Bacterial Communities Along Environmental Gradients in Tropical Soda Lakes

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Research Article

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Bacterial communities along environmental gradients in tropical soda lakes

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Abstract

Soda lakes environment is known to be variable and can have distinct differences according to geographical location. In this study, we investigated the effect of different environmental conditions of six adjacent soda lakes 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 in 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, that 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 linked to 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 less stressed lake conditions. Stress response metabolism was overrepresented in ET and OT lakes and underrepresented in CVO lakes. Altogether, this study illustrated 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, extreme environment, saline-alkaline lakes, cyanobacterial blooms

Introduction

Extreme or hostile environments are characterized by severe physicochemical conditions that reduce the growth of organisms [1]. Despite these harsh conditions, such

habitats can provide a diverse ecological niche for a wide range of microorganisms [2]. For example, sediments of hypersaline soda lakes host bacteria affiliated with candidate phyla radiation, an important and little explored group of fermentative microorganisms with a possible role in primary carbon degradation [2,3]. In addition, this role of aforementioned phyla highlights the importance of microbial activity in biogeochemical cycles in environments where other life forms are limited. The higher productivity observed in hypersaline soda lakes is sustained by microbial activity. Specifically, the cyanobacterial group acts as key species in biogeochemistry and ecosystem functioning due to primary productivity [4,5].

Soda lakes are natural environments with rich carbonate and bicarbonate waters, comprising saline and hypersaline alkaline waters with elevated pH (ranging from 9.5–11) and salinities approaching saturation [4,6]. Several studies had attempted to unravel and map microbial communities inhabiting some soda lakes complexes as the East African Rift Valley, Carpathian Basin, Kulunda Steppe, and Cariboo Plateau [4,7,8]. The prokaryotic community identified in these lakes comprises Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria, and some archaeal groups as Euryarchaeota [5,7,9,10].

The Brazilian Pantanal biome (specifically in the Nhecolândia sub-region) hosts hundreds of pristine soda lakes (ca. 500–600) concentrated in a 27,000 km² area, and its microbial community remains underexplored [11]. Nuanced interactions between abiotic parameters, such as seasonal and spatial variations and resident microbial composition, may manifest in distinct Pantanal soda lake patterns [10,11]. The seasonality of Nhecolândia soda lakes is characterized by heavy rainfall during summer, followed by a strong evaporation process in the rest of the year, directly affecting the water level [10,12]. 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 affected water chemistry and biotic compartments. To accomplish these goals, we analyzed microbial communities from six lakes in contrasting periods of the hydrological cycle using a combination of metagenomics and flow cytometry, concomitantly with limnological variables and water chemistry.

2. Methods

2.1. Sample site and Collection

The lakes studied here are located in the São Roque Reserve in the Nhecolândia sub-region, Mato Grosso do Sul State, Brazil. These 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 1.5 m deep), and round or irregular-shaped lakes [10,11]. 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°C and 32°C during the dry to wet period [13]. The annual precipitation in southwest Nhecolândia varies from 710 to 1200 mm in the south-southwest [14,15].

Surface waters were collected from six lakes (Fig. 1), with four replicates separated by at least 100 m. Sampling was carried out under both wet and dry conditions (Sep-2018 and Sep-2019, respectively) (Figure S1). Although a previous long-term survey has reported that rains are concentrated from October to March [12], the intra-annual rainfall variability can be pronounced, as was observed in both of the sampling years (Fig. S1).

102 *2.2. Data Acquisition*

103 Monthly accumulated rainfall and land surface temperature (LST) data were obtained
104 from the Climate Hazards Group Infrared Precipitation [16] and MODIS LST datasets
105 respectively, using the Google Earth Engine platform. The water surface area (km^2) and
106 water perimeter (km) were measured using PlanetScope imagery. The lake water volume
107 (m^3) was obtained by multiplying the water surface area (m^2) with the average water depth
108 (m) of each lake.

109 Lake depth and water transparency were measured using a *Secchi* disc. The water
110 temperature, electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured
111 *in situ* using multiparameter probes (YSI-6600 V2 -Yellowspring, OH, USA and Horiba
112 U50, Kyoto, Japan) and interference by turbulence and bubbles were avoided. Water (10
113 L) was collected in a sterile container and stored in a 500 mL polyethylene bottle at 4°C
114 until further analysis.

115

116 *2.3. Flow cytometry and pigment analyses*

117 To determine heterotrophic prokaryote (HP) abundance, 1.2 mL water samples were
118 fixed in 1% formaldehyde, immediately flash-frozen in liquid nitrogen, and stored at –
119 80°C until analysis. Phototrophic picoplankton (PPP) cells were detected by
120 autofluorescence using a flow cytometer (see details in Supplementary Text S1).
121 Cytometrically defined populations among phototrophic picoplankton were classified
122 into five groups: phycocyanin-rich picocyanobacteria (PcyPC), phycoerythrin-rich
123 picocyanobacteria type I and II (PcyPE_1 and PcyPE_2), picoeukaryotes (Peuk),
124 nanoeukaryotes (Neuk), and phycoerythrin-rich eukaryotes or Cyanobacteria (Perec)
125 [17,18]. Chlorophyll-*a* (Chl-*a*) was extracted using 90% acetone and determined by
126 spectrophotometry using the EPA 446.0 method [19].

127

128 *2.4. Microscopic identification of bloom-forming cyanobacteria*

129 Water samples were observed under an optical microscope (Axioskop 40, Carl Zeiss,
130 Jena Germany) to identify the dominant bloom-forming cyanobacteria in each lake.
131 Morphological identification was performed based on the method described by Komárek
132 and Anagnostidis [20].

133

134 *2.5. Metagenomic DNA extraction and sequencing*

135 Environmental total DNA was extracted from lyophilized water sample (0.5 g)
136 obtained in triplicate from each lake using the PowerLyzer PowerSoil DNA isolation kit
137 (Qiagen, Hilden, Germany). The integrity of the extracted DNA was checked using
138 agarose gel electrophoresis (1% w/v) and quantified with the Qubit 2.0 fluorometer
139 (Thermo Fisher Scientific, Waltham, MA, USA). A total of 36 DNA samples were
140 obtained for shotgun-sequencing (6 lakes × 3 replicates × 2 seasons). Libraries were
141 generated using the Nextera XT DNA Sample Preparation kit (Illumina, Inc., San Diego,
142 CA, USA), following the manufacturer's recommendations, and sequenced on the
143 Illumina HiSeq 2500 platform.

144

145 *2.6 Bioinformatic analyses*

146 The raw sequence adapters were removed using CutAdapt [21] and quality
147 controlled using FastQC 0.10.1 [22]. The merging of paired ends reads was performed
148 using PEAR software [23]. Sequences smaller than 50 pb and Phred < 20 were removed
149 using Seqyclean 1.3.12 [24].

150 Metagenome reads were submitted for taxonomic and functional annotation
151 (RefSeq and SEED subsystems database) via the MG-RAST bioinformatics pipeline [25].

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%.

2.7. 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 [26]. 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 analyses [27]. Orthophosphate (oPO_4^{3-}) concentrations were quantified using the ascorbic acid method [28]. Alkalinity was analyzed with 0.1 mol L^{-1} hydrochloric acid solution titration. Total dissolved solids were determined using the Environmental Protection Agency method 1684 [29]. Water salinity was estimated from the total amount of inorganic dissolved solids in water samples [30]. 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).

2.8. Data analysis

Analyses of variance were measured using Tukey or Kruskal-Wallis tests and applied to test for significant differences among lakes using the Multicomp package [31]. All statistical assumptions were considered and are detailed described 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. Further, principal coordinate analysis (PCoA) was conducted to explore microbial profiles with metagenomic data (class taxonomy level) among lake typology using the FactoExtra package [32]. Alpha diversity, Chao1 richness, and Shannon diversity analyses were performed using MicrobiomeAnalyst [33], with the data rarefied to the minimum library size and scaled using the total sums.

3. Results

3.1 Lake typology

The six soda lakes showed remarkable differences in their watercolor, limnological and cytometric profiles. In general, evaluated lakes showed a saline-alkaline condition, with a pH gradient varying between 8.62 to 10.05 and salinity from 0.41 to 1.72 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 ortophosphate (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 1.18 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).

3.2. An overview of metagenomic data and the microbiological observation

Metagenomic sequencing recovered a total of 10,507,992 and 23,850,354 sequences after trimming and removal of low-quality sequences for the wet and dry periods, respectively (Table S1). The average sequence size varied between 105 and 107 bp for wet and 102 and 103 bp for dry conditions, whereas the GC content ranged from 48% to 55% in wet and 46% to 58% in dry periods.

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).

The PCoA from taxonomic data followed the clustering observed for limnologic and cytometric data (ET, OT, and CVO groups) with a clear separation between the dry and wet periods (x-axis, 48.5%) (Fig. 3B). However, an overlap between one lake ET (05SR) and one lake OT (01SR) was observed. In the wet period, 05SR lake was more similar to OT lakes than ET, while in the dry period, 01SR lake was more similar to ET lakes.

The main differentiating factor between ET from OT and CVO lakes was the presence of bloom-forming cyanobacteria. Trichomes of *Arthrospira platensis* (Oscillatoriales) and *Anabaenopsis elenkinii*. (Nostocales) (observed under an optical

226 microscope) were predominated during the dry period. A few unicellular cyanobacterium
227 *Geminocystis* sp. (Chroococcales) was also observed in samples from the ET lakes.
228
229 *3.3. Specificity of each lake's group*
230 *3.3.1. Eutrophic turbid lakes (ET)*
231 ET lakes presented a natural cyanobacterial bloom from *A. elenkinii* or *A. platensis*
232 species, resulting in greenish-colored waters. These lakes had high pH, EC, salinity, and
233 alkalinity as compared with other lakes. Moreover, all these parameters were higher in
234 the dry season than in the wet period (Table 1 and Table S2). High concentrations of
235 nitrogen (TN, TDN, and NH_4^+) and phosphorus lead to low TN:TP ratios. The nutrient-
236 richest lake was 04SR, covering 17.49 to 90.43 mg L^{-1} for TN and from 3.35 to 22.81 mg
237 L^{-1} for TP.
238 Cyanobacterial blooms reduced light penetration associated with high PPP abundance,
239 DOC, DO, and Chl-*a* concentrations (Table 1 and Table S4). Chl-*a* and DOC
240 concentrations were enhanced during the dry period. Field measurements detected oxic
241 conditions in all lakes during the dry period, while lakes 05SR and 08SR had anoxic
242 conditions during the wet period as a consequence of the absence or low presence of light.
243 The HP and PPP population abundance, bacterial diversity, and richness index values
244 (Table S4) were significantly higher in ET than those in OT and CVO, with slight
245 fluctuations in seasonality (Fig. S2). However, the 05SR and 08SR lakes showed a higher
246 diversity index during the dry period, whereas 04SR showed a higher diversity during the
247 wet period (Fig. S2). Lakes 04SR and 05SR had higher richness index values during the
248 dry period, whereas that for 08SR lake was observed during the wet period (Fig. S2).
249 The main bacterial taxa identified were Actinobacteria [21.82% (wet), 8.49% (dry)],
250 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). The "Carbohydrates" and "Virulence Diseases and Defense" were predominant in the wet period compared to the dry period (Fig. 3C). The ordination analysis clustered the 05SR lake together with the OT and CVO (07SR) lakes as well as 08SR in the wet period. This clustering could occur as a result of the differential abundance of "Carbohydrates" (higher in 04SR and 08SR_dry) and "Membrane transport" (enriched on 04SR and 08SR_dry) functions (Fig S4).

3.3.2. 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 and Fig. S4). Although these lakes (01SR and 06SR) showed similarities in their limnological parameters, they host a different bacterial communities composition. Lake 06SR was enriched in Actinobacteria and Proteobacteria (Betaproteobacteria class), while the 01SR lake was enriched in low-frequency organisms ("Others") (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 on wet period while the "Phages, Prophages, Transposable Elements, Plasmids" function was enriched on dry period. Seasonality affected the distribution of potential functional genes in 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).

3.3.3. 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 than the other two 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 peuk and PcyPE_2 organisms were more abundant, while this lake had the lowest bacterial diversity (Table S4; Fig. S2A).

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 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 on the wet period (Fig. 3C). The CVO lake clustered with the 05SR wet sample (ET lake) (Fig. S4).

4. 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 cyanobacterium biomass of *Anabaenopsis elenkinii* or *Arthrospira platensis* and their positive correlation with TP, TN, DOC, EC, and pH as observed on PCA plot and correlation matrix. These eutrophic conditions favor cyanobacterial blooms and promote changes in the environmental and ecological conditions, as previously observed in other aquatic ecosystems [34]. The two planktonic cyanobacteria have been reported as common inhabitants of several Nhecolândia soda lakes and are important primary producers in these extreme habitats [35, 36]. Furthermore, both cyanobacterial genera are associated with the occurrence of blooms in other soda lakes [4].

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, with some of them exhibiting aquatic plants and other organisms, such as amphibians, slugs, snails, and insects (field observations). Both lakes were positively correlated with depth, volume, and water surface area and water surface perimeter relationship. Furthermore, OT lakes were correlated with 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 [37,38]. Lakes are intimately connected to their surrounding land, showing significant correlations between physicochemical and geomorphological variables (especially water volume and altitude) [39, 40]. Notably, the enrichment of

eukaryote and phycoerythrin-rich organisms in the CVO lake could be directly correlated to the runoff, as observed in other studies [41,42].

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 to other soda lakes worldwide, especially due to their remarkable seasonality [11]. Changes in intra-annual rainfall and long periods of drought can alter the water volume in lakes, and in the Nhecolandia region, some lakes can be completely dry, as occurred in 2017, 2020, and 2021. Hydrology is a key driver of phytoplankton and heterotrophic bacterial communities in tropical freshwater lakes, as well as soda lakes [43,44]. Water dynamics impact nutrient concentration and its flux by modulating the diverse components of the system [45]. Seasonality is a determinant of the structure 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 [46,47]. 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. *A. platensis* appears to tolerate high salinity and grows at high nitrogen and phosphorus availability (low N:P ratios) [48]. In contrast, *A. elenkinii* requires a lower salinity level and nutrient concentration to flourish [10, 49, 50].

The top-level heterotrophic bacteria of the ET lakes were Actinobacteria, Bacteriodetes, Proteobacteria (Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria) and Planctomycetacia. These bacterial groups have already been described in association with cyanobacterial blooms in other soda lakes [4,8,51]. Cyanobacteria release labile DOC through exudation during the bloom, thus stimulating the proliferation of heterotrophic bacteria [52]. Lakes 04SR and 08SR had a similar

374 composition of heterotrophic bacterial community with a slight difference from 05SR, as
375 evidenced by PCoA analysis. This difference could be a result of the complex interactions
376 established between biotic (cyanobacteria and heterotrophic bacteria) and abiotic factors,
377 which modulate how these bacteria adapt to stress conditions and overcome this adversity
378 [52,53].

379 OT and CVO lakes with absence of cyanobacterial blooms were colonized mainly
380 by Actinobacteria, Proteobacteria (Betaproteobacteria) and Planctomycetacia. A higher
381 abundance of these phyla when Cyanobacteria population is low has been already
382 described under oligotrophic conditions [10,54]. During the dry period,
383 Alphaproteobacteria and Gammaproteobacteria were the most abundant. Members of
384 these two bacterial classes are commonly reported in soda lakes of various salinity levels,
385 and with the potential to use sulfur compounds as a primary or secondary energy source
386 [4,5,7].

387 The Nhecolândia soda lakes have unique abiotic conditions encompassing
388 eutrophic conditions, hyposaline to saline content, and a low water level, which has never
389 already been described all together for other soda lakes region. ET lakes showed
390 enrichment of bacterial functions associated with iron acquisition, motility, chemotaxis,
391 virulence, and membrane transport. Some bacterial species have the potential to
392 metabolize iron and other metals that can be discharged during rainfall runoff [55, 56].
393 Moreover, cyanobacteria benefit from as they require ten-fold more iron than that
394 required by heterotrophic bacteria to drive many processes, such as photosynthesis and
395 nitrogen fixation [57, 58]. OT and CVO lakes were supplied with a high relative
396 abundance of genes associated with the metabolism of nitrogen, phosphorus, protein, and
397 respiration, which may potentially compensate for their oligotrophic conditions. A study
398 on Arctic microbial mats demonstrated that microorganisms could maintain a nutrient-

rich environment by promoting recycling and scavenging processes, intensifying genes related to light, nitrogen, and phosphorus-related process [59]. In extreme environments, a well-adapted microbial community has special machinery to maintain important biogeochemical processes, even under stress conditions [7,60,61]. Stress response metabolism was overrepresented in the ET and OT lakes. The stress metabolism category encompasses the responses of osmotic, oxidative, heat shock, and detoxification [62].

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 subregion over the last decade. The dry or wet periods may be intensified in a warming climate, resulting in short- and long-term impacts on lake biogeochemistry and regional carbon budgets.

Supplementary Information

The online version contains supplementary material available at:

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Author Contributions

MFF and TAP conceived the study. TAP, JSC, HS and ED collected the samples. TP, SC, JSC, HS and ED analyzed the data. All authors were involved in writing the paper and had final approval of the manuscript.

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Competing interests

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

Data Availability

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

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Table 1. Synthesis 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	$\mu g\ L^{-1}$	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	$cell\ mL^{-1}$	$1.59x10^6 \pm 1.82x10^6$	$5.51x10^7 \pm 1.12x10^8$	$1.67x10^5 \pm 1.86x10^5$	$3.23x10^6 \pm 3.02x10^6$	$1.03x10^4 \pm 7.69x10^2$	$2.70x10^4 \pm 1.88x10^3$
HP		$3.44x10^7 \pm 4.42x10^7$	$2.00x10^8 \pm 2.30x10^8$	$1.01x10^7 \pm 1.08x10^7$	$8.41x10^6 \pm 8.83x10^6$	$2.33x10^6 \pm 6.76x10^4$	$5.18x10^5 \pm 1.39x10^5$

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; N.A.: Not available data.

Figure legends

Figure 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).

Figure 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 (B). 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). Illustrative scheme for each lake type and physical-chemical patterns (C).

Figure 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 “class level” of each lake type (B). Heatmap of functional genes (SEED subsystem database level 1) of each lake type.

666 Figure 1.

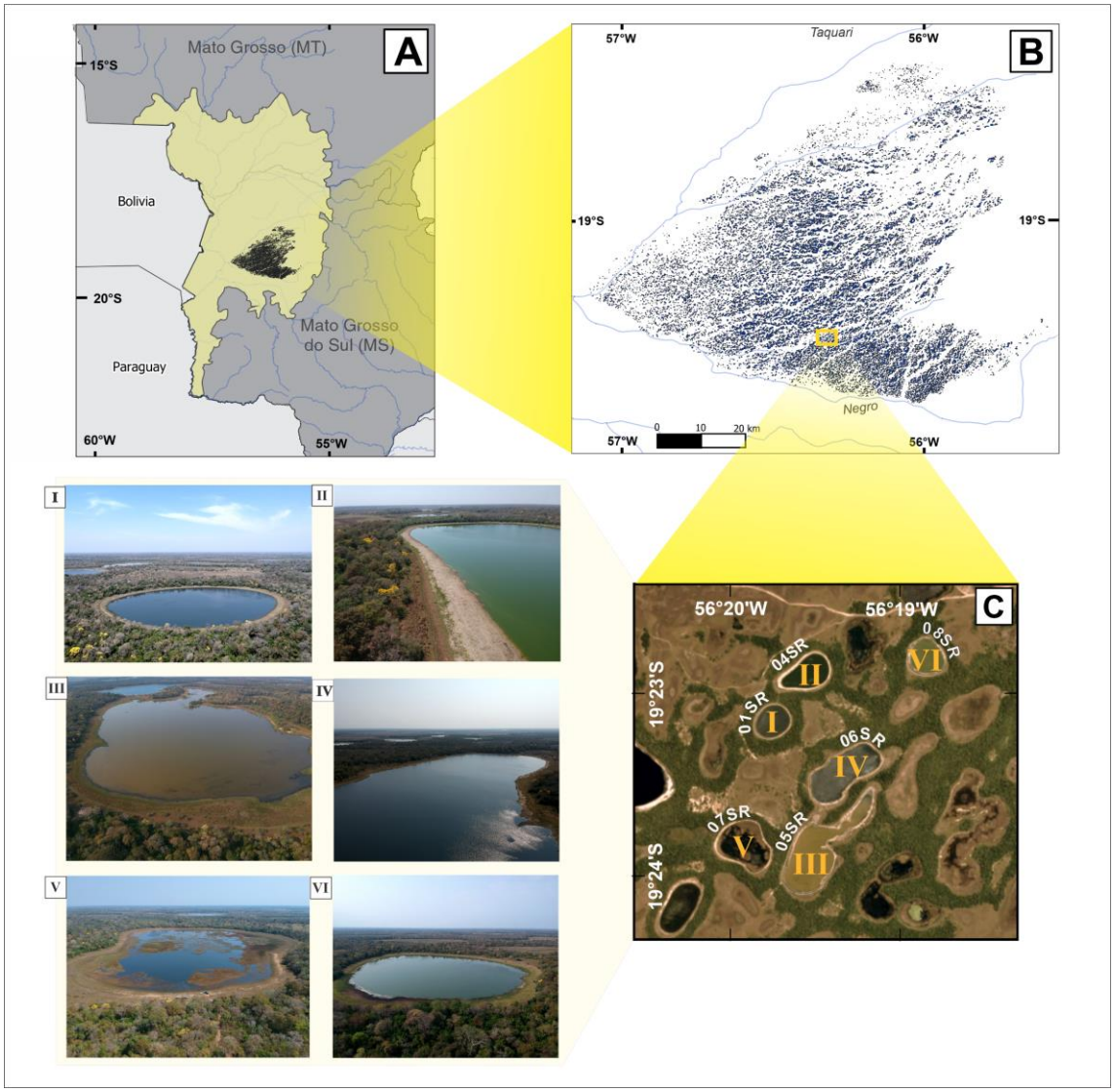

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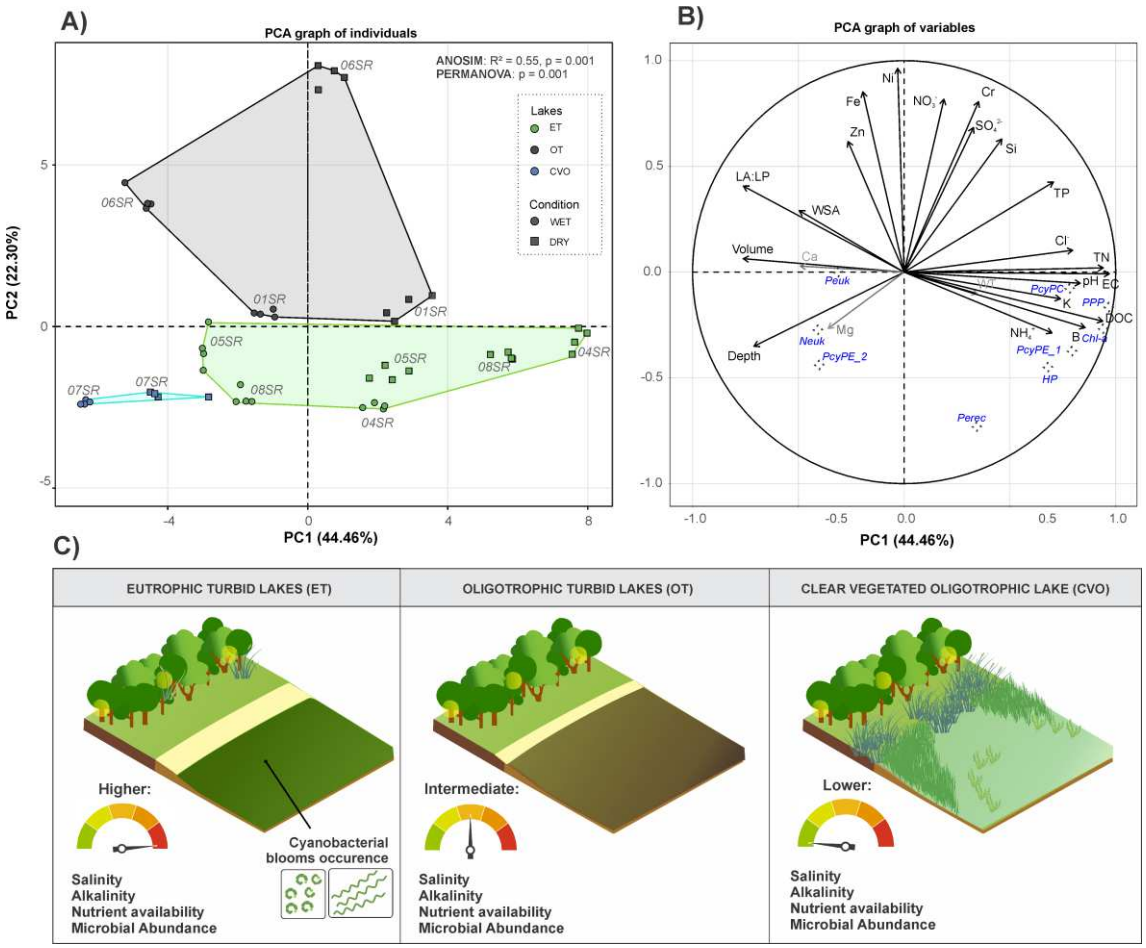
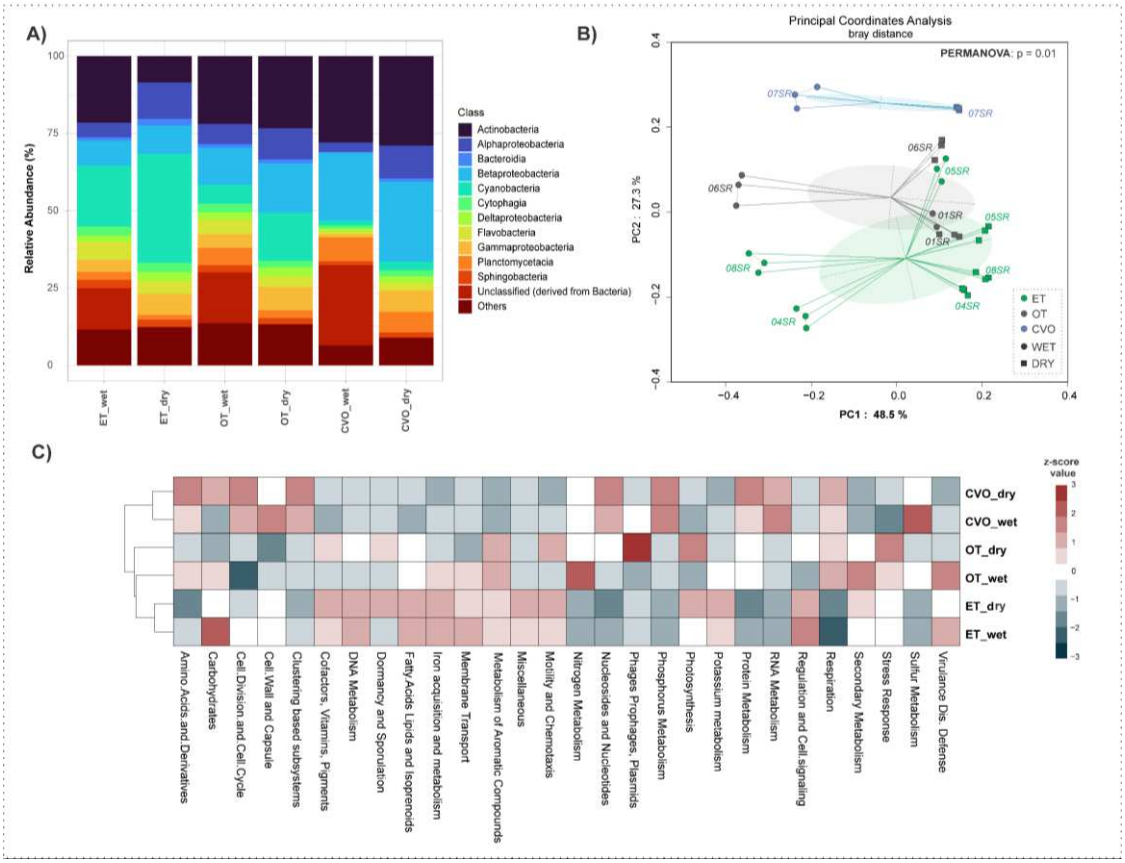
Figure 2.


A)
PCA graph of individuals
ANOSIM: R² = 0.55, p = 0.001
PERMANOVA: p = 0.001
Lakes
ET
OT
CVO
Condition
WET
DRY
PC2 (22.30%)
PC1 (44.46%)
B)
PCA graph of variables
PC1 (44.46%)
C)
EUTROPHIC TURBID LAKES (ET)
OLIGOTROPHIC TURBID LAKES (OT)
CLEAR VEGETATED OLIGOTROPHIC LAKE (CVO)
Higher:
Intermediate:
Lower:
Cyanobacterial blooms occurence
Salinity
Alkalinity
Nutrient availability
Microbial Abundance

673 Figure 3.



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