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Bacterial Communities Along Environmental Gradients in Tropical Soda Lakes

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Abstract

Soda lake environments are known to be variable and can have distinct differences according to geographical location. In this study, we investigated the effects of different environmental conditions of six adjacent soda lakes in the Pantanal biome (Mato Grosso do Sul state, Brazil) on bacterial communities and their functioning using a metagenomic approach combined with flow cytometry and chemical analyses. Ordination analysis using flow cytometry and water chemistry data from two sampling periods (wet and dry) clustered soda lakes into three different profiles: eutrophic turbid (ET), oligotrophic turbid (OT), and clear vegetated oligotrophic (CVO). Analysis of bacterial community composition and functioning corroborated this ordination; the exception was one ET lake, which was similar to one OT lake during the wet season, indicating drastic shifts between seasons. Microbial abundance and diversity increased during the dry period, along with a considerable number of limnological variables, all indicative of a strong effect of the precipitation-evaporation balance in these systems. Cyanobacteria were associated with high electric conductivity, pH, and nutrient availability, whereas Actinobacteria, Alphaproteobacteria, and Betaproteobacteria were correlated with landscape morphology variability (surface water, surface perimeter, and lake volume) and with lower salinity and pH levels. Stress response metabolism was enhanced in OT and ET lakes and underrepresented in CVO lakes. The microbiome dataset of this study can serve as a baseline for restoring impacted soda lakes. Altogether, the results of this study demonstrate the sensitivity of tropical soda lakes to climate change, as slight changes in hydrological regimes might produce drastic shifts in community diversity.

Keywords Microbial ecology · Metagenomics · Saline-alkaline lakes · Cyanobacterial blooms · Flow cytometry

Introduction

Extreme or hostile environments are characterized by harsh physicochemical conditions that inhibit the growth of organisms [1]. Soda lakes are naturally occurring water bodies rich in carbonates and bicarbonates, comprising saline and hypersaline alkaline waters with an elevated pH (ranging from 9.5–11) and salinities that can approach saturation [2, 3]. This environment requires that the microbial inhabitants develop several strategies to deal with issues related to pH homeostasis and the intracellular osmotic pressure (e.g., osmoprotectant synthesis) [2, 4]. Several studies have attempted to establish and map the microbial communities inhabiting soda lake complexes, such as the East African Rift Valley, Carpathian Basin, Kulunda Steppe, and Cariboo Plateau [2, 5, 6]. The prokaryotic community identified in these lakes comprises Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria, and some archaeal groups, such as Euryarchaeota [4, 5, 7, 8].

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Microbial activity in soda lakes contributes to high productivity and to several critical steps in the biogeochemical cycles. Specifically, the cyanobacterial group acts as key taxa in biogeochemistry and ecosystem functioning due to its role as a primary producer [2, 7]. Moreover, several unexplored organisms can be found in soda lake-associated microbiomes, for example, the candidate phyla radiation found in soda lake sediments represents an important fermentative microorganism with a possible role in primary carbon degradation [2, 9, 10].

The Brazilian Pantanal biome (specifically the Nhecolândia sub-region) is considered the largest tropical wetland in the world and the most conserved biome in Brazil [11]. Nhecolândia hosts hundreds of pristine soda lakes (ca. 500–600) concentrated in a 27,000 km^2 area, and its microbial community remains underexplored [12]. Previous studies indicated that even nearby lakes harbor distinct bacterial communities and biogeochemical functioning due to different metabolic processes [8, 12]. Nuanced interactions between abiotic parameters, such as seasonal and spatial variations and resident microbial composition, may manifest in distinct Pantanal soda lake patterns [8, 12]. The seasonality of Nhecolândia soda lakes is characterized by heavy rainfall during summers, followed by a strong evaporation process during the rest of the year, directly affecting the water level [8, 12]. Therefore, the aims of this study were (1) to establish a lake typology for Nhecolândia soda lakes, integrating limnological, chemical, and microbiological data; (2) to evaluate the environmental variables that drive microbial communities in Nhecolândia soda lakes; and (3) to evaluate how seasonal variability in the hydrological balance affects water chemistry and the biotic components of the lake. To accomplish these goals, we analyzed microbial communities from six lakes during contrasting periods of the hydrological cycle using a combination of metagenomics and flow cytometry, concomitantly with the investigation of the limnological variables and water chemistry.

Methods

Sample Site and Collection

The soda lakes studied here are located in the São Roque Reserve in the Nhecolândia sub-region, Mato Grosso do Sul State, Brazil. This location is situated in a remote area with very difficult access by small dirt roads easement and relies on yearly weather conditions. The long rainy periods and the consecutive months of flooding restrict terrestrial access. Consequently, the sampling was conducted during the months when the conditions were favorable and access by cars was feasible. The sampled soda lakes have relatively closed drainage without direct connection to major fluvial systems and are described as small (500- to 1000-m diameter), shallow (0.5- to 2-m deep), and round or irregular-shaped lakes [8, 12]. The region is classified as a tropical savanna climate with a dry-winter period ("Aw" type) based on the Köppen classification, with an average air temperature ranging between 21 and 32 °C during the dry to wet period [13]. The annual precipitation in south-southwest Nhecolândia varies from 710 to 1200 mm in south-southwest [14, 15]. Although a previous long-term survey had reported that rains are concentrated from October to March [16], the intra-annual rainfall variability can be pronounced, as was observed in both sampling years (Fig. S1).

Surface waters (0–15 cm) were collected from six lakes (Fig. 1), with each lake sampled in four distinct points spatially separated by at least 100 m. Sampling was carried out under both wet and dry conditions (Sep-2018 and Sep-2019, respectively) (Figure S1). A total of 48 water samples were collected (6 lakes × 4 samples × 2 seasons). Water (10 L) was collected in a container and one aliquot (500 mL) of each sample was preserved in polyethylene bottles at 4 °C for chemical analysis. Additional aliquots of 50 mL for DNA extraction and 1.2 mL (fixed in situ with 1% formaldehyde) for flow cytometry analyses were stored in situ at − 80 °C in liquid nitrogen.

Data Acquisition

Monthly accumulated rainfall and land surface temperature (LST) data were obtained from the Climate Hazards Group Infrared Precipitation [17] and MODIS LST datasets respectively, using the Google Earth Engine platform. The water surface area (km^2) and water perimeter (km) were measured using PlanetScope imagery. The lake water volume (m^3) was obtained by multiplying the water surface area (m^2) with the average water depth (m) of each lake.

Lake depth and water transparency were measured using a *Secchi* disk. The water temperature, electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured in situ using multiparameter probes (YSI-6600 V2 -Yellowspring, OH, USA and Horiba U50, Kyoto, Japan) and interference by turbulence and bubbles were avoided.

Flow Cytometry and Pigment Analyses

To determine heterotrophic prokaryote (HP) abundance, 1.2-mL water samples were thawed, stained in the dark at room temperature for 15 min using SYBR Green I (Thermo Fisher Scientific, MA, USA) and examined in a flow cytometer (Accuri™ C6, BD Biosciences, Ann Arbor, MI, USA). Phototrophic picoplankton (PPP) cells were detected by autofluorescence in the flow cytometer. Both HP and PPP

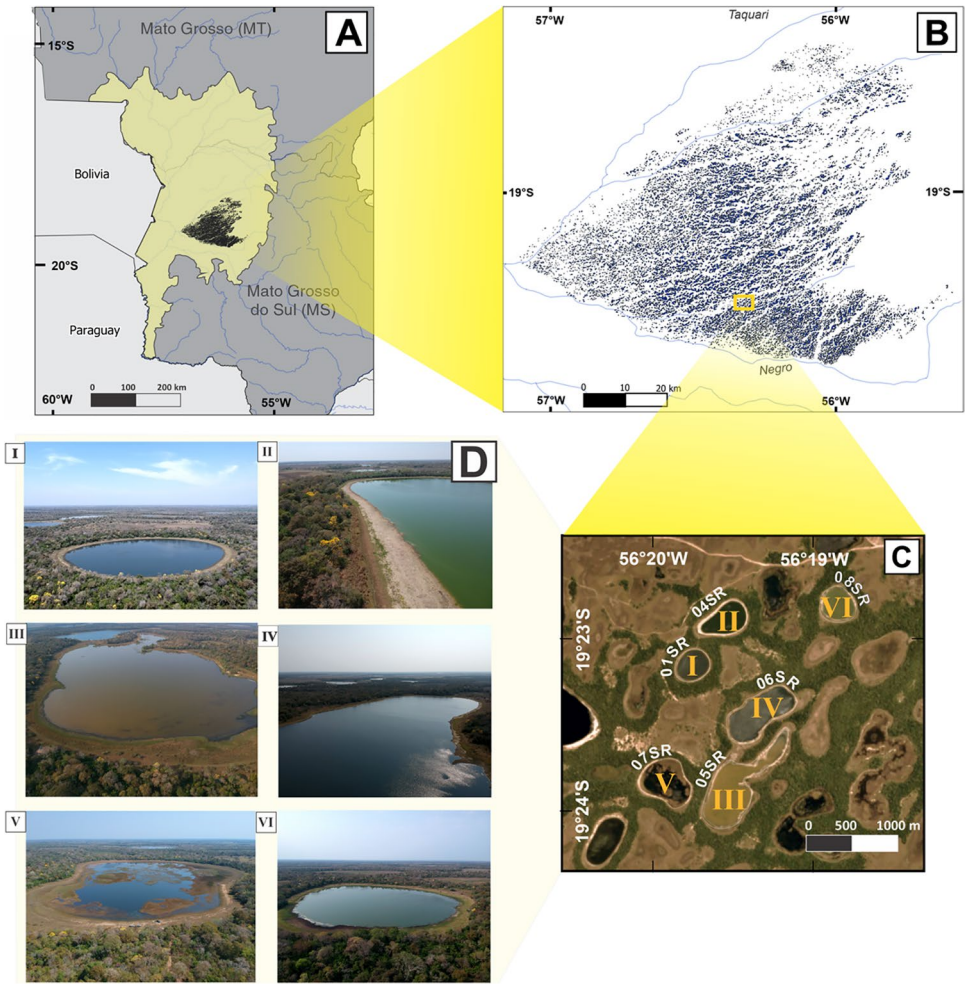

Fig. 1 Geographic distribution of Pantanal in South America (**A**); localization of Nhecolândia sub-region in Pantanal (**B**); lakes complex distribution in Nhecolandia sub-region with studied area highlighted (**C**); satellite image of sampled lakes in studied area (**D**); aerial photography of sampled lakes:01SR (I); 04SR (II); 05SR (III); 06SR (IV); 07SR (V); 08SR (VI)

cells were detected and quantified using four channels at 533 nm, 585 nm, 670 nm, and 675 nm [18, 19]. A detailed description of this analysis can be found in Supplementary Text (S1). Cytometrically defined populations among phototrophic picoplankton were classified into five groups: phycocyanin-rich picocyanobacteria (PcyPC), phycoerythrin-rich picocyanobacteria types I and II (PcyPE_1 and PcyPE_2), picoeukaryotes (Peuk), nanoeukaryotes (Neuk), and phycoerythrin-rich eukaryotes or Cyanobacteria (Perec) [18, 19]. Chlorophyll-*a* (Chl-*a*) was extracted using 90% acetone and determined by spectrophotometry using the EPA 446.0 method [20].

Microscopic Identification of Bloom-Forming Cyanobacteria

Water samples were observed under an optical microscope (Axioskop 40, Carl Zeiss, Jena, Germany) to identify the dominant bloom-forming cyanobacteria in each lake. Morphological identification was performed based on the method described by Komárek and Anagnostidis [21].

Metagenomic DNA Extraction and Sequencing

Environmental total DNA was extracted from 50 mL of a lyophilized unfiltered water sample (0.5 g) using the PowerLyzer PowerSoil DNA isolation kit (Qiagen, Hilden, Germany). Due to the variability in the quality and quantity of the extracted DNA across repeat samples, only three replicates with the best quality and quantity were sequenced. The integrity of the extracted DNA was determined using agarose gel electrophoresis (1% w/v) and quantified with the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Thirty-six DNA samples were subjected to shotgun sequencing (6 lakes × 3 samples × 2 seasons). The DNA libraries were prepared using an Illumina Nextera XT DNA Library Preparation kit (Illumina, Inc., San Diego, CA, USA), following the manufacturer's recommendations, and sequenced in

paired-end reads in 2 × 100 bp (200 cycles) on an Illumina HiSeq 2500 platform.

Bioinformatic Analyses

The raw sequence adapters were removed using CutAdapt 1.18 [22] and quality controlled using FastQC 0.10.1 [23]. The merging of paired-end reads was performed using PEAR software 0.9.6 [24]. Sequences smaller than 50 pb and Phred < 20 were removed using Seqyclean 1.3.12 [25].

Metagenome reads were submitted for taxonomic and functional annotation (RefSeq and SEED subsystems databases) via the MG-RAST bioinformatics pipeline 4.0.3 [26]. Hierarchical taxonomic and functional abundance profiles were generated using Best Hit Classification, with a minimum alignment length of 15 bp, minimum e-value cutoff of 10^{-5}, and a minimum percentage identity cutoff of 60%.

Water Chemistry Analyses

Water samples were divided into three sub-samples for chemical analysis: unfiltered, filtered through a glass microfiber with a pore size of 0.7 µm (Whatman GF/F, Sigma-Aldrich, St. Louis, MO, USA) and filtered through a 0.45-µm pore size ester-cellulose membrane (Merck Millipore, Billerica, MA, USA). Unfiltered sub-samples were used to determine total nitrogen (TN) and total phosphorus (TP) content using the persulfate method for simultaneous determination, following the American Public Health Association method 4500-P J [27]. Filtered GF/F sub-samples were used to analyze dissolved organic and inorganic carbon (DOC and DIC, respectively) and total dissolved nitrogen (TDN) by combustion (Shimadzu model TOC-5000A analyzer). Sub-samples filtered through a 0.45-µm ester-cellulose membrane were used to determine the concentration of the following ions: NH_4^+, NO_3^-, NO_2^-, by flow injection analysis [28]. Orthophosphate (oPO_4^{3-}) concentrations were quantified using the ascorbic acid method [29]. Alkalinity was analyzed with a 0.1 mol L^{-1} hydrochloric acid titration. Total dissolved solids were determined using the Environmental Protection Agency method 1684 [30]. Water salinity was estimated from the total amount of inorganic dissolved solids in water samples [31]. Concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} were analyzed by ICS-90 ion chromatography (Dionex, Sunnyvale, CA, USA). Trace elements such as Al, B, Cu, Fe, Mn, Ni, Si, and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP/OES, JY ULTIMA 2000, Longjumeau, France).

Data Analysis

All data analysis was performed in R 4.1.2 [31]. Analyses of variance were measured using the Tukey or Kruskal–Wallis tests and applied to test for significant differences among lakes using the Multicomp package [33]. All statistical assumptions were considered and are detailed in Supplementary Text S2. Principal component analysis (PCA) was performed using FactoMineR, with environmental variables set as explanatory variables and cytometric data as supplementary variables. Furthermore, non-metric multidimensional scaling (NMDS) was performed to access microbial profiles with metagenomic data (genus taxonomic level) among the typologies of the lakes using the Vegan package [34]. The functional profile of each lake was plotted in a heatmap using the Pheatmap R package [35]. Z-score transformations were applied using the scale function available in the R core base package [32]. The alpha diversity, Chao1 richness, and Shannon diversity analyses were performed using MicrobiomeAnalyst [36], with the data rarefied to the minimum library size of 181,230 and scaled using the total sums.

Results

Lake Typology

The six soda lakes showed remarkable differences in their water coloring, limnological, and cytometric profiles. In general, evaluated lakes showed a saline-alkaline condition, with a pH gradient varying between 8.62 and 10.26 and salinity from 0.41 to 2.42 g L^{-1} (Table 1). A high to moderate productivity was observed, as evidenced by high chl-*a* (up to 4123 µg L^{-1}), DOC (16 to 252 mg L^{-1}), TN (2.22 to 90 mg L^{-1}), and TP (0.02 to 22.81 mg L^{-1}) concentrations. Dissolved organic nitrogen (0.50 to 45 mg L^{-1}) was the major source of N, followed by inorganic forms such as NH_4^+ (0.0194 to 1.14 mg L^{-1}) and NO_3^- (up to 0.98 mg L^{-1}).

Seasonality was evident at the water column level, which ranged from 88 to 109 cm in wet and 53 to 77 cm in dry periods (Table 1), promoting changes in water chemistry and cytometric abundance. These variables were plotted in a PCA ordination, and three distinct groups were observed (ANOSIM $R^2 = 0.55$, $p = 0.001$; PERMANOVA, $p = 0.01$): the first group (eutrophic turbid (ET)) was composed of lakes 04SR, 05SR, and 08SR, the second group (oligotrophic turbid (OT)) was composed of 01SR and 06SR, and the third group (clear vegetated oligotrophic (CVO) was composed exclusively of lake 07SR (Fig. 2A).

An Overview of Metagenomic Data and the Microbiological Observation

The two metagenomic sequencing generated 10,507,992 and 23,850,354 sequences after cutting and removing

Table 1 Synthesis of physical and chemical variables of sampled lakes ($n=48$) in Nhecolândia, during dry and wet periods with mean of each period ($n=24$ per period). Average data are presented with standard deviation (values after ±)

Variables	Units	ET_w	ET_d	OT_w	OT_d	CVO_w	CVO_d
Depth	cm	94.83 ± 19.94	52.92 ± 5.42	88.37 ± 9.61	61.25 ± 3.53	109.5 ± 7.37	77.5 ± 50
WSA	km^2	0.22 ± 0.12	0.17 ± 0.11	0.19 ± 0.09	0.17 ± 0.08	0.19 ± 0.00	0.19 ± 0.00
WSP	km	2.15 ± 1.10	2.03 ± 1.14	1.69 ± 0.54	1.59 ± 0.51	1.72 ± 0.00	1.69 ± 0.00
WT	°C	25.84 ± 0.76	29.92 ± 4.05	25.93 ± 0.52	29.30 ± 4.24	27.10 ± 0.25	27.41 ± 0.15
Secchi	(cm)	5 ± 0.00	7 ± 3.72	15.37 ± 9.26	6.75 ± 3.28	109.50 ± 7.37	77.50 ± 5.00
pH		9.75 ± 0.27	10.05 ± 0.16	9.18 ± 0.11	9.56 ± 0.12	8.62 ± 0.05	9.05 ± 0.01
DO	mg L^{-1}	11.71 ± 3.67	26.70 ± 19.87	5.99 ± 0.58	12.18 ± 4.44	6.60 ± 0.58	10.32 ± 1.31
EC	mS cm^{-1}	1.24 ± 0.35	2.70 ± 0.39	0.90 ± 0.24	1.64 ± 0.39	0.56 ± 0.00	0.69 ± 0.00
DOC	mg L^{-1}	51.37 ± 1.08	164.50 ± 67.58	21.65 ± 10.25	55.18 ± 21.98	15.95 ± 1.12	33.96 ± 14.72
TN		9.51 ± 6.22	56.01 ± 28.76	4.00 ± 2.31	21.81 ± 7.85	2.23 ± 0.08	3.54 ± 0.34
TP		1.41 ± 1.45	10.65 ± 9.16	0.72 ± 0.63	7.75 ± 7.82	0.06 ± 0.04	0.02 ± 0.01
TN:TP ratio		14.01 ± 15.13	7.40 ± 3.91	22.74 ± 27.44	27.70 ± 31.74	41.34 ± 16.85	146.11 ± 28.00
Chl-a	µg L^{-1}	61.90 ± 44.86	1814.12 ± 1724.20	18.80 ± 9.60	67.86 ± 48.15	5.90 ± 0.93	60.87 ± 3.30
PPP	cell mL^{-1}	$1.59\times10^{6}\pm1.82\times10^{6}$	$5.51\times10^{7}\pm1.12\times10^{8}$	$1.67\times10^{5}\pm1.86\times10^{5}$	$3.23\times10^{6}\pm3.02\times10^{6}$	$1.03\times10^{4}\pm7.69\times10^{2}$	$2.70\times10^{4}\pm1.88\times10^{3}$
HP	cell mL^{-1}	$3.44\times10^{7}\pm4.42\times10^{7}$	$2.00\times10^{8}\pm2.30\times10^{8}$	$1.01\times10^{7}\pm1.08\times10^{7}$	$8.41\times10^{6}\pm8.83\times10^{6}$	$2.33\times10^{6}\pm6.76\times10^{4}$	$5.18\times10^{5}\pm1.39\times10^{5}$

WSA, water surface area; *WSP*, water surface perimeter; *WT*, water temperature; *DO*, dissolved oxygen; *EC*, electric conductivity; *DOC*, dissolved organic carbon; *TN*, total nitrogen; *TP*, total phosphorus; *Chl-a*, chlorophyll-*a*; *PPP*, photoautothrophic picoplankton; *HP*, heterotrophic prokaryotes

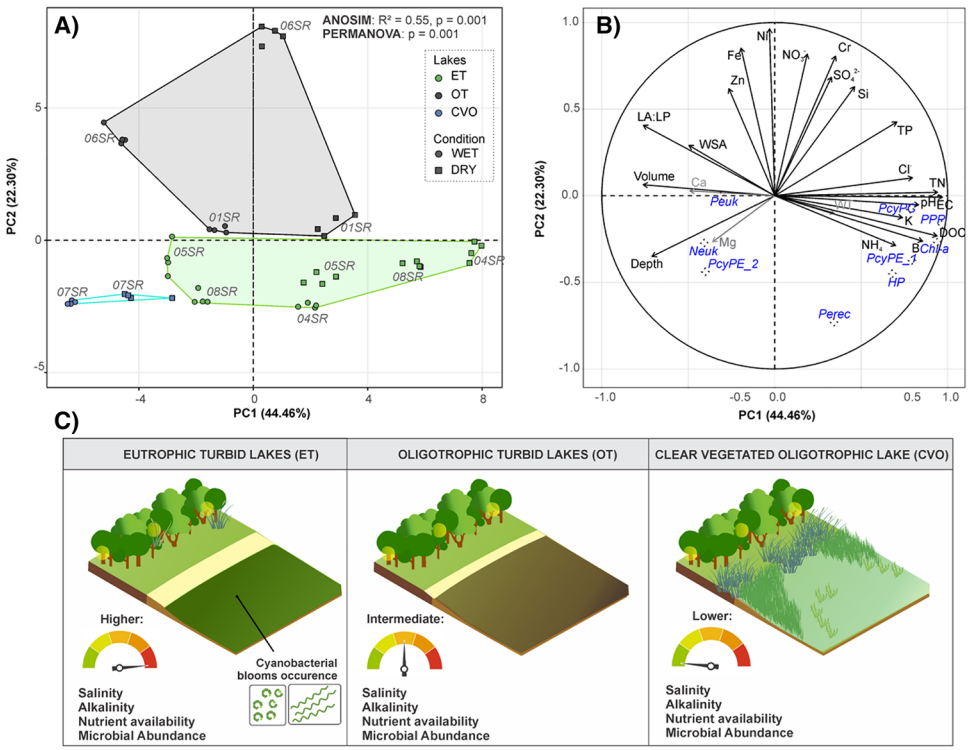

Fig. 2 Principal component analysis (PCA) of individuals considering the lake types (**A**). PCA of variables with supplementary information such as of chlorophyll-a (Chl-a), cytometric population abundances. Phycocyanin-rich picocyanobacteria (PcyPC), phycoerythrin-rich picocyanobacteria type I and II (PcyPE_1 and PcyPE_2), picoeukaryotes (Peuk), nanoeukaryotes (Neuk) and phycoerythrin-rich eukaryotes or cyanobacteria (Perec) (**B**). Illustrative scheme for each lake type and physical–chemical patterns (**C**)

low-quality sequences (Table S1). The average sequence size varied between 102 and 107 bp, whereas the GC content ranged from 46 to 58%.

The most representative bacterial taxa (above 3%) were Actinobacteria (19.80%), Cyanobacteria (17.70%), Betaproteobacteria (12.93%), Alphaproteobacteria (7.98%), Gammaproteobacteria (5.37%), Flavobacteria (4.07%), and Planctomycetacia (3.54%) (Fig. 3A). In general, the taxonomic ordination of the bacterial community followed the clustering observed for the limnologic and cytometric data (ET, OT, and CVO groups) (Fig. 3B). However, an overlap was observed between one ET lake (05SR-wet) and the OT group.

The main factor differentiating ET lakes from OT and CVO lakes was the presence of bloom-forming filamentous

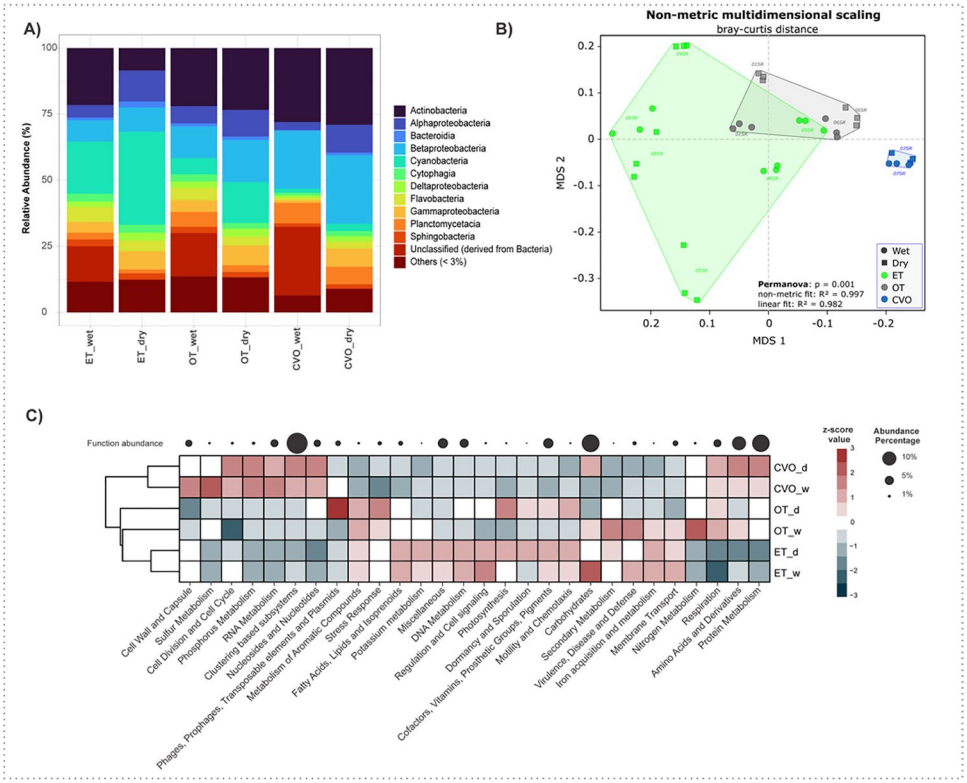

Fig. 3 Bar plot of microbial community relative abundance of each lake type and seasonal condition at class level (**A**). Principal coordinate analysis of community structure considering "genus level" of each lake type using bray distance (**B**). Heatmap of functional genes (SEED subsystem database level 1) of each lake type with a relative abundance (**C**)

cyanobacteria in the ET lakes. Trichomes of *Arthrospira platensis* (Oscillatoriales order) and *Anabaenopsis elenkinii* (Nostocales order) (morphologically identified under an optical microscope) predominated during the dry period. In ET and OT lakes (01SR) few unicellular cyanobacteria, members of the *Geminocystis* genus (Chroococcales order) were detected (also identified morphologically under an optical microscope). Representatives of these three taxa were isolated and cultured, and their 16S rRNA gene sequences were analyzed, confirming their morphological identity (data not shown). Moreover, Cyanobacteria displayed important roles in metabolic functions in the ET lakes, whereas in the other lakes the main metabolic processes were attributed to Actinobacteria, and alpha, beta, and Gammaproteobacteria (Fig. S5 and S6).

Specificity of Each Lake's Group

Eutrophic Turbid Lakes (ET)

ET lakes presented a natural cyanobacterial bloom from *A. elenkinii* or *A. platensis* species, resulting in greenish-colored waters. These lakes had high pH, EC, salinity, and alkalinity as compared with other lakes. Moreover, all these parameters were higher in the dry season than in the wet period (Table 1 and Table S2). High concentrations of nitrogen (TN, TDN, and NH_4^+) and phosphorus lead to low TN:TP ratios. The most nutrient-rich lake was 04SR, covering 17.49 to 90.43 mg L^{-1} for TN and from 3.35 to 22.81 mg L^{-1} for TP.

Cyanobacterial blooms reduced light penetration associated with high PPP abundance, DOC, DO, and Chl-*a* concentrations (Table 1 and Table S4). Chl-*a* and DOC concentrations were enhanced during the dry period. Field measurements detected oxic conditions in all lakes during the dry period, while lakes 05SR and 08SR had anoxic conditions during the wet period due to the absence or low presence of light.

The HP and PPP population abundance, bacterial diversity, and richness index values (Table S4) were significantly higher in ET than those in OT and CVO, with slight fluctuations in seasonality (Fig. S2). Specifically, the 05SR and 08SR lakes showed a higher diversity index during the dry period, whereas the 04SR showed a higher diversity index during the wet period (Fig. S2). Lakes 04SR and 05SR had higher richness index values during the dry period, whereas lake 08SR had higher richness index values during the wet period (Fig. S2).

The main bacterial taxa identified were Actinobacteria (21.82% (wet), 8.49% (dry)), Cyanobacteria (19.96% (wet), 35.23% (dry)), Betaproteobacteria (8.24% (wet), 9.20% (dry)), Flavobacteria (5.76% (wet), 4.10% (dry)), Alphaproteobacteria (4.79% (wet), 11.77% (dry)), and Gammaproteobacteria (4.11% (wet), 6.91% (dry)) (Fig. 3A and Fig. S3). The relative abundances of Alphaproteobacteria, Betaproteobacteria, and Cyanobacteria were lower

during the wet period than during the dry period. For the Actinobacteria, Flavobacteria, and Planctomycetacia, this pattern was the opposite (Fig. 3A).

The identified prevalent functions were "Fatty Acids, Lipids and Isoprenoids," "Iron acquisition and metabolism," "Regulation and Cell signaling," "Potassium metabolism," "Miscellaneous," "Photosynthesis," and "Dormancy and sporulation" (Fig. 3C and Fig. S4). "Carbohydrates" and "Virulence Diseases and Defense" were predominant in the wet period compared to the dry period (Fig. 3C). According to the ordination analysis, the potential bacterial functionality of 05SR and 08SR lakes was heavily influenced by seasonality, while that of 04SR lake was less influenced (Fig. S4). The 05SR lake during the wet period was closer to the OT group due to the differential relative abundance of "Respiration" and "Phages, Prophages, Transposable Elements, Plasmids" functions.

Oligotrophic Turbid Lake (OT)

OT lakes were characterized by turbid waters due to the high concentration of mineral-associated organic matter resulting in blackish-colored waters. The pH, EC, salinity, and alkalinity of these lakes were lower than those in the ET lakes (Table 1 and Table S2). Reduced concentrations of DOC and TN were found during the dry period. The major N source varied between the lakes, where 06SR was enriched in nitrate and 01SR was enriched in ammonium. In contrast, high values of TP and low to moderate N:P ratios showed that P was not a limiting nutrient in these lakes. High concentrations of SO_4^{2-}, Cl^-, Al, Fe, Cu, Mn, and Si were detected especially under dry conditions (Table S3).

PPP and HP abundances were reduced compared to ET lakes (Table S4 and Table S5), but they were affected by seasonality. The bacterial richness had an intermediate level when compared to ET and CVO lakes, and in contrast to ET lakes, this index was higher during the wet period (Fig. S2A). The exception was the 01SR lake, where the bacterial diversity was higher in the dry period than in the wet period (Fig. S2B).

The prevalent bacterial classes found in these lakes were similar to those observed in ET lakes, but Actinobacteria (22.29% (wet), 22.62% (dry)), Betaproteobacteria (12.13% (wet), 15.45% (dry)), and Planctomycetacia (5.71% (wet), 2.44% (dry)) had a higher relative abundance when compared to ET lakes. The seasonality effect was noticeable in the relative abundance of Cyanobacteria (lower in the wet period) (Fig. 3A, Fig. S4, Fig. S5). Although these lakes (01SR and 06SR) showed similarities in their limnological parameters, they host different bacterial community compositions. Lake 06SR was enriched in Actinobacteria and Proteobacteria (Betaproteobacteria class), while the 01SR lake was enriched in low-frequency organisms ("Others," relative abundance below 3%) (Fig. S3).

The prevalent potential bacterial functions were "Metabolism of Aromatic Compounds," "Respiration," "Secondary Metabolism," and "Stress Response" (Fig. 3C). The "Nitrogen Metabolism" function was enriched in the wet period while the "Phages, Prophages, Transposable Elements, Plasmids" function was enriched in the dry period. Seasonality affected the distribution of potential functional genes in the 01SR lake. During the dry period, the 01SR lake samples were clustered with 05SR_dry and 08SR_wet lakes, whereas the 01SR_wet samples were clustered with 04SR (both dry and wet) and 08SR_dry samples (Fig. S4).

Clear Vegetated Oligotrophic Lake (CVO)

The CVO lake had crystalline water owing to its low turbidity and high light penetration. This lake demonstrated the lowest concentrations of ions, pH, and EC of the three lake types (Table 1). As observed for the previous group of lakes, these variables were slightly increased in dry conditions. In contrast, the salinity and alkalinity were higher during the wet period. In addition, the higher TN:TP ratios indicated a low availability of TN and TP, resulting in low microbial abundance. The lowest bacterial diversity was found in the CVO lake (Fig. S2A), and the Peuk and PcyPE_2 organisms were the most abundant and associated with water surface area, volume, and depth (Table S4; Fig. 2A and 2B).

The bacterial composition of the CVO lake was remarkably different from that of the previous lake types. A higher relative abundance of Actinobacteria (28.28% (wet), 28.58% (dry)), Proteobacteria (Betaproteobacteria) (22.14% (wet), 25.57% (dry)), and Planctomycetacia (7.85% (wet), 6.54% (dry)) were observed. The relative abundances of Actinobacteria and Proteobacteria (Betaproteobacteria) were reduced during the wet period, whereas that of Planctomycetacia was increased (Fig. 3A).

The prevalent functional genes found were "Protein Metabolism," "Nucleosides and Nucleotides," "Amino Acids and Derivatives," "Clustering based subsystem," "Phosphorus metabolism," "RNA Metabolism," and "Respiration" (Fig. 3C and Fig. S4). "Cell wall and Capsule" and "Sulfur Metabolism" functions were enriched in the wet period (Fig. 3C). The CVO lake clustered with the 05SR wet sample (ET lake) (Fig. S4).

Discussion

This study, which used a detailed set of limnologic parameters, sheds light on the typology of Brazilian tropical soda lakes. Statistical and ordination analyses of the limnological dataset clustered the lakes into three categories: ET, OT, and CVO. Bacterial community composition also validated the division of these categories.

The ET lakes were well defined by the dense filamentous cyanobacterium biomass of *Anabaenopsis elenkinii* or *Arthrospira platensis* and their positive correlation with TP, TN, DOC, EC, and pH as observed on the PCA plot. These eutrophic conditions favor cyanobacterial blooms and promote changes in the environmental and ecological conditions, as previously observed in other aquatic ecosystems [37]. These two planktonic cyanobacteria have been reported as common inhabitants of several Nhecolândia soda lakes and are important primary producers in these extreme habitats [38, 39]. Furthermore, both cyanobacterial genera are associated with the occurrence of blooms in other soda lakes [2].

The main differentiation factor between CVO and OT lakes was the higher abundance of eukaryotic and phycoerythrin-rich organisms (prevalent in CVO), in addition to the metal concentration and particulate solids in suspension (prevalent in OT). Compared to ET, the OT and CVO lakes had less stressful environmental conditions (lowest salinity and pH levels), with some of them exhibiting aquatic plants and other organisms, such as amphibians, slugs, snails, and insects (field observations). Both lakes were associated with high values of depth, volume, and water surface area. Furthermore, OT lakes had high concentrations of some ions, such as Zn, Fe, Ni, NO_3^-, SO_4^{2-}, and Si, indicating a more mineralized environment. The input of nutrients such as organic matter, nitrogen, phosphorus, calcium, and iron ions, among others, may occur due to the infiltration of runoff into the lakes, which could be intensified during heavy rainfall in the wet period. This edge effect has been described for lakes, including soda lakes in Russia [40, 41]. In freshwater lakes, terrestrial organic matter and iron loads have been shown to modify the color of the water in a process known as "brownification" [42–44]. Lakes are intimately connected to their surrounding land, showing significant correlations between physicochemical and geomorphological variables (especially water volume and altitude) [45, 46]. Notably, the enrichment of eukaryotic and phycoerythrin-rich organisms in the CVO lake appeared directly associated with the runoff. This event could be a consequence of allochthonous nutritional inputs caused by surface runoff, water transparency, and reduced salinity levels. In nutrient-limiting environments, the allochthonous nutritional sources from the watershed contribute significantly to bacterial abundance [47]. Moreover, the transparency of the water and the reduction of salinity levels result in an enrichment of phycoerythrin-rich cells and a reduction of eukaryotic diversity respectively [48, 49].

Although clustered lakes suggested a uniform chemical and biological composition, each of them was highly diverse and preserved its traits. These soda lakes have unique features compared with other soda lakes worldwide, especially due to their remarkable variability influenced by seasonality [12]. Considering potential future anthropogenic disturbances such as cattle expansion and vegetation burns, our study may serve as a starting point for restoration practices by providing a detailed description of these pristine water environments. The resident microbial community is well known for its importance in setting revitalization goals and is frequently used to track the progress of ecological restoration [50]. By establishing a community reference, it can be applied for site reconnaissance (a set of ecological measurements considered as a reference), assessing the resilience to changes in natural conditions, or when constant parameters are disturbed (e.g., by anthropogenic activity). For example, soil-associated microbial taxonomic and functional profile were evaluated in human-disturbed environments (e.g., forest-to-pasture conversion, mining activities) in order to track the progress of ecological restoration practices [51, 52]. Changes in intra-annual rainfall and long periods of drought can alter the water volume in lakes, and in the Nhecolândia region, some lakes can be completely dry, as occurred in the recent years. Hydrology is a key driver of phytoplankton and heterotrophic bacterial communities in tropical freshwater lakes, as well as soda lakes [53, 54]. Water dynamics impact nutrient concentration and its flux by modulating the diverse components of the system [55]. Seasonality is determinant to the composition of the inhabiting microorganisms of soda lakes. The dry period was characterized by a high concentration of nutrients, light intensity, and temperature. These factors favor the occurrence of cyanobacterial blooms [56, 57]. The bloom of the cyanobacterium *A. platensis* occurred in 04SR and 08SR lakes, while *A. elenkinii* blooms were observed only in lake 05SR under dry conditions. *Arthrospira platensis* appears to tolerate high salinity and grows at high nitrogen and phosphorus availability (low N:P ratios) [58]. In contrast, *A. elenkinii* requires a lower salinity level and nutrient concentration to flourish [8, 59, 60].

The most abundant phyla after the Cyanobacteria in the ET lakes, i.e., Actinobacteria, Bacteroidetes, Proteobacteria (Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria), and Planctomycetacia have already been associated with cyanobacterial blooms in other soda lakes [2, 6, 61]. Cyanobacteria release labile DOC through exudation during the bloom, thus stimulating the proliferation of heterotrophic bacteria [62]. Lakes 04SR and 08SR had a similar composition of bacterial communities with a slight difference from 05SR, as evidenced by NMDS analysis. This difference could be a result of the complex interactions established between biotic (cyanobacteria and other bacteria) and abiotic factors, which modulate how these bacteria adapt to stress conditions and overcome this adversity [62, 63].

OT and CVO lakes with the absence of filamentous cyanobacterial blooms were colonized predominantly by Actinobacteria, Proteobacteria (Betaproteobacteria), and Planctomycetacia. A higher abundance of these phyla

when the Cyanobacteria population is low has been already described under oligotrophic conditions [8, 64]. During the dry period, Alphaproteobacteria and Gammaproteobacteria were the most abundant. Members of these two bacterial classes are commonly reported in soda lakes of various salinity levels, and with the potential to use sulfur compounds as a primary or secondary energy source [2, 5, 7].

The Nhecolândia soda lakes have a combination of eutrophic conditions, variable salinities, and a low water level that has never been previously described. ET lakes showed enrichment of bacterial functions associated with iron acquisition, motility, chemotaxis, virulence, secondary metabolism, and membrane transport. Some bacterial species have the potential to metabolize iron and other metals that can be discharged during rainfall-runoff, such as Proteobacteria, an enriched bacterial group in these lakes [65, 66]. Moreover, Cyanobacteria members benefit from additional iron, as they require tenfold more iron than other bacteria phyla to drive several processes, such as photosynthesis and nitrogen fixation [67, 68]. Furthermore, Cyanobacteria that dominate the ET lakes are known as an important source of secondary metabolites with biotechnological interest, such as antibiotics, pigments, and enzymes, among others, which may also be produced by Bacteroidetes and Actinobacteria [69–71]. Adaptive advantage functions were also associated with Cyanobacteria, such as stress response, nitrogen, phosphorus, and sulfur, virulence disease and defense, and dormancy and sporulation. OT and CVO lakes were supplied with a high relative abundance of genes associated with the metabolism of nitrogen, phosphorus, protein, amino acids, and respiration, which may potentially compensate for their oligotrophic conditions. The enrichment of Actinobacteria, Proteobacteria, and Planctomycetes reinforces this pattern of oligotrophic conditions; as aforementioned, these bacterial groups are adapted to nutrient-limited conditions [8, 64].

A study on Arctic microbial mats demonstrated that microorganisms could maintain a nutrient-rich environment by promoting recycling and scavenging processes and intensifying genes related to light, nitrogen, and phosphorus-related processes [72]. In extreme environments, a well-adapted microbial community has special machinery to maintain important biogeochemical processes, even under stress conditions [5, 73, 74]. Stress response metabolism was enriched in the ET and OT lakes, specifically in lakes 01SR and 05SR in the dry season and 08SR in the wet season. The stress response metabolism encompasses the responses of osmotic and oxidative stress, heat shock, and detoxification [75]. The features of these lakes (high pH and salinity) select microorganisms that are able to thrive in these conditions. These organisms present several cell and bioenergetic adaptations (inorganic salt accumulation in the cytoplasm and modifications in the structural membrane, and the production of compatible solutes, among others) that permit their survival [2]. As expected, the main characteristics showing enrichment in these lakes were those belonging to the oxidative and osmotic stress categories (data not shown).

The division of the lakes in the three typologies agreed with the variation in bacterial composition. However, some lakes (01SR and 05SR) may shift their status from ET to OT and vice versa, seasonally, depending on the hydrological balance. Changes in water level promoted by the precipitation-evaporation balance alter nutrient availability in the lakes, which favors cyanobacterial blooms in ET lakes and consequently modifies the heterotrophic bacterial composition. The hydrological cycle has been relatively unstable from year to year in the Nhecolândia sub-region over the last decade. The intensification of cattle production and unsustainable land use in these areas have also contributed to environmental disturbances. This anthropogenic pressure affects the natural healthy function of the whole biome. The dry or wet periods (specifically extreme rainfalls) may be intensified in a warming climate, resulting in short- and long-term impacts on lake biogeochemistry and regional carbon budgets [11, 76]. The regulation of Pantanal's conservation needs to be prioritized for conservation at a regional scale [77]. The robust dataset of chemical and microbial community profiles from several pristine Nhecolândia soda lakes provided in this study, which demonstrated the effect of environmental factors on these lakes, may be used in the future as a reference of undisturbed lake conditions in a possible scenario associated with climate change or anthropogenic impacts.

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Author Contribution M. F. F. and T. A. P. conceived the study. T. A. P., J. S. C., H. S., and E. D. collected the samples. T. P., S. C., J. S. C., H. S., and E. D. analyzed the data. All authors were involved in writing the paper and had final approval of the manuscript.

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Data Availability The sequence data (total of 36 metagenomes) have been deposited in the MG-RAST database under the project name Pantanal and accession numbers: mgp88859 (2018) and mgp92377 (2019).

Declarations

Competing Interests The authors declare no competing interests.

References

1. Gil JF, Mesa V, Estrada-Ortiz N, Lopez-Obando M, Gómez A, Plácido J (2021) Viruses in extreme environments, current overview, and biotechnological potential. Viruses 13(1):81
2. Sorokin DY, Berben T, Melton ED, Overmars L, Vavourakis CD, Muyzer G (2014) Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18:791–809. https://doi.org/10.1007/s00792-014-0670-9
3. Tindall BJ (1988) Prokaryotic life in the alkaline, saline, athalassic environment. Halophilic bacteria 1:31–67
4. Szabó A, Korponai K, Kerepesi C, Somogyi B, Vörös L, Bartha D, Felföldi T (2017) Soda pans of the Pannonian steppe harbor unique bacterial communities adapted to multiple extreme conditions. Extremophiles 21(3):639–649
5. Zorz JK, Sharp C, Kleiner M, Gordon PM, Pon RT, Dong X, Strous M (2019) A shared core microbiome in soda lakes separated by large distances. Nat Commun 10(1):1–10
6. Felföldi T (2020) Microbial communities of soda lakes and pans in the Carpathian Basin: a review. Biologia Futura. 71(4):393–404
7. Vavourakis CD, Andrei AS, Mehrshad M, Ghai R, Sorokin DY, Muyzer G (2018) A metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake sediments. Microbiome 6(1):1–18
8. Andreote AP, Dini-Andreote F, Rigonato J, Machineski GS, Souza BC, Barbiero L, Fiore MF (2018) Contrasting the genetic patterns of microbial communities in soda lakes with and without cyanobacterial bloom. Front Microbiol 9:244
9. Shu WS, Huang LN (2021) Microbial diversity in extreme environments. Nat Rev Microbiol.
10. Danczak RE, Johnston MD, Kenah C, Slattery M, Wrighton KC, Wilkins MJ (2017) Members of the candidate phyla radiation are functionally differentiated by carbon-and nitrogen-cycling capabilities. Microbiome 5(1):1–14
11. Guerreiro RL, Bergier I, McGlue MM, Warren LV, de Abreu UGP, Abrahão J, Assine ML (2019) The soda lakes of Nhecolândia: a conservation opportunity for the Pantanal wetlands. Perspectives in ecology and conservation 17(1):9–18
12. Barbiero L, Neto MS, Braz RR, do Carmo JB, Rezende Filho AT, Mazzi E, Camargo PB, (2018) Biogeochemical diversity, O_2-supersaturation and hot moments of GHG emissions from shallow alkaline lakes in the Pantanal of Nhecolândia, Brazil. Sci Total Environ 619:1420–1430
13. IBGE 2003 Integrated digital cartographic database of Brazil at 1:1,000,000 for ArcGis Desktop-ArcView. In: IBGE, editor
14. Zhou L, Lau KM (1998) Does a monsoon climate exist over South America? J Clim 11:1020–1040
15. Assine ML, Merino ER, Pupim FN, Warren LV, Guerreiro RL, McGlue MM (2015) Geology and geomorphology of the Pantanal basin. In: Bergier, I., Assine, M.L. (Eds.), Dynamics of the Pantanal wetland in South America. Springer International, Switzerland, pp. 23–50
16. Thielen D, Schuchmann K-L, Ramoni-Perazzi P, Marquez M, Rojas W, Quintero JI, Marques MI (2020) Quo vadis Pantanal? Expected precipitation extremes and drought dynamics from changing sea surface temperature. PLoS ONE 15(1):e0227437
17. Funk C, Peterson P, Landsfeld M, Pedreros DV, Shukla J, Husak S, Rowland G, Harrison J, Hoell L, Michaelsen A, J, (2015) The climate hazards infrared precipitation with stations—a new environmental record for monitoring extremes. Scientific data 2(1):1–21
18. Sarmento H, Unrein F, Isumbisho M, Stenuite S, Gasol JM, Descy J-P (2008) Abundance and distribution of picoplankton in tropical, oligotrophic Lake Kivu, eastern Africa. Freshw Biol 53:756–771
19. Gasol JM, Del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64(2):197–224
20. Arar EJ (1997) Method 446.0: In vitro determination of chlorophylls a, b, c + c and pheopigments in 1 2 marine and freshwater algae by visible spectrophotometry. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/005
21. Komarek J, Anagnostidis K (1989) Modern approach to the classification system of cyanophytes 4-nostocales. Archiv für Hydrobiologie Supplementband Monographische Beiträge 82(3):247–345
22. Martin M (2011) Cutadapt removes adapter sequences from high throughput sequencing reads. EMBnet Journal 17:10–12
23. Andrews S (2010) FastQC: a quality control tool for high throughput sequence data
24. Zhang J, Kobert K, Flouri T, Stamatakis A (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30(5):614–620
25. Zhbannikov IY, Hunter SS, Foster JA, Settles ML (2017) SeqyClean: a pipeline for high-throughput sequence data preprocessing. In Proceedings of the 8th ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics (pp. 407–416)
26. Meyer F, Paarman D, D'SouzaM OR, Glass EM, Kubal M, EdwardsRA, (2008) The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9(1):1–8
27. Federation WE, Association APH (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, DC, USA
28. Ŗužička J, Hansen EH (1975) Flow injection analyses Part I A new concept of fast continuous flow analysis. Analytica Chimica Acta. 78(1):145–157
29. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36
30. Telliard WA (2001) Method 1684: Total, fixed, and volatile solids in water, solids, and biosolids. US Environmental Protection Agency, Washington
31. Williams WD, Sherwood JE (1994) Definition and measurement of salinity in salt lakes. Int J Salt Lake Res 3(1):53–63
32. Team, RC (2013) R: A language and environment for statistical computing
33. Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, Hothorn MT, 2016 Package 'multcomp'. Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria
34. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara, RB, Oksanen, MJ, (2013) Package 'vegan.' Community ecol packag version 2(9):1–295
35. Kolde R, Kolde MR (2015) Package 'pheatmap.' R package 1(7):790
36. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J (2017) MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45(W1):W180–W188
37. Paerl HW, Xu H, McCarthy MJ, Zhu G, Qin B, Li Y, Gardner WS (2011) Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N and P) management strategy. Water Res 45(5):1973–1983

38. Genuário DB, Andreote APD, Vaz MGMV (2017) Fiore MF (2017) Heterocyte-forming cyanobacteria from Brazilian saline-alkaline lakes. Mol Phylogenet Evol 109:105–112
39. Santos KRS, Hentschke GS, Andreote APD, Laughinghouse HD IV, Ballot A, Novelo E, Fiore MF, Sant'Anna CL, (2018) Polyphasic characterization of newly isolated *Anabaenopsis* (Cyanobacteria) strains from tropical Brazil and Mexico. Phytotaxa 367(1):001–012
40. Sinyukovich VN, Latysheva IV., Makukhin VL (2020) Catastrophic floods on the southern tributaries of Lake Baikal and features of the atmospheric circulation. Limnology and Freshwater Biology. 564–565
41. Borzenko SV, Zamana LV, Usmanova LI (2018) Basic formation mechanisms of Lake Doroninskoye soda water, East Siberia. Russia Acta Geochimica 37(4):546–558
42. Kritzberg ES, Ekström SM (2012) Increasing iron concentrations in surface waters–a factor behind brownification? Biogeosciences 9(4):1465–1478
43. Xiao Y, Riise G (2021) Coupling between increased lake color and iron in boreal lakes. Sci Total Environ 767:145104
44 Kritzberg ES, Hasselquist EM, Škerlep M, Löfgren S, Olsson O, Stadmark J, Laudon H (2020) Browning of freshwaters: consequences to ecosystem services, underlying drivers, and potential mitigation measures. Ambio. 49(2):375-390.t
45. Coskun M, Musaoglu N (2004) Investigation of rainfall-runoff modelling of the Van Lake catchment by using remote sensing and GIS integration. In International Archives of Photogrammetry Remote Sensing Commission VII, XXth ISPRS Congress, Istanbul (pp. 12–23)
46. Klimaszyk P, Brzeg A, Rzymski P, Piotrowicz R (2015) Black spots for aquatic and terrestrial ecosystems: impact of a perennial cormorant colony on the environment. Sci Total Environ 517:222–231
47. Kritzberg ES, Cole JJ, Pace MM, Granéli W (2006) Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: results from whole-lake 13C addition experiments. Ecosystems 9(3):489–499
48. Vo¨ro¨s L, Gulyas P, Nemeth J (1991) Occurrence, dynamics and production of picoplankton in Hungarian shallow lakes. Int Rev Gesamten Hydrobiol 76:617–629
49. Menéndez-Serra M, Triadó-Margarit X, Casamayor EO (2021) Ecological and metabolic thresholds in the bacterial, protist, and fungal microbiome of ephemeral saline lakes (Monegros Desert, Spain). Microb Ecol 82(4):885–896
50. Hart M, Cross A, D'Agui H, Dixon K, Van der Heyde M, Moreira-Grez B, Whitely A (2020) Only sequence at your peril: examining assumptions of soil microbial ecology in the monitoring of ecological restoration. Ecological Solutions and Evidence
51. Pedrinho A, Mendes LW, Merloti LF, Da Fonseca MDC, Cannavan FDS, Tsai SM (2019) Forest-to-pasture conversion and recovery based on assessment of microbial communities in Eastern Amazon rainforest. FEMS microbiology ecology. 95(3):fiy236
52. Liang JL, Liu J, Jia P, Yang TT, Zeng QW, Zhang SC, Li JT (2020) Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. ISME J 14(6):1600–1613
53. Musil N-M, Raitt CF, L, (2010) A review of phytoplankton dynamics in tropical African lakes. S Afr J Sci 106(1):13–18
54. Freitas R, Vieira HH, de Moraes GP, de Melo ML, Vieira AAH, Sarmento H (2018) Productivity and rainfall drive bacterial metabolism in tropical cascading reservoirs. Hydrobiologia 809(1):233–246
55. Costa NB, Kolman MA, Giani A (2016) Cyanobacteria diversity in alkaline saline lakes in the Brazilian Pantanal wetland: a polyphasic approach. J Plankton Res 38(6):1389–1403
56. Bakker ES, Hilt S (2016) Impact of water-level fluctuations on cyanobacterial blooms: options for management. Aquat Ecol 50(3):485–498
57. Brasil J, Attayde JL, Vasconcelos FR, Dantas DD, Huszar VL (2016) Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. Hydrobiologia 770(1):145–164
58. Kebede E (1997) Response of Spirulina platensis (= Arthrospira fusiformis) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. J Appl Phycol 9(6):551–558
59. Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA, Pflugmacher S (2004) Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita. J Plankton Res 26(8):925–935
60. Krienitz L, Dadheech PK, Kotut K (2013) Mass developments of the cyanobacteria Anabaenopsis and Cyanospira (Nostocales) in the soda lakes of Kenya: ecological and systematic implications. Hydrobiologia 703(1):79–93
61. Kolmonen E, Sivonen K, Rapala J, Haukka KJAME (2004) Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas. Finland Aquatic microbial ecology 36(3):201–211
62. Alvarenga DO, Andreote APD, Branco LHZ, Fiore MF (2017) Kryptousia macronema gen nov sp nov and Kryptousia microlepis sp nov nostocalean cyanobacteria isolated from phyllospheres. International journal of systematic and evolutionary microbiology 67(9):3301–3309
63. Nelson C, Garcia-Pichel F (2021) Beneficial cyanosphere heterotrophs accelerate establishment of cyanobacterial biocrust. Appl Environ Microbiol 87(20):e01236-e1321
64. Ji B, Qin H, Guo S, Chen W, Zhang X, Liang J (2018) Bacterial communities of four adjacent fresh lakes at different trophic status. Ecotoxicol Environ Saf 157:388–394
65. Zavarzina DG, Kolganova TV, Boulygina ES, Kostrikina NA, Tourova TP, Zavarzin GA (2006) Geoalkalibacter ferrihydriticusgen. nov. sp. nov., the first alkaliphilic representative of the family Geobacteraceae isolated from a soda lake. Microbiology 75:673–682
66. Oremland RS, Saltikov CW, Stolz JF, Hollibaugh JT (2017) Autotrophic microbial arsenotrophy in arsenic-rich soda lakes. FEMS microbiology letters. 364:15
67. Molot LA, Watson SB, Creed IF, Trick CG, McCabe SK, Verschoor MJ, Schiff SL (2014) A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. Freshw Biol 59(6):1323–1340
68. Qiu GW Koedooder C Qiu BS Shaked Y Keren N (2021) Iron transport in cyanobacteria–from molecules to communities. Trends in Microbiology
69. Shishido TK, Popin RV, Jokela J, Wahlsten M, Fiore MF, Fewer DP, Sivonen K (2019) Dereplication of natural products with antimicrobial and anticancer activity from Brazilian cyanobacteria. Toxins 12(1):12
70. Sorokin DY, van Pelt S, Tourova TP, Evtushenko LI (2009) Nitriliruptor alkaliphilus gen. nov., sp. nov. a deep lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. Int J Syst Evol Microbiol 59:248–253
71. Kalwasińska A, Jankiewicz U, Felföldi T, Burkowska-But A, Swiontek-Brzezinska M (2018) Alkaline and halophilic protease production by Bacillus luteus H11 and its potential industrial applications. Food Technol Biotechnol 56:553–561Return to ref 2018 in article
72. Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2010) Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnol Oceanogr 55(5):1901–1911

73. Jeffries TC, Seymour JR, Newton K, Smith RJ, Seuront L, Mitchell JG (2012) Increases in the abundance of microbial genes encoding halotolerance and photosynthesis along a sediment salinity gradient. Biogeosciences 9(2):815–825
74. Lay CY, Mykytczuk NC, Yergeau Ï, Lamarche-Gagnon G, Greer CW, Whyte LG (2013) Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring. Appl Environ Microbiol 79(12):3637–3648
75. Mangrola AV, Dudhagara P, Koringa P, Joshi CG, Patel RK (2015) Shotgun metagenomic sequencing based microbial diversity assessment of Lasundra hot spring, India. Genomics data 4:73–75
76. Guerra A, de Oliveira PTS, de Oliveira Roque F, Rosa IM, Ochoa-Quintero JM, Guariento RD, Garcia LC (2020) The importance of Legal Reserves for protecting the Pantanal biome and preventing agricultural losses. J Environ Manage 260:110128
77. Harris MB, Tomas W, Mourão G, Da Silva CJ, Guimaraes E, Sonoda F, Fachim E (2005) Safeguarding the Pantanal wetlands: threats and conservation initiatives. Conserv Biol 19(3):714–720