated (340–386 µM O_2), while oxygen concentration decreased below that level. Within the depth interval of 21–24 m, oxygen concentration decreased from 17.8 to <2.8 µM. Methane concentration in the epilimnion (0–17 m) was in the range of 0.03–0.4 µM with a small increase (0.3–0.4 µM CH_4) in the upper 0–5 m layer (Fig. 3A). Below 21 m, methane content increased from 4.5 to 920 µM (at 33 m) (Figs. 1 & 2A). Traces of sulfide (0.25 µmol l^{-1}) were detected at 21 m. A small sulfide peak (0.85 µM) was observed at 22 m, while at 23–35 m the concentration increased gradually from 0.5 to 2 µM (Fig. 1). The highest Fe^{3+} concentrations were observed at the chemocline lower border (23 m) and in the hypolimnion (33 m) (Table 1).

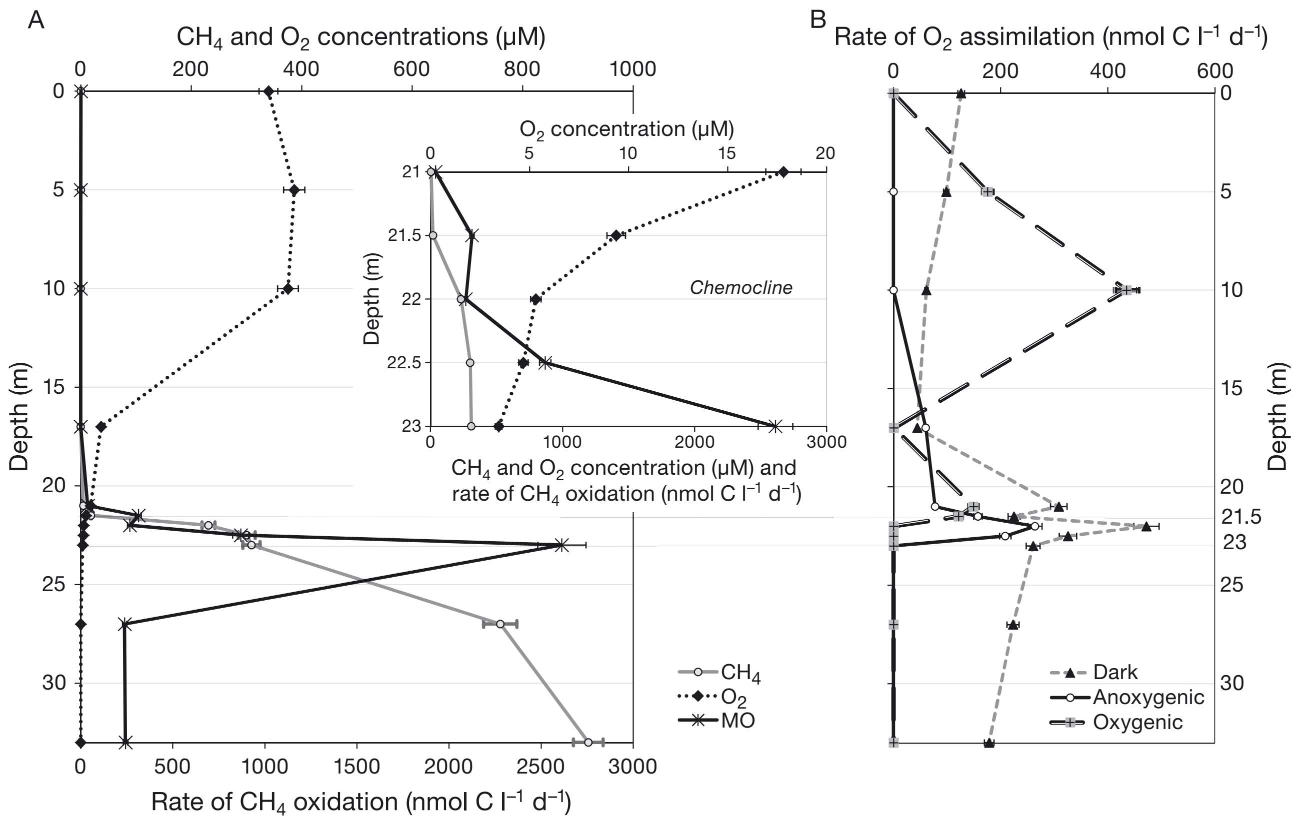

Fig. 2. Rates of microbial processes in the water column of Lake Svetloe. Methane oxidation (MO) rate profile determined with (A) $^{14}CH_4$, methane, and oxygen concentrations, and (B) oxygenic and anoxygenic photosynthesis, and dark CO_2 assimilation measured with $NaH^{14}CO_3$. Inset represents a detailed view for methane and oxygen concentrations and MO rates in the chemocline zone. Values are shown as averages ± SD (n = 3)

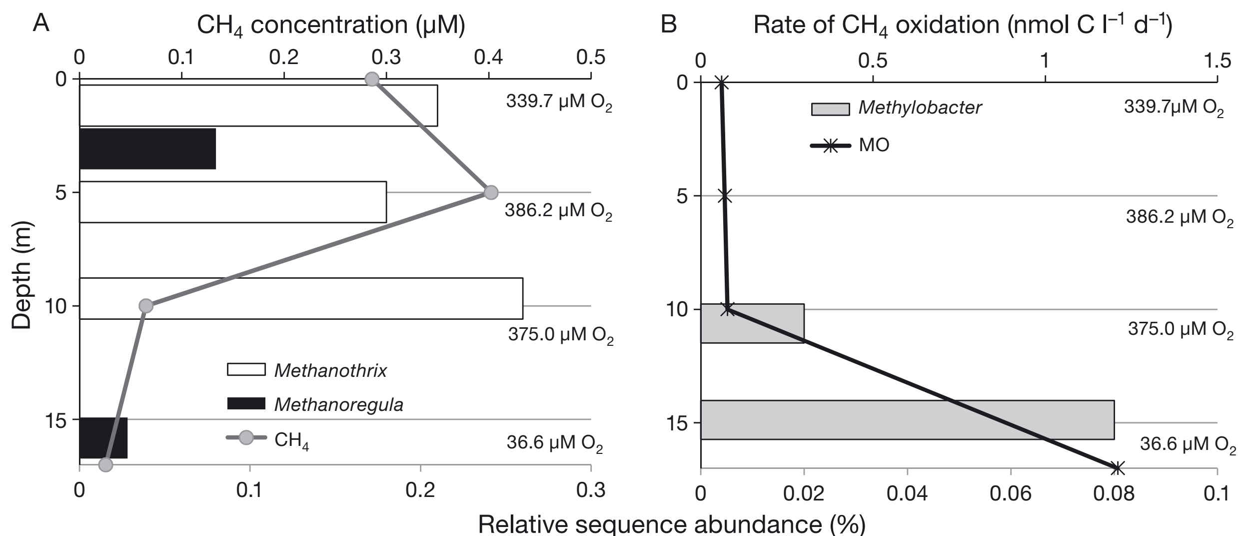

Fig. 3. Methane-associated microorganisms in the oxygen-saturated epilimnion of Lake Svetloe. (A) Relative abundance of methanogens toward the methane concentration and (B) relative abundance of aerobic methanotrophs toward the MO rates. Numbers on each panel show oxygen concentration

Total microbial numbers increased sharply in the chemocline with the maximum at 23 m. Microbial numbers in the hypolimnion were lower than in the chemocline, but higher than in the epilimnion (Fig. 1).

Rates of microbial processes

Data on the rates of dark and light CO_2 assimilation revealed 2 layers in the chemocline zone, differing in the character of microbial processes. The lower epilimnion and upper chemocline horizons (17–21.5 m) were the zones where oxygenic phototrophs developed, with maximum activities (150 and 120 nmol C l^{-1} d^{-1}) at 21 and 21.5 m depth, respectively (Fig. 2B). In the lower chemocline (21.5–23 m), anoxygenic phototrophs developed, with maximum activity (264 nmol C l^{-1} d^{-1}) at 22 m depth. The profile of the rates of dark CO_2 assimilation also had 2 maxima, which coincided with the peaks of oxygenic and anoxygenic photosynthesis. Another maximum of oxygenic phototrophic activity (435 nmol C l^{-1} d^{-1}), i.e. 3 times the rate in the chemocline, was located in the epilimnion, at 10 m. No activity of oxygenic or anoxygenic phototrophs was detected at 22 and 23 m, respectively, and below.

MO activity was very low (<0.1 nmol C l^{-1} d^{-1}) in the oxygen-saturated epilimnion (Fig. 3B), and started to increase below 10 m depth (Figs. 2A & 3B). The first small peak of MO rate (313 nmol C l^{-1} d^{-1}) was revealed in the chemocline zone at 21.5 m and coincided with the zone of highest activity of oxygenic phototrophs (Fig. 2B). Oxygen concentration in this layer was 9.4 µM (Fig. 2A). The highest MO rate of 2.6 µmol C l^{-1} d^{-1} was found in the chemocline, at 23 m. While oxygen (3.4 µM) was present in this horizon, radiotracer techniques did not reveal activity of oxygenic phototrophs (Fig. 2B). MO also occurred in the anaerobic hypolimnion, and the rates of this process (220–243 nmol C l^{-1} d^{-1}) were lower than in the chemocline (313–2611 nmol C l^{-1} d^{-1}) (Fig. 2A).

Light-dependent MO was confirmed at 21 and 21.5 m by $^{14}CH_4$ radiotracer experiments with transparent and darkened vials incubated directly at the sampled layers of the water column. Compared with the dark vials, MO rate in the light increased by almost 40% at 21 m and 30% at 21.5 m (*t*-test, p-value = 0.003). No stimulation of MO by light was observed in the presence of DCMU, an inhibitor of photosystem II in oxygenic phototrophs (Fig. 4). Low MO at 21 m, where oxygen supply was more favorable for development of aerobic methanotrophs, was probably due to low concentration of available methane (4.5 µM at 21 m compared with 18.5 µM at 21.5 m), rather than any other factor. No significant difference (*t*-test, p-value = 0.86) among light and dark MO rates was found for the 22 m depth sample, suggesting that the MO process occurred at this depth without contribution of oxygenic phototrophs. These results coincided with the absence of activity of oxygenic phototrophs measured with $NaH^{14}CO_3$ at 22 m and below (Fig. 2B).

The rates of methanogenesis are considered underestimated since they were measured only for hygro-

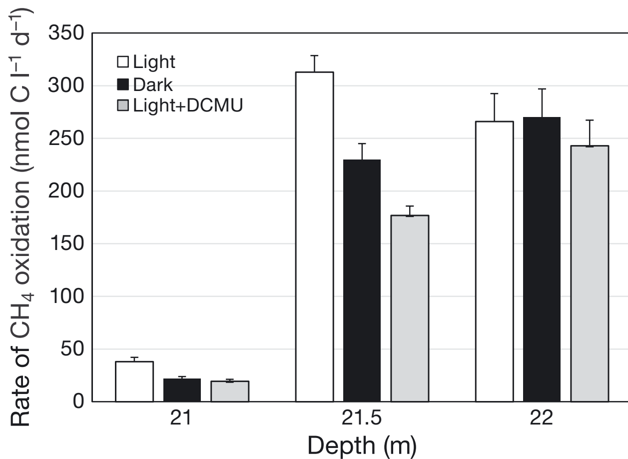

Fig. 4. Effect of light on the MO rate in the chemocline of Lake Svetloe. MO rates were determined by a radiotracer technique; the water samples with $^{14}CH_4$ were incubated *in situ* in transparent, transparent with addition of DCMU (inhibitor of photosystem II in oxygenic phototrophs), and darkened vials. Values are shown as averages ± SD (n = 3)

genotrophic methanogenesis with H_2/CO_2 as a substrate. Very low rates of H_2/CO_2 methanogenesis (1.8–6.2 nmol C l^{-1} d^{-1}) in the hypolimnion indicated that other substrates could possibly be involved in methane production, e.g. acetate, formate, or C1-methylated compounds.

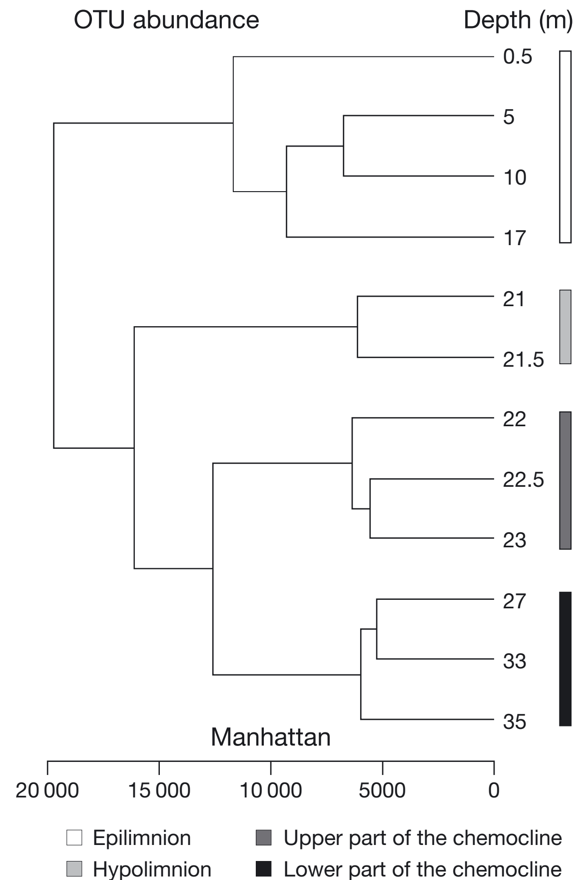

Fig. 5. Hierarchical cluster analysis of microbial communities' compositional profile in Lake Svetloe water column. Four zones (epilimnion, upper and lower chemocline, and hypolimnion) were revealed based on differences in microbial communities composition. Hierarchical cluster analysis was carried out using R language (hc function); Manhattan distance was used for the construction of the operational taxonomic unit (OTU) tree

Composition of microbial communities

Molecular genetic analysis resulted in detailed description of the microbial communities' composition throughout the water column of Lake Svetloe. Hierarchical cluster analysis of microbial community compositional profiles showed that microbial communities differed between 4 water layers: epilimnion (0.5–17 m), upper chemocline (21–21.5 m), lower chemocline (22–23 m), and hypolimnion (27–35 m) (Fig. 5), which was in agreement with the results of radiotracer measurements of the rates of the processes (Fig. 2).

The epilimnion microbial communities were typical of those from oxygen-saturated lake environments (Newton et al. 2011). Members of the phyla *Actinobacteria* (27–32% of the total number of the 16S rRNA gene sequences), *Bacteroidetes* (30–36%), *Planctomycetes* (2–3%), *Alphaproteobacteria* (2–4%), *Betaproteobacteria* (7–20%), and *Verrucomicrobia* (2–6%) were predominant (Fig. 6A).

Cyanobacteria became numerous at 17 m (12% of the total number of the 16S rRNA reads); their share in the upper layers did not exceed 3%. Microalgae were probably the major phototrophs in the epilimnion, since their chloroplast sequences were detected in these horizons. Cyanobacteria belonged mostly to the genera *Synechococcus* and, to a lesser degree, *Pseudoanabaena*. The shares of cyanobacteria and gammaproteobacteria were highest in the upper chemocline layers, reaching 15–17 and 11–12%, respectively, at 21–21.5 m (Fig. 6A). The gammaproteobacteria in the chemocline were represented almost exclusively by aerobic methanotrophs closely related to *Methylobacter psychrophilus* (99% identity of the 16S rRNA gene fragments), while their contribution to the microbial community of the oxygen-saturated epilimnion above 17 m was very low (up to 0.08%) (Fig. 3B). *Methylobacter* sp. was highly abundant at 21 and 21.5 m depths (11 and 10%, respectively). Another smaller peak of its abundance appeared at 23 m depth. These maxima matched the peaks of MO rates, although they were opposite in

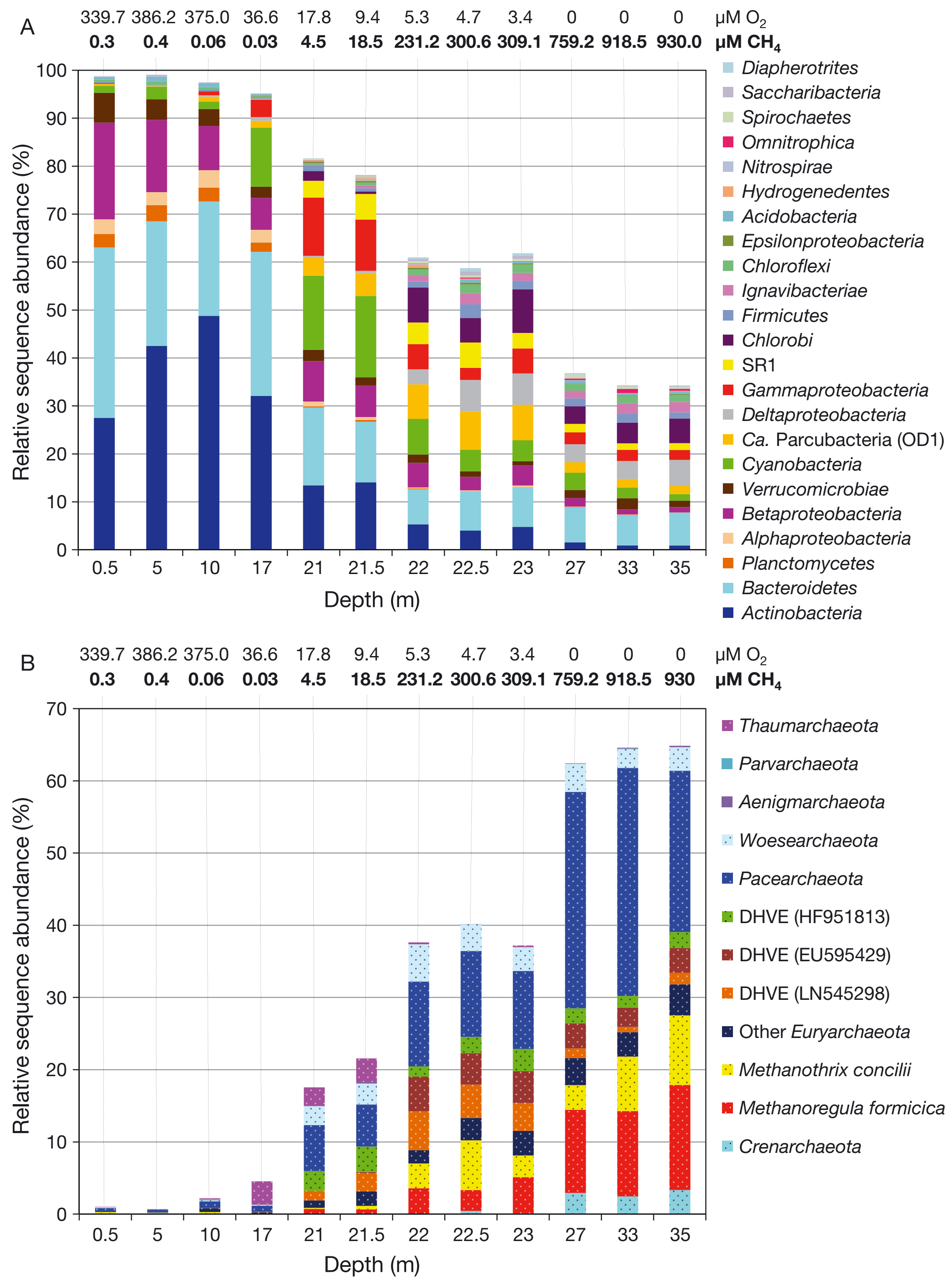

Fig. 6. Composition of microbial communities in Lake Svetloe water column determined by high-throughput sequencing of 16S rRNA genes. (A) Bacterial phyla. (B) Most abundant archaea. Numbers are given as percentages of total number of the 16S rRNA gene sequences. Numbers on each panel show oxygen (regular font) and methane (bold font) concentrations

direction: the highest MO activity was instead found at 23 m, and the smaller peak appeared at 21.5 m (Fig. 7). Few sequences of aerobic methanotrophs of the genera *Methylomonas*, *Methylomarinum*, *Methylococcus*, and *Methylocystis* were also detected in the chemocline.

Positive correlations were revealed between relative abundances of methanotrophic *Methylobacter*

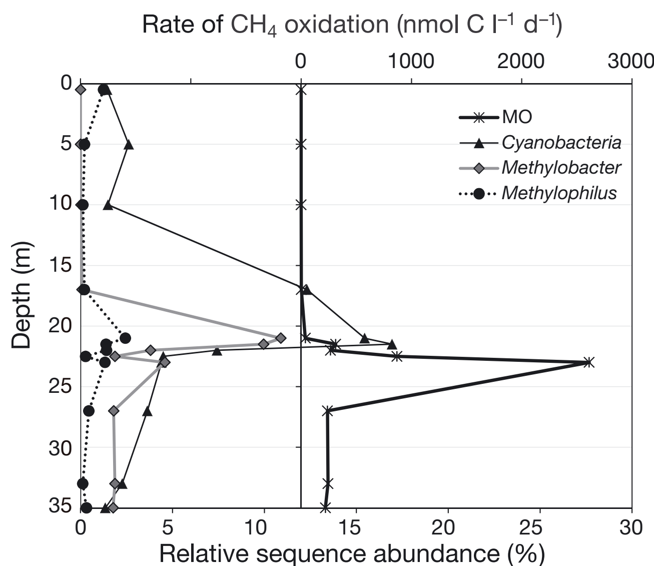

Fig. 7. Depth profiles for relative abundances of aerobic methanotrophs *Methylobacter* sp., methylotrophs *Methylophilus* sp., and oxygenic phototrophs of *Cyanobacteria* phylum in Lake Svetloe water column compared with the MO rates

sp. and cyanobacteria (ρ = 0.79, p-value = 0.0025), as well as with obligate methylotrophic betaproteobacteria *Methylophilus methylotrophus* (ρ = 0.81, p-value = 0.0015) (Fig. 7). It should be noted that the sequences of aerobic methanotrophs, methylotrophs, and cyanobacteria were also found in the anoxic hypolimnion. While their share in the hypolimnion was lower than in the chemocline, they still constituted a significant portion of the microbial community: up to 1.8% for *Methylobacter* sp., 1.35% for *Synechococcus* sp., and 0.3% for *Methylophilus* sp. at 35 m (Fig. 7).

Betaproteobacteria related (98% similarity of the 16S rRNA gene sequences) to non-photosynthetic facultatively anaerobic bacteria *Rhodoferax* (*Albidoferax*) *ferrireducens* capable of Fe^{3+} to Fe^{2+} reduction were revealed in the chemocline. While they were almost absent in the oxic epilimnion, their share was as high as 3.6% in the chemocline and not more than 0.3% in the anoxic hypolimnion. The share of anoxygenic green phototrophic bacteria *Chlorobium ferrooxidans*, which oxidize Fe^{2+} to Fe^{3+}, also increased to 9% in the lower chemocline layer (Fig. 6A).

Relative abundance of uncultured bacteria of the candidate division OD1 ('*Candidatus* Parcubacteria') increased in the lower chemocline layer (22–23 m). Their share reached 7.2–8.0% of the total number of the 16S rRNA gene reads in the zone of maximal MO (22–23 m), while it did not exceed 2% in the epilimnion and hypolimnion. Members of the candidate phylum SR1 were also present in the chemocline (3–5% of total 16S rRNA gene reads), but there was only a trace abundance in the epi- and hypolimnion (Fig. 6A). Relative abundance of *Deltaproteobacteria*, represented by sulfate reducers of the orders *Desulfobacteraceae* and *Syntrophaceae* (genus *Desulfomonile*), also increased (to 6.5%) in the lower chemocline layer, where a small peak of sulfide occurred (Fig. 1).

Among archaea, the organisms closely related (97–100% similarity of the 16S rRNA gene sequences) to deep-sea hydrothermal vent *Euryarchaeota* (DHVE) (GenBank accession nos. HF951813, EU595429, and LN545298) contributed significantly to the chemocline community, with their highest total share (11.3–11.6%) revealed in the lower chemocline horizons (22–23 m) (Fig. 6B). They were almost absent in the epilimnion, and their share in the hypolimnion was lower than in the chemocline (5–7%).

Microbial communities of the anoxic hypolimnion (27–35 m) were characterized by predominance of archaea, which were responsible for 62–65% of the total number of microbial 16S rRNA sequences. The 3 predominant archaeal phyla were *Euryarchaeota* (25–36%), *Pacearchaeota* (22–32%), and *Woesearchaeota* (3–4%) (Fig. 6B). The phylum *Euryarchaeota* was represented mainly by methanogens of the orders *Methanomicrobiales* and *Methanosarcinales* in almost equal amounts. Obligate aceticlastic methanogens *Methanothrix* (*Methanosaeta*) *concilii* (99% 16S rRNA similarity) were responsible for 7–9% of the total number of microbial sequences, while hydrogenotrophic *Methanoregula formicica* (97% similarity) constituted 11–13%. It should be noted that the sequences of anaerobic methanogens *Methanothrix* sp. and *Methanoregula* sp. were also detected in the oxygen-saturated epilimnion, where they constituted up to 0.26 and 0.08% of the total microbial 16S rRNA reads, respectively (Fig. 3B).

No sequences of the known microorganisms involved in AOM, i.e. archaea of the ANME clusters, including '*Candidatus* Methanoperedens nitroreducens,' and bacteria of the NC10 phylum ('*Candidatus* Methylomirabilis oxyfera'), were revealed in the water column of Lake Svetloe.

Incubation experiments

Addition of Fe^{3+} did not result in detectable methane consumption under anoxic conditions for any of the studied samples (chemocline and near-bottom water and the upper sediments). Contrary to

expectations, stimulation of methanogenesis was revealed for the sediment samples incubated with ferrihydrite ($Fe(OH)_3$) compared with the control without external Fe^{3+} (*t*-test, p-value = 0.006). Methanogenesis occurred in the sediments in all variants, both experimental with Fe^{3+} addition and control ones, with the lowest amount of methane (1.75 times lower than in the control) formed in the variant with addition of $Fe_2(SO_4)_3$ (Fig. 8). Incubation of water samples with Fe^{3+} resulted in neither methane consumption nor production.

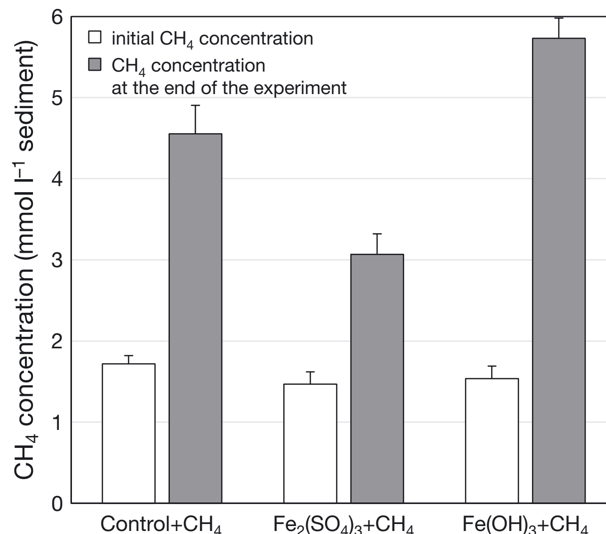

Fig. 8. Net methane production in the upper (0–5 cm) sediment samples from Lake Svetloe. Methane production was revealed in laboratory incubations under anaerobic conditions with methane and Fe^{3+} addition; methanogenic substrates were not supplemented. Values are shown as averages ± SD (n = 3)

DISCUSSION

Diverse microbial communities, which differed in composition between the oxic epilimnion, chemocline, and the anoxic hypolimnion, were revealed by 16S rRNA gene profiling in the water column of the meromictic subarctic freshwater Lake Svetloe (Fig. 5). Thirty high-level bacterial and archaeal taxa, including as yet uncultured candidate phyla, were detected (Fig. 6). In contrast to high overall diversity of microbial communities, the groups involved in the methane cycle processes, specifically methanogenesis and aerobic MO, were uniform throughout the water column and were represented by few genera of well-known methanogens and aerobic methanotrophs, typical inhabitants of freshwater lakes.

Based on molecular analyses, it can be concluded that methane was produced in the water column of Lake Svetloe via both aceticlastic and hydrogenotrophic methanogenesis, since all detected methanogens were almost equally represented by only 2 genera: *Methanothrix* (*Methanosaeta*) and *Methanoregula*. Acetate is the only growth substrate for obligate aceticlastic *Methanothrix* sp., which exhibits high substrate affinity and predominates in the environments with low (<1 mM) acetate concentrations (Jetten et al. 1992, Welte & Deppenmeier 2014). Another methanogen abundant in the Lake Svetloe is *Methanoregula* sp. belonging to obligate hydrogenotrophic cytochrome-lacking methanogens of *Methanomicrobiales* order, which due to their biochemical properties can use either H_2/CO_2 and/or formate (formate is transformed into H_2 by intracellular formate lyase) for growth and methane production (Thauer et al. 2008). *Methanoregula formicica*, the closest relative to those detected in Lake Svetloe, uses both H_2/CO_2 and formate (Yashiro et al. 2011). Due to very low (1.8–6.2 nmol C l^{-1} d^{-1}) rates of H_2/CO_2 methanogenesis determined in the hypolimnion of Lake Svetloe by radiotracer analyses, we suppose that formate was a preferable substrate for *Methanoregula* sp. The hydrogenotrophic *Methanoregulaceae* and aceticlastic *Methanosaetaceae* are the most frequently detected methanogens in freshwater lakes (Borrel et al. 2011), including boreal ones (Peura et al. 2015, Rissanen et al. 2017).

An ever-increasing number of recent studies indicate that methane production is not restricted to anaerobic conditions. Since this process is also widespread in oxygenated subsurface sea- and freshwaters, it can globally contribute to methane emission (Bogard et al. 2014, Repeta et al. 2016, DelSontro et al. 2017, Donis et al. 2017). Among the current explanations for this methane paradox are methane production by aerobic bacteria from methylphosphonates (Repeta et al. 2016), occurrence of anoxic microniches in surface waters, and involvement of some algal metabolites as precursors for methane production, etc. (see references within Donis et al. 2017). It was demonstrated for a shallow boreal oligo-mesotrophic lake that methane production in oxygenated waters is driven by aceticlastic methanogenesis and associated with algal dynamics (Bogard et al. 2014). Methanogenic archaea have been found in various oxic habitats (see references within Bogard et al. 2014), and antioxidant genes have been detected in their genomes (Lyu & Lu 2018). A small increase in methane concentration was also observed in oxygenated water layers of Lake Svetloe, and sequences of methanogenic archaea, particularly aceticlastic

Methanothrix sp., were detected in these horizons (Fig. 3). However, we cannot directly link anaerobic aceticlastic methanogens with increased methane concentration in subsurface waters, since we did not measure the aceticlastic activity *in situ*.

MO was revealed by radiotracer analysis in both oxic and anoxic water layers of Lake Svetloe. In oxygen-containing layers, MO can be attributed to aerobic psychrophilic methane-oxidizing gammaproteobacteria *Methylobacter psychrophilus*, as it was the major species among known methanotrophs detected throughout the water column by molecular analyses. Aerobic methanotrophs of genera *Methylomonas*, *Methylomarinum*, *Methylococcus*, and *Methylocystis* were also found, but their relative abundances were very low. *Methylobacter* spp. are considered as typical inhabitants of various freshwater habitats, including boreal lakes, as these methanotrophs are the ones most commonly detected in such environments (Borrel et al. 2011, He et al. 2012, Peura et al. 2012, Crevecoeur et al. 2017, Martinez-Cruz et al. 2017). Moreover, *Methylobacter* sp. was recently shown to be also involved in AOM in sediments of a thermokarst arctic lake (Martinez-Cruz et al. 2017).

It was previously discovered that aerobic methanotrophic gammaproteobacteria could play a principal role in MO in anoxic waters of freshwater stratified lakes (Milucka et al. 2015, Oswald et al. 2015, 2016a,b, 2017). One of the possibilities, so-called light-dependent MO, implies trophic relationships between aerobic methanotrophic gammaproteobacteria and oxygenic phototrophs below the oxycline. The latter produce oxygen, which may be used by aerobic methanotrophs for MO (Milucka et al. 2015, Oswald et al. 2015, 2016b). Light-dependent MO was hypothesized in the course of the present study and was confirmed for the upper chemocline layer (21–21.5 m) of Lake Svetloe by radiotracer *in situ* incubations (Fig. 4) and by obtaining a correlation ($\rho = 0.79$, p-value = 0.0025) between relative abundances of *Methylobacter* sp. and *Cyanobacteria* (Fig. 7). Sequences of cyanobacteria were also revealed in the anoxic hypolimnion of Lake Svetloe together with the sequences of *Methylobacter* sp. and methylotrophic *Methylophilus* sp., both known as aerobes. Based on the molecular analysis results, it was impossible to determine whether these microorganisms were physiologically active or were a suspension of inactive and dead cells. Cyanobacteria were most probably inactive, since no oxygenic CO_2 assimilation was detected below 22 m by radiotracer analysis. Due to a relatively high abundance of autofluorescent microorganisms, attempts at fluorescence in situ hybridization determination of the numbers of physiologically active aerobic methanotrophs in the water column proved unsuccessful. High autofluorescence in Lake Svetloe has been reported previously (Savvichev et al. 2017).

Methanotrophic *Methylobacter* sp. and methylotrophic *Methylophilus* sp. exhibited similar relative abundance profiles ($\rho = 0.81$, p-value = 0.0015) throughout the epilimnion, chemocline, and hypolimnion of Lake Svetloe (Fig. 7). It should be noted that correlation does not imply dependence between the parameters, but only indicates that 2 parameters vary according to the same pattern. This implies the existence of a third factor affecting both parameters so that they vary according to the same pattern and show correlation. Based on data from the current literature, we suppose, however, that positive correlation in relative abundances found for *Methylobacter* and *Methylophilus* in Lake Svetloe means that these microorganisms are associated. Under hypoxic conditions, classical type I methanotrophs of the family *Methylococcaceae* often occur together with methylotrophic bacteria of the family *Methylophilaceae*, which utilize methanol and methylamines, but not methane (Chistoserdova 2015). A good linear relationship between the relative abundances of methanotrophs and methylotrophs in pyrosequencing reads was revealed for the microbial community of arctic lake sediments that derived carbon from methane (He et al. 2012). *Methylococcaceae* and *Methylophilaceae* relative abundances increased in several ice-covered boreal lakes where MO occurred (Denfeld et al. 2016). Sequences of *Methylophilaceae* were also abundant together with *Methylobacter* sp. in ^{13}C DNA enrichment from the sediments of a thermokarst Alaskan lake (Martinez-Cruz et al. 2017). Predominance of these organisms in metagenomes and their coordinated response to stimulation with methane and nitrate may indicate that under oxygen limitation some species of methanotrophs and methylotrophs may be involved in cooperative cross-feeding interactions (He et al. 2012, Beck et al. 2013, Oshkin et al. 2015). Oxygen availability is one of the main factors determining their partnership in the course of MO (Oshkin et al. 2015). The question is, do these relations enhance MO meaningfully under hypoxic conditions or not?

The highest MO rate was measured at the lower chemocline zone (23 m) of Lake Svetloe, where traces of oxygen (3.4 µM) were still present. We failed to find positive correlation between MO and relative abundance of *Methylobacter* sp. for the chemocline zone of the lake: the highest peak of MO rate

matched the lowest peak in *Methylobacter* sp. abundance and vice versa (ρ = 0.16, p-value = 0.63) (Fig. 7). The first, smaller peak of MO rate detected at 21.5 m can be attributed to *Methylobacter* sp. However, at the lower chemocline layer (23 m) other microorganisms might contribute to MO together with aerobic methanotrophs. Among different taxa with a total share in the community over 1%, a correlation was revealed (with ρ > 0.55) between MO rates and relative abundances of *Chlorobi* (ρ = 0.72, p-value = 0.008), *Deltaproteobacteria* (ρ = 0.68, p-value = 0.014), '*Candidatus* Parcubacteria' (OD1) (ρ = 0.66, p-value = 0.02), *Chloroflexi* (ρ = 0.61, p-value = 0.034), and the weakest correlation with DHVE archaea (accession no. EU595429) (ρ = 0.57, p-value = 0.054). None of the listed taxa, except sulfate-reducing *Deltaproteobacteria*, have yet been proved as directly participating in either aerobic or anaerobic MO. We could only speculate about their possible involvement in MO by providing some intermediates that could be further used by methanotrophic microorganisms. For example, in our studies, the phylum *Chlorobi* was represented by *Chlorobium ferrooxidans* (99–100% 16S rRNA similarity), which is known to oxidize Fe^{2+} to Fe^{3+} (Heising et al. 1999), and therefore could provide Fe^{3+} for methanotrophs as an electron acceptor for AOM. The sulfate-reducing members of *Deltaproteobacteria*, whose relative abundances also matched the peak of sulfide concentration in the chemocline, might be involved in sulfate-dependent AOM. In our case, however, ANME archaea were not detected in Lake Svetloe. Members of the novel candidate division '*Candidatus* Parcubacteria' (OD1) are globally distributed in marine and terrestrial habitats, including freshwater lakes, and appear to be mainly present in anoxic environments (Elshahed et al. 2005, Luef et al. 2015). Since the overall representation of this group was positively correlated with dissolved organic carbon and methane concentrations in the suboxic hypolimnion, it was hypothesized that these bacteria were anaerobic and probably played a role in MO (Peura et al. 2012). Recent genomic studies indicated the possibility of their anaerobic fermentative metabolism; the data on their possible growth substrates are, however, contradictory (Kantor et al. 2013, León-Zayas et al. 2017). The *Chloroflexi* detected in Lake Svetloe are represented by members of the family *Anaerolineaceae*, which are known as saccharolytic anaerobes (Yamada et al. 2007). The DHVE group includes the *Euryarchaeota* from diverse cold and terrestrial environments (Plasencia et al. 2011). Their role in communities is presently not established. Previous studies on marine planktonic *Euryarchaeota* have suggested either a putative anaerobic respiration physiology or the potential to carry out a photoheterotrophic metabolism (Restrepo-Ortiz & Casamayor 2013). It is also possible that microbial groups potentially involved in MO in Lake Svetloe were missed at the stage of DNA extraction or PCR amplification due to some limitations of protocols or primers.

While AOM was revealed in the hypolimnion of Lake Svetloe by radiotracer analyses, it is still unclear what microbial groups were responsible for this process. Involvement of the known sulfate-, nitrate-, and nitrite-dependent anaerobic methane oxidizers can most likely be ruled out, since these organisms were not detected by molecular genetic techniques, and concentrations of sulfate, nitrite, and nitrate in the water column were low (Table 1). *Methylobacter* sp. might be involved in AOM, since methanotrophic gammaproteobacteria were previously hypothesized to couple MO with reduction of manganese and iron oxides or nitrate/nitrite in the anoxic hypolimnion of freshwater lakes (Oswald et al. 2016a, 2017). However, the mechanism underlying this process remains unclear, as well as the physiological state of *Methylobacter* sp. detected in the hypolimnion of Lake Svetloe. Participation of Fe^{3+} as an electron acceptor for AOM has been strongly suggested for various freshwater basins, including ferruginous and boreal lakes (Crowe et al. 2011, Norði, et al. 2013, Ettwig et al. 2016, Oswald et al. 2016a,b, Rissanen et al. 2017, Timmers et al. 2017, He et al. 2018, Sturm et al. preprint doi:10.5194/bg-2015-533), and was hypothesized in the course of the present study. Since taxonomic affiliation of the microorganisms responsible for metal-dependent AOM has not been clearly defined, we conducted incubations with Fe^{3+} additions to enrich, if possible, the microbial groups involved. However, the effect of Fe^{3+} was unclear. We did not detect methane consumption in any of the water and sediment samples. Instead, a net methane production was observed for sediment samples, which, however, cannot completely rule out AMO activity, since more sensitive measurements are required for AOM quantification, e.g. addition of labeled CH_4 to track the amount of CH_4 being oxidized from the total CH_4 (added and produced by methanogenesis). The sulfate ion derived from $Fe_2(SO_4)_3$ was initially supposed to be an electron acceptor for AOM in addition to Fe^{3+}. Instead, $Fe_2(SO_4)_3$ supply most probably led to competition between sulfate reducers and methanogens, which resulted in lower methane production compared with the control and treatment with ferrihydrite (Fig. 8). Our data were similar to the results of

Rissanen et al. (2017), who also did not observe stimulation of potential MO in anaerobic incubations supplemented with Fe^{3+} for the sediments of 2 iron-enriched shallow boreal lakes. In the case of methanogenesis, however, our results contradicted: in the work of Rissanen et al. (2017), Fe^{3+} addition decreased the potential net methane production rates. The suppression of methanogenesis was attributed in this study to protection of organic matter from degradation caused by Fe^{3+} supply (Rissanen et al. 2017). AOM coupled to Fe^{3+} reduction has also been suggested for the ferruginous Lake La Cruz, but the authors did not establish a clear link between MO and concurrent metal reduction (Oswald et al. 2016b). Based on our incubation experiments and referred results of other researchers, it is also reasonable to suggest that in Lake Svetloe, Fe^{3+} is not involved directly (as an electron acceptor) in AMO, and conversion of Fe^{3+} to Fe^{2+} and vice versa occurred mostly due to activity of *Rhodoferax ferrireducens* and *C. ferrooxidan*s, both abundant in the chemocline. *R. ferrireducens* reduces Fe^{3+} to Fe^{2+} by using various organic compounds as electron donors (Finneran et al. 2003, Risso et al. 2009). Since AOM rates were relatively low (~10% of the rate of AMO at 23 m), TMO by methanogenic archaea (Timmers et al. 2017) might also be suggested for Lake Svetloe.

CONCLUSION

Meromictic ferruginous subarctic Lake Svetloe is an example of a freshwater basin with extremely low diversity of microorganisms involved in the aerobic MO and methanogenesis. These microorganisms are uniform throughout the water column and represented by only few genera (aerobic *Methylobacter*-related methanotrophs, aceticlastic *Methanothrix*- and hydrogenotrophic *Methanoregula*-related methanogens). Detection of methanogens in the oxygenated epilimnion, together with an increase in methane concentration suggests archaeal origin of epilimnetic methane. This methane, while small, could be a potential source of emission due to very low MO rates and low abundance of aerobic methanotrophs in oxygenated waters. The light-dependent MO detected in the Lake Svetloe upper chemocline suggests trophic relations between aerobic methanotrophs and cyanobacteria similar to those found in a number of lakes located in the temperate climatic zone. This further suggests ubiquity of light-dependent MO in stratified freshwater basins. Special attention must be paid to the lower chemocline layers of meromictic freshwater lakes where oxygenic photosynthesis is absent and oxygen is close to the detection limit, while MO is high. The question of which microorganisms are responsible for MO in the lower chemocline and hypolimnion of Lake Svetloe is still open. It is very attractive to attribute the highest MO rate exclusively to aerobic *Methylobacter* sp., since it was the only known methanotroph detected. However, the absence of positive correlation between MO rates and *Methylobacter* relative abundance suggested that other microorganisms might contribute to this process together with *Methylobacter*. Microbial groups and electron acceptors driving AOM require more detailed research, since known ANME archaea and NC10 bacteria were not detected in the hypolimnion of Lake Svetloe, and incubations with Fe^{3+} did not reveal methane consumption under anoxic conditions. Other questions for future studies are why did ferrihydrite addition promote methanogenesis, and what underlies this stimulation?

Acknowledgements. We are grateful to I. Yu. Oshkin (Winogradsky Institute of Microbiology, Research Center of Biotechnology, Russian Academy of Sciences) for determination of the total microbial numbers, A. V. Chupakov (N. Laverov Federal Center for Integrated Arctic Research) for assistance in field work, and M. V. Glagolev (Lomonosov Moscow State University) for assistance in data analyses. We are grateful to the 3 anonymous reviewers and the editor for their valuable and constructive comments and recommendations. The work was performed using the scientific equipment of Core Research Facility 'Bioengineering' and supported by the Russian Science Foundation, project no. 16-14-10201.

LITERATURE CITED

Bastviken D, Ejlertsson J, Tranvik L (2002) Measurement of methane oxidation in lakes: a comparison of methods. Environ Sci Technol 36:3354–3361

Bastviken D, Cole J, Pace M, Tranvik L (2004) Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochem Cycles 18:GB4009

Bastviken D, Tranvik LJ, Downing JA, Crill PM, Enrich-Prast A (2011) Freshwater methane emissions offset the continental carbon sink. Science 331:50

Beck DA, Kalyuzhnaya MG, Malfatti S, Tringe SG and others (2013) A metagenomics insight into freshwater methane-utilizing communities and evidence for cooperation between the *Methylococcaceae* and the *Methylophilaceae*. PeerJ 1:e23

Biderre-Petit C, Jézéquel D, Dugat-Bony E, Lopes F and others (2011) Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. FEMS Microbiol Ecol 77:533–545

Bogard MJ, del Giorgio PA, Boutet L, Chaves MC and others (2014) Oxic water column methanogenesis as a major component of aquatic CH_4 fluxes. Nat Commun 5:5350

Borrel G, Jézéquel D, Biderre-Petit C, Morel-Desrosiers N and others (2011) Production and consumption of methane in freshwater lake ecosystems. Res Microbiol 162:832–847

Camacho A, Walter XA, Picazo A, Zopfi J (2017) Photoferrotrophy: remains of an ancient photosynthesis in modern environments. Front Microbiol 8:323

Canfield DE, Rosing MT, Bjerrum C (2006) Early anaerobic metabolisms. Philos Trans R Soc Lond B Biol Sci 361: 1819–1836

Chistoserdova L (2015) Methylotrophs in natural habitats: current insights through metagenomics. Appl Microbiol Biotechnol 99:5763–5779

Chupakov AV, Chupakova AA, Moreva OYu, Shirokova LS and others (2017) Allochthonous and autochthonous carbon in deep, organic-rich and organic-poor lakes of the European Russian subarctic. Boreal Environ Res 22: 213–230

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14: 454–458

Cole JR, Wang Q, Cardenas E, Fish J and others (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37: D141–D145

Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292

Costello AM, Auman AJ, Macalady JL, Scow KM, Lindstrøm ME (2002) Estimation of methanotroph abundance in a freshwater lake sediments. Environ Microbiol 4:443–450

Crevecoeur S, Vincent WF, Comte J, Matveev A, Lovejoy C (2017) Diversity and potential activity of methanotrophs in high methane-emitting permafrost thaw ponds. PLOS ONE 12:e0188223

Crowe SA, Katsev S, Leslie K, Sturm A and others (2011) The methane cycle in ferruginous Lake Matano. Geobiology 9:61–78

Danilova OV, Suzina NE, Van De Kamp J, Svenning MM and others (2016) A new cell morphotype among methane oxidizers: a spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. ISME J 10:2734–2743

DelSontro T, del Giorgio PA, Prairie YT (2017) No longer a paradox: the interaction between physical transport and biological processes explains the spatial distribution of surface water methane within and across lakes. Ecosystems, doi:10.1007/s10021-017-0205-1

Denfeld BA, Ricão Canelhas M, Weyhenmeyer GA, Bertilsson S and others (2016) Constraints on methane oxidation in ice-covered boreal lakes. J Geophys Res Biogeosci 121: 1924–1933

Donis D, Flury S, Stöckli A, Spangenberg JE and others (2017) Full-scale evaluation of methane production under oxic conditions in a mesotrophic lake. Nat Commun 8:1661

Duc NT, Crill P, Bastviken D (2010) Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. Biogeochemistry 100: 185–196

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

Egger M, Rasigraf O, Sapart CJ, Jilbert T and others (2015) Iron-mediated anaerobic oxidation of methane in brackish coastal sediments. Environ Sci Technol 49:277–283

Elshahed MS, Najar FZ, Aycock M, Qu C and others (2005) Metagenomic analysis of the microbial community at Zodletone Spring (Oklahoma): insights into the genome of a member of the novel candidate division OD1. Appl Environ Microbiol 71:7598–7602

Erkkilä KM, Ojala A, Bastviken D, Biermann T and others (2018) Methane and carbon dioxide fluxes over a lake: comparison between eddy covariance, floating chambers and boundary layer method. Biogeosciences 15:429–445

Ershova AA, Vorobyeva TYa, Moreva OYu, Chupakov AV and others (2015) Hydrochemical and microbiological research of a nitrogen cycle in freshwater meromictic lake Svetloe (the Arkhangelsk region). Reg Environ Issues 5:44–50

Ettwig KF, Butler MK, Le Paslier D, Pelletier E and others (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Ettwig KF, Zhu B, Speth D, Keltjens JT and others (2016) Archaea catalyze iron-dependent anaerobic oxidation of methane. Proc Natl Acad Sci USA 113:12792–12796

Finneran KT, Johnsen CV, Lovley DR (2003) *Rhodoferax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). Int J Syst Evol Microbiol 53:669–673

Fu L, Li SW, Ding ZW, Ding J and others (2016) Iron reduction in the DAMO/*Shewanella oneidensis* MR-1 coculture system and the fate of Fe(II). Water Res 88:808–815

Gies EA, Konwar KM, Beatty JT, Hallama SJ (2014) Illuminating microbial dark matter in meromictic Sakinaw Lake. Appl Environ Microbiol 80:6807–6818

Gorlenko VM, Vainstein MV, Chebotarev EN (1980) Bacteria involved in turnover of sulfur and iron metabolism in meromictic Lake Kuznechikha with low sulfate content. Microbiology 49:804–812

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Haroon MF, Hu S, Shi Y, Imelfort M and others (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 500:567–570

He R, Wooller MJ, Pohlman JW, Quensen J and others (2012) Shifts in identity and activity of methanotrophs in arctic lake sediments in response to temperature changes. Appl Environ Microbiol 78:4715–4723

He Z, Zhang Q, Feng Y, Luo H and others (2018) Microbiological and environmental significance of metal-dependent anaerobic oxidation of methane. Sci Total Environ 610–611:759–768

Heising S, Richter L, Ludwig W, Schink B (1999) *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a '*Geospirillum*' sp. strain. Arch Microbiol 172:116–124

Jetten MSM, Stams AJM, Zehnder AJB (1992) Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. FEMS Microbiol Rev 88:181–197

Kankaala P, Taipale S, Nykänen H, Jones RI (2007) Oxidation, efflux, and isotopic fractionation of methane during autumnal turnover in a polyhumic, boreal lake. J Geophys Res 112:G02033

Kantor RS, Wrighton KC, Handley KM, Sharon I, and others (2013) Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla. MBio 4:e00708-13

Knittel K, Boetius A (2009) Anaerobic oxidation of methane:

progress with an unknown process. Annu Rev Microbiol 63:311–334
Laurion I, Vincent WF, MacIntyre S, Retamal L and others (2010) Variability in greenhouse gas emissions from permafrost thaw ponds. Limnol Oceanogr 55:115–133
Lehours AC, Borrel G, Morel-Desrosiers N, Bardot C and others (2016) Anaerobic microbial communities and processes involved in the methane cycle in freshwater lakes — a focus on Lake Pavin. In: Sime-Ngando T, Boivin P, Chapron E, Jezequel D, Meybeck M (eds) Lake Pavin. Springer, Cham, p 255–284
León-Zayas R, Peoples L, Biddle JF, Podell S and others (2017) The metabolic potential of the single cell genomes obtained from the Challenger Deep, Mariana Trench within the candidate superphylum *Parcubacteria* (OD1). Environ Microbiol 19:2769–2784
Lidström ME, Somers L (1984) Seasonal study of methane oxidation in Lake Washington. Appl Environ Microbiol 47:1255–1260
Liu Y, Whitman WB (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 1125:171–189
Llirós M, García-Armisen T, Darchambeau F, Morana C and others (2015) Pelagic photoferrotrophy and iron cycling in a modern ferruginous basin. Sci Rep 5:13803
Lofton DD, Whalen SC, Hershey AE (2015) Vertical sediment distribution of methanogenic pathways in two shallow Arctic Alaskan lakes. Polar Biol 38:815–827
Luef B, Frischkorn KR, Wrighton KC, Holman HY and others (2015) Diverse uncultivated ultra-small bacterial cells in groundwater. Nat Commun 6:6372
Lyu Z, Lu Y (2018) Metabolic shift at the class level sheds light on adaptation of methanogens to oxidative environments. ISME J 12:411–423
Martinez-Cruz K, Sepulveda-Jauregui A, Anthony KW, Thalasso F (2015) Geographic and seasonal variation of dissolved methane and aerobic methane oxidation in Alaskan lakes. Biogeosciences 12:4595–4606
Martinez-Cruz K, Leewis MC, Herriott IC, Sepulveda-Jauregui A and others (2017) Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. Sci Total Environ 607-608:23–31
McAuliffe CC (1971) GC determination of solutes by multiple phase equilibration. Chem Technol 1:46–51
Milucka J, Kirf M, Lu L, Krupke A and others (2015) Methane oxidation coupled to oxygenic photosynthesis in anoxic waters. ISME J 9:1991–2002
Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49
Norði K, Thamdrup B, Schubert CJ (2013) Anaerobic oxidation of methane in an iron-rich Danish freshwater lake sediment. Limnol Oceanogr 58:546–554
Oshkin IY, Beck DA, Lamb AE, Tchesnokova V and others (2015) Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. ISME J 9:1119–1129
Oswald K, Milucka J, Brand A, Littmann S and others (2015) Light-dependent aerobic methane oxidation reduces methane emissions from seasonally stratified lakes. PLOS ONE 10:e0132574
Oswald K, Milucka J, Brand A, Hach P and others (2016a) Aerobic gammaproteobacterial methanotrophs mitigate methane emissions from oxic and anoxic lake waters. Limnol Oceanogr 61:S101–S118
Oswald K, Jegge C, Tischer J, Berg J and others (2016b) Methanotrophy under versatile conditions in the water column of the ferruginous meromictic lake La Cruz (Spain). Front Microbiol 7:1762
Oswald K, Graf JS, Littmann S, Tienken D, and others (2017) *Crenothrix* are major methane consumers in stratified lakes. ISME J 11:2124–2140
Peura S, Eiler A, Bertilsson S, Nykänen H and others (2012) Distinct and diverse anaerobic bacterial communities in boreal lakes dominated by candidate division OD1. ISME J 6:1640–1652
Peura S, Sinclair L, Bertilsson S, Eiler A (2015) Metagenomic insights into strategies of aerobic and anaerobic carbon and nitrogen transformation in boreal lakes. Sci Rep 5:12102
Pimenov NV, Bonch-Osmoloyskaya EA (2006) In situ activity studies in thermal environments. In: Rainey F, Oren A (eds) Methods in microbiology, Vol 35. Elsevier, Amsterdam p 29–53
Pimenov NV, Kallistova AYu, Rusanov II, Yusupov SK and others (2010) Methane formation and oxidation in the meromictic oligotrophic Lake Gek-Gel (Azerbaijan). Microbiology 79:247–252
Plasencia A, Baneras L, Lliros M, Casamayor EO, Borrego C (2011) Maintenance of previously uncultured freshwater archaea from anoxic waters under laboratory conditions. Ant Leeuwenhoek 99:403–408
Repeta DJ, Ferrón S, Sosa OA, Johnson CG and others (2016) Marine methane paradox explained by bacterial degradation of dissolved organic matter. Nat Geosci 9: 884–887
Restrepo-Ortiz CX, Casamayor EO (2013) Environmental distribution of two widespread uncultured freshwater *Euryarchaeota* clades unveiled by specific primers and quantitative PCR. Environ Microbiol Rep 5:861–867
Rissanen AJ, Karvinen A, Nykänen H, Peura S and others (2017) Effects of alternative electron acceptors on the activity and community structure of methane-producing and consuming microbes in the sediments of two shallow boreal lakes. FEMS Microbiol Ecol 93:fix078
Risso C, Sun J, Zhuang K, Mahadevan R and others (2009) Genome-scale comparison and constraint-based metabolic reconstruction of the facultative anaerobic Fe(III)-reducer *Rhodoferax ferrireducens*. BMC Genomics 10:447
Saunois M, Bousquet P, Poulter B, Peregon A (2016) The global methane budget 2000–2012. Earth Syst Sci Data 8: 697–751
Savvichev AS, Kokryatskaya NM, Zabelina SA, Rusanov II and others (2017) Microbial processes of the carbon and sulfur cycles in an ice-covered, iron-rich meromictic lake Svetloe (Arkhangelsk region, Russia). Environ Microbiol 19:659–672
Schloss PD, Westcott SL, Ryabin T, Hall JR and others (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
Sepulveda-Jauregui A, Walter Anthony KM, Martinez-Cruz K, Greene S, Thalasso F (2015) Methane and carbon dioxide emissions from 40 lakes along a north–south latitudinal transect in Alaska. Biogeosciences 12:3197–3223
Sundh I, Bastviken D, Tranvik LJ (2005) Abundance, activity, and community structure of pelagic methane oxidizing bacteria in temperate lakes. Appl Environ Microbiol 71:6746–6752

Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol 6: 579–591

Timmers PH, Welte CU, Koehorst JJ, Plugge CM and others (2017) Reverse methanogenesis and respiration in methanotrophic archaea. Archaea 2017:1654237

Viollier E, Inglett PW, Hunter K, Roychoudhury AN, Van Cappellen P (2000) The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. Appl Geochem 15:785–790

Vonk JE, Tank SE, Bowden WB, Laurion I and others (2015) Reviews and syntheses: effects of permafrost thaw on Arctic aquatic ecosystems. Biogeosciences 12:7129–7167

Walter KM, Smith LC, Chapin FS (2007) Methane bubbling from northern lakes: present and future contributions to the global methane budget. Philos Trans A Math Phys Eng Sci 365:1657–1676

Walter XA, Picazo A, Miracle MR, Vicente E and others (2014) Phototrophic Fe(II)-oxidation in the chemocline of a ferruginous meromictic lake. Front Microbiol 5:713

Welte C, Deppenmeier U (2014) Bioenergetics and anaerobic respiratory chains of aceticlastic methanogens. Biochim Biophys Acta 1837:1130–1147

Welte CU, Rasigraf O, Vaksmaa A, Versantvoort W and others (2016) Nitrate- and nitrite-dependent anaerobic oxidation of methane. Environ Microbiol Rep 8:941–955

Wik M, Varner RK, Walter Anthony K, MacIntyre S, Bastviken D (2016a) Climate-sensitive northern lakes and ponds are critical components of methane release. Nat Geosci 9:99–105

Wik M, Thornton BF, Bastviken D, Uhlbäck J, Crill PM (2016b) Biased sampling of methane release from northern lakes: a problem for extrapolation. Geophys Res Lett 43:1256–1262

Wilson K (2003) Preparation of genomic DNA from bacteria. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Current protocols in molecular biology. Wiley & Sons, New York, NY, 2.4.1–2.4.5

Yamada T, Imachi H, Ohashi A, Harada H and others (2007) *Bellilinea caldifistulae* gen. nov., sp. nov. and *Longilinea arvoryzae* gen. nov., sp. nov., strictly anaerobic, filamentous bacteria of the phylum *Chloroflexi* isolated from methanogenic propionate-degrading consortia. Int J Syst Evol Microbiol 57:2299–2306

Yashiro Y, Sakai S, Ehara M, Miyazaki M and others (2011) *Methanoregula formicica* sp. nov., a methane-producing archaeon isolated from methanogenic sludge. Int J Syst Evol Microbiol 61:53–59

Yu Y, Lee C, Kim J, Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng 89:670–679

Zabelina SA, Pokrovsky OS, Klimov SI, Chupakov AV and others (2013) The first results of the methane concentration in the stratified lakes of the River Svetlaya (the catchment area of the White Sea). In: Lisitzin AP, Politova NV, Shevchenko VP (eds) Geology of seas and oceans. Proc 20th Int Sci Conf on Marine Geology, Vol III. GEOS, Moscow, p 156–160

Zavarzina DG, Chistyakova NI, Shapkin AV, Savenko AV and others (2016) Oxidative biotransformation of biotite and glauconite by alkaliphilic anaerobes: the effect of Fe oxidation on the weathering of phyllosilicates. Chem Geol 439:98–109

Editorial responsibility: Paul del Giorgio, Montreal, Quebec, Canada

Submitted: September 27, 2017; Accepted: May 28, 2018
Proofs received from author(s): August 21, 2018