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ORIGINAL PAPER

Microbial communities involved in the methane cycle in the near-bottom water layer and sediments of the meromictic subarctic Lake Svetloe

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Abstract Although arctic and subarctic lakes are important sources of methane, the emission of which will increase due to the melting of permafrost, the processes related to the methane cycle in such environments are far from being comprehensively understood. Here we studied the microbial communities in the near-bottom water layer and sediments of the meromictic subarctic Lake Svetloe using high-throughput sequencing of the 16S rRNA and methyl coenzyme M reductase subunit A genes. Hydrogenotrophic methanogens of the order *Methanomicrobiales* were abundant, both in the water column and in sediments, while the share of acetoclastic *Methanosaetaceae* decreased with the depth of sediments. Members of the *Methanomassiliicoccales* order were absent in the water but abundant in the deep sediments. Archaea known to perform anaerobic oxidation of methane were not found. The bacterial component of the microbial community in the bottom water layer included oxygenic (*Cyanobacteria*) and anoxygenic (*Chlorobi*) phototrophs, aerobic Type I methanotrophs, methylotrophs, syntrophs, and various organotrophs. In deeper sediments the diversity of the microbial community decreased, and it became dominated by methanogenic archaea and the members of the *Bathyarchaeota*, *Chloroflexi* and Deltaproteobacteria. This study shows that the sediments of a subarctic meromictic lake contain a taxonomically and metabolically diverse community potentially capable of complete mineralization of organic matter.

Keywords Freshwater lake · Microbial diversity sediments · Methanogenesis

Introduction

About one-third of total emissions of methane, the second most important greenhouse gas after carbon dioxide, originate from natural environments, primary wetlands. Although lakes cover less than 1% of the Earth's surface, their estimated contribution to total natural methane emissions is between 6 and 16% (Bastviken et al. 2004; Kirschke et al. 2013), which is much higher than emissions from the world's oceans. Arctic and subarctic lakes are important sources of

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methane, and their role in the global methane cycle is ever increasing due to the melting of permafrost and, as a consequence, increased methane production via methanogenesis (Walter et al. 2007; Wik et al. 2016; Martinez-Cruz et al. 2017). Although thermokarst lakes attract the most scientific attention due to their high methane emission rates (Wik et al. 2016; Carnevali et al. 2018), glacial and post-glacial lakes emit overall more methane due to their larger total area (Wik et al. 2016).

Biogenic methane is mostly produced by methanogenic archaea performing the terminal stage of decomposition of organic matter in anoxic environments (Conrad 2009; Borrel et al. 2011). In sulphate-poor freshwater lakes methane production rates are maximal in the upper sediments at the water–sediment interface, while in meromictic lakes significant methane production occurs also in the permanently anoxic hypolimnion (Borrel et al. 2011). Up to 80–90% of produced methane is subsequently oxidized by aerobic and anaerobic methanotrophs that utilise methane as a carbon and energy source (Knittel and Boetius 2009; Ettwig et al. 2010; Chistoserdova 2015). Aerobic methane oxidation is most active at the oxic-anoxic interface, where methane and oxygen form steep counter gradients. Aerobic methane oxidation also occurs in the near-bottom water layer and the upper layer of the sediments in oxygenated lakes, or in the chemocline zone in stratified lakes (Borrel et al. 2011; Lidström and Somers 1984; Sundh et al. 2005).

However, the processes related to the methane cycle in lakes are far from being fully understood, which is evident from such findings as active methane production in oxygenated waters (Schulz et al. 2001; Grossart et al. 2011; Tang et al. 2016) and methane oxidation in anoxic zones (Crevecoeur et al. 2017; Blees et al. 2014; Rissanen et al. 2018). The coupling of methane oxidation to nitrate reduction under hypoxia was recently shown in the Gammaproteobacterium *Methylomonas denitrificans*, assigning this species a previously missed role in connecting the carbon and nitrogen cycles (Kits et al. 2015). Methanotrophic Gammaproteobacteria were reported to be involved in methane oxidation in anoxic zones, probably due to interactions with methylotrophic bacteria of the family *Methylophilaceae* (Chistoserdova 2015) or the ability to use Fe^{3+}, Mn^{4+}, or nitrate as electron acceptors (Oswald et al. 2016).

Previously, we investigated the microbial processes of the methane cycle in the ferruginous Lake Svetloe in the Arkhangelsk region of the Russian Federation (Kallistova et al. 2019). Lake Svetloe represents a very rare type of freshwater meromictic lakes, as it has high concentrations of ferrous iron and dissolved methane and low concentrations of sulphate and sulphide in the anoxic hypolimnion (Savvichev et al. 2017). Iron-enriched and sulphur-depleted meromictic lakes with conditions suitable for photoferrotrophy are considered modern analogues of the ancient Archean ocean (Canfield et al. 2006; Crowe et al. 2011; Camacho et al. 2017). A combination of physicochemical and radiotracer analysis, and 16S rRNA gene profiling was used to characterise microbial communities in the water column of Lake Svetloe and to correlate the community profile and methane cycle processes (Kallistova et al. 2019). Both hydrogenotrophic and acetoclastic methanogenic archaea were responsible for methane production in the anoxic zone (monimolimnion). Methane oxidation was detected by radiotracer analysis both in oxic and anoxic zones. The maximum levels occurring at the chemocline were attributed to activity of aerobic *Methylobacter* trophically interacting with cyanobacteria (Kallistova et al. 2019). However, the mechanisms of methane oxidation in the anoxic zone are unclear, since no known anaerobic methanotrophs were detected (Kallistova et al. 2019). Moreover, microbial communities in the sediments of Lake Svetloe, which most likely make a major contribution to overall methane production, have not been studied previously.

Microbial communities in the sediments of arctic and subarctic lakes are generally understudied relative to their counterparts in the lakes located in the temperate and tropical areas, making information about them sparse (Matheus Carnevali et al. 2018; Ruuskanen et al. 2018; He et al. 2012; Rissanen et al. 2019). In this work, to complete the description of the methane cycle in Lake Svetloe and further investigate the sediment microbial communities of high-latitude lakes, we carried out molecular analysis of the microbial community in the bottom water layer and the different layers of sediments at Lake Svetloe.

Materials and methods

Sampling and isolation of DNA

The freshwater meromictic Lake Svetloe is located in the Arkhangelsk region (N 65°04.98′, E 41°06.26′) of the Russian Federation. Lake Svetloe has a maximum depth of about 39 m, and the chemocline zone is located at a depth of 20–24 m. The water has a neutral pH and a constant temperature of about 4 °C at depths below 8 m (Savvichev et al. 2017). The physicochemical parameters of the lake have been measured in previous studies (Kallistova et al. 2019; Savvichev et al. 2017). The concentration of sulphate in the upper oxygenated zone is 40–50 μM and decreases in the zone of the monimolimnion, reaching 2 μM in the bottom water layer (Savvichev et al. 2017). The nitrite concentration in the bottom water layer is 2.0–2.9 μM, nitrate is 8.3–20.2 μM, and ammonium is 143–214 μM (Ershova et al. 2015). The concentration of ferrous iron in the monimolimnion is as high as 240 μM in the bottom water layer, while Fe^{2+} concentration is below 7.3 μM (Savvichev et al. 2017). Methane concentrations increase below the chemocline, reaching 920 μM at 33 m. The oxygen concentration in the bottom water layer is below 2.8 μM (Kallistova et al. 2019). The concentration of dissolved organic carbon increases with depth up to 230 μM in the bottom water layer (Kallistova et al. 2019).

A sample of water from the bottom water layer from a depth of 35 m (sample W-35) was collected in May 2016 with a bathometer that was lowered from the ice surface. Simultaneously, a sample of bottom sediments was taken with a stratometer-type tube corer. For the analysis, slices of the sediment core corresponding to depths of 0–5 cm (sample S0-5), 20–25 cm (sample S20-25) and 35–40 cm (sample S35-40) were chosen. The collected samples were divided each into three subsamples and stored at − 20 °C. Genomic DNA was isolated using the CTAB/NaCl method (Wilson 2003). For sediment samples, equal amounts of DNA extracted from three subsamples were pooled to minimise spatial and technical variations.

16S rRNA gene sequencing and analysis

PCR amplification of 16S ribosomal RNA gene fragments containing the V3–V4 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; Kallistova et al. 2019). The PCR fragments were sequenced with a GS FLX sequencing system (Roche, Switzerland), using the Titanium Sequencing Kit XL+. Construction of the library, its amplification, and sequencing on the GS FLX were carried out by following the relevant protocols provided by Roche.

For subsequent analysis, the reads containing the forward primer sequences were selected with Mothur v.1.42.1 (Schloss et al. 2009) and trimmed to a length of 200 bp, then low-quality reads were removed prior to clustering using Usearch v.10 (Edgar 2010). High-quality reads were clustered to operational taxonomic units (OTUs) at a distance of 0.03 using the Usearch algorithm; at this step chimeric sequences and singletons were removed. Initial reads, including low-quality and singleton reads, were mapped back to OTUs at 97% identity to calculate OTU sizes using Usearch. The final datasets representing OTUs consisted of 12851, 5961, 8308, and 7436 of 16S rRNA reads for the samples W-35, S0-5, S20-25 and S35-40, respectively.

Taxonomic classification of OTUs was performed using the SINA online alignment and classification platform and the v 1.2.11 database with default parameters (Pruesse et al. 2012), and by searches against the NCBI NR database using BLASTN.

Diversity indices were calculated at a 97% OTU cut-off level using Usearch. To avoid sequencing depth bias, the number of reads generated for each sample were randomly sub-sampled to the size of the smallest set (reads from S0-5) using the "otutab_rare" command of Usearch (Edgar 2010).

Sequencing and analysis of mcrA gene sequences

The DNA fragments encoding the *mcrA* (methyl coenzyme M reductase subunit A) gene were amplified using PCR with the universal primers mlas-mod-F (5′-GGY GGT GTM GGD TTC ACM CAR TA) and mcrA-rev-R (5′-CGT TCA TBG CGT AGT TVG GRT AGT) (Angel et al. 2012; Steinberg and Regan 2008). The following program was used: 96 °C for 2 min, followed by 40 cycles of 96 °C for 30 s, 58 °C for 40 s and 72 °C for 60 s, and a final elongation at 72 °C for 5 min.

We also attempted to amplify the *mcrA* gene fragments using *Bathyarchaeota*-specific primers Bathy-mcrA-2/3F (5′-GCT KGG RTT YTA CAT GAG) and Bathy-mcrA-2R (5′-GGG TAG TTA AGG

CCT CTC) (McKay et al. 2017) and the following PCR protocol: 96 °C for 2 min, followed by 40 cycles of 40 s at 96 °C, 40 s at an annealing temperature (45 °C, 46 °C, 49 °C, 51 °C and 53 °C tested) and 60 s at 72 °C, and a final elongation at 72 °C for 5 min. PCR product was not obtained in any case.

The PCR fragments obtained with the MCRf/MCRr primer pair were sequenced on a GS FLX sequencing system (Roche, Switzerland) as described above for the 16S rRNA gene fragments. Clustering of the nucleotide sequences of *mcrA* was carried out at a distance of 0.1 in the same manner as for the 16S rRNA gene sequences. Sequences of obtained *mcrA* OTUs were translated to amino acids and aligned by the Muscle algorithm using MEGA7 (Kumar et al. 2016). The alignments were manually inspected and non-McrA sequences were discarded. Finally, a total of 3418, 8983, 8918, and 7389 of *mcrA* sequences represented OTUs for the samples W-35, S0-5, S20-25, and S35-40, respectively.

For phylogenetic analysis the deduced amino acid sequences of *mcrA* OTUs and McrA sequences of known methanogenic lineages were aligned using MEGA7. The maximum likelihood phylogenetic tree was constructed using PhyML v. 3.3 with default parameters (Guindon et al. 2010). The *mcrA* OTUs were taxonomically assigned using BLASTP searches against the NCBI non-redundant protein sequence database and further analysis of the phylogenetic tree.

Data availability

All data supporting the findings of this study are available within the article and its supporting information files. The raw data generated from 16S rRNA and *mcrA* gene sequencing are available from the NCBI SRA repository through BioProject PRJNA407875.

Results and discussion

Various groups of methanogens predominated in the bottom water layer and sediments

Archaea represented about 70% of all the 16S rRNA gene sequences in the bottom water layer and 40 to 50% of all sequences in the sediments at different depths (Table 1). These data are in agreement with previous findings of the prevalence of Archaea over Bacteria in the lake monimolimnion (Kallistova et al. 2019). Diversity indices (Shannon and Inverse Simpson) indicated high bacterial and lower archaeal diversity in both water and sediments, with diversity and richness estimators decreasing with sediment depth (Table 1).

The canonical groups of methanogens accounted for about 20% of all 16S rRNA gene sequences, both in the bottom water layer and in the sediments. The abundances of different groups of methanogens, however, varied in different samples (Fig. 1 and Supplemental Table S1). The relative abundance of 16S rRNA gene sequences of hydrogenotrophic methanogens of the orders *Methanocellales* and *Methanomicrobiales* in the sediments was more than twice as high as in the bottom water layer. The higher relative abundance of hydrogenotrophic methanogens in the sediments was due to the higher content of *Methanomicrobiales* (mostly the genus *Methanoregula*), while *Methanocellales* were nearly absent in the sediments. Acetoclastic methanogens of the genus *Methanosaeta* (order *Methanosarcinales*) made up the majority of 16S rRNA gene sequences of methanogens in the water, but in the sediments their proportion decreased sharply. The predominance of *Methanomicrobiales* and *Methanosaetaceae* among methanogens in freshwater sediments, as well as a decrease of the relative abundance of *Methanosaetaceae* with depth, has been noted in other studies. The latter may be explained by the faster decrease of the fermentative formation of acetate than that of hydrogen with depth (Chan et al. 2005; Zeleke et al. 2013).

Another group of methanogens, members of the order *Methanomassiliicoccales* that use hydrogen and methanol for production of methane, were not found in the bottom water layer, but were present in sediments, where their proportion increased with depth and reached 5.9% of the 16S rRNA gene sequences in sample S35-40. Methanol formation in the sediments can result from anaerobic degradation of plant components such as pectin (Schink and Zeikus 1982). Notably, we found no organisms related to known anaerobic methane oxidizers (ANME-1, 2, 3).

To support phylogenetic affiliation of the 16S rRNA gene sequences to particular methanogenic archaeal lineages, we amplified and sequenced the libraries targeting the genes coding for a conserved

Table 1 Relative abundance and diversity of Archaea and Bacteria

Diversity and abundance	Sample			
	W-35	S0-5	S20-25	S35-40
Relative abundance of Archaea (% of all 16S rRNA gene sequences)	69.76	40.95	49.89	47.03
Chao1				
Archaea	361.7	219.4	149.2	117.6
Bacteria	639.6	687.8	357.1	345.7
Shannon				
Archaea	4.17	4.0	3.34	3.04
Bacteria	4.97	5.71	4.41	4.7
Inverse simpson				
Archaea	25.19	21.19	12.05	9.71
Bacteria	41.32	135.14	24.45	53.19

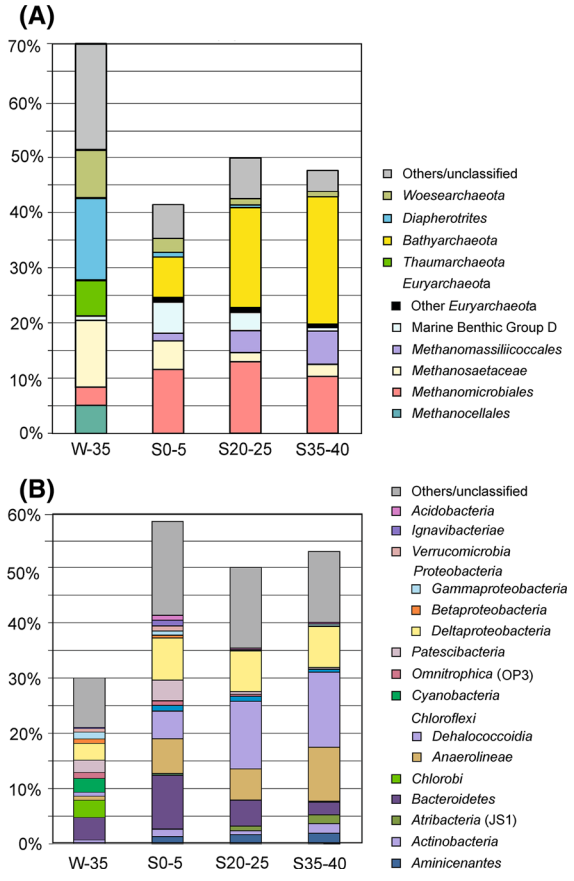


Fig. 1 Microbial community structures based on 16S rRNA gene sequences. Relative abundancies of taxonomic groups of Archaea (**a**) and Bacteria (**b**) (% of the total 16S rRNA gene sequences). Composition of Euryarchaeota is shown at the order level. Proteobacteria are shown at the class level. Other lineages are shown at the phylum level

region of the alpha subunit of the methyl coenzyme M reductase gene (*mcrA*). Like 16S rRNA genes, the *mcrA* genes can be used in the reconstruction of phylogenetic relationships among methanogens (Luton et al. 2002). Phylogenetic analysis of deduced amino acid sequences for McrA confirmed the presence of *Methanomicrobiales, Methanosaetaceae* and *Methanomassiliicoccales* (Table 2 and Fig. 2), lineages of methanogens also identified by the 16S rRNA gene analysis. In addition, a minor fraction of methylotrophic methanogens of the phylum *Verstraetearchaeota* (Vanwonterghem et al. 2016) were identified in the sediments and accounted for up to 0.26% of the *mcrA* sequences. Consistent with the results of 16S rRNA gene profiling, the fraction of *mcrA* sequences of *Methanosaetaceae* in the near-bottom water column was higher than in the sediments, while the opposite trend was observed for *Methanomassiliicoccales*, which became more abundant in deep sediments (Table 2).

Diversity of non-methanogenic archaeal lineages

Uncultured archaea of the superphylum DPANN (comprising the *Micrarchaeota, Diapherotrites, Aenigmarchaeota, Nanohaloarchaeota, Parvarchaeota, Nanoarchaeota, Pacearchaeota*, and *Woesearchaeota* (Rinke et al. 2013)) are widespread in various aquatic ecosystems, including boreal lakes (Carnevali et al. 2018; Llirós et al. 2008; Restrepo-Ortiz and Casamayor 2013; Ortiz-Alvarez and

Casamayor 2016). This monophyletic group includes archaea with small cell sizes and reduced genomes (Castelle et al. 2015). Members of two DPANN phyla, *Diapherotrites* and *Woesearchaeota,* were found in Lake Svetloe. These phyla accounted, respectively, for 14.7% and 8.6% of all 16S rRNA gene sequences in the water, but in the sediments their relative abundances were several times lower and decreased with depth (Fig. 1). Genomic analyses of *Diapherotrites* have shown that these archaea are probably able to utilize and ferment a limited set of substrates (Youssef et al. 2015). *Woesearchaeota* have small genomes and are devoid of many important biosynthetic pathways, and therefore they are considered as obligate symbionts or parasites (Castelle et al. 2015). The decrease in the relative abundances of these groups in the sediments could be simply due to the lack of potential hosts.

Archaea of the genus *Nitrosopumilus* of the phylum *Thaumarchaeota* were abundant in the near-bottom water layer (6.6% of all 16S rRNA gene sequences), but almost absent in the sediments. The current known members of this genus are autotrophs, which obtain energy from aerobic oxidation of ammonia (Walker et al. 2010). The concentration of ammonium in the water of Lake Svetloe reaches its maximum in the bottom layer (143-214 μM, (Ershova et al. 2015)) and is sufficient for the growth of ammonia-oxidizing archaea. The lack of oxygen most likely explains the absence of *Thaumarchaeota* in the completely anoxic sediments.

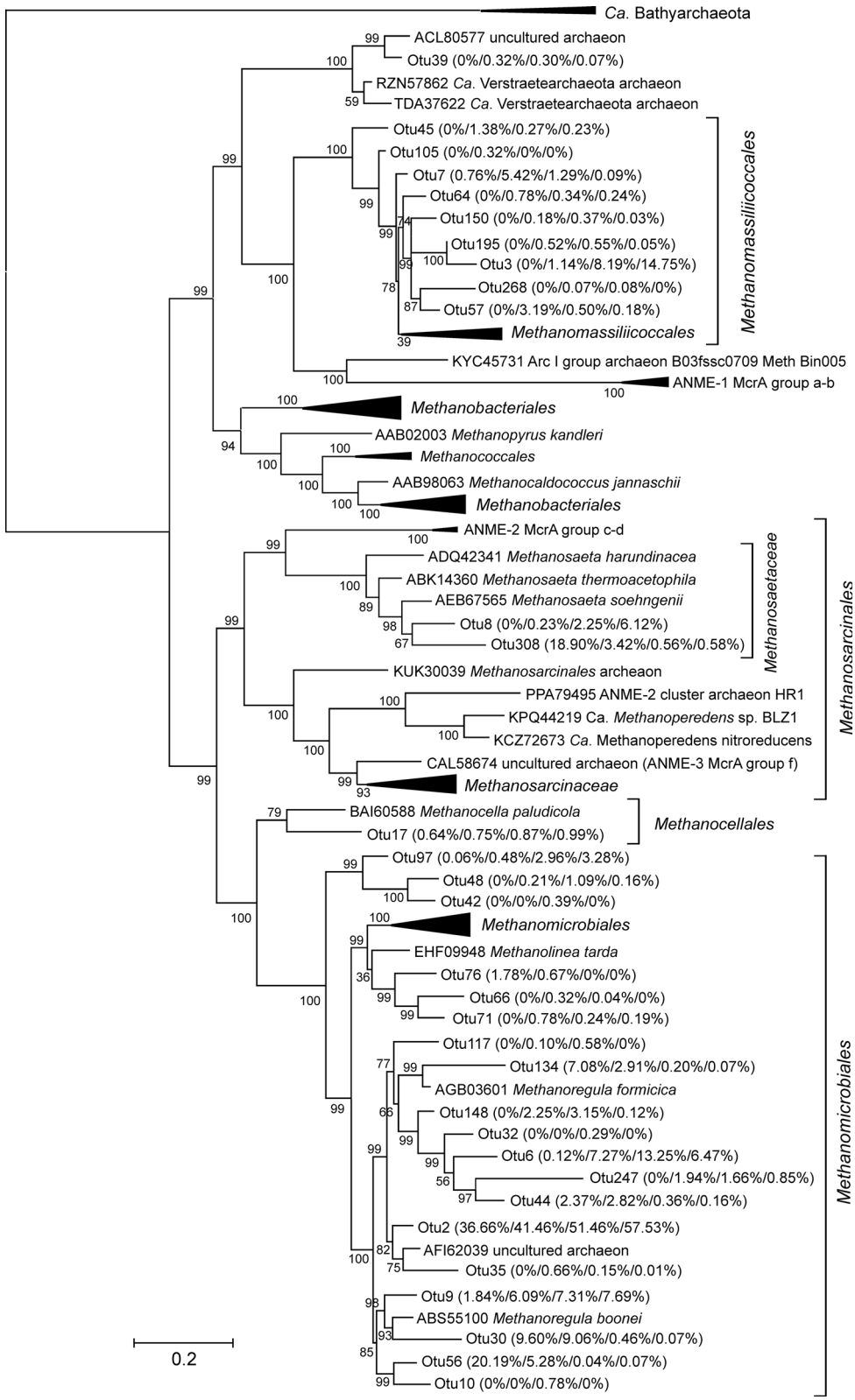
The relative abundance of the 16S rRNA gene sequences assigned to the uncultured lineage Marine Benthic Group D (MBG-D) was maximal in the upper sediment layer (5.5%) and decreased in deeper layers; in the bottom water layer the share of this group was also small. This archaeal lineage, initially found in coastal marine sediments (Vetriani et al. 1999), is phylogenetically related to the order *Thermoplasmatales* of the phylum *Euryarchaeota*. Analysis of the genomes of these archaea assembled from metagenomes suggested that they are fermenters capable of degrading detrital proteins by deploying secreted proteases, but are unable to use carbohydrates (Lazar et al. 2017). Their genomes also encode the full set of enzymes of the Wood-Ljungdal pathway, which can be used for autotrophic fixation of CO_2 with hydrogen consumption and acetate formation, but it can also function in the opposite direction (Schuchmann and Müller 2016). It is likely that MBG-D archaea degrade and ferment the proteinaceous substrates of a dead biomass that has fallen to the bottom of the lake, and provide substrates for methanogens.

The archaea of the uncultured phylum *Bathyarchaeota* (MCG) were not found in the bottom water layer, but accounted for a significant fraction of 16S rRNA gene sequences in the sediments, increasing in the deeper layers, and reaching 22.7% at a depth of 35-40 cm. According to genomic data, these archaea are organoheterotrophs with various metabolic capacities and are able to use as substrates, apart from detrital proteins, also polysaccharides of plant origin (Lazar et al. 2016). Recently *Bathyarchaeota* in marine sediments were even shown to be capable of utilising lignin as an energy source (Yu et al. 2018). *Bathyarchaeota* are often found in freshwater sediments as one of the dominant groups of archaea (Ma

Fig. 2 Phylogenetic tree based on the deduced amino acid sequences of *mcrA* genes found in this work and representatives of known methanogenic and ANME lineages. The support values for the internal nodes were estimated by approximate Bayes test in PhyML. Numbers in parentheses after OTU ID indicate the relative abundances (%) of a given OTU in four analysed samples (W-35/S0-5//S20-25/S35-40). GenBank accession numbers are shown before the isolate/clone names ▶

Table 2 Relative abundance of phylogenetic groups carrying the *mcrA* gene in water and sediment samples

Phylogenetic assignment	Relative abundance (% of *mcrA* sequences)			
	W-35	S0-5	S20-25	S35-40
Methanomicrobiales	79.70	82.28	84.43	76.67
Methanosarcinales (fam. *Methanosaetaceae*)	18.90	3.65	2.81	6.70
Methanomassiliicoccales	0.76	13.00	11.58	15.57
Methanocellales	0.64	0.75	0.88	0.99
Verstraetearchaeota	0.00	0.32	0.30	0.07


Ca. Bathyarchaeota
ACL80577 uncultured archaeon
Otu39 (0%/0.32%/0.30%/0.07%)
RZN57862 Ca. Verstraetearchaeota archaeon
TDA37622 Ca. Verstraetearchaeota archaeon
Otu45 (0%/1.38%/0.27%/0.23%)
Otu105 (0%/0.32%/0%/0%)
Otu7 (0.76%/5.42%/1.29%/0.09%)
Otu64 (0%/0.78%/0.34%/0.24%)
Otu150 (0%/0.18%/0.37%/0.03%)
Otu195 (0%/0.52%/0.55%/0.05%)
Otu3 (0%/1.14%/8.19%/14.75%)
Otu268 (0%/0.07%/0.08%/0%)
Otu57 (0%/3.19%/0.50%/0.18%)
Methanomassiliicoccales
Methanomassiliicoccales
KYC45731 Arc I group archaeon B03fssc0709 Meth Bin005
ANME-1 McrA group a-b
Methanobacteriales
AAB02003 Methanopyrus kandleri
Methanococcales
AAB98063 Methanocaldococcus jannaschii
Methanobacteriales
ANME-2 McrA group c-d
ADQ42341 Methanosaeta harundinacea
ABK14360 Methanosaeta thermoacetophila
AEB67565 Methanosaeta soehngenii
Otu8 (0%/0.23%/2.25%/6.12%)
Otu308 (18.90%/3.42%/0.56%/0.58%)
Methanosaetaceae
KUK30039 Methanosarcinales archeaon
PPA79495 ANME-2 cluster archaeon HR1
KPQ44219 Ca. Methanoperedens sp. BLZ1
KCZ72673 Ca. Methanoperedens nitroreducens
CAL58674 uncultured archaeon (ANME-3 McrA group f)
Methanosarcinaceae
Methanosarcinales
BAI60588 Methanocella paludicola
Otu17 (0.64%/0.75%/0.87%/0.99%)
Methanocellales
Otu97 (0.06%/0.48%/2.96%/3.28%)
Otu48 (0%/0.21%/1.09%/0.16%)
Otu42 (0%/0%/0.39%/0%)
Methanomicrobiales
EHF09948 Methanolinea tarda
Otu76 (1.78%/0.67%/0%/0%)
Otu66 (0%/0.32%/0.04%/0%)
Otu71 (0%/0.78%/0.24%/0.19%)
Otu117 (0%/0.10%/0.58%/0%)
Otu134 (7.08%/2.91%/0.20%/0.07%)
AGB03601 Methanoregula formicica
Otu148 (0%/2.25%/3.15%/0.12%)
Otu32 (0%/0%/0.29%/0%)
Otu6 (0.12%/7.27%/13.25%/6.47%)
Otu247 (0%/1.94%/1.66%/0.85%)
Otu44 (2.37%/2.82%/0.36%/0.16%)
Otu2 (36.66%/41.46%/51.46%/57.53%)
AFI62039 uncultured archaeon
Otu35 (0%/0.66%/0.15%/0.01%)
Otu9 (1.84%/6.09%/7.31%/7.69%)
ABS55100 Methanoregula boonei
Otu30 (9.60%/9.06%/0.46%/0.07%)
Otu56 (20.19%/5.28%/0.04%/0.07%)
Otu10 (0%/0%/0.78%/0%)
Methanomicrobiales
0.2

et al. 2016; Kadnikov et al. 2012; Borrel et al. 2012; Wurzbacher et al. 2017; Rissanen et al. 2019), and their relative abundance increases with depth (Wurzbacher et al. 2017; Rissanen et al. 2019). Similar to the MBG-D archaea, *Bathyarchaeota* have the Wood-Ljungdal pathway and, depending on environmental conditions, can use it either for acetogenesis (He et al. 2016) or in reverse, using acetate to form H_2 and CO_2. Moreover, the genomes of two members of *Bathyarchaeota* harbor divergent genes for methane metabolism and it was suggested that they could be capable of methyl-dependent hydrogenotrophic methanogenesis (Evans et al. 2015). However, it remains to be determined whether these *Bathyarchaeota* are indeed methanogens or their *mcrA* genes are involved in short-chain alkane oxidation (Borrel et al. 2019). Probably, the metabolic versatility of *Bathyarchaeota* allowed them to dominate in the energy-limited conditions of deep lake sediments.

The McrA sequences of *Bathyarchaeota* were not found in the McrA dataset obtained with the universal McrA primers, and we also failed to amplify the PCR fragments using *Bathyarchaeota*-specific primers. However, the *Bathyarchaeota* detected by 16S rRNA profiling were phylogenetically distant from putative methanogenic members of this phylum (< 90% of 16S rRNA identity with *Candidatus* Bathyarchaeota archaeon BA1 and *Candidatus* Bathyarchaeota archaeon BA2). Therefore, it seems more likely that *Bathyarchaeota* in Lake Svetloe belong to non-methanogenic lineages of this candidate phylum.

A significant fraction of the obtained archaeal 16S rRNA gene sequences could not be classified even at the phylum level. The relative abundance of such unclassified archaeal lineages was about 19.2% in the bottom water layer and from 4 to 7% in the sediments.

Bacteria in the near-bottom water layer and sediments

Bacteria accounted for about 30% of the 16S rRNA gene sequences in the near-bottom water layer and for more than a half in the sediments (Table 1). In the bottom water layer the most abundant groups of bacteria belonged to the phyla *Bacteroidetes* (4.1% of all 16S rRNA reads), *Chlorobi* (3.1%), *Proteobacteria* of the classes delta (3.06%), gamma (1.25%), and beta (0.79%), *Cyanobacteria* (2.52%), *Patescibacteria* (also known as Candidate Phylum Radiation, 2.24%), and *Omnitrophica* (OP3, 1.09%) (Fig. 1 and Table S1). The most numerous phylum, *Bacteroidetes*, was represented by the families *Sphingobacteriaceae* and *Flavobacteriaceae*, whose heterotrophic species are common in aquatic environments. *Bacteroidetes* were also abundant in the upper sediments, but their share decreased with depth.

The relative abundance of *Cyanobacteria* and *Chlorobi* sharply decreased in the sediments consistently with their photosynthetic lifestyle. The phylum *Cyanobacteria* was mostly represented by the genus *Cyanobium*. Most of *Chlorobi* belonged to the species *Chlorobium ferrooxidans* that was shown to be capable of phototrophic Fe(II) oxidation at low light intensity (Hegler et al. 2008; Heising et al. 1999). This anoxygenic phototrophic bacterium was found in the chemoclines of Lake Svetloe (Kallistova et al. 2019) and of the ferruginous sulphate-poor meromictic lake La Cruz in Spain (Walter et al. 2014).

Oxygen generated by photosynthetic *Cyanobacteria* in the bottom water layer could be used for aerobic oxidation of methane. Consistently, Type I methanotrophs, Gammaproteobacteria of the family *Methylococcaceae* (1.11% of 16S rRNA gene reads) were found in the bottom water sample. Most of them belonged to the genus *Methylobacter*. Some Type I methanotrophs appear to be adapted to cold environments, such as high-latitude tundra soils (Graef et al. 2011) and lakes (Crevecoeur et al. 2017), and could be active under low-oxygen conditions (Crevecoeur et al. 2017; Blees et al. 2014; Rissanen et al. 2018). Members of *Methylococcaceae* could cooperate with methylotrophic Betaproteobacteria of the family *Methylophilaceae* (Chistoserdova 2015), also present in the water sample, although in minor amounts (0.17%). The co-occurrence of *Methylophilaceae* and *Methylococcaceae* in oxygen-limited zones was reported in several lakes, including the meromictic Lake Pavin (Corinne et al. 2018).

Deltaproteobacteria were abundant both in the bottom water layer (3.06% of the 16S rRNA reads) and in the sediments (7–8%). In the water sample, most of the Deltaproteobacteria belonged to the family *Syntrophaceae* (Kuever 2014). The presence of *Syntrophaceae* indicates that syntrophic processes, probably linked to methanogenesis, could contribute to decomposition of organic matter in the bottom water layer. In the sediments the relative abundance of *Syntrophaceae* decreased, and uncultured lineages of

the Deltaproteobacteria became more numerous. Particularly, the Sva0485 clade accounted for 3.17% of the 16S rRNA sequences in the upper sediments and for about 5% in deeper layers. This yet uncultured lineage was assumed to be capable of sulphate reduction (Concheri et al. 2017) and has been found in various environments, including the sediments of the ferruginous Lake Towuti, Indonesia (Vuillemin et al. 2018). Although sulphate concentration in Lake Towuti sediments is within the low μM range, it has been proposed that sulphate-reducers participate in cryptic sulphur cycling supported by re-oxidation of sulphide with ferric iron (Vuillemin et al. 2018).

Other participants of the sulphur cycle could be members of the phylum *Omnitrophica* (OP3). Genome analysis of a member of this division, *Candidatus* Omnitrophus magneticus SKK-01 suggested that this is a magnetotactic bacterium capable of oxidising reduced sulphur compounds under anaerobic conditions using Fe(III) as an electron acceptor (Kolinko et al. 2012). The latter is likely a result of phototrophic oxidation of Fe(II) by *Chlorobium ferrooxidans*.

Members of the Candidate Phyla Radiation (CPR) group of Bacteria accounted for 2.24% of 16S rRNA gene sequences in the bottom water layer and for 3.79% in the upper sediments; in the deeper sediments their relative abundance decreased to 0.27%. Most of them belonged to the candidate division *Absconditabacteria* (SR1), widespread in hydrothermal habitats, freshwater lakes, and groundwater (Davis et al. 2009). Like Archaea of the superphylum DPANN, CPR bacteria have small genomes, lack many biosynthetic pathways, and probably grow in association with other microorganisms in a symbiotic manner (Kantor et al. 2013; Castelle et al. 2018).

The relative abundance of 16S rRNA gene sequences of three bacterial lineages was much higher in the sediments than in the near-bottom water layer and further increased with depth: *Chloroflexi* (from 1.45% in W-35 to 23.36% in the S35-40 sample), *Atribacteria*/JS1 (from 0 to 1.58%), and *Aminicenantes* (from 0.01 to 1.92%). Detected *Chloroflexi* belonged to the classes *Anaerolineae* and *Dehalococcoidia*. Members of *Anaerolineae* are metabolically versatile chemoorganoheterotrophs capable of degrading plant-derived polysaccharides, performing fermentation and aerobic or anaerobic respiration (Yamada et al. 2006). Cultured species of *Dehalococcoidia* have been described as anaerobic bacteria dedicated to the transformation of various chlorinated organic compounds via reductive dechlorination (Maymó-Gatell et al. 1997). Members of *Dehalococcoidia* have been detected in a number of anoxic freshwater and marine sediment samples (Kittelmann and Friedrich 2008; Kadnikov et al. 2012). While the presence of halogenated compounds is usually associated with industrial contamination, natural sources are also known, for example these compounds can be produced by algae (Haggblom et al. 2003). However, *Dehalococcoidia* detected in Lake Svetloe were phylogenetically distant from cultured species of this lineage (91–93% 16S rRNA sequence identity).

Bacteria of the candidate phylum *Aminicenantes* were frequently found in various aquatic habitats including freshwater lakes and groundwater (Farag et al. 2014). Metabolic reconstruction of near-complete genomes assembled from metagenomes revealed that *Aminicenantes* are anaerobes that could utilise various proteinaceous substrates and carbohydrates in fermentative metabolism, while known pathways of aerobic or anaerobic respiration were missing (Robbins et al. 2016; Kadnikov et al. 2019). The lack of respiratory capacities and specialization in fermentation of carbohydrates and/or syntrophic oxidation of organic acids have been predicted for members of Atribacteria-JS1 lineage (Nobu et al. 2016; Lee et al. 2018). Both these groups could be involved in decomposition of organic matter through fermentation, thus providing the substrates for methanogens.

Tentative microbial processes in the bottom water and sediments of Lake Svetloe

This study revealed the presence of a taxonomically and metabolically diverse community in the sediments of Lake Svetloe. About a third of the 16S rRNA gene sequences assigned to Archaea both in the bottom water layer and in the sediments were related to known methanogenic groups, but the relative abundance of individual groups varied with depth. The decrease with depth in the share of acetoclastic methanogens of the family *Methanosaetaceae* may reflect a decrease in the availability of acetate due to competition with other potentially acetate-consuming microorganisms, such as members of the *Bathyarchaeota*. The low availability of sulphate ($\sim$ 2 μM) and nitrate (8.3–20.2 μM) appears to determine the lack of ANME archaea, most of which oxidize methane by

forming partnerships with sulfate and/or nitrate reducing bacteria. The second abundant physiological group of archaea, anaerobic fermenters with the capability to degrade carbohydrates and proteinaceous substrates derived from sunken remnants of plant and animal biomass, was mostly represented by *Bathyarchaeota* and MBGD.

The bacterial component of the microbial community in the bottom water layer appeared to be more diverse taxonomically and potentially functionally. It included oxygenic (*Cyanobacteria*) and anoxygenic (*Chlorobi*) phototrophs, aerobic methanotrophs, syntrophs and various organotrophs. It is tempting to suggest that cyanobacteria produce oxygen, which is then rapidly consumed by methanotrophic Gammaproteobacteria and ammonium-oxidizing *Thaumarchaeota*. Significant cyanobacterial activity in near-bottom water layer of Lake Svetloe is, however, doubtful due to extremely low light intensity at this horizon. Thus, the brown-coloured green sulphur bacteria *Chlorobi*, which are the most efficient bacteria at utilising low light fluxes (Caumette 1984; Imhoff 2014), were found to occur at the depth of 24–27 m, which is 8 m above the near-bottom water horizon (Savvichev et al. 2017). Moreover, radiotracer experiments utilising $^{14}CO_2$ assimilation revealed the same values of light and dark assimilation below 25 m (Kallistova et al. 2019), a finding that indicates an absence of high cyanobacterial activity. Thus, it may be suggested that methanotrophs and ammonium-oxidizing *Thaumarchaeota* could use electron acceptors other than oxygen, most likely Fe^{3+}. Both Fe^{3+} and Fe^{2+} were shown to occur in the Lake Svetloe water column reaching, respectively, 7.3 μM and 240 μM in the bottom water layer (Kallistova et al. 2019). Confirmation of the possibility of this process requires, however, additional research.

In anoxic sediments the diversity of the microbial community decreased and it became dominated by only a few taxonomic and metabolic groups. These groups include methanogens (mostly *Methanomicrobiales* and *Methanomassiliicoccales*), presumably fermentative organisms (*Bathyarchaeota, Anaerolineae*), *Chloroflexi* of the class *Dehalococcoidia*, and the Sva0485 lineage of the *Deltaproteobacteria*. Although cultivated *Dehalococcoides/Dehalogenimonas* spp. relies on an obligate dehalogenating lifestyle (Taş et al. 2010), genomic studies of Dehalococcoidia-related *Chloroflexi* from marine sediments provided no support for a metabolism based on reductive dehalogenases (Kaster et al. 2014) and revealed homoacetogenesis via a Wood-Ljungdahl pathway (Sewell et al. 2017). Metabolic capabilities of the Sva0485 lineage are not definitively known, although it is assumed to be capable of sulphate reduction. If this is the case, then a cryptic sulphur cycle involving sulphate reduction and re-oxidation of sulphide with Fe^{3+} could occur in the sediments.

The prevalence of fermentative and methanogenic microorganisms and low abundance of methanotrophs in the sediments of Lake Svetloe suggests that the sediments of arctic and subarctic meromictic lakes are important carbon storages and potential methane emitters. Further study of the microbial community of Lake Svetloe using metagenomic approaches will enable better understanding of the genetic potential and the likely functional role of uncultured archaeal lineages, particularly of *Bathyarchaeota* that accounted for almost a half of Archaea in the deep sediments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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