Environmental drivers define contrasting microbial habitats, diversity, and community structure in Lake Baikal, Siberia

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ABSTRACT

Understanding how microbial communities respond to environmental change requires knowing the main drivers of their structure, diversity and potential resilience. Lake Baikal is the world's most ancient, deep, voluminous, and biodiverse lake, holding 20 percent of unfrozen fresh water that is undergoing rapid warming. Despite its global importance, little is known about Baikal's bacterioplankton communities and their drivers. In this extensive survey, we show that temperature, along with stratification, nutrients, and dissolved oxygen, but not geographic distance, define major microbial habitats and community similarity. Mixed layer and deep water communities exhibited contrasting patterns of richness, diversity and evenness, and comprised different cohesive modules in the whole Baikal OTU co-occurrence network. The network's small-world properties indicated likely resistance to perturbations but sensitivity to abundance changes of central, most connected OTUs. Previous studies showed Baikal water temperature rising by over 1.2°C since 1946, and this trend is predicted to accelerate. Because temperature emerged as the most significant predictor of the mixed layer community structure, we hypothesize that it is most likely to drive future community changes. Understanding how temperature and other abiotic factors structure microbial communities in this and other rapidly

29 changing ecosystems will allow better predictions of ecosystem responses to anthropogenic
30 stressors.
31

32 **INTRODUCTION**

33 The ecological importance of microorganisms in aquatic systems has been recognized at least
34 since the appearance of "ooze" in Lindeman's trophic energy transfer diagram [1]. Their central
35 place in material and energy fluxes is now recognized for nearly all nutrient cycles, with greater
36 relative importance of prokaryotic organisms in more oligotrophic systems [2]. The advent of
37 next-generation sequencing, combined with environmental monitoring, enabled new discoveries
38 of microbial diversity and function in various aquatic habitats. However, the environmental
39 drivers of microbial community diversity, community structure, function, and stability remain
40 poorly characterized in many aquatic ecosystems, including the world's most ancient (25 My)
41 lake Baikal – a UNESCO heritage site and known hotspot for endemism of its biota. Baikal is the
42 world's deepest (1643 m) and most voluminous lake, holding about 20% of world's surface
43 unfrozen freshwater [3]. Of the approximately 2600 plant and animal species in the lake, two-
44 thirds are endemic, including the dominant primary producers, phytoplankton, zooplankton
45 grazers, benthic and pelagic fish and the top predator – world's only freshwater seal [3, 4].

46 Hampton *et al.* [4] showed that Baikal water temperatures have risen by 1.2 °C over 60 years
47 in a high-resolution time series, contributing to an increase in the abundance of non-endemic
48 zooplankton and algal species, with potential consequences for nutrient cycling, food web
49 structure [3] and microbial communities. Moreover, Lake Baikal region is predicted to warm by
50 3-4°C in the next century [5], with ongoing changes likely to continue and even accelerate.
51 Because the biota of Lake Baikal, including microbial communities, are adapted to cold

52 temperatures, it may be especially vulnerable to warming. Additionally, other changing
53 environmental factors may also alter the lake's microbial communities.
54 Despite the remarkable size of this freshwater ecosystem, its uniqueness and global
55 significance as a natural evolution experiment and the single largest reservoir of unfrozen
56 freshwater, the microbial communities in the lake are just beginning to be characterized.
57 Historically, culture-dependent efforts focused on sewage runoff monitoring, with one
58 amplicon effort using a taxonomically targeted approach [6]. Culture-independent efforts have
59 explored oil seeps [7, 8], sediments, invertebrate gut [9], diatom-associated [10] and pelagic
60 communities, with the latter on Sanger [11, 12] and 454 [13–15] platforms. Kurilkina and
61 colleagues sampled a one station depth profile in September and June [14]. In both seasons, the
62 main taxonomic groups included Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes,
63 Chloroflexi, Acidobacteria, Cyanobacteria, while Caldiserica was substantially represented in
64 September only.
65 Recent culture-independent studies emphasized analyses of metagenome-assembled genomes
66 (MAGs), where contigs assembled from metagenomic shotgun sequences are binned into draft
67 genomes. While these approaches offer additional insights into genes and function, they have
68 been limited in scope to 1-5 samples per study. This lack of replication inherently limits the
69 ability to connect genomic data with environmental covariates, as well as investigate co-
70 occurrence patterns between taxa.
71 An amplicon-based survey with good spatial coverage was recently reported by Mikhailov *et*
72 *al.* (2019), where the authors examined the possible biotic and abiotic influences on prokaryotic
73 and eukaryotic communities. The survey data were from a June cruise, thus capturing the non-
74 stratified homothermic period between spring and summer phytoplankton blooms. The study

found correlation between overall community structure and environmental variables in multivariate space, as well as a few interesting cross-kingdom co-occurrences.

Here we present a comprehensive report of microbial communities during summer stratification in Lake Baikal. Our survey and analyses of microbial plankton span all three basins, from open waters to shallow bays, and multiple depths, including above and below thermocline, down to 500 m. We reveal major community composition trends that correlate with continuous environmental gradients in a spatial context. Multiple linear regression and model averaging reveals which specific environmental covariates are associated with the two most fundamental measures of diversity: richness and evenness.

We then identify strongest environmental covariates to community composition in a multivariate framework. Finally, we use co-occurrence network analyses to reveal clusters of OTUs with contrasting environmental associations and identify clades and taxa most responsible for maintaining the structure of each network cluster. We conclude by suggesting that temperature may be the main driver of the microbial community changes in Baikal in response to global environmental change.

MATERIALS AND METHODS

Our survey was guided by the recorded natural history of Baikal [3, 16, 17] that divides the lake into eight distinct regions (Fig. 1a, S1). Among them, are the open Baikal basins, Chivyrkuy Bay, Proval Bay, and Selenga river plume. Chivyrkuy Bay was sampled extensively to capture transition from the shallow innermost bay (9 m depth) to open waters. Proval Bay and Selenga plume stations represented the two most eutrophic areas in Baikal. In total, we collected samples from 24 stations, of which 10 were sampled at various depths for a total of 46 samples.

98 Temperature and dissolved oxygen profiles were measured with a YSI Instruments sonde (YSI,
99 Inc., Yellow Springs, OH, USA). Whole water was taken using a Van Dorn sampler (Wildco,
100 Inc., Yulee, Florida, USA), and 5 L were filtered onto 3 μm and 0.22 μm mixed nitrocellulose
101 acetate membranes (EMD Millipore, Billerica, MA, USA) and stored at −20°C in RNAlater
102 (Life Technologies, Grand Island, NY, USA) to capture particle-attached (3 μm filter) and free-
103 living (0.22 μm filter) fractions of microorganisms. Genomic DNA was extracted using the Mo-
104 Bio PowerSoil Kit (Mo-Bio Laboratories, Carlsbad, CA), following manufacturer's protocol.
105 Then, the V4 region of the 16S rRNA gene was amplified using 515F–806R dual-index primers
106 sequenced on a MiSeq platform (250PE), as described previously [18]. Raw sequences and
107 metadata needed to reproduce our results are available in GenBank under BioProject
108 PRJNA530071.
109 Sequences were processed generally following the *de novo* clustering mothur SOP. Statistical
110 analyses were performed in the R (3.2.2) environment, unless noted otherwise. Multiple
111 regression search and model selection were done with the glmulti package [19]. Distance
112 matrices, ordinations, and correlations with environmental variables were calculated with vegan
113 [20] and phyloseq [21] packages. We constructed the co-occurrence network using sparCC [22],
114 following recommended best practices by Berry and Widder [23]. Next, we identified network
115 modules with the optimum modularity algorithm in the iGraph package [24]. We followed the
116 WGCNA package workflow [25] to generate eigenvectors for each module and correlate the first
117 eigenvector (first principal component, PC1) with environmental variables to generate plots and
118 a heatmap (Fig. 5).
119
120 **RESULTS AND DISCUSSION**

121 **Taxonomic diversity.** At a depth of 28059 sequences per sample, we detected 38457 OTUs in
122 the combined 3.0 μm and the 0.22 μm fractions. Non-singletons (6099 in 3.0 μm and 3346 in
123 0.22 μm) were classified into 61 phyla, dominated by typical freshwater Actinobacteria,
124 Bacteroidetes, Cyanobacteria, Proteobacteria and Verrucomicrobia (Fig. 1).
125 Microbial community composition differed from some previous studies of Lake Baikal,
126 which explored pelagic communities [11–15]. In a recent effort, Kurilkina et al. [14] sampled
127 one station depth profile in September and June to reveal the main taxonomic groups as
128 Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Acidobacteria, and
129 Cyanobacteria in both seasons, and Caldiserica in September only. Our study did not detect
130 Caldiserica and revealed only a very minor presence of Chloroflexi, while showing a substantial
131 Verrucomicrobia presence (absent in Kurilkina *et al.*) in every sampled region (Fig. 1).
132 Interestingly, in the most recent amplicon survey of the lake [15], Mikhailov and colleagues
133 report Verrucomicrobia relative abundance up to 59% in integrated water column samples taken
134 across 0-25 m. The differences may be sensitive to the choice of amplicon primer sets, as
135 Kurilkina *et al.*, Mikhailov *et al.*, and present study used three highly cited but ultimately
136 different sets.
137 Our study revealed compositional differences between the 0.22 μm (free-living) and 3 μm
138 (mostly particle-attached) fractions. Actinobacteria were significantly enriched in the 0.22 μm
139 fraction (Table S1), similar to results from lakes in Michigan, USA [26]. Proteobacteria were
140 only marginally more prevalent in the 0.22 μm fraction. We also found enrichment of
141 Bacteroidetes and Cyanobacteria on the 3 μm fraction, the latter owing to filamentous taxa.
142 Indeed, the top three most differentially abundant taxa identified with permutation-based
143 analyses [27] were all classified as the Nostocales genus *Dolichospermum* and contributed up to

90% of Cyanobacteria in the 3 µm fraction. The high abundance of a nitrogen-fixing cyanobacterium confirms the significance of N limitation in the lake (O'Donnell et al. 2017).

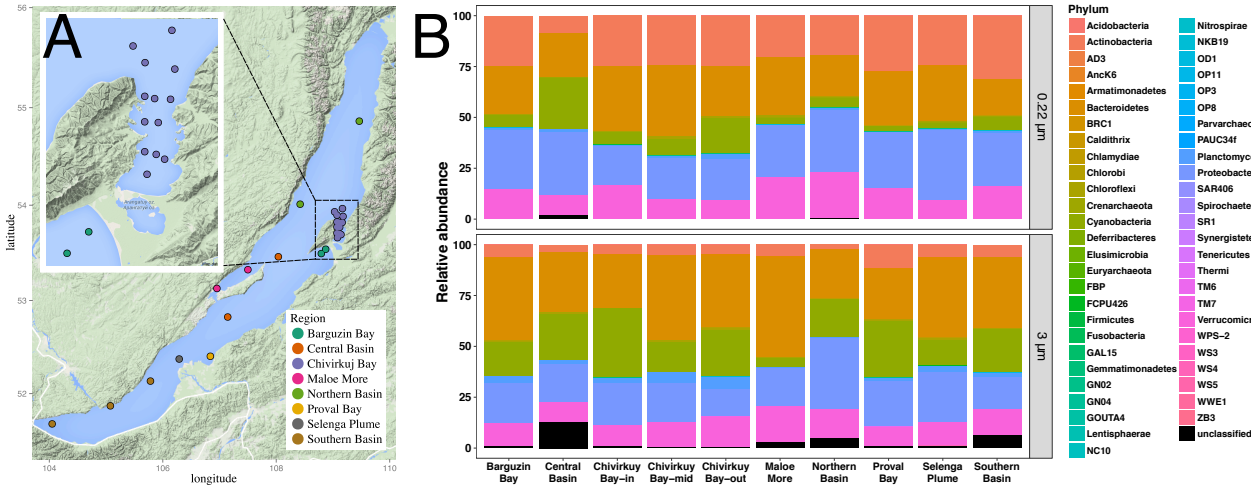


Figure 1: (A) Lake Baikal sampling locations. Stations in outside of Chivyrkuy Bay, Proval Bay, and Selenga Plume were sampled at multiple depths. (B) Taxonomic composition of the free-living (0.22 µm, top) and particle-attached (3 µm, bottom) size fractions across surface samples. While both fractions were mostly dominated by common freshwater phyla, Actinobacteria were enriched in the 3 µm fraction.

Free-living (0.22 µm) surface samples in the open Baikal were dominated by betaproteobacterium *Limnohabitans* sp. Littoral zones were more variable, represented by multiple OTUs classified as *Limnohabitans*, *Synechococcus* and *Actinobacteria* acI. Surface samples of the shallow Proval Bay and the Selenga river plume represented the extreme end of the eutrophic gradient and were dominated by Actinobacteria acI and Verrucomicrobia *Chthoniobacter*. A representative from this genus is reportedly incapable of growth on amino acids or organic acids other than pyruvate [28]. Indeed, organic acids are among the most oxidized organic compounds that can be seen in a lake and would be found in areas where labile carbon is exhausted. *Chtinobacter*, therefore was found where expected - in eutrophic areas with ample labile carbon. Together with Bacteroidetes *Sediminibacterium* and *Cytophagia*, and Betaproteobacteria *Limonhabitans* and *Polynucleobacter*, the six OTUs make up ~46% of each

164 Proval Bay and Selenga River plume's relative abundance. The deepest samples in this study,
165 collected at 500 m and 300 m, were dominated by Actinobacteria acI and acIV clades as well as
166 ammonia oxidizing Group 1a Crenarchaeota *Nitrosopumilus*. The latter has been found in
167 various environments. However, its type species is ubiquitous specifically in oligotrophic oceans,
168 albeit in the surface layer and not at 500 and 300m [29].
169
170 **Community Richness and Diversity.** Free-living community diversity showed opposing trends
171 in the upper mixed layer (ML) and deeper waters (DW). Overall, richness and evenness strongly
172 correlated with temperature in the ML and with depth in DW. For each sample, we calculated the
173 effective number of species (ENS), a measure of diversity [30–32]. Hill [30] identified ENS as
174 the number of equally-common species that yields a given value of a diversity index, such as the
175 Shannon H'. Jost *et al.* [31] have argued that because ENS scales linearly with richness of
176 equally-common species, it is the preferred metric for quantitative analyses (see Supplementary
177 Methods). We show that the ENS increased with depth in DW (Fig. 2A) and was driven by an
178 increase in evenness (Fig. 2C) with no accompanying trend in OTU richness (Fig. 2B). In the
179 ML, ENS showed a modest increase at the surface in samples collected at 0 m (Fig. 2B). Unlike
180 in DW, higher ML diversity was generated by higher OTU richness (Fig. 2B), while evenness
181 remained unaffected. Mixed layer diversity was positively correlated with temperature (Fig. 2D),
182 along with OTU richness (Fig. 2E). Deep water diversity and richness were marked by high
183 overall variability, and richness showed a marginally significant increase in samples from cooler
184 water. Evenness was not directly correlated with temperature in either layer (Fig. 2F).
185 Furthermore, in mixed layer, richness had a greater response (slope) to temperature than
186 diversity did, suggesting that samples at higher temperatures and shallower depths, while

supporting the greatest richness, were dominated by a few successful OTUs. This could be due to variable and high resource conditions leading to coexistence of more taxa, including the persistence of rare taxa.

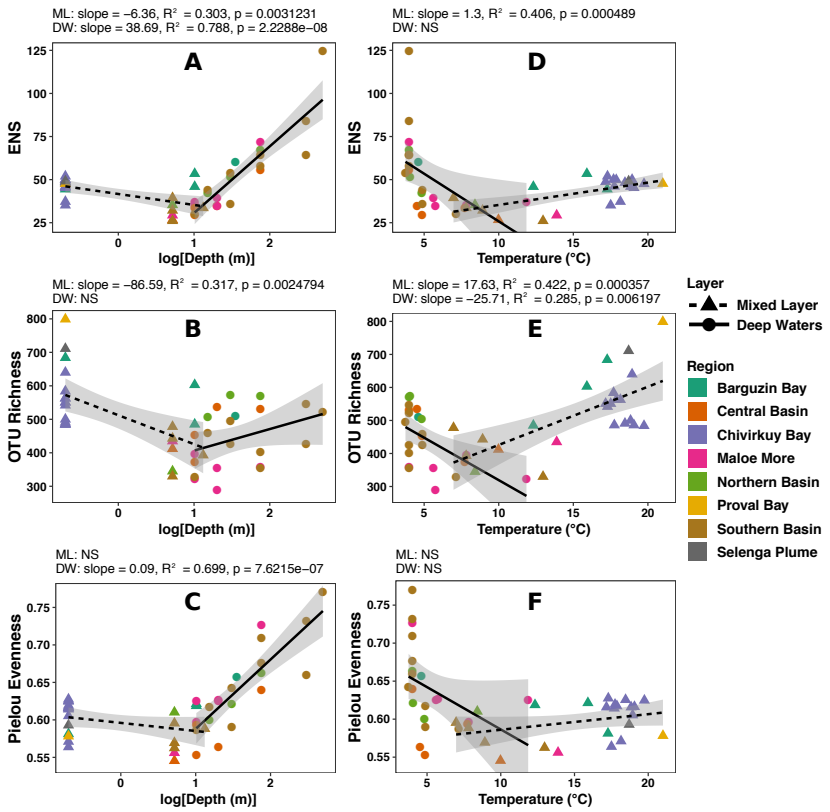
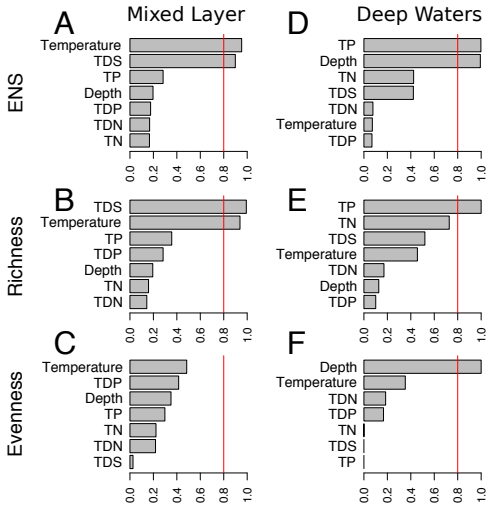


Figure 2: Diversity and evenness trends across depth and temperature in the upper mixed layer and deep waters. Effective Number of Species (ENS) was driven by richness in mixed layer and by evenness in deep waters. Effect size from Pearson correlation are above the panels.

Deep waters offer the opposite story. While richness had a significant but weak relationship with depth, ENS and Pielou evenness were very strongly positively correlated with depth (R^2=0.79, p=2.2x10^{-8}; R^2=0.70, p=7.6x10^{-7}), indicating that a substantial increase in diversity in the deep hypolimnion was not driven by richness but by an even community structure. These trends reveal an intriguing picture: warmer shallower part of mixed layer and deeper parts of the deep water layer both support more diverse (higher ENS) communities. But ML diversity, while rich in OTUs, is highly uneven, whereas the deep water communities achieve diversity by

202 increasing evenness. The trends were similar for the 3 μm size fraction (Fig. S7). To better
203 resolve underlying drivers that underpin these contrasting community features, we modeled ENS
204 diversity, richness, and evenness with measured environmental variables using iterative multiple
205 regression and model averaging [19].



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207 **Figure 3:** Model-averaged importance of environmental predictors for ENS diversity (top), OTU
208 richness (middle) and Pielou Evenness (bottom) in the upper mixed layer (left) and the deeper
209 waters (right). Tested predictors included total dissolved silica (TDS), total and dissolved
210 phosphorus (TP, TDP), and total and dissolved nitrogen (TN, TDN).
211
212 Diversity trends in the ML were different, compared with deep waters. Diversity in the mixed
213 layer correlated the most with temperature and total dissolved silica (TDS) (Fig 3A). These
214 covariates were also significant for the OTU richness, while no measured environmental variable
215 was a reliable predictor of ML evenness (Fig. 3B). Depth was not an important predictor for any
216 ML community feature (Fig. 3C), possibly because depth variation was not large. These results
217 highlighted the importance of temperature and silica in predicting the ML diversity, by elevating
218 OTU richness. In the deep waters, total phosphorus (TP) and depth correlated with ENS (Fig.
219 3D). Furthermore, only TP was a significant predictor for the deep water OTU richness, while
220 only depth was for evenness (Fig. 3E, F). Temperature, when controlling for other covariates,

221 was not an important community feature predictor in deep water. The importance of temperature
222 in ML and depth in DW was not unexpected, considering that the surveyed mixed layer spanned
223 hundreds of kilometers across all three Baikal's basins and multiple bays, where temperature
224 ranged from a median 9.5°C in open waters to 21°C in Proval Bay (Fig. S1), while deep waters
225 were characterized by a large range of depths from 10 to 500 m and a relatively constant
226 temperature environment. Additionally, nutrients appeared to associate specifically with OTU
227 richness. Dissolved silica, combined with light availability in ML, is known to promote diatom
228 growth. Diatom exudates, acting as resources, could create additional niches, enabling
229 coexistence of a greater number of OTUs. In deep waters, TP could promote OTU richness by
230 supporting higher phosphorus demands often attributed to fast-growing copiotrophic organisms
231 [33]. Logue and colleagues [34] found TP alone to be significantly correlated with OTU richness
232 in a survey of Swedish lakes. Our results suggest that eutrophication leads primarily to an
233 increase in the overall number of OTUs, but also to dominance of those with opportunistic
234 lifestyles.

235 **Multivariate trends.** Ordination of the Bray-Curtis dissimilarity matrix of the 0.22 μm
236 samples revealed clear grouping, which we designated into four significant clusters (Fig. 4A,
237 permANOVA $p<0.001$). The first cluster (eutrophic) comprised samples from shallow and warm
238 areas, specifically inner Chivyrkuy and Proval bays. The second cluster (transition) included
239 samples from the Chivyrkuy Bay to open lake Baikal gradient, the main tributary Selenga River
240 plume, and the two samples collected in Barguzin Bay – the deepest and most open of the three
241 examined bays. The third cluster (open) contained the bulk of our samples collected in open
242 waters that largely separated in ordination space along a depth gradient. The last cluster (deep)
243 comprised the three deep water samples from 300 and 500 m. *Post hoc* pairwise comparisons of

cluster centroids revealed significant differences between each cluster pair (FDR corrected $p<0.05$). Thus, the clusters broadly captured the different habitats of lake Baikal, separated by depth and the transitions between open lake waters and bays. Given the confounding effects of abiotic forcing and spatial autocorrelation, we used reciprocal causal modeling [35, 36] to test whether selection by abiotic environment or spatial dispersal/distance better explained community composition trends in mixed layer.

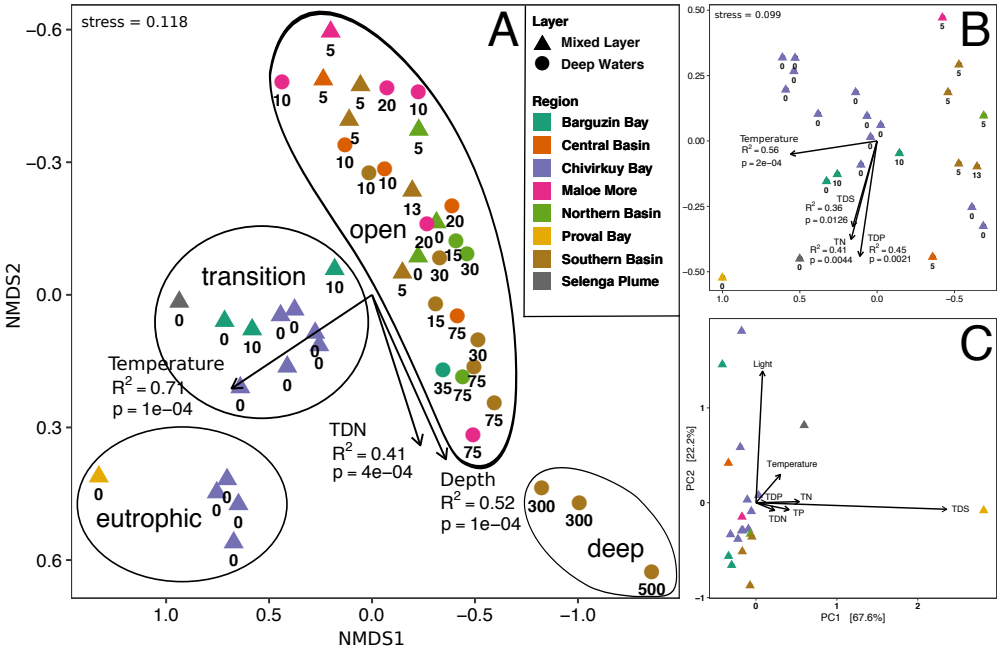

Figure 4: Ordination of the Bray-Curtis dissimilarity matrix for all 0.22 µm fraction samples (A) and the mixed layer only samples (B). (C) Principal component analysis of the mixed layer samples in environmental space using all measured covariates. In all panels, colors reflect major recognized regions of lake Baikal (15, 22). In (A) and (B), numbers below bubbles indicate sample depth (m), and arrows show correlation of environmental covariates with the layout of sample points in ordination space. Each arrow length = R^2; p-values were obtained using permutations. In (A), ellipses were drawn to aid visualization.

Environmental conditions, and not the distance, played a dominant role in structuring free-living mixed layer communities. Reciprocal causal effects models test the hypotheses for significant correlation between the community dissimilarity matrix and each of the geographic distance and environmental distance matrices, while controlling for the other. We found that,

263 when controlling for environment, geographic distance had no correlation with community
264 dissimilarity. In contrast, when controlling for geographic distance, community structure showed
265 a significant correlation with the environment (R^2=0.33, $p<1x10^{-6}$). Our results are consistent
266 with other studies in freshwater [37, 38] and marine systems [39], which usually note stronger
267 effects of environment on species composition, compared with dispersal, suggesting that
268 dispersal is likely not limiting community assembly at this scale.

269 Temperature, depth, and total dissolved nitrogen (TDN) were the strongest environmental
270 covariates with multivariate dissimilarity trends among free-living communities (Fig. 4). The
271 temperature vector (Fig 4A, R^2=0.71, $p=1x10^{-4}$) indicated the greatest temperature variability
272 along the open waters to bays gradient. We also confirmed the presence of the depth covariate
273 among samples collected in the open waters (R^2=0.52, $p=1x10^{-4}$). Interestingly, open water
274 community dissimilarities also revealed correlation with TDN in approximately the same
275 direction as depth. Because depth can confound the effects of various environmental factors, we
276 further considered an ordination of samples just from the mixed layer (Fig. 4B). Among those
277 samples, temperature was still the strongest predictor of community dissimilarity (R^2=0.56,
278 $p=2x10^{-4}$), and TDS was also significant (R^2=0.36, p=0.012). Notably, these were also the only
279 two model-averaged important predictors of OTU richness and ENS (Fig. 2A, B). However, TN
280 and TDP were also significant predictors of community dissimilarity (Fig. 4B), although they did
281 not show an association with alpha diversity metrics (Fig. 2A, B, C). As expected, depth was a
282 not a significant predictor of community dissimilarity in the mixed layer. Lastly, we investigated
283 whether the same environmental variables that predicted differences in microbial community
284 structure also accounted for major abiotic differences between sampled sites. For this, we
285 constructed a PCA of mixed layer samples using all measured environmental factors (Fig. 4C).

286 The first two principal components captured 89.8% of the variation. TDS and light had the
287 highest scores of all environmental variables. Furthermore, they were almost parallel to PC1 and
288 PC2, respectively, suggesting that the two were responsible for explaining most of the measured
289 abiotic differences between sampled ML sites. While TDS was a significant environmental
290 covariate in ordination of biotic community structure, light was not (Fig. 4B). Similarly, light
291 was not a significant predictor of biotic alpha diversity metrics either (Fig 2). TDP was the least
292 important predictor of abiotic differences in ML (Fig. 4C); however, it was the second most
293 significant predictor of biotic dissimilarity in ML (Fig. 4C). These features highlight that
294 changes in microbial community structure were not correlated with simply the most variable
295 environmental factors, and that significant associations of particular environmental factors
296 warrant attention in further studies.
297
298 **OTU co-occurrence networks.** To gain more insight into bacterial community structure in Lake
299 Baikal and its dependence on the abiotic drivers and to identify OTUs that tend to co-occur, we
300 constructed an OTU co-occurrence network. Co-occurrence networks enable data-guided
301 identification of OTU assemblages and correlation of their abundance with continuous
302 environmental variables. In contrast to ANOVA-based permutation procedures, networks do not
303 carry a user bias of arbitrarily defining sample categories, which, even if chosen wisely, result in
304 information loss.
305 Our network captured the bulk of Baikal's OTUs. We constructed a co-occurrence network
306 for OTUs present in at least 80% of samples (105 nodes and 819 edges, Fig. 5; see
307 Supplementary Methods). Importantly, although 105 OTUs appeared to be a large reduction
308 from the total >38,000 detected in Baikal, the network OTUs amounted to over >81% of

cumulative relative abundance of every sample in >85% of samples. The remainder of the samples had 63% ± 17% of relative abundance included in the networks. Environments that were better sampled had more OTUs in the networks. Lower coverage environments included the notable outliers collected at 500 m, 300 m in Southern Basin and at the surface of the eutrophic Proval Bay and Selenga River plume. Thus, with network analyses we used non-categorized continuous data to directly capture the vast majority of microbial community and environmental covariate data, revealing the dominant groups of co-occurring OTUs and their association with major environmental drivers.

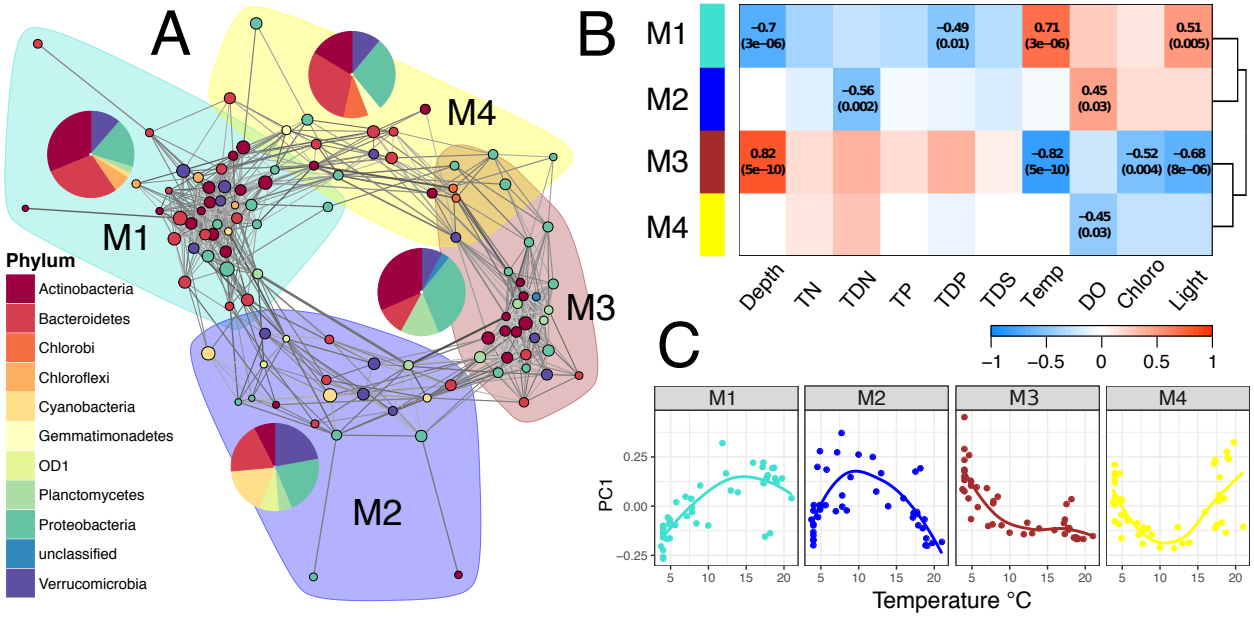

Figure 5: (A) Co-occurrence network of OTUs across all samples. Edge grayscale hue reflects pairwise correlation strength (darker edges show stronger correlations), and thickness indicates edge betweenness score. Modules were defined using maximum modularity optimization. For each module, the first principal component (eigenvector, PC1) of just that module's constituent OTUs abundance matrix was used to summarize the dominant abundance trends across sampled sites. (B) PC1 trends are summarized in a heatmap, where numbers are Spearman correlation coefficients with p-values in parentheses below. Empty cells indicate non-significant results. (C) Example PC1 trends are shown with respect to temperature.

The resulting network exhibited the "small world" properties (Fig. S10). Small world networks are characterized by high connectivity between neighboring nodes and low

329 connectivity between distant nodes [40], creating clusters or modules of consistently co-
330 occurring OTUs. Within each module, OTUs with many connections (central nodes) are thought
331 to reflect the processes that bring together module members. Small world networks may be more
332 robust to perturbations but changes to the abundances of the central, well-connected OTUs may
333 disproportionately affect the whole modules [41–43].

334 Using the optimal modularity approach [24, 44], we identified four modules of OTUs that
335 tend to co-occur across sampled sites (Fig. 5A). Strong cohesion (see Supplementary Methods)
336 within Module 1 (M1; clustering coefficient, CC_{M1}=0.68; Table S1) and Module 3 (M3;
337 CC_{M3}=0.82) suggested common drivers for the consistently co-occurring OTUs. In contrast, low
338 cohesion within Module 2 (M2; CC_{M2}=0.54) and Module 4 (M4; CC_{M4}=0.58) pointed to a looser
339 internal structure.

340 Module eigenvectors [25] of M1 and M3 revealed opposing monotonic relationships with
341 respect to environmental covariates (detailed introduction in Supplementary Methods). M1
342 showed a negative monotonic relationship with depth (Spearman ρ=-0.70, $p=3x10^{-6}$) and a
343 positive relationship with temperature (ρ=0.71, $p=3x10^{-6}$). In contrast, M3 had a strong positive
344 relationship with depth (ρ=0.82, $p=5x10^{-10}$) and a negative relationship with temperature (ρ=-
345 0.82, $p=5x10^{-10}$). As expected, the direction of the opposing trends for the two clusters was
346 reversed for light, owing to its inverse relationship with depth (Fig. 5B). Thus, we found that a
347 large fraction of Baikal's planktonic prokaryotes can be classified into one of the two cohesive
348 clusters: mixed layer "warm" M1 and deeper waters "cold" M3 cluster.

349 Modules M2 and M4 (Fig. 5a) showed inverse opposing unimodal (non-monotonic) trends
350 with depth, temperature and light (Fig. 5B, C). Weaker clustering within these modules
351 suggested less cohesion, possibly due to the inclusion of taxa with weaker habitat preferences or

352 terrestrial or riverine dispersal. M2 showed highest cumulative relative abundance at an
353 intermediate temperature of about 10°C, with a strongly positive relationship with oxygen. In
354 contrast, M4 showed a negative relationship with oxygen, abundance peaks at low and high
355 temperature extremes and minimum abundance at approximately 11°C. Specifically, M4 was
356 high in abundance in the immediate surface (0 m) and deep water, but with a sharp drop in
357 abundance at 5 m depth.
358
359 **Phylogenetic signal in modules.** Membership in the modules had a weakly significant
360 phylogenetic signal (Fig. S11). This, combined with different habitat preferences among the
361 modules, suggests that different taxonomic groups displayed preferential environmental
362 associations, thus supporting the importance of niche differences and phylogenetic niche
363 conservatism.
364 M1 and M4 had a high percentage of *Bacteroidetes*, known as opportunistic degraders of
365 high molecular weight organic matter, such as proteins and carbohydrates, with genomes
366 containing numerous carbohydrate-active enzymes covering a large spectrum of substrates from
367 plant, algal and animal origin [45]. In aquatic systems, *Bacteroidetes* have been noted to follow
368 pulses of organic matter inputs and cyanobacterial blooms [46]. This further casts M1 as a warm
369 water ML module and M4 as the loose product of sediment and terrestrial input.
370 M1 is the only module to contain members (three OTUs) of the Chloroflexi phylum.
371 Although Chloroflexi have been found in diverse environments, including oxygenated (its type
372 genus) and anoxic [47] hot springs, the CL500-11 clade – a subclass of the deep-ocean SAR202
373 clade – has been suggested as characteristic of oxygenated hypolimnia in deep lakes, including
374 Crater Lake [48], Lake Biwa [49] and the Laurentian Great Lakes [50]. However, the three

375 Chloroflexi OTUs detected in our Lake Baikal network were from the Chloroflexi class (two
376 OTUs) and Roseiflexales order (one OTU), and, as part of M1, appear to prefer warmer and
377 shallower environments in the lake.

378 The cold deep-water cluster M3 had the highest relative abundance of OTUs in unclassified
379 phyla. While the module also contained characteristically terrestrial Gammaproteobacteria
380 *Pseudomonas* and *Acinetobacter*, their low connectivity suggested they are unlikely to play a
381 central role in M3 processes.

382 M2 is the only module with Cyanobacteria; the module's three most abundant OTUs –
383 indeed, second and fourth most abundant in the entire >38,000 OTU dataset – classified as
384 *Synechococcus*. Cumulative relative abundance of *Synechococcus* OTUs peaked at ~15 m,
385 reflecting the approximate location of deep chlorophyll maximum at stratified stations. To
386 further understand internal structure of the four modules, we focused on central OTUs that
387 played important roles in creating the module structure.

388

389 **Central OTUs in each module reflect module ecology.** The meaning of a node (in our case
390 OTU) position in the network is the subject of much discussion. A central OTU has a significant
391 positive correlation with a large number of that cluster's OTUs. We offer one abiotic and one
392 biotic interpretation, resulting from two explanations for the presence of network clusters in the
393 first place. First, clusters of OTUs that correlate with important environmental covariates, like
394 temperature, could reflect abiotic habitat filtering. In this case, OTUs in modules with higher
395 clustering coefficients would more consistently reflect environmental conditions associated with
396 that module. For example, every OTU in M3, which has the highest clustering coefficient (Table
397 S1), shows clear negative relationship with temperature (Fig. S12). A second explanation

398 requires an assumption that co-occurrences reflect biotic species interactions. Then, a central
399 OTU could be hypothesized to directly increase the abundance of other OTUs, e.g., by
400 synthesizing or otherwise making available a limiting resource. It is important to point out that
401 the two explanations are not mutually exclusive and could both be parts of ecological
402 mechanisms that give rise to observed clusters of co-occurring microbial species.

403 The most globally central (most connected) OTUs belong to the most populous module with
404 the greatest number of connections (M1). The most central OTU in the network is OTU137,
405 classified as an autotrophic methylotroph LD19, which was previously reported as a summer-fall
406 bloomer in Lakes Michigan and Muskegon, MI [51]. Although not very abundant in our dataset,
407 its high centrality suggests it is indicative of an ecological process that is at least partially
408 responsible for supporting other OTUs in the ML.

409 OTU001 (*Limnohabitans*) – also in M1 – has a high overall abundance and the network
410 position that straddles the balance between centrality in M1 and connectedness to M2. Its high
411 relative abundance and ubiquity across the lake suggest important roles in lake ecology.
412 Connectedness to two modules could reflect this ubiquity and further indicate an influence of
413 OTU001 on OTUs that occupy both niches. More broadly, *Limnohabitans* genus has many
414 ecotypes. Its fine-scale phylogeny and functioning, shown experimentally with isolates [52],
415 revealed diverse lifestyles. In fact, three OTUs out of 105 in our network were classified as
416 *Limnohabitans*, and one of the other OTUs (OTU104) was in the hypolimnetic M3. Separation of
417 different *Limnohabitans* OTUs into M1 and M3 with opposing spatial occurrences and
418 environmental preferences suggests they are indeed ecotypes [53] with distinct functions. Future
419 studies can experimentally assess *Limnohabitans* ecotype responses to temperature and other
420 environmental drivers.

421 The most connected OTU in the hypolimnetic M3 was Planctomycetes OTU022, classified
422 into the Phycisphaerales order. Little is known about the ecological role of Planctomycetes in
423 aquatic environments [46]. The order is known for its distinct visual appearance, and a recent
424 report of a 3D reconstruction of Phycisphaerales cellular membrane revealed characteristic deep
425 invaginations in the cellular envelope [54]. It is possible the increased surface area of
426 Phycisphaerales becomes useful in the oligotrophic hypolimnion of Baikal. In contrast to the
427 most central but not abundant OTU in M1, OTU022 is also the most abundant in M3, which is
428 the most tightly clustered module in the network (Table S1). These data suggest Phycisphaerales
429 may substantially contribute to nutrient cycling below the thermocline with direct impacts for the
430 rest of M3.

431 The most recent amplicon survey of Baikal included co-occurrence network analyses of
432 prokaryotes from the photic layer [15], calculated across samples collected at integrated depths
433 from 0 to 25 m. Authors reported a similar phylogenetic makeup in a network that was
434 characterized by predominantly positive interactions. Our sampling plan comprised separate
435 samples from discrete depths in both the photic and abyssal layers, making it possible to resolve
436 network clusters associated with the different conditions within the water column.

437 In summary, our survey of Lake Baikal's bacterioplankton revealed that temperature, along
438 with nutrients, are the major drivers of the microbial community composition and diversity,
439 consequently, the anthropogenic changes in these factors would likely significantly alter
440 planktonic microbial communities. The OTU co-occurrence network analysis identified two
441 major clusters, associated with the upper mixed layer and deep waters, with a detectable
442 phylogenetic signal in each cluster composition. The "small world" properties of the network
443 suggest that the communities may be resilient to perturbations but the changes in abundances of

the central OTUs may result in network rearrangement and substantial community shifts. Further studies, including controlled field and laboratory experiments, are needed to experimentally test the effects of different temperatures and other abiotic factors on the structure and diversity of microbial communities in Lake Baikal.

Conflict of Interest Statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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