

## Article

# Bacterial Communities in a Gradient of Abiotic Factors Near a Sulfide Thermal Spring in Northern Baikal

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**Abstract:** The structure and diversity of microbial communities developing in the combined gradient of temperature (44–19 °C), as well as concentration of oxygen (0–10 mg/L) and hydrogen sulfide (33–0.7 mg/L), were studied in the thermal sulfide spring on the coast of Northern Lake Baikal. The predominance of bacteria participating in sulfur and nitrogen cycles and significant changes in the composition of microbial communities were noted at changing physicochemical conditions. *Thiovirga* sp. (sulfur-oxidizing bacteria, up to 37%) and *Azonexus* sp. (nitrogen-fixing bacteria, up to 43%) were dominant at high temperatures and concentrations of hydrogen sulfide in two hydrotherms. In addition, a significant contribution of the *Rhodocyclaceae* family (up to 51%) which is involved in the denitrification processes, and *Acetoanaerobium* sp. (up to 20%) fixing carbon oxide were found in the spring water. In the stream, mainly oxygenic cyanobacteria (up to 56%) developed at a temperature of 33 °C, in the presence of hydrogen sulfide and oxygen. In addition, sulfur bacteria of the genus *Thiothrix* (up to 48%) found in epibiotic communities of benthic animals of Lake Baikal were present here. *Thiothrix* sp. formed massive fouling in the zone of mixing lake and thermal waters with a significant contribution of hydrogen-oxidizing bacteria of the genus *Hydrogenophaga* (up to 22.5%). As well as chemolitho- and phototrophic bacteria, chemoorganotrophs (phyla *Firmicutes*, *Chloroflexi*, *Desulfobacterota*, *Nitrospirota*, *Fibrobacterota*, etc.) have been identified in all communities. The chemical parameters of water in spring and coastal zones indicate a significant change in the composition of thermal waters occurring with the participation of diverse microbial communities that contribute to the assimilation of inorganic components of mineral thermal waters.

**Keywords:** microbial communities; sulfur bacteria; hot spring; molecular genetic analysis; Baikal



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## 1. Introduction

Hot springs are widespread throughout the world, most commonly in the areas of high volcanic and geothermal activity. Their physical and chemical features depend on the particulars of the surrounding territory, geological composition and the hydrochemical processes both on surface and deeper in the crust. The study of hot springs became particularly active after the discovery of previously unknown thermophilic microorganisms inhabiting their waters [1]. A number of papers shows that diversity and composition of a microbial community depends on the spring type [2–5]; typically, chemolithoautotrophic metabolism is associated with extreme temperatures and pH, and the concentration of inorganic ions.

Yellowstone National Park hot springs (YNP, USA) are among the most well-studied. For example, in three hot alkaline springs of the Heart Lake Geyser Basin (southern YNP) the dominant bacterial community changed from moderately thermophilic and photosynthetic members (*Cyanobacteria* and *Chloroflexi*) at 44 °C to a mixed photosynthetic and thermophilic community (*Deinococcus-Thermus*) at 63 °C, and further to a non-photosynthetic thermophilic community at 75 °C. The archaeal community was predomi-

nantly a methanogenic community in the 44 and 63 °C springs, and a thermophilic community in the 75 °C spring [2]. Composition of microbial community in thermal springs with pH < 4 was significantly different from that in alkaline ones: in the former, cyanobacteria were not detected at all, while archaea and bacteria were dominated by those using H<sub>2</sub>, S<sup>0</sup> and Fe<sup>2+</sup> as electron donors [3,4].

In Russia, hot springs are present in volcanically active Kuril Islands and Kamchatka, as well as in areas of tectonic activity (such as Buryatia, Altai Krai, Khabarovsk krai and others) [5]. Most well-studied ones are located in the Uzon caldera, where a relatively small area hosts all major types of geothermal activity. The high temperature of these springs leads to the development of photoautotrophic microorganisms utilizing reduced sulfur compounds, hydrogen and other volcanic gases [6]. Archaeal nitrification, carboxydrotrophy and iron oxidation are predominant, but methanogenic, sulfur- and sulfate-reducing microorganisms were observed as well [7]. Most springs are dominated by chemoautotrophic phylum *Aquificae*, which form massive grey-white growths [8]. Sulfide hot spring Termofil'nyi (55–75 °C, pH 6.2) contains white growths of sulfur bacterium *Thermotrix thiopara* and deposits of native sulfur [9,10]. At temperatures below 60 °C, anaerobic phototrophs and cyanobacteria start appearing as well. With further decrease in temperature and sulfur concentration, accompanied by oxygen concentration increase, cyanobacteria become predominant and form massive algobacterial mats with various other bacteria [7].

Northern Caucasus (Russia) contains multiple thermal springs, most of them moderately warm. Some of these springs were shown to host sulfur-oxidizing and phototrophic bacteria [11]. A unique *Nitrospira* strain was isolated and shown to perform the complete nitrification process [12]. Despite their diversity and popularity, hydrothermal springs in this area remain poorly studied.

The Baikal rift zone (BRZ, Russia) also contains a number of thermal springs, typically alkaline with temperatures below 50 °C [13]. The vents of all hydrothermal waters with temperatures below 70 °C host massive microbial mats; these vary in their composition, but mostly consist of cyanobacteria [14–16]. Many thermal springs of BRZ also contain a significant number of microorganisms involved in the sulfur cycle. Usually, the areas of mixture between sulfur-rich thermal water and colder river or lake water are colonized at temperatures below 40 °C by massive growths of colorless sulfur bacteria from the genus *Thiothrix*. Where iron appears in significant amounts, these are joined by microaerophilic iron(II)-oxidizers with less abundant iron(III)-reducers, sulphate-reducers and phototrophic and nitrate-reducing iron(II)-oxidizers [17].

Several hot springs are close to the coastline of Lake Baikal (East Siberia, Russia) with a varying distance to the lake itself. The hot spring in Zmeinaya bay is just several meters away from the water line. Several vents are present, two of which are capped and used as hot baths. As thermal waters move to the lake along the outflow, they gradually cool down and form morphologically different microbial communities. Cyanobacterial mats form within the outflow, while white bacterial growths are more common on rocks and plants near the edge of water. Previously, only cyanobacteria, methanotrophs and methylotrophs have been studied in this location [18,19].

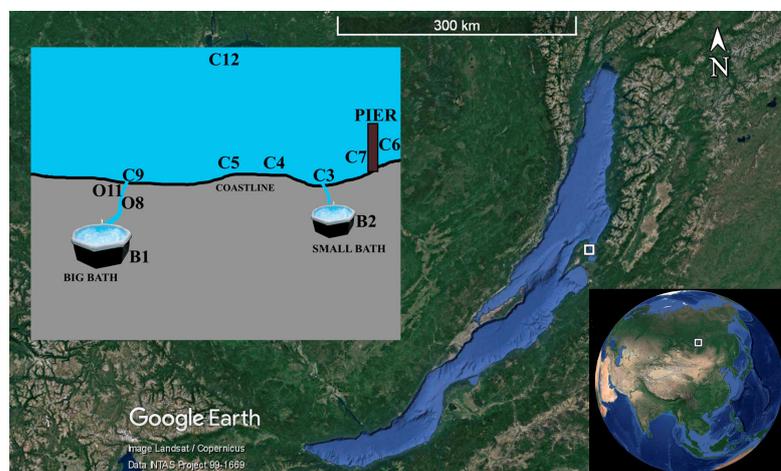
The goal of this work was to study the diversity and structure of bacterial communities within a hot spring and the littoral area of Zmeinaya bay under the gradient of oxygen and sulfide concentrations, temperature and other abiotic factors using NGS technologies, as well as to find out whether hot spring microorganisms are present in the littoral area of Lake Baikal.

## 2. Materials and Methods

### 2.1. Characteristics of the Study and Sampling Areas

Samples of water and bacterial mats were taken in July 2019 at various sites in Zmeinaya bay: in baths at the vents (B1, B2); along the outflow leading from vent to Lake Baikal (O8, O11); in littoral area 2–3 m away from water edge, where white growths were found on rocks (C3, C5, C6); and vascular plants (C4, C9); and over the black sed-

iment (C7) (Figure 1). Bacterial mats forming along the outflow (O11) were sampled in layer-wise manner, separately isolating white (O11/1), white-green (11/2), purple (O11/3), and blue-green (O11/4) layers. Sample C12 was taken from Lake Baikal 10 m away from the coast, where no bacterial growths were observed.



**Figure 1.** Schematic map of the study area and sampling sites in Zmeinaya bay (Lake Baikal, Russia). Maps were taken from the Google Earth 7.3.4.8248 Pro software <https://www.google.com/intl/ru/earth/versions/#earth-pro> (accessed on 12 October 2021).

## 2.2. Physical and Chemical Parameters of Sites

Chemical analysis was carried out as described previously [20]. Anion concentrations were measured by means of liquid chromatography on a Milichrom-2A chromatograph with a relative error of 5–10%. The samples for cation analysis were stored in polystyrene vials pre-acidified with 50  $\mu\text{L}$  of ultrapure concentrated  $\text{HNO}_3$  and stored at 4  $^\circ\text{C}$  prior to analysis. Cation ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) concentrations were determined by means of atomic absorption on an AAS-30 Carl Zeiss Jena spectrophotometer, as well as by flame emission methods with a relative error of 3–5% [21]. Concentrations of  $\text{O}_2$  were measured by Winkler method, hydrogen sulfide—by spectrophotometry [22]. Physicochemical characteristics (pH, Eh, T) were measured with a WTW pH 3310 device at all sampling sites. The main characteristics are presented in Supplementary Table S1.

## 2.3. DNA Extraction and Sequencing

Water samples from both baths (5 L) were filtered using nitrocellulose filters (25 mm diameter, 0.2  $\mu\text{m}$  pore size; “Millipore”, Darmstadt, Germany) using a diaphragm pump. Filters were placed in a TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA, pH 8.0) and frozen at  $-20\text{ }^\circ\text{C}$ . DNA from filters and bacterial fouling was extracted according to the modified method of phenol-chloroform extraction [23]. The extracted DNA was kept at  $-70\text{ }^\circ\text{C}$  for further use. For PCR amplification of the bacterial 16S rRNA gene fragments, including the V2–V3 variable region, B\_V2F (5′-AGTGGCGGACGGGTGAGTAA) and B\_V3R (5′-CCGCGGCTGCTGGCAC) primers were used [24]. Sequencing was performed at the Illumina MiSeq platform in (Collective Instrumental Centre of All-Russian Research Institute of Agricultural Microbiology, Saint-Petersburg). Raw sequences are available in the NCBI SRA archive under the BioProject number PRJNA801752.

## 2.4. Bioinformatics Analysis

Paired-end sequencing reads were trimmed and filtered in Trimmomatic version 0.39 [25] to remove positions in which more than 25% of the reads had bases with relative quality scores  $< 20$ . Then the R1 and R2 sequences were merged into contigs, filtered by size and average quality score ( $\geq 25$ ), aligned and checked for chimeras against SILVA 138\_1 databases (<http://www.arb-silva.de> (accessed on 8 October 2021)) using Mothur [26].

Thereafter, gaps were re-moved and sequences were tested for presence of respective forward/reverse primers, allowing two mismatches between forward and reverse primer and sequence. Further rRNA sequence processing was performed according to MiSeq SOP recommendations [27]. The filtered sequences were aligned, clustered into OTUs (operational taxonomic units) at genetic distances of 0.03 and identified taxonomically using the SILVA 138.1 databases. Rarefaction curves were calculated for each sample based on the identified OTUs using the package Mothur. All calculations were performed with HPC-cluster «Akademik V.M. Matrosov» (Irkutsk).

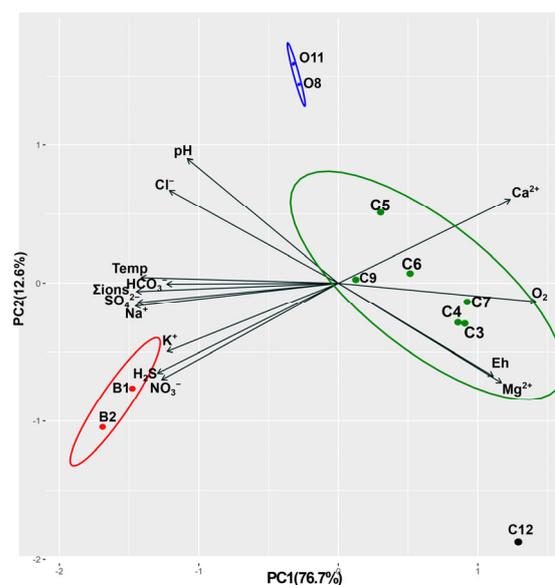
The OTUs were randomly resampled to normalize the reads for each sample, then the richness (ACE, Chao) and diversity estimators (Simpson's inverse index) were calculated with using web-based tool <https://www.microbiomeanalyst.ca> (accessed on 8 October 2021). The application <http://bioinformatics.psb.ugent.be/webtools/Venn/> (accessed on 23 September 2021) was used to search for overlapping OTUs. The metabolic functions were predicted using the phylogenetic investigation of communities' reconstruction of unobserved states (PICRUSt2) pipeline (<http://picrust.github.io/picrust/> (accessed on 8 October 2021)) using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The Mantel test was used to evaluate the correlation of environmental factors and microbial community structure and was calculated using R (9999 permutations).

Before statistical analysis, all data on the concentration of ions in samples, as well as OTU relative abundance, were normalized by unitization with a zero minimum [(x-min)/range]. Comparisons of environmental parameters, alpha and beta diversity of microbial communities using principal coordinate analysis (PCA), boxplots, heat maps and nonmetric multidimensional scaling (NMDS), as well as statistical tests, were performed in R with the *vegan*, *stats*, *cluster*, *pheatmap*, *ggplot2*, and *ggbiplot* packages [28].

### 3. Results

#### 3.1. Chemical Composition

Multidimensional scaling of chemical and physical environmental values using principal coordinate analysis (PCA) detected three major clusters, excluding sample C12 (Figure 2). This latter sample was taken from Lake Baikal 10 m away from the coast, and its chemical composition is similar to the pelagic waters of the lake [29].



**Figure 2.** Principal components analysis (PCA) plot describing the differences of water samples based on the concentration of sum of ions, nutrients, the oxygen concentration ( $O_2$ ), pH, Eh, T.

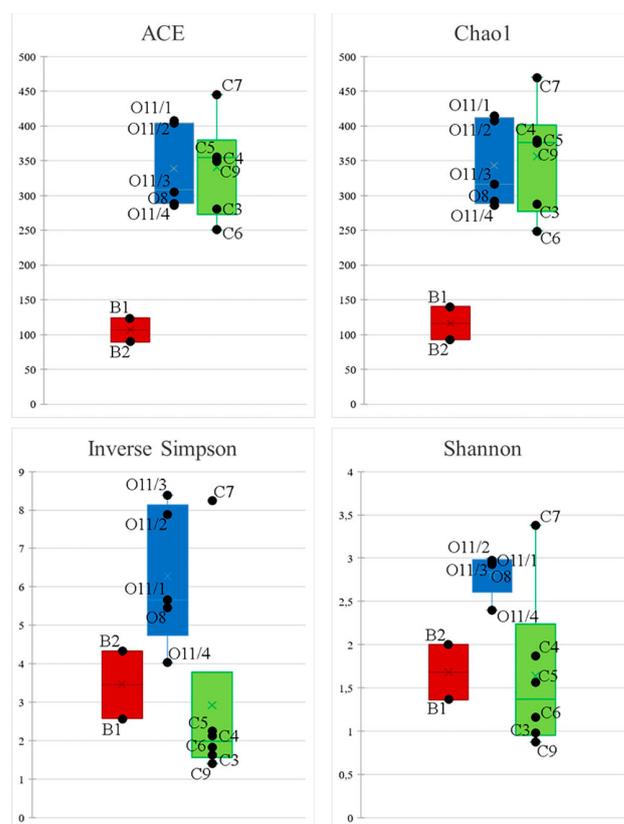
Cluster I included water samples from the baths (B1 and B2), which featured high temperatures (42.6° and 39 °C), absence of oxygen, high concentration of hydrogen sulfide (33 mg/L) and total ion concentration (up to 426 mg/L), pH 9.1–9.3, Eh = −422 mV.

Cluster II contained samples along the outflow (O8, O11) where green cyanobacterial mats with white and purple inclusions were observed. In this area, water temperature was lower, at 33 °C, hydrogen sulfide concentration was only 1.4 mg/L, and total mineralization was 242 mg/L. Water Eh (−426 mV) was close to bath samples and points to reducing conditions. The water pH was more alkaline than it was at the vent, reaching 9.6.

Finally, cluster III consisted of samples taken in coastal Lake Baikal where hydrothermal waters mix with those of the lake (C3–C9). Water temperature in this area varied between 18° and 24 °C, oxygen concentration increased to 9 mg/L, hydrogen sulfide concentration decreased to 0.7 mg/L and total mineralization to 74–220 mg/L. The water pH was still alkaline at 8.3–8.9, and Eh varied between −153 and −230 mV.

### 3.2. Alpha Diversity Differences among Sites

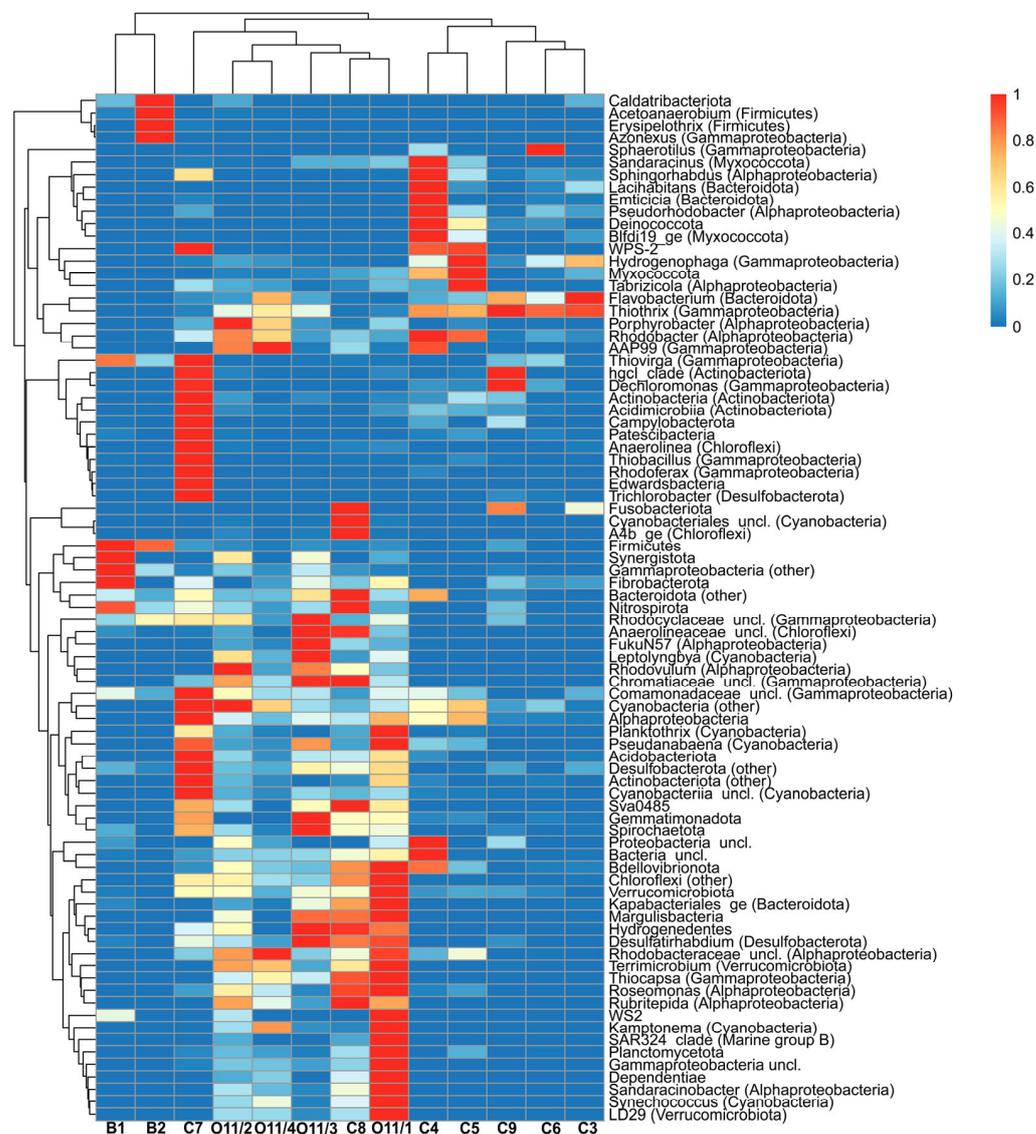
Sequencing produced a total of 255,271 reads, which were separated into 922 OTUs from 29 phyla. Rarefaction curves for all samples reached plateau (Figure S1). The total abundance of OTUs observed in 16S sequencing data is closest to real diversity in bath samples (ACE = 89 and sobs = 79 in B2). The number of OTUs observed here is lower than in other libraries, more so in the smaller bath (B2, Chao1 index 89) than in the larger one (B1, Chao index 123) (Figure 3). The distribution is most even in the second cluster and sample C7 (inverse Simpson index 8.2). The highest number of OTUs was observed in sample C7 (415 OTUs).



**Figure 3.** Box plots show bacterial alpha diversity (ACE and Chao1, Shannon Index and inverse Simpson Index); variation across samples based on non-rarefied data (for a cluster distance of 0.03).

### 3.3. Beta Diversity Differences between Sites

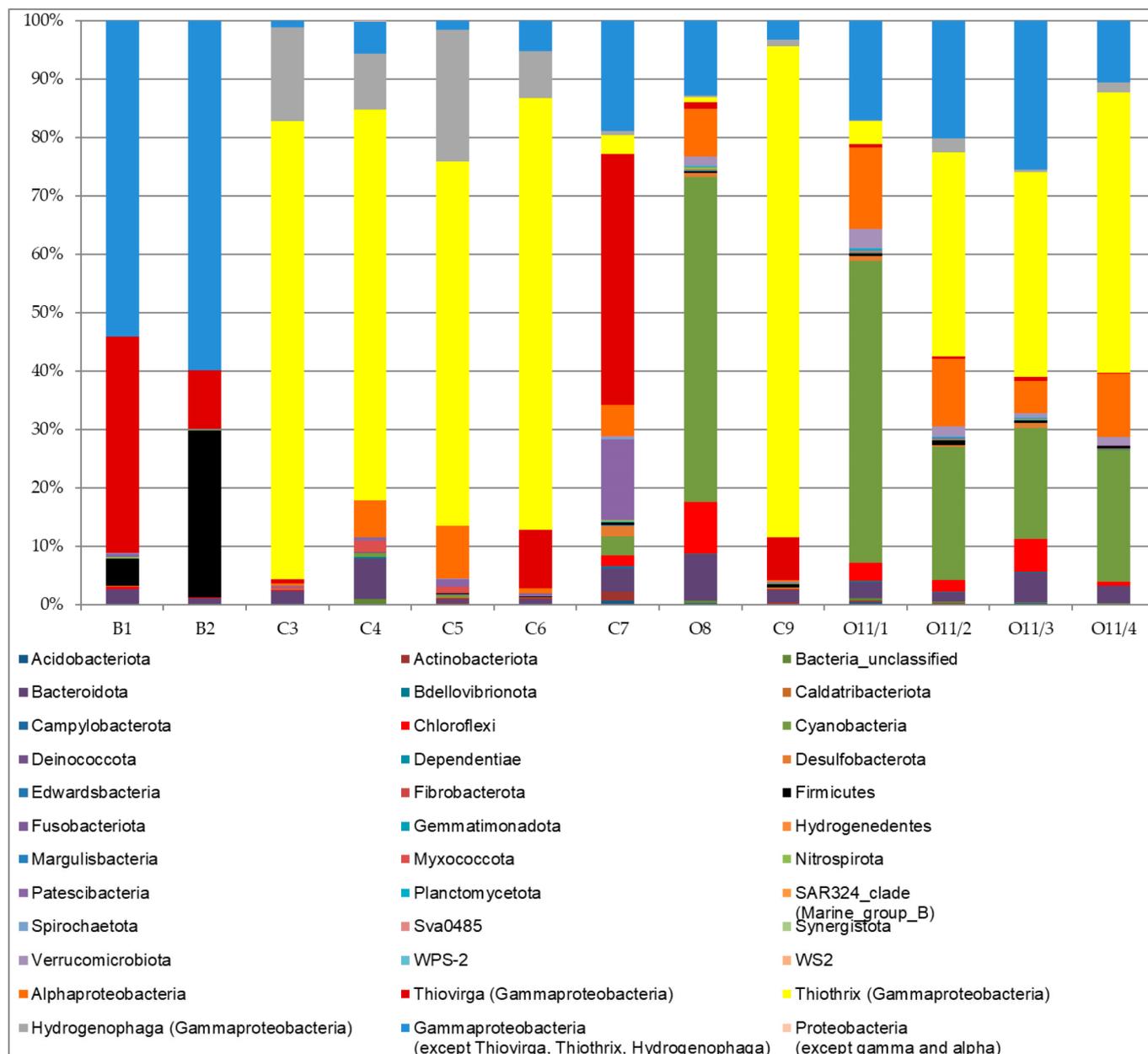
Clustering samples by their taxonomic composition at phylum or genus rank with un-weighted pair group method with arithmetic mean based on Bray-Curtis distance measures generated three clusters identical to those observed from abiotic data. However, sample C7, with its high diversity, is somewhat distant from the rest of cluster III (Figure 4).



**Figure 4.** Heat map demonstrating the distribution of bacterial phylum and dominant genus.

Microbial communities of cluster I (B1 and B2) were dominated by *Gammaproteobacteria* (91% and 70%, respectively, Figure 5). This class was predominantly represented by uncultured members of the family *Rhodocyclaceae* (51% and 14%, respectively). This family is metabolically diverse, including photoautotrophic, photoheterotrophic and photoorganotrophic bacteria. The large bath (B1) community was also dominated by sulfur oxidizer genus *Thiovirga*, family *Halothiobacillaceae* (37%), which only reached approx. 10% abundance in the small bath (B2). In contrast, the small bath community contained numerous nitrogen-fixing *Azonexus*, family *Rhodocyclaceae* (43%), as well as some *Firmicutes* (now *Bacillota* [30]) from genera *Thermobranchium* (3.3%) and *Acetoanaerobium* (20%). *Thermobranchium* are proteolytic, while *Acetoanaerobium* produce acetate from H<sub>2</sub> and CO<sub>2</sub>. Phylum *Bacteroidota* was the third most abundant in these two baths (2.7 and 11%), but it was mostly represented by unclassified and uncultured species. In minor abundances

(under 1%), cluster I samples also contained *Chloroflexi*, *Desulfobacterota*, *Patescibacteria*, *Nitrospira* and *Fibrobacteria*. Some sequences of *Verrucomicrobia*, *Synergistota*, *Spirochaetota* and *Caldatribacteriota* were observed as well.



**Figure 5.** Taxonomic composition of bacterial communities in different sites of Zmeinaya bay.

Cluster II communities, which develop under lower temperature and hydrogen sulfide concentration, were different: autotrophic cyanobacteria became the dominant phylum (8–56% and 11/1–52%). Out of cyanobacteria, genus *Synechococcus* was most abundant, reaching 39% in O11/1, and genus *Cyanobium* was most abundant (present in all samples with abundances up to 0.3%). These communities also included the representatives of genera *Leptolyngbya* (16% in O11/3), *Kamptonema* (3.6% in O11/1), *Pseudanabaena* (up to 1%), *Limnothrix* and others.

Class *Gammaproteobacteria* also showed a significant abundance in 16S rRNA libraries for this cluster (between 15% and 62%). Besides colorless sulfur bacteria (0.9–48%), purple sulfur bacteria *Allochromatium* and *Thiocapsa* (both up to 5.1%), as well as other minor genera, were observed. Pigmented *Alphaproteobacteria* that actively participate in sulfur and

nitrogen cycles also play a significant role in these communities. In particular, red-colored *Rubritepida* (up to 1.1%) can oxidize reduced sulfur compounds to sulphate. Furthermore, we detected a remarkable abundance of unclassified purple non-sulfur bacteria (up to 7.8%) and members of genus *Rhodovulum* (up to 0.6%). Finally, phyla SAR324 (Marine\_group\_B) and *Margulisbacteria* were observed in small amounts. The latter are genetically close to cyanobacteria and have not been previously observed in Lake Baikal. They are thought to be involved in the formation of aerobic lifestyle [31].

The final cluster contained samples taken from the area where lake and hot spring water mix, creating an environment rich in both oxygen and hydrogen sulfide. These communities were heavily dominated by phylum *Proteobacteria* (96–98%), with genus *Thiothrix* comprising from 62% to 85% of all reads. Another abundant genus was hydrogen-oxidizing *Hydrogenophaga* (22.5% in C5). This cluster was the only one to feature methylotrophic *Methylophilus* and *Methylotenera* (also involved in nitrate reduction), although at abundances below 0.3%. In addition, we observed numerous *Alphaproteobacteria*, including aerobic anoxygenic phototrophs *Tabrizicola* (up to 2.4%), phototrophic *Rhodobacter* (up to 1.1%) and *Cereibacter* (under 0.1%), and chemoorganotrophic *Roseomonas* (under 0.1%).

Communities from all three clusters also contained members of phyla *Bacteroidota* (0.7–6.9%), *Nitrospirota* (up to 0.4%), and *Desulfobacterota* (under 1.8%). Clusters II and III also contained members of *Fusobacteriota* and *Gemmatimonadota* (both around 0.1%).

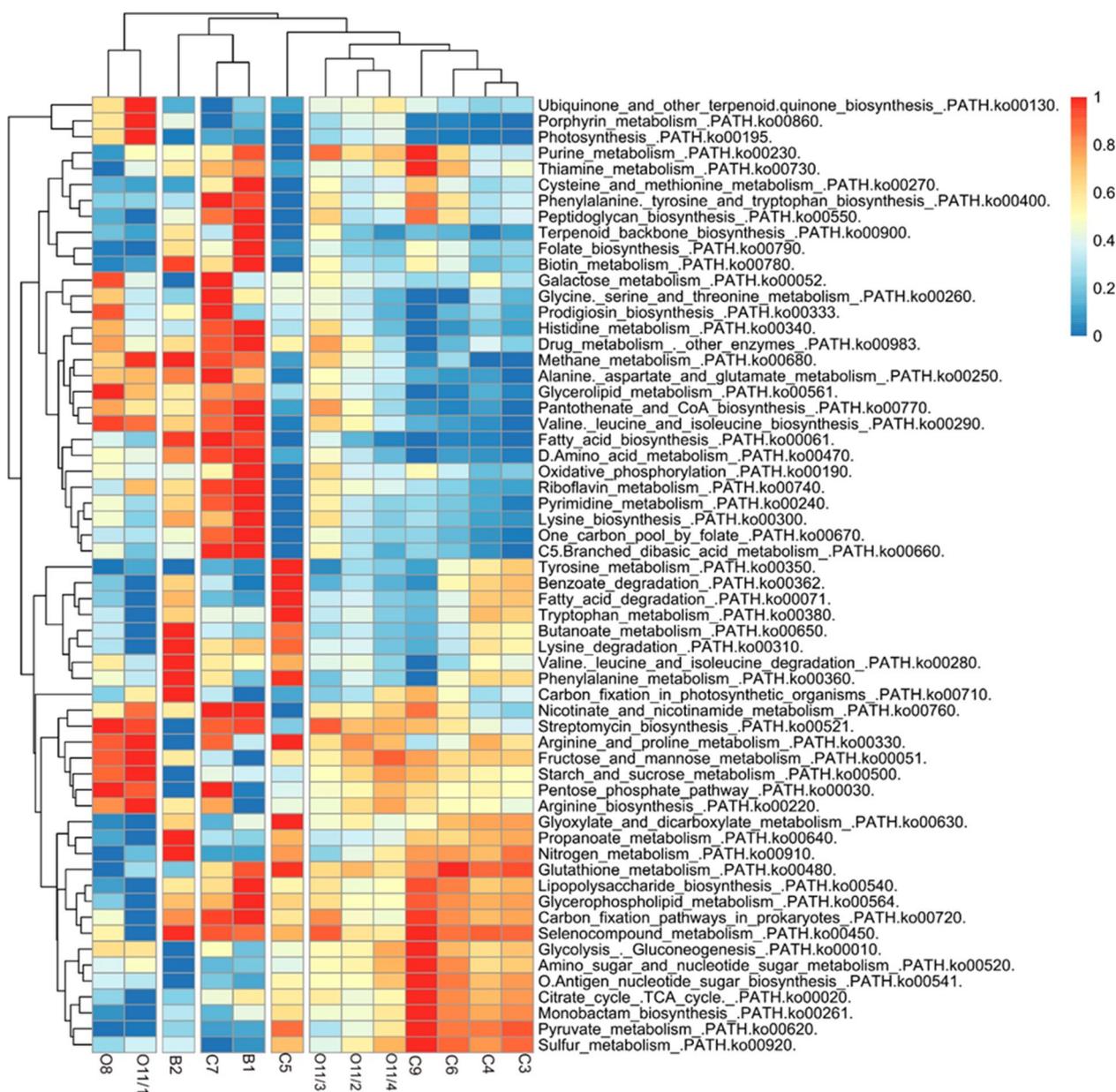
### 3.4. Comparative Community Analysis

Using the Venn diagrams application, we compared bacterial communities from each sample, taking into account OTUs. According to this tool, 49 OTUs were unique for bath samples, and 19 of those were shared between B1 and B2 (Supplementary Table S2). Most of these belonged to genus *Thiovirga* and one is from genus *Desulfomicrobium* (*Desulfobacteriota*). B1 had six unique OTUs, two of which belonged to *Brevinema* (*Spirochaeta*) and *Tepidomonas* (*Gammaproteobacteria*). B2 had 29 unique OTUs, nine of which belonged to genus *Azonexus* and five to *Acetonanaerobium*. OTUs shared between the cluster II communities belonged to the phylum *Dependentiae* (formerly known as TM6). According to metagenomic data, parasitism is widespread in this lineage [32] and its presence is significantly higher in mineral nitrogen-treated plots [33]. Samples O11/1-O11/4 had 32 unique OTUs, three of which belong to genera *Thermoflexibacter* (*Bacteroidota*), *Ochrobactrum*, and *Elioraea* (*Alphaproteobacteria*). Members of phylum *Fibrobacterota*, capable of degrading plant-based cellulose, were shared between large bath and cluster III. Finally, a total of 13 unique OTUs were detected in cluster III. The highest number of unique OTUs, 78, was observed in community C7. Only one OTU, chemolithoautotrophic sulfur-oxidizing *Thiovirga* sp., was shared between this sample and all others.

### 3.5. Predicted Metabolic Functions

The putative metabolic functions of the microbial communities developing in Zmeinaya bay were predicted from 16S rDNA amplicon sequence data using the PICRUSt2 tool (Figure 6). The estimated KEGG orthologues were found to be involved in the main pathways of metabolism. Most of the 60 pathways were associated with the metabolism of energy, carbohydrates, amino acids, lipids and glycan biosynthesis. Xenobiotic biodegradation was found in a small number of studied samples and was linked to the degradation of benzoate. The predicted energy metabolic pathways in the bacterial communities from hot spring in Zmeinaya bay involved oxidative phosphorylation and the metabolism of sulfur, methane and nitrogen. Methane metabolism was found in both baths and in the outflow, and metabolism of sulphur and nitrogen were found in microbial communities of the coastal area. However, in the small bath (B2) community, the nitrogen metabolism pathway was predicted. Carbon fixation in prokaryotes was found in almost every sample, but the carbon fixation pathway in photosynthetic organisms was found only in microbial communities of the small bath (B2). Secondary metabolite pathways for streptomycin, monobactam and prodigiosin were predicted for microbial communities from clusters

II and III. Terpenoid backbone biosynthesis was predicted only for both baths (B1, B2). Metabolism of cofactors and vitamins also was found in microbial communities of baths and C7.



**Figure 6.** Heat map of the top 60 abundant putative pathways of microbial communities from the hot spring area in Zmeinaya bay. The pathways are presented according to KEGG.

### 3.6. Interaction between Microbial Diversity and Abiotic Factors

Analysis of Spearman correlations between relative abundances of major taxa (phyla, families and genera) and various abiotic environmental factors has detected multiple significant correlations, both positive and negative. The negative effect on some taxa was caused by temperature, pH, and concentrations of  $K^+$  and hydrogen sulfide (Figure S2). Significant positive correlations were observed between: genera *Tabrizicola*, *Rhodobacter*, *Sphingorhabdus* and nitrate availability; genera *Thiocapsa*, *Desulfatirhabdium* and  $Ca^{2+}$ ; and genera *Thiocapsa*, *Trichlorobacter* and Eh. Phylum *Deinococcota*, genera *Sphaerotilus* (*Gammaproteobacteria*) and *Lacihabitans* (*Bacteroidota*) correlated negatively with  $Ca^{2+}$  concentration. The latter genus

also shows negative correlation with Eh and positive correlation with temperature and hydrogen sulfide concentration.

The Mantel test based on the Bray–Curtis method was used to evaluate the effect of environmental factors on the microbial community structure near the hot spring in Zmeinaya bay. All factors positively correlated with the bacterial community in studied area, among which the correlations of sodium and sulfate-ions were the most significant (Table 1,  $R = 0.814$  and  $R = 0.786$ ,  $p < 0.05$ ). The effect of environmental factors on the microbial community structure also is shown with using NMDS (Figure S3).

**Table 1.** Mantel analysis of the relationship between environmental factors and microbial community from the area of hot spring in Zmeinaya bay.

Factor	R Value	p Value
T°	0.741	$1 \times 10^{-4}$
pH	0.398	$6.7 \times 10^{-3}$
Eh	0.350	$1.4 \times 10^{-2}$
HCO <sub>3</sub> <sup>−</sup>	0.457	$8.5 \times 10^{-3}$
Cl <sup>−</sup>	0.432	$2.6 \times 10^{-3}$
NO <sub>3</sub> <sup>−</sup>	0.604	$8 \times 10^{-4}$
SO <sub>4</sub> <sup>2−</sup>	0.786	$1 \times 10^{-4}$
Na <sup>+</sup>	0.814	$1 \times 10^{-4}$
K <sup>+</sup>	0.515	$1 \times 10^{-3}$
Ca <sup>2+</sup>	0.711	$1 \times 10^{-4}$
Mg <sup>2+</sup>	0.509	$8 \times 10^{-4}$
H <sub>2</sub> S	0.664	$1 \times 10^{-4}$
O <sub>2</sub>	0.790	$1 \times 10^{-4}$
Σions	0.799	$1 \times 10^{-4}$
total	0.867	$1 \times 10^{-4}$

#### 4. Discussion

As our study shows, the water of hot spring Zmeinaya bay is the closest in temperature to other thermal springs of the BRZ (for example, Davsha) and Caucasus region [11], but has higher concentration of hydrogen sulfide and more alkaline pH. It is also obvious that neither temperature nor hydrogen sulfide concentration are constant [13,34,35], likely due to changing trajectory and speed of fluid flows. Previously, the effect of fluid flows on the microbial communities was shown in a study of sediment microbes in Posolsk Bank [36]. It is possible that thermal waters of the two Zmeinay vents pass through different subsurface materials, taking with them whatever gases and autochthonous microorganisms are present in these subterranean habitats. This could explain the different composition of dominant taxa in two baths: one (B1) was dominated by sulfur-oxidizing *Thiovirga* and the other (B2) by nitrogen-fixing *Azonexus*, although the vents are close to each other and contain chemically similar water. We should also note that all samples contained OTUs of *Thiovirga*, also observed in a sublacustrine thermal spring of Yellowstone Lake with similar temperature and hydrochemistry [37].

Both the abiotic factors and microbial communities have changed along the way to the lake littoral; the latter varied both in composition and predicted metabolic capacity. As our data show, the community composition is most affected by changing temperature and concentrations of hydrogen sulfide and oxygen. This conclusion is supported both by statistically significant correlation value (Figure S2) and by similar observations in other hot springs of the world [2,6,7,37,38]. Communities in hot and highly mineralized waters

(outflow) were dominated by nitrogen fixers, acetogenic bacteria and aerobic sulfur oxidizers perhaps associated with the autotrophic denitrification [39]. These were predominantly mesophilic anaerobic or microaerophilic chemo- or organotrophic bacteria, most of whom utilize sulfur compounds in their metabolism. As the water moves towards the lake, it cools down and somewhat decreases in mineralization, which leads to increasing diversity and changing dominant taxa. Mat-forming cyanobacteria become major players, and a variety of bacteria involved in mat formation may take part in increasing water alkalinity. Major metabolic processes in this area are photosynthesis, nitrogen fixation and sulfide oxidation. Finally, the water and the community it hosts change once again after mixing with cold, oxygen-rich and poorly mineralized waters of Lake Baikal. Now, the communities are dominated by hydrogen-reducing bacteria and consumers of organic matter. In similar ecotopes, the oxidation of sulfur compounds is the main oxygen sink, which is compatible with our data and potentially explains the low abundance of sulfate-reducing and methanotrophic bacteria within the spring.

Microbial communities inhabiting the hot spring in Zmeinaya bay from vent to water edge are similar in their diversity and structure to microbiomes that form in other sulfide springs under temperature gradient. Typically, sulfide-rich waters below 50° are dominated by cyanobacteria and sulfur bacteria. 16S rRNA libraries of the spring we studied contained various cyanobacterial taxa. In particular, we have observed members of the family *Leptolyngbyaceae* at 44 °C and 33 mg/L sulfide concentration; thus, this family may have wider tolerance than previously thought. At lower temperature and sulfide concentrations (33 °C and 3 mg/L, respectively), genera *Synechococcus* and *Leptolyngbya* thrive and form layered deposits, including colorless sulfur bacteria. It is possible that the latter use cyanobacteria-produced oxygen rather than rely on atmospheric supply. The littoral area near the spring, where geothermal and lacustrine waters mix and cool to 19 °C, is dominated by colorless sulfur bacteria *Thiothrix* and trichome-forming cyanobacterial genera *Calothrix*, *Ancylothrix* and *Limnothrix*. White thread-like growths were previously observed near multiple BRZ hot spring at water temperature below 38 °C: Bauntovski, Kotelnikovskiy, Bolsherechenskiy and others [14,40,41] and sulfide concentration below 41.5 mg/L [42]. It is also important to note that *Thiothrix* was also detected in epibiotic consortia on oligochaetes from Gorevoi Utes oil seep and ostracodes from Goloustnoe methane seep in Lake Baikal [43,44], where sulfide concentration was below the detection threshold using the same methods as here. These data show that genus *Thiothrix* has a wide range of acceptable temperatures, oxygen and hydrogen sulfide concentrations; they also do not depend on the light.

All clusters and thus all areas contained bacteria that are capable of disproportionating thiosulfate and elemental sulfur (phylum *Nitrospirota*), as well as those that reduce sulfates to sulfides and degrade organics to acetate and CO<sub>2</sub> (phylum *Desulfobacterota*). Members of phylum SAR324 found in cluster II may play a role in sulfur cycle, as well. According to metapangenomic data, these bacteria exhibit high metabolic plasticity: they use sulfur-based chemolithoautotrophy in the absence of light and are photoheterotrophic when light is available [45]. Phylum *Margulisbacteria* has also been detected in cluster II. This phylum is known to be involved in sulfur oxidation and hydrogen cycle; it is also closely related to *Cyanobacteria* [46].

Since the sulfur cycle is linked to the iron cycle, littoral microbial communities contain a remarkable abundance of heterotrophic iron bacteria *Sphaerotilus* sp. (*Gammaproteobacteria*) that accumulate sulfur granules in their cells [47]. Microbial mats formed by simultaneously *Thiothrix* and *Sphaerotilus* are usually observed in the activated sludge of treatment facilities and in the surface layers of fresh water heavily contaminated with agricultural waste. Growths of *Sphaerotilus natans* and *Thiothrix* sp. were originally discovered in sulfur springs of Northern Caucasus, with the former dominating under low concentration of hydrogen sulfide and disappearing under higher ones [48]. Another sulfur-oxidizing bacterium in our dataset is *Sideroxydans* sp., which is detected in sample C7.

Besides primary producers, the microbial communities of Zmeinaya bay contain bacteria involved in various destruction processes. The littoral area hosts the members of family

*Saprospiraceae* (phylum *Bacteroidota*) capable of algicidal activity against cyanobacteria, lytic destruction of some bacterial cells, and degradation of complex macromolecules. Clusters II and III contain predatory bacteria *Bdellovibrio* (*Bdellovibrionota*) that consume external biopolymers such as proteins and nucleic acids. Members of phylum *Patescibacteria* are present as well, and the genomes of this group suggest the capacity for degrading organic matter [49]. Previously, *Patescibacteria* (CPR) were only present significantly in the deep aphotic layer of Lake Baikal [50]. Almost all samples also contain a small abundance (up to 0.04%) of cellulose destructor phylum *Fibrobacterota*, which was never before documented in the lake itself, but present in the microbial community of BRZ hot spring Kuchiger [51].

The baths in Zmeinaya bay are a popular tourist destination, particularly during the summer. Thus, it is not surprising that some opportunistic pathogens are present in studied microbial communities. In particular, the smaller bath contains genus *Erysipelothrix* (5.5%, phylum *Firmicutes*), which includes skin pathogens. This genus is also present near the larger bath (0.01%) and along the coastline (up to 0.14%). It is interesting that the majority of potential pathogens have been detected in cluster III, presumably due to the more tolerable temperature. Microbial communities of this cluster contain predatory phylum *Myxococcota* at 0.1–19% abundance. Members of this phylum take part in sulphate and nitrite reduction, and some of them are pathogenic for humans. The same communities also include *Roseomonas* sp. (*Alphaproteobacteria*), some strains of which are opportunistic pathogens. Other members of this genus have also been found in Baikal sediment cores [52] and epilithic biofilms on quartz [53].

Thus, the waters of hot spring in Zmeinaya bay contain a peculiar microbial community whose metabolic potential is based on chemolithoautotrophs utilizing sulfur and nitrogen compounds as an energy source. On the way from spring issue to Lake Baikal, the community composition repeatedly shifts, from chemoautotroph-dominated baths to photoautotrophs in the outflow and chemoheterotrophs near outflow mouth. In turn, the bacterial community affects the chemical composition of spring water. This effect of the microbiome on water chemistry has also been described in the lake itself, although it has a completely different microbial community with different metabolic capacities. It is obvious that some bacteria are moved from the spring to the lake, but their habitat is localized and does not extend far beyond outflow mouth, presumably due to lower temperature and oligotrophic conditions.

## 5. Conclusions

It is obvious that the mineralized water of the geothermal spring in Zmeinaya bay has a local effect on the ecosystem of Lake Baikal, which is quickly neutralized by the dissolution of geothermal water in the lake and consumption of  $N_2$ ,  $CO_2$ ,  $H_2$ ,  $H_2S$  and other reduced sulfur compounds by microbial communities. These bacteria serve as a sort of filter, preventing toxic compounds from reaching the wider Baikal ecosystem. Gradients of temperature, sulfide concentration and oxygen concentration lead to the formation of multiple distinct bacterial communities in a small patch of lake coast. We should also note the high number of sequences with low homology to studied species, suggesting high potential for the discovery of novel bacteria.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15020298/s1>, Figure S1: Rarefaction curves plotting the number observed OTUs as a function of the number of sequences defined at genetic distance level of 0.03; Figure S2: A heat map (with Spearman's rank correlation coefficient) showing correlation between dominant OTUs (97% identity) with abiotic factors. Colors indicate the  $r$  values of Spearman's rank correlation coefficient. The influence of a factor was assumed to be reliable if the  $p$ -value of the Spearman statistics was  $<0.05$ ; Figure S3: Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities shows relationships between the relative bacterial abundance of each sample and environmental parameters; Table S1: Physical and chemical parameters of water in Zmeinaya bay (concentration of ion-mg/L). Table S2: Total and unique OTUs in samples.

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