# Comparative Analysis of Microbial Community Composition in Tropical Aquatic Ecosystems

**Hazrat Ali[1], Mohammad Moniruzzaman[2], Nafisa Azmuda[1], Nihad Adnan[1], Anowar Khasru Parvez[1], Abdul Khaleque[3], Salma Akter[1] & Md. Firoz Ahmed[1]**

Affiliations:

1. Department of Microbiology, Jahangirnagar University, Dhaka, Bangladesh.
2. Department of Marine Biology and Ecology, Rosenstiel School of Marine, Atmospheric, and Earth Science, University of Miami, USA.
3. Department of Biochemistry and Microbiology, North South University, Dhaka, Bangladesh.

* Corresponding author

Email: firoz@juniv.edu (MFA)

## Abstract

Aquatic ecosystems provide vital ecological services to global health through facilitating biogeochemical cycles, providing water for drinking & industrial usage, supporting fisheries, and preserving biodiversity. Critical elements such as climate change and growing anthropogenic pressures are causing changes in microbial communities, leading to far-reaching consequences for human health. Systematic analysis of microbial communities based on overall genomic diversity reveals the interplay between microorganisms and environmental factors, responsible for indicative features of aquatic ecosystems. In this study, we conducted a 16S and 18S rRNA gene amplicon-based metagenomic analysis across four types of tropical aquatic habitats. 16S data showed the bacterial phylum Pseudomonadota dominated 38% to 83% of the total prokaryotic communities with *Limnohabitans* and *Marinobacterium* being the most abundant genera across all the aquatic habitats except for the pond, which was dominated by the phylum Bacteroidota around 42% with the genera *Macellibacteroides*. For eukaryotic communities, 18S data showed that a phylum of single-celled fungi Cryptomycota was the most dominant in the pond, brown algae Ochrophyta was dominant in around half of the canal and lake ecosystems. Conversely, the

green algae Chlorophyta was the predominant eukaryotic phylum in marine ecosystems. *Poteriospumella*, *Spumella* and *Chrysamoeba* were the most abundant eukaryotic genera across all habitats, while more than 60% of eukaryotic genera remained unclassified, particularly in marine samples. Our findings provide a comprehensive picture of the diverse freshwater and marine microbiomes, highlighting the differential abundance, taxonomic distribution, community structure, and potential functional roles of microbial assemblages across diverse tropical aquatic habitats. These patterns are influenced by environmental factors and geographic location, laying the foundation for future ecological and conservation studies. Moreover, understanding these microbial communities can offer valuable insights into ecosystem health and potential pathogen reservoirs, contributing to improved strategies for environmental monitoring and public health protection.

***Keywords:*** *Tropical aquatic ecosystem, Microbial diversity, 16S and 18S metagenome, Freshwater & Marine microbiome, Geographic microbial variability.*

# 1. Introduction

Aquatic ecosystems are complex environments harboring diverse microbial communities that drive fundamental biogeochemical processes and provide vital services(1,2). Microorganisms, including bacteria, archaea, and microscopic members of the eukaryota, are significant elements of aquatic ecosystems(3). They play a critical role in biogeochemical cycling pathways, transportation of nutrients, and mitigation of pollutants, driving most processes in the natural world, including nutrient cycling and energy flow(4,5).

Understanding aquatic microbial ecology is crucial for several reasons. Firstly, microorganisms respond quickly to environmental changes, making them key indicators of ecosystem health(3,6). Secondly, they play a vital role in ecological processes such as the biodegradation of pollutants that impact water quality(7–9). Lastly, they are essential for the maintenance of healthy aquatic ecosystems. In addition to understanding

the ecology of these microorganisms, it is also important to study other environmental matrices, as they provide the context in which these microorganisms exist and interact(10,11). For instance, water quality parameters can influence microbiome assemblage, and different water bodies and sediments can host different functional microbial communities(12,13). The study of aquatic microbial ecology has reached a significant milestone with the advent of new technologies such as high-throughput sequencing technologies, multi-omics, bioinformatics, and their integrated analysis(14–16). These advancements have allowed for a more in-depth understanding of microbial impacts on ecological restoration.

Bangladesh, a deltaic nation crisscrossed by an intricate network of rivers, estuaries, and coastal regions, hosts a diverse array of aquatic habitats(17). These water bodies are integral components of the country's ecosystem, impacting both its economy and ecological balance e(18,19). Despite their ecological and economic significance, comprehensive studies on microbial communities in these environments remain limited. Few existing research has largely focused on individual water bodies or employed traditional methods, such as culture-based studies, which fail to capture the full diversity of microbial communities(20–24). However, integrated molecular studies across diverse aquatic habitats in Bangladesh are scarce, leaving gaps in our understanding of microbial ecology and its interaction with environmental factors. So, we focus on several distinct types of aquatic environments within Bangladesh like ponds, canals, lakes, and marine, aiming to unravel their microbial communities and ecological patterns.

These ecosystems were selected for their ecological diversity and contrasting physicochemical characteristics. Urban ponds, for example, are frequently exposed to nutrient enrichment and anthropogenic pollution, while ponds within university campuses or protected zones offer relatively undisturbed conditions (24,25). Lakes influenced by waterfalls and canals linked to river networks serve as reservoirs with varied hydrological and nutrient profiles (26–28). In contrast, coastal habitats connected to the Bay of Bengal are shaped by tidal forces and saline intrusions, supporting unique microbial assemblages adapted to fluctuating salinity and organic matter inputs (15,29,30).

Firstly, a pond is a small, stationary body of water within an area with or without external/anthropogenic disturbances(31). Ponds, often nestled within urban landscapes, serve as microcosms of aquatic ecosystems(32,33). These small, shallow water bodies exhibit varying sizes and harbor diverse biotic communities, including microbial populations, algae, aquatic plants, and invertebrates (34). Abiotic factors such as water quality, temperature, and nutrient availability influence their dynamics (25,35). Urban ponds in Bangladesh often experience nutrient enrichment and contamination due to anthropogenic activities, impacting microbial diversity and function(24,25). Ponds within universities or restricted areas offer unique research opportunities, allowing scientists to explore microbial diversity, nutrient cycling, and ecosystem functioning (23,36). Secondly, canals and lakes, which act as natural reservoirs, are frequently subject to eutrophication and varying pollutant loads (26). Natural waterfall-associated lakes, often originating from mountain streams, play a vital role in maintaining the hydrological balance as sources or recipients of waterfall outflows, contributing to the overall ecosystem (37–39). These lakes receive direct water input from waterfalls, influencing their water levels and chemistry. Rich in biodiversity, these dynamic systems support aquatic life, including fish, amphibians, and invertebrates (27,28). Lastly, the coastline along the Bay of Bengal connects it to the vast Indian Ocean, shaped by tidal dynamics and anthropogenic pressures, exhibits unique microbial assemblages influenced by salinity, nutrient availability, and pollution (15,29). Water quality and pollution significantly influence microbial community structure in aquatic ecosystems (8,30). Although a number of studies have reported on water quality and microbial diversity within individual aquatic habitats in Bangladesh, comprehensive comparative analyses across diverse ecosystem types remain scarce (22,40). Such comparative investigations are crucial for disentangling both the shared and environment-specific patterns of microbial community composition, which are shaped by variations in physicochemical parameters, hydrological connectivity, and anthropogenic pressures. A deeper understanding of these microbial assemblages is fundamental not only for advancing ecological theory in tropical aquatic systems but also for informing ecosystem management, improving water quality surveillance, and assessing potential risks to public health (32–35,41,42).

This study addresses these knowledge gaps by employing next-generation sequencing (NGS)-based 16S and 18S rRNA gene amplicon metagenomics to investigate microbial community composition across ponds, lakes, canals, and marine environments in Bangladesh. The use of 16S and 18S rRNA gene markers allows for a high-resolution and culture-independent assessment of both prokaryotic and eukaryotic microbial communities, capturing the breadth of taxonomic diversity often overlooked by traditional methods (43–46). This dual-marker approach not only enables a comprehensive perspective on ecosystem-wide microbial assemblages but also facilitates comparisons of cross-domain community structures under varying environmental conditions (47). Moreover, the integration of microbial community profiles with physicochemical parameters enhances the ability to identify key ecological drivers of community composition and diversity, offering critical insights into the processes shaping aquatic microbial ecosystems in tropical regions. The findings of this research are particularly relevant for public health, ecosystem management, and biodiversity conservation in Bangladesh. For example, understanding microbial communities in urban ponds can inform strategies for mitigating the impacts of nutrient pollution, while insights from coastal microbial ecology can aid in managing fisheries and preserving marine biodiversity. By applying high-throughput sequencing and advanced bioinformatics tools, this study offers a novel framework to explore species-environment relationships in Bangladesh's aquatic ecosystems, providing critical data for sustainable management and ecological restoration efforts. This research not only bridges the gap in microbial ecological studies in Bangladesh but also sets a foundation for future investigations on the role of microorganisms in maintaining ecosystem services, resilience to environmental changes, and responses to anthropogenic pressures.

# 2. Materials and methods

## 2.1 Study site and sample collection

Water samples were collected in March 2022 from eight strategically selected sites representing four distinct aquatic ecosystems in Bangladesh: pond (BD1, BD5), canal (BD2, BD8), lake (BD3, BD4), and marine (BD6, BD7) environments (Fig 1). The sites spanned five geographically and anthropogenically diverse locations, capturing a gradient of pollution and human disturbance. At each site, 1-liter surface water samples (~30 cm depth) were collected using sterile polypropylene bottles (PP-5). Physicochemical parameters including pH, temperature, total dissolved solids (TDS), and salinity (NaCl concentration) were measured in situ using a portable multiparameter meter (HI98194, Hanna Instruments, USA). Samples were transported to the laboratory under aseptic conditions within 24-48 hours, maintained at 4 °C in insulated containers for downstream analyses (S1 Table).

## 2.2 Sample processing and DNA extraction

Upon laboratory arrival, samples were pre-filtered using Whatman No. 1 filter paper to remove large particulate matter. Subsequently, 1 liter of each sample was passed through a 0.2 µm membrane filter using a Millipore filtration unit. DNA was extracted directly from the 0.2 µm membrane filters using the DNeasy PowerWater Kit (Qiagen, USA) according to the manufacturer's protocol. DNA concentration and purity (A260/280) were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The extracted DNA was sent to EzBiome (USA) for high-throughput sequencing targeting the 16S and 18S rRNA genes.

## 2.3 PCR amplification and next generation sequencing

A two-step PCR protocol was employed. The V3–V4 region of the 16S rRNA gene was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACHVGGGTATCTAATCC-3′), while the V9 region of the 18S rRNA gene was amplified using primers 1391F (5′-GTACACACCGCCCGTC-3′) and EukBr (5′-TGATCCTTCTGCAGGTTCACCTAC-3′), following previously published protocols (22). In the second PCR step, Illumina Nextera XT adapters and dual indices were incorporated. Libraries were sequenced using the Illumina MiSeq platform with 2×300 bp paired-end reads.

## 2.4 Bioinformatics and statistical analysis

Raw sequence quality was assessed using FastQC (v0.12.1)(48). Trimming of low-quality reads was performed with Trimmomatic (v0.39.1) (49), using the parameters: HEADCROP:7, LEADING:20, SLIDINGWINDOW:4:20, TRAILING:25, and MINLEN:50 (Phred score cutoff: $Q < 20$). Processed reads were analyzed in QIIME2 (v 2024.10) (50) using the VSEARCH pipeline (51) for read merging, dereplication, OTU clustering (99% similarity), and chimera removal. Taxonomic classification was performed using a Naïve Bayes classifier trained on the SILVA_138.2_SSURef_NR99_tax database (52,53). Annotation was conducted separately for 16S and 18S rRNA datasets using the classify-sklearn algorithm. Functional potential of microbial communities was predicted using PICRUSt2 (v2.6.1) (54) for both 16S and 18S data. For the latter, FUNGuild (v1.1) (55) was additionally applied to account for eukaryotic profiles. All statistical analyses and visualizations were performed in R (v4.4.2) using RStudio (v2024.09.1+394). Mapping and visualization of sampling sites and Sankey diagrams were generated using the *"maps"*, and *"highcharter"* packages (56,57). The Venn diagram was drawn using the *"ggvenn"* package to show the shared OTUs at the species level among ecosystems (58). Alpha diversity indices (Shannon, Chao1, Inverse Simpson, Pielou's evenness) were calculated using *"phyloseq", "vegan", "ggplot2",* and *"ggpubr"*. Group differences were tested using Kruskal-Wallis and one-way ANOVA, followed by Tukey's

HSD post-hoc analysis. Beta diversity was assessed using Bray-Curtis dissimilarities, with group separation tested using PERMANOVA (adonis2, *"vegan"* package). Pairwise comparisons employed the *"RVAideMemoire"* package with FDR correction. Ordination analyses such as Principal Coordinates Analysis (PCoA), Non-metric Multidimensional Scaling (NMDS) and Detrended Correspondence Analysis (DCA) were conducted using *"phyloseq"* and *"vegan"*, with 95% confidence ellipses. Community structure comparisons were further supported by ANOVA, Kruskal–Wallis, and PERMANOVA tests. Data were normalized via Total Sum Scaling (TSS). Heatmaps were generated using *"pheatmap"* package(59), while *"circlize"* was used to construct Circos plots of the top 25 genera (60,61). PCA was performed with *"FactoMineR",* and *"factoextra"* (62,63) and and UpSet plots were generated using *"UpSetR"* (64). Correlations between dominant genera and physicochemical parameters were assessed using Pearson's correlation, visualized with *"corrplot", "ggplot2",* and *"ggpubr"* (65,66).

# 3. Results

## 3.1 Physicochemical characteristics of aquatic environments

Water samples were collected from four distinct aquatic ecosystems in Bangladesh like pond, canal, lake, and marine (Saint Martin's coastal region) between March 1 and March 15, 2022. Among the non-saline freshwater sources (pond, canal, lake; $n = 6$), pH levels were generally slightly acidic (mean = 6.0), except sample BD1 (pH = 8.0). In contrast, marine samples ($n = 2$) exhibited slightly basic pH values (mean = 7.4). Water temperatures across all environments ranged from 28 to 31 °C (mean = 29.41 °C), with no significant differences among ecosystem types. Marked differences were observed in total dissolved solids (TDS) and salinity across ecosystem types. Freshwater samples exhibited low TDS (mean = 80 ppm) and near-zero salinity (mean = 0.07%). Conversely, marine samples demonstrated substantially elevated TDS (mean > 6000 ppm) and salinity levels (mean = 3.6%). These differences were statistically significant between marine and all freshwater ecosystems (TDS: $p < 5.9\times10^{-5}$;

197 salinity: $p < 1.5\times10^{-6}$, Bonferroni-adjusted t-test), while no significant variation was found among pond,
198 canal, and lake samples (S1 Table and Fig 1).
199
200 **Fig 1. Geographic distribution and physicochemical profiling of sampling sites across aquatic**
201 **ecosystems in Bangladesh.** (A) Map illustrating the eight sampling sites representing four aquatic
202 ecosystems: pond (BD1, BD5), canal (BD2, BD7), lake (BD3, BD4), and marine (BD6, BD8). Sites were
203 strategically selected to capture a gradient of anthropogenic impact across five geographically diverse
204 locations. (B) Summary of in-situ physicochemical parameters like pH, temperature, total dissolved solids
205 (TDS), and salinity (NaCl) measured at each sampling site. Here, Jahangirnagar University is presented as
206 JU and Bangladesh Livestock Research Institute as BLRI.
207

208 # 3.2 Microbial diversity and community composition

209 ## 3.2.1 Operational Taxonomic Unit (OTU) distribution across ecosystems

210 High-throughput sequencing of the 16S rRNA gene V3-V4 regions yielded a total of 979
211 prokaryotic OTUs across the eight aquatic sites. Among these, canal samples harbored the highest number
212 of unique OTUs (179 OTUs; 18.28%), indicating elevated microbial richness. Marine and Lake samples
213 exhibited 152 (15.53%) and 126 (12.87%) unique OTUs, respectively. Pond samples presented the lowest
214 taxonomic uniqueness, with only 70 distinct OTUs (7.15%). Importantly, 44 OTUs (4.49%) were shared
215 among all four ecosystem types, representing a core microbial community with potential ecological
216 significance (Fig 2 A).

217 Amplicon-based analysis of the 18S rRNA gene V9 region identified 466 eukaryotic OTUs. Not similar to
218 the prokaryotic patterns, pond samples demonstrated the highest eukaryotic richness (65 unique OTUs;
219 13.95%), followed by canal (58 unique OTUs; 12.45%), lake (57 OTUs; 12.23%) and marine habitats (47

OTUs; 10.09%). A total of 26 eukaryotic OTUs (5.58%) were ubiquitously detected across all four ecosystems, indicating the presence of cosmopolitan eukaryotic taxa with broad environmental tolerance (Fig 2 B).

**Fig 2. Distribution of operational taxonomic units (OTUs) among four distinct aquatic habitats in Bangladesh.** The Venn diagram depicts both habitat-specific and cosmopolitan (A) Prokaryotic and (B) Eukaryotic taxa among the sampled ecosystems.

## 3.2.2 Microbial diversity patterns across aquatic habitats

To evaluate microbial diversity within and between aquatic ecosystems, both alpha and beta diversity analyses were conducted on the 16S (prokaryotic) and 18S (eukaryotic) rRNA gene amplicon datasets. Alpha diversity indices, including Shannon diversity index, Chao1 richness, Inverse Simpson index, and Pielou's evenness, were calculated to assess within-sample diversity. Although numerical variation was observed across the four habitat types (pond, canal, lake, and marine), statistical analysis did not reveal significant differences (S2 Table).

For the 16S rRNA dataset, Kruskal-Wallis tests indicated no statistically significant differences across habitats for any of the alpha diversity indices ($p > 0.05$). These results were corroborated by one-way ANOVA and Tukey's HSD post hoc tests, all of which failed to detect significant pairwise differences among groups ($p > 0.05$). A similar trend was observed for the 18S rRNA dataset, where no significant differences in alpha diversity metrics were detected among habitat types (Kruskal-Wallis and ANOVA; all $p > 0.05$). These results suggest that while diversity indices vary among samples, the observed intra-habitat variability exceeds the inter-habitat variation, precluding statistical significance under the current sampling regime (Fig 3 A-B).

To explore compositional differences between microbial communities across habitats, beta diversity was assessed using Bray-Curtis dissimilarity matrices. Principal coordinates analysis (PCoA), non-metric multidimensional scaling (NMDS), and detrended correspondence analysis (DCA) were used for ordination visualization. PERMANOVA (adonis2) analysis of 16S rRNA data revealed statistically significant differences in bacterial community structure across the four habitats ($R^2 = 0.635$, $F = 2.3237$, $p = 0.046$), indicating that environmental context substantially influenced prokaryotic community composition (Fig 3 C). For the 18S rRNA data, PERMANOVA yielded a borderline result ($R^2 = 0.635$, $F = 2.3237$, $p = 0.054$), suggesting that eukaryotic community structuring may also vary by habitat, though statistical power may be limited due to sample size. Subsequent pairwise PERMANOVA comparisons revealed trends toward differentiation between specific habitat pairs; however, none reached statistical significance after false discovery rate (FDR) correction (adjusted $p > 0.05$) (Fig 3 F).

NMDS plots showed partial clustering of microbial communities by habitat, particularly for marine samples, which tended to separate from freshwater environments (Fig 3 D and G). NMDS stress values were below 0.2 for both datasets, indicating a valid two-dimensional representation of community dissimilarity. DCA analyses revealed compositional gradients consistent with habitat-driven ecological structuring. Marine samples were clearly distinct, suggesting salinity and associated abiotic factors as potential drivers of community separation, while pond and canal samples exhibited overlapping clustering patterns, possibly reflecting shared microbial assemblages or similar environmental conditions (Fig 3 E and H). Collectively, these results show that aquatic microbial communities exhibit both shared and habitat-specific composition, with stronger structuring apparent in bacterial than eukaryotic communities. Although alpha diversity did not differ significantly across habitats, beta diversity patterns highlight the influence of ecosystem type on microbial community assembly.

**Fig 3. Microbial diversity analysis of distinct aquatic ecosystems in Bangladesh.** Violin plots representing alpha diversity metrics including Shannon diversity index, Chao1 richness, Inverse Simpson

index, and Pielou's evenness for prokaryotic (A) and eukaryotic (B) microbial communities. Each plot illustrates the richness and evenness within samples from pond, canal, lake, and marine habitats. Statistical comparisons were conducted using the Wilcoxon rank-sum test, and p-values are indicated above each pairwise comparison. Beta diversity of prokaryotic communities assessed via Bray-Curtis dissimilarity and visualized using Principal Coordinate Analysis (PCoA) (C), Non-metric Multidimensional Scaling (NMDS) (D), and Detrended Correspondence Analysis (DCA) (E). Corresponding ordination plots for eukaryotic microbial communities using the same beta diversity methods: PCoA (F), NMDS (G), and DCA (H).

### 3.2.3 Microbial composition and diversity at phylum, class and order level

Amplicon-based sequencing of the 16S and 18S rRNA gene regions revealed substantial microbial taxonomic diversity across the four aquatic habitats studied. Across all samples, 43 bacterial phyla were identified from the 16S rRNA gene dataset, illustrating high taxonomic richness. Among these, Pseudomonadota was the most dominant and universally present phylum, accounting for over half of the total prokaryotic sequences (51.17%). Other prominent phyla included Bacteroidota (19.03%), Actinomycetota (7.67%), Verrucomicrobiota (4.68%), and Cyanobacteriota (3.63%). Habitat-specific patterns were evident. Canal water exhibited the highest phylum-level richness, with four unique phyla (10.53%) and a community dominated by Pseudomonadota (52.5%), Bacteroidota (12.4%), Patescibacteria (12.0%), and Actinomycetota (9.2%). Marine and lake habitats both harbored three unique phyla (7.89%), and they were both dominated by Pseudomonadota (83.3% and 37.89%). Pond samples exhibited dominance of Bacteroidota (41.7%) and Pseudomonadota (31.3%). Overall, 14 phyla (36.84%) were shared across all environments, representing a conserved core bacterial microbiome (Fig 4 A).

Eukaryotic community profiling using 18S rRNA gene sequences revealed 56 phyla, though a notable proportion (9.65%) of sequences remained unclassified at the phylum level. Twenty-one phyla (37.50%)

were shared across all habitats, suggesting a cosmopolitan eukaryotic microbial core. The most abundant phylum was Ochrophyta (42.11%), followed by Cryptomycota (11.91%), Ciliophora (9.31%), Alveolata (8.20%), and Chlorophyta (4.26%). Distinct community structures emerged at the habitat level. Canal and lake environments were strongly dominated by Ochrophyta (53.53% and 47.83%), whereas pond samples exhibited higher relative abundances of Cryptomycota (27.23%) and Ochrophyta (20.3%). Marine samples were unique in their dominance by Chlorophyta (37.0%) and Dinoflagellata (18.5%), with 33.9% of sequences remaining unclassified, indicating potential for novel eukaryotic taxa in saline environments (Fig 4 B).

At the class level, 80 distinct bacterial classes were identified, with canal samples again exhibiting the highest richness (14 unique classes; 17.5%), followed by lake (7), marine (3), and pond (2). Twenty classes (25%) were common to all ecosystem. The five dominant bacterial classes such as Gammaproteobacteria, Alphaproteobacteria, Bacteroidia, Actinobacteria, and Verrucomicrobiia together accounted for over 80% of total bacterial community at class level. Marine samples were dominated by Alphaproteobacteria (45.4%) and Gammaproteobacteria (37.9%), whereas Bacteroidia prevailed in pond samples (41.7%). Lake and canal samples showed more even class-level distributions, suggesting more complex or heterogeneous ecological conditions (Fig 4 C).

Similarly, a total of 99 eukaryotic microbial classes were identified. Pond samples showed the highest richness (10 unique classes; 9.90%), followed by marine (8), lake (7), and canal (2). Twenty-four classes (23.76%) were shared across all ecosystems. The dominant classes across all samples included Chrysophyceae (40.48%), Intramacronucleata (9.29%), and Perkinsea (8.20%). Canal and lake samples were particularly rich in Chrysophyceae, whereas pond and marine samples harbored large proportions of unclassified taxa (47.42% and 72.34%, respectively), underscoring the potential for underexplored or novel microeukaryotic diversity in these ecosystems. Interestingly, MALV-I (Marine Alveolate Group I), a taxon typically associated with marine environments, was highly abundant (18.3%) in marine samples, aligning with known ecological distributions and salinity preferences (Fig 4 D).

**Fig 4. Taxonomic composition of microbial communities at the phylum and class level across four aquatic ecosystems in Bangladesh**. Heatmaps showing the relative abundance of the top 25 most abundant phyla for prokaryotic (A) and eukaryotic (B) communities across individual water samples from pond, canal, lake, and marine environments. These heatmaps reveal patterns of dominant phyla and their variation across habitats. Bar charts illustrating the relative abundance (%) of prokaryotic (C) and eukaryotic (D) microbial classes identified through 16S and 18S rRNA gene amplicon sequencing, respectively. Only taxa with ≥1% relative abundance are included, providing insight into the dominant microbial groups at the class level within each aquatic ecosystem.

The prokaryotic community at the order level exhibited strong structuring, with dominance by specific lineages primarily affiliated with the Proteobacteria and Bacteroidota phyla, along with a considerable fraction of unclassified taxa (7.1%), emphasizing the presence of undercharacterized microbial diversity. A total of 178 bacterial orders were identified across all samples, with Burkholderiales emerging as the most abundant, comprising 15.4% of all sequences. Other prominent orders included Rhodobacterales (12.2%), Flavobacteriales (6.8%), and Pseudomonadales (6.7%) (Fig 5 A). Habitat-specific taxonomic patterns were evident. In pond ecosystems, the community was dominated by Burkholderiales (18.1%) and Bacteroidales (16.0%), followed by Flavobacteriales (13.3%) and Cytophagales (6.0%). Canal and lake waters showed the greatest order-level richness, concealing 29 (16.29%) and 25 (14.04%) unique bacterial orders, respectively, whereas marine and pond environments contained fewer unique taxa (11 and 7, respectively). Notably, 39 bacterial orders (21.91%) were common among all habitats, forming a taxonomically consistent core community across ecosystems.

Distinct order-level signatures also emerged with environmental context. Burkholderiales was especially dominant in freshwater environments (canal and lake), whereas Rhodobacterales and Enterobacterales were notably enriched in marine samples, reflecting habitat-specific ecological selection and potential salinity-driven differentiation. Principal Component Analysis (PCA) of order-level bacterial relative abundances

elucidated major gradients in community composition. The first three principal components collectively explained 70.5% of the total variance (PC1: 32.1%, PC2: 22.4%, PC3: 16.1%). PC1 distinguished freshwater habitats (particularly pond and canal samples) and was driven by orders such as Chitinophagales, Enterobacterales, Burkholderiales, and Rhodobacterales. PC2 separated more oligotrophic or saline environments, influenced by Pelagibacterales, Synechococcales, and Microtrichales, commonly associated with marine and lake samples. PC3 highlighted differentiation within pond communities, shaped by Erysipelotrichales, Bacteroidales, and Oscillospirales, possibly reflecting nutrient-enriched or organically loaded conditions (Fig 5 B-C).

Eukaryotic microbial communities were similarly diverse, with 152 distinct orders identified across the aquatic systems. Pond ecosystems displayed the greatest richness at the order level, with 19 unique orders (12.5%), whereas marine and canal environments each contained 11 unique orders (7.24%). Lake samples harbored 13 unique orders (8.55%). A total of 21 eukaryotic orders (13.82%) were common across all four habitats, portentous a conserved core microbiota despite environmental heterogeneity (Fig 5 D). However, 32.6% of eukaryotic orders remained unclassified, highlighting the significant presence of poorly characterized or novel taxa within aquatic environments. Among classified taxa, Chromulinales dominated overall (28.0%), followed by Ochromonadales (9.2%) and Perkinsea (8.2%). These groups likely represent key phototrophic and parasitic lineages contributing to primary productivity and trophic interactions in the water column. PCA of eukaryotic order-level composition further illustrated habitat-specific assemblages (Fig 5 E-F). The first three principal components accounted for 83.0% of total variation (PC1: 41.1%, PC2: 25.6%, PC3: 16.3%). PC1 was predominantly shaped by heterotrophic protist orders such as Dactylopodida, Prokinetoplastina, and Cercomonadidae, with sample BD3 exhibiting a distinct community profile along this axis. PC2 emphasized ciliate and cryptophyte lineages (Spirotrichea, Eubodonida, Cryptophyceae), with sample BD8 showing strong separation. PC3 highlighted variability driven by Ploimida, Perkinsea, and Colpodellida, suggesting niche differentiation among specific pond and lake samples (BD2 and BD8) (Fig 5 E-F).

**Fig 5. Microbial community structure and interrelationships at the order level across diverse aquatic ecosystems in Bangladesh.** The Upset plots show how the prokaryotic (A) and eukaryotic (D) microbes at the order level are distributed across the four water bodies. Principal Component Analysis (PCA) biplots illustrating the ordination of water samples based on the top 25 most abundant prokaryotic (B) and eukaryotic (E) microbial orders, revealing community structure and sample-wise variation and Corresponding scree plots for prokaryotic (C) and eukaryotic (F) PCA analyses showing the proportion of variance explained by each principal component, indicating the dimensional contribution of microbial orders to overall community composition.

## 3.2.4 Microbial composition and diversity at family and genus level

By the comprehensive analysis of the 16S rRNA data at the family level, a total of 284 distinct bacterial families were recognized across all aquatic samples. Canal waters exhibited the highest family richness, with 37 unique families (13.03%), whereas pond environments showed the lowest richness with only 13 unique families (4.58%). Marine and lake habitats contained 36 and 33 unique families, respectively. Markedly, 35 families (12.32%) were shared across all four ecosystems, demonstrating a potential core microbiome.. A large portion (14.1%) of the overall bacterial abundance, however, remained unclassified at the family level, highlighting gaps in current taxonomic resolution. Among the classified taxa, Paracoccaceae (12.2%) and Comamonadaceae (8.7%) were the most dominant across samples, followed by Flavobacteriaceae (4.0%), Vibrionaceae (3.8%), Chitinophagaceae (3.7%), and Sporichthyaceae (3.4%). These distributions reflect the predominance of Proteobacteria-associated families and emphasize the coexistence of both well-characterized and yet-uncultured groups shaping microbial community dynamics (Fig 6 A).

The eukaryotic community contained 175 distinct families, of which 35.45% remained unclassified. Pond samples demonstrated the highest family richness, harboring 21 unique families (12%), while marine environments presented the lowest richness with 14 unique families (7.91%). Lake and canal samples contained 19 (10.85%) and 17 (9.71%) unique families, respectively. A total of 19 families were common among all four ecosystems, telling a degree of taxonomic consistency despite environmental variability. Among the identified groups, Chromulinales dominated with an abundance of 26.9%, followed by Ochromonadales (9.2%) and Perkinsidae (8.2%). Other notable groups included Hypotrichia (3.9%) and Oligohymenophorea (2.5%), known for their roles in trophic transfer and nutrient cycling in aquatic systems (Fig 6 B).

**Fig 6. Microbial Community Composition, Distribution, and Comparative Analysis at the Family Level Across Tropical Aquatic Ecosystems in Bangladesh.** Boxplots displaying the relative abundance of the top 15 prokaryotic (A) and eukaryotic (B) microbial families identified across pond, canal, lake, and marine water samples. Statistical comparisons were performed using the Wilcoxon rank-sum test to evaluate differences in family-level diversity among habitats. P-values shown above each plot indicate no statistically significant variation in family abundance across the different aquatic environments.

At a finer taxonomic resolution, 534 distinct bacterial genera were identified. Marine environments unveiled the highest genus richness with 105 unique genera (19.66%), while ponds showed the lowest diversity, harboring only 36 unique genera (6.74%). Canals and lakes contained 99 (18.54%) and 70 (13.11%) unique genera, respectively. Only 13 genera (2.43%) were shared across all environments, suggesting a small but potentially ecologically significant core microbiota. However, 43.08% of all bacterial genera remained unclassified, further highlighting taxonomic knowledge gaps. Among the dominant classified genera, *Limnohabitans* (4.36%), *Acinetobacter* (2.71%), *Macellibacteroides* (2.50%), *Luteolibacter* (2.29%), and *Flavobacterium* (2.28%) were most abundant (Fig 7 A). In pond habitats,

418 *Macellibacteroides* (10.6%), *Flavobacterium* (6.8%), and *Cloacibacterium* (6.1%) prevailed. Canal waters
419 were dominated by *Limnohabitans* (12.6%) and *Acinetobacter* (8.9%), alongside *Luteolibacter* (5.8%) and
420 *Cypionkella* (4.4%). Lake samples featured *Limnohabitans* (4.6%) and *Cyanobium* (4.0%) as dominant
421 genera, with notable contributions from *Methylotenera* (2.9%) and *Prosthecobacter* (2.8%). In marine
422 environments, *Marinobacterium* (6.5%), *Catenococcus* (6.3%), *Marinomonas* (4.7%), and *Cognatishimia*
423 (4.5%) were most prevalent.

424 Eukaryotic communities comprised 284 distinct genera, with ponds displaying the highest richness (45
425 unique genera; 15.85%) and marine habitats the lowest (27 unique genera; 9.51%). Lakes and canals
426 contained 36 and 35 unique genera, respectively. Only seven genera (2.46%) were shared among all
427 environments, while a striking 60.66% of the total eukaryotic genera remained unclassified, indicating a
428 vast unexplored eukaryotic diversity. Among the identified genera, *Poteriospumella* was the most abundant
429 (19.14%), followed by *Spumella* (4.69%), *Chrysamoeba* (2.26%), and *Adriamonas* (1.02%) (Fig 7 B). In
430 pond habitats, *Chrysamoeba* (7.86%), *Poteriospumella* (4.35%), and *Cyclopoida* (3.32%) were prominent.
431 Canal samples were dominated by *Poteriospumella* (14.21%), *Spumella* (9.72%), and *Oligohymenophorea*
432 (1.63%). In lakes, *Poteriospumella* reached an exceptionally high abundance (39.33%), suggesting its
433 ecological dominance, possibly due to its bacterivorous nature. Other genera, including *Adriamonas*
434 (3.25%), *Ichthyobodo* (1.63%), and *Rhynchobodo* (1.54%), hinted at the presence of parasitic or symbiotic
435 protists. In contrast, marine samples had the highest proportion of unclassified eukaryotic genera (93.32%),
436 highlighting a substantial gap in reference databases. The most abundant identified marine taxa were
437 *Pseudobodo* (1.34%), *Neobodo* (1.29%), and *Paraphysomonas* (0.85%).

438

## 3.3 Influence of physicochemical factors on the abundance and function of microbial communities across diverse aquatic habitats

To assess the environmental drivers of microbial community composition, we conducted Pearson correlation analyses between physicochemical parameters (pH, temperature, total dissolved solids [TDS], and salinity) and the relative abundances of key microbial genera. The results revealed distinct associations that highlight how environmental gradients influence the structure and distribution of both prokaryotic and eukaryotic communities. Several bacterial genera displayed significant correlations with environmental parameters. *Acinetobacter* showed a moderate positive correlation with pH ($r = 0.64$, $p = 0.015$), suggesting a preference for more alkaline environments. *Alteromonas* also correlated positively with pH ($r = 0.62$, $p = 0.019$), reinforcing its ecological tolerance for elevated pH levels. Conversely, *Limnohabitans* exhibited a significant negative correlation with pH ($r = -0.65$, $p = 0.014$), indicating a preference for more acidic or neutral waters. A particularly strong positive correlation was observed between *Catenococcus* and pH ($r = 0.79$, $p = 0.001$), reflecting its affinity for high-pH environments. Temperature was another key factor shaping bacterial distributions. Both *Alteromonas* ($r = 0.75$, $p = 0.002$) and *Catenococcus* ($r = 0.81$, $p = 0.001$) showed strong positive correlations, suggesting thermal preference for warmer waters. In contrast, *Limnohabitans* displayed a strong negative correlation with temperature ($r = -0.78$, $p = 0.001$), implying a niche adapted to cooler aquatic systems. TDS also exerted differential effects on bacterial genera. *Catenococcus* exhibited a robust positive correlation with TDS ($r = 0.85$, $p = 0.0003$), reflecting its adaptability to solute-rich environments. *Limnohabitans* showed a strong negative correlation ($r = -0.81$, $p = 0.001$), indicating a preference for fresher waters. *Acinetobacter* demonstrated a moderate negative correlation with TDS ($r = -0.59$, $p = 0.029$), suggesting a limited tolerance for high dissolved solids. Salinity, as expected, strongly influenced the distribution of marine-associated taxa. *Catenococcus* ($r = 0.85$, $p = 0.0002$), *Alteromonas* ($r = 0.75$, $p = 0.002$), and *Acinetobacter* ($r = 0.65$, $p = 0.012$) all showed strong to moderate positive correlations with salinity, supporting their presence in brackish or saline conditions. In

463 contrast, *Limnohabitans* was negatively correlated with salinity (r = -0.80, p = 0.001), affirming its
464 freshwater origin.
465
466 **Fig 7. Community Composition and Environmental Correlations of Dominant Microbial Genera**
467 **Across Aquatic Ecosystems in Bangladesh.** Circos plots illustrate the relative abundance and distribution
468 of the top 25 prokaryotic (A) and eukaryotic (B) genera in pond, canal, lake, and marine water samples,
469 with normalized read counts (divided by 100) for enhanced visual comparison. Heatmaps display the
470 Pearson correlation between the top 20 most abundant prokaryotic (C) and eukaryotic (D) genera and key
471 physicochemical parameters measured across the sampling sites. Genera were selected based on pooled
472 abundance across all ecosystems. Statistical significance is indicated as follows: *** $p < 0.001$, ** $p < 0.01$,
473 * $p < 0.05$.
474
475 In addition to environment-genus relationships, genus-to-genus interactions revealed potential ecological
476 co-occurrence or niche separation. *Acinetobacter* and *Alteromonas* were strongly positively correlated (r =
477 0.88, p = 0.0001), suggesting cohabitation in similar environments with elevated pH and salinity. A similar
478 positive correlation was observed between *Catenococcus* and *Alteromonas* (r = 0.83, p = 0.0004),
479 reinforcing their co-occurrence in high-salinity and high-TDS waters. In contrast, *Limnohabitans* and
480 *Catenococcus* exhibited a strong negative correlation (r = -0.86, p = 0.0002), indicating distinct
481 environmental preferences and potential ecological competition (Fig 7 C)**.** Eukaryotic genera also
482 demonstrated key associations with environmental parameters. *Bodo* showed a strong positive correlation
483 with pH (r = 0.77, p < 0.01), implying a preference for alkaline conditions. *Cryptomonas* was positively
484 correlated with temperature (r = 0.75, p < 0.01), suggesting its proliferation in warmer waters. *Euplotes*
485 demonstrated a strong positive correlation with TDS (r = 0.80, p < 0.01), indicating its adaptability to
486 environments with high solute concentrations. *Spumella* showed a weaker but significant positive
487 correlation with salinity (r = 0.45, p < 0.05), suggesting moderate salt tolerance. Several genera, such as
488 *Daphnia* and *Spumella*, showed moderate correlations with multiple environmental variables, highlighting

their adaptability to a broad range of conditions. *Bodo*, in contrast, exhibited weak correlations with most factors (aside from pH), indicating a more specialized ecological niche. Correlation analysis also discovered interactions among eukaryotic genera. A strong positive correlation between *Cryptomonas* and *Spumella* ($r = 0.77$, $p < 0.01$) suggests co-existence in warm, moderately saline environments. *Euplotes* and *Daphnia* were moderately positively correlated ($r = 0.65$, $p < 0.01$), indicating a possible shared ecological preference for high-TDS habitats. Additionally, a moderate positive correlation between *Spumella* and *Bodo* ($r = 0.72$, $p < 0.01$) points toward potential co-occurrence in ecosystems characterized by variable pH and temperature. Interestingly, *Euplotes* and *Bodo* displayed a weak negative correlation ($r = -0.38$, $p = 0.06$), hinting at possible niche differentiation (Fig 7 D). These findings collectively highlight how physicochemical gradients such as pH, temperature, TDS, and salinity shape the abundance, diversity, and potential ecological interactions of microbial taxa in diverse aquatic systems of Bangladesh. They also suggest that certain microbial genera may serve as bioindicators of environmental change or habitat conditions.

## 3.4 Comparative pathway analysis and functional diversity of microbial communities in four tropical aquatic habitats

The comparative analysis of pathway abundance derived from 16S and 18S rRNA metagenomic data across four distinct aquatic environments revealed pronounced differences in microbial functional potential. These variations reflect the influence of local ecological conditions on microbial metabolic strategies and community structure in tropical aquatic habitats of Bangladesh. In the Pond habitat, microbial communities showed elevated abundances of pathways involved in fatty acid and amino acid biosynthesis, such as *oleate biosynthesis IV* and *L-valine biosynthesis*. These findings suggest a metabolic orientation toward lipid production and essential amino acid synthesis, likely facilitated by the availability of organic substrates and relatively stable environmental conditions. Additionally, high representation of the *fatty acid salvage* and *TCA cycle* pathways indicates an energy-efficient metabolic framework, possibly supporting

514 microbial growth in nutrient-enriched but low-oxygen pond ecosystems. Similarly, the Marine environment
515 (samples BD6 and BD7) exhibited high abundances of lipid-related pathways, including *palmitoleate*
516 *biosynthesis I* and *L-isoleucine biosynthesis*, suggesting an analogous reliance on lipid metabolism and
517 amino acid synthesis. The presence of pathways such as *fatty acid salvage* and *TCA cycle* further
518 underscores the central role of aerobic respiration and energy metabolism in supporting marine microbial
519 life, likely influenced by the relatively stable salinity and moderate nutrient levels typical of coastal marine
520 environments.
521
522 In contrast, the Canal habitat displayed a distinct metabolic profile characterized by elevated activities in
523 energy-generating pathways such as *TCA cycle II* and the *superpathway of glycolysis*. These observations
524 point to enhanced microbial participation in energy production, possibly driven by fluctuating oxygen
525 levels, nutrient enrichment, and organic matter accumulation in canal waters. Additionally, increased
526 representation of *nucleotide degradation* and *biosynthesis pathways* in canals may reflect active recycling
527 of genetic materials, potentially as a response to environmental stressors or dynamic flow conditions. The
528 Lake environment exhibited a functional emphasis on organic matter degradation and nucleotide
529 metabolism. Pathways such as *phytol degradation*, *guanosine nucleotide degradation II*, and *adenosine*
530 *deoxyribonucleotide de novo biosynthesis II* were prominent, suggesting specialized microbial roles in
531 breaking down complex organic compounds. The presence of biosynthetic pathways like *L-serine* and
532 *glycine biosynthesis* points toward enhanced metabolic interdependence and resource competition among
533 microbial taxa. These features may be indicative of more stratified nutrient cycling and diverse microbial
534 trophic interactions typical of lentic lake ecosystems.
535 Overall, the differences in pathway abundances across these habitats highlight distinct microbial metabolic
536 strategies. While Pond and Marine habitats are predominantly associated with lipid and amino acid
537 biosynthesis, the Canal and Lake systems are more engaged in energy production and organic matter
538 turnover. These functional profiles provide crucial insights into the biogeochemical roles of microbes across
539 contrasting aquatic systems (Fig 8 A-B).

**Fig 8. Heatmap Visualization of the Top 50 Metabolic and Functional Pathways in Microbial Communities Across Tropical Aquatic Ecosystems in Bangladesh.** The heatmaps represent the relative abundance of the top 50 predicted metabolic and functional pathways identified from (A) 16S rRNA and (B) 18S rRNA metagenomic data across pond, canal, lake, and marine habitats. Pathway predictions were derived from amplicon-based functional profiling, highlighting key ecological functions and metabolic capabilities of prokaryotic and eukaryotic microbial assemblages in each aquatic environment.

Beyond pathway-level comparisons, our investigation also identified a wide array of microbial genera exhibiting diverse functional traits. Several genera demonstrated notable metabolic versatility, including *Acinetobacter*, *Alteromonas*, *Marinomonas*, *Cercomonas*, and *Poteriospumella*. These taxa are capable of aerobic metabolism, heavy metal resistance, and active participation in nutrient cycling, enabling them to persist across varying environmental gradients and contribute to overall ecosystem functioning (S3 Table). Importantly, the presence of pathogenic genera was also detected, including *Acinetobacter*, *Clostridium*, *Legionella*, *Mycobacterium*, *Pseudomonas* and *Vibrio* (S4 Table). The occurrence of these genera highlights potential risks to aquatic ecosystem health and public safety, particularly in regions where water bodies may serve recreational, agricultural, or domestic functions. Their presence highlights the critical need for continuous microbial monitoring and water quality management strategies to mitigate potential outbreaks and ensure ecosystem resilience.

# 4. Discussion

This study aimed to explore the microbial diversity and community composition in various tropical aquatic ecosystems in Bangladesh using 16S and 18S rRNA gene amplicon metagenomics. The results discovered a rich and diverse microbial community, characterized by distinct prokaryotic and eukaryotic taxonomic distributions across different water bodies, including ponds, lakes, canals, and marine

environments. Our findings reveal not only the richness and structural variability of microbial communities but also demonstrate how environmental parameters shape microbial distributions and functions across freshwater and marine systems which contribute to the growing body of knowledge on aquatic microbiota, help to understand the complex relationships between environmental factors and microbial communities and provide insights into the ecological roles and potential health implications of these microbial communities.

Our combined analysis of alpha and beta diversity metrics provides valuable insights into how microbial assemblages are structured across these tropical aquatic habitats. The alpha diversity indices, including species richness and diversity estimators, suggested no statistically significant differences between habitats for either prokaryotic (16S) or eukaryotic (18S) communities. This outcome implies that microbial communities in these aquatic systems possess comparable levels of within-sample diversity, despite the contrasting physical and chemical conditions expected between freshwater and marine habitats. In contrast, beta diversity analysis clearly demonstrated significant differences in microbial community structure across habitats, particularly for the 16S rRNA dataset. The statistically significant PERMANOVA result ($p = 0.046$) indicates that environmental conditions exert selective pressure on bacterial community assembly, likely driven by factors such as salinity, nutrient concentrations, and hydrological connectivity. The 18S rRNA dataset also showed a borderline significant pattern ($p = 0.054$), suggesting that eukaryotic microbial communities are influenced by habitat type, although the current sample size might limit the power to detect strong differentiation. The NMDS and DCA ordination plots further illustrated this trend. While alpha diversity showed no significant differences between ecosystems, beta diversity analyses revealed distinct prokaryotic and eukaryotic community structures influenced by the unique environmental conditions of each aquatic habitat. These findings highlight the importance of evaluating both taxonomic richness and compositional turnover to comprehensively understand microbial diversity patterns across ecosystems.

Our results of microbial composition demonstrated the prevalence of specific bacterial and eukaryotic phyla across different aquatic ecosystems, with Pseudomonadota (Proteobacteria) and Ochrophyta emerging as dominant phyla in the prokaryotic and eukaryotic communities, respectively. The bacterial phylum Proteobacteria was found to be the most abundant across all ecosystems, representing at least over half of the total microbial composition (51.2%). The dominance of Proteobacteria across all sampled water bodies corroborates previous research emphasizing their ubiquitous presence in aquatic ecosystems, where it is known to play crucial roles in nitrogen cycling, organic matter degradation, and other biogeochemical processes. The dominance of Proteobacteria in aquatic environments could be attributed to its metabolic flexibility, enabling it to thrive in diverse conditions (67–69). Other notable bacterial phyla such as Bacteroidetes, Actinomycetota (Actinobacteria), and Cyanobacteria were also prevalent, suggesting their significant ecological roles in nutrient cycling and carbon fixation (69). Eukaryotic communities were equally diverse, with Ochrophyta emerging as the most abundant group, comprising 42.1% of the total eukaryotic composition. Ochrophyta, which includes diatoms and brown algae, is typically more dominant in temperate and cold-water environments, rather than in tropical aquatic habitats. In tropical regions, algal groups such as Chlorophyta (green algae) and Rhodophyta (red algae) are generally more prevalent due to their adaptability to warmer temperatures and higher light availability (70,71). The unexpectedly high abundance of Ochrophyta in our study suggests potential shifts in environmental conditions, particularly an increase in earth surface temperatures, which may be creating more favorable niches for these taxa. While some Ochrophyta species are known to tolerate warmer conditions, their overall diversity and dominance in tropical regions are usually limited. Thus, their prevalence in our samples could be indicative of changing climatic patterns or altered nutrient regimes, warranting further investigation into their ecological drivers and implications. Cryptomycota (12%) emerging as the second most abundant eukaryotic phylum in our tropical habitats. While Cryptomycota is a relatively recently discovered phylum of fungi, its presence in aquatic environments has been documented in other studies. The abundance of Cryptomycota in our study suggests that fungi might play an essential role in nutrient cycling and decomposition in aquatic ecosystems. Some studies reported the dominance of

aerobic denitrifying bacterial genera like, Acinetobacter, Arthrobacter, Pseudomonas, Elizabethkingia, Pantoea along with anaerobic community at the subsoil level comprising of the prevalence of Cryptomycota among eukaryotic phyla highlights its significance in structuring microbial communities, potentially serving as a bioindicator of environmental health (72–74). The biotrophic parasite Cryptomycota plays important role in organic carbon and energy transfer from primary producer host organism to grazing zooplanktons. The presence of shared microbial signatures and the interconnectedness of eukaryotic communities across diverse aquatic habitats further accentuate the complexity of microbial dynamics in these ecosystems (75). Additionally, the prevalence of Ciliophora (9.30%) and Euglenozoa (1.9%) aligns with findings from other aquatic microbiome studies, where these taxa are considered key players in microbial food webs, acting as grazers on bacteria and algae (76).

At the class level, we observed the highest bacterial class richness in canal water, which contained 14 unique classes, compared to the lower richness in pond samples. This variation in bacterial class diversity could be attributed to environmental factors such as nutrient availability, water temperature, and oxygen levels, which differ significantly between these ecosystems. Similarly, the presence of certain classes like Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria reflects the high metabolic diversity within the aquatic microbiota and their adaptation to various environmental niches. The significant correlation between these classes and environmental factors, such as water temperature and salinity, further supports the idea that bacterial communities are highly responsive to environmental conditions (77). The dominance of α, β and γ-proteobacteria corroborates with other publications reporting microbial community in freshwater ecosystem of Bangladesh (40,67). The eukaryotic class diversity was also high, with Chrysophyceae being the most abundant class, with previous studies that highlight the prevalence of golden algae in freshwater ecosystems, where they contribute to primary production and organic matter cycling (71,78). Additionally, the presence of Spirotrichea, a class of ciliates, suggests that grazing on bacterial populations might be a significant ecological function in these aquatic systems (76).

642 At the order level, we found that Burkholderiales and Rhodobacterales were among the most abundant
643 bacterial orders, particularly in canal and lake samples. These orders are known for their roles in nitrogen
644 fixation, bioremediation, and organic matter degradation. The high relative abundance of these orders in
645 specific aquatic environments suggests that they may be adapting to localized environmental conditions
646 that favor their growth, such as the availability of nitrogen-rich organic compounds (79,80). Chromulinales
647 and Ochromonadales were the most abundant eukaryotic orders, particularly in pond, canal and lake
648 samples that were reported before (81,82). Genus-level patterns such as the dominance of *Limnohabitans*
649 in canals and lakes, and *Marinobacterium* and *Marinomonas* in marine samples, highlight niche partitioning
650 among taxa. Among eukaryotes, ponds and lakes showed higher genus richness, with taxa like
651 *Poteriospumella* and *Spumella* dominating, suggesting strong trophic interactions showcasing their
652 significant roles in shaping microbial communities across diverse aquatic habitats, aligning with previous
653 research highlighting the influence of habitat characteristics on microbial community structure (34,83).
654
655 Our study also revealed significant proportions of unclassified taxa at various taxonomic levels mostly at
656 the genus level, particularly among the eukaryotic communities. This highlights the vast unexplored
657 diversity in tropical aquatic ecosystems and emphasizes the limitations of current taxonomic databases. The
658 unclassified taxa may represent novel species or groups that have yet to be identified and could provide
659 opportunities for future research into the discovery of new microbial lineages with potential ecological or
660 biotechnological applications.
661
662 Our findings are consistent with previous research on microbial communities in aquatic environments. For
663 instance, Proteobacteria and Bacteroidetes have been frequently reported as dominant phyla in aquatic
664 microbiomes (39,84), similar to our observations in tropical ecosystems. Additionally, studies have also
665 highlighted the importance of eukaryotic groups such as Cryptomycota and Ciliophora in aquatic
666 microbiomes, supporting the ecological roles we propose for these taxa (85). According to previous reports,
667 several pathogenic genera of gamma-proteobacteria have been detected in lakes of Dhaka city, with

seasonal fluctuation. However, in this study no pathogenic strains of *E. coli- Shigella* group have been identified, but presence of *Acinetobacter* has been identified in the bioinformatics analysis. The pre-enrichment and enrichment of water samples plays a crucial role for identifying pathogenic genera like *E. coli, Shigella* etc., which was not performed in our study. Notably, the lakes under this study are in an isolated region, which are not contaminated by municipal wastes like the lakes of Dhaka city (86). This may lead to presence of human gut microbiota in lake water samples of Dhaka. Moreover, the ponds are located inside the campus of residential university, but the microbiome composition reveals absence of human gut microbiota from organic wastes. These ponds are habitat of indigenous wild birds, fishes and animals, contributing to a diverse microbial population of the aquatic environments (23,27,33,87).

Furthermore, our study displayed significant correlations between microbial community composition and physicochemical factors and the correlation between the composition itself. The observed correlations between microbial genera and environmental parameters provide insights into the adaptive strategies employed by microorganisms to bloom in diverse ecosystems. These findings provide a representation of the functional diversity and interconnectivity of microbial communities in aquatic ecosystems. Functional prediction revealed significant differences in metabolic potential across habitats. Pond and Marine environments were enriched in lipid and amino acid biosynthesis pathways, such as oleate and palmitoleate biosynthesis, indicating a focus on growth and energy storage under relatively stable conditions. Conversely, the Canal habitat showed elevated activity in glycolysis and nucleotide metabolism, suggesting higher energy turnover and microbial stress responses, likely due to organic pollution or fluctuating physicochemical conditions. Lake samples exhibited signatures of complex organic molecule degradation and nucleotide biosynthesis, indicating microbial adaptation to nutrient-limited but structurally complex ecosystems. Importantly, the detection of potential pathogenic genera including *Acinetobacter, Pseudomonas, Vibrio*, and *Tremula* raises concerns regarding public health risks associated with waterborne diseases, especially in areas where water is used for domestic or recreational purposes (40,88). The metabolic versatility of these genera, including resistance to environmental stress and roles in nutrient

cycling, suggests their potential to persist and proliferate under changing environmental conditions. Understanding these functional relationships is essential for elucidating ecosystem dynamics, predicting ecosystem responses to environmental changes, and informing management strategies for preserving aquatic biodiversity and ecosystem health (77,89,90). Our results are steady with previous reports highlighting the influence of environmental factors on microbial community dynamics. The identification of pathogenic and potentially pathogenic genera emphasizes the importance of monitoring water quality to safeguard public health and ecosystem integrity.

While our study provides valuable insights into microbial ecology in aquatic ecosystems, it is not without limitations. The sample size of eight water samples may not fully capture the full diversity of microbial communities across all aquatic ecosystems in Bangladesh. A larger sample size, including samples from additional water bodies and different seasonal periods, would provide a more comprehensive understanding of microbial diversity. The limited spatial and temporal scale of sampling may not fully capture the complexity of microbial dynamics in these habitats. Additionally, our study focused on prokaryotic and eukaryotic community composition and diversity at the various taxonomic levels, which may not fully reflect the functional roles of microbes in the ecosystem. This study has numerous important implications for understanding microbial ecology in aquatic ecosystems. The dominance of specific bacterial and eukaryotic taxa in different water types suggests that microbial communities are finely tuned to their environments, with implications for ecosystem functioning and environmental management. For example, the high abundance of nitrogen-fixing bacteria such as Burkholderiales in certain water bodies could have implications for nutrient cycling and water quality in these ecosystems. Moreover, the findings suggest that aquatic microbiomes in Bangladesh, like those in other tropical regions, may harbor significant microbial diversity that is yet to be fully explored. This diversity holds potential for biotechnological applications, including the discovery of novel enzymes, bioactive compounds, and microorganisms with industrial or medical uses.

Future research should incorporate larger sample sizes and longitudinal monitoring to improve our understanding of microbial community responses to environmental variability. For instance, investigating the impact of human activity, such as agricultural runoff or industrial pollution, on microbial diversity could provide valuable insights into the environmental health of aquatic ecosystems. Additionally, functional analysis of microbial communities could elucidate their roles in nutrient cycling and ecosystem functioning.

# 5. Conclusions

In short, our study delivers a comprehensive analysis of microbial diversity in tropical aquatic ecosystems in Bangladesh, highlighting the diversity, composition and ecological significance of prokaryotic and eukaryotic microbial communities. With the integration of environmental parameters with microbial community data, it provides critical insights into the taxonomic distribution, community structure, and functional roles of aquatic microbes in these ecosystems. The findings also emphasize the importance of tropical aquatic environments as hotspots for microbial diversity and their potential vulnerability to environmental changes. This research not only boosts our understanding of microbial ecosystems in tropical water bodies but also lays the foundation for future studies focusing on the functional and ecological aspects of microbial communities. Continued investigation into the relationships between microbial diversity and environmental variables, particularly under the influence of anthropogenic and climatic changes, will be crucial for developing strategies to conserve and sustainably manage these vital ecosystems.

# 6. Data availability

The 16S and 18S sequence data are available in the NCBI database under the BioProject accession numbers PRJNA1091616 and PRJNA1091622 respectively. Additionally, all supplementary files associated with the manuscript have been uploaded alongside the manuscript.

## 7. Funding Information

Professor Md. Firoz Ahmed received a grant from University Commissions of Bangladesh (2021-22) and and Dr. Salma Akter received the special research allocation (FY 2022-23, SRG-223499) from the Ministry of Science & Technology, Govt. of Bangladesh. The funds were utilized for sample collection, travel to collection sites, purchase of kits, reagents, consumables and sequencing services. No funding was available for publications costs.

## 8. Acknowledgement

We acknowledge the kind support for the centrifuge and nanodrop access for the extraction and quantification of DNA samples, generously provided by Professor Dr. Sabita Rezwana Rahman, Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.

## 9. Supporting information

S1 Table. Demographic and physicochemical profile of water samples collected from four tropical aquatic sources in Bangladesh.

S2 Table. Comparative alpha diversity metrics of prokaryotic and eukaryotic communities of tropical aquatic habitats in Bangladesh

S3 Table. List of prokaryotic genera with their metabolic and functional characteristics found from distinct water samples in Bangladesh

S4 Table. List of the pathogenic genera that are associated with human diseases found in distinct water samples in Bangladesh

S1 File. Abundance of the top ten prokaryotic and eukaryotic taxa of each tropical aquatic habitat in Bangladesh

# References


1. Shi J, Zhang B, Liu J, Fang Y, Wang A. Spatiotemporal dynamics in microbial communities mediating biogeochemical cycling of nutrients across the Xiaowan Reservoir in Lancang River. Science of The Total Environment. 2022 Mar 20;813:151862.

2. Chandra P, Enespa, Kumar M. Contribution of Microbes in the Renovation of Wetlands. In: Restoration of Wetland Ecosystem: A Trajectory Towards a Sustainable Environment. Springer Singapore; 2020. p. 101–24.

3. Sagova-Mareckova M, Boenigk J, Bouchez A, Cermakova K, Chonova T, Cordier T, et al. Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. Vol. 191, Water Research. Elsevier Ltd; 2021.

4. Zhang X, Ward BB, Sigman DM. Global Nitrogen Cycle: Critical Enzymes, Organisms, and Processes for Nitrogen Budgets and Dynamics. Vol. 120, Chemical Reviews. American Chemical Society; 2020. p. 5308–51.

5. Madsen EL. Microorganisms and their roles in fundamental biogeochemical cycles. Vol. 22, Current Opinion in Biotechnology. 2011. p. 456–64.

6. Sehnal L, Brammer-Robbins E, Wormington AM, Blaha L, Bisesi J, Larkin I, et al. Microbiome Composition and Function in Aquatic Vertebrates: Small Organisms Making Big Impacts on Aquatic Animal Health. Vol. 12, Frontiers in Microbiology. Frontiers Media S.A.; 2021.

7. Ahmad HA, Ahmad S, Cui Q, Wang Z, Wei H, Chen X, et al. The environmental distribution and removal of emerging pollutants, highlighting the importance of using microbes as a potential degrader: A review. Vol. 809, Science of the Total Environment. Elsevier B.V.; 2022.

8. Yao X, Cao Y, Zheng G, Devlin AT, Yu B, Hou X, et al. Use of life cycle assessment and water quality analysis to evaluate the environmental impacts of the bioremediation of polluted water. Science of the Total Environment. 2021 Mar 20;761.

9. Bilal M, Iqbal HMN. Microbial bioremediation as a robust process to mitigate pollutants of environmental concern. Case Studies in Chemical and Environmental Engineering. 2020 Sep 1;2.

10. Mony C, Vandenkoornhuyse P, Bohannan BJM, Peay K, Leibold MA. A Landscape of Opportunities for Microbial Ecology Research. Vol. 11, Frontiers in Microbiology. Frontiers Media S.A.; 2020.

11. Sokol N, Pett-Ridge J, Blazewicz S, Hestrin R, Slessarev E, Marschmann G, et al. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. 2021.

12. Jing X, Su S, Zhang C, Zhu J, Hou Y, Li Z, et al. Dynamic changes in microbial community structure in farming pond water and their effect on the intestinal microbial community profile in juvenile common carp (Cyprinus carpio L.). Genomics. 2021 Jul 1;113(4):2547–60.

13. Manirakiza B, Zhang S, Addo FG, Yu M, Alklaf SA. Interactions between water quality and microbes in epiphytic biofilm and superficial sediment of lake in trophic agriculture area. Science of The Total Environment. 2024 Feb 20;912:169321.

804 14. Dai X, Shen L. Advances and Trends in Omics Technology Development. Vol. 9, Frontiers in
805 Medicine. Frontiers Media S.A.; 2022.

806 15. Rogers AD, Appeltans W, Assis J, Ballance LT, Cury P, Duarte C, et al. Discovering marine
807 biodiversity in the 21st century. Adv Mar Biol. 2022 Jan 1;93:23–115.

808 16. O'Donnell ST, Ross RP, Stanton C. The Progress of Multi-Omics Technologies: Determining
809 Function in Lactic Acid Bacteria Using a Systems Level Approach. Vol. 10, Frontiers in
810 Microbiology. Frontiers Media S.A.; 2020.

811 17. Parven A, Pal I, Witayangkurn A, Pramanik M, Nagai M, Miyazaki H, et al. Impacts of disaster
812 and land-use change on food security and adaptation: Evidence from the delta community in
813 Bangladesh. International Journal of Disaster Risk Reduction. 2022 Aug 1;78:103119.

814 18. Haque MN, Saroar M, Fattah MA, Morshed SR. Environmental benefits of blue ecosystem
815 services and residents' willingness to pay in Khulna city, Bangladesh. Heliyon. 2022 May 1;8(5).

816 19. Bir J, Zaman S, Golder R. A review on blue economy in Bangladesh: prospects and challenges.
817 2020; Available from: https://www.researchgate.net/publication/345814617

818 20. Fakruddin M. Methods for analyzing diversity of microbial communities in natural environments.
819 In: Microbes in Soil and Their Agricultural Prospects. Nova Science Publishers, Inc.; 2015. p.
820 123–51.

821 21. Douterelo I, Boxall JB, Deines P, Sekar R, Fish KE, Biggs CA. Methodological approaches for
822 studying the microbial ecology of drinking water distribution systems. Water Res. 2014 Nov
823 15;65:134–56.

824 22. Akter S, Rahman MS, Ali H, Minch B, Mehzabin K, Siddique MM, et al. Phylogenetic diversity
825 and functional potential of the microbial communities along the Bay of Bengal coast. Sci Rep.
826 2023 Dec 1;13(1).

827 23. Das DK, Feeroz MM, Begum S. Diversity and population density of birds at the Jahangirnagar
828 University campus, Bangladesh. Bangladesh J Zool. 2021 Apr 11;48(2):289–300.

829 24. Oertli B, Parris KM. Review: Toward management of urban ponds for freshwater biodiversity.
830 Ecosphere. 2019 Jul 1;10(7).

831 25. Hill MJ, Biggs J, Thornhill I, Briers RA, Gledhill DG, White JC, et al. Urban ponds as an aquatic
832 biodiversity resource in modified landscapes. Glob Chang Biol. 2017 Mar 1;23(3):986–99.

833 26. Eslamian S. Eutrophication : The Major Threat to Water Bodies [Internet]. 2013. Available from:
834 https://www.researchgate.net/publication/332523300

835 27. Islam MS, Chakma NB, Chakma P, Kabir MH, Shammi RS, Hoque MMM, et al. Seasonal
836 Variation of Water Qualities in River, Lake and Waterfall in Rangamati and Khagrachhari Hill
837 Tracts, Bangladesh. Grassroots Journal of Natural Resources. 2022 Jun 1;5(2):59–79.

838 28. Reddy MT, Sivaraj N, Kamala V, Pandravada SR, Sunil N, Dikshit N. Classification,
839 Characterization and Comparison of Aquatic Ecosystems in the Landscape of Adilabad District,
840 Telangana, Deccan Region, India. OAlib. 2018;05(04):1–49.

29. Bhattacharya P, Kumar A. ADBI Working Paper Series DRIVERS OF BLUE ECONOMY IN ASIA AND PACIFIC ISLAND COUNTRIES: AN EMPIRICAL INVESTIGATION OF TOURISM AND FISHERIES SECTORS Asian Development Bank Institute [Internet]. 1996. Available from: https://www.adb.org/publications/drivers-blue-economy-asia-pacific-island-countries

30. Ramljak A, Žučko J, Lučić M, Babić I, Morić Z, Fafanđel M, et al. Microbial communities as indicators of marine ecosystem health: Insights from coastal sediments in the eastern Adriatic Sea. Mar Pollut Bull. 2024 Aug 1;205.

31. Magyari EK, Szabó Z, Falus G, Móra A, Szalai Z, Hamerlik L, et al. Large shallow lake response to anthropogenic stressors and climate change: Missing macroinvertebrate recovery after oligotrophication (Lake Balaton, East-Central Europe). Science of The Total Environment. 2024 Oct 10;946:174191.

32. Termaat T, Ketelaar R, van Kleef HH, Verberk WCEP, van Grunsven RHA, WallisDeVries MF. Spearhead blues: How threats to the damselfly Coenagrion hastulatum changed over time. J Insect Conserv. 2024 Apr 1;28(2):211–24.

33. Gillis LG, Viana IG, De Los Santos CB. A research blueprint: Plant trait responses to stress and effects on blue carbon storage.

34. Vijayan J, Nathan VK, Ammini P, Ammanamveetil AMH. Bacterial diversity in the aquatic system in India based on metagenome analysis—a critical review. Vol. 30, Environmental Science and Pollution Research. Springer Science and Business Media Deutschland GmbH; 2023. p. 28383–406.

35. Bornette G, Puijalon S. Response of aquatic plants to abiotic factors: A review. Vol. 73, Aquatic Sciences. Birkhauser Verlag AG; 2011. p. 1–14.

36. Das C, Jolly SN. Composition and Diversity of Algal Flora in Jahanara Imam-Pritilota Hall Sorobar (JP) Lake of Jahangirnagar University Campus, Savar, Dhaka, Bangladesh. Annu Res Rev Biol. 2023 May 6;61–79.

37. Alotaibi MO, Mohammed AE, Eltom KH. Metagenomic analysis of bacterial communities of Wadi Namar Lake, Riyadh, Saudi Arabia. Saudi J Biol Sci. 2022 May 1;29(5):3749–58.

38. Saleem F, Azim MK, Mustafa A, Kori JA, Hussain MS. Metagenomic profiling of fresh water lakes at different altitudes in Pakistan. Ecol Inform. 2019 May 1;51:73–81.

39. Wu K, Zhao W, Wang Q, Yang X, Zhu L, Shen J, et al. The relative abundance of benthic bacterial phyla along a water-depth gradient in a plateau lake: Physical, chemical, and biotic drivers. Front Microbiol. 2019;10(JULY).

40. Hossain MdMM, Mojumdar S, Farjana N, Sharmeen Moon R, Islam MS, Rahman N, et al. Diversity of bacterial communities in the Sundarbans mangrove, Bangladesh, with special focus on pathogens affecting aquatic organisms. Comparative Immunology Reports. 2024 Jun;6:200131.

41. Magyari EK, Szabó Z, Falus G, Móra A, Szalai Z, Hamerlik L, et al. Large shallow lake response to anthropogenic stressors and climate change: Missing macroinvertebrate recovery after oligotrophication (Lake Balaton, East-Central Europe). Science of the Total Environment. 2024 Oct 10;946.

42. Ahmad T, Gupta G, Sharma A, Kaur B, El-Sheikh MA, Alyemeni MN. Metagenomic analysis exploring taxonomic and functional diversity of bacterial communities of a Himalayan urban fresh water lake. Vol. 16, PLoS ONE. Public Library of Science; 2021.

43. Mu Y, Su W, Mu Y, Jiang L. Combined Application of High-Throughput Sequencing and Metabolomics Reveals Metabolically Active Microorganisms During Panxian Ham Processing. Front Microbiol. 2020 Jan 10;10.

44. Oliveira C, Shakiba E, North D, McGraw M, Ballard E, Barrett-D'amico M, et al. 16S rRNA Gene-Based Metagenomic Analysis of Rhizosphere Soil Bacteria in Arkansas Rice Crop Fields. Agronomy. 2022 Jan 1;12(1).

45. White RA, Chan AM, Gavelis GS, Leander BS, Brady AL, Slater GF, et al. Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community. Front Microbiol. 2016;6(JAN).

46. Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A. Metagenomics: The next culture-independent game changer. Vol. 8, Frontiers in Microbiology. Frontiers Media S.A.; 2017.

47. Song D, Huo T, Zhang Z, Cheng L, Wang L, Ming K, et al. Metagenomic Analysis Reveals the Response of Microbial Communities and Their Functions in Lake Sediment to Environmental Factors. Int J Environ Res Public Health. 2022 Dec 1;19(24).

48. Simon Andrews. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom. 2010;

49. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014 Aug 1;30(15):2114–20.

50. Hall M, Beiko RG. 16S rRNA Gene Analysis with QIIME2. In: Methods in Molecular Biology. Humana Press Inc.; 2018. p. 113–29.

51. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: A versatile open source tool for metagenomics. PeerJ. 2016;2016(10).

52. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006 Jul;72(7):5069–72.

53. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013 Jan 1;41(D1).

54. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol [Internet]. 2020;38(6):685–8. Available from: https://doi.org/10.1038/s41587-020-0548-6

55. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, et al. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 2016 Apr 1;20:241–8.

56. Title Draw Geographical Maps. 2024.

57. Package “highcharter” Title A Wrapper for the “Highcharts” Library. 2022.

58. Package “ggvenn” [Internet]. 2023. Available from: https://orcid.org/0000-0002-4990-6239

59. Package “pheatmap.” 2022.

60. Gu Z, Gu L, Eils R, Schlesner M, Brors B. Circlize implements and enhances circular visualization in R. Bioinformatics. 2014 Apr 2;30(19):2811–2.

61. Package “igraph” Title Network Analysis and Visualization Version 2.1.2. 2024.

62. Husson F, Josse J, Le S, Mazet J. Package “FactoMineR” Title Multivariate Exploratory Data Analysis and Data Mining [Internet]. 2024. Available from: http://factominer.free.fr

63. Package “factoextra” Type Package Title Extract and Visualize the Results of Multivariate Data Analyses [Internet]. 2022. Available from: https://github.com/kassambara/factoextra/issues

64. Conway JR, Lex A, Gehlenborg N. UpSetR: An R package for the visualization of intersecting sets and their properties. Bioinformatics. 2017 Sep 15;33(18):2938–40.

65. Package “corrplot” Title Visualization of a Correlation Matrix [Internet]. 2024. Available from: https://github.com/taiyun/corrplot/issues

66. Type Package Title “ggplot2” Based Publication Ready Plots. 2023.

67. Azmuda N, Fakruddin M, Khan SI, Birkeland NK. Bacterial community profiling of tropical freshwaters in Bangladesh. Front Public Health. 2019;7(May).

68. Yan L, Kuang Y, Xie X, Peng K, Deng Y, Gan Y, et al. Insights into nitrogen biogeochemical cycling in mangrove wetland from Genome-Resolved metagenomic sequencing. J Hydrol (Amst). 2024 Aug 1;640:131741.

69. Ge H, Li C, Huang C, Zhao L, Cong B, Liu S. Bacterial community composition and metabolic characteristics of three representative marine areas in northern China. Mar Environ Res. 2025 Feb 1;204:106892.

70. Mateo-Cid LE, Mendoza-Onzález ÁC, Casas CMH. Diversity of brown algae (Ochrophyta, phaeophyceae) of sian ka´an reserve biosphere, mexican caribbean. Pak J Bot. 2019 Feb 1;51(1):361–6.

71. Korneva LG, Solovyeva V V. Golden algae (Chrysophyta) in plankton of the Volga River reservoirs: Taxonomic structure, dynamics of diversity, and abundance. Inland Water Biol. 2017 Apr 1;10(2):168–75.

72. Zhang H, Wang Y, Chen S, Zhao Z, Feng J, Zhang Z, et al. Water bacterial and fungal community compositions associated with urban lakes, Xi’an, China. Int J Environ Res Public Health. 2018 Mar 7;15(3).

73. Samson R, Rajput V, Shah M, Yadav R, Sarode P, Dastager SG, et al. Fungal communities (Mycobiome) as potential ecological indicators within confluence stretch of Ganges and Yamuna Rivers, India [Internet]. 2019. Available from: http://biorxiv.org/lookup/doi/10.1101/848259

74. Grossart HP, Hassan EA, Masigol H, Arias-Andres M, Rojas-Jimenez K. Inland Water Fungi in the Anthropocene: Current and Future Perspectives. In: Encyclopedia of Inland Waters, Second Edition. Elsevier; 2022. p. 667–84.

75. Gleason FH, Carney LT, Lilje O, Glockling SL. Ecological potentials of species of Rozella (Cryptomycota). Vol. 5, Fungal Ecology. Elsevier Ltd; 2012. p. 651–6.

76. Weisse T, Montagnes DJS. Ecology of planktonic ciliates in a changing world: Concepts, methods, and challenges. Vol. 69, Journal of Eukaryotic Microbiology. John Wiley and Sons Inc; 2022.

77. Akter N, Wahiduzzaman M, Yeasmin A, Islam KS, Luo JJ. Spatial modelling of bacterial diversity over the selected regions in Bangladesh by next-generation sequencing: Role of water temperature. Applied Sciences (Switzerland). 2020 Apr 1;10(7).

78. Lengyel E, Barreto S, Padisák J, Stenger-Kovács C, Lázár D, Buczkó K. Contribution of silica-scaled chrysophytes to ecosystems services: a review. Hydrobiologia. 2023 Jul 1;850(12–13):2735–56.

79. Arsyadi A, Guo Y, Ebihara A, Sakagami N, Sakoda M, Tago K, et al. A Nitrate-Transforming Bacterial Community Dominates in the Miscanthus Rhizosphere on Nitrogen-Deficient Volcanic Deposits of Miyake-jima. Microorganisms. 2023 Feb 1;11(2).

80. Demtröder L, Pfänder Y, Schäkermann S, Bandow JE, Masepohl B. NifA is the master regulator of both nitrogenase systems in Rhodobacter capsulatus. Microbiologyopen. 2019 Dec 1;8(12).

81. Kumar Mozumder P, Niamul Naser M, Tweb A, Ahmed A. ABUNDANCE OF ZOOPLANKTON AND PHYSICO-CHEMICAL PARAMETERS OF A POLYCULTURE FISH POND OF MANIKGANJ, BANGLADESH. Vol. 42, Bangladesh J. Zool. 2014.

82. Boden L, Klagus C, Boenigk J. Insights from single-strain and mixed culture experiments on the effects of heatwaves on freshwater flagellates. PeerJ. 2024;12(9).

83. Kasalický V, Jezbera J, Hahn MW, Šimek K. The Diversity of the Limnohabitans Genus, an Important Group of Freshwater Bacterioplankton, by Characterization of 35 Isolated Strains. PLoS One. 2013 Mar 7;8(3).

84. Stevens H, Stübner M, Simon M, Brinkhoff T. Phylogeny of Proteobacteria and Bacteroidetes from oxic habitats of a tidal flat ecosystem. FEMS Microbiol Ecol. 2005 Nov 1;54(3):351–65.

85. Finkel Z V., Follows MJ, Liefer JD, Brown CM, Benner I, Irwin AJ. Phylogenetic diversity in the macromolecular composition of microalgae. PLoS One. 2016 May 1;11(5).

86. Faria FF, Ariyan TN, Mia MY. Trophic status analysis and nutrient source allocation in urban lakes of Dhaka, Bangladesh: a comprehensive approach to eutrophication monitoring. Environ Monit Assess. 2024 Dec 1;196(12).

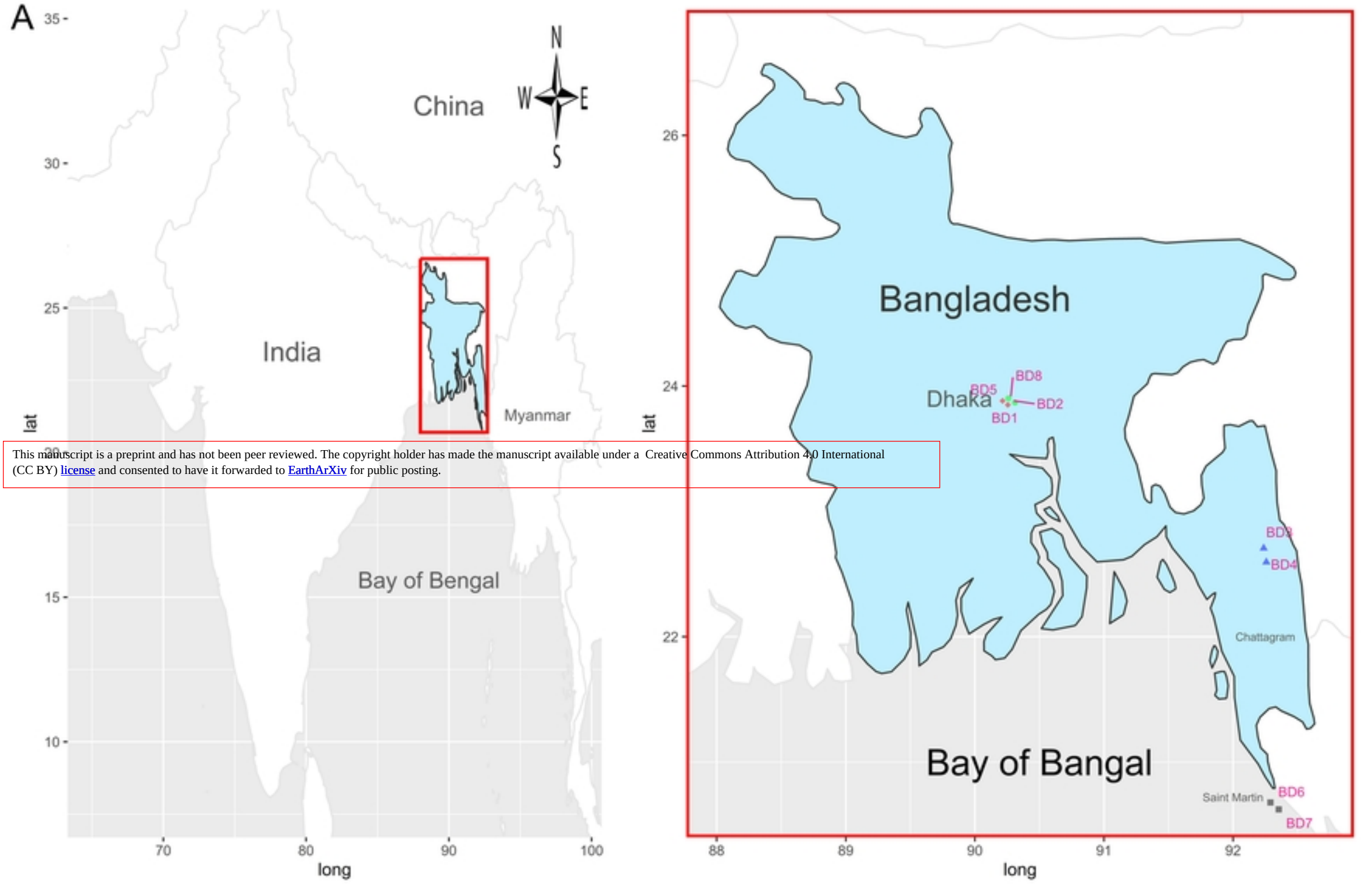
87. Razzak MA, Awwal MdS, Rahman KMdZ, Bashar K. Diversity and abundance of soil arthropods in Jahangirnagar University campus, Dhaka, Bangladesh. International Journal of Fauna and Biological Studies. 2022 Nov 1;9(6):07–12.

88. Loo KY, Law JWF, Tan LTH, Pusparajah P, Wong SH, Chan KG, et al. The Burden of Vibrio sp. Infections – A Scoping Review. Vol. 6, Progress in Microbes and Molecular Biology. HH Publisher; 2023.

89. Dory F, Nava V, Spreafico M, Orlandi V, Soler V, Leoni B. Interaction between temperature and nutrients: How does the phytoplankton community cope with climate change? Science of the Total Environment. 2024 Jan 1;906.

90. Ramos JL, de Lorenzo V, López P. Meeting report: microbes as safeguards of the environment. Sustainable Microbiology. 2024 Jan 17;1(1).

A




B

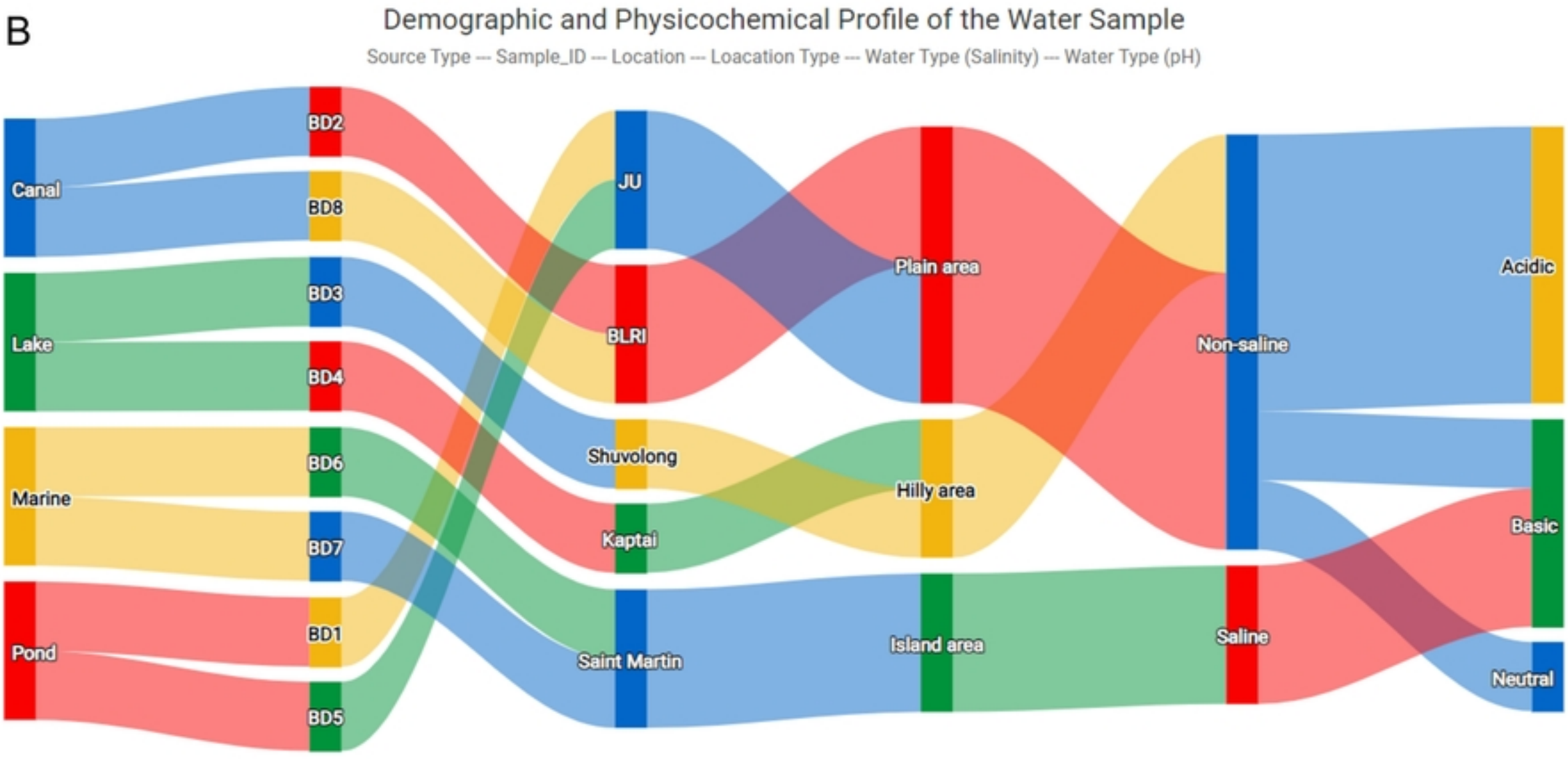



Figure-1

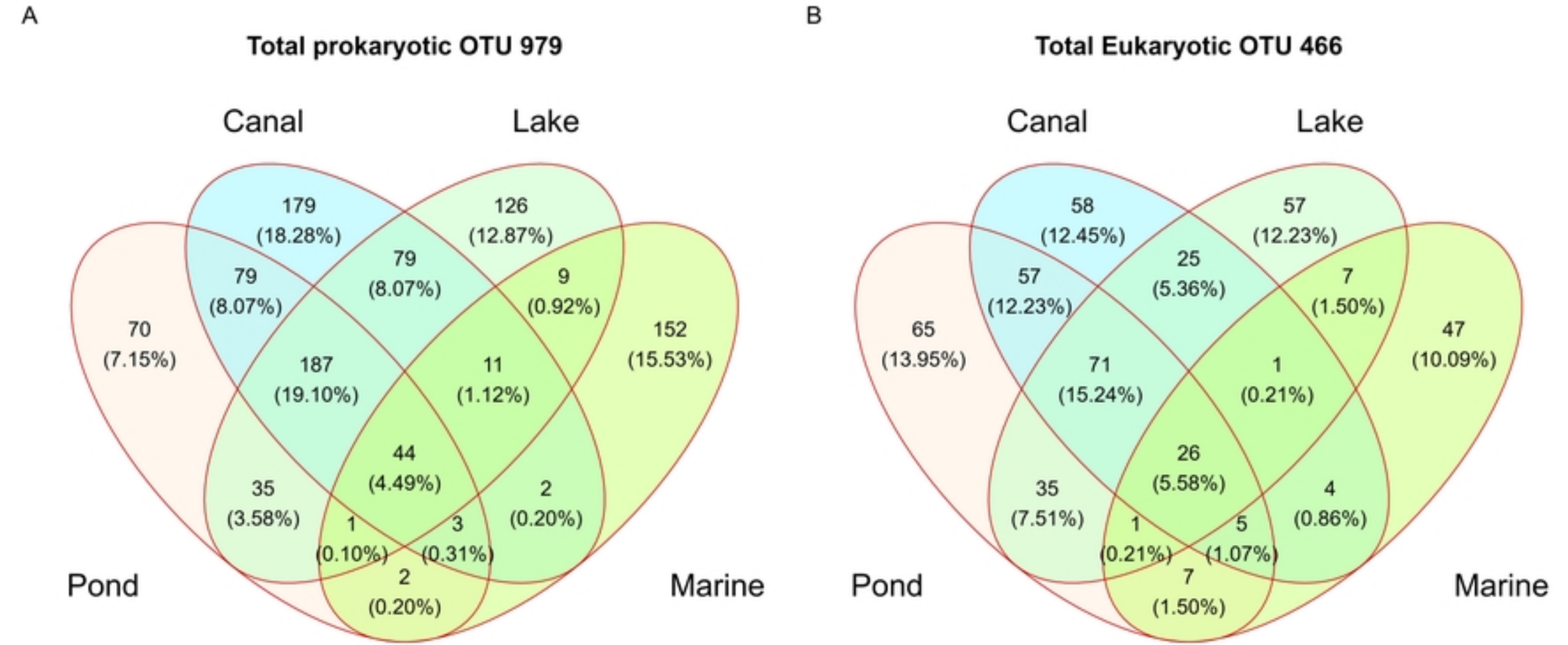

A
Total prokaryotic OTU 979
Canal
Lake
Pond
Marine
179
(18.28%)
126
(12.87%)
79
(8.07%)
79
(8.07%)
9
(0.92%)
70
(7.15%)
187
(19.10%)
11
(1.12%)
152
(15.53%)
44
(4.49%)
35
(3.58%)
1
(0.10%)
3
(0.31%)
2
(0.20%)
2
(0.20%)
B
Total Eukaryotic OTU 466
Canal
Lake
Pond
Marine
58
(12.45%)
57
(12.23%)
57
(12.23%)
25
(5.36%)
7
(1.50%)
65
(13.95%)
71
(15.24%)
1
(0.21%)
47
(10.09%)
26
(5.58%)
35
(7.51%)
1
(0.21%)
5
(1.07%)
4
(0.86%)
7
(1.50%)

Figure-2

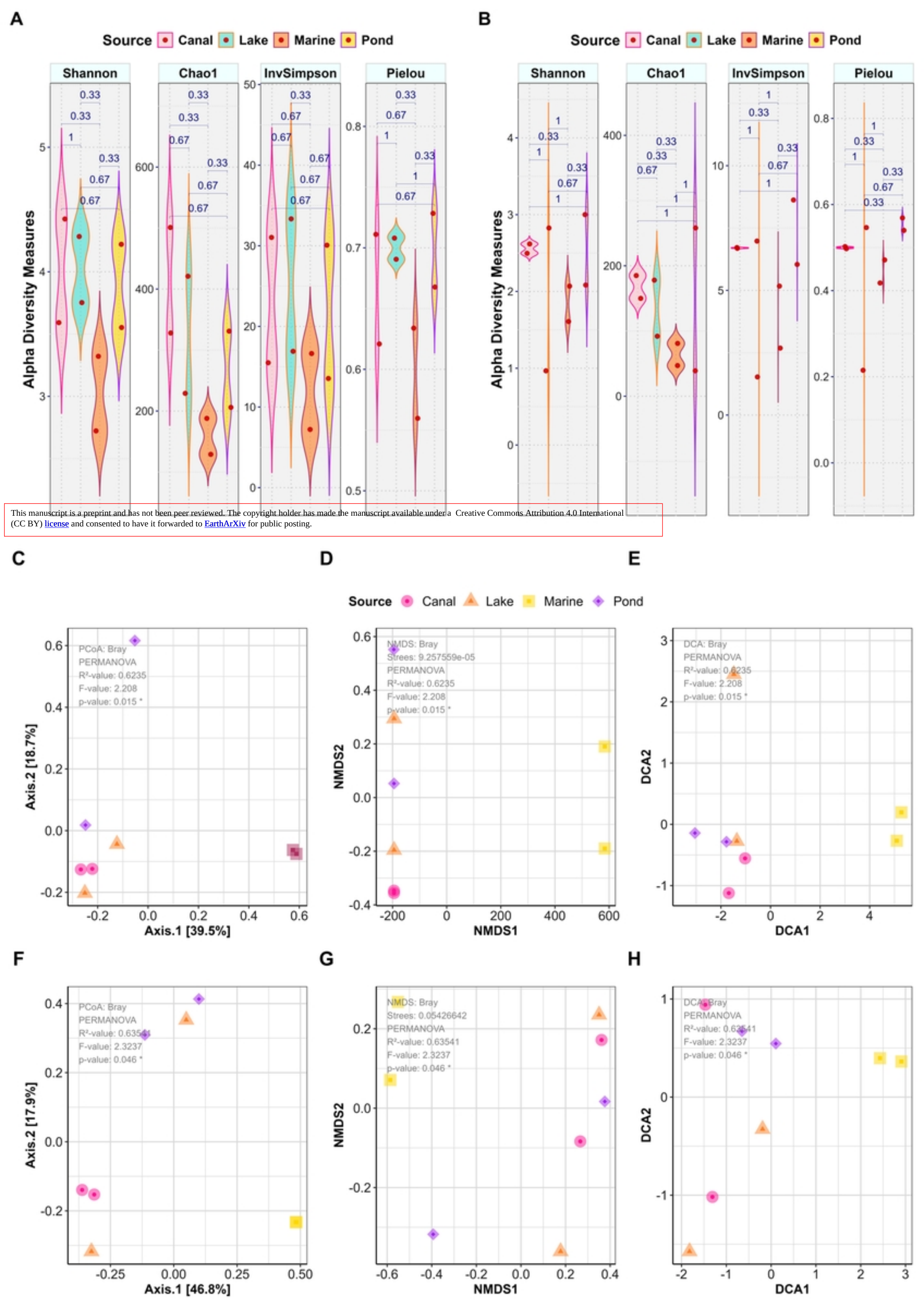



Figure-3

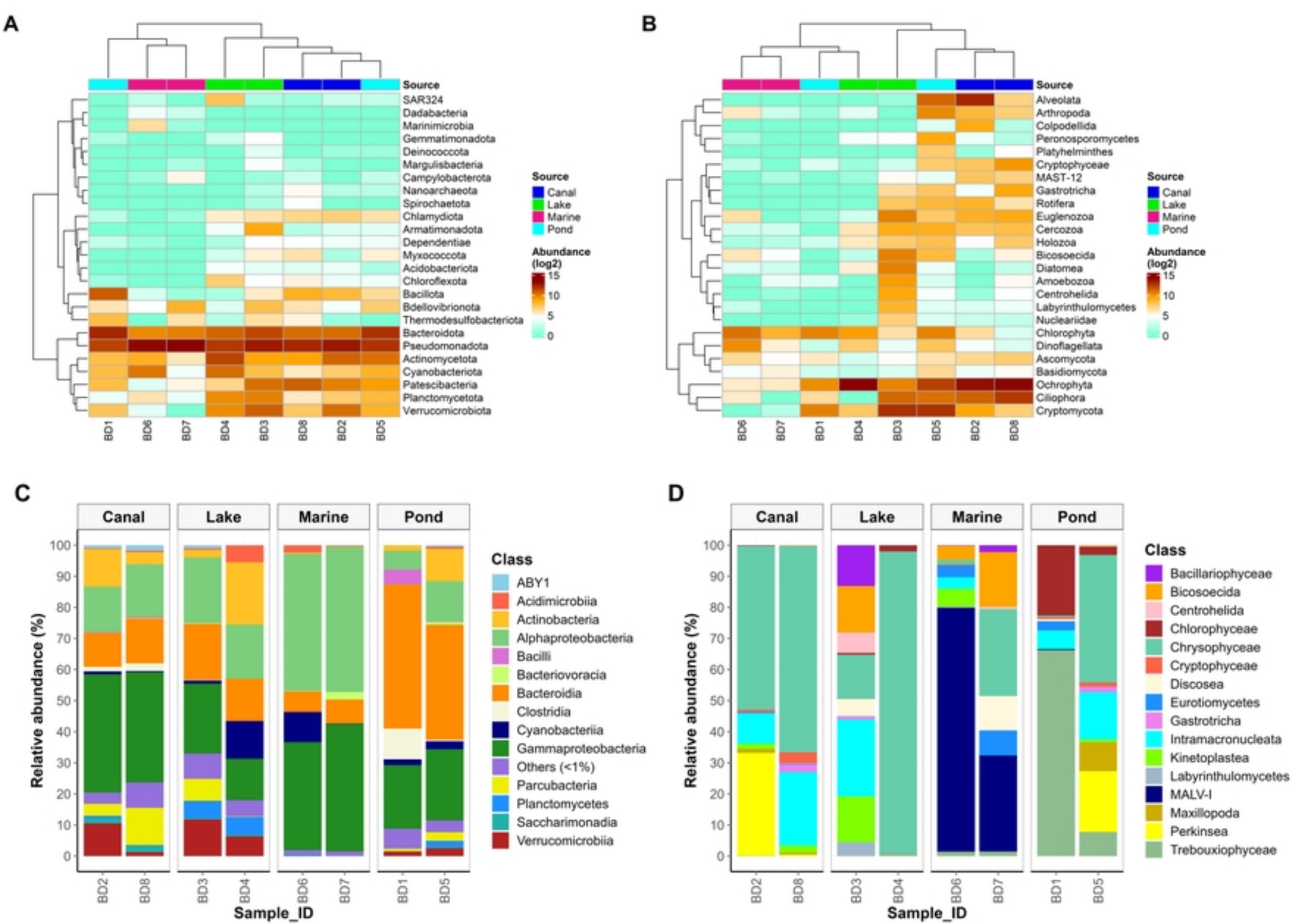

A
Source
SAR324
Dadabacteria
Marinimicrobia
Gemmatimonadota
Deinococcota
Margulisbacteria
Campylobacterota
Nanoarchaeota
Spirochaetota
Chlamydiota
Armatimonadota
Dependentiae
Myxococcota
Acidobacteriota
Chloroflexota
Bacillota
Bdellovibrionota
Thermodesulfobacteriota
Bacteroidota
Pseudomonadota
Actinomycetota
Cyanobacteriota
Patescibacteria
Planctomycetota
Verrucomicrobiota
BD1 BD6 BD7 BD4 BD3 BD8 BD2 BD5
Source
Canal
Lake
Marine
Pond
Abundance (log2)
15
10
5
0
B
Source
Alveolata
Arthropoda
Colpodellida
Peronosporomycetes
Platyhelminthes
Cryptophyceae
MAST-12
Gastrotricha
Rotifera
Euglenozoa
Cercozoa
Holozoa
Bicosoecida
Diatomea
Amoebozoa
Centrohelida
Labyrinthulomycetes
Nucleariidae
Chlorophyta
Dinoflagellata
Ascomycota
Basidiomycota
Ochrophyta
Ciliophora
Cryptomycota
BD6 BD7 BD1 BD4 BD3 BD5 BD2 BD8
Source
Canal
Lake
Marine
Pond
Abundance (log2)
15
10
5
0
C
Canal
Lake
Marine
Pond
Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
BD2 BD8 BD3 BD4 BD6 BD7 BD1 BD5
Sample_ID
Class
ABY1
Acidimicrobiia
Actinobacteria
Alphaproteobacteria
Bacilli
Bacteriovoracia
Bacteroidia
Clostridia
Cyanobacteriia
Gammaproteobacteria
Others (<1%)
Parcubacteria
Planctomycetes
Saccharimonadia
Verrucomicrobiia
D
Canal
Lake
Marine
Pond
Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
BD2 BD8 BD3 BD4 BD6 BD7 BD1 BD5
Sample_ID
Class
Bacillariophyceae
Bicosoecida
Centrohelida
Chlorophyceae
Chrysophyceae
Cryptophyceae
Discosea
Eurotiomycetes
Gastrotricha
Intramacronucleata
Kinetoplastea
Labyrinthulomycetes
MALV-I
Maxillopoda
Perkinsea
Trebouxiophyceae

Figure-4

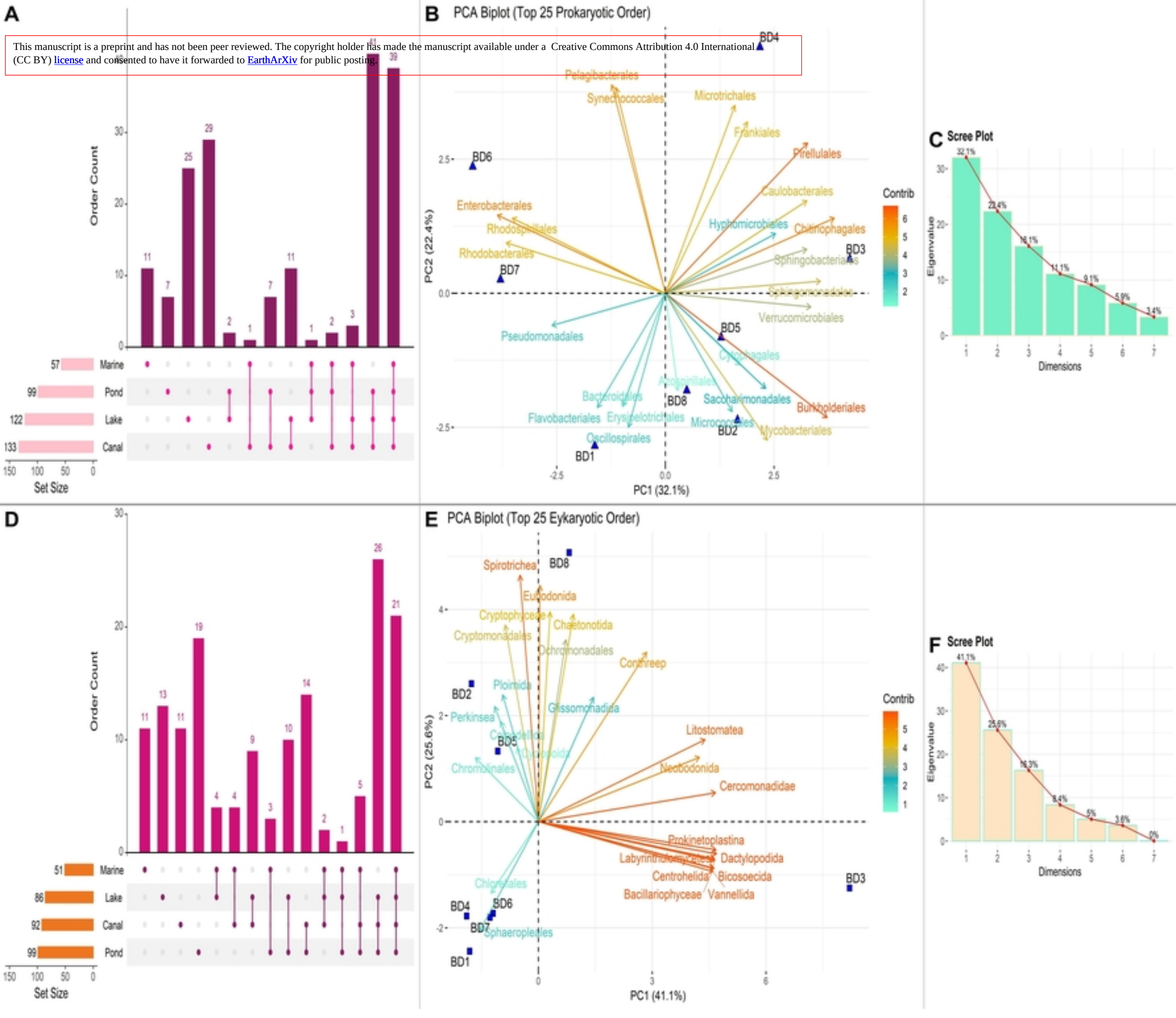



Figure-5

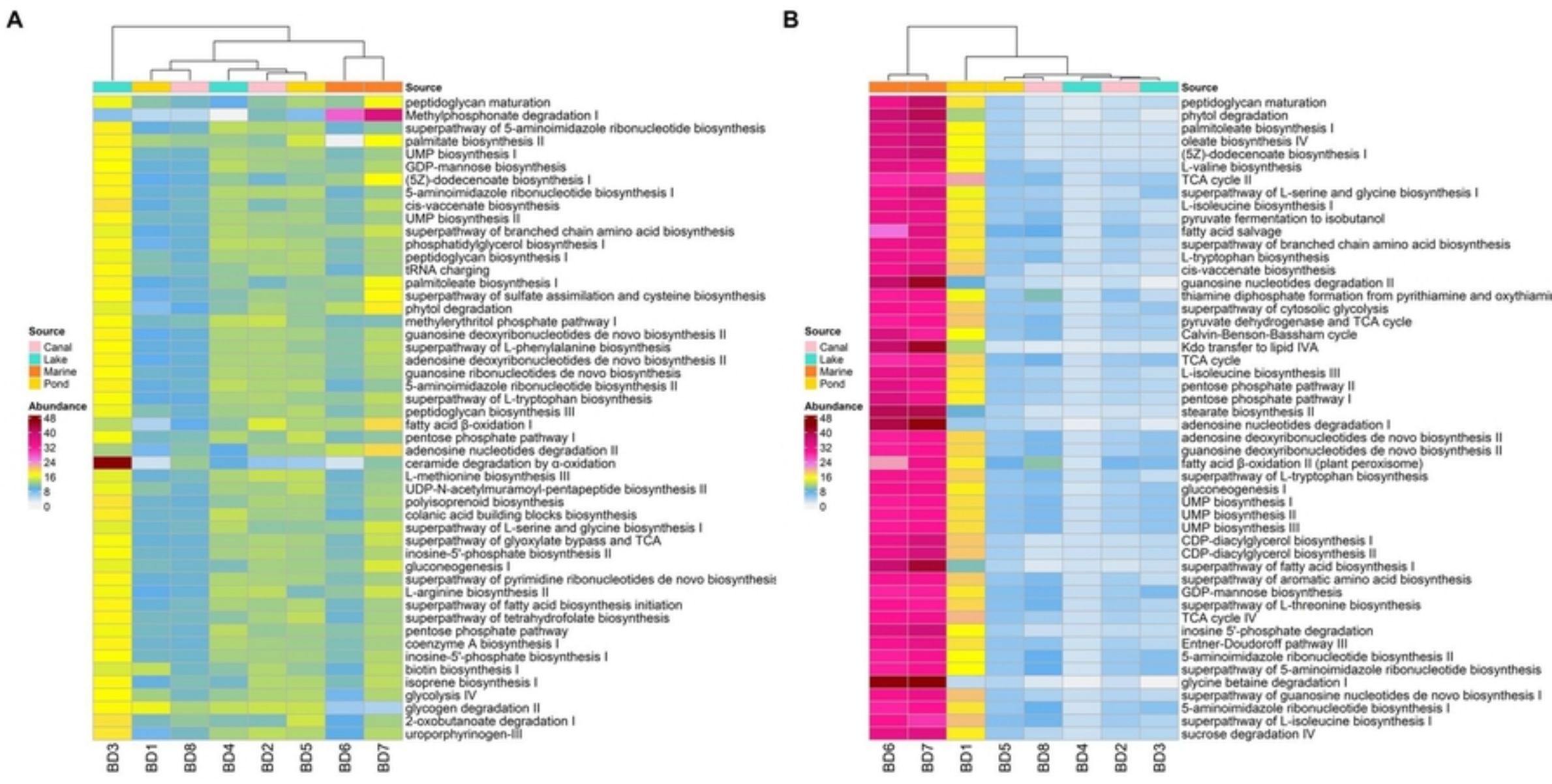

A
Source
peptidoglycan maturation
Methylphosphonate degradation I
superpathway of 5-aminoimidazole ribonucleotide biosynthesis
palmitate biosynthesis II
UMP biosynthesis I
GDP-mannose biosynthesis
(5Z)-dodecenoate biosynthesis I
5-aminoimidazole ribonucleotide biosynthesis I
cis-vaccenate biosynthesis
UMP biosynthesis II
superpathway of branched chain amino acid biosynthesis
phosphatidylglycerol biosynthesis I
peptidoglycan biosynthesis I
tRNA charging
palmitoleate biosynthesis I
superpathway of sulfate assimilation and cysteine biosynthesis
phytol degradation
methylerythritol phosphate pathway I
guanosine deoxyribonucleotides de novo biosynthesis II
superpathway of L-phenylalanine biosynthesis
adenosine deoxyribonucleotides de novo biosynthesis II
guanosine ribonucleotides de novo biosynthesis
5-aminoimidazole ribonucleotide biosynthesis II
superpathway of L-tryptophan biosynthesis
peptidoglycan biosynthesis III
fatty acid β-oxidation I
pentose phosphate pathway I
adenosine nucleotides degradation II
ceramide degradation by α-oxidation
L-methionine biosynthesis III
UDP-N-acetylmuramoyl-pentapeptide biosynthesis II
polyisoprenoid biosynthesis
colanic acid building blocks biosynthesis
superpathway of L-serine and glycine biosynthesis I
superpathway of glyoxylate bypass and TCA
inosine-5'-phosphate biosynthesis II
gluconeogenesis I
superpathway of pyrimidine ribonucleotides de novo biosynthesis
L-arginine biosynthesis II
superpathway of fatty acid biosynthesis initiation
superpathway of tetrahydrofolate biosynthesis
pentose phosphate pathway
coenzyme A biosynthesis I
inosine-5'-phosphate biosynthesis I
biotin biosynthesis I
isoprene biosynthesis I
glycolysis IV
glycogen degradation II
2-oxobutanoate degradation I
uroporphyrinogen-III
Source
Canal
Lake
Marine
Pond
Abundance
48
40
32
24
16
8
0
BD3
BD1
BD8
BD4
BD2
BD5
BD6
BD7
B
Source
peptidoglycan maturation
phytol degradation
palmitoleate biosynthesis I
oleate biosynthesis IV
(5Z)-dodecenoate biosynthesis I
L-valine biosynthesis
TCA cycle II
superpathway of L-serine and glycine biosynthesis I
L-isoleucine biosynthesis I
pyruvate fermentation to isobutanol
fatty acid salvage
superpathway of branched chain amino acid biosynthesis
L-tryptophan biosynthesis
cis-vaccenate biosynthesis
guanosine nucleotides degradation II
thiamine diphosphate formation from pyrithiamine and oxythiamine
superpathway of cytosolic glycolysis
pyruvate dehydrogenase and TCA cycle
Calvin-Benson-Bassham cycle
Kdo transfer to lipid IVA
TCA cycle
L-isoleucine biosynthesis III
pentose phosphate pathway II
pentose phosphate pathway I
stearate biosynthesis II
adenosine nucleotides degradation I
adenosine deoxyribonucleotides de novo biosynthesis II
guanosine deoxyribonucleotides de novo biosynthesis II
fatty acid β-oxidation II (plant peroxisome)
superpathway of L-tryptophan biosynthesis
gluconeogenesis I
UMP biosynthesis I
UMP biosynthesis II
UMP biosynthesis III
CDP-diacylglycerol biosynthesis I
CDP-diacylglycerol biosynthesis II
superpathway of fatty acid biosynthesis I
superpathway of aromatic amino acid biosynthesis
GDP-mannose biosynthesis
superpathway of L-threonine biosynthesis
TCA cycle IV
inosine 5'-phosphate degradation
Entner-Doudoroff pathway III
5-aminoimidazole ribonucleotide biosynthesis II
superpathway of 5-aminoimidazole ribonucleotide biosynthesis
glycine betaine degradation I
superpathway of guanosine nucleotides de novo biosynthesis I
5-aminoimidazole ribonucleotide biosynthesis I
superpathway of L-isoleucine biosynthesis I
sucrose degradation IV
Source
Canal
Lake
Marine
Pond
Abundance
48
40
32
24
16
8
0
BD6
BD7
BD1
BD5
BD8
BD4
BD2
BD3

Figure-8

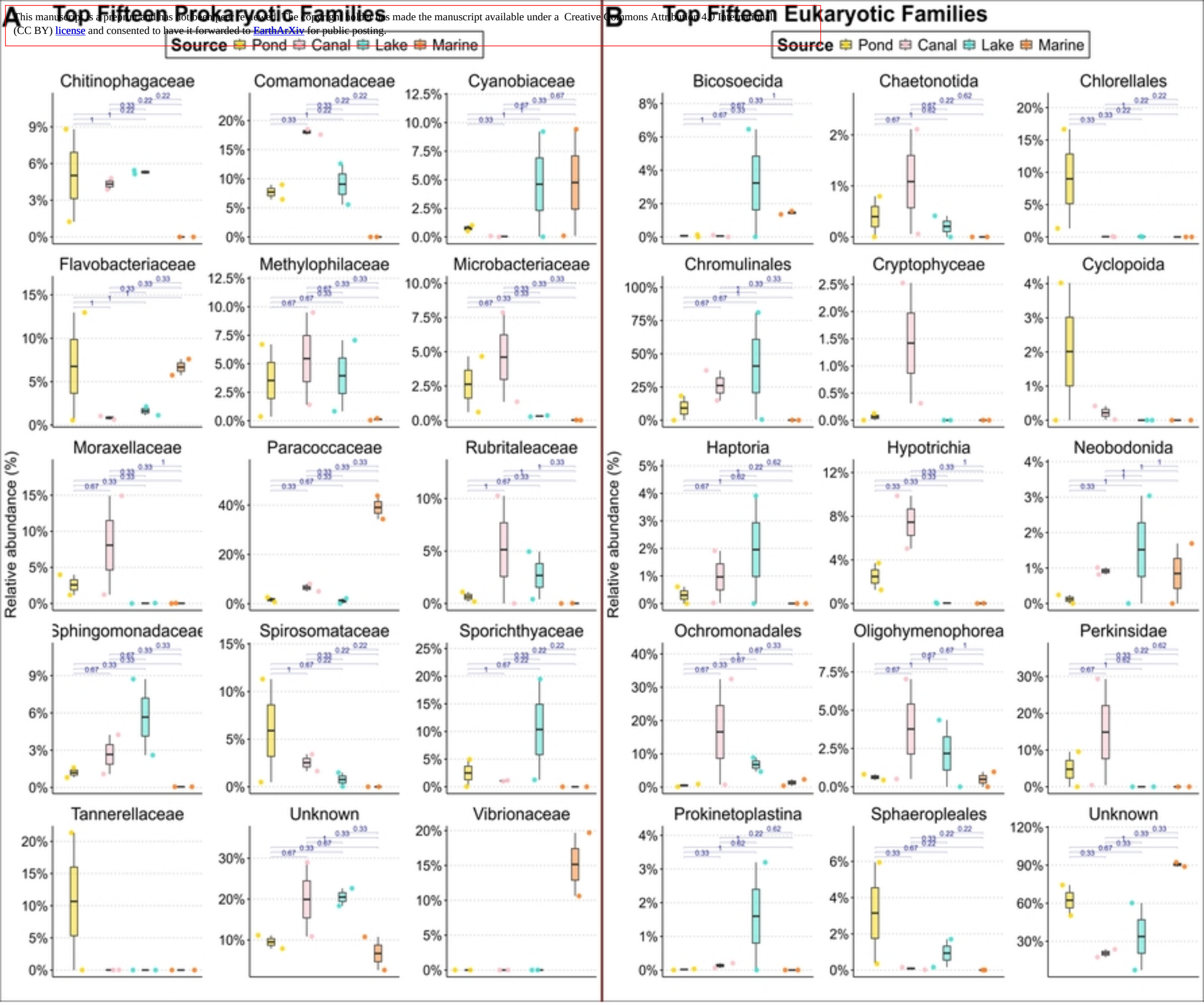
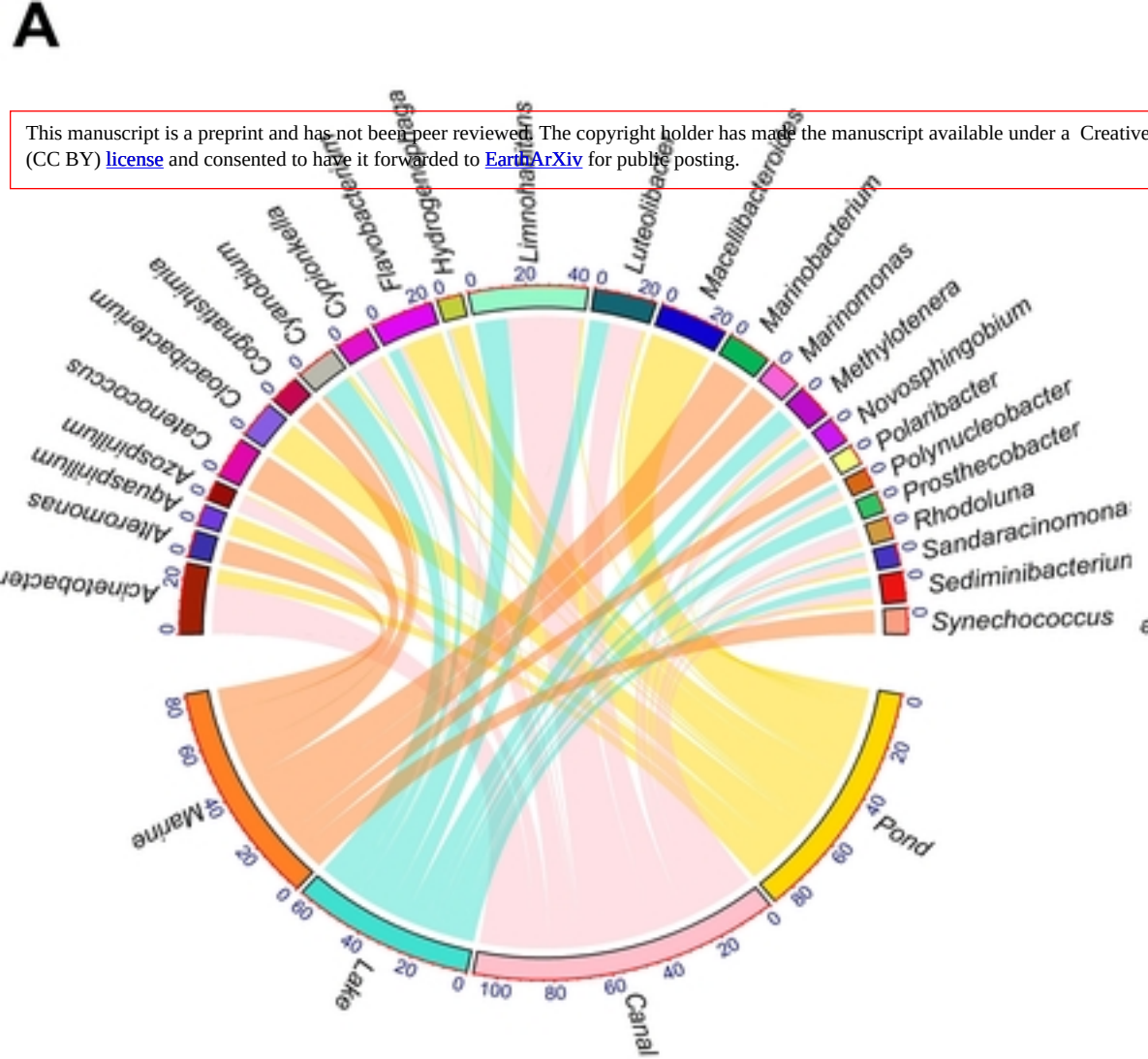
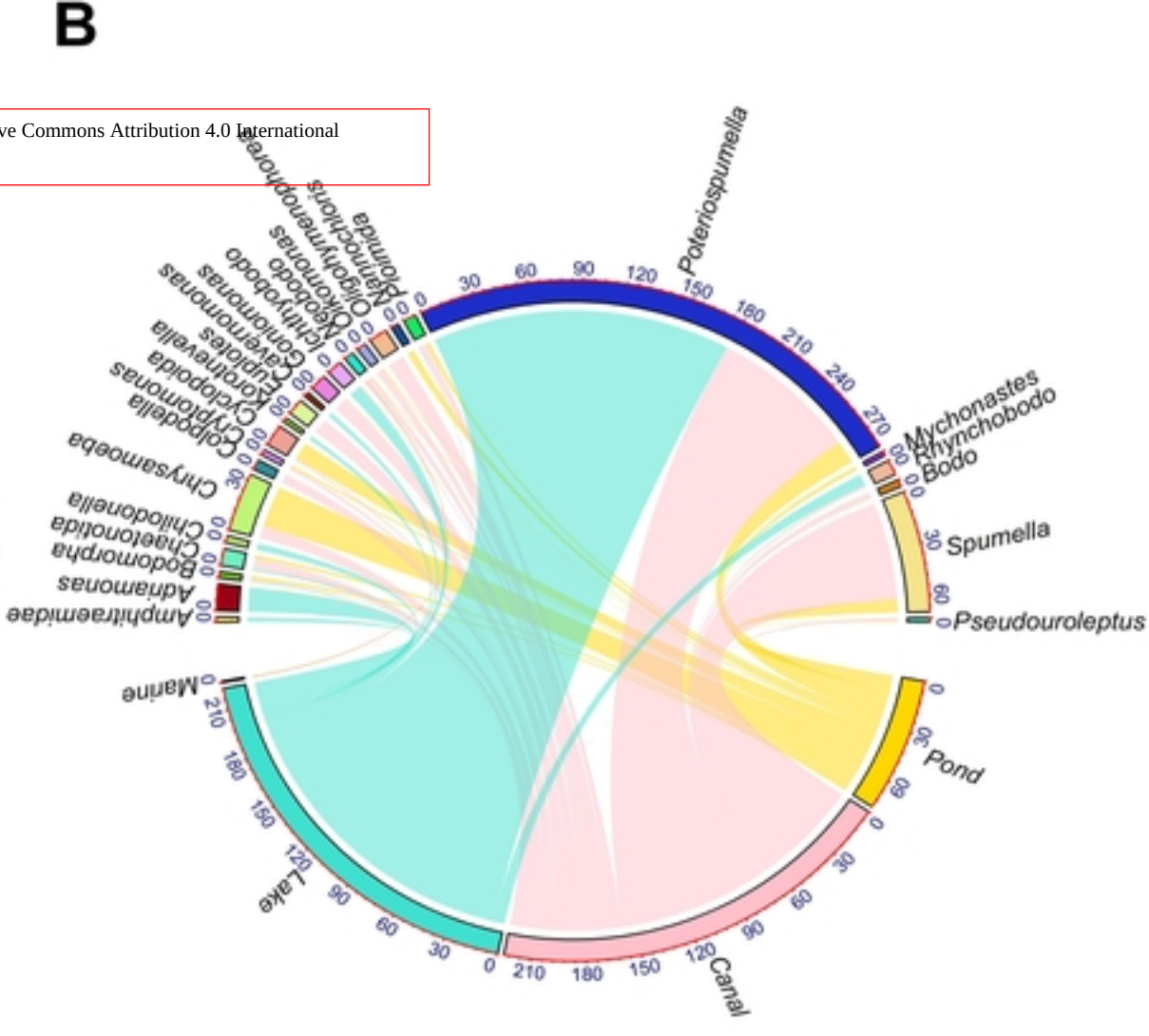
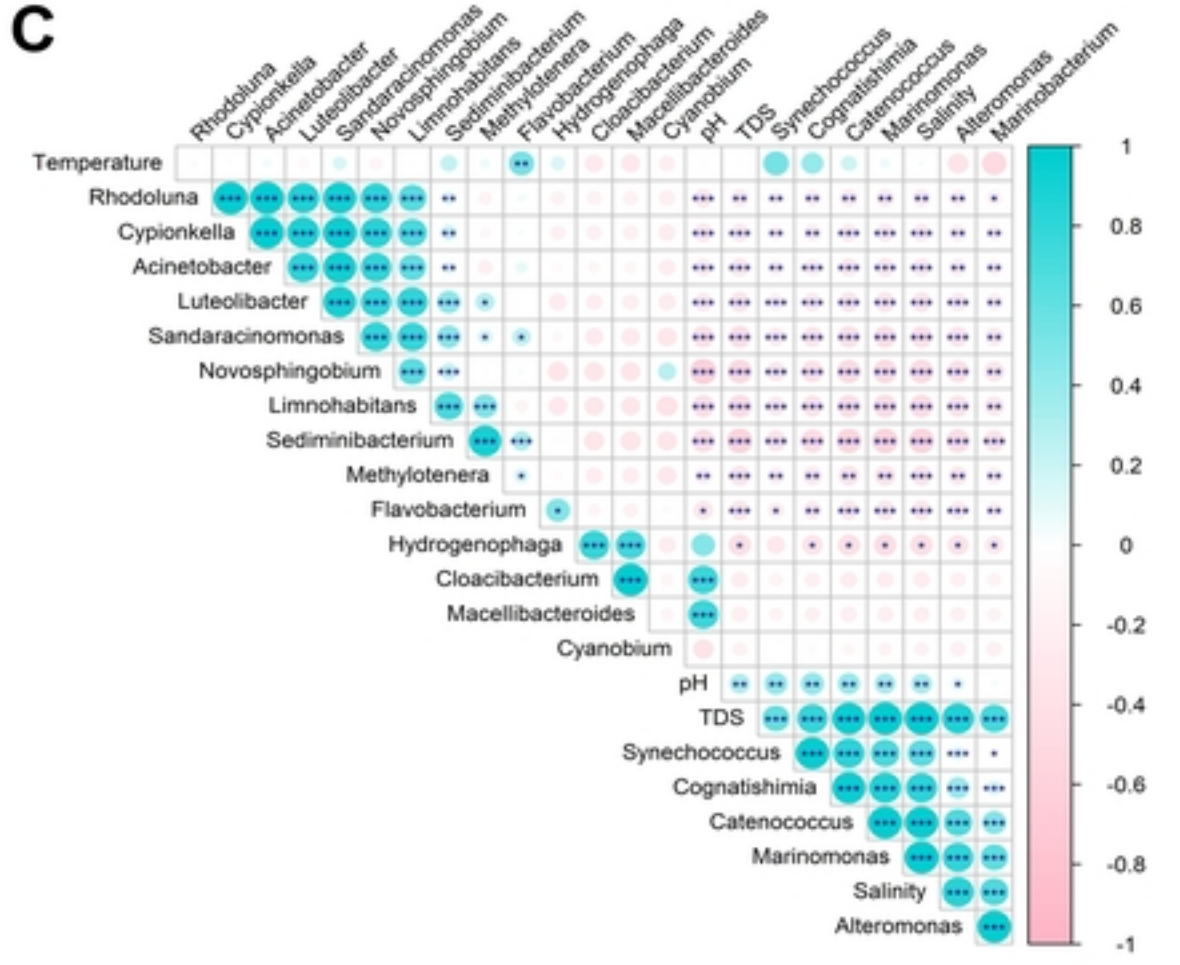



Figure-6_resized



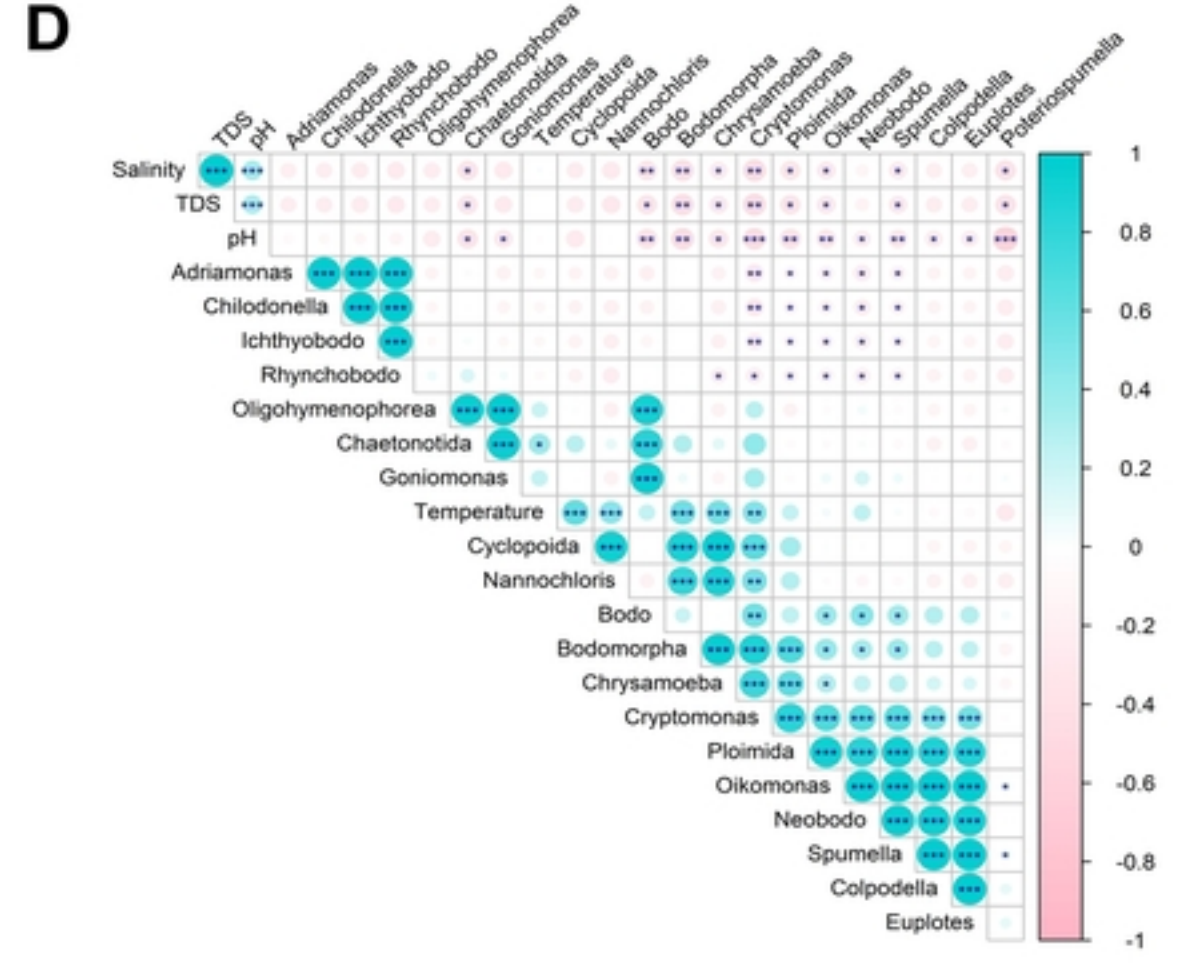




Figure-7_resized