Adaptive strategies of the microbial community in meromictic soda Lake Doroninskoye (Transbaikalia, Russia)

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Abstract. The adaptive strategies of biogeochemical systems are considered as a criterion for natural fluctuations of the territory using the example of the microbial community in meromictic soda Lake Doroninskoye (Transbaikalia, Russia). The features of the phylogenetic and functional diversity of the microbial community of the lake have been investigated. In the off-season, a change in the dominant component of the community in the chemocline of the lake was established. During the ice period, the lake is dominated by metabolically flexible, anoxygenic, photoheterotrophic non-sulfuric purple bacteria Rhodospirillaceae and Rhodobacteraceae (class Alphaproteobacteria), which can switch from anoxic photosynthesis to aerobic chemotrophic metabolism. During the open water period, facultative aerobic bacteria of the families Enterobacteriaceae (class Gammaproteobacteria) and Alcaligenaceae (class Betaproteobacteria) predominate, which switch to denitrification in the absence of oxygen.

1. Introduction
Adaptive strategies of living organisms are an important part of the evolution of biogeochemical systems and can be an important criterion for short- and long-term natural fluctuations. Modern climate changes require the development of effective indicators for predicting these fluctuations. Landscape indication methods using biological systems have been successfully used to detail the hydrological, hydro-permafrost, and hydrogeological conditions in some regions of Transbaikalia [1].

The work investigates the inter-seasonal dynamics of the phylogenetic and functional diversity of the microbial community of meromictic soda Lake Doroninskoye (Transbaikalia) and shows the possibility of using these changes as natural model systems to create effective landscape indicators.

Meromictic soda Lake Doroninskoye is one of the three well-known meromictic reservoirs of Siberia. Microorganisms in the lake are active under special environmental conditions [2]. The lake is located in an area with a sharply continental climate, in a permafrost zone, and has specific physical and chemical conditions such as alkaline pH, relatively high salinity of water, and bottom sediments up to 35.0 g/L [3]. The lake differs from other meromictic water bodies of the world in certain parameters, including a low level of illumination of the chemocline (0.001%) [2] and a rare type of alkaline water formed under conditions of evaporative concentration of sedimentary rocks [3].

Stable in space and time meromictic conditions in the lake are characterized by high microbial diversity with a maximum in the chemocline zone. The Shannon index is higher here than in the mixo- and monimolimnion [2, 4].
The chemocline of meromictic lakes is a special zone where key biogeochemical transformations are carried out by the dominant bacteria of the sulphur cycle. The predominance of bacteria with certain metabolic strategies in the chemocline zone can be associated with both a combination of functional characteristics and physiology of these organisms and favourable environmental conditions for the implementation of their main ecological role.

To understand these important positions, we conducted a study of the dominant composition of microorganisms of the chemocline in meromictic soda Lake Doroninskoye in the winter under-ice (March) and autumn open (September) periods using high-throughput sequencing.

2. Materials and methods
Lake Doroninskoye is a meromictic soda lake. Its natural water column is usually divided into three zones: the upper oxygen mixolimnion (from the surface to 3.15 m in March and up to 4.0 m in September), a sharp chemocline (thickness 0.15–0.20 m, depth depending on the season from 3.15 m in March and 4.0 m in September), and the lower oxygen-free monimolimnion (from 3.2 to 6.2 m in March and from 4.1 to 6.2 m in September). During the study periods, the stable chemocline was located at a depth of 3.15 m in March and 4.0 m in September and was characterized by a high content of sulphates and a sharp gradient of oxygen and sulphides [2, 4].

Samples were taken at the central station of the lake (51°25’ N; 112°28’ E, depth 6.2 m) according to standard hydrobiological methods (March, September 2013). Sampling was carried out using an underwater submersible sampler probe specially made for thin layer sampling [5]. Water temperature, conductivity, oxygen, pH, and salinity were measured using a Miltu-340 field station (Germany). For metagenomic analysis, 200 mL of water samples were filtered sequentially through 0.65 and 0.22 μm pore size polycarbonate filters (Millipore) and stored at −20°C. Genomic DNA was extracted from both filters in two replicates and pooled to construct a single library from each water sample using commercial kits according to the manufacturer’s protocols: Bacterial Genomic DNA kit (Axygen, USA) and DNA-sorb B (AmpliSens, Moscow). The V3 and V4 hypervariable regions of the 16S rRNA gene were sequenced (2 × 300 bp) using the MiSeq Reagent Kit v3 (Illumina) at the SB RAS’ Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia). Data obtained in this study have been deposited in the NCBI database using accession number PRJNA420191. Bioinformatics and statistical analysis included the online services Mothur 1.31.2, RDP, QIIME, and others.

3. Results and Discussion
The 16S rRNA gene amplicons metasequencing revealed a high phylogenetic diversity of the studied zone in the interseasonal aspect. We found that the microbial community of the chemocline (water layer at a depth of 3.15 m in March and water layer at a depth of 4.0 m in September) of the lake during the study period was characterized by high taxonomic diversity.

Representatives of 10 phyla of eubacteria have been identified in March: Actinobacteria, Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, Verrucomicrobia, along with two candidate groups, Candidatus Saccharibacteria (TM7), and Candidatus Absconditabacteria (SR1). Eight phyla of eubacteria were identified in September: Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria, Spirochaetes, Tenericutes, Verrucomicrobia, two candidate groups of eubacteria, Candidatus Absconditabacteria (SR1), Candidatus Cloacimonetes (WWE1), one archaeal phylum, Euryarchaeota, and two candidate groups of archaea, Candidatus Pacearchaeota and Candidatus Woesearchaeota.

The share of detected phyla in the microbial community of the lake chemocline in March and September is presented in table 1. In all periods of research, eubacteria phyla remained dominant: Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria [2, 4].
| Phylum                          | Chemocline March Fraction in the community, % | Chemocline September Fraction in the community, % |
|------|------------------------------------------|-----------------------------------------------|
| **Eubacteria**                  |                                          |                                               |
| Proteobacteria                  | 27.5                                     | 60.4                                          |
| Actinobacteria                  | 9.1                                      | 3.1                                           |
| Cyanobacteria                   | 12.1                                     | 3.9                                           |
| Firmicutes                      | 13.4                                     | 2.5                                           |
| Bacteroidetes                   | 29.9                                     | 1.3                                           |
| Spirochaetes                    | 1.6                                      | 0.3                                           |
| Tenericutes                     | 0.2                                      | 2.2                                           |
| Verrucomicrobia                 | 0.6                                      | 0.1                                           |
| Deinococcus-Thermus             | <0.1                                     | ND*                                           |
| Planctomycetes                  | <0.1                                     | ND*                                           |
| **Candidate eubacteria groups** |                                          |                                               |
| Candidatus Saccharibacteria (TM7)| <0.1                                     | ND*                                           |
| Candidatus Absconditabacteria (SR1)| <0.1                                   | <0.1                                          |
| Candidatus Cloacimonetes (WWE1)  | ND                                      | <0.1                                          |
| Parcubacteria group (OD1)       | <0.1                                     | <0.1                                          |
| Unclassified Bacteria           | 5.4                                      | 25.1                                          |
| **Archaea**                     |                                          |                                               |
| Euryarchaeota                   | <0.1                                     | 0.2                                           |
| **Candidate archaean groups**   |                                          |                                               |
| Candidatus Pacearchaeota        | ND                                      | 0.4                                           |
| Candidatus Woesearchaeota       | ND                                      | 0.4                                           |
| unclassified Euryarchaeota      | <0.1                                     | ND*                                           |

* ND – microorganisms were not detected

Microorganisms of the phyla *Deinococcus-Thermus* and *Planctomycetes* and one candidate group of eubacteria *Candidatus Saccharibacteria* (TM7) were detected only in March in the chemocline of the lake. Microorganisms of the candidate group of eubacteria, *Candidatus Cloacimonetes* (WWE1), and two candidate groups of archaean, *Candidatus Pacearchaeota* and *Candidatus Woesearchaeota*, were detected only in September. It is interesting to note that the share of unclassified bacteria in September increased almost five times as compared to their share in the microbial community in March.

A meta-analysis of the 16S rRNA gene amplicons has established that metabolically flexible, anoxygenic, photoheterotrophic purple non-sulfur bacteria *Rhodospirillaceae* and *Rhodobacteraceae* (class *Alphaproteobacteria*), which can switch from anoxygenic to aerobic metabolism, prevailed in chemocline during the ice period.

In the autumn period, a change in the dominant bacteria of this water layer was revealed. Bacteria of the families *Enterobacteriaceae* (class *Gammaproteobacteria*) and *Alcaligenaceae* (class *Betaproteobacteria*) dominated the chemocline of Lake Doroninskoye. These bacteria are known as facultative aerobic bacteria which switch to denitrification in the absence of oxygen.

It should be noted that the analysis of the dominant composition of microorganisms in meromictic lakes in the world [2], as a rule, shows the temporal stability of this parameter. The change in the dominant microorganisms for the meromictic lakes of the world is known only for Lake Kadagno, during the period when the limnic status of the lake changed to holomictic [6].
Inter-seasonal studies show that a change in the ecological conditions of Lake Doroninskoye leads not only to a change in the dominant chemocline bacteria of the lake but also to an increase in the proportion of chemoorganotrophic bacteria. Based on a comprehensive understanding of the functioning of the microbial community in the lake ecosystem and molecular genetic studies, the main groups of microorganisms associated with key metabolic processes in the chemocline of the meromictic soda Lake Doroninskoye (Transbaikalia) were identified and their share in the community in March and September was determined (Appendix, figure A1).

According to the data (figure 1), Bacteroidetes, Gammaproteobacteria, Firmicutes accounted for a significant proportion among the main functional groups of microorganisms in the chemocline of the lake in March – 29.9, 13.6, and 13.4% respectively.

During this period, the main key processes in the microbial community of the lake were associated with heterotrophy, anoxygenic phototrophy, nitrification, fermentation, and sulphate/nitrate/metal reduction. The share of Cyanobacteria, which is associated with the key processes – oxygenic photosynthesis and N₂ fixation – was also high and amounted to 11.1%. As shown earlier [2], March is the period of early under-ice vegetation of photosynthetic organisms, which is associated with the zonal features of the lake. The share of Actinobacteria, another dominant main functional group of microorganisms, reached 9.1% in the community; Actinobacteria are associated with key metabolic processes and heterotrophy.

In September, changes occur in the composition of the main functional groups of microorganisms and the key metabolic processes associated with them. During this period, the share of Gammaproteobacteria increases 2.6 times and represents 36.5% of the total diversity. The share of another one of the main functional groups, Betaproteobacteria, also grows from 1.3 to 19.9% (15.3 times) (Appendix, figure A1). The main key processes in the lake are processes associated with heterotrophy, anoxygenic phototrophy, nitrification, nitrate/DMSO/metal reduction, and anoxygenic phototrophy.

4. Conclusions
The biodiversity of meromictic soda lakes, limited by extreme conditions, makes them ideal model systems for studying the influence of microbes on the geochemistry of the reservoir since it is possible to cover most of the existing diversity and associate taxa with specific processes and functions [7].

By understanding the functional relationships between ecosystem components in these lakes, it is possible to track the state of the ecosystem and use the data to create effective landscape indications. As they are particularly sensitive to climatic influences, these lakes function as sentinels for observing climate change.

The adaptive strategy of using living systems of the lake, specifically the change of the dominant in the microbial community in the chemocline of the lake, requires further detailed study. The study of the relationship between environmental parameters and microbial diversity in the chemocline zone of lakes can become promising for creating predictive models of changes in the ecological conditions of natural ecosystems.

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| Fraction in the community in March, % | Key metabolic processes (Taxonomy and functional characteristic of main groups of microorganisms) | Fraction in the community in September, % |
|-------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------|
| 11.1                               | Oxygenic photosynthesis and N₂ fixation *(Cyanobacteria)*                      | 3.9                                         |
| 4.0                                | Heterotrophy and aerobic anoxygenic phototrophy *(Alphaproteobacteria* belonged to the class of purple non-sulfur bacteria, PNSB; aerobic BChl-containing bacteria, ABC) | 0.4                                         |
| 1.3                                | Heterotrophy, nitrification, and anoxygenic phototrophy *(Betaproteobacteria* belonged to the class of purple non-sulfur bacteria, PNSB; aerobic BChl-containing bacteria, ABC) | 19.9                                        |
| 13.6                               | Heterotrophy, anoxygenic phototrophy, nitrification, and nitrate/DMSO/metal reduction *(Gammaproteobacteria* belonged to the class of purple sulfur bacteria, PSB; aerobic BChl-containing bacteria, ABC) | 36.5                                        |
| 6.7                                | Dissimilatory sulfur/sulphate reduction *(Deltaproteobacteria)*                 | 1.6                                         |
| 29.9                               | Heterotrophy *(Cytophaga–Flavobacterium–Bacteroides, CFB group)*                | 1.3                                         |
| 13.4                               | Fermentation and sulfate/nitrate/metal reduction *(Firmicutes)*                 | 2.5                                         |
| 9.1                                | Heterotrophy *(Actinobacteria)*                                                 | 3.1                                         |

Figure A1. Short-term fluctuations of the main groups of microorganisms associated with key metabolic processes in the chemocline zone of Lake Doroninskoye.
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