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Marine signature taxa and core microbial community stability along latitudinal and vertical gradients in sediments of the deepest freshwater lake

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Running title: Benthic microbial communities of Lake Baikal

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Lake Baikal is the deepest (~1.6 km) and most voluminous freshwater reservoir on Earth. Compared to plankton, its benthos remains poorly explored. Here, we ask whether latitude and/or depth determine benthic microbial community structure and how Baikal communities compare to those of other freshwater, brackish and marine sediments. To answer, we collected sediment upper layers (0-1 cm) across a ~600 km North-South transect covering the three basins of the lake and from littoral to bathybenthic depths (0.5 to 1450 m). Metabarcoding of 16S and 18S rRNA genes revealed communities with high richness and evenness where rare operational taxonomic units (OTUs) collectively dominated. Archaea represented up to 25% of prokaryotic sequences. Baikal sediments harbored typically marine eukaryotic and prokaryotic OTUs recently identified in some lakes (diplonemids, Bolidophyceae, Mamiellales, SAR202, marine-like *Synechococcus*, Pelagibacterales) but also SAR324, Syndiniales and Radiolaria. We hypothesize that, beyond the salinity barrier, adaptation to oligotrophy explains the presence of these otherwise typically marine lineages. Baikal core benthic communities were relatively stable across sites and seemed not determined by depth or latitude. Comparative analyses with other freshwater, brackish and marine prokaryotic sediment communities confirmed the distinctness of Baikal benthos, which include elements of similarity to marine and hydrothermally-influenced systems.

Keywords: Lake Baikal; benthos; 16S/18S rRNA metabarcoding; archaea; bacteria; protist; marine-freshwater transition

45 Lake Baikal in Southern Siberia is the oldest (~30 Myr), deepest (~1650 m maximum and 750 m and
average depth), and largest (by volume, ~23000 km³) freshwater lake on Earth. This unique ecosystem
is akin, in several respects, to sea environments [1]. Its surface freezes in winter, which triggers coastal
downwelling and deep-water ventilation. Consequently, its water body remains cold (~4°C), oxygen-
rich and ultra-oligotrophic [2]. Deep, cold and associated with a rifting zone, Lake Baikal host methane
50 hydrates [3]. Geographically, the Academician Ridge and the Selenga river delta delimitate three basins:
Northern, Central, and Southern [1]. Lake Baikal hosts many endemic metazoan species [2]. Molecular
analyses of prokaryotic (e.g.[4-6]) and microbial eukaryotic (e.g.[7-9]) diversity have been mostly
carried out in plankton. Comparatively, benthic microbial communities remain poorly known, with few
studies of archaea and bacteria in bottom, often seepage-influenced, sediments [10] and of sediment-
55 associated eukaryotes [7]. Here, we asked how benthic communities vary spatially along latitudinal and
vertical gradients in Lake Baikal and how they compare to those of other freshwater, brackish and
marine ecosystems. To answer, we carried out a comparative study of Baikal benthic prokaryotic and
eukaryotic microbial communities across a ~600 km latitudinal North-South gradient traversing the
three lake basins and from surface (littoral sediment) to the greatest depths (>1400 m) using a
60 metabarcoding approach (Fig.1A; Supplementary Text). We purified DNA from the upper sediment
layer (ca. 0-1 cm) and massively sequenced amplicons of 16S (V4-V5 region) and 18S (V4 region) rRNA
genes. High-quality reads clustered in 33943 prokaryotic and 3351 eukaryotic SWARM-derived
operational taxonomic units (OTUs), which did not reach saturation in accumulation curves
(Supplementary Fig.1; Supplementary Text). Computed from rarefied sequence matrices, eukaryotic
65 and, especially, prokaryotic communities exhibited high richness, diversity and evenness scores
(Supplementary Tables 1-2). Therefore, Baikal sediments seem to harbor complex communities with
no clear dominant species.

Archaea reached up to 25% relative abundance and, on average, 20% of the OTUs (Supplementary
Fig.2). They encompassed diverse phyla, consistent with recent studies of seepage areas [10], with
70 DPANN (Woesearchaeota and Pacearchaeota, now included in Nanoarchaeota) and TACK
(Nitrososphaeria-Thaumarchaeota and, in some samples Bathyarchaeota) being the most abundant,
followed by Euryarchaeota (Thermopfundales/Marine Benthic Group-D and locally
Methanofastidiosales/WS2A) (Fig.1A; Supplementary Figs.3-4; Supplementary Table 3). Free-living
Thermopfundales and Thaumarchaeota might be potential hosts for the parasitic/symbiotic DPANN
75 [11]. Bacteria comprised a wide variety of phyla likely involved in complex N, S and C cycling. PVC
members (Verrucomicrobia and Planctomycetes) and Acidobacteriota were the most relatively
abundant, followed by FCB (notably Bacteroidota, Latescibacteria, Gemmatimonadota),
Proteobacteria, Chloroflexi and Nitrospinota (Fig.1A; Supplementary Fig.5). Proteobacteria (ca. 15%)

were little abundant compared to lake or marine surficial sediments, but akin subseafloor sediments
80 [12]. Some biomass-degrading lineages, e.g. Phycisphaerae [13], Ignavibacteria [14] or Latescibacteria
[15], suggest the contribution of thermophilic or seepage-associated microbes to Baikal benthic
communities, possibly along faulting zones. Benthic protists were dominated by Stramenopiles and
Alveolata (Fig.1A). Alveolates comprised mostly ciliates, dinoflagellates and Syndiniales (Supplementary
Table 4). Stramenopiles included heterotrophic lineages (labyrinthulids, amphifilidae/thraustochytrid-
85 like, oomycetes, MAST) and ochrophyte algae (chrysophytes, diatoms), which sediment from the water
column [16]. Compared to prokaryotes, benthic protists were rare in seeping zones (BK22S), as
obtaining amplicons was difficult. The abundance (percentage of reads) and diversity (percentage of
OTUs) patterns of rare versus abundant OTUs across phyla were rather similar for eukaryotes and rare
prokaryotic OTUs, whereas the diversity of abundant prokaryotic OTUs was remarkably constant across
90 sites (Supplementary Figs.6-7), suggesting a relatively stable core of dominant OTUs.

To see whether the depth and/or the latitude determined benthic microbial community structure,
we performed an NMDS analysis based on dissimilarity matrices of OTU frequencies. However, we
observed no obvious pattern discriminating samples according to basin or depth (Fig.1B). Although two
samples of intermediate depth (100-800 m) appeared to segregate on along axis 1, surface (<100 m)
95 and deep (>800 m) samples appeared mixed. This pattern was almost superimposable to that observed
for prokaryotic OTUs, whereas eukaryotic OTUs seemed to segregate better surface from deep samples
(Supplementary Fig.8). PERMANOVA analyses confirmed no significant discrimination of prokaryotic
and eukaryotic communities at the OTU level by latitude and only marginal significance for depth
(prokaryotes, $R^2=0.23$, $p=0.01$; eukaryotes, $R^2=0.19$, $p=0.07$) (Supplementary Table 5). Since this
100 marginal effect of depth might be due to the large collective dominance of rare OTUs (~40% prokaryotic
and ~85% eukaryotic OTUs; Fig.1C, upper-right inset), we also carried out NMDS and PERMANOVA
analyses on i) dissimilarity matrices at high-rank taxon, rather than OTU, level, ii) Jaccard distance
matrix, iii) Unifrac distance matrix and iv) Null model Raup-Crick index. Again, prokaryotic and
eukaryotic communities did not segregate by depth (Supplementary Figs.9-12). Phyla and depth
105 categories did not significantly correlate for eukaryotes and only marginally for prokaryotes
(Supplementary Tables 5-6). This situation contrasts with that of stratified plankton communities [8]
and might be due to similar sediment environmental conditions, notably temperature, across lake
depths. We detected a core of 44 prokaryotic OTUs shared by all the sediment samples that
represented between ~15% and ~40% of prokaryotic abundance (Fig.1C). Their phylogenetic affiliation
110 fitted the overall prokaryotic phyla distribution, suggesting that, accompanying widely diverse rare
OTUs, there is a stable core of benthic prokaryotic communities across basins and depths in Lake Baikal.

At odds with the idea that marine-freshwater transitions rare [17], we identified 332 OTUs belonging to typical marine taxa across the lake (Fig.2A; Supplementary Table 7). Several of them belonged to groups already observed in freshwater systems, such as MAST clades, relatively abundant in Baikal, but also rarer diplomonads, Radiolaria (for which we identified several OTUs; Supplementary Fig.13), Mamiellales, Bolidophyceae and, among bacteria, marine-like *Synechococcus*, SAR202 Chloroflexi and Pelagibacterales. Some of them were recently observed in Lake Baikal [8, 9, 18]. Others have never been reported in freshwater ecosystems, such as diverse Syndiniales and SAR324 Deltaproteobacteria, metabolically flexible sulphur metabolizers often associated with submarine hydrothermal plumes [19]. To eliminate potential cross-contamination with marine samples during collection, handling or sequencing, we mined for other typical abundant marine taxa in our datasets. We detected neither thraustochytrids nor *Prochlorococcus* and *Alteromonas* (Fig.2A), reinforcing the conclusion that Baikal 'marine' OTUs are indeed autochthonous. Our study confirms and extends the presence of several typically marine prokaryotes and eukaryotes at low abundances in Lake Baikal, reinforcing the idea that transition frequency between marine and freshwater habitats is underestimated [20]. This opens questions about the specific molecular adaptations to the very low salinity of Lake Baikal salinity (0.0 PSU) and suggests that oligotrophy and deep waters might be more important drivers than salinity for these lineages.

How do benthic Baikal communities compare to those of other aquatic ecosystems? Does the presence of Baikal marine taxa indicate intermediate ecological features between freshwater and marine environments? To answer, we retrieved 16S rRNA gene metabarcoding data from other freshwater, brackish and marine sediment samples (Supplementary Text; Supplementary Tables 8-9). An NMDS plot based on the high-rank taxa dissimilarity matrix showed that Lake Baikal sediment samples clustered away from other samples, albeit closer to freshwater systems (Fig.2B). PERMANOVA analyses revealed a significant effect of the size and salinity category ($R^2 = 0.45$; p -value = 10^{-4}) (Supplementary Table 5), partly driven by Acidobacteriota, PVC, Nitrospirota and Chloroflexi (Fig.2B-C). Therefore, the comparison of communities associated to upper-layer sediment in Lake Baikal and other benthic ecosystems across different depth and salinity ranges set it apart from other freshwater and marine systems.

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Author contributions PLG, DM and NVA designed the work and organized the limnological cruise. PLG, GR, NVA and KEV collected sediment samples. PB and GR purified DNA and carried out PCR reactions for metabarcoding. GR carried out the bioinformatic analysis of amplicon sequences, statistical analyses and wrote an early draft of the manuscript. PLG wrote the final manuscript. All authors read, critically commented and approved the final manuscript.

Compliance with ethical standards

155 **Conflict of interest** The authors declare that they have no conflicts of interest.

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Figure Legends

Fig.1. Sampling points and overall prokaryotic and eukaryotic diversity in Baikal sediments. **A**, Bathymetric map of Lake Baikal showing the sampling sites and depths along the three major basins of the lake extending along the North-South latitude axis (left panel). Relative abundance of clean merged reads (CMRs) representing the major prokaryotic and eukaryotic taxa for each sampling location. The asterisk shows the average diversity derived from two replicates from the same sampling site, after nested PCR amplification. **B**, Non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities based on OTU frequencies of both prokaryotic and eukaryotic OTUs. Each point represents a different sample. Ellipses enclose all points per depth category: shallow (<100 m), medium (100-800 m), deep (>800 m). Samples from the different Baikal basins are indicated with different marker shapes. NMDS for only prokaryotic and eukaryotic communities are presented in Supplementary Figure 8. **C**, UpSetR plot (central panel) showing the number, phylogenetic affiliation (taxa indicated in bold in A) and relative abundance of OTUs within the core prokaryotic community shared by all Baikal sediment samples (left bar) or all the sediment samples but one (light grey dot; bars on the right). The bottom-left histogram shows the relative proportion (CMRs) of the prokaryotic core community in the total prokaryotic community of each sediment sample. The upper right inset shows the total number of shared prokaryotic and eukaryotic OTUs per groups of sediment samples.

Fig.2. Marine signature taxa and comparison of Lake Baikal with other freshwater, brackish and marine sediment communities. **A**, Presence (light blue) / absence (white) matrix of typical marine taxa identified in Lake Baikal sediments. Each row represents a sampling location and each column a taxon. The barcharts represent the sum of the detected CMRs (dark red) and OTUs (light red) per typical marine taxon (top) and sampling location (right). **B**, NMDS of sediment samples based on Bray-Curtis dissimilarities of bacterial high-rank taxa. Colored ellipses and symbols correspond to Baikal (light blue squares), other freshwater sediments (light green squares), brackish (red dots) and marine (dark blue triangles) sediment samples. **C**, Diversity barchart displaying the relative abundance of bacterial sequences in the different sediment samples (left) and the dendrogram (right) resulting from the corresponding clustering analysis based on the Bray-Curtis dissimilarity. Dendrogram leaves represent the NMDS points depicted in (B).

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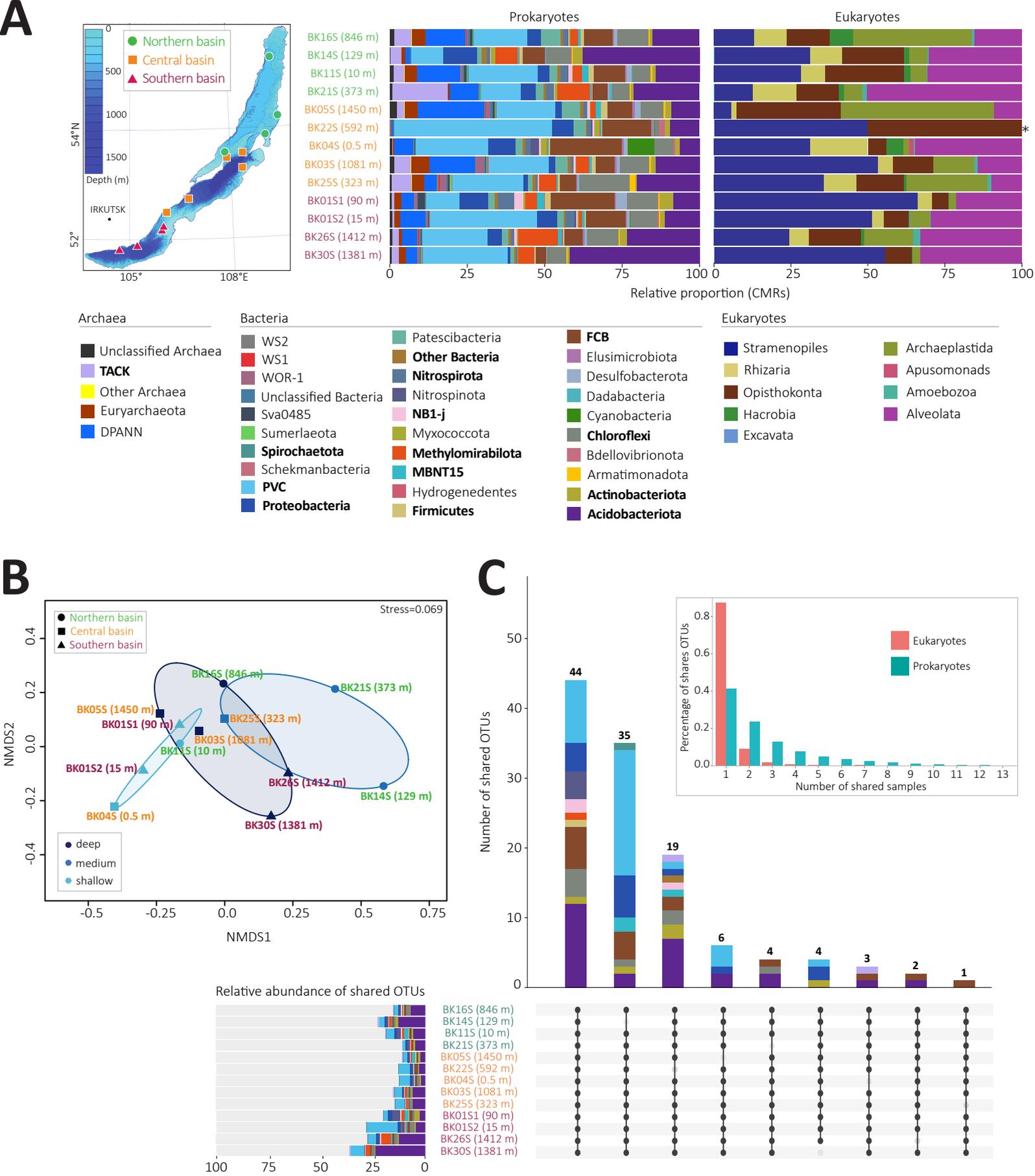


Figure 1. Reboul et al.

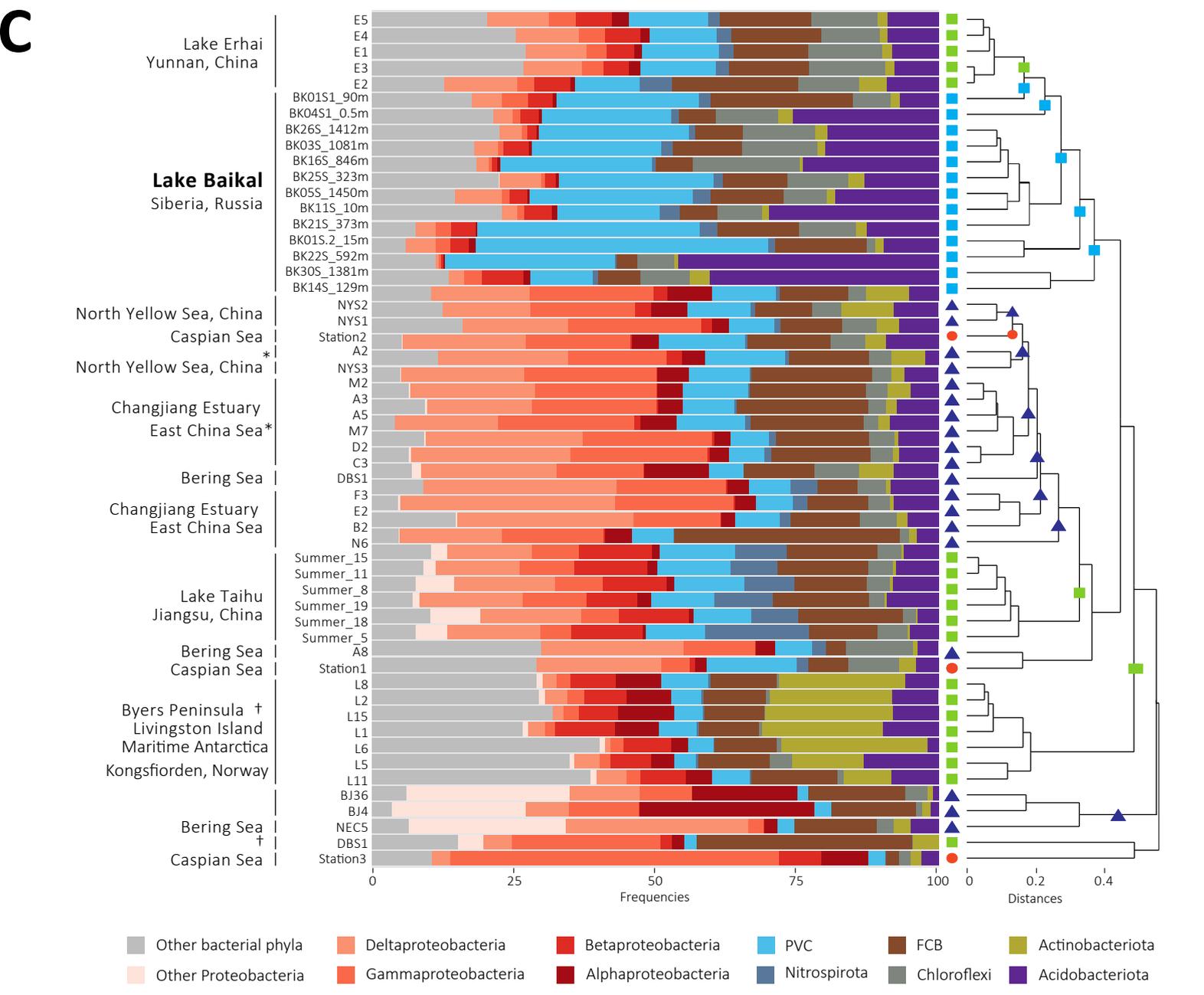
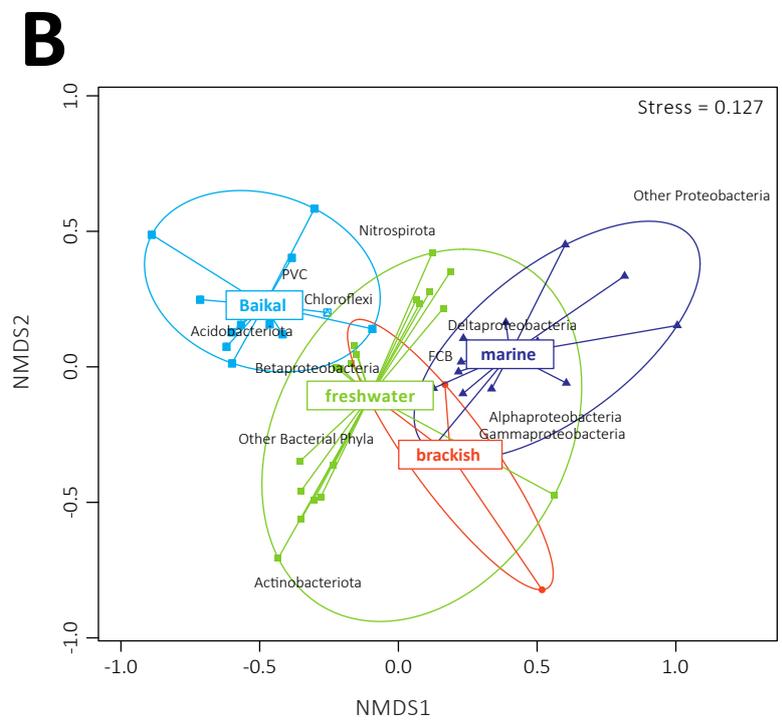
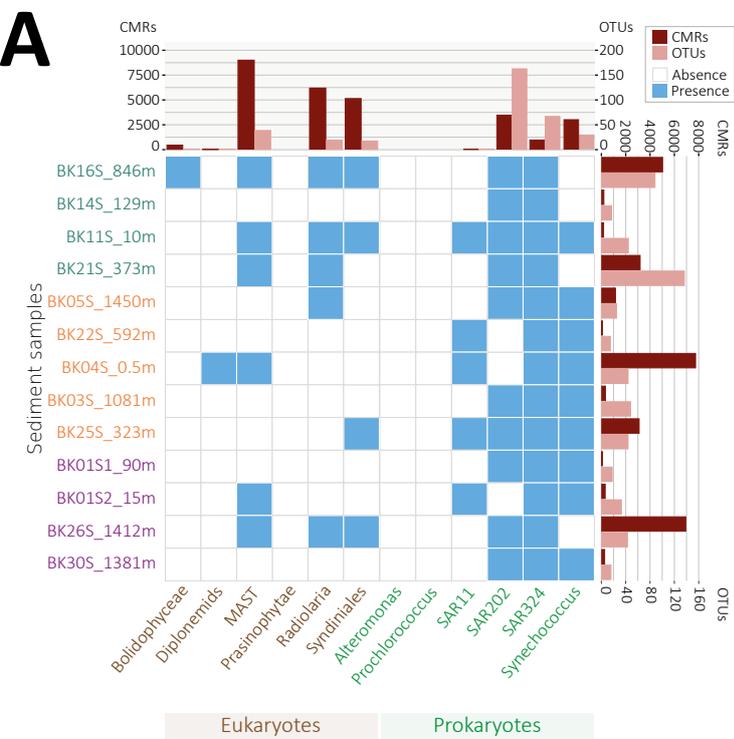


Figure 2. Reboul et al