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Composition of Microbial Communities in Sediments from Southern Baikal Containing Fe/Mn Concretions

T. I. Zemskaya[a, *], **A. V. Lomakina**[a], **E. V. Mamaeva**[a], **A. S. Zakharenko**[a], **A. V. Likhoshvai**[a], **Yu. P. Galach'yants**[a], **and B. Müller**[b]

[a]*Limnological Institute, Siberian Branch, Russian Academy of Sciences, Irkutsk, Russia*

[b]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland*

**e-mail: tzema@lin.irk.ru*

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Abstract—Massive parallel sequencing of the 16S rRNA gene fragments was used to investigate the composition and diversity of microbial communities in sediments from Southern Baikal to a depth of 9 cm with 1-cm step. In the layers from the sediment surface to the lower border of oxygen penetration (2 cm), organotrophic bacteria with high similarity to the heterotrophic species *Luteolibacter luojiensis* constituted the largest fraction of the community. In the formation zone of Fe/Mn crusts (3–5 cm), *Proteobacteria* and *Actinobacteria* predominated in the community, while the share of *Cyanobacteria* was considerable. The lower reduced layers showed an increased contribution of the *Bacteroidetes*, while the shares of the taxa predominant in the higher layers remained significant. Analysis of archaeal 16S rRNA gene amplicons revealed predominance of the soil and aquatic *Thaumarchaeota* (Marine Group I lineage), which are involved in anaerobic ammonium oxidation, practically in all sediment layers. The buried oxidized layer (6–7 cm), where members of the uncultured Marine Benthic Group D lineage of the order *Thermoplasmatales* (*Euryarchaeota*) predominated, was an exception in this regard. Small numbers of archaea of the Baikal-1 lineages (below 1%) were observed in the communities from the 6–7 and 7–8 cm layers, while the archaea involved in anaerobic methane oxidation (including the ANME-2d group) were not detected.

Keywords: Lake Baikal, bottom sediments, Fe/Mn crusts, microbial communities, analysis of the 16S rRNA gene amplicons

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In the recent decades, application of massive parallel sequencing has greatly increased our knowledge of the diversity of microbial communities in diverse ecosystems, including the deep and cold-water Lake Baikal. Based on analysis of the 16S rRNA gene amplicons, taxonomic compositions of ice and planktonic microbial communities (Zakharova et al., 2013; Mikhailov et al., 2015; Bashenkhaeva et al., 2015), as well as of prokaryotic communities in bottom sediments of discharge areas of gas-saturated high-mineral fluids were investigated (Kadnikov et al., 2012; Zemskaya et al., 2015; Chernitsyna et al., 2016; Lomakina et al., 2018). The structure of the microbial communities in different ecotopes of Lake Baikal is typical of freshwater lakes at the high taxonomic level (Newton et al., 2011), with the most significant differences observed in the areas with a high content of nutrients, including the areas of discharge of mineralized fluids, oil and other hydrocarbons, as well as areas of algal accumulation, especially after their massive development. Bacteria capable of utilization of petroleum hydrocarbons predominated in microbial communities of the sediments of natural oil ingress area, while the share of bacteria involved in methane oxidation was considerable in the communities in the areas of methane discharge (Kadnikov et al., 2012; Zemskaya et al., 2015). Specific migration routes of mineralized fluids or deposits of gas hydrate fields determined an increase in the share of members of the phyla *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, as well as of the candidate phyla *Atribacteria*, *Saccharibacteria*, and *Latescibacteria* (Kadnikov et al., 2012; Chernitsyna et al., 2016).

Formation of iron-manganese crusts, or concretions, is one of the interesting phenomena observed in the sediments of some areas of Lake Baikal (Dubinina, 1976; Granina, 2008; Zakharova et al., 2010). Formation of iron-manganese concretions is associated with the activity of microorganisms, which is confirmed by cultivation experiments with bacteria able to precipitate oxidized Fe and Mn (Gorlenko et al., 1977; Sommers et al., 2002). Representatives of the phylum NC10, known as anaerobic methane oxidizers, and of *Nitrospira* species exhibiting the greatest similarity with bacteria from Fe-rich seeps predominated in microbial communities of iron-rich sediments of Lake

Superior (Dittrich et al., 2015). Fe-rich layers, where iron transformation was mediated by anaerobic nitrate-dependent oxidation, were detected in the sediments of Lake Constance (Hauck et al., 2001). The hypothesis of diagenetic formation of Fe/Mn layers caused by biogenic activity in freshwater sediments of Lake Baikal was confirmed by Dubinina (1976), Granina (2008), and Zakharova et al. (2010). In these works, the presence of Fe- and Mn-oxidizing bacteria in the bottom sediments of Lake Baikal and dependence of distribution and abundance of these bacteria on the ambient redox conditions, organic matter content, and the presence of dissolved Fe and Mn in the pore water were demonstrated. Representatives of six genera, *Metallogenium*, *Leptothrix*, *Siderocapsa*, *Naumaniella*, *Bacillus*, and *Pseudomonas*, were involved in oxidation of Fe(II) and Mn(II) in the sediments (Zakharova et al., 2010). Studies of the composition of microbial communities in the zones of iron-manganese crusts formation based on high-throughput sequencing platforms have not previously been performed.

Several cores of the sediments containing iron-manganese crusts were collected during the Swiss-Russian expedition to Southern Baikal in 2010 (station A) (Och et al., 2012). In this study, the composition of pore water (concentration of some ions, oxygen, and methane) was reported and the basic chemical processes providing formation of iron-manganese layers and crusts were considered.

The goal of the present work was to study the composition and diversity of microbial populations in different layers of the sediments in one of the cores, including the zone of formation of iron-manganese concretions using analysis of the 16S rRNA gene fragments.

MATERIALS AND METHODS

Sampling of the sediment was performed in Southern Baikal (51°45′36.38 N; 105°01′28.51 E, depth of 1397 m) using an UWITEC multi-coring device (www.uwitec.at), which provides for simultaneous collection of nine sediment cores of 60 cm in length. One of the cores was used for molecular biological analysis, whereas others were used for the lithological description of the sediment and investigation of the chemical composition of pore waters. The results of these studies were reported in the article (Och et al., 2012), and the data on O_2, Fe, Mn, SO_4^{2-}, and CH_4 concentrations were used in the present study for statistical analysis and evaluation of the correlation between individual OTUs and chemical parameters.

Total DNA extraction and pyrosequencing. Sediment samples for DNA isolation were collected from the middle part of the core from the surface to a depth of 9 cm with a 1-cm step; extraction was carried out directly on board the ship under sterile conditions. Total DNA was isolated from the sediments by the phenol-chloroform method described previously (Shubenkova et al., 2005).

PCR amplification of bacterial 16S rRNA gene fragments including the variable V3 region was performed using universal primers U341F (5'-CCTACGGGRSGCAGCAG-3') and U515R (5'-TTACCGCGGCKGCTGVCAC-3') according to the following program: 96°C, 3 min; 96°C, 30 s; 55°C, 30 s; 72°C, 40 s (30 cycles); 72°C, 10 min. PCR amplification of archaeal 16S rRNA gene was performed using the A2Fa (5'-TTCCGGTTGATCCYGCCRGA-3') and A519R (5'-GGTDTTACCGCGGCKGCTG-3') primers covering the V1–V3 variable regions (Teske and Sørensen, 2008; Kim et al., 2011) according to the following program: 96°C, 2 min; 96°C, 30 s; 58°C , 45 s; 72°C, 40 s (30 cycles); 72°C, 10 min. Pyrosequencing of obtained PCR fragments was performed using 454 Genome Sequencer FLX Titanium (Roche) and Titanium series reagents GS FLX according to the Titanium Sequencing Method Manual protocol following the manufacturer's recommendations.

Diversity and taxonomic composition of microbial communities of the sediments was analyzed using the Mothur software package v. 1.31.2 (http://www.mothur.org). Chimeric sequences were revealed and removed using the UCHIME algorithm with the standard parameters (Edgar et al., 2011). OTUs containing single sequences and sequences less than 150 bp in length were excluded from the analysis. To determine the diversity of the community and to characterize its taxonomic composition, alignment of the sequences and cluster analysis were performed. The sequences were clustered into operational taxonomic units (OTUs) at a genetic distances of 0.03, 0.05, and 0.1 corresponding to different taxonomic levels. Taxonomic affiliation of representative sequences of the clusters with genetic distance of 0.03 was determined using several databases of the 16S rRNA gene sequences: SILVA, Greengenes, RDP, and NCBI. Taxonomic complexity of community was evaluated using Rarefaction software, a part of the Mothur software package, analyzing the rarefaction curves demonstrating dependence of the number of detected phylotypes (i.e., clusters) on the number of analyzed sequences for genetic distances of 0.1 to 1.0 corresponding to different taxonomic levels. Taxonomic identification of representative sequences of the clusters was performed using the BLASTN algorithm by means of comparison with those deposited in the database of the 16S rRNA gene sequences in GenBank. Phylogenetic trees were constructed based on the neighbor-joining method with the Kimura two-parameter model using MEGA v. 5.1 software (Tamura et al., 2011).

Comparison of the composition and diversity of the communities in different sediment layers was performed using the principal component analysis

(PCoA) method. Correlation between individual OTUs and chemical parameters (NMDS) was evaluated using the Grapher 9 software. The numbers of common and unique OTUs in each studied sample were obtained by analysis of the Venn diagrams (not shown) constructed using the Mothur software.

In total, 25915 bacterial and 30423 archaeal sequences were obtained. The sequences were deposited in the GenBank database, Sequence Read Archive (SRA), under accession nos. SRR3156030 (Bacteria) and SRR3156034 (Archaea).

RESULTS

Chemical composition of pore waters. The presence of manganese- and iron-rich layers and crusts occurring at depths of 2–5 cm and 6–7 cm was detected in the studied core (Och et al., 2012). The upper limit of the Fe/Mn layer (2 cm) coincided with the lower border of oxygen penetration, where Mn(II) oxidation occurred, resulting in formation of Fe/Mn crusts. Mn^{2+} concentration in the pore water at depths of 3–4 cm increased up to 1.9 mmol/L (the minimum value reached 0.9 mmol/L). According to the previously obtained data, at a depth of 4–5 cm, Mn(IV) was reduced to Mn(II), which, in turn, diffused in the upper layers and was oxidized by O_2 (Och et al., 2012). Buried Fe/Mn layers undergo slow reductive dissolution, which, in the authors' opinion, is mainly due to anaerobic CH_4 oxidation. Profiles of Fe concentration (0.49 to 0.65 mmol/L) were characterized by the presence of two peaks at depths of 4–5 cm and 7–8 cm. SO_4^{2-} and CH_4 concentrations varied slightly and were 55.5–77.5 μmol/L and 4.6–7.5 mmol/L, respectively.

Analysis of the microbial community composition in different sediment layers using the 16S rRNA gene sequencing. Comparative analysis of the libraries of the 16S rRNA gene fragments from different layers of the sediment and of the Venn diagrams showed that the greatest number of common OTUs was observed in bacterial communities of the oxidized layers (3.8–7.2%), while in reduced layers it was of 0.6 to 4.3%. In the communities from surrounding layers of the sediment, common OTU number was higher than in those from the sediment layers more distant from each other. In archaeal communities, common OTU number in the oxidized surface layer of the sediment was 0 to 23.5%, while in reduced layers it was 0.7 to 4.1%.

Analysis of the 16S rRNA gene libraries of bacterial communities revealed a change of predominant taxa (Fig. 1) depending on the sediment depth. In the communities from the oxygen penetration zone and the layer located 1 cm lower (1–3 cm), representatives of the phyla *Verrucomicrobia*, *Cyanobacteria*, *Proteobacteria*, and unclassified microorganisms predominated. It should be noted that numerous OTUs were not assigned to any known bacterial taxa represented in the databases. These OTUs were also numerous in the sediment layers with a high content of manganese ions (3–4 cm), while the shares of *Cyanobacteria*, *Proteobacteria*, and *Actinobacteria* were also considerable, and the total fraction of other OTUs comprised less than 10% of the total number of the sequences. In bacterial communities from deeper layers of the core (4–5 and 5–6 cm), the share of unclassified sequences was also high and the share of bacteria of the phylum *Nitrospirae* increased; members of this phylum were detected in Fe-rich seeps in Lake Superior (Dittrich et al., 2015). Representatives of the phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Proteobacteria* predominated in bacterial communities in the buried oxidized layer (6–7 cm). In the deeper layers of the sediment (7–8 and 8–9 cm) under sufficiently homogeneous geochemical conditions, community structures also differed. Representatives of *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, and unclassified microorganisms predominated at a depth of 7–8 cm, whereas *Proteobacteria, Bacteroidetes* and unclassified were predominant at a depth of 8–9 cm.

Representatives of the phylum *Verrucomicrobia*, the major group in bacterial communities of the surface layers of the sediment, had 99% identity of 16S rRNA genes with the species *Luteolibacter luojiensis*, which was isolated from the tundra soil of Svalbard (Norway). This species is a heterotroph and utilizes a wide range of polysaccharides, including those produced during degradation of algal biomass (Cardman et al., 2014).

The ratio of representatives of different classes of *Proteobacteria* also varied considerably. In all layers of the studied core, representatives of α- and β-*Proteobacteria* were more numerous than δ- and γ-*Proteobacteria*. The share of β-*Proteobacteria* was the greatest in the community of the 8–9 cm layer (23.8%) and 19.6% of them belonged to the family *Comamonadaceae*. Representatives of this family were organo- and chemoheterotrophs, involved in denitrification and considered one of the major taxa in the communities of freshwater ecosystems (Hahn, 2006). They predominated in the sediments of Lake Geneva (Haller et al., 2011) and in the bottom area of the St. Petersburg methane seep (Kadnikov et al., 2012). Bacteria of the genus *Hydrogenophaga* belonging to this family utilize some carbohydrates and a wide range of organic acids, including amino acids (Willems et al., 1991). Some representatives of the genus *Rhodoferax* can ferment various carbohydrates under anaerobic conditions and in the absence of light (Finneran et al., 2003). Cultured obligately lithotrophic iron-oxidizing bacteria from freshwater habitats growing in media with neutral pH also belong to β-*Proteobacteria* (Emerson et al., 2010).

The share of α-*Proteobacteria* increased in communities of the sediment from the depths below 4 cm, with the maximum observed at a depth of 8–9 cm

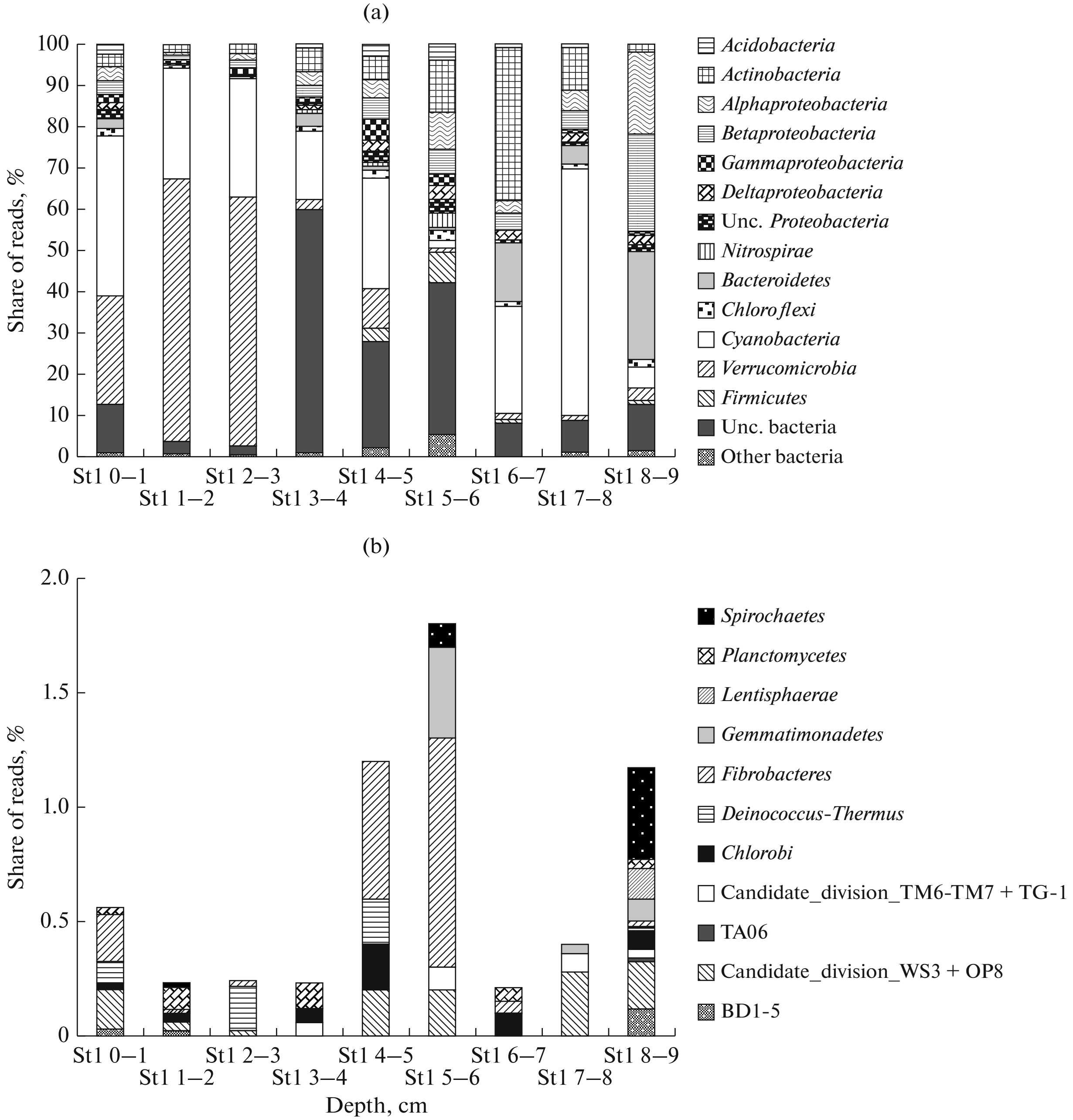

Fig. 1. The taxonomic composition of bacterial communities of the bottom sediments of Southern Baikal: more than 98% reads (a) and less than 2% reads (b).

(19.9%). Representatives of the orders *Rhodobacterales*, *Rhodospirillales*, *Rhizobiales*, and *Sphingomonadales* were predominant among them. Cultured representatives of these taxa were chemoorganoheterotrophs, chemolithotrophs, and facultative photoorganoheterotrophs occurring in various habitats.

Representatives of γ-*Proteobacteria*, including aerobic methanotrophs of the order *Methylococcales*, comprised a small share in the communities of all layers of the sediment and *Methylococcales* were not detected in 8–9 cm layer. Representatives of δ-*Proteobacteria* were detected in the community of the surface layer of the sediment and in the layer below the border of oxygen penetration (Fig. 1). They were present in small numbers and most of them had low similarity to bacteria of the families *Sorangiineae*, *Desulfurellaceae*, and *Bacteriovoraceae*.

The number of representatives of the phylum *Bacteroidetes* increased in the lower layers of the sediment. Their share was the greatest in the community from the 6–7 cm layer (14.5%). The sequences belonged to the families *Flavobacteriaceae* and *Sphin-*

gobacteriaceae, and some of them were unclassified. In the 8–9 cm layer, sequences of the genera *Paludibacter*, *Flavobacterium*, and *Sediminibacterium*, as well as unclassified sequences predominated among *Bacteroidetes*.

The share of representatives of the phylum *Firmicutes*, which included bacteria involved in Fe(II) and Mn(II) oxidation in Lake Baikal (Zakharova et al., 2010), was minor in the community from the upper layer of Fe/Mn crusts (3–4 cm) and was more considerable (7.6% of the total sequence number) in its lower layer (5–6 cm). Most of them belonged to the order *Bacillales* and class *Clostridia*, the identity of which to cultivated iron and manganese bacteria from Lake Baikal was not confirmed.

The archaeal community in different layers of the studied core included OTUs assigned to the following taxa: *Thaumarchaeota* (1–100%), *Euryarchaeota* (0–98.3%), *Bathyarchaeota* (0–2.5%), candidate phyla *Aenigmarchaeota* (0–2.3%), *Aigarchaeota* (0–12.4%), and *Woesearchaeota* (less than 1%) (Fig. 2). Sequences of Marine Group I (MGI) of the phylum *Thaumarchaeota* predominated almost in all layers of the core, with the only exception of the community from the 7–8 cm layer, where archaea of the phylum *Euryarchaeota* (MBG-D) were predominant. Representation of minor archaeal taxa increased in manganese- and iron-rich layers of the sediment (4–5, 5–6, 6–7, and 7–8 cm, from 2 to 9%). Small fractions of representatives of the following taxa were detected in the communities of different layers: *Methanomicrobia*, *Bathyarchaeota*, *Aenigmarchaeota*, *Woesearchaeota*, Group C3, and SCG (*Thaumarchaeota*). The OTU belonging to the candidate phylum *Aigarchaeota* (superphylum TACK) and uncultured line South_African_-Gold_Mine_Gp_1 (SAGMEG-1) were found among the minor identified taxa. Representatives of the predominant line (MGI) of the phylum *Thaumarchaeota* are widely distributed in marine environments, soil, and coastal waters, where they play an important ecological role as autotrophic aerobic ammonium oxidizers (Walker et al., 2010). Some of obtained sequences were clustered together with the cultured species *Nitrosopumilus maritimus* of the MGI lineage, while most of the sequences formed a separate cluster together with uncultured members of the phylum (Fig. 3). It should be noted that closely related sequences belonging to these lineages were detected in the surface and deep layers of the sediments in areas of methane seeps and mud volcanoes of Lake Baikal (Lomakina et al., 2018). The share of another cluster of this phylum that included representatives of uncultured lineage SAGMEG-1 was 3 to 10% of the total number of archaeal sequences in the studied core. This group was originally found in the gold mines of South Africa, and recently representatives of this taxon have been detected in terrestrial soils, marine sediments containing gas hydrates, and in sediments of freshwater lakes (Takai et al., 2001; Inagaki et al., 2003). Sequences of the uncultured lineage Soil Crenarchaeotic Group (SCG) were detected in the surface and several deep layers of the studied core, while sequences of the candidate phylum *Aigarchaeota* were found at a depth of 5–6 cm and those of the Group C3 (previously assigned to the phylum *Crenarchaeota*) were detected at depths of 6–7 and 7–8 cm (Fig. 3). Representatives of the latter taxon were clustered together with sequences of the Baikal-1 line typical of communities of the areas of hydrocarbon fluids discharge (Fig. 3) (Kadnikov et al., 2012). Representatives of SCG were found in soils, activated sludge of anaerobic wastewater treatment plants, and in various extreme ecosystems (Beal et al., 2009).

Representatives of *Euryarchaeota* were detected in the sediments at depths greater than 4 cm, while the shares of methanogenic archaea were minor or they were not detected (Fig. 4). OTUs of the archaea predominant in the community of the buried oxidized layer (6–7 cm) were represented by the sequences of uncultured Marine Benthic Group D (MBG-D) of the order *Thermoplasmatales*. The sequences of this group were also detected in the community of the deeper layer at a depth of 7–8 cm. The MBG-D archaea are widely distributed in freshwater and marine habitats (Schubert et al., 2011), their share is considerable in the community of methane seeps (Beal et al., 2009) and sediments from Lake Pavin (Borrel et al., 2012). It is assumed that they can degrade proteins in marine sediments, and are also involved in methanogenesis or anaerobic methane oxidation (AOM) (Schubert et al., 2011). A cultured representative of this class, *Methanomassiliicoccus luminyensis* (Group E2), was recently shown to produce methane from methanol in the presence of hydrogen (Dridi et al., 2012). The sequences obtained in the present work had low similarity with this species (82%) and were identical with the sequences of uncultured bacteria found in surface sediments of the freshwater Hong Lake and Lake Shallow (Laguna de Carizo, Central Spain).

Representatives of the new archaeal phylum *Bathyarchaeota* (formerly cluster *Crenarchaeota* MCG) were detected in archaeal communities at the depths of 4–5, 6–7, and 7–8 cm (Fig. 3). Archaea belonging to this taxon are considered heterotrophic anaerobes capable of utilizing organic carbon in buried sediments, and identical sequences have often been found in anoxic habitats (Teske and Sørensen, 2008). Members of this phylum are widespread in subsurface marine sediments. It is assumed that the metabolism of certain lineages of this phylum is similar to that of homoacetogenic bacteria involved in acetogenesis and fermentation of various organic substrates (He et al., 2016). In archaeal 16S rRNA gene libraries from the same layer of the sediment, minor amounts (about 2%) of the sequences of another uncultured archaeal lineage, the candidate phylum *Aenigmarchaeota* (for-

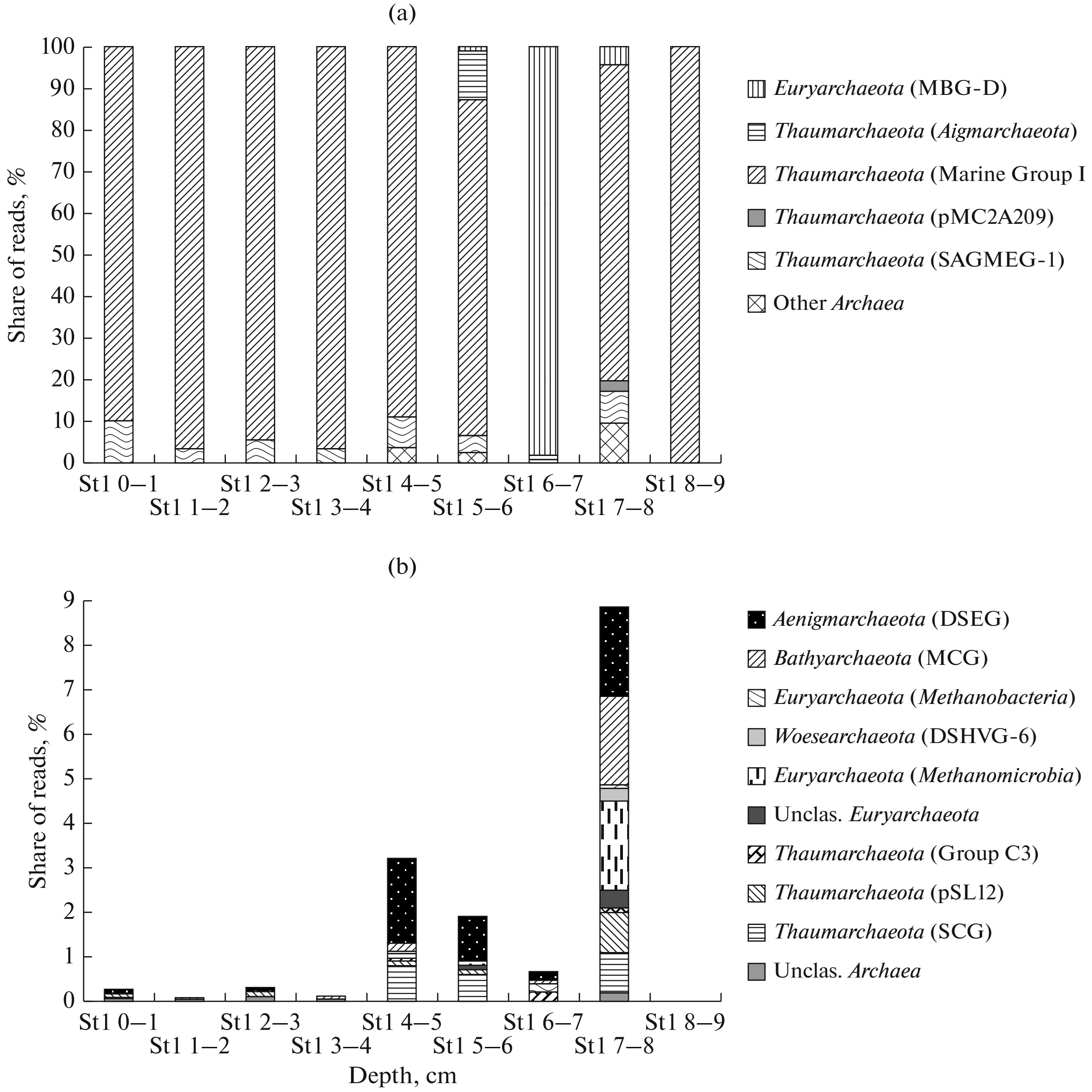

Fig. 2. The taxonomic composition of archaeal communities of the bottom sediments of Southern Baikal: more than 98% reads (a) and less than 2% reads (b).

merly Deep Sea Euryarcheotic Group), were found (Fig. 4).

The difference in taxonomic composition and diversity of the communities of bacteria and archaea in different layers of the sediment was confirmed using principal component analysis (PCoA) (Figs. 5a and 5b). Bacterial communities in the oxygen penetration zone were the most similar in the species composition and representation of the phylotypes, while the communities from the areas with the maximum of manganese (3–4 cm) and iron (4–5 and 7–8 cm) concentrations considerably differed from other communities. The composition and representation of archaeal phylotypes in different layers also differed, despite predominance of the sequences of the phylum *Thaumarchaeota* almost in all layers of the studied core. Bacterial phylotypes associated with the presence of iron ions were detected in the communities using nonmetric multidimensional scaling (NMDS) (Fig. 6a). For example, positive correlation was revealed between the iron ion content and the most represented taxa of *Actinobacteria* (hgcI_clade, marine group), *Bacteroidetes* (*Flavobacterium*, *Sediminibacterium*), *Cyanobacteria*, *Nitrospirae* (*Nitrospira*), and *Betaproteobacteria* (*Rhodoferax*). The sequences of *Verrucomicrobia* (*Luteolibacter*), some *Actinobacteria* (*Actinomycetales*), and *Cyanobacteria* as well as unclassified ones, positively correlated with total carbon content (TCC), SO_4^{2-}, and Mn^{2+}. A significant correlation was

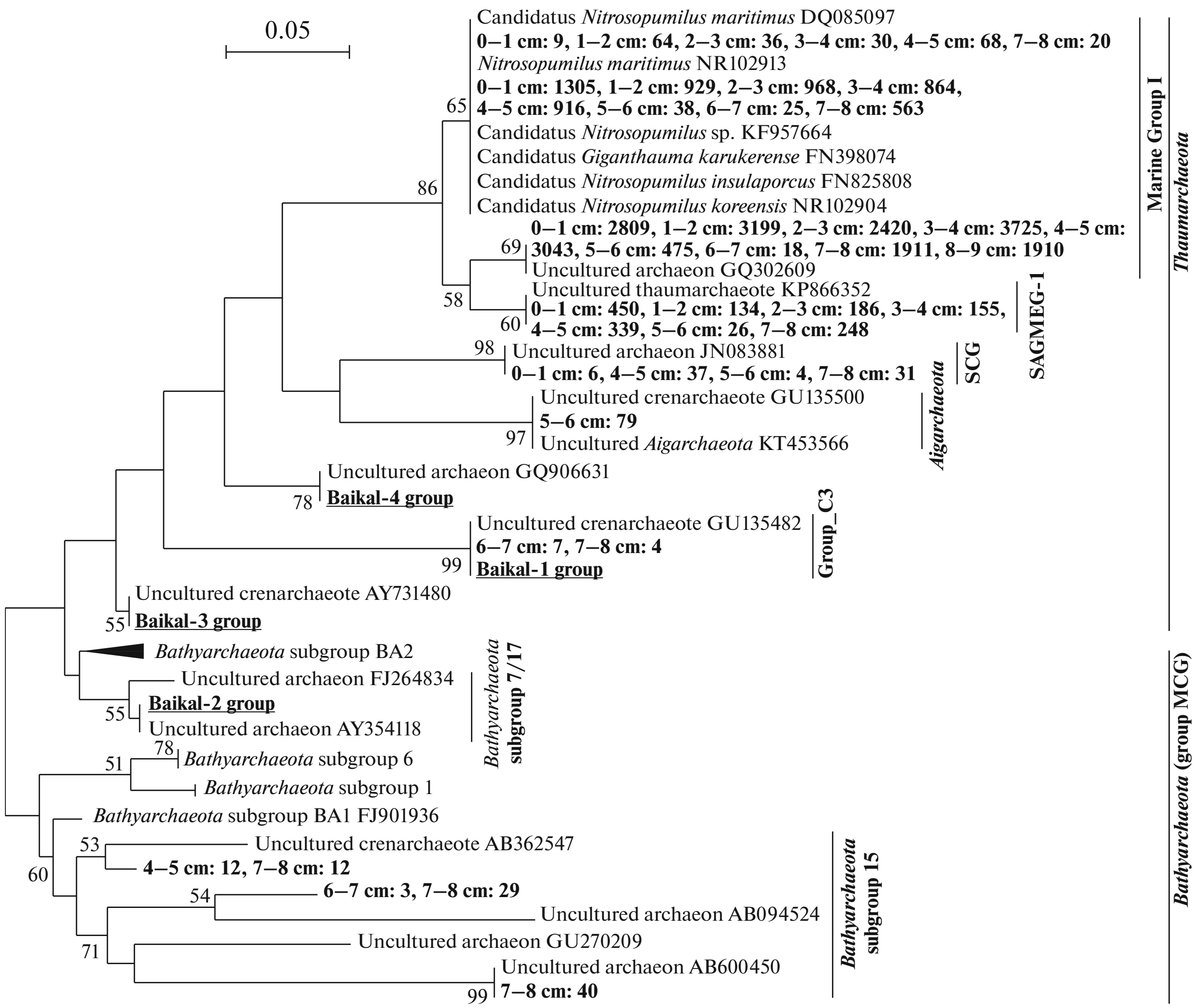

Fig. 3. Phylogenetic tree of the members of the phyla *Bathyarchaeota* and *Thaumarchaeota* constructed based on comparative analysis of nucleotide sequences of the 16S rRNA genes from different layers of the studied layer (depth (cm) and number of sequences are indicated) and the Baikal uncultured lineages of archaea (Baikal-1, Baikal-2, Baikal-3, and Baikal-4). Scale bar corresponds to 5 substitutions per 100 nucleotides (evolutionary distances). The numerals indicate bootstrap support values based on the analysis of 100 alternative trees.

observed between TCC and several OTUs of the phylum *Thaumarchaeota* of the group SAGMEG-1, as well as between the methane concentration and the *Thaumarchaeota* phylotypes present almost in all layers of the studied core (Fig. 6b).

DISCUSSION

In the core of Southern Baikal sediments characterized by the presence of iron-manganese concretions, the composition of microbial communities changed at different depths, probably due to the changes in the geochemical conditions. The most considerable differences in the composition of the dominant bacterial taxa were observed below the border of oxygen penetration (3–4 cm) and in the buried oxidized layer of the sediment (6–7 and 7–8 cm), while the composition of archaeal taxa differed in the buried oxidized layer. Bacterial taxa common in freshwater lakes (Newton et al., 2011) and involved in the initial stages of the degradation of organic compounds (*Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*) predominated in the oxidized layer of the sediment to a depth of 3 cm. The sequences of the two first phyla were found in bacterial communities from other areas of the lake, while predominance of the phylum *Verrucomicrobia* in the communities has not been previously detected (Kadnikov et al., 2012; Zemskaya et al., 2015). Phylogenetic analysis of the most numerous OTUs demonstrated no similarity to acidophilic representatives of the phylum, which are known to be capable of methane oxidation (Dunfield et al., 2007). Representatives of this taxon are most likely involved in the degradation of diatoms annually settling from the photic zone to the sediments after their intense

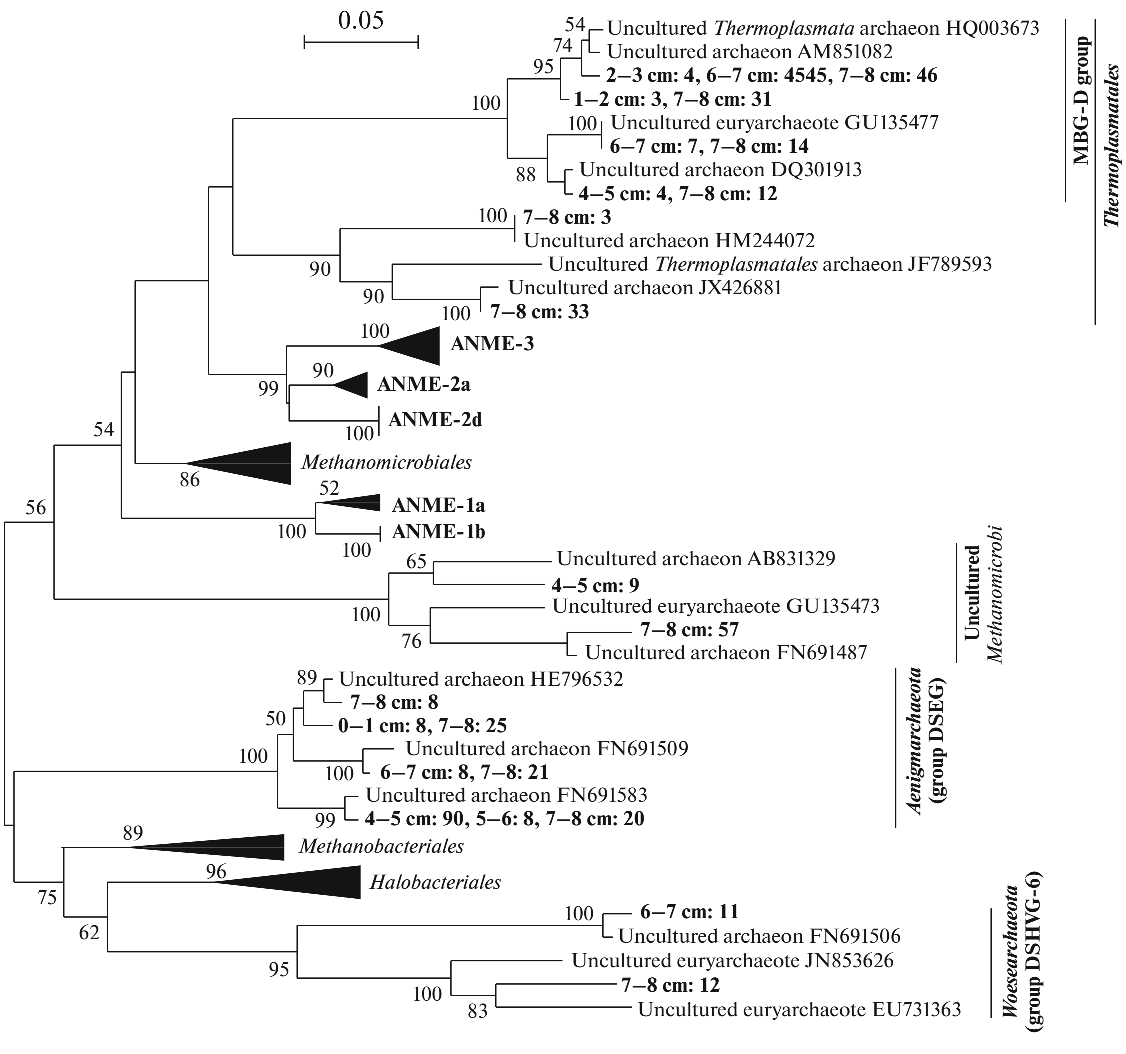

Fig. 4. Phylogenetic tree of the representatives of the phylum *Euryarchaeota* and of the novel phyla *Aenigmarchaeota* and *Woesearchaeota*, constructed based on comparative analysis of nucleotide sequences of the 16S rRNA genes from different layers of the studied layer (depth (cm) and number of sequences are indicated). Scale bar corresponds to 5 substitutions per 100 nucleotide (evolutionary distances). The numerals indicate bootstrap support values based on the analysis of 100 alternative trees.

development in spring and autumn (Zakharova et al., 2013). The changes in the structure of bacterial communities were also evident in the zone of the iron-manganese layer, where the sequences with unknown taxonomic affiliation associated with the Mn concentration were predominant. According to the hypothesis (Torres et al., 2014), Mn(IV) and, potentially, Fe(III) may act as oxidants for microbiologically mediated nitrification process. Increase in the share of nitrite-oxidizing bacteria of the phylum *Nitrospirae* and the presence of archaea of the phylum *Thaumarchaeota* oxidizing ammonium to nitrite were indeed revealed in microbial communities below the border of oxygen penetration. Furthermore, in the communities of the Fe/Mn layer, significant numbers of the α- and β-*Proteobacteria* sequences positively correlated with the content of iron ions were detected. The presence of increased concentrations of these ions could induce various decomposition processes, in which these compounds could act as electron acceptors for the microorganisms participating in reduction/oxidation of various substrates and involved in formation of iron-manganese crusts.

Previously it was assumed that reductive dissolution of buried Fe/Mn layers occurred due to anaerobic CH_4 oxidation (Och et al., 2012). Comparison of methane profiles with the composition of archaeal communities did not clarify this assumption. Significant changes in the taxonomic composition were detected only in the community of the buried oxidized layer (6–7 cm). Representatives of the order *Thermoplasmatales* (uncultured lineage MBG-D) with unknown type of metabolism predominated among the archaea in this community and only a small part of the sequences was identical to methanogenic repre-

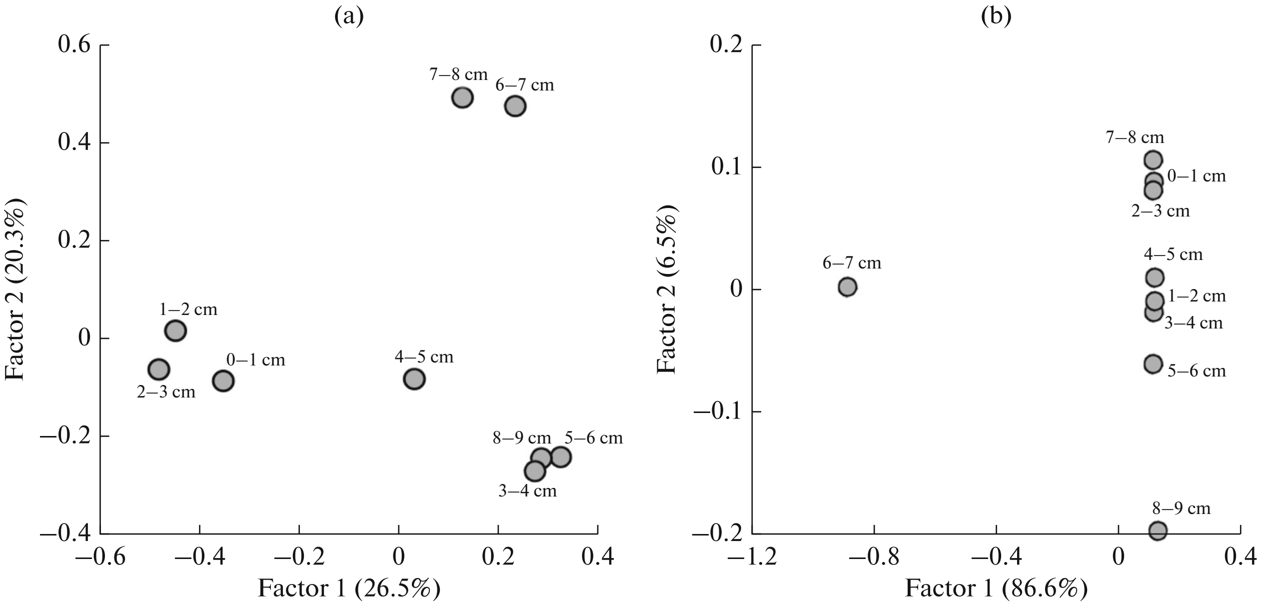

Fig. 5. Plot constructed using principal component analysis (PCoA) demonstrating the correlation between OTUs of bacterial (a) and archaeal (b) 16S rRNA gene libraries in different layers of the studied core.

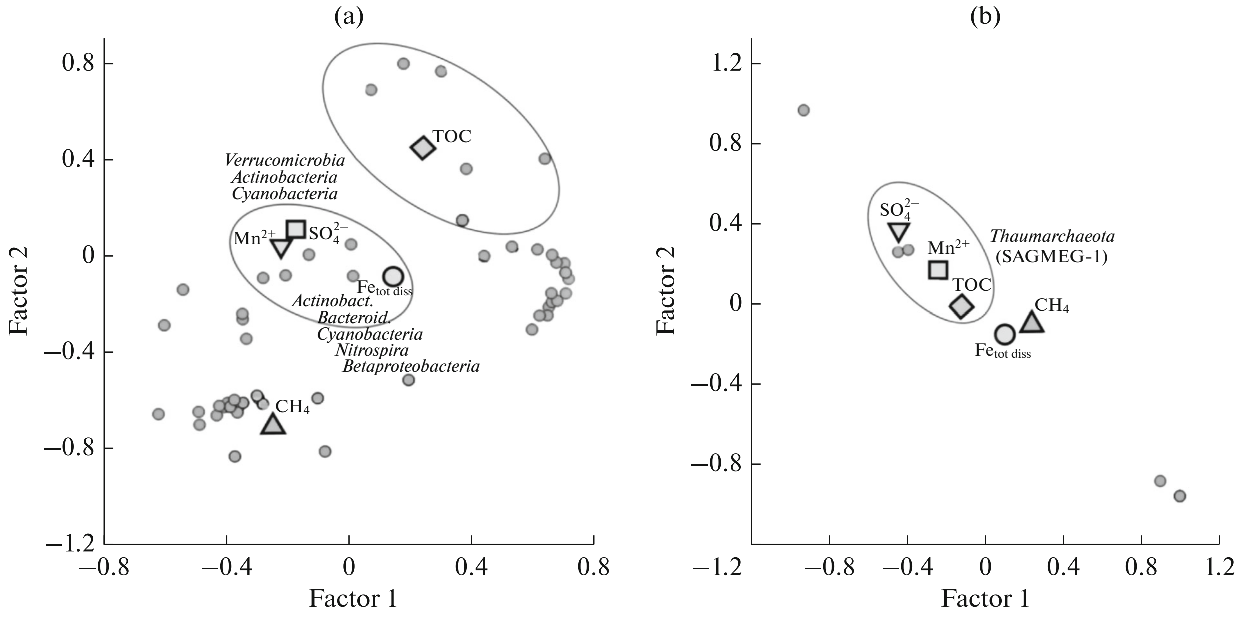

Fig. 6. Plot of non-metric multidimensional scaling (NMDS) demonstrating the correlation between OTUs of bacterial (a) and archaeal (b) 16S rRNA gene libraries and the chemical parameters of pore waters in different layers of the studied core.

sentatives of *Euryarchaeota*. Phylogenetic analysis of archaeal 16S rRNA gene libraries demonstrated no presence of the sequences closely related to ANME groups, including the sequences assigned to the ANME-2d group typical of freshwater ecosystems, which have been detected in other areas of Lake Baikal (Lomakina et al., 2018). The most significant methane decline was observed below the border of the oxidized layer, where changes in the structure of the bacterial community, but not of the archaeal one, were detected. As was noted above, involvement of the members of the predominant phylum *Verrucomicrobia* in methane oxidation was also doubtful, because most of the sequences had a high identity (99%) with the heterotrophic species *Luteolibacter luojiensis* (Cardman et al., 2014), rather than with acidophilic species capable of methane oxidation. Investigation of the microorganisms in the sediments of a marine methane seep in the Eel River basin in California demonstrated not only the archaea, but also bacteria to play a considerable role in manganese-dependent AMO (Beal et al., 2009). It is possible that the unclassified organisms, sequences of which comprised a significant part in the communities of the Fe/Mn layers, may be responsible for AOM. Nevertheless, this hypothesis requires further experimental confirmation to reveal the microorganisms involved in this process in Lake Baikal.

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