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Co-occurrence Networks Among Bacteria and Microbial Eukaryotes of Lake Baikal During a Spring Phytoplankton Bloom

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Abstract

The pelagic zone of Lake Baikal is an ecological niche where phytoplankton bloom causes increasing microbial abundance in spring which plays a key role in carbon turnover in the freshwater lake. Co-occurrence patterns revealed among different microbes can be applied to predict interactions between the microbes and environmental conditions in the ecosystem. We used 454 pyrosequencing of 16S rRNA and 18S rRNA genes to study bacterial and microbial eukaryotic communities and their co-occurrence patterns at the pelagic zone of Lake Baikal during a spring phytoplankton bloom. We found that microbes within one domain mostly correlated positively with each other and are highly interconnected. The highly connected taxa in co-occurrence networks were operational taxonomic units (OTUs) of Actinobacteria, Bacteroidetes, Alphaproteobacteria, and autotrophic and unclassified Eukaryota which might be analogous to microbial keystone taxa. Constrained correspondence analysis revealed the relationships of bacterial and microbial eukaryotic communities with geographical location.

Keywords Co-occurrence network · Bacteria · Microbial eukaryotes · Community · Lake Baikal · Pyrosequencing

Introduction

Individual organisms coexist in natural ecosystems, forming webs of ecological interactions. These associations mediate the impact of biodiversity on ecosystem function [1]. Microbes (bacteria, archaea, and microbial eukaryotes) significantly contribute to the biodiversity of aquatic ecosystems and play an important role in food webs and biogeochemical cycles [2–5]. The structure of microbial communities is affected by interspecies interactions and local physicochemical and regional conditions [6–9]. Phytoplankton bloom is one of the major factors affecting the composition of bacterial communities, as shown in marine [10–12] and freshwater [13, 14] ecosystems. Co-occurrence and correlation patterns revealed on the basis of next generation sequencing data can be used to predict positive and negative ecological interactions between various species and environmental parameters in aquatic ecosystems [12, 14–16]. Correlation network analysis is applied to understand the organization of microbial communities and interactions between their components. Studying the central nodes of the network may identify potential target microbes (keystone species) whose disappearance might lead to the disturbance of a mature community [16–18].

Lake Baikal is located in the rift valley in the south-east of Siberia (Fig. 1) and is the deepest, largest by volume freshwater lake in the world. The lake stretches almost 4° in latitude from the south-west to north-east, which results in non-uniform warming of the southern and northern parts of the lake during spring and summer [19]. Phytoplankton blooms during the spring period from when the lake surface is covered with ice (March to May) to after the ice cover is gone (May to June) [20]. At that time, diatoms [20], dinoflagellates [21], green algae [22], eustigmatophytes [23], and protozooplankton [24] develop in plankton of Lake Baikal. In addition, using NGS,

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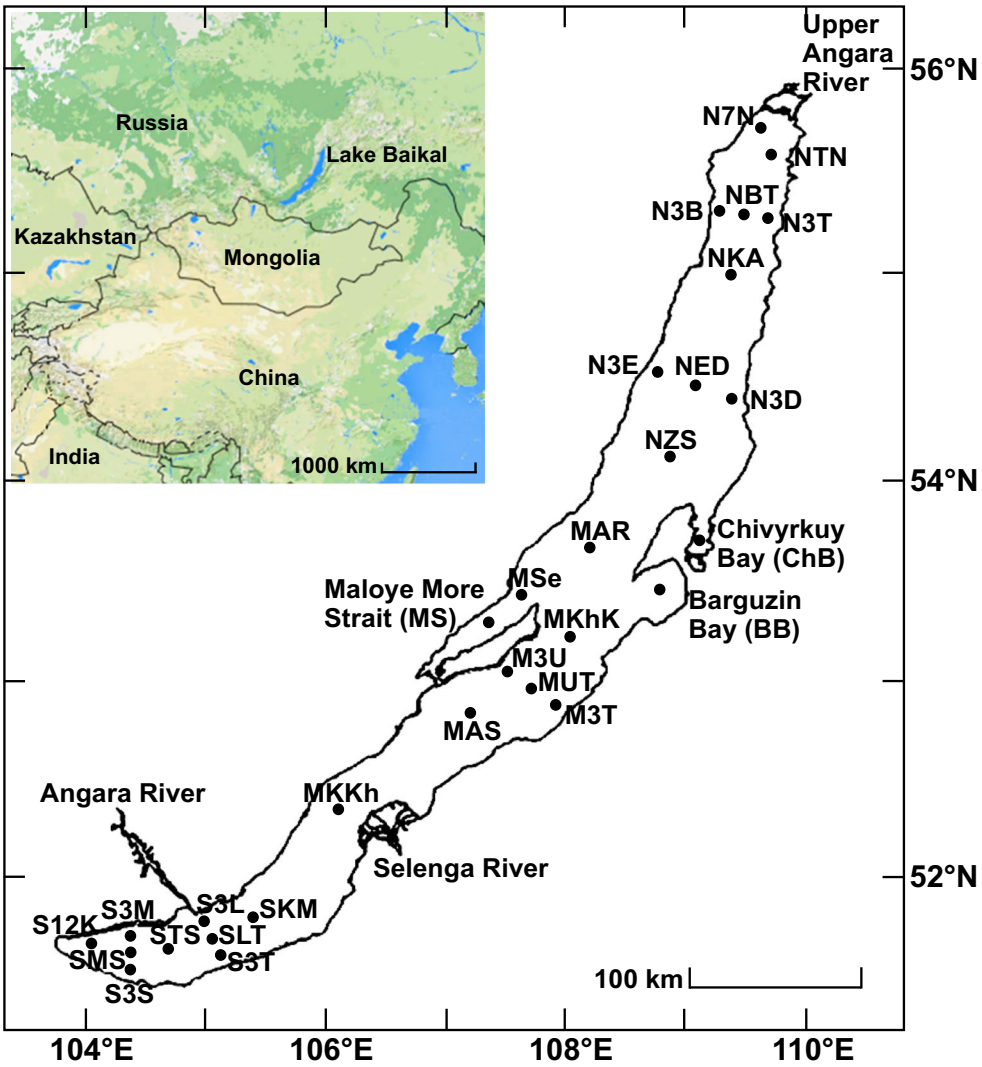

Fig. 1 Map of sampling stations at Lake Baikal. South Basin (SB): S12K, S3M, SMS, S3S, STS, S3L, SLT, S3T, and SKM; Middle Basin (MB): MKKh, MAS, M3U, MUT, M3T, MKhK, MSe, and MAR; North Basin (NB): NZS, N3E, NED, N3D, NKA, N3B, NBT, N3T, NTN, and N7N; Bays and strait: BB, ChB, and MS

the composition of microbial eukaryotes in water column and near-bottom of southern basin of Lake Baikal was analyzed [25]. NGS analysis showed that bacterial communities mostly included Actinobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, Acidobacteria, and Cyanobacteria in local sites of the littoral area [26], the deep near-bottom [27], sub-ice [28], and the water column [29] of the lake.

However, co-occurrence patterns and linkages between microbes and the environmental parameters of Lake Baikal had not been previously studied. The study aimed to identify co-occurrence patterns between bacteria, microbial eukaryotes, and environmental factors of the upper water layer of Baikal's pelagic zone during spring phytoplankton bloom. We hypothesized that (i) bacterial or microbial eukaryotic groups had predominant positive linkages and were interconnected in communities of the lake, and (ii) the composition of bacterial and microbial eukaryotic communities were affected by environmental factors and geographical locations.

Materials and Methods

Study Area and Sampling

Water samples were collected from the RV "Akademik V.A. Koptyug" with an SBE 32 Carousel water sampler (Sea-Bird Electronics, USA) into 0.5-L polyethylene bottles from the upper 0, 5, 10, 15, 20, and 25 m layers. Samples were obtained from 27 stations within the pelagic zone of the lake, two stations in Barguzin Bay (BB) and Chivurkuy Bay (ChB) and one station in Maloe More Strait (MS) during a 1-week period in early June (June 3 to 10) 2012 (Fig. 1).

Analysis of Environmental Parameters

Dissolved oxygen was measured using iodometry according to Winkler, with a relative error of 1% [30], performed immediately after sampling. A spectrophotometric method, based on the formation of phosphomolybdic heteropolyacid

followed by reduction with in chloride, was used to measure phosphate concentrations (method error 5%). Nitrate was determined with sulfophenic acid (method error 5%) [31]. A method based on measuring the intensity of yellow silicomolybdic heteropolyacid coloring (method error 5%) was used to detect silicic acid [32]. Temperature measurements were obtained using a high-precision SBE 25 Sealogger CTD profiler (Sea-Bird Electronics) to an accuracy of 0.002 °C. Data on phytoplankton biomass and total bacterial abundance analyzed by microscopy were previously obtained [33].

DNA Extraction, PCR Amplification, and 454 Pyrosequencing

In order to extract DNA, integrated water samples (200 ml from each sample collected from 0, 5, 10, 15, 20, and 25-m depths, totaling 1.2 L) were filtered through 0.2 μm polycarbonate filters (Millipore, Ireland), then the biomass was collected and washed into sterile bottles with 5 ml TE buffer (1 mM EDTA and 10 mM Tris-HCl; pH 7.5) and stored at − 20 °C. DNA was extracted by the lysozyme–SDS–phenol/chloroform method [27]. The V3–V4 region of the 16S rRNA gene was amplified using universal primers U341F (5′-CCTACGGGRSGCAGCAG-3′) and U785R (5′-GGACTACCVGGGTATCTAAKCC-3′) [34], and the V3 region of the 18S rRNA gene was amplified with forward (5′-ATTAGGGTTCGATTCCGGAGAGG-3′) and reverse (5′-CTGGAATTACCGCGGSTGCTG-3′) broad eukaryotic primers [35] using a thermocycler (BIS M-111; BIS-N, Novosibirsk, Russia). Amplicons for pyrosequencing were made in four replicates of 20 μL, then pooled into one sample. Pyrosequencing was completed in two runs and was performed on a GS 454 FLX genome sequencer (Roche, USA) at the Limnological Institute of the Siberian Branch of RAS. The first run of 16S rRNA samples had been published [33]. The second run of 18S rRNA samples was completed using Titanium reagents, according to the manufacturer's GS FLX Titanium Sequencing Method Manual.

Pyrosequencing Data Analysis

Analysis of pyrosequencing data was performed using Mothur 1.19.0 software [36]. The obtained sequences were processed using the PyroNoise algorithm [37] to remove sequencing errors, then sequences of an average length of 180 bp were aligned with 18S rRNA gene sequences from the SILVA database [http://www.mothur.org/wiki/Silva_reference_files]. Pre-clustering was performed to simplify the dataset by grouping sequences that differed by two nucleotides. Chimeric sequences were identified using the Chimera UCHIME algorithm [38] with standard parameters. The obtained sequences were classified by SILVA with bootstrap support of at least 80%. The sequences were grouped into operational taxonomic units (OTUs) with 97% similarity. The sequences were deposited in the NCBI Short Read Archive (SRA) under the accession numbers SRR1806738 for 16S rRNA and SRR2027823 for 18S rRNA. The OTU numbers and richness (Chao1) and diversity estimators (Shannon index) were calculated for each sample based on the OTUs identified using the Mothur package.

Statistical Analysis

Before statistical analysis, an array of reads of bacterial and microbial eukaryotic in samples was examined for representativeness of samples using a bootstrap index [39]. The bootstrap index determines the proportion of underestimated species (OTUs). If the proportion of underestimated species exceeded 10%, then the sample was removed from statistical analysis. Estimation was carried out using the "vegan" package. The analysis of representativeness of samples, which characterize the number of reads per OTU, was conducted for dominant OTUs, which comprise 90% of the data pool. For bacterial communities (Table S2), the proportion of underestimated OTUs exceeded 10% in three samples (S12K, NED, NTN) out of total 30. These samples were removed from analysis. As for data on the composition of eukaryotic communities (Table S3), the proportion of underestimated species was less than 10% in all samples. Clustering of communities was performed using Bray–Curtis dissimilarity matrix with UPGMA. The uncertainty of hierarchical clustering was assessed by multiscale bootstrap resampling in "pvclust" [40] with 1000 bootstrap replicates. Exploratory analysis of OTU abundance and their correspondence to environmental factors was performed with "phyloseq" v.1.16.2 [41] and "vegan" [42] packages, respectively. For multivariate statistical analyses and hypothesis testing, abundance counts were log transformed. Ordination was performed by NMDS and CCA using Bray–Curtis index to calculate matrix of pairwise community dissimilarities. In order to better identify those OTUs, their sequences were classified manually using the SINA aligner and SILVA database, in addition to BLASTN and NCBI. Distribution of the relative abundance of 16S rRNA and 18S rRNA OTUs were visualized with the help of dot plots constructed using the "ggplot" package [43] in R software. For the correlation analysis, the data on the number of sequences of microbial OTUs in the samples was recalculated into relative abundance. We performed preliminary analysis of samples using the Shapiro–Wilk test [44], which showed that not all samples were normally distributed. Thus, we used correlation analysis and Spearman's r correlation coefficient [45] to identify pairwise associations between the relative abundance of bacterial and microbial eukaryotic OTUs and environmental parameters. The accuracy of the correlation coefficients was determined

using "W" Spearman's statistics [45], for which the null hypothesis was rejected if its probability was lower than the critical level where $\alpha = 0.05$. p values were corrected for the false discovery rate in multiple comparisons using the Benjamini–Hochberg equation [46]. The correlation matrix was visualized by a heat map generated using the "gplot" package [47] in R. The lines and columns on the heat map were clustered by the "complete" method using the Euclidean distance metric. Clustering of the correlations on heat maps was tested for accuracy using the bootstrap method (1000 replacements) [40]. Also, the correlation matrix was visualized through a network using "igraph" packages in R [48]. The network topology is based on the number of links and correlation coefficients between neighboring nodes. The more links the nodes form among themselves, the closer they are placed in the network. To identify keystone taxa in co-occurrence networks for each node of the network, there was accounted betweenness centrality. The higher the value of a network node indicator, the more significant a taxon in the relevant pelagic community of the lake. The calculations were carried out using the "igraph" package [48] in R.

Results

Limnological Data

In early June 2012, the temperature profile of upper water layer reflected its spring homothermic period. The temperature of the upper water layer (0 m) was slightly higher (3.87–5.42 °C) at the stations located 3 km from the east coast of the middle (M3T) and northern (N3D, N3T, NTN, and N7N) basins, compared to other stations (2.42–3.63 °C). The concentration of dissolved oxygen in the photic layers ranged between 11.58 and 13.49 mg L^{-1}, the concentration of phosphates (PO_4^{3-}) and nitrates (NO_3^-) were 0.017–0.031 mg L^{-1} and 0.18–0.39 mg L^{-1}, respectively, and the pH was 7.82–8.19 (Table 1S). The concentration of silicon (Si) in the northern basin (up to 1 mg L^{-1}) was slightly higher than that measured in the middle (up to 0.8 mg L^{-1}) and southern (up to 0.64 mg L^{-1}) basins, due to the incoming streams from the lake tributaries. Unlike the deep water stations, the water temperature in the shallow ChB was higher (8.3 °C), while concentrations of Si, PO_4^{3-}, and NO_3^- were lower than in other parts of the lake (Table S1).

Structure of Bacterial Communities

Pyrosequencing of the amplicon 16S rRNA gene V3–V4 fragment for 30 samples generated a total of 107,996 sequences with a mean length of 420 bp. After processing 87,951 sequences were generated for Bacteria (51.1%), Archaea (0.04%), and chloroplasts (48.8%). The latter was excluded from the analysis. Using a genetic distance of 97%, a total of 867 OTUs were obtained, of which 399 OTUs were single sequences. The number of OTUs, the Chaol estimator, and Shannon index for bacterial communities varied between 51 and 439, 72 and 625, and 2.07–4.22, respectively (Table S4). The assessment of community similarities was performed using the Bray–Curtis distance. A majority of bacterial communities formed one general cluster. In some cases, communities located in close proximity shared the most similarities (Fig. 2a). Unconstrained community ordination with NMDS did not show dependence on physico-chemical parameters. Fitting showed that the environment variables did not significantly affect the ordination. The only significant factor is basin variable ($p = 0.015$). NMDS showed that the samples of the northern basin formed a diffuse cluster; the samples of the remaining two did another one (Fig. 2b). Constrained correspondence analysis of the model built with basin variable produced significant results ($p = 0.001$) (Fig. S1). The identified sequences were assigned that the Bacteria consists predominantly of Actinobacteria (9–57%), Bacteroidetes (11–46%), Verrucomicrobia (0.5–59%), Proteobacteria (6–24%), Acidobacteria (0.4–45%), Cyanobacteria (0.1–26%), and unclassified Bacteria (0.2–5.4%) (Fig. 3a). Actinobacteria were comprised of *Ilumatobacter* (4–40%), unclassified Actinobacteria (4–28%), *Actinomycetales* (0.4–8%), and *Acidimicrobineae* (up to 2%) (Fig. S2). Bacteroidetes were represented by *Flavobacterium* (1–29%), *Cryomorphaceae* (1–11%), *Sediminibacterium* (up to 6.5%), *Chitinophagaceae* (up to 5%), *Cytophagaceae* (up to 4%), *Algoriphagus* (up to 2.6%), and *Fluviicola* (up to 1%) (Fig. S2). Verrucomicrobia included *Spartobacteria* (up to 59%), subdivision 3 (up to 6.4%), and *Luteolibacter* (up to 1%). Acidobacteria was classified as the Gp6 subgroup (0.2–43%) and unclassified Acidobacteria (up to 3%). Betaproteobacteria (2–18%) were represented by *Limnohabitans* (0.4–8.8%), *Comamonadaceae* (0.2–6.6%), unclassified *Betaproteobacteria* (up to 2%), *Albidiferax* (up to 4.2%), *Polynucleobacter* (up to 3.3%), and *Alcaligenaceae* (up to 0.6%). Alphaproteobacteria (1.5–8%) included *Rhodobacter* (up to 5%), *Acetobacteraceae* (up to 2.3%), *Sphingorhabdus* (up to 2.2%), *Rhizobiales* (up to 1.4%), and *Pelagibacter* (up to 0.8%) (Fig. S2). Gammaproteobacteria (up to 3.5%) and Deltaproteobacteria (0.4%) were found in small quantities (Fig. 3a). Cyanobacteria were classified as *Synechococcus* (Fig. S2).

Structure of Microbial Eukaryotic Communities

Pyrosequencing of the amplicon 18S rRNA gene V3 region generated sequences with an average length of 180 bp. After alignment, pre-clustering, and removal of chimeric sequences for 30 samples, 420,772 sequences were generated for Eukaryota. After removing Metazoa, 260,064 of sequences remained, represented by 2442 OTUs (genetic distance of

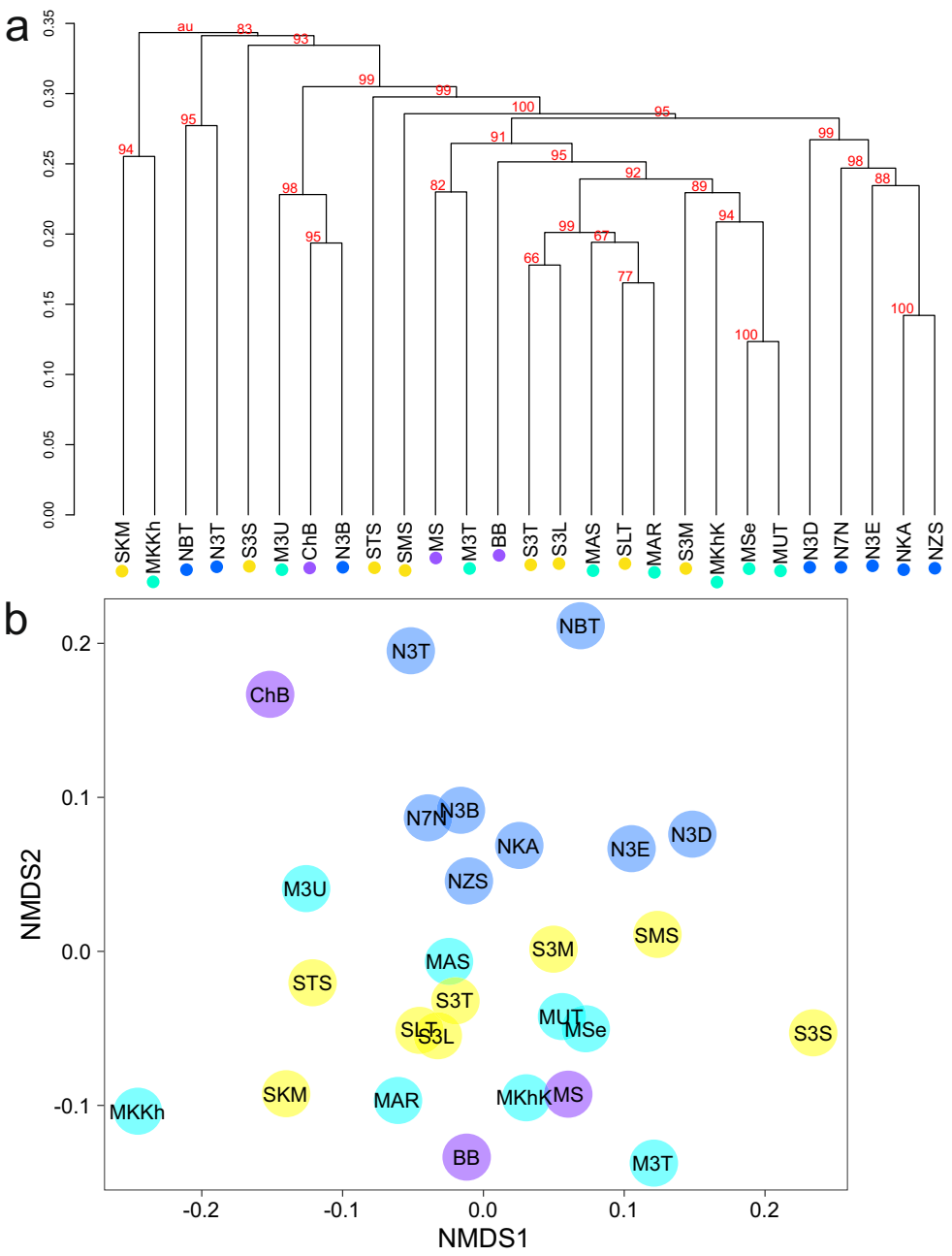

Fig. 2 Cluster analysis (**a**) and NMDS biplot (**b**) completed for OTUs (97% sequence identity) of bacterial communities with Bray–Curtis dissimilarity index. Red numbers along dendrogram are approximately unbiased support values. Yellow, green, blue, and purple circles show stations of the north, south, middle Baikal basins, and the bays and strait, respectively

97%), of which 1438 OTUs were single sequences. The number of OTUs, the Chaol estimator, and Shannon index of microbial eukaryotic communities in the lake varied in the ranges of 49–427, 79–990, and 2.16–3.84, respectively (Table S5). Community clustering revealed a group of samples from the northern basin, whereas communities from the southern and middle basins, along with BB and MS formed the second cluster. NTN, ChB, and N3B were positioned separately from other stations (Fig. 4a). Unconstrained ordination of communities with NMDS revealed correlation with pH ($r^2 = 0.49$, $p = 0.001$), NO_3 ($r^2 = 0.47$, $p = 0.002$), temperature ($r^2 = 0.38$, $p = 0.005$), Si ($r^2 = 0.3$, $p = 0.008$), PO_4 ($r^2 = 0.22$, $p = 0.026$), and O_2 ($r^2 = 0.2$, $p = 0.044$) (Fig. 4b). Basin factor variable also possesses significant correlation ($r^2 = 0.29$, $p = 0.005$) with community structure. Constrained correspondence analysis of the model built with basin variable ($r^2 = 0.42$, $p = 0.001$) and pH ($r^2 = 0.48$, $p = 0.001$) produced significant correlations with communities (Fig. S3).

The identified sequences were assigned that Eukaryota was composed of Dinophyceae (11–56%), unclassified Eukaryota (3.4–45%), Chrysophyceae (4–31%), Chlorophyta (0.7–41%), Ciliophora (1.3–28%), Fungi (0.2–45%), Cryptophyta (1.6–17%), Haptophyceae (0.4–21%), Cercozoa (0.2–10%), Eustigmatophyceae (0.2–9%), Dictyochophyceae (up to

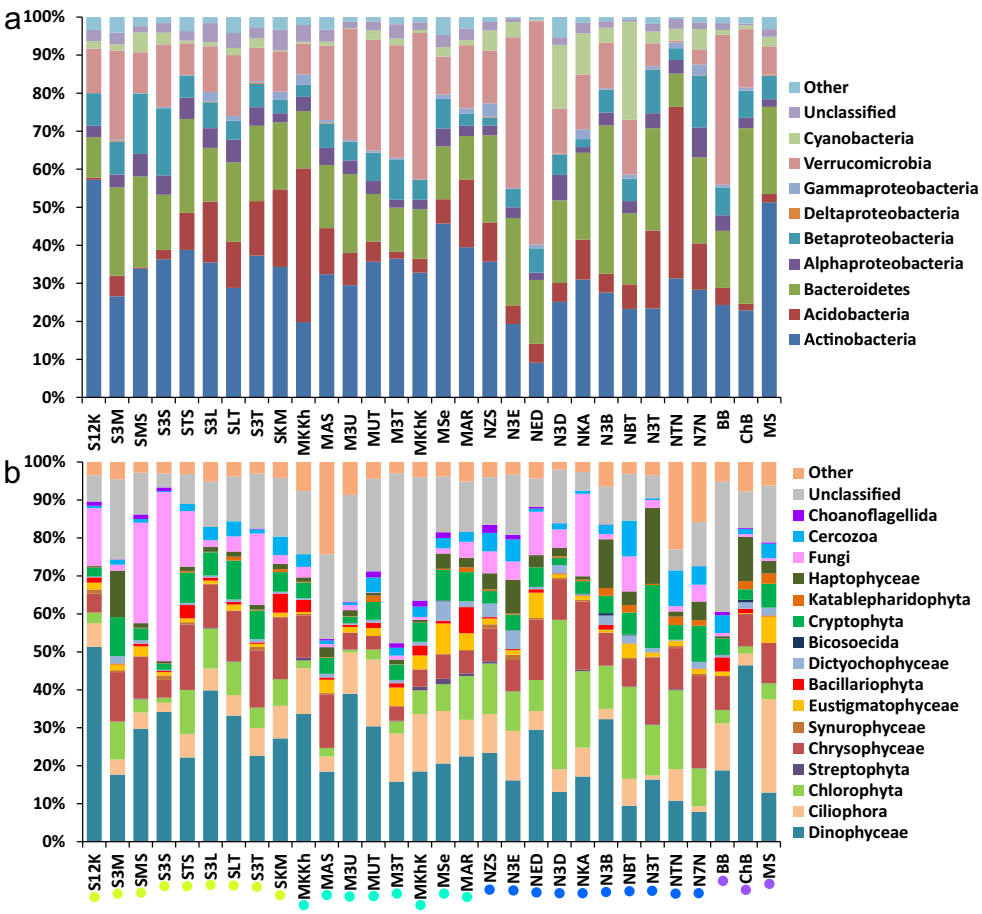

Fig. 3 The relative abundance of reads at the domains Bacteria [33] (**a**) and Eukaryota (**b**). Yellow, green, blue, and purple circles show stations of the north, south, middle Baikal basins, and the bays and strait, respectively

5.4%), Bacillariophyta (up to 8%), Katablepharidophyta (up to 3.1%), Choanoflagellida (up to 2.4%), Synurophyceae (up to 1.6%), Streptophyta (up to 1.3%), and Bicosoecida (up to 0.4%) (Fig. 3b). Taxa with a small number of sequences and singletons accounted for 5% of total sequences. Dinophyceae were represented by *Gyrodinium* (2–37%) and unclassified *Dinophyceae* (3.3–44%) (Fig. S4). Chrysophyceae were classified as *Spumella* (2–22%) and unclassified *Chrysophyceae* (0.4–14%); Eustigmatophyceae as *Nannochloropsis*, Dictyochophyceae as *Apedinella*, and Chlorophyta as *Koliella* (0.2–39%); *Chlamydomonas* (up to 4%); and unclassified *Trebouxiophyceae* (0.1–3%). Ciliophora were comprised of *Halteria* (0.1–23%), *Stokesia* (up to 11.5%), *Tintinnidium* (up to 9.3%), and *Tintinnopsis* (up to 4.6%). Fungi were classified as unclassified Fungi (up to 45%), *Chytridiomycota* (up to 4%), and *Cryptococcus* (up to 3%). Cryptophyta were represented by *Teleaulax* (0.6–15%), *Cryptomonas* (up to 5.3%), *Chroomonas* (up to 2.2%), and unclassified *Cryptophyta* (up to 2.4%). Haptophyceae included *Chrysochromulina* (up to 20%) and unclassified *Haptophyceae* (up to 2.2%). Cercozoa (0.2–10%) were classified as *Cryothecomonas*, Bacillariophyta as *Synedra*, Katablepharidophyta as *Katablepharidaceae*, and Choanoflagellida as *Sphaeroeca* (Fig. S4). All sequences of each OTU for Synurophyceae (*Chrysosphaerella*), Ciliophora (*Strombidium*, *Histiobalantium*, *Cyclotrichium*, *Didinium*, and unclassified *Strombidiidae*), and Bacillariophyta (*Urosolenia*) accounted for less than 0.5%.

Co-occurrence Networks of Bacteria and Microbial Eukaryotes

The co-occurrence network constructed for strong ($r \geq |0.53|$) and significant ($\alpha = 0.05$) correlations between the relative abundance of bacterial OTUs in samples consist of 60 nodes and 149 edges (Fig. 5). The correlations identified by the analysis were predominantly positive. The following OTUs had the highest values of betweeness centrality: OTU 8 *Flavobacterium* (0.183), OTU 53 Actinomycetales (0.154), OTU 4 Actinobacteria unclassified (0.149), OTU 65 *Pelagibacter* (0.133), OTU 20 *Sediminibacterium* (0.128), OTU 33 *Ilumatobacter* (0.105), OTU 77 *Flavobacterium* (0.104), OTU 13 Cryomorphaceae (0.103), OTU 76 Rhizobiales (0.1), OTU 1 *Ilumatobacter* (0.088), OTU 10 Actinobacteria unclassified (0.088), and OTU 59 Acetobacteraceae (0.082). Acidobacteria comprised a distinct cluster, whose members had positive correlations with each other and with Alphaproteobacteria. Verrucomicrobia had only few

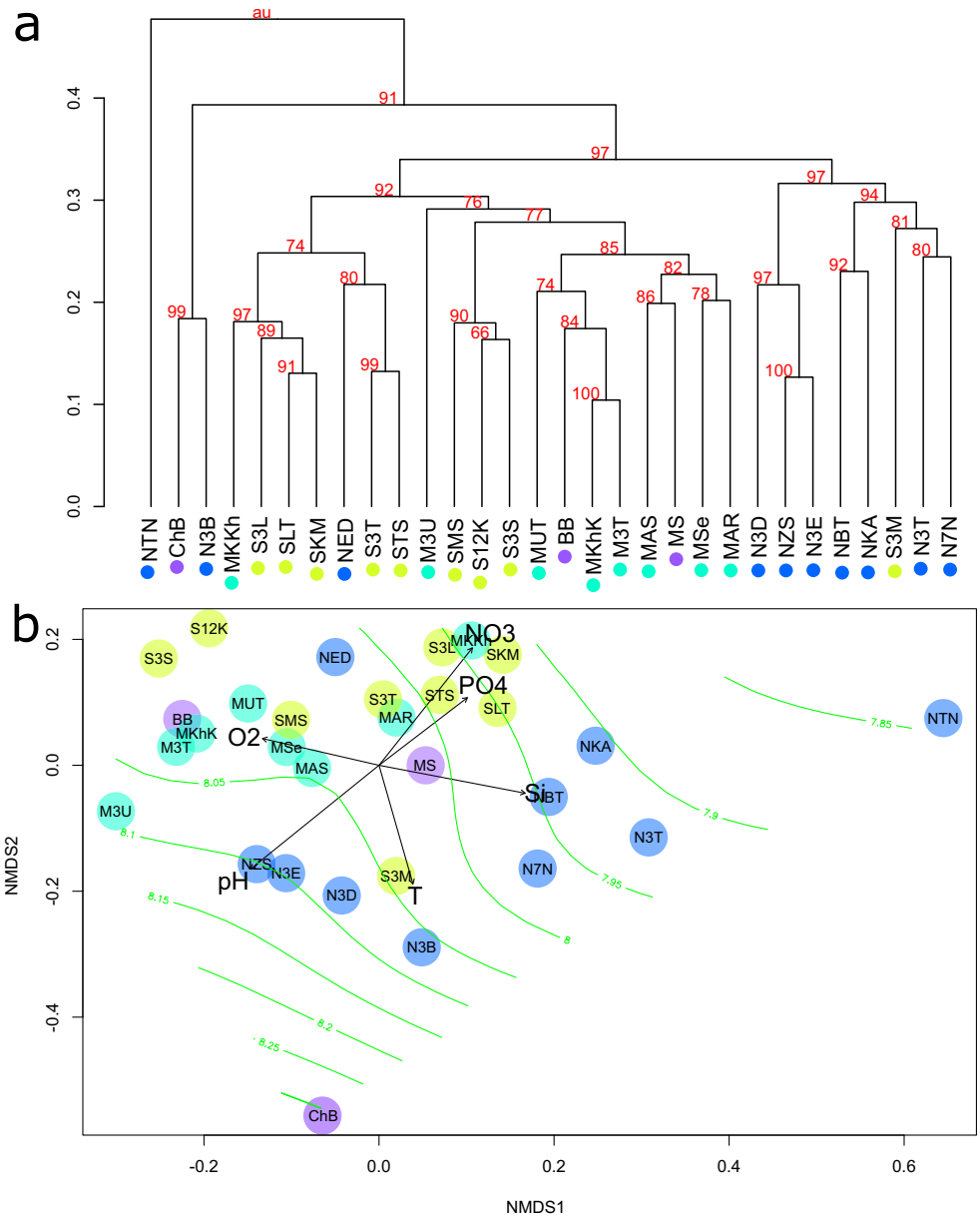

Fig. 4 Cluster analysis (**a**) and NMDS biplot (**b**) completed for OTUs (97% sequence identity) of microbial eukaryotic communities with Bray–Curtis dissimilarity index. Red numbers along dendrogram are approximately unbiased support values. The fitted vectors show physicochemical variables. Yellow, green, blue, and purple circles show stations of the north, south, middle Baikal basins, and the bays and strait, respectively. Green lines show pH gradient

connections. OTU 3 Spartobacteria had negative correlations with OTU 59 Acetobacteraceae and OTU 61 Acidobacteria subgroup Gp6, and positive correlations with *Luteolibacter*. Generally, phylogenetically related bacterial OTUs were correlated with a similar complex of bacterial OTUs (Fig. 5).

The co-occurrence network of microbial eukaryotes constructed from correlations, where $r \geq |0.49|$ and $\alpha = 0.05$, had 31 nodes and 55 edges (Fig. 6). The correlations identified were predominantly positive. Unclassified Eukaryota and autotrophic eukaryotes had the highest values of betweenness centrality, including unclassified Eukaryota OTU 29 (0.348); *Nannochloropsis* (0.217); *Apedinella* (0.208); *Chlamydomonas* (0.123); *Chroomonas* (0.114); unclassified Eukaryota OTU 16 (0.103), as well as phagotrophic *Stokesia* (0.205); and *Halteria* (0.105). The network of correlations ($r \geq |0.63|$ and $\alpha = 0.05$) between the relative abundance of bacterial and microbial eukaryotic OTUs included 12 nodes and 11 edges (Fig. 7). Some microbial eukaryotic OTUs were correlated with a complex of several bacterial OTUs, which were specific only to them (Fig. 7).

Correlations between bacterial OTUs and environmental parameters were insignificant. A relatively small number of microbial eukaryotic OTUs correlated with environmental parameters. Microbial eukaryotic OTUs formed two clusters with one of them positively correlated with O_2, pH, total biomass of phytoplankton (TBP), and total bacterial abundance (TBA) and negatively correlated with Si, temperature, NO_3, and PO_4, while another one correlates oppositely (Fig. 8). Positive correlations were observed between *Teleaulax*, *Cryptococcus*, and unclassified Eukaryota OTU 16 with

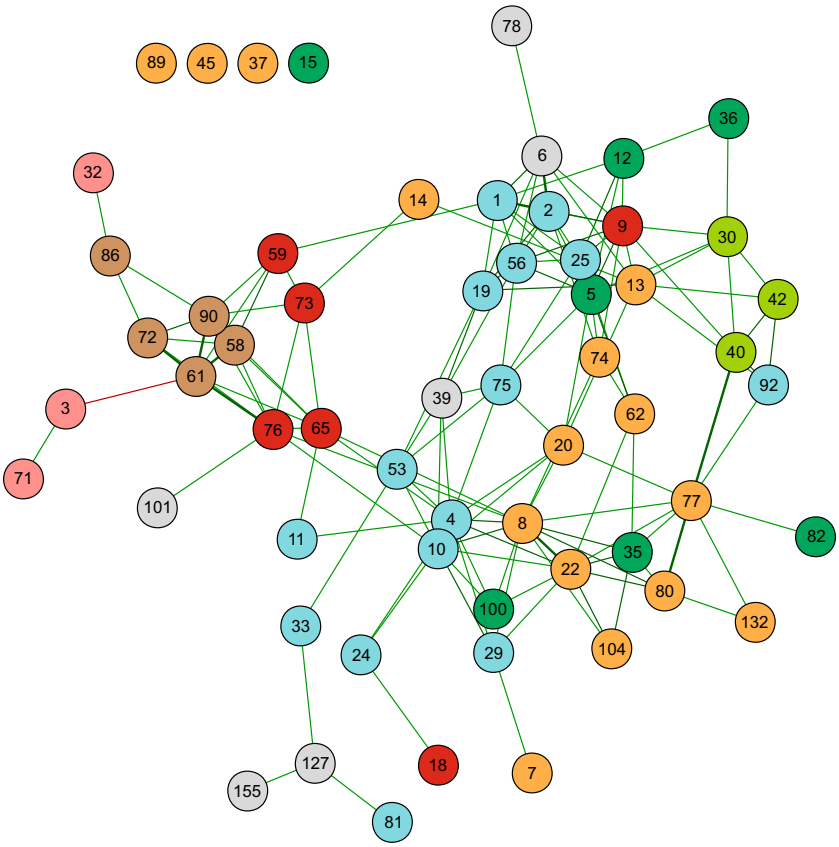

Fig. 5 Correlation network of top 60 bacterial OTUs in the photic layer of Lake Baikal during spring phytoplankton bloom. The network shows strong and significant correlations ($r \geq |0.53|$, $\alpha = 0.05$). Numbers in the circles are OTU numbers. Green lines show positive correlations; red lines show negative correlations. Circles are bacterial OTUs: blue—Actinobacteria, orange—Bacteroidetes, red—Alphaproteobacteria, dark green—Betaproteobacteria, pink—Verrucomicrobia, light green—Cyanobacteria, brown—Acidobacteria, gray—unclassified Bacteria

NO_3 ($r = 0.56$), and unclassified Fungi with O_2 ($r = 0.57$). *Sphaeroeca* was positively correlated with pH and O_2 ($r = 0.55$) and negatively correlated with NO_3 ($r = -0.59$) (Fig. 8). Analysis of the associations between species of phytoplankton revealed that the biomass of *Koliella longiseta* (Vischer) Hindák showed negative correlations with the

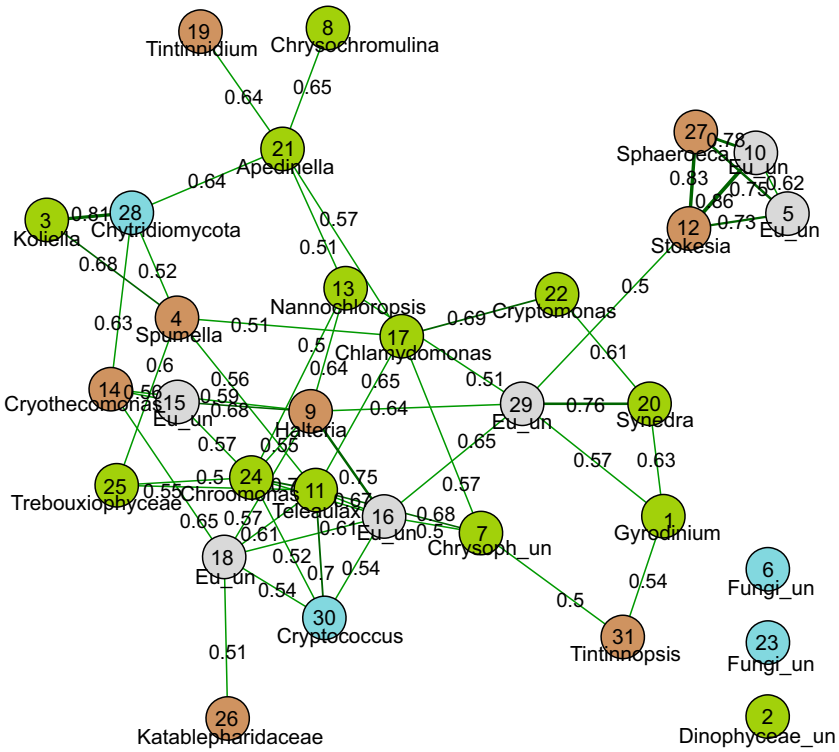

Fig. 6 Correlation network of top 31 OTUs of microbial eukaryotes in the photic layer of Lake Baikal during spring bloom. The network shows correlations ($r > |0.49|$, $\alpha = 0.05$) between OTUs of microbial eukaryotes. Numbers in the circles show OTU numbers. Green lines show positive correlations; red lines show negative correlations. Correlation coefficients are displayed on the lines. Circles are eukaryotic OTUs: green—photosynthesizing, brown—heterotrophic, blue—fungi, gray—unclassified eukaryotes (EU_un)

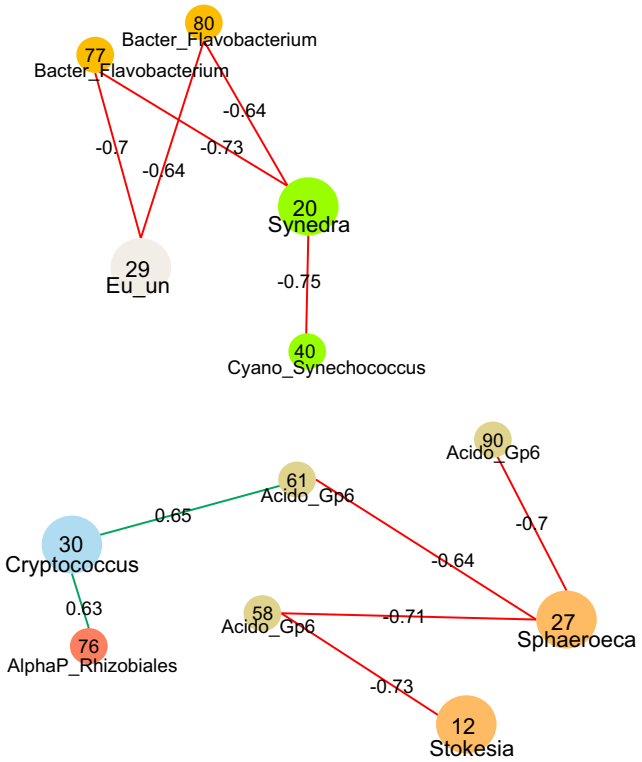

Fig. 7 Correlation network between bacterial OTUs and OTUs of microbial eukaryotes in the photic layer of Lake Baikal during spring bloom. The network shows significant correlations ($r > |0.63|$, $\alpha = 0.05$) between bacterial OTUs and OTUs of microbial eukaryotes. Numbers in the circles denote OTU numbers. Green lines indicate positive correlations, while red lines indicate negative ones. Correlation coefficients are displayed on the lines

biomass of the diatom *Aulacoseira baicalensis* (K. Meyer) Simonsen ($r = -0.63$). TBP was correlated positively with pH ($r = 0.5$) and negatively with PO_4 ($r = -0.51$). TBA were correlated positively with pH ($r = 0.45$) and negatively with NO_3 ($r = -0.65$) and PO_4 ($r = -0.53$).

Discussion

Relationship Between the Structure of Microbial Metacommunity and Environmental Parameters

We have studied relationship between structures of microbial communities and environmental parameters in the pelagic zone of Lake Baikal during a spring phytoplankton bloom using pyrosequencing of 16S rRNA and 18S rRNA genes and statistical analysis. Dominant bacterial and microbial eukaryotic taxa were distributed across the lake, but their ratio in different areas varied (Fig. 3, S1, S3). Within the metacommunity, local communities are connected through multiple potentially interacting species, and the process of community assembly is affected by both local interactions and regional processes [49, 50]. Analysis of the beta diversity revealed that communities from common basins were the most similar. However, in some instances, communities from distant sampling sites, such as from the southern and northern basins, showed high similarity (Figs. 2 and 4). The similarity between these communities may be due to the connections between them and similar environmental factors at sampling sites. Vertical mixing of water caused by homothermal conditions [51, 52] and continuous inter-basin horizontal water exchange due to wind currents [53] in Lake Baikal during spring contributed to create the associations between organisms within metacommunity. CCA showed relationship between the communities with pH and geographical location (Figs. S1, S3). Total biomass of phytoplankton correlated positively with pH and negatively with PO_4. The two clusters that were identified among microbial eukaryotic OTUs, which were inversely correlated with the two groups of environmental parameters (Fig. 8), may indicate their preference for a particular host of factors conducive for their growth and differences in strategies for their existence and functioning in the lake ecosystem. Hydrophysical processes, solar radiation, and the nutrients have been found to be major factors affecting the growth and distribution of phytoplankton in Lake Baikal [19, 54].

The phyla such as Actinobacteria, Bacteroidetes, Verrucomicrobia, Proteobacteria, and Acidobacteria were found in the photic layer of the pelagic zone of the lake (Fig. 3a). Actinobacteria are abundant in freshwater ecosystems [55], including low-nutrient oligotrophic lakes [56], accounting for as much as 50% of all bacteria in the surface layer (the epilimnion) of the lake [57, 58]. The small size of freshwater Actinobacteria protects them from predation by heterotrophic flagellates [59]. Members of Bacteroidetes probably utilize organic matter produced by different types of phytoplankton such as Bacillariophyta, Dinophyceae, Haptophyceae, Cryptophyta, Eustigmatophyceae, Synurophyceae, Chrysophyceae, and Chlorophyta (Fig. 3b). *Flavobacteria* are abundant in aquatic ecosystems during periods of high primary production [60], they are associated with phytoplankton, and also participate in the cleavage of polysaccharides [11, 12, 61, 62]. Verrucomicrobia were dominant at some stations in the northern basin (Fig. 3a). Members of Verrucomicrobia (21–55%) have been reported to be dominant in microbial communities in the euphotic zone of ultraoligotrophic Crater Lake (OR, USA) where Bacillariophyta, Chlorophyta, Chrysophyta, and Dinophyta bloomed [63, 64]. Ciliophora were distributed across pelagic zone of the lake (Fig. 5). In Lake Baikal, the biomass of plankton ciliates is correlated with the dynamics of phytoplankton blooms, and ciliates may contribute 40–80% of zooplankton biomass in the 0–50-m layer during spring [24, 65]. We identified heterotrophic nanoflagellates Cercozoa, Katablepharidophyta, Choanoflagellida, and Bicosoecida. Heterotrophic nanoflagellates in marine and freshwater pelagic ecosystems were dominated by chrysomonads and bicosoecids (20–50%), followed by choanoflagellates (5–40%), and

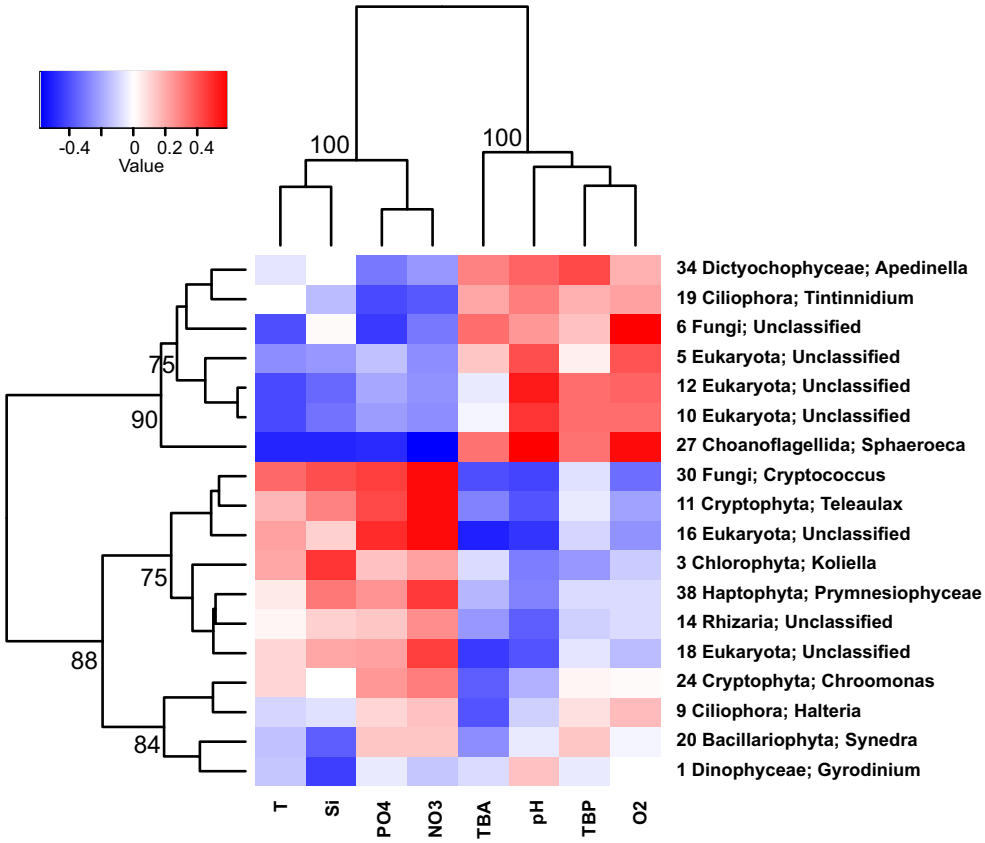

Fig. 8 A heat map showing correlation between dominant OTUs (97% sequence identity) of microbial eukaryotes with abiotic and biotic factors. All displayed OTUs have at least one correlation coefficient that is ≥ 0.35. Colors indicate the *r* values of Spearman's rank correlation coefficients. Bootstrap supports in percent are shown on the dendrogram

kathablepharids (10–25%) [66]. The highest number of Acidobacteria sequences was in the MKKh and NTN stations, located across river outlets (Fig. 3a). Acidobacteria are one of the dominant groups making up microbial communities in soil, rhizosphere [67, 68], and deep-sea [69] ecosystems. Acidobacteria (Gp6) has been previously reported to comprise 6.9% of the bacterial community in the near-bottom layer in Lake Baikal, which contains diatom cells [27]. Members of Alphaproteobacteria, such as *Rhodobacter* and Rhizobiales, had positive correlations with *Synechococcus* (Fig. 5) and *Cryptococcus* (Fig. 7), respectively. Alphaproteobacteria have been previously reported to be dominant in bacterial communities before a phytoplankton bloom in the North Sea, and their abundance was found to double during the spring phytoplankton bloom [11]. Relationships between microbes and concentrations of nutrients could pinpoint species that are involved in biogeochemical cycling [18]. The biomass of phytoplankton in the lake is dominated by diatoms [33], but analysis 18S reads the diatom contribution was small. Probably, it is necessary to use specific primers to identify diatoms.

Co-occurrence Networks of Bacteria and Microbial Eukaryotes

The structure of co-occurrence networks gave insights into the organization of microbial communities. Among the microbial eukaryotic OTUs, autotrophic eukaryotes and unclassified Eukaryota had the highest values of betweeness centrality (Fig. 6), which may point to their important role in communities. Members of the Bacteroidetes (OTU 8 and OTU 77 *Flavobacterium*, OTU 20 Sediminibacterium, OTU 13 Cryomorphaceae), Actinobacteria (OTU 53 Actinomycetales, OTU 4 and OTU 10 Actinobacteria unclassified, OTU 33 and OTU 1 *Ilumatobacter*), and Alphaproteobacteria (OTU 65 *Pelagibacter*, OTU 76 Rhizobiales, OTU 59 Acetobacteraceae) had the highest values of betweeness centrality. The highly connected taxa (hub) in network might be analogous to microbial keystone species [17]. Keystone taxa have disproportionately important roles in maintaining network structure relative to the other taxa in the network [18, 70]. The disappearance of the putative keystone taxa may cause networks to disassemble [71], and thus, keystone taxa may play a role in maintaining ecosystem stability [70].

Co-occurrence correlation analysis showed that the microbes of one domain had significant and predominantly positive correlations between each other (Figs. 5 and 6). This may indicate the existence of mature microbial communities, niche separation, and absence of competition between microbes of one domain in the lake. Co-occurrence network analysis is used to predict direct associations between taxa and can be used to determine the general preference of taxa for specific environmental factors, when environmental parameters are

included [15, 18, 72]. Microbes also may positively or negatively correlate for indirect reasons, based on their environmental preferences [73]. Phylogenetically related bacterial OTUs were positively correlated with a similar set of bacterial (Fig. 5) and microbial eukaryotic OTUs (Fig. 7). Similarly, phylogenetically related bacteria have been shown to be positively correlated with the environmental parameters of the Baltic Sea during a cyanobacteria bloom [10]. Phylogenetically related bacteria can have common preferences for specific environmental conditions [15, 17].

Flavobacterium and Cryomorphaceae were positively correlated with *Synechococcus* ($r = 0.76$ and $r = 0.65$, respectively) (Fig. 5). The abundance of Bacteroidetes has been reported to increase during the spring diatom bloom in the North Sea [11] and during the phytoplankton bloom in pre-alpine Lake Zurich (Switzerland), which indicates that bacteria consumed exudates of phytoplankton [74]. Actinomycetales were positively correlated with *Synechococcus* ($r = 0.72$) (Fig. 5). Genome-based analysis of freshwater Actinobacteria identified genes involved in carbohydrate acquisition, which points toward an important role in carbon cycling in freshwater ecosystems [75]. *Limnohabitans* and *Albidiferax* were positively correlated with *Synechococcus* ($r = 0.53$ and $r = 0.56$, respectively) (Fig. 5). *Limnohabitans* was reported to be one of the dominant among Betaproteobacteria in the epilimnion of 72 freshwater systems (lakes and ponds) with different environmental conditions [76]. It is important to note that not all algae–bacteria associations can be identified using the correlation approach, and correlations may not necessarily result from associations between populations [14].

An assumption can be made that the strong ($r = 0.83$) and significant ($\alpha = 0.05$) correlations between *Stokesia* and *Sphaeroeca* (Fig. 6) demonstrated mutualism. Heterotrophic nanoflaggelates showed positive associations with phototrophic and phagotrophic plankton species, as well as with Fungi (Fig. 6). Negative correlations may suggest competition or predation among taxa. Strong negative correlations between *Stokesia*/*Sphaeroeca* with Acidobacteria Gp6 may reflect predator–prey relationships, as these protists are phagotrophic. Bacterial consumer *Halteria* dominated among ciliates of the lake (Fig. S4). *Halteria* are dominant in ciliate communities in freshwater ecosystems with a different tropic status, as they have efficient uptake of prey of a large-size spectrum (approximately 0.4 to 5 μm) and high growth rate [77] and are important bacterial consumers in freshwater communities [78]. The negative correlations between phototrophic prokaryotes *Synechococcus* with phototrophic eukaryotes *Synedra* (Fig. 7) may suggest competition between species of these genera. *Synechocystis limnetica* and *Synechococcus* spp. are the main members of autotroph picoplankton in the lake [79, 80]. *Cyanobacteria* proliferate when the autotrophic microplankton biomass in the pelagic zone of the lake is low during spring and summer, reaching their maximum growth rate in the northern basin [79].

Mycoplankton composed of unclassified Fungi, Chytridiomycota, and *Cryptococcus* correlated with other microbes (Figs. 6 and 7) and environmental factors (Fig. 8). Aquatic fungi potentially play an important role in nutrient and carbon cycles and also interact with other organisms, which affects the food web dynamics in ecosystems [81]. We found that Chytridiomycota were positively correlated with phototrophic and heterotrophic eukaryotes, including *Koliella* ($r = 0.81$, $\alpha = 0.05$), *Apedinella*, *Cryothecomonas*, and *Spumella* (Fig. 6). Parasitic Chytridiomycota (chytrids) tend to affect freshwater phytoplankton [82]. In the meso-oligotrophic Lake Pavin and eutrophic Lake Aydat (France), hosts of various chytrids were found to be composed of diatoms, Chlorophyta, and Cyanobacteria. Through algae destruction, chytrids emit dissolved substrates for microbiological processes [83].

Conclusions

In the present study, we found that members of the microbial metacommunity had multiple positive associations within one domain during the spring phytoplankton bloom in Lake Baikal. The highly connected taxa (hub) in networks were OTUs of Actinobacteria, Bacteroidetes, Alphaproteobacteria, and autotrophic and unclassified Eukaryota which might be analogous to microbial keystone taxa. Phylogenetically related bacterial OTUs correlated with a similar set of bacterial and microbial eukaryotic OTUs. Positive correlations between some bacterial and microbial eukaryotic OTUs may serve as evidence of mutualistic interactions, whereas negative correlations may indicate competition. The structure of microbial communities was affected with geographical location.

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