Original Article

Microbial communities in the estuarine water areas of the rivers in the southeastern part of Lake Baikal

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ABSTRACT. Using the Illumina MiSeq platform, we have studied the diversity of bacteria and archaea in three rivers of the southeastern end of Lake Baikal in the under-ice period of 2018. In analysed 16S rRNA gene libraries of all rivers, we have identified sequences of 12 bacterial phyla: *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Elusimicrobia*, *Epsilonbacteraeota*, *Fibrobacteres*, *Firmicutes*, *Omnitrophicaeota*, *Patescibacteria*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia*. The contribution of minor taxa to the microbiomes from the estuaries of the rivers Solzan and Bolshaya Osinovka is more significant. Three phyla (*Thaumarchaeota*, *Euryarchaeota* and *Crenarchaeota*) and one superphylum (DPANN) represent archaea. The diversity of bacteria and archaea in the investigated ecotopes has its specifics and is different to that found in the pelagic zone of Southern Baikal. Bacteria show phylogenetic diversity at the level of families and genera, whereas archaea – at the level of phyla. In the microbiomes, we have identified microorganisms involved in various stages of transformation of organic and inorganic substances.

1. Introduction

Rivers and their estuarine water areas are zones, where transformation and accumulation of various substances from shores and river waters take place (Newton et al., 2013; 2015; Cloutier et al., 2015). The depth of information acquired by using advanced molecular genetic approaches provides a means to characterize the microbial composition, distribution, and transportation pathways in the environment and to relate them to understand pollution mechanisms (Newton et al., 2013; Halliday et al., 2014). There was the active destruction of various substances in the estuarine water areas of large tributaries of Lake Baikal: the rivers Selenga and Upper Angara (Maksimenko et al., 2008; 2012; Sorokovikova et al., 2012), and to a lesser extent – in water areas small inflowing tributaries (Maksimov, 1995). There were practically no studies concerning the diversity of microbial communities in these areas; only the taxa ratio at the phylum level were investigated in rivers and estuarine water areas of the rivers Selenga and Upper Angara. At the same time, some works state that precipitation (Khodzher et al., 2002; Sorokovikova et al., 2002; Tomberg et al., 2016) as well as domestic sewage (Drucker et al., 1993; Shtykova et al., 2016) and groundwater runoff from

sludge and lignin storage pits of the closed Baikalsk Pulp and Paper Mill (State Report…, 2017) influence the chemical composition in the rivers of Southern Baikal.

In the under-ice period of 2018, we conducted comprehensive studies at estuaries and estuarine water areas of small rivers in the southeastern part of Lake Baikal (Zemskaya et al., 2019). We found that acidity of snow cover and waters of the Pereyomnaya River did not adversely affect the abundance of microorganisms and their productiveness. The high values of dark carbon dioxide fixation in the water of the estuaries of the rivers in comparison with the estuarine zone were a distinctive feature of these water areas, and the high P/B coefficients (specific bacterioplankton production) with a significantly lower total microbial count indicated a high percentage of metabolically active cells that ensure the inclusion of carbon not only in the bacterioplankton biomass but also other organic compounds. Therefore, we were interested in the study of the diversity and structure of microbial communities at the estuary and an estuarine water area of the Pereyomnaya River as well as estuaries of the rivers Solzan and Bolshaya Osinovka having the acidity of snow cover and inflow of wastewater from sludge and lignin storage pits, using the Illumina MiSeq platform.

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2. Material and methods
2.1. Study areas and sampling

The water studies were carried out in March 2018 at estuaries and an estuarine water area of the Pereyomnaya River as well as the estuaries of the rivers Solzan and Bolshaya Osinovka (Southern Baikal). Water was sampled using a Niskin bottle at the estuaries of the rivers and a distance of 100 and 200 m from the estuaries. A description of the stations, methods for studying chemical parameters and pH are shown in (Zemskaya et al., 2019).

2.2. DNA extraction

We obtained five DNA samples from the surface water. Water samples (5 L) were filtered on nitrocellulose filters (25 mm diameter, 0.2 micron pore size; "Millipore", Germany) using a diaphragm pump. The filter was then placed in a TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA, pH 8.0) and frozen at -20°C. Then, it was transported to the laboratory. DNA was extracted according to the modified method of phenol-chloroform extraction (Sambrook et al., 1989). The extracted DNA was kept at -70°C for further use. The universal primers U341F (5'-CCTACGGGRSGCAGCAG-3') and U785R (5'- GACTACHVGGGTATCTAATCC -3') as well as a program: 96°C for 3 min; 96°C for 30 s; 55°C for 30 s; 72°C for 40 s (25 cycles); and 72°C for 10 min, were used for PCR amplification of 16S rRNA gene fragments of bacteria, including the variable region V3–V4. The primers that included the variable region V2–V3: A113F (5'-ACKGCTSAGTAACACGTGG-3') and A520R (5'-TACGGCGGCKGCTGGCA-3') (Whitehead and Cotta, 1999; Kim et al., 2011), as well as a program: 96°C for 2 min; 96°C for 30 s, 58°C for 45 s, 72°C for 40 s (25 cycles); and 72°C for 10 min, were used for PCR amplification of 16S rRNA gene fragments of archaea. The libraries were analysed using Illumina MiSeq Standard Kit v.3 (Illumina) at the Genomics Core Facility of the Evrogen Join Stock company (Moscow).

2.3. Data preparation and filtering: Bioinformatics analysis

Paired-end sequencing reads were trimmed and filtered by quality using leading and sliding window trimming with the average Phred-value = 25 and window size = 13-15 bases in Trimmomatic version 0.39 (Bolger et al., 2014). The R1 and R2 sequences corresponding to ribosomal RNA amplicons were merged into contigs with the mothur merge.contigs command. The fragments obtained were filtered by size and tested on the contents of respective forward/ reverse primers with allowing two mismatch between forward and reverse primer and sequence. Further rRNA sequence processing was performed using mothur v.1.34.4 software (Schloss et al., 2009) according to MiSeq SOP recommendations (Kozich et al., 2013). To compare the microbial diversity among the samples, the bacterial read numbers of each sample were subsampled to those of the sample with the smallest number of reads by random removal of sequencing reads using the sub. sample command of the mothur program. The filtered sequences were aligned, clustered, and identified taxonomically using the SILVA 132 databases (http://www.arb-silva.de). To estimate similarity among bacterial communities, the samples were analysed using non-metric multidimensional scaling and clustered on the basis of the Euclidean distance matrix by the Vegan and Cluster packages in R. For statistical analysis and assessment of the association of individual OTUs with chemical parameters, the concentrations of O_2, POB, SO_4^{2-}, and CH_4 shown in (Zemskaya et al., 2019) were used. The closest homologues of each gene fragment sequence were found with a BLAST search against the NR database (http://blast.ncbi.nlm.nih.gov). The 16S rRNA sequences were deposited in the NCBI's Sequence Read Archive (Accession number: Bioproject PRJNA556789).

3. Results and discussion

The obtained rarefaction curves (data not shown) indicate that the sequencing volume reached in the analysis of the samples is satisfactory for the complete characterisation of diversity of bacterial communities from river samples but insufficient for the reference station. In the latter sample, we obtained the sequences of almost one taxon, cyanobacteria, which differed significantly from the data simultaneously obtained in the pelagic zone of Southern Baikal (Cabello-Yeves et al., 2018). In river microbiomes, the Chao1 and Shannon indices were maximum for 16S rRNA gene libraries of bacteria from the Solzan River (4471.3 and 6.8, respectively) as well as for archaea from the estuarine water area of the Pereyomnaya River (138 and 4.2, respectively, Table 1). The number of archaeal species varied less significantly; the highest values of the Shannon index (4.2) were in the estuarine water area of the Pereyomnaya River, and the lowest ones (3.3) – at the estuary of the Solzan River. Notably, the lower number of the obtained archaeal sequences in comparison with bacterial ones may be due to their low abundance and an insufficient amount of investigated DNA.

Analysis of the 16S rRNA gene libraries of bacteria and archaea in the rivers and estuarine water areas indicated a different taxonomic composition of the communities. OTUs of bacteria and archaea formed two clusters each on dendrograms. The first one included sequences from the estuary and estuarine water area of the Pereyomnaya River, and the second – from the estuaries of the rivers Solzan and Bolshaya Osinovka (Fig. 1A, 1B). Based on the intracluster distances, the diversity of bacterial communities was more similar in the microbiomes from the estuarine water area of the Pereyomnaya River than between microbiomes from the estuary and estuarine water area. According to intercluster distances, the differences in the composition of archaeal communities were more obvious in comparison with bacterial communities.

In the analysed 16S rRNA gene libraries of all rivers, there were sequences of 12 bacterial

phyla: *Actinobacteria, Bacteroidetes, Cyanobacteria, Elusimicrobia, Epsilonbacteraeota, Fibrobacteres, Firmicutes, Omnitrophicaeota, Patescibacteria, Planctomycetes, Proteobacteria,* and *Verrucomicrobia* (Fig. 2A). The contribution of minor taxa varied: their diversity and percentage of sequences were lower in the microbiome from the water area of the Pereyomnaya River and higher at the estuaries of the rivers Solzan and Bolshaya Osinovka (4.7 and 2.1%) (Fig. 2B). In the microbiome from the estuary of the Pereyomnaya River, the sequences of *Proteobacteria* (46%), *Epsilonbacteraeota* (21%) and *Bacteroidetes* (11.2%) as well as *Cyanobacteria* (7.8%) and *Patescibacteria* (4.1%) had the highest percentage. At a distance of 100 m from the river estuary, the ratio of the dominant taxa in the microbiomes changed, with an increase in the contribution of the members of the phyla *Firmicutes* (8.5%), *Bacteroidetes* (18.2%) and *Cyanobacteria* (18.3%) and decrease in *Proteobacteria* (25.1%). At a distance of 200 m from the river estuary, the ratio of taxa in the microbiomes again changed; the members of *Bacteroidetes* (26.3%), *Cyanobacteria* (25.5%) and *Gammaproteobacteria* (25.3%) had the highest percentage, and the sequences of *Firmicutes* (0.2%) and *Epsilonbacteraeota* (4.1%) were minor. Microorganisms of the *Gammaproteobacteria* (20.0-32.4%) mainly represented Proteobacteria with a large contribution of the sequences of the order *Betaproteobacteriales*. Among them, we identified the sequences of methano- and methylotropic bacteria, in particular, the members of the families *Methylophilaceae* (genus *Methylotenera*) and *Methylomonaceae* (genus *Methylobacter*). The presence of a great number of sequences of these taxa at the estuary of the Pereyomnaya River is likely due to their inflow with the runoff of the river, which flows
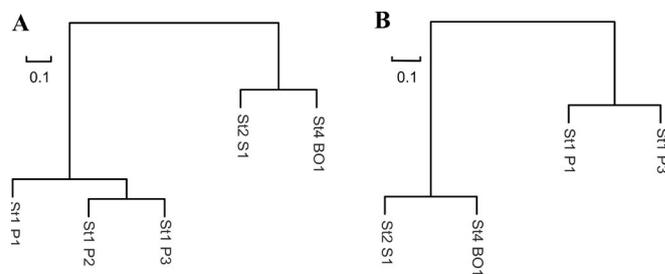
Fig.1. Dendrogram of similarity and difference of bacterial (A) and archaeal (B) communities based on Euclidean distance matrix

through wetlands. Moreover, the microbiome of this river (11.7%) had a high percentage of the sequences of the genus *Methylobacterium* (*Alphaproteobacteria*), which also participate in the methane cycle, ensuring the methanol oxidation. In the communities of the estuarine water area of the Pereyomnaya River (2.8 and 4.8%, respectively), the members of this genus were a minor component. The bacterial sequences of the genus *Sphingomonas* (*Alphaproteobacteria*), which closest homologues have proteo- and lipolytic activity and are capable of surviving under low concentrations of nutrients (Yoon et al., 2008), had a large contribution in the microbiomes of all investigated samples.

Members of the *Deltaproteobacteria* class in the Pereyomnaya River and its water area were not numerous (1.9% or less) and included both aerobic predatory bacteria of the genus *Bdellovibrio* and anaerobic sulphate- (genus *Desulfobulbus*) and metal-reducing microorganisms (genus *Geobacter*). The sequences of the phylum *Bacteroidetes* (11.2-22.5%), which belong to both planktonic (*Flavobacterium, Fluviicola, Arcicella,* and *Parasediminibacterium*) and

Table 1. Indices of species richness and diversity (at a cluster distance of 0.03) of bacteria and archaea in the 16 rRNA gene libraries of microbial communities from the rivers Pereyomnaya, Solzan and Bolshaya Osinovka.

Sampling area, sample		Number of reads	Coverage, %	Number of OTU$_{0.03}$	ACE	Chao1	Simpson's Inverse Index	Shannon
Bacteria								
estuary of the Pereyomnaya River	St1 P1	148453	99.6	3821	4110.4	4171.2	15.0	4.67
100 m from the estuary	St1 P2	106195	99.1	3086	3781.9	3701.2	12.4	4.24
200 m from the estuary	St1 P3	100175	99.1	3301	3986.3	3987.0	14.4	4.66
estuary of the Solzan River	St2 S1	162003	99.9	4432	4471.3	4509.7	218.1	6.79
estuary of the Bolshaya Osinovka River	St4 BO1	104767	99.4	4110	4375.9	4442.7	97.6	6.40
reference station	St5 Fon	2941	99.9	8	11.8	9.0	1.0	0.05
Archaea								
estuary of the Pereyomnaya River	St1 P1	1117	99.2	80	83.9	86.0	15.0	3.6
200 m from the estuary	St1 P3	1670	99.4	123	125.6	138.0	37.4	4.2
200 m from the estuary	St2 S1	1278	99.4	66	69.1	70.6	15.2	3.3
estuary of the Bolshaya Osinovka River	St4 BO1	637	98.9	80	81.9	82.3	27.7	3.9
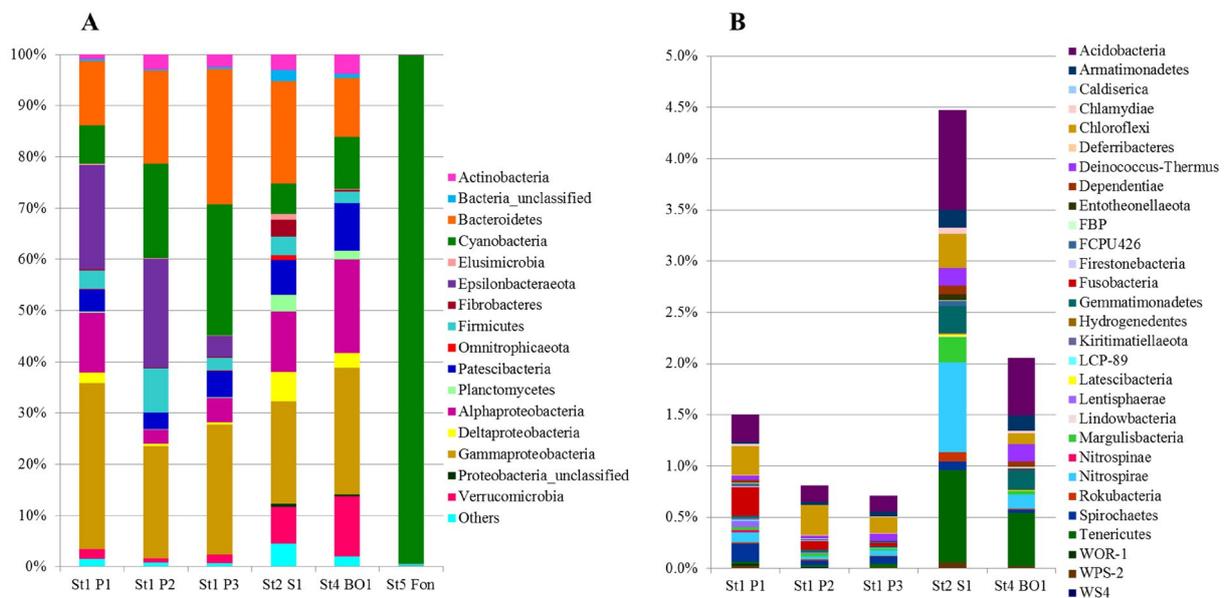
Fig.2. The composition of the bacterial community in the water of the rivers Pereyomnaya, Solzan and Bolshaya Osinovka according to the analysis of sequences of 16S rRNA gene fragments: A – dominant phyla (share of sequences >95.0%); B – minor taxa (share of sequences <5.0%) The composition of the bacterial community in the water of the rivers Pereyomnaya, Solzan and Bolshaya Osinovka according to the analysis of sequences of 16S rRNA gene fragments: A – dominant phyla (share of sequences >95.0%); B – minor taxa (share of sequences <5.0%)

benthic microorganisms (*Paludibacter*), were more diverse and numerous in the microbiomes of this area. The identified bacteria of the genera *Flavobacterium* and *Fluviicola* are capable of decomposing plant polysaccharides, such as starch, cellulose, xylans and pectins, as well as photosynthesizing due to the presence of rhodopsins in the cells (Humphry et al., 2001; Martinez-Garcia et al., 2011; Feng et al., 2015; Park et al., 2017). Additionally, bacteria of the genera *Arcicella* and *Parasediminibacterium* play an important role in protein metabolism (Sheu et al., 2010; Kang et al., 2016), whereas the *Paludibacter* members ferment sugars (Ueki, 2006).

Microbiome in the Solzan River had a wider range of bacterial taxa (Fig. 2A), including dominant *Gammaproteobacteria* (20%), *Alphaproteobacteria* (11.8%) and *Bacteroidetes* (20.1%). In the microbiome of this river, the contribution of *Patescibacteria* (6.8%) and *Verrucomicrobia* (*Opitutaceae* family, 7.2%) increased, and that of *Cyanobacteria* (5.9%) decreased. Like in the microbiomes of the Pereyomnaya River, members of the order *Betaproteobacteriales*, in particular, the sequences of the genera *Paucibacter* and *Rhodoferax*, were numerous among *Gammaproteobacteria*. The strains of the genus *Paucibacter* from lake sediments can cleave cyanobacterial hepatotoxins, microcystins and nodularin (Rapala et al., 2005). In the microbiome of the Solzam River, the share of minor taxa increased (4.5%), among which the intracellular parasites of arthropod from the phylum *Tenerecutes* (*Mycoplasmataceae* family, 0.9%) (Kostanjsek et al., 2007) as well as ammonia- and nitrite-oxidizing bacteria of the phylum *Nitrospirae* (genus *Nitrospira*, 0.9%) (Koch et al., 2015) and *Acidobacteria* (approximately 1%) were the most representative. Furthermore, in the minor component of the microbiome, there were members of the phyla *Planctomycetes*, *Omnitrophicaeota*, *Fibrobacteres*, and

Firmicutes. A wider range of taxa in the microbiome of this river may be due to the influx of water and microorganisms from sludge and lignin storage pits remaining after the closure of Baikalsk Pulp and Paper Mill.

The structure and diversity of the microbiome from the Bolshaya Osinovka River were similar to those from the Solzan River. *Gammaproteobacteria* (24.7%), *Alphaproteobacteria* (18.3%), *Bacteroidetes* (11.6%), *Verrucomicrobia* (11.6%), and *Patescibacteria* (9.3%) were among the dominant phyla. The contribution of minor taxa did not exceed 2%. Notably, in the microbiomes of the Solzan and Bolshaya Osinovka Rivers, there was a decrease in the contribution of the sequences of sulphur-oxidizing bacteria (genus *Sulfurospirillum*) from the phylum *Epsilonbacteraeota* (0.01%), which had a high percentage in the microbiomes from the Pereyomnaya River (up to 21%).

The taxonomic composition of archaea was less diverse (Fig. 3) in the investigated areas. We identified members of three phyla (*Thaumarchaeota*, *Euryarchaeota*
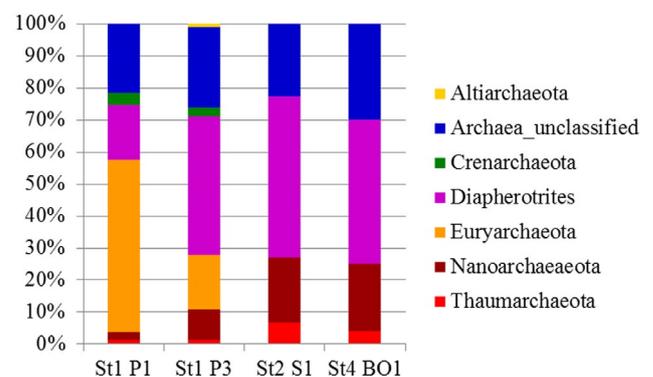
Fig.3. The composition of archaeal community in the water of the rivers Pereyomnaya, Solzan and Bolshaya Osinovka according to the analysis of the sequences of 16S rRNA gene fragments
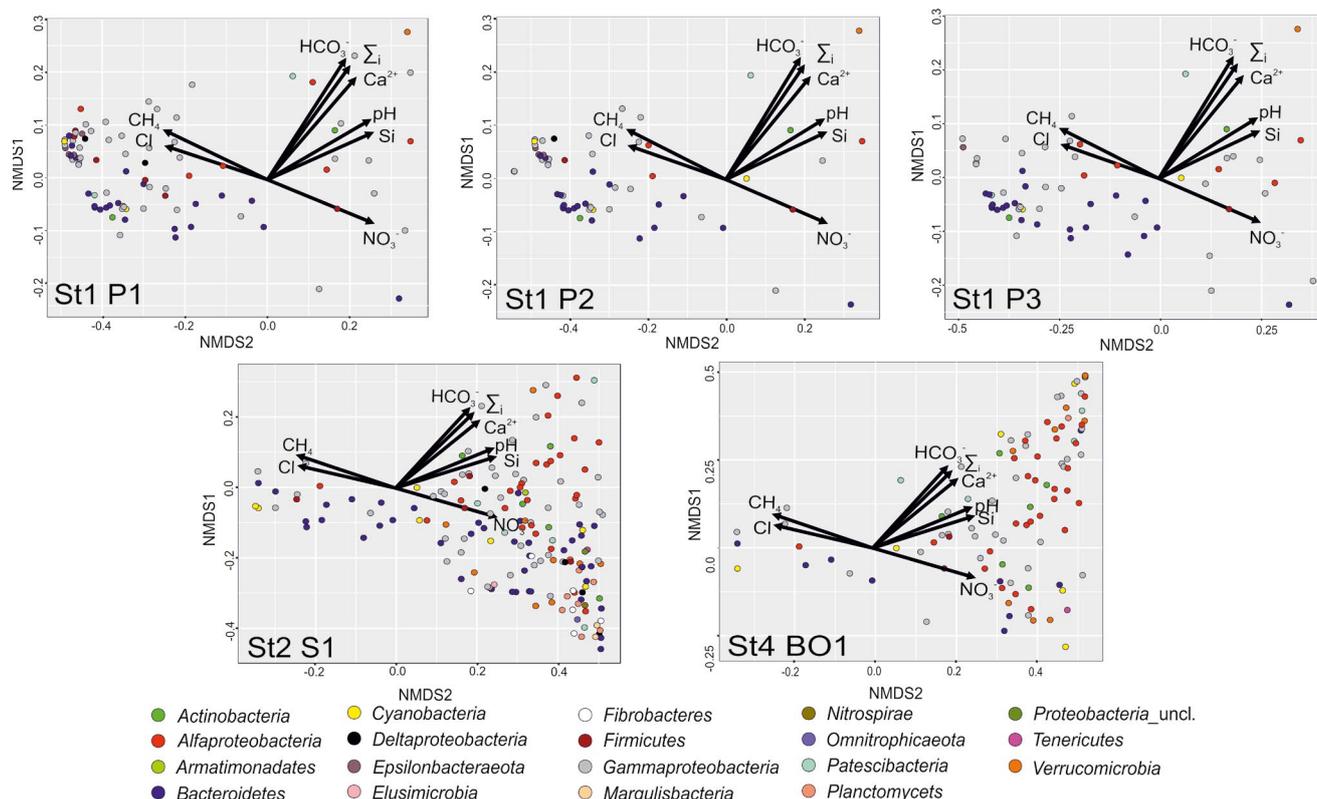
Fig.4. Non-metric multidimensional scaling (NMDS) ordination of bacterial community composition in each sample based on Euclidean distances calculated for abundant OTUs with more than 150 sequences (stress = 1.37). Vectors show correlations of environmental variables (p < 0.05)

and *Crenarchaeota*) and one superphylum (DPANN) of archaea. Unlike the archaeal community from the photic layer of the pelagic zone in Southern Baikal, where there were only members of the phylum *Thaumarchaeota* (Cabello-Yeves et al., 2018), microbiomes of the studied rivers had a more diverse taxonomic composition. The contribution of the *Thaumarchaeota* members is insignificant (1.1-6.7%), and the revealed sequences belong to ammonium-oxidizing microorganisms of the families *Nitrosopumilaceae* and *Nitrosotaleaceae* (Park et al., 2012). The microbiome from the water area of the Pereyomnaya River had the greatest number of archaeal taxa, and at the estuary, the bulk of sequences belonged to the methanogenic archaea of the phylum *Euryarchaeota* (53.9%); in the estuarine water area, their contribution was much smaller (16.9%), and they were not detected in the communities from other rivers. The members of *Crenarchaeota* were also found in minor quantities only in the microbiomes from the Pereyomnaya River (3.8-2.6%), and the *Diapherotrites* and *Nanoarchaeota* members of the DPANN superphylum dominated the microbiomes of the rivers Solzan and Bolshaya Osinovka (from 20.3 to 50.4%). Notably, the sequences of the DPANN superphylum were observed only in the near-bottom area of the pelagic zone in Southern Baikal (Cabello-Yeves, personal communication).

The nonmetric multidimensional scaling technic evaluated the association of the individual taxonomic units and chemical indicators in the studied biotopes (Fig. 4, Fig. 5). The analysis included OTUs having more than 150 sequences. To construct dendrograms for data array on bacteria, we used such parameters as

decomposable organic matter (DOM), a total number of ions, the concentration of major ions as well as pH. The dendrograms show the data on the association of chemical parameters with the individual OTUs of bacteria (Fig. 4) and archaea (Fig. 5). It is obvious that three clusters of bacterial OTUs form in each water area. In the microbiomes of the Pereyomnaya River and its water area, the OTU group associated with the concentration of Cl^- and CH_4 ions is the most evident. This cluster includes members of such taxa as *Bacteroidetes*, *Alpha-* and *Gammaproteobacteria*, among which the most representative are the sequences of the families *Methylophilaceae* (genus *Methylotenera*) and *Methylomonaceae* (genus *Methylobacter*) involved
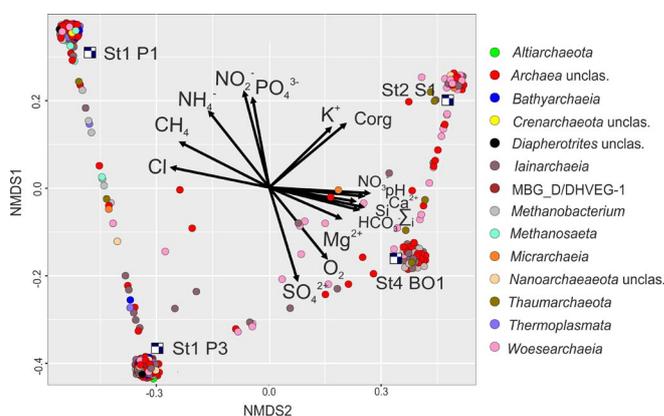
Fig.5. Non-metric multidimensional scaling (NMDS) ordination of archaeal community composition in each sample based on Euclidean distances calculated for abundant OTUs with more than 50 sequences (stress = 1.74). Vectors show correlations of environmental variables

in various stages of methane oxidation. Among *Bacteroidetes*, the members of the genera *Paludibacter, Arcicella* and *Flavobacterium* are numerous. In the microbiomes of the rivers Solzan and Bolshaya Osinovka, a large number of OTUs is associated with the total number of ions, the concentration of Ca^{2+}, Si and HCO_3^- ions as well as the pH of the water. In this cluster, the most representative sequences are *Alpha-* (families *Caulobacteraceae, Hyphomonadaceae, Paracaedibacteraceae, Rhodobacteraceae,* and *Rickettsiaceae*), *Gammaproteobacteria* (*Burkholderiaceae, Chitinibacteraceae, Rhodocyclaceae, Cellvibrionaceae,* and *Pseudomonadaceae*) and *Actinobacteria* (*Microbacteriaceae* and *Solirubrobacteraceae*). The third OTU cluster is the most numerous in the microbiomes of the rivers Solzan and Bolshaya Osinovka, and it correlates with the concentration of nitrate ion. This cluster has OTUs of *Gammaproteobacteria* (*Burkholderiaceae; Xanthomonadaceae*), *Alphaproteobacteria* (*Hyphomonadaceae; Rhodobacteraceae; Sphingomonadaceae*), *Tenerecutes, Verrucomicrobia* (*Rubritaleaceae*), *Planctomycetes* (*Pirellulaceae*), and *Nitrospiraceae* (*Nitrospira*).

The analysis of data array of the archaeal OTUs indicates their different contribution to the composition of the communities in the studied water areas (Fig. 5) and the lack of reliable associations with most on the analysed parameters. This is most evident for the archaeal community from the water area of the Pereyomnaya River, where we have determined no reliable correlation between dominant OTUs with any of the parameters. The individual OTUs of archaea from the Bolshaya Osinovka River are associated with nitrates, the total number of the Si and HCO_3^- ions as well as the pH of the water (Fig. 5), whereas in the Solzan River – with the concentration of DOM and potassium ions.

4. Conclusions

The studied rivers have different salinity, the pH of the water, the concentration of DOM and individual ions, which affected the diversity of microbial communities. The phylogenetic analysis has shown that their diversity and structure in the rivers are not the same and differ from those simultaneously observed in the pelagic zone (Cabello-Yeves et al., 2018). The level of families and genera mostly shows the taxonomic difference in bacteria, and the level of phyla – in archaea. In the communities of the Pereyomnaya River and its water area, where the waters have the lowest salinity and mildly acidic pH of the environment, the sequences of bacteria involved in various stages of methane oxidation predominated in the microbiomes. This is consistent with higher methane concentrations at the estuary and estuarine water of this river in comparison with other rivers. The taxonomic composition of the communities in the estuarine water area is similar to riverine and pelagic communities. At a distance from the river estuaries, the contribution of the members of *Patescibacteria* and *Epsilonbacteraeota*, as well as minor taxa, decreases. Despite the smaller watershed basin and

runoff in comparison with the Pereyomnaya River, the microbiomes of the rivers Solzan and Bolshaya Osinovka have a more diverse phylogenetic composition. This is most likely due to the inflow of wastewater from the sludge and lignin storage pits of Baikalsk Pulp and Paper Mill, which ensure the activity of a wide range of microorganisms. Moreover, the structure of bacterial communities in the rivers differs from the structure of the communities from the photic layer of the pelagic zone in Southern Baikal (5 and 20 m) (Cabello-Yeves et al., 2018). In all microbiomes, the contributions of *Gammaproteobacteria, Alphaproteobacteria* and *Bacteroidetes* are comparable, whereas in the microbiomes of rivers, the phyla *Patescibacteria* and *Epsilonbacteraeota* are more representative, and *Actinobacteria* are less representative. Probably, the small representation of the latter ones in the river biotopes during the under-ice period is due to a dense snow cover that does not let in sunlight and, hence, limits the development of photoheterotrophic bacteria. In the pelagic zone of Southern Baikal, where the ice was less covered with snow, actinobacteria with a photoheterotrophic type of metabolism prevailed, whereas, in the river microbiomes, chemoorganotrophic microorganisms capable of using various substrates as well as methane were the most representative. High metabolic activity in the river samples compared to those from the estuarine water area of the Pereyomnaya River implies the lack of a mixing zone typical of large tributaries, which serves as a kind of biofilter that impedes the influx of various compounds in the lake waters.

Acknowledgements

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