Microbiological indicators for assessing the ecological status of freshwater lakes in Buryatia

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Abstract. Possible complex approaches for assessing the condition of freshwater lakes using data on microbial diversity, obtained by high-throughput sequencing, were considered. The structural features of microbial communities, associated with increased anthropogenic impact, have been revealed. We identified some microbial taxa, which can be considered as indicators of the environmental status of freshwater bodies.

1. Introduction
An assessment of the status of freshwater ecosystems is an important part of environmental management programmes, in predicting possible risks to human health and in developing recommendations for effective management of freshwater resources. Traditional studies to assess the condition of freshwater ecosystems have focused on monitoring physicochemical parameters (due to the ease and accessibility of detection methods), control of sanitary-epidemiological groups of microorganisms, or on determining the impact of various pollutants on biological objects [1, 2]. The ecological approach to assessing the state of ecosystems is reflected in numerous biodiversity and biotic indices [3].

Microorganisms are a key component of freshwater ecosystems, participating in the production and decomposition of organic matter. The microbial community is closely related to environmental conditions and hydrochemical parameters, and is also able to quickly respond to negative changes in the ecological state of water bodies [4]. Therefore, studies related to the use of microorganisms as indicators of changes in the ecosystem, including anthropogenic ones, are relevant. The widespread use of modern high-throughput sequencing methods for assessing the microbial diversity in aquatic ecosystems makes it possible to conduct a more informative analysis of water and sediments microbial communities. The anthropogenic effect on structural changes in the microbial community has been well studied in recreational and urban freshwater ecosystems, where changes in the bacterial composition were observed along the concentration gradients of organic matter, nitrogen, phosphorus, etc. [5-7]. The aim of this study was to comprehensively assess the ecological state of large freshwater lakes in Buryatia: Gusinoe, Shchuchye, Kotokel, Isinga, Gunda, Sosnovoye and Bolshoye Yeravnoye, using data on the diversity of microorganisms, based on high-throughput sequencing.

2. Materials and Methods
Water temperature, total dissolved solids (TDS), pH, concentrations of nutrients and dissolved oxygen were measured by portable instruments; cation and anion content, total organic matter concentrations were determined in the laboratory in accordance with standard protocols.
Microbial diversity was studied by high-throughput sequencing using Illumina MiSeq platform in three technical replicates as described earlier [7]. The research was done using equipment of the Core Centrum ‘Genomic Technologies, Proteomics and Cell Biology’ in ARRIAM. Bioinformatics analysis was performed using QIIME ver. 1.9.1 and SILVA software [9].

The aquatic microorganism-based bacterial eutrophic index (BEI) was determined as the function of temperature and abundances of *Cyanobacteria* and *Actinobacteria* [3].

## 3. Results and Discussion

Environmental parameters of the water samples are presented in Table 1. In general, dissolved oxygen had normal variations without significant differences among the seven lakes. However, high content of organic matter, increased value of nitrates and phosphates were found in lakes: Gunda, Bolshoye Yeravnoye and Isinga, as well as and in the ‘Kotokel 1’ sample after accelerated growth of *Cyanobacteria*. The total dissolved solids of Lake Sosnovoye tended to be higher than those of the other lakes ($P < 0.1$).

| Lake           | TDS, mg/l   | NO$_3$, mg/l | P$_2$O$_5$, mg/l | C$_{org}$, mg/l | O$_2$, mg/l |
|----------------|-------------|--------------|------------------|----------------|-------------|
| Gusinoe        | 373.2±8.7   | 0.36±0.1     | 0.09±0.04        | 5.75±0.30      | 12.9±1.3    |
| Shchuchye      | 278.7±3.4   | 0.6±0.16     | 0.09±0.01        | 6.6±0.4        | 14.1±1.1    |
| Kotokel        | 128.8±14.3  | 2.93±3.6     | 0.10±0.03        | 10.5±4.2       | 12.7±0.1    |
| Gunda          | 365.7±4.7   | 0.73±0.19    | 0.09±0.04        | 17.1±1.7       | 10.0±0.5    |
| Bolshoye Yeravnoye | 301.8±40 | 1.0±0.2      | 0.14±0.50        | 19.1±0.1       | 8.96±0.30   |
| Isinga         | 279.3±4     | 1.81±0.1     | 0.37±0.50        | 21.7±0.8       | 11.3±1.5    |
| Sosnovoye      | 583.8±7.5   | 1.36±02      | 0.09±0.04        | 5.75±0.30      | 12.8±1.3    |

Biological diversity was associated with water quality parameters and environmental conditions. At phylum level, the seven freshwater lakes analysed had a similar microbial community structure with the dominance of the few typical freshwater bacterial groups even though conspicuous variations in their relative abundance were also observed. Distribution of 17 common freshwater lineages/genera cosmopolite of freshwater lakes revealed some trends in habitats with a high anthropogenic impact (beaches, places of urban drainage, etc.) and ecotopes without significant changes.

| Lake           | Temperature, °C | Cyanobacteria abundance, (%) | Actinobacteria abundance, (%) | BEI | Eutrophication level       |
|----------------|-----------------|------------------------------|-------------------------------|-----|---------------------------|
| Gusinoe        | 23.2±2.3        | 13.3±2.3                     | 17.96±7.40                   | 1.03±0.30 | middle eutrophic/ hyper eutrophic |
| Shchuchye      | 23.40±1.36      | 9.57±0.36                    | 20.65±1.75                   | 0.59±0.05 | light eutrophic           |
| Kotokel        | 19.50±0.98      | 12.4±15.7                    | 10.97±4.00                   | 1.23±1.20 | oligo-trophic/ middle eutrophic/ hyper eutrophic |
| Gunda          | 21.4±0.5        | 1.7±0.8                      | 17.2±6.7                     | 0.12±0.03 | oligotrophic              |
| Bolshoye Yeravnoye | 23.1±2.5 | 3.83±2.00                   | 16.7±7.7                    | 0.35±0.29 | oligotrophic/ middle eutrophic |
| Isinga         | 21.4±0.1        | 1.9±0.57                     | 17.44±16.20                  | 0.24±1.70 | light eutrophic           |
| Sosnovoye      | 21.6±0.6        | 5.6±1.4                      | 9.5±2.3                      | 0.74±0.20 | light eutrophic/ middle eutrophic |
In particular, representatives of the genera Flavobacterium and Rhodoferax demonstrated a high correlation between the abundance and high nutrient content in the areas of lakes associated with cyanobacterial blooms. The genera Rhodobacter, Polynucleobacter, and Luteolibacter preferred environments with a higher trophic level and were also indicators of possible anthropogenic impact. Our results confirmed potential relationships between microbial community composition and the environmental parameters measured in freshwater ecosystems [5, 6]. Such results, together with the ecological characteristics of common freshwater taxa, their wide and even cosmopolitan distribution range, high abundance, and sensitivity to environmental changes, allow us to consider them as indicators of the water quality in freshwater lake ecosystems.

One of the possible methods for classifying eutrophication of freshwater ecosystems is bacterial eutrophic index based on microbial diversity [3]. This method has been proposed to assess the water quality of global freshwater ecosystems and has allowed the trophic status of different lakes to be determined. As shown in Table 2, BEI was calculated for the studied lakes, and the classification of eutrophication in terms of aquatic microorganisms was determined. BEI values range from 0.1 in oligothrophic Lake Gunda to 2.5 in the sample ‘Kotokel 3’ with significant level of eutrophication due to cyanobacterial bloom. Another example of high BEI value was found in Lake Gusinoe, sampled in the warm water discharge and was influenced by increased temperature of water.

### Table 3. Distribution of fam. *Enterobacteriaceae* in water samples of the studied lakes.

| Sample, location | Average abundance, % | Dominant genera |
|------------------|----------------------|-----------------|
| Shch1, west part of Lake Shchuchye | 0.59 | - |
| Shch2, the centre of Lake Shchuchye | 4.05 | Klebsiella, Serratia, Enterobacter |
| Gus4, Lake Gusinoe beach | 0.37 | - |
| Kotokel 1, the beach | 0.01 | - |
| Kotokel 2, south-west part of the lake | 1.69 | Escherichia-Shigella |
| Isinga 1 | 15.4 | Klebsiella |
| Isinga 3 | 1.22 | Klebsiella |
| Gunda 1 | 28.0 | Klebsiella |
| Gunda 2 | 12.3 | Klebsiella |

Qualitative and quantitative determination of fecal indicator bacteria (total and fecal coliforms, Escherichia coli, and enterococci), thermotolerant coliform bacteria, coliphages, salmonella, and alternative indicators (Enterococcus, Staphylococcus, Streptococcus, Bacteroides, Clostridium, Finegoldia, Burkholderia, Klebsiella, and bacteriophages) are routinely used in the assessment of sanitary quality of freshwater ecosystems [8]. Among analysed water samples, total coliform and thermotolerant coliform bacteria were detected most consistently in lakes Gunda (200 colony forming unit/100 ml), Isinga (60-120 CFU/100 ml), Sosnovoye (60 CFU/100 ml) and Bolshoye Yeravnoye (60 CFU/100 ml). In lakes Gusinoe, Shchuchye, Kotokel total coliform and thermotolerant coliform bacteria were detected least frequently.
However, the results of high-throughput sequencing revealed the presence of a significant number of sequences representing genus Klebsiella in lakes Shchuchye (4% of total bacteria), Isinga (1.22-15.4%), and Gunda (12.3-28% of total bacteria). The genera Staphylococcus and Streptococcus were found in high abundance in Lake Isinga (3.4 and 5% of total bacteria, accordingly). The genus Escherichia-Shigella had the greatest occurrence in Lake Kotokel (Table 3). It is likely that the microbial communities in these lakes, especially in lakes Gunda and Isinga, were affected by external bacterial loading via fecal contamination due to intensive grazing. So, complicated models combined with environmental parameters and complex of microbial indicators (e.g., BEI, analysis of common freshwater lineages/genera distribution) are recommended for more effective water quality evaluation of a freshwater ecosystem.

4. Conclusion
The methods based on aquatic microorganisms should be considered when assessing the water quality of freshwater lakes. The structural features of microbial communities associated with an increased anthropogenic load have been revealed. We have identified some microbial taxa that can serve as indicators of the quality in freshwater bodies.

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