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Microbial communities and functional genes involved in nutrient cycling in saline alkaline lakes of the Pantanal of Nhecolândia (MS)

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"Rejoice with your family in the beautiful land of life." –

Albert Einstein

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"Intelligence is based on how efficient a species became at doing the things they need to survive."

(**Charles Darwin | 1809 – 1882)**

ABSTRACT

PELLEGRINETTI, T.A. **Microbial communities and functional genes involved in nutrient cycling of Pantanal of Nhecolândia - MS**. 2022. 128 p. Tese (Doutorado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2022.

Nhecolândia (MS), one important sub-region of the Pantanal, consists of 12,150 lakes among which approximately 600 lakes can be described as saline alkaline (soda lakes). Soda lakes present high concentrations of carbonates and sodium bicarbonates, resulting in high pH (above 9) and salinities (up to 35 mS cm^{-1}). Even under hostile conditions, this ecosystem hosts a rich biodiversity of microorganisms. In this study, an approach that included molecular and chemical analyses was used to evaluate the composition (taxonomic and functional) and lifestyle of bacterial communities in the surface water of this environment. Through ordination analysis using limnological and metagenomic data, it was possible to group the soda lakes into three distinct profiles: eutrophic turbid (ET), oligotrophic turbid (OT) and clear vegetated oligotrophic (CVO). Seasonality was a crucial factor in the dynamics of the bacterial community. During the dry season, the water level reduced drastically due to intense evaporation periods, leading to an increase in salinity, pH and nutrient concentration, which in turn enhanced the diversity and relative abundance of microorganisms. This scenario led to the predominance of cyanobacterial blooms in ET lakes, mainly associated with *Artrospira platensis* or *Anabaenopsis elenkinii*. This predominance could be justified by the investment in mechanisms of nutrient acquisition by these organisms. In the OT and CVO lakes, there was a predominance of Actinobacteria, Alphaproteobacteria and Betaproteobacteria and these organisms possibly adopted strategies associated with nutrient uptake. It is interesting to note that nutrient acquisition genes were predominant in ET lakes, with high relative abundance of genes associated with biological fixation of CO_2, N_2, alkaline phosphatase and sulfate reduction. In OT and CVO lakes, we observed a higher abundance of low frequent processes such as nitrification and methanogenesis while genes associated with nitrate and nitrite reduction, and denitrification were more abundant. Moreover, the OT and CVO lakes presented prevalence of genes related to phosphate transport, phosphorus regulation as well as oxidation of sulfide and thiosulfate. Altogether, this study illustrates the taxonomic and functional diversity in tropical soda lakes, as well as how microorganisms behave under distinct

environmental conditions. Moreover, the dataset obtained in this study may provide guidelines for management practices and conservation of the Pantanal's biodiversity.

Keywords: Metagenomic. Microbial ecology. Biogeochemical cycles. Cyanobacterial blooms.

RESUMO

PELLEGRINETTI, T.A. **Comunidades microbianas e genes funcionais envolvidos na ciclagem de nutrientes em lagoas salino alcalinas do Pantanal da Nhecolândia – MS**. 2022. 128 p. Tese (Doutorado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2022.

A Nhecolândia (MS), uma importante sub-região do Pantanal, é composta por 12.150 mil lagoas dentre as quais aproximadamente 600 lagoas podem ser descritas como salino alcalinas ("soda lakes"). As lagoas salino alcalinas apresentam altas concentrações de carbonatos e bicarbonatos de sódio, resultando em altos valores de pH (acima de 9) e salinidade (até 35 mS cm^{-1}). Mesmo apresentando condições consideradas extremas, este ecossistema possui uma rica biodiversidade de microrganismos. Neste trabalho, foram adotadas estratégias que combinaram análises moleculares e químicas com o objetivo de avaliar a composição (taxonômica e funcional) e o estilo de vida das comunidades bacterianas nas águas superficiais deste ambiente. Por meio de análises de ordenação utilizando dados limnológicos e metagenômicos, foi possível agrupar as lagoas salino alcalinas avaliadas em três perfis distintos: túrbidas eutróficas (ET), túrbidas oligotróficas (OT) e cristalinas oligotróficas vegetadas (CVO). Um fator preponderante na dinâmica da comunidade bacteriana foi a sazonalidade. Durante a estação seca, o nível da água reduziu consideravelmente devido a intensos períodos de evaporação, levando a um aumento nos níveis de salinidade, pH e concentração de nutrientes, o que por sua vez aumentou a diversidade e a abundância relativa dos microrganismos. Esse cenário propiciou a ocorrência de florações de cianobactérias nas lagoas ET, principalmente associadas aos táxons *Arthrospira platensis* ou *Anabaenopsis elenkinii*. Essa predominância pode ser justificada pelo investimento por mecanismos de aquisição de nutrientes por esses organismos. Nas lagoas OT e CVO houve uma predominância de Actinobacteria, Alphaproteobacteria e Betaproteobacteria e esses organismos possivelmente adotaram estratégias associadas a captação de nutrientes. É interessante observar que os genes de aquisição de nutrientes foram predominantes nas lagoas ET, com elevada abundância relativa de genes associados a fixação biológica de CO_2, N_2, fosfatase alcalina e redução de sulfato. Em lagoas OT e CVO, observamos uma baixa ocorrência de genes associados a nitrificação e metanogênese enquanto genes associados a redução de nitratos e nitritos, e a desnitrificação foram mais abundantes. Em condições oligotróficas genes relacionados com o transporte de fosfato, a regulação de fósforo como também a oxidação de sulfeto e tiossulfato foram abundantes. Ao todo, este estudo desvenda a diversidade taxonômica

e funcional em lagoas salino alcalinas tropicais, como também explora como os microrganismos se comportam em condições ambientais distintas. Além disso, este estudo servirá como ponto de partida para as práticas de manejo e medidas de conservação da biodiversidade do Pantanal.

Palavras-chave: Metagenômica. Ecologia microbiana. Ciclos biogeoquímicos. Florações de cianobactéria.

SUMMARY

1 INTRODUCTION

The Brazilian Pantanal is the world's largest tropical wetland (≈ 150,000 km^2 of area). This Brazilian biome has a complex hydrological system driven by the Paraguay river dynamics and hosts species that are endemic in this region. Its large extension promotes a subdivision of Pantanal in several subregions, where the Nhecolândia is one of these subregions. Nhecolândia comprises 12,150 lakes encompassing saline alkaline and freshwaters environments. Especially, saline-alkaline lakes (soda lakes) measure approximately 1 km² in width and range from 1 to 2 meters in depth. Flooding periodically connects freshwater lakes and lagoons in Pantanal, whereas soda lakes stay outside of flood zones coexisting nearby with lakes varying in physical, chemical, and biological properties.

The Pantanal's soda lakes are rich in sodium carbonates and bicarbonates resulting in a high pH level (ranging between 9 and 11). The surrounding soils of these lakes have an elevation of three meters (known as '*cordilheiras*') creating an "island effect" and contributing to their isolation during the floods. The biogeochemical functioning of soda lakes is highly dependent on hydrological regimes due to their low connectivity with the watershed. The high evaporation ratios (directly correlated with the seasonality) promote variation in the water column influencing the soil composition and water chemistry. The variability in the water column concentrates or dilutes nutrients and ions, which influence the functioning of lakes.

Beyond the impact on soil composition and water chemistry, the processes of dilution or concentration of nutrients and ions influence the resident microbial community. As microorganisms drive essential biogeochemical processes and are the basis of the food web, it is crucial to understand the role of the microbial community in these lakes and how they respond to this situation of pronounced seasonality in a harsh environment (high pH and salinity).

Thus, the purpose of this study was to investigate the microbial community resident in these lakes, particularly the bacterial community, and evaluate their composition and contribution to the nutrient cycling. Based on this goal, three central objectives were proposed: (*i*) describe the bacterial community composition and understand the correlation between the bacterial community and the water-chemistry differentiation observed on these lakes; (*ii*) identify the bacterial behavior on these lakes and its correlation to the distribution of bacterial communities in tropical soda lakes; and (*iii*) understand the contribution of bacterial community to the occurrence of biogeochemical cycles.

Pantanal biome and landscapes

The Pantanal is the world's largest continuous tropical wetland with an important role in supporting the biodiversity of South America (JUNK; CUNHA, 2012; IVORY et al., 2019). It is a vast and well-preserved wetland distributed in three countries: Brazil (≈ 140,000 km²), Bolivia (≈ 15,000 km²), and Paraguay (≈ 5000 km^2) (GARCIA et al., 2021) and is a continuous sedimentary plain formed thousands of years ago by the upheaval of the Andes Mountains (ALHO; GONÇALVES, 2005). The hydrology of this floodplain-savanna type ecosystem is controlled by the Upper Paraguay River, which flows along the western margin of the basin (MCGLUE et al., 2011).

The climate of the region is described as tropical humid with rainy summers and dry winters, corresponding to type Aw in the Köpen climate classification (ALVARES et al., 2014). Low altitudes (80 to 150 m) combined with an extremely low slope (0.03 to 0.5 m/km) favor the flooding in almost all regions of Pantanal (FRANCO; PINHEIRO, 1982; ALHO, 2008). The average air temperature ranges from 21ºC to 32ºC and precipitation ranges from 1000 to 1400 mm throughout the winter and summer seasons, respectively (RICHTER et al., 2019). However, 80% of precipitation occurs during the summer months, specifically in December and January. During rainy seasons, the intense precipitation causes flooding over the sub-regions of the Pantanal that extends for many months. This flooding promotes nutrient exchanges across Pantanal's compartments such as soils, plants, freshwater lakes, rivers, and groundwaters. In addition, this phenomenon controls ecological relationships and biogeochemical processes, such as floodplain primary production, carbon cycling, fish spawning and bird migratory patterns, and predator-prey interactions (HAMILTON, 2002; GUERREIRO et al., 2019).

Although the Pantanal is defined as a flooded zone, this wetland hosts thousands of lakes with diverse origins, morphologies, and aquatic properties (POR, 1995). Flooding episodes annually link the largest and deepest (up to 9m) lakes in the Pantanal to the Upper Paraguay river at the western boundary of the basin (MCGLUE et al., 2011; LO et al., 2017). In contrast, the Nhecolândia subregion, is remote and separated from the flood pulse of Upper Paraguay River (GUERREIRO et al., 2019). Nhecolândia is one of the five subregions that comprise the Pantanal. It encompasses approximately 26,900 km², located between the Taquari and Negro rivers, and is characterized by a large lake system comprised of about 12,150 lakes (PEREIRA et al., 2020). Periodically, the freshwater lakes got flooded while the soda lakes are physically isolated from the floodplains by a surrounding sandy ridge covered by trees known as '*cordilheira*' (GUERREIRO et al., 2019; FREITAS et al., 2019). Approximately 600 soda

lakes with high salinity levels and pH values (> 9) (COSTA et al., 2015; PEREIRA et al, 2020) are chemically comparable to those found in Africa, Asia, Australia, and Canada (VAVOURAKIS et al., 2019; ZORZ et al., 2019; BURGANSKAYA et al., 2018; SOROKIN et al., 2014).

Nhecolândia soda lakes

The Nhecolândia soda lakes are popular known as '*salinas'* due to their high salinity and pH levels (MARIOT et al., 2007). In general, these soda lakes are rich in sodium carbonate and bicarbonate, resulting in a pH close to or above 10, high salinity (up to 35 mS cm^{-1}) and low calcium and magnesium concentrations (BARBIERO et al., 2018). They are shallow (less than 2 m in depth), small (between 0.5 and 1 km in diameter), hydrologically isolated, and bordered by 'cordilheiras'. *Cordilheira* acts as a physical barrier to preventing floods in soda lakes (FREITAS et al., 2019). This physical isolation increases the ions concentration in water, making these lakes directly impacted by the yearly ratio of precipitation to evaporation as well as the soil system composition (MARTINS, 2012; PARIZOTO, 2012; FURIAN et al., 2013). Therefore, seasonality (associated with rainfall and evaporation ratios) has a remarkable impact on the hydrology of these lakes, resulting in waters where the nutrients and ions are diluted during wet seasons and concentrated during dry seasons (BARBIERO et al., 2007; MARIOT et al., 2007).

In contrast to the macrophyte-covered freshwater lakes of Nhecolandia and the deep lakes located along the Paraguay River, only microbes inhabit the soda lakes (GUERREIRO et al., 2019; BENITO et al., 2018). Its harsh conditions, which include a high pH, electrical conductivity, and a marked change in nutrient concentration, substantially reduce the presence of aquatic plants, benthic macroinvertebrates, and fishes, hence restricting their biological diversity to microorganisms. Furthermore, natural cyanobacteria blooms are regularly seen and may be seasonal or permanent (ANDREOTE et al., 2018; BARBIERO et al., 2018).

Several metabolic activities, including sulfur-oxidizing, sulfate reduction, nitrification, denitrification, methanogenesis and phototrophy, have been identified in the microbial communities of soda lakes (ANTONY et al., 2013). Besides providing novel research possibilities linked with extremophile life and biotechnology, the study of microbial communities and their role in soda lakes contribute to the knowledge of the greenhouse gas (GHG) dynamics of the Pantanal. Studies described that Nhecolândia soda lakes have substantially lower CH_4 fluxes than freshwater lakes, and they can act as a CO_2 sink, while freshwater lakes are a source of CO_2 to the atmosphere (BERGIER; ASSINE, 2015;

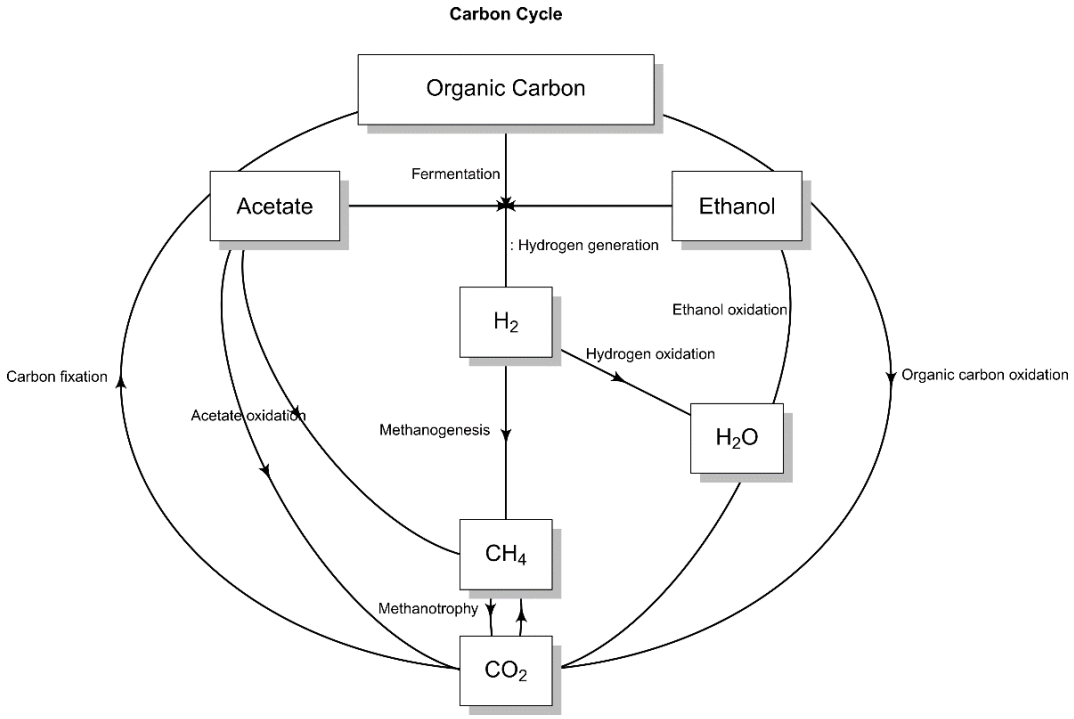
BARBIERO et al., 2018). However, the extent to which these gas fluxes and other biogeochemical processes in soda lakes are orchestrated by microorganisms, particularly regarding nutrients such as carbon, nitrogen, phosphorus and sulfur, is currently understudied.

Nutrient cycling in soda lakes

Carbon

The carbon cycle is a set of natural processes, where the carbon is transported between its primary reservoirs, such as the atmosphere, aquatic and terrestrial biomes, and living organisms (POST et al., 1990; SCHMITZ et al., 2014). The carbon cycle begins with the fixation of atmospheric carbon dioxide (CO_2) by autotrophic organisms including plants, algae, and microorganisms (such as Cyanobacteria, Alphaproteobacteria, Betaproteobacteria, Firmicutes, among others) (HU et al., 2019). A wide range of microorganisms can assimilate CO_2 into biomass such as anoxygenic (Chlorobi, Chloroflexi, Firmicutes and Alphaproteobacteria) and oxygenic (Cyanobacteria) photosynthetic bacteria, and non-photosynthetic bacteria and Archaea (Clostridia, Actinobacteria, Archaea) (SALEHIZADEH et al., 2020). The carbon cycle may be summed up by the inorganic carbon fixation performed by primary producers and the return of CO_2 or CH_4 to the atmosphere as a final product of metabolism (organic carbon oxidation, fermentation and methanogenesis) (Figure 1) (ZHOU et al., 2022). Carbon fixation may occur through six distinct mechanisms: (i) the Calvin–Benson–Bassham (Calvin) cycle, (ii) the reductive citric acid cycle, (iii) the reductive acetyl-coenzyme A pathway, (iv) the 3-hydroxypropionate bicycle, (v) the hydroxypropionate–hydroxybutyrate cycle, and (vi) the dicarboxylate–hydroxybutyrate cycle (DURALL; LINDBLAD, 2015).

Figure 1. Major microbial mediated carbon transformation
(Adapted from ZHOU et al., 2021)



In soda lakes, carbon fixation is a marked process, being performed by haloalkaliphilic oxygenic Cyanobacteria, anoxygenic purple sulfur bacteria, and chemolithotrophs (SOROKIN et al., 2014). The prevalence of Cyanobacteria in several soda lakes is well documented. In tropical environments, filamentous cyanobacteria were mainly affiliated with *Arthrospira*, *Anabaenopsis* and *Cyanospira*, besides benthic and planktonic, other filamentous and unicellular morphotypes (KRIENITZ et al., 2013; ANDREOTE et al., 2014; 2018; GENUARIO et al., 2017). Another important primary producer in soda lakes is the anoxygenic phototrophs from Gammaproteobacteria, Alphaproteobacteria and Gemmatimonadetes (GORLENKO, 2007; ANDREOTE et al., 2018; ZORZ et al., 2019). In Cyanobacteria mats from Cariboo Plateau (Canada) soda lakes, the occurrence of *Nodosilinea*, *Phormidium*, *Spirulina*, *Cyanobium* and *Geitlerinema* had documented as important photosynthetic bacteria (ZORZ et al., 2019). Moreover, the Cyanobacteria *Nodosilinea sp* and *Geitlerinema sp*, as well as eukaryotic algae such as *Ctenocladus*, *Picocystis salinarium*, and *Dunaliella viridis* were important primary producers at high salinity levels (up to 250 g L^{-1}) in Kulunda Steppe soda lakes (Russia) (SAMYLINA et al., 2014; SAMYLINA et al., 2019).

As consumers, the heterotrophic bacteria will oxidize organic matter produced by autotrophic organisms. They could use this organic matter as an electron donor or acceptor depending on their metabolism (aerobe, facultative anaerobe, microaerophilic and anaerobe) (SOROKIN et al., 2014). It is worth highlighting that it was already described a high prevalence of fermentative organisms in soda lakes such as Actinobacteria, Bacteroidetes, Clostridia and, Firmicutes and methanogenic Archaea (SOROKIN et al., 2014). Methanogenesis is known to occur in saline habitats either by a non-competitive (methylotrophic) pathway, hydrogen or acetate oxidation (SOROKIN et al., 2015; PAUL ANTONY et al., 2013).

The nitrogen cycle

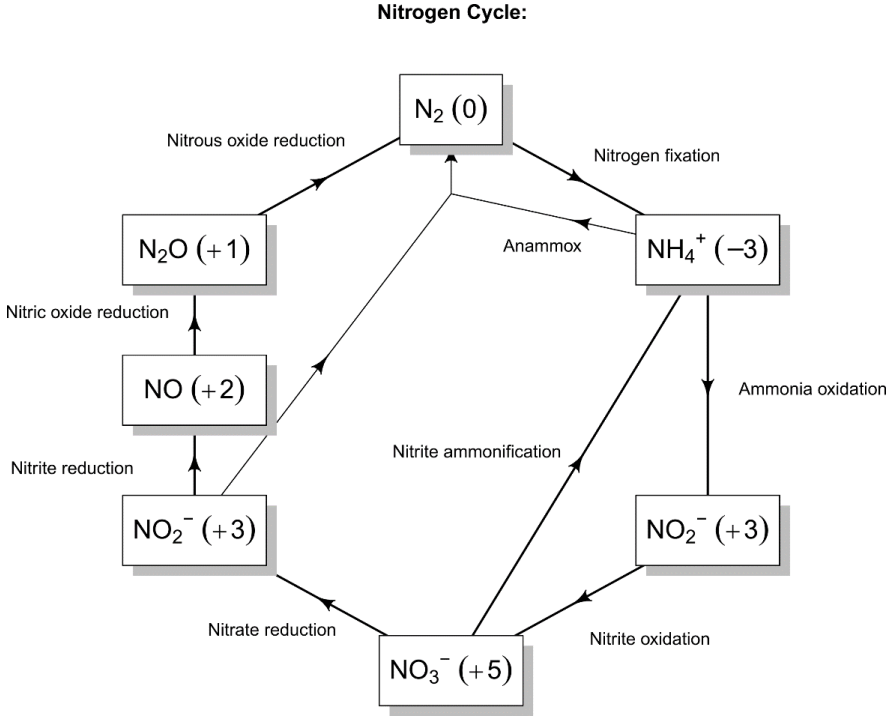
Nitrogen is the second most abundant element and occurs in a diverse number of natural forms due to its high variability in redox states (rainging from -3 to +5). It is a common electron acceptor and donor for energy metabolism and is found on most nitrogen-containing structures (protein, nucleic acids) in living organisms (ZEHR; KUDELA, 2011; FURNAS, 2018; ZHANG et al., 2018). The nitrogen transformations between compartments are entirely controlled by living organisms including archaea, bacteria and eukaryotes (FURNAS, 2018). Ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) are the primary sources from which microorganisms assimilate inorganic nitrogen, while urea [$CO(NH_2)_2$] and dissolved organic nitrogen are less frequently utilized (ZEHR; KUDELA, 2011; FURNAS, 2018). The nitrogen cycle can be separated into oxidative and reductive processes. In oxidative processes, nitrification may proceed either through the sequential oxidation of ammonia to nitrite and nitrite to nitrate, or directly from ammonia to nitrate by complete ammonia oxidation (commamox) (MYROLD, 2021). The anaerobic ammonia oxidation combines the oxidation of ammonium with nitrite reduction in a process named annamox (KRAFT; STROUS, 2011). Considering the reductive processes, nitrate reduction can involve multiple pathways including assimilatory nitrate reduction to ammonium (ANRA), dissimilatory nitrate reduction to ammonium (DNRA) and denitrification (HENSON et al., 2017).

The pathways of nitrogen transformation in soda lakes are crucial to ecosystem functioning. However, few studies focused in elucidate the microbial community involved in this element transformations (SOROKIN et al., 2014; ZORZ et al., 2019). The microbial species inhabiting soda lakes evolved strategies to fix atmospheric nitrogen, making them advantageous in these nutrient-limiting environments. Recent studies indicate that nitrogen fixation is a relevant pathway in soda lakes and is frequently performed by Cyanobacteria, Alphaproteobacteria, Gammaproteobacteria, Desulfobacteriota, Actinomycetacia,

Gemmatimonadota, Myxococcota and Firmicutes (ANDREOTE et al., 2018; ZORZ et al., 2018). Moreover, other species were also described as nitrogen-fixers in soda lakes as iron reducer bacteria *Geoalkalibacter* sp. (ZAVARZINA et al., 2006) and cellulolytic *Clostridium* sp. (ZHILINA et al., 2009).

The conversion of ammonia to nitrite and nitrate (nitrification) (Figure 2) is frequently inhibited by high salt concentrations and high concentrations of ammonium (toxic to ammonia oxidizers), features associated with soda lakes (higher than 1M Na) (SOROKIN, 1998). Moreover, at high pH (above 9) a significant amount of ammonium may be converted to gaseous ammonia, which is lost to the environment. However, in Russian soda lakes the occurrence of nitrification was detected, where the alkali-tolerant *Nitrosomonas* perform the oxidation of ammonia to nitrite, while the transformation of nitrite to nitrate is mediated by alkali-tolerant *Nitrobacter* (SOROKIN et al., 2015b; VAVOURAKIS et al., 2018).

Figure 2. Major microbial mediated nitrogen transformation
(Adapted from ZHOU et al., 2022)



The DNRA was described in soda lakes on the Cariboo Plateau in Canada, where it was associated with metagenome-assembled genomes (MAGs) belonging to the Gammaproteobacteria, Bacteroidota, and Desulfobacterota (ZORZ et al., 2018). In the Nhecolândia soda lakes, it was observed that the nitrate and nitrite ammonification were

associated to Spirochaetia, Cyanobacteria, Gammaproteobacteria and Actinomycetacia (ANDREOTE et al., 2018). Regarding the denitrification process, the microorganisms associated with this function were affiliated specially to Gammaproteobacteria (SHAPOVALOVA et al., 2008; SOROKIN et al., 2015). However, in Nhecolândia and Cariboo Plateau this function was low prevalent as denitrification depends on anoxic local conditions to be performed and on both lakes, the occurrence of these anoxic sites is limited by their low depth (ANDREOTE et al., 2018; ZORZ et al., 2018).

Phosphorus cycle

Phosphorus (P), alongside carbon and nitrogen is a key nutrient in living systems, essential for cellular structure and function (BERGKEMPER et al., 2016). It is an essential component of ATP, nucleic acids, and phospholipids, and it is required for other processes essential to life (LEBRUN et al., 2018). Despite their importance, phosphorus is considered a limiting factor for primary production in natural aquatic environments (HÅKANSON et al., 2007) and the ability to utilize non-conventional phosphorus sources may represent a significant competitive advantage (KOPEJTKA et al., 2018).

In general, phosphorus can be divided into three major compounds: dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP), and particulate-bound phosphorus (Part-P) (KARL; BJÖRKMAN, 2001). Considering the total P-pool, most of phosphorus is in the particulate fraction, where inorganic forms are dominant, and commonly associated with ions such as Mg^{2+}, Ca^{2+} and Fe^{3+} (SLOMP, 2011).

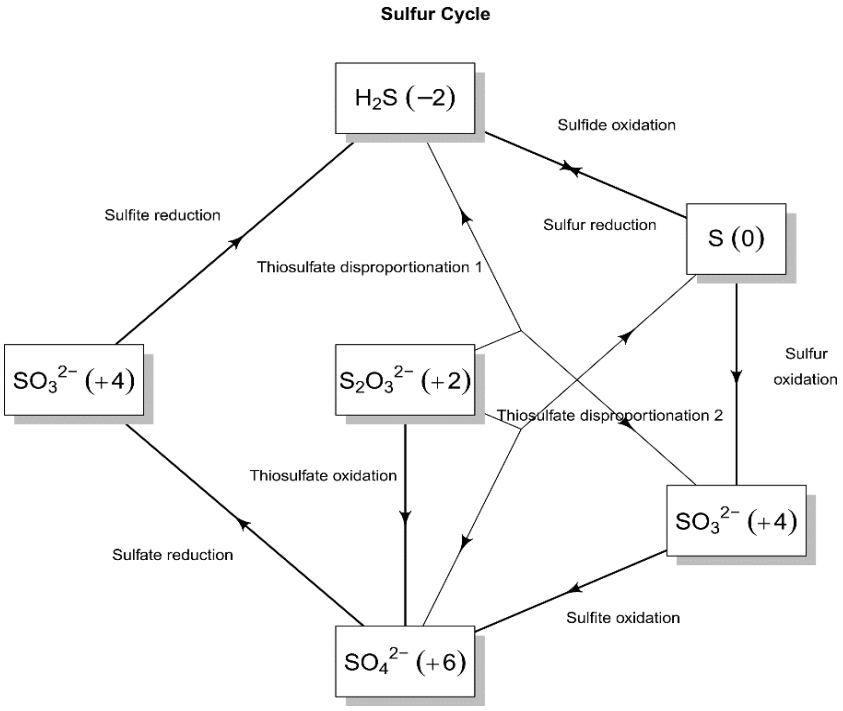
Microorganisms effectively mineralize organic and solubilize precipitated forms of phosphorus, where the phosphate ion could be released into the environment or immobilized into the biomass (BERGKEMPER et al., 2016). Several processes are included in phosphorus cycle, such inorganic phosphorus solubilization (*gcd, ppa, ppx*), organic phosphorus mineralization (*phoA*, *phoD*, *phnGHIJNOPW*), regulatory genes (*phoR*, *phoB*), and phosphorus transporters (*pstSABC*, *phnCE*, *ugpABCEQ*) (WU et al., 2022). The essential *phoA* gene, which encodes a well-characterized alkaline phosphatase that hydrolyzes phosphate esters for assimilation, is required for organic phosphorus utilization (SEBASTIAN; AMMERMAN, 2009). The *ppa* gene is responsible to convert inorganic pyrophosphate (PPi), a common biosynthesis product (such as DNA, peptidoglycan, and other biopolymers), to orthophosphate and release substantial amount of energy to support microbial growth (LAHTI, 1983; KHMELENINA et al., 2018). Genes involved in hydrolyzing of polyphosphate (*ppk*, *ppx*), phosphonate C-P lyase (*phnGHIJNOPW*), and phosphate transporters

(*pstSABC* and *pit*) are known to be associated with a strategy to survive at phosphorus limited environments (RUVINDY et al., 2016; YAO et al., 2016; FANG et al., 2021). Microorganisms can perform organic phosphorus mineralization using glycerophosphoryl diester phosphodiesterase (UgpQ) (OHSHIMA et al., 2008; LIANG et al., 2020). Even though the importance of P, only one study evaluated the dynamic of this element in soda lakes which demonstrated that alkaline phosphatases was important to enzyme that allowed to bacterial community to utilize dissolved organic phosphorus under P limitation (VALDESPINO-CASTILLO et al., 2014).

Sulfur cycle

Sulfur cycling is closely related with other essential element cycles (carbon, nitrogen, iron, manganese) and thus has substantial effects on cellular- and ecosystem-level processes (WASMUND et al., 2017). The sulfur cycle (Figure 3) is a complex network of oxi-reduction mechanisms dependent of chemical (temperature and pH) and microbial reactions (VAVOURAKIS et al., 2019). Sulfur occurs in a range of oxidation states with -2 (sulfide and reduced organic sulfur), 0 (elemental sulfur) and +6 (sulfate), being the last (sulfate) the most prevalent in nature (TANG et al., 2009). Like nitrogen, the sulfur cycle could be organized into two major groups: oxidative and reductive pathways (WASMUND et al., 2017). Diverse microorganisms can convert sulfide into sulfur or sulfate, and sulfate-reducing bacteria can reverse this process (TANG et al., 2009). A wide range of bacteria utilize sulfate as an electron acceptor and convert it to sulfide in a variety of metabolic processes. In addition, sulfide is an electron source for phototrophic or chemolithotroph bacteria, which converts it to sulfate or elemental sulfur by the process of oxidation of reduced sulfide (TANG et al., 2009). Moreover, sulfate can be assimilated both to bacteria, phytoplankton and macrophytes (FURNAS, 2018).

Figure 3. Major microbial mediated sulfur transformation
(Adapted from ZHOU et al., 2022)



In soda lakes, the inorganic sulfur transformation is one of the most active and well-studied processes. This process is driven by high sulfate concentrations and primary productivity (SOROKIN et al., 2014; SOROKIN et al., 2015b; VAVOURAKIS et al., 2019). Dissimilatory reduction of oxidized sulfur compounds, such as sulfate, sulfite, thiosulfate, and sulfur, and production of sulfide have been recorded in soda lakes (SOROKIN et al., 2014). At high pH, deprotonated hydrosulfide (HS) is the main form of H_2S, and sulfidogenesis is not inhibited by H_2S, differently that occurs at acidic or neutral pH (VAVOURAKIS et al., 2019). Moreover, the high alkalinity of soda lakes increased the chemical stability of polysulfides in anoxic sediments, resulting in dissimilatory sulfate reduction. The microbial community of sediments rapidly oxidized $S_2O_3^{2-}$ in the presence of oxygen, but this oxidation was partially inhibited by light (VAVOURAKIS et al., 2019).

1.1 Objectives

1.1.1 General objectives

The general objective of this thesis was to investigate the bacterial composition and identify the processes that control differences in the biogeochemical functioning of Nhecolândia soda lakes, taking into account environmental factors and nutrient dynamics, as well as to comprehend the cycles of these elements in these systems.

1.1.2 Specific objectives

To achieve the general objective of this thesis the following specific objectives were considered:

- To determine the community structure and the functional genes in distinct soda lakes by applying a DNA shotgun metagenomic approach to analyze samples collected in several soda lakes during the dry and wet seasons.
- To understand the factors associated with the bacterial community composition and its correlation with its lifestyle.
- To elucidate the occurrence of the biogeochemical cycles in soda lakes (focusing on carbon, nitrogen, phosphorus, and sulfur cycles) and correlate these findings with the greenhouse gas dataset.

1.2 The organization of this thesis

This thesis contains three studies presented in scientific manuscript format written in the English language covering the specific objectives listed above. The first manuscript is under evaluation on Microbial Ecology Journal (*under review*) while the second manuscript was published in Scientific Reports (doi.org/10.1038/s41598-022-12046-2). The third manuscript is being finalized and will be submitted to the Science of the Total Environment Journal (IF=7.963). The supplementary materials referenced in each chapter are available in the Appendix section.

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2 BACTERIAL COMMUNITIES ALONG ENVIRONMENTAL GRADIENTS IN TROPICAL SODA LAKES[1]

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

2.1 Introduction

Extreme or hostile environments are characterized by harsh physicochemical conditions that inhibit the growth of organisms (GIL et al., 2021). Soda lakes are naturally occurring water bodies rich in carbonates and bicarbonates, comprising saline and hypersaline alkaline waters

[1] Pellegrinetti, T.A.; Cotta, S.R.; Sarmento, H.; Costa, J.S.; Delbaje, E.; Montes, C.R.; Camargo, P.B.; Laurent Barbiero, L.; Rezende-Filho, A.T.; Fiore, M.F. Bacterial communities along environmental gradients in tropical soda lakes. **Microbial Ecology**, 2022. In pre-pirnt.

with an elevated pH (ranging from 9.5–11) and salinities that can approach saturation (TINDALL, 1988; SOROKIN et al., 2014). 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) (SOROKIN et al., 2014; SZABÓ et al., 2017). Several studies have attempted to establish and map the microbial communities inhabiting soda lakes complexes, such as the East African Rift Valley, Carpathian Basin, Kulunda Steppe, and Cariboo Plateau (SOROKIN et al., 2014; ZORZ et al., 2019; FELFÖLDI et al., 2020). The prokaryotic community identified in these lakes comprises Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria, and some archaeal groups, such as Euryarchaeota (SZABÓ et al, 2017; ZORZ et al., 2019; VAVOURAKIS et al., 2018; ANDREOTE et al., 2018).

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 (SOROKIN et al., 2014; VAVOURAKIS et al., 2018). 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 (SOROKIN et al., 2014; SHU et al., 2021; DANEZAK et al., 2017).

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 (GUERREIRO et al., 2019). Nhecolândia hosts hundreds of pristine soda lakes (ca. 500–600) concentrated in a 27,000 km² area, and its microbial community remains underexplored (BARBIERO et al., 2018). Nuanced interactions between abiotic parameters, such as seasonal and spatial variations and resident microbial composition, may manifest in distinct Pantanal soda lake patterns (ANDREOTE et al., 2018; BARBIERO et al 2018). The seasonality of Nhecolândia soda lakes is characterized by heavy rainfall during summer, followed by a strong evaporation process during the rest of the year, directly affecting the water level (ANDREOTE et al., 2018; BARBIERO et al., 2018). 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.

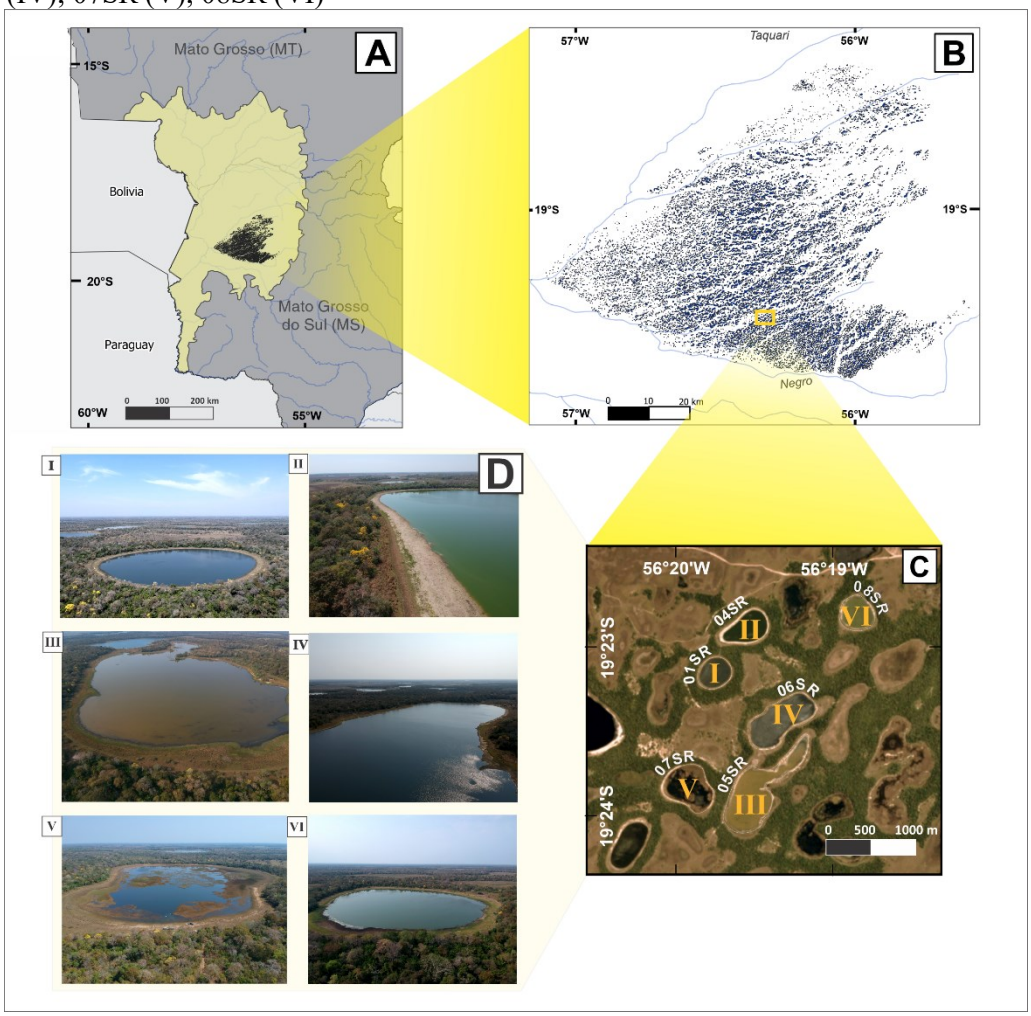
2.2 Methods

2.2.1 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 (ANDREOTE et al., 2018; BARBIERO et al., 2018). 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 (IBGE, 2003). The annual precipitation in south-southwest Nhecolândia varies from 710 to 1200 mm in the south-southwest (ZHOU; LAU, 1998; ASSINE et al., 2015).

Surface waters (0-15 cm) were collected from six lakes (Figure 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). A total of 48 water samples were collected (6 lakes × 4 replicates × 2 seasons). One aliquot (500 mL) of each sample was preserved in polyethylene bottles at 4°C for chemical analysis and another aliquot (50 mL) was stored in nitrogen liquid (-80 ºC) for DNA extraction. Although a previous long-term survey had reported that rains are concentrated from October to March (THIELEN et al., 2020), the intra-annual rainfall variability can be pronounced, as was observed in both sampling years (Figure S1).

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)



2.2.2 Data Acquisition

Monthly accumulated rainfall and land surface temperature (LST) data were obtained from the Climate Hazards Group Infrared Precipitation (FUNK et al., 2015) 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* disc. 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.

2.2.3 Flow cytometry and pigment analyses

To determine heterotrophic prokaryote (HP) abundance, 1.2 mL water samples were fixed *in situ* with 1% formaldehyde, immediately flash-frozen in liquid nitrogen, and stored at –80°C until analysis. Thawed samples were 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 cells were detected and quantified using four channels at 533 nm, 585 nm, 670 nm, and 675 nm. Cytometrically defined populations among phototrophic picoplankton were classified into five groups: 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) (GASOL; DEL GIORGIO, 2000; SARMENTO et al., 2008). Chlorophyll-*a* (Chl-*a*) was extracted using 90% acetone and determined by spectrophotometry using the EPA 446.0 method (ARAR, 1997).

2.2.4 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 (KOMAREK; ANAGNOSTIDIS, 1989).

2.2.5 Metagenomic DNA extraction and sequencing

Environmental total DNA was extracted from 50 mL of 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 the subjected to shotgun sequencing (6 lakes × 3 replicates × 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.

2.2.6 Bioinformatic analyses

The raw sequence adapters were removed using CutAdapt 1.18 (MARTIN, 2011) and quality controlled using FastQC 0.10.1 (ANDREWS, 2010). The merging of paired ends reads was performed using PEAR software 0.9.6 (ZHANG et al., 2014). Sequences smaller than 50 pb and Phred < 20 were removed using Seqyclean 1.3.12 (ZHBANNIKOV et al., 2017).

Metagenome reads were submitted for taxonomic and functional annotation (RefSeq and SEED subsystems database) via the MG-RAST bioinformatics pipeline 4.0.3 (MEYER et al., 2008). 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%.

2.2.7 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 (APHA, 2005). 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 (ŔUŽIČKA; HANSEN, 1975). Orthophosphate (oPO_4^{3-}) concentrations were quantified using the ascorbic acid method (MURPHY; RILEY, 1962). Alkalinity was analyzed with 0.1 mol L^{-1} hydrochloric acid titration. Total dissolved solids were determined using the Environmental Protection Agency method 1684 (TELLIARD, 2001). Water salinity was estimated from the total amount of inorganic dissolved solids in water samples (WILLIAMS; SHERWOOD, 1994). 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).

2.2.8 Data analysis

All data analysis was performed in R 4.1.2 (TEAM, 2013). Analyses of variance were measured using Tukey or Kruskal-Wallis tests and applied to test for significant differences among lakes using the Multicomp package (HOTHORN et al., 2016). Principal component analysis (PCA) was performed using FactoMineR, with environmental variables set as explanatory variables and cytometric data as supplementary variables. Further, 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 (OKSANEN et al., 2013). The functional profile of each lake was plotted in a heatmap using Pheatmap R package (KOLDE; KOLDE, 2015). Z-score transformations were applied using the scale function available in the R core base package (TEAM, 2013). Alpha diversity, Chao1 richness, and Shannon diversity analyses were performed using MicrobiomeAnalyst (DHARIWAL et al., 2017), with the data rarefied to the minimum library size of 181230 and scaled using the total sums.

2.3 Results

2.3.1 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 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 orthophosphate (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}).

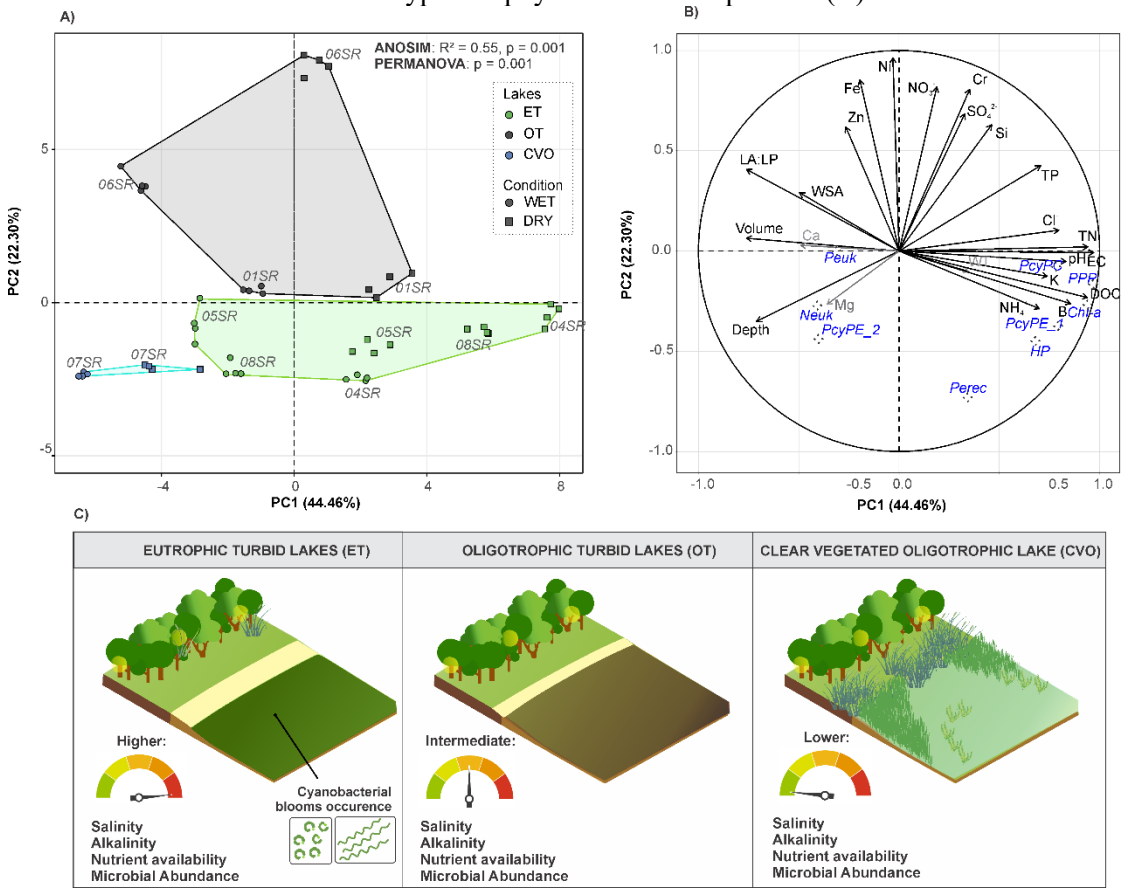
Table 1. Synthesis physical and chemical variables of sampled lakes (n=48) in Nhecolândia, during dry and wet periods with mean of each period (n=24 per period). Average data are presented with standard deviation (values after ±)

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.

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 (Figure 2A).

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)



2.3.2 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 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%) (Figure 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) (Figure 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 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) were detected 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 Gamma Proteobacteria (Figure S5 and S6).

2.3.3 Specificity of each lake's group

2.3.3.1 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.

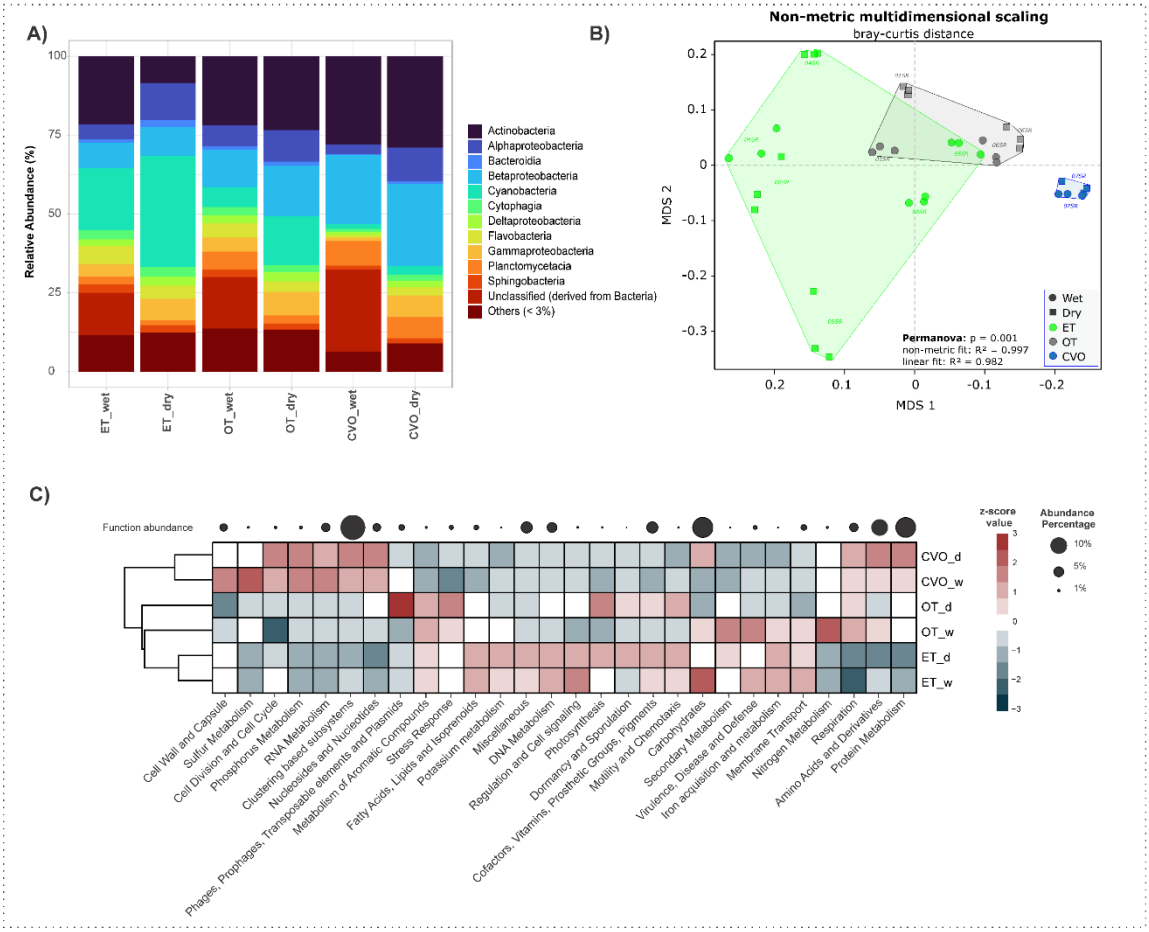
Cyanobacterial blooms reduced light penetration associated with high PPP abundance, DOC, DO, and Chl-*a* concentrations (Table 1 and Table S4). Chl-*a* and DOC concentrations were enhanced during the dry period. Field measurements detected oxic conditions in all lakes during the dry period, while lakes 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 (Figure S2). Specifically, the 05SR and 08SR lakes showed a higher diversity

index during the dry period, whereas 04SR showed a higher diversity during the wet period (Figure 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 (Figure 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)] (Figure 3A and Figure 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 (Figure 3A).

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 "genus level" of each lake type using bray distance (B). Heatmap of functional genes (SEED subsystem database level 1) of each lake type with relative abundance



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” (Figure 3C and Figure S4). The “Carbohydrates” and “Virulence Diseases and Defense” were predominant in the wet period compared to the dry period (Figure 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 (Figure 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.

2.3.3.2 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 (Figure S2A). The exception was the 01SR lake, where the bacterial diversity was higher in the dry period than in the wet period (Figure 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) (Figure 3A, Figure S4, Figure 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%) (Figure S3).

The prevalent potential bacterial functions were “Metabolism of Aromatic Compounds,” “Respiration,” “Secondary Metabolism,” and “Stress Response” (Figure 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 (Figure S4).

2.3.3.3 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 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 (Figure S2A), and the Peuk and PcyPE_2 organisms were the most abundant and associated with water surface area, volume, and depth (Table S4; Figure 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 (Figure 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” (Figure 3C and Figure S4). “Cell wall and Capsule” and “Sulfur Metabolism” functions were enriched on the wet period (Figure 3C). The CVO lake clustered with the 05SR wet sample (ET lake) (Figure S4).

2.4 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 filamentous 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 (Figure S5). These eutrophic conditions favor cyanobacterial blooms and promote changes in the environmental and ecological conditions, as previously observed in other aquatic ecosystems (PAERL et al., 2011). The two planktonic cyanobacteria have been reported as common inhabitants of several Nhecolândia soda lakes and are important primary producers in these extreme habitats (GENUÁRIO et al., 2017; SANTOS et al., 2018). Furthermore, both cyanobacterial genera are associated with the occurrence of blooms in other soda lakes (SOROKIN et al., 2014).

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 (SINYUKOVICH et al., 2020). In freshwater lakes, terrestrial organic matter and iron load have been shown to modify the color of the water in a process known as “brownification” (KRITZBERG; EKSTRÖM, 2012; XIAO; RIISE, 2021; KRITZBERG et al., 2020). Lakes are intimately connected to their surrounding land, showing significant correlations between physicochemical and geomorphological variables (especially water volume and altitude) (COSKUN; MUSAOGLU, 2004; KLIMASZYK et al., 2015). Notably, the enrichment of eukaryotic and phycoerythrin-rich organisms in the CVO lake appeared directly associated to 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 (KRITZBERG et al., 2006). Moreover, the transparency of the water and the reduction of salinity levels results in an enrichment of phycoerythrin-rich cells and a reduction of eukaryotic diversity respectively (VÖRÖS; GULYAS, 1991; MENÉNDEZ-SERRA et al., 2021).

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 (BARBIERO et al., 2018). Considering potential future anthropogenic disturbances such as cattle expansion and vegetation burns, our study may serve as a starting point for restoration practice 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 (HART et al., 2020). 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 2017, 2020, and 2021. Hydrology is a key driver of phytoplankton and heterotrophic bacterial communities in tropical freshwater lakes, as well as soda lakes (NDEBELE-MURISA et al., 2010; FREITAS et al., 2018). Water dynamics impact nutrient concentration and its flux by modulating the diverse components of the system (COSTA et al., 2016). Seasonality is a determinant of 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 (BAKKER et al., 2016; BRASIL et al., 2016). 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) (KEBEDE, 1997). In contrast, *A. elenkinii* requires a lower salinity level and nutrient concentration to flourish (BALLOT et al., 2004; KRIENITZ et al., 2013; ANDREOTE et al., 2018).

The most abundant phyla after the Cyanobacteria in the ET lakes, i.e. Actinobacteria, Bacteriodetes, Proteobacteria (Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria) and Planctomycetacia have already been associated with cyanobacterial blooms in other soda lakes (KOLMONEN eta l., 2004; SOROKIN et al., 2014; FELFÖDI, 2020). Cyanobacteria release labile DOC through exudation during the bloom, thus stimulating the proliferation of heterotrophic bacteria (ALVARENGA et al., 2017). Lakes 04SR and 08SR

had a similar composition of bacterial community 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 (ALVARENGA et al., 2017; JI et al., 2018).

OT and CVO lakes with absence of filamentous cyanobacterial blooms were colonized predominatly by Actinobacteria, Proteobacteria (Betaproteobacteria) and Planctomycetacia. A higher abundance of these phyla when Cyanobacteria population is low has been already described under oligotrophic conditions (ANDREOTE et al., 2018; JI et al., 2018). 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 (SOROKIN et al., 2014; ZORZ et al., 2019; VAVOURAKIS et al., 2018).

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 on these lakes (ZAVARZINA et al., 2006; OREMLAND et al., 2017). Moreover, Cyanobacteria members benefit from additional iron, as they require ten-fold more iron than other bacteria phyla to drive several processes, such as photosynthesis and nitrogen fixation (MOLOT et al., 2014; QIU et al., 2021). 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 (SOROKIN et al., 2009; KALWASINSKA et al., 2018; SHISHIDO et al., 2019). 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 reinforce this pattern of oligotrophic conditions, as aforementioned, these bacterial groups are adapted to nutrient-limited conditions (ANDREOTE et al., 2018; JI et al., 2018).

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 (VARIN et al., 2010). In extreme environments, a well-adapted microbial community has special machinery to maintain important biogeochemical processes, even under stress conditions (ZORZ et al., 2019; JEFFRIES et al., 2012; LAY et al., 2013). 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 (MANGROLA et al., 2015). 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 (SOROKIN et al., 2014). As expected, the main characteristics showing enrichement 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 subregion 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 (GUERREIRO et al., 2019; GUERRA et al., 2020). The regulation of Pantanal's conservation needs to be prioritized for conservation at regional scale (HARRIS et al., 2005). The robust dataset of chemical and microbial community profiles from several pristine Nhecolândia soda-lakes provided in this study, which also demonstrated the effect of environmental factors on these lakes, can be used as baseline for future changes associated with climate change or human-induced perturbations.

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3 DISENTANGLING THE LIFESTYLE OF BACTERIAL COMMUNITIES IN TROPICAL SODA LAKES[2]

Abstract

Microbial lifestyles may reveal niche-specific signatures and can contribute to detecting the effects of abiotic fluctuations on biogeochemical cycles. Microorganisms make a tradeoff between optimizing nutrient uptake, improving biomass yield, and overcoming environmental changes according to environmental hostility. Soda lakes are natural environments rich in carbonate and bicarbonate water, resulting in elevated pH and salinities that frequently approach saturation. We hypothesized that during the dry period (elevated pH and salinity), microorganisms try to overcome this harshness by allocating energy to the cellular maintenance process. As these environmental conditions improve during the wet period, microorganisms will begin to invest in nutrient uptake. To test this hypothesis, we evaluated four soda lakes in two different seasons by applying metagenomics combined with flow cytometry (estimate heterotrophic bacterial biomass). The natural occurrence of cyanobacterial blooms in some lakes is the main driver of carbon. These primary producers provide organic carbon that supports heterotrophic bacterial growth and, consequently, a high biomass yield. Under harsh conditions (dry season), cyanobacteria invest in nutrient uptake mechanisms, whereas heterotrophic bacteria allocate energy to survive at the expense of biomass yield. Lakes without cyanobacteria blooms invest in nutrient uptake independent of environmental hostility. This study clarifies the microbial tradeoffs in hostile environments and the impact of this choice on carbon and energy flux in tropical alkaline lakes.

3.1 Introduction

Knowledge of the microbial metabolic pathways may be critical for improving the prognosis of the effects of abiotic fluctuations on biogeochemical cycles. Under ever-changing environmental conditions in time and space, microorganisms must constantly adapt their functionality to guarantee continuity (WALLENSTEIN et al., 2012; CHEN et al., 2021).

Life strategies represent sets of traits that tend to correlate physiological or evolutionary tradeoffs with strategies that are favored under different environmental conditions (KRAUSE et al. 2014, MALIK et al., 2020). Three main microbial life-history strategies were recently

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described by Malik and coworkers (MALIK et al., 2020); a high yield life strategy (Y), a resource acquisition life strategy (A), and a stress tolerance life strategy (S). The high-yield strategist maximizes resource uptake and allocates it to biosynthetic processes, whereas the resource acquisition strategist is based on competition for nutrient acquisition. Stress tolerance involves the production and secretion of components or cellular structures associated with overcoming stress conditions (MALIK et al., 2020).

The Brazilian Pantanal biome (specifically in the Nhecolândia sub-region) contains hundreds of soda lakes with high pH values (ranging from 9.5 to 11) and salinities approaching saturation. In contrast to other Pantanal sub-regions, Nhecolândia soda lakes are mainly influenced by the evaporation ratio (high water evaporation relative to precipitation, with no input from rivers or flooding) (TARIFA, 1986; FURIAN et al., 2013; GUERREIRO et al., 2019). During the dry season, alkaline lakes remain isolated from the other lakes due to the low permeability of soil horizons (especially the silica layer), promoting solute concentration[8]. However, intensive rainfall (during the wet season) causes a rise in the water level on the subsurface that could connect these lakes, bypassing the silica layer (BARBIERO et al., 2008; FURIAN et al., 2013; GUERREIRO et al., 2019). Furthermore, these lakes are separated from the regional drainage system by “cordilheiras” (narrow and elongated sand hills covered by savanna vegetation) that are 2-3 m higher than adjacent plains. This barrier maintains alkaline waters and shows a high amount of organic matter that is more dependent on local cycles than terrestrial inputs by annual flooding (BARBIERO et al., 2018; PEREIRA et al., 2020).

Despite harsh conditions (high pH and salinity), alkaline lakes are remarkably productive because of elevated temperatures and luminosity (JONES; GRANT, 1999). These lakes host diverse microbial communities and frequently experience seasonal or permanent cyanobacterial blooms (AGUIRRE-GARRIDO et al., 2016; ANDREOTE et al., 2018). These cyanobacterial blooms generate a pulse of organic carbon that affects the carbon turnover (ZHENG et al., 2019). Approximately 20% of photosynthetic carbon is released in aquatic systems, and this organic matter supports a substantial portion of the heterotrophic community (BERTILSSON et al., 2003; MORANA et al., 2014; LINZ et al., 2020). Photosynthetic primary production is the driving force behind nutrient recycling in marine and freshwater environments (CARLSON et al., 2007; MORANA et al., 2014; LINZ et al., 2020). However, the carbon and energy fluxes in alkaline lakes have not been explored. In a hostile environment, it has been suggested that bacterial growth efficiency (BGE) is reduced, and energy is allocated to non-growth reactions that help cells maintain their molecular, cellular, and functional integrity, an equivalent of the S-strategy (CARLSON et al., 2007; MALIK et al 2020).

Based on this, we hypothesized that microorganisms try to overcome this harshness during the dry season to allocate energy to the cellular maintenance process. The improvement in these environmental conditions during the wet season allows the microorganism to invest in nutrient uptake. To test this hypothesis, we evaluated four shallow, alkaline lakes on the Pantanal during two seasons, wet and dry, by applying metagenomics and flow cytometry.

3.2 Material and Methods

3.2.1 Data collection

We evaluated four lakes located in the São Roque Reserve in the Nhecolândia sub-region of Mato Grosso do Sul State, Brazil. Recently, soda lakes found in this region were categorized into three groups: eutrophic turbid (ET), oligotrophic turbid (OT), and clear vegetated oligotrophic (CVO) (PELLEGRINETTI et al., 2022). ET lakes presented a natural cyanobacterial bloom from *Anabaenopsis elenkinii* (Nostocales) or *Arthrospira platensis* (Oscillatoriales) species, resulting in greenish waters. We selected two lakes belonging to the ET group (04SR and 08SR), one belonging to the OT group (06SR), and one belonging to the CVO group (07SR). Samples were collected from each lake with replicates separated by at least 50 m during the dry period and approximately 100 m during the wet period.

The physical and chemical features of water were defined as described by Pellegrinetti et al. (2022). Briefly, total nitrogen (TN) and total phosphorus (TP) contents were simultaneously detected using the persulfate method. Dissolved organic and inorganic carbon (DOC and DIC, respectively) and total dissolved nitrogen (TDN) were quantified by combustion (Shimadzu model TOC-5000A analyzer). The ion concentrations (NH_4^+, NO_3^-, and NO_2^-) were determined by flow injection analyses. Orthophosphate (oPO_4^{3-}) concentrations were quantified using the ascorbic acid method. Alkalinity was analyzed using 0.1 mol L^{-1} hydrochloric acid solution titration. Total dissolved solids were determined using the Environmental Protection Agency method 1684. Water salinity was estimated from the total amount of dissolved inorganic solids in the water samples. Concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and SO_4^{2-} were analyzed using ICS-90 ion chromatography. 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)[38]. The main physical and chemical characteristics of the samples are listed in Table 1.

As seasonality is an important phenomenon, sampling was conducted in October 2017 during the dry season and in September 2018 during the wet season. The seasonal rainfall cycle frequently concentrates on the rain between September and April (http://www.dsr.inpe.br/laf/series/). During the dry season, these lakes presented low depth (0.01 to 0.05 m), an apparent absence of stratification, widths ranging from 100 to 220 m, and lengths ranging from 100 to 450 m. In the wet season, these lakes also present an absence of stratification but an increase in depth (0,08 to 0,11 m), with widths ranging from 300 to 400 m and lengths ranging from 450 to 840 m. The main lake characteristics are listed in Table 1 and Supplementary Table 1.

3.2.2 DNA extraction

Total DNA extraction was carried out using lyophilized material (0.5 g) from each lake sample using the PowerLyzer PowerSoil DNA isolation kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. The amount and quality of DNA were measured by 1% (w/v) agarose gel electrophoresis, and the final concentrations were quantified with a Qubit (Qubit® 2.0 Fluorometer, Life Technologies).

3.2.3 Sequencing of DNA from water from soda lakes

A total of 24 samples were shotgun-sequenced (four lakes × three replicates × two seasons). Libraries were generated using the Nextera XT DNA Sample Preparation kit for paired-end fragments of 100 bp and sequenced on the Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA, USA) following the manufacturer's recommendations. Sequencing procedures were performed at the Laboratory for Functional Genomics Applied to Agriculture (http://www.esalq.usp.br/genomicafuncional/) located at the Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, SP, Brazil).

3.2.4 Sequence analysis

The quality of the raw sequences was checked via FastQC 0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The merging pair-end reads were realized using the pair-end read mergeR (PEAR) software (ZHANG et al., 2014). After the merging process, sequences smaller than 50 bp and Phred < 20 were removed using Seqyclean 1.3.12 (http://cores.ibest.uidaho.edu/software/seqyclean) (ZHBANNIKOV et al., 2017). The resulting sequences were uploaded to the MG-RAST bioinformatics pipeline (http: //metagenomics. anl. gov) for analysis (MEYER et al., 2008).

3.2.5 Data Availability

These datasets were publicly available at MG-RAST (http://metagenomics.anl.gov). Sequences derived from the dry season are available under the codes [04SR_ET (mgm4810104.3, mgm4810097.3, and mgm4810100.3), 06SR_OT (mgm4810099.3, mgm4810102.3, and mgm4810101.3), 07SR_CVO (mgm4810094.3, mgm4810095.3 and, mgm4810107.3), 08SR_ET (mgm4810106.3, mgm4810093.3, and mgm4810096.3)] while the sequences derived from the wet season are available under the code mgp88859.

3.2.6 The taxonomic and functional analysis

Taxonomic profiling of the microbiomes based on ribosomal RNA sequences was performed using the databases available in MG-RAST (Annotation source: Silva database (ssu); Max e-value cutoff: 1e−5, min % identity cutoff: 60%). To avoid errors associated with the difference in the sequencing depth of samples, they were normalized using the package edgeR (library size) (ROBINSON et al., 2010) in R software (TEAM, 2017).

Taxonomic patterns were compared among the lake microbiomes based on principal correspondence analysis (PCoA) considering the Bray-Curtis distance using the vegan package (vegdist, betadisper, and anosim) (OKSANEN et al., 2017). The differential abundance of taxa was evaluated using the edgeR package. The counts were normalized using the relative log expression (RLE) method, and the p-values were corrected using the Benjamini-Hochberg (BH) method (MCMURDIE et al., 2014). All graphics were generated using the ggplot2 package (WICKHAM et al., 2016).

Functional annotation was performed using the databases available in MG-RAST (Annotation source: SEED database; Max e-value cutoff: 1e−5, min % identity cutoff: 60%). Functional grouping was evaluated by heatmap construction using the function qplot on the ggplot2 package (WICKHAM et al., 2016). The enrichment of traits was calculated using differential expression analysis based on negative binomial distribution using the edgeR package (MCMURDIE et al., 2014). This analysis was carried out using two datasets: (*i*) the whole bacterial community and (*ii*) the bacterial community, except for the phylum Cyanobacteria. Then, the enriched traits were grouped according to life history strategy (Y, A, or S strategy) as described by Malik et al (2020). The classifications are presented in Supplementary Table 1.

3.2.7 Correlation between abiotic and biotic features of the lakes

To understand the correlations between abiotic (physical and chemical features) and biotic (bacterial community composition) factors, we applied the db-RDA analysis followed by the forward selection test using the function ordistep in the vegan package. Thus, we determined the set of parameters that best explained the variation in community composition (VALVERDE et al., 2015). These selected parameters were correlated with the differentially abundant bacterial taxa using the package corrplot (WEI; SIMKO, 2017).

3.2.8 Heterotrophic prokaryotes biomass

The heterotrophic bacterial biomass (HBB) was estimated by heterotrophic prokaryote counts (cells.mL^{-1}) using flow cytometry (FCM) (BD Accuri C6). Samples of 1.2 mL of lake water were fixed with formaldehyde (1% final concentration), flash-frozen in liquid nitrogen, and stored at -80°C until analysis in the laboratory. Samples were stained with SYBR Green I at a final concentration of 1:10,000 for 15 min in the dark at room temperature. HP detection was evaluated using the FL1 channel (533 nm) of the fluorescence sets following the Gasol and del Giorgio (GASOL; DEL GIORGIO, 2000) protocol adapted for freshwater. The bacterial cell size (*V*) (in μm^3 cell^{-1}) was estimated using the relationship between the average bacterial cell size and the average fluorescence of the sample relative to the beads (FL1 bacteria/FL1 beads), as reported previously by Gasol and Del Giorgio (2000), as follows: V = 0.0075 + 0.11 × (FL1 bacteria/FL1 beads). The bacterial biomass (BB) (in pg C cell^{-1}) was calculated by using the carbon-to-volume (V) (in μm^3 cell^{-1}) relationship derived previously by Norland (1993) from the data of Simon and Azam (1989), as follows: BB 0.12 × V$^{0.7}$.

3.3 Results

Here, we adopted metagenomic and flow cytometry approaches to estimate the lifestyle of bacterial populations that allow them to overcome the harsh conditions observed in tropical soda lakes.

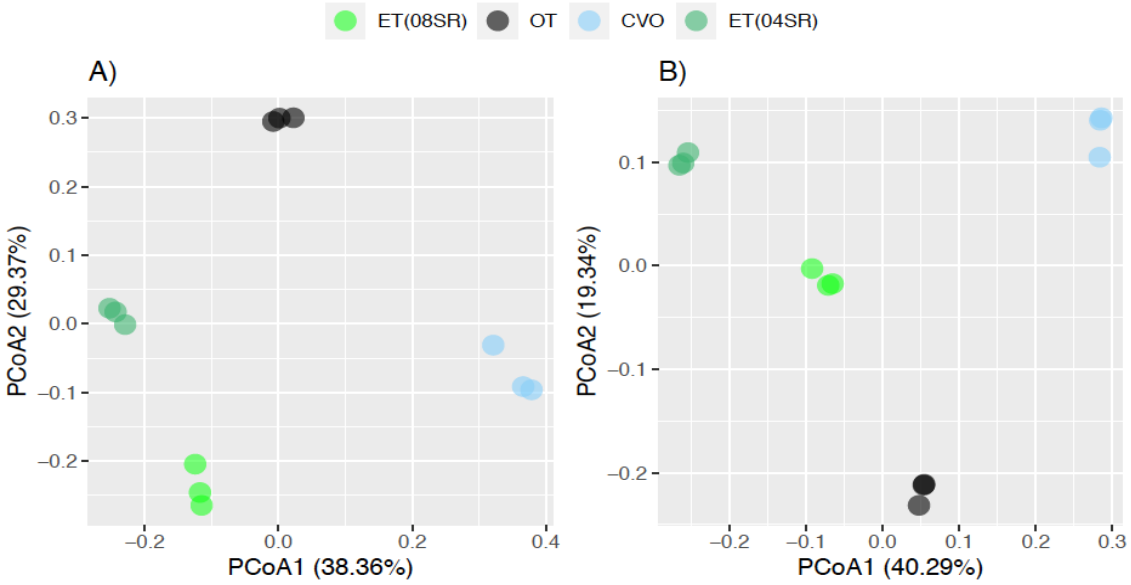
3.3.1 Metagenomic datasets

After trimming and removing low-quality sequences, 39,324,182 to 48,803,399 million reads were recovered during the dry season, and 34,694,196 to 41,212,766 million reads were recovered during the wet season. The replicates were reproducible, showing slight variation in the number of reads obtained (data not shown).

3.3.2 Bacterial communities' composition in Nhecolândia alkaline shallow lakes

The bacterial communities in the alkaline lakes were structurally different (Figure 1). During the dry season (Figure 1A), the first axis separated the lakes with the occurrence of cyanobacterial blooms from the lakes without the bloom (38.36 %). The second axis separated lake OT (oligotrophic turbid) from the other lakes by 29.37% (ANOSIM, global R= 1.00; p-value: 1e-04, PERMANOVA, R^2= 0.79881; p = 0.0001) (Figure 1A). This pattern was similar during the wet season (Figure 1B). The first axis separated the lakes without bloom (OT and CVO, clear vegetated oligotrophic lake) from the lakes with the occurrence of blooms [ET – eutrophic turbid lake (04SR) and ET (08SR)] by 40.28%. The second axis separated lake CVO from the other lakes by 19.34% (ANOSIM, global R= 1.00; p-value: 2e-04; PERMANOVA, R^2=0.75129; p-value=0.0001) (Figure 1B).

Figure 1. The Principal Coordinates Analysis (PCoA) of shallow alkaline lakes. The letter A corresponds to the dry season, while the letter B corresponds to the wet season

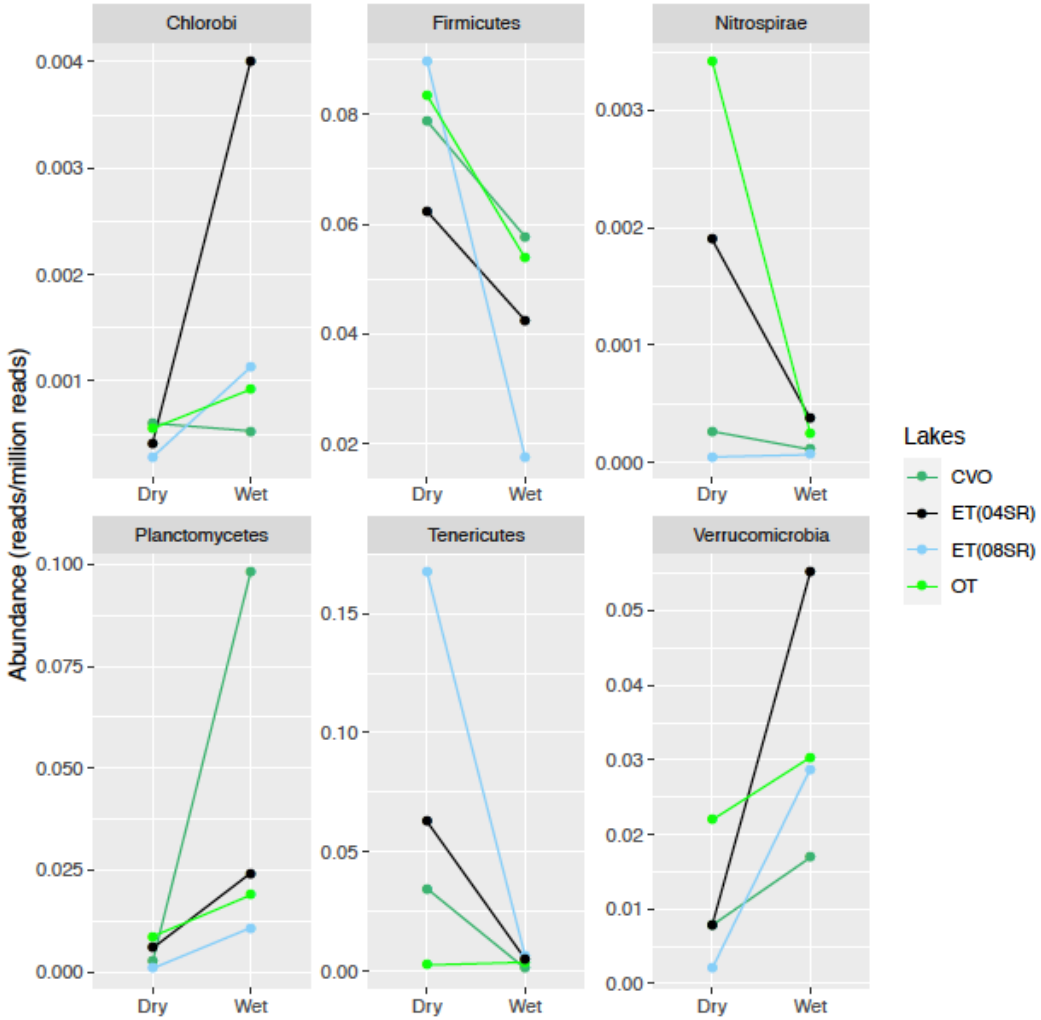


At a high phylogenetic level, the lakes harbor a similar bacterial community composition. Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria, Tenericutes, and Verrucomicrobia were the most abundant phyla identified in the alkaline lakes (relative abundance > 2%). The bacterial composition was similar between the lakes and seasons (Supplementary Figure 1). The differences observed in the PCoA analysis described above could be explained by the differential abundance of some bacterial groups.

3.3.3 Differential abundance of bacterial community

Although the bacterial composition was similar between lakes and between the seasons evaluated, it was possible to detect some groups whose abundance varied between the lakes and over time. Considering the bacterial differential abundance detected between the seasons, it is interesting that some groups increased during the dry season while others were enriched during the wet season. Chlorobi, Planctomycetes, and Verrucomicrobia were enriched during the wet period, whereas Firmicutes, Nitrospirae, and Tenericutes were more abundant during the dry season (Figure 2).

Figure 2. The differential abundances of bacterial groups associated with seasonality per lake. It was represented only in the groups whose abundance differed significantly between the harvest period ($p < 0.05$)



During the dry season, the abundances of Actinobacteria, Bacteroidetes, Cyanobacteria, Fusobacteria, Lentisphaerae, Nitrospira, Tenericutes, and Verrucomicrobia varied among the lakes. Actinobacteria, Bacteroidetes, Fusobacteria, Lenthisphaerae, and Tenericutes were abundant in lakes with blooms (ET), while Actinobacteria, Nitrospirae, and Verrucomicrobia were abundant in lakes without blooms (CVO and OT). The abundance of Cyanobacteria was reduced in lake CVO (Supplementary Figure 2).

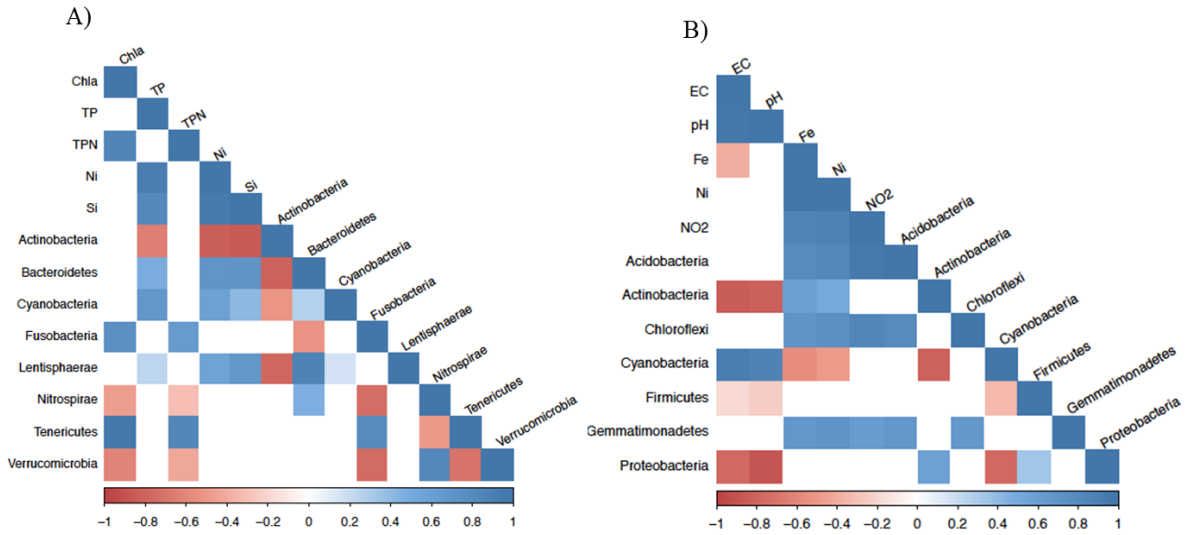
During the wet season, the abundance of Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, and Proteobacteria fluctuated between lakes. Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, and Proteobacteria were more abundant in lakes without blooms, while Cyanobacteria were abundant in lakes with blooms. Firmicutes were reduced in the ET (08SR) lake (Supplementary Figure 3).

3.3.4 Correlation between abiotic and biotic factors

Chemical and physical characteristics were relevant factors that explained the changes in bacterial community composition and functionality. Here, only the environmental variables significantly explained the variability in bacterial community structure based on db-RDA analysis (data not shown). For the dry season, the environmental variables that best explained the variability in the bacterial community were chlorophyll-*a*, TP, TPN, Si, and Ni ($P < 0.05$), while for the wet season, the environmental variables were E.C., pH, Fe, Ni, and NO_2^- ($P < 0.05$). These variables were correlated with the differentially abundant bacterial groups previously described.

During the dry season, Actinobacteria, Nitrospirae, and Verrucomicrobia were negatively correlated with the evaluated environmental features. Actinobacteria were negatively correlated with TP, Ni, and Si, whereas Nitrospirae and Verrucomicrobia were negatively correlated with chlorophyll-a and TPN (Figure 3A). Bacteriodetes, Cyanobacteria, Fusobacteria, Lenthisphaerae, and Tenericutes were positively correlated with the chemical characteristics of the lakes. Bacteriodetes, Cyanobacteria, and Lenthisphaerae were positively correlated with TP, Ni, and Si. Fusobacteria and Tenericures were positively correlated with chlorophyll-a and TPN (Figure 3A).

Figure 3. The correlation plot between abiotic parameters with differential abundant groups. The blue squares represent positive correlations, and the red squares represent the negative correlations. The white squares represent the absence of significant correlation ($p < 0.05$). The letter A corresponds to the dry period, while the letter B corresponds to the wet period



As observed in the dry season, in the wet season, some bacterial groups were positively correlated with some abiotic conditions, while others were negatively correlated. Acidobacteria, Chloroflexi, and Gemmatimonadetes were positively correlated with Fe, Ni, and NO_2^-. Actinobacteria were positively correlated with Fe and Ni but negatively correlated with E.C. and pH; an opposite trend was observed for Cyanobacteria. The phylum Cyanobacteria was positively correlated with E.C. and pH, and negatively correlated with Fe and Ni. Firmicutes and Proteobacteria were negatively correlated with E.C. and pH (Figure 3B).

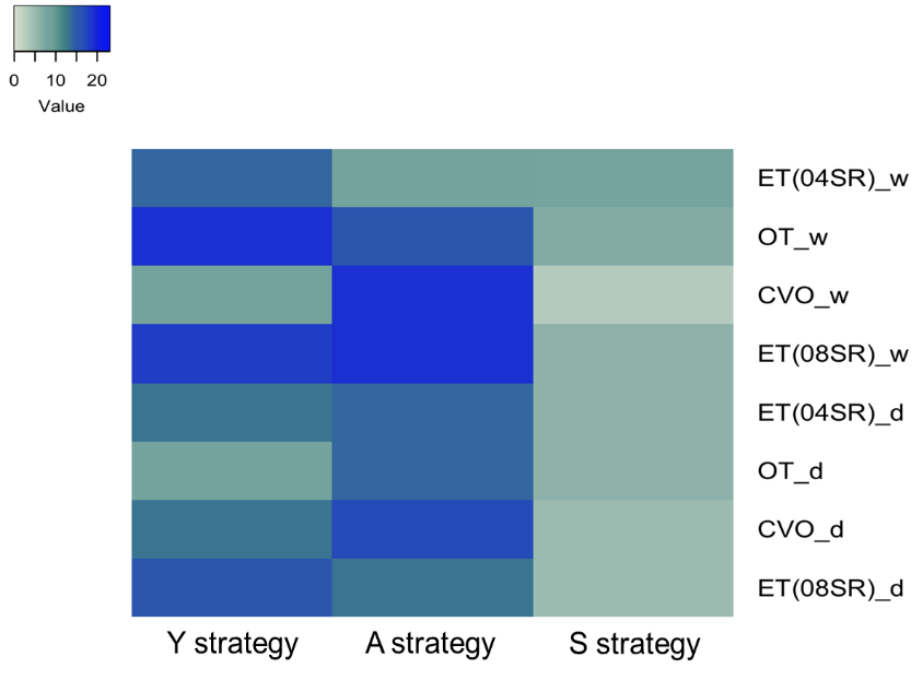
3.3.5 Distribution of prevalent functions on alkaline lakes

The prevalent functions (> 3%) associated with alkaline lakes were Carbohydrates, Protein Metabolism, and Amino Acids (and their derivatives) (Supplementary Figure 4). Following these functions, RNA Metabolism, Respiration, Nucleosides and Nucleotides, Cell Wall and Capsule, cofactors (cofactors, vitamins, prosthetic group, pigments), DNA metabolism, and Membrane transport were also abundant. The Phages, Prophages, Transposable elements, and Plasmids categories were prevalent specifically for lake ET(08SR) during the dry season (Supplementary Figure 4).

3.3.6 Bacterial trait-based framework

Based on the trait-based framework (the statistically significant functions were grouped following the categorization suggested in Supplementary Table 01), it was possible to observe that seasonality and the presence of bloom had a remarkable effect on the bacterial community tradeoff. Considering the whole bacterial community, inhabiting organisms of lakes OT and CVO preferentially adopted an A strategy (e.g., enrichment of ABC transporters), while the local bacterial community on ET lake preferentially adopted a Y strategy (e.g., enrichment of Di – oligosaccharides function) during the dry season (Figure 4; Supplementary Table 2). However, this tradeoff was modified in the wet season, when the bacterial community on the lakes preferentially adopts a Y strategy (e.g., enrichment of aminosugar function). The exception was the lake CVO, where the dwelling bacterial community continued to adopt an A strategy (e.g., enrichment of protein translocation in the plasmatic membrane and sugar phosphotransferase functions) (Supplementary Table 2; Figure 4).

Figure 4. Heatmap of the number of traits affiliated with each life strategy. The letters D and W represent samples from the dry and wet seasons, respectively



To understand how bacterial communities are affected by the presence of Cyanobacteria, we removed sequences associated with this phylum to perform a trait-based framework analysis. After removing the Cyanobacteria sequences from the analysis, it is interesting to note that traits associated with the S strategy were enriched in ET (04SR) and OT lakes during the dry season. In the wet season, the bacterial community tradeoff was similar to that observed in the presence of Cyanobacteria (Supplementary Figure 5A and C). The preferential use of Y-strategies in ET(04SR) and 08SR lakes was corroborated by the results obtained by determining heterotrophic bacterial biomass. Lakes where blooms occurred [ET(04SR) and ET(08SR)] had a higher biomass of heterotrophic bacteria than the other lakes, and this was independent of seasonality (Supplementary Figures 5B and D). Furthermore, absolute quantification of the microbial community by flow cytometry showed that during the dry period, heterotrophic bacteria (HB) were abundant in ET lakes compared to OT and CVO lakes. This pattern remained in the wet period when HB was prevalent, while OT Lake showed the lowest abundance. Notably, picoeukaryotes were prevalent in the CVO Lake during the wet period (Table 1).

3.4 Discussion

The associated microbiome of alkaline lakes has been described in several ecosystems worldwide. In general, the major phyla associated with alkaline lakes were Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, and Verrucomicrobia, a similar bacterial community composition observed on this study (AGUIRRE-GARRIDO et al., 2016; VAVOURAKIS et al., 2016). This similar frequency of bacterial community composition could be associated with niche conservatism, whereby species present traits that allow them to cope with certain environmental conditions (LENNON et al., 2012).

However, this bacterial composition pattern was not static. It is possible to detect fluctuations in bacterial abundance and composition over time and space. This shift is associated with the selection of populations that are more suitable for the given abiotic conditions (WALLENSTEIN et al., 2012). During the dry season, it was possible to observe an increase in nutrient and ion concentrations owing to the water evaporation rate. This increase in nutrient concentrations allows organisms to grow in environments where resources are abundant (OKIE et al., 2020). Usually, these organisms have high growth and metabolic rates, which can be disadvantageous in stable, nutrient-poor environments (OKIE et al., 2020).

During the dry season, a differential abundance of Firmicutes, Nitrospirae, and Tenericutes was observed. Specifically, Nitrospirae was abundant in Lake 06SR, where water showed a high concentration of particulate material resulting in a black color (low irradiance). During this season, this lake had a high concentration of nitrite and nitrate, which are important elements associated with the physiology of this bacterial group (ANDREOTE et al., 2018). Organisms belonging to the phylum Tenericutes are frequently described as obligate symbionts because of their small genomes. However, they are resistant to osmotic lysis and show an enrichment in DNA repair mechanisms on their genomes, a feature associated with stress tolerance (WANG et al., 2020). Interestingly, Tenericutes was positively correlated with chlorophyll-a, indicating a possible association with phytoplankton (mainly cyanobacteria).

However, the increase in rainfall during the wet season results in the dilution of nutrients in alkaline lakes, promoting a shift in bacterial abundance and composition. The reduction in nutritional status selects for organisms well-adapted to nutrient-poor environments, where resource uptake is prioritized in relation to biomass growth (OKIE et al., 2020). Chlorobi, Planctomycetes, and Verrucomicrobia were enriched during this period. Although the nutritional status was reduced during the wet season, the pH increased slightly. This increase in pH promotes an increase in organic carbon availability, which could stimulate Planctomycetes metabolism (POLLET et al., 2014; ANDREI et al., 2019). Planctomycetes have slow growth taxa that compensate for this through the efficient use of organic matter (ANDREI et al., 2019).

This increase in pH could be associated with the carbon concentration mechanism (CCM). Some autotrophic organisms, especially Cyanobacteria, enhance carbon fixation during photosynthesis through the uptake of inorganic carbon (CO_2, HCO^-_3, and CO_2^{-3}) (LONG et al., 2016; ZORZ et al., 2019). As a result, the environmental pH increases owing to the excretion of OH- and the generation of pericellular $CaCO_3$ precipitation (ALMEIDA et al., 2011; KUPRIYANOVA et al., 2015). Cyanobacteria are described as environmental engineers because they have strong effects on higher trophic levels and ecosystems functioning as critical drivers of bacterial assembly.

Microorganisms exhibit versatile metabolism, and this variability modulates the organization and functioning of communities. The trait-based approach, which analyses trait variation, is widely being adopted in ecology because it can clarify the microbial adaptations that permit the colonization of a specific niche and how these organisms will respond to environmental change (GREEN et al., 2008; LITCHMAN et al., 2008). Some criteria have been suggested to organize and classify the traits in the function of different microbial lifestyle

strategies. However, this is not a consensus, and it is continuously updating (WOOD et al., 2018; MALIK et al., 2020).

Resource utilization and competition for nutrients are important factors that shape phytoplankton communities (TILMAN et al., 1982; LITCHMAN et al., 2015). Resource availability in the aquatic environment is directly associated with spatiotemporal variations and is dependent on the quantity and quality of these resources. During the dry season, the bacterial community preferentially adopted an A-strategy [except for ET(08SR) lake], wherein the bacterial groups enhance nutrient acquisition at the expense of growth yield (MALIK et al., 2020). Although a high nutrient concentration was promoted by the high evaporation ratio, the quality and availability of these nutrients could be low, enhancing the necessity to efficiently uptake nutrients rather than microbial biomass production.

Notably, the tradeoff between nutrient uptake and biomass production was modified if the target was exclusively the heterotrophic bacterial community. Heterotrophic bacteria preferentially adopt an S-strategy when subjected to hostile environments. This tradeoff results in a direct energy flux for cell maintenance at the expense of bacterial growth efficiency (BGE). This mechanism is well known in freshwater and marine environments (CARLSON et al., 2007). Therefore, the adoption of the A-strategy by the whole bacterial community during the dry season is predominantly associated with cyanobacteria. The CCM mechanism described above is an important process of CO_2 uptake and an important strategy for adapting to the major changes in CO_2 availability that can be encountered during cyanobacteria blooms (JI et al., 2020).

However, these microbial tradeoffs change during the wet season, and this change represents niche differentiation among species, which emerges from individual-level constraints within an environmental context (KNEITEL et al., 2004). During the wet season, microbial communities adopt the Y-strategy, especially those inhabiting lakes with blooms. Primary productivity drives the energy flux through food webs, supporting the respiration and yield of heterotrophic bacteria (GIORGIO; PETERS, 1993). Lakes with cyanobacterial blooms showed eutrophic characteristics, such as a high concentration of carbon, nitrogen, and phosphorus nutrients, which stimulate the microbial community to grow and consequently increase biomass production (BERGIER; ASSINE, 2015).

3.5 Conclusion

Therefore, cyanobacterial blooms mediate carbon and flux energy in tropical alkaline lakes. During the dry season, cyanobacteria can adapt to harsh environmental conditions (e.g., high UV irradiation) through CO_2 uptake mediated by the CCM mechanism. As a consequence, these alternative processes for CO_2 fixation could promote alkalization of the water, driving heterotrophic bacteria to adopt a strategy focused on maintaining cellular functioning over the biomass yield. When environmental conditions become more favorable during the wet period, cyanobacteria support bacterial growth. This "cyanobacteria factor" is evident in the CVO lake where cyanobacteria are absent. Independent of the sampling period, the heterotrophic bacterial community inhabiting the CVO lake took up nutrients to support cellular functioning, which compromised biomass yields.

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4. BIOGEOCHEMICAL CYCLES AND ECOLOGY OF TROPICAL SODA LAKES

Abstract

Soda lakes are extreme habitats due to the high salinity and alkaline pH. Despite these harsh conditions, evolutionary cell adaptations and a metabolic versatility enables the microbial community of soda lakes to thrive in this environment. Here, the biogeochemical functioning and the functional associated taxonomy of three distinct soda lakes are described. Even at short distances (less than 1 km) the lake composition (functional and taxonomy) varied considerably. The eutrophic turbid lake (ET) showed a higher functional diversity with slight fluctuations over the seasons, suggesting a functional robustness and resilience. This trend was closely associated with natural blooms of Cyanobacteria which increased nutrients availability by several pathways such as carbon and nitrogen fixation, nitrate and sulfate reduction and phosphorus mineralization. In contrast, oligotrophic turbid lake (OT) and clear vegetated oligotrophic lake (CVO) had a lower functional diversity that changed significantly across seasons. Due to the predominance of heterotrophic over autotrophic metabolism, both OT and CVO lakes exhibited a metabolism related with carbon and nitrogen assimilation. Since autotrophic metabolism was low abundant, the microbial abundance in OT and CVO lakes was directly associated to nutrient availability. In this condition, an efficient growth rate and nutrient uptake mechanism would be required. The biogeochemical functional genes suggested that the ET lake had a greater potential to consume CO_2 and N_2 while OT and CVO had the potential to produce these gases. Our study provides insights into soda lake ecology, as well as unraveling the biogeochemical cycles mediated by microorganisms in contrasted seasons.

4.1 Introduction

Microorganisms play an important role in aquatic ecosystems by providing a wide range of ecological services, such as primary productivity, nutrient transformation in biogeochemical cycles, and the production and consumption of greenhouse gas (GHG) (COTNER; BIDDANDA, 2002; STEIN, 2020). Since these processes are impacted by abiotic and biotic factors, the concentration of these gases in the atmosphere is a dynamic system shaped by both anthropic and naturally occurring processes (FIERER, 2008; SADEGHI et al., 2021). Despite its relevance, it is unclear how the biogeochemical cycle works under hostile conditions such as high pH (up to 10.5) and salinities approaching saturation, particularly in tropical regions.

Important processes associated with an efficient renovation of nutrients are inhibited under these conditions, as nitrification is inhibited in high salinities (SOROKIN et al., 2014).

Soda lakes are home to a diverse microbial community that performs essential ecological functions associated to energy uptake and cell maintenance such as cycling of carbon, nitrogen, sulfur, and phosphorus (SOROKIN et al., 2011; SOROKIN et al., 2014; VAVOURAKIS et al., 2018). Several evolutionary cell adaptations and the ability to utilize a variety of energy and nutritional sources enables the microbial community of soda lakes to thrive in this environment (SOROKIN et al., 2014). This kind of lake is widely distributed, occurring in Russia, Africa, Canada, and the Brazilian Pantanal (SOROKIN et al., 2014; ANDREOTE et al., 2018, ZORZ et al., 2019).

The tropical soda lakes of the Brazilian Pantanal (in the sub-region Nhecolândia) stand out because they are shallower, smaller, and have lower salinity levels (up to 35 $mS.cm^{-1}$) than the large majority of other soda lakes in the world. Although the high density of soda lakes in the Nhecolândia subregion (i.e., 600 lakes in 24,000 km^2) (BECKER et al., 2018; PEREIRA et al., 2020), they are geographically separated from each other and the surrounding landscape, resulting in broad range of physical, chemical, and biological ecosystems. Despite their variability, these lakes may be classified into three distinct profiles based on their ionic and nutritional, and microbial composition: eutrophic turbid (ET), oligotrophic turbid (OT) and clear vegetated oligotrophic (CVO) lakes (PELLEGRINETTI et al., 2022).

The widespread distribution of soda lakes ecosystems not only sustains local and global food webs, but also affects the gas fluxes of GHG (CH_4, CO_2, and N_2O), and other ecologically significant compounds (NO, H_2S, NOx, SOx) (ANTONY et al., 2013). Carbon and nitrogen might be stored or released as CO_2, CH_4 and N_2O depending in the predominant type of bacterial metabolism (SOROKIN et al., 2014, ANDREOTE et al., 2018; BARBIERO et al., 2018). Recently, studies in Nhecolândia's soda lakes revealed that OT lakes are probable CO_2 and CH_4 sources while ET lakes (rich in Cyanobacteria) are CO_2 and N_2O sinks but CH_4 sources (BARBIERO et al., 2018). Moreover, current findings demonstrated that significant CH_4 emissions could occur in the oxic layers of aquatic environments due to phosphonate cycling (C-P lyases) from Cyanobacteria metabolism (BIŽIĆ et al., 2020).

To investigate the role of bacterial functioning in biogeochemical processes in tropical soda lakes, the relative abundance of functional genes associated with ecologically relevant processes in the carbon, nitrogen, phosphorus, and sulfur cycles was assessed using DNA metagenomic sequencing.

4.2 Methods

4.2.1 The description of sampling sites

Three soda lakes located in the São Roque Reserve in the Nhecolândia sub-region of Mato Grosso do Sul State, Brazil were investigated (Fig. 1A). It was selected one representative lake of each soda lakes' group according to the previous classification [eutrophic turbid (ET) – 04SR, oligotrophic turbid (OT) – 06SR and clear vegetated oligotrophic (CVO) – 07SR]. It is worth highlighting that ET lakes have a natural bloom occurrence of filamentous Cyanobacteria (alternating between *Anabaenopsis elenkinii* and *Arthospira platensis*) (PELLEGRINETTI et al., 2022). Sampling expeditions were conducted in three distinct years (Oct 2017, Sep 2018 and Sep 2019). In each sampled year, different conditions were found; Oct-2017 was in the end of dry period with low water level (≈ 20 to 56 cm); Sep-2018 was after the wet period, with high water level (≈ 86 to 130 cm); Sep-2019 was an intermediary period between the others (≈ 60 to 67 cm). The physical and chemical characteristics of each lake are described in Supplementary Table S1. Surface waters were collected between 0-10 cm deep in replicates (three replicates) equidistantly separated by at least 100 meters. For more details about the sampling methodology see Pellegrinetti et al (2022).

4.2.2 Metagenomic DNA extraction and sequencing

The total environmental DNA of water samples was extracted from lyophilized water (50 mL, 0.5 g) using the PowerLyzer PowerSoil DNA isolation kit (Qiagen, Hilden, Germany). The integrity of the extracted DNA was evaluated with agarose gel electrophoresis (1% w/v) and quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The shotgun sequencing was realized in 27 DNA samples (3 lakes x 3 replicates x 3 sampling periods). 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. The obtained sequences were deposited at MG-RAST server and are publicly available under projects "*mgp86324*", "*mgp88859*" and "*mgp92377*" for respectively years (2017,2018 and 2019).

4.2.3 Bioinformatic analyses

The raw sequence adapters were trimmed using CutAdapt 1.18 (MARTIN, 2011) and their quality was inspected with FastQC 0.10.1 (ANDREWS, 2010). The merging of paired ends reads was performed using PEAR software 0.9.6 (ZHANG et al., 2014). Sequences smaller than 50 pb and Phred < 20 were removed using Seqyclean 1.3.12 (ZHBANNIKOV et al., 2017). The DiTing pipeline 0.9 (XUE et al., 2021) was used to determine the relative abundance and occurrence of metabolic and biogeochemical functional pathways of carbon, nitrogen, sulfur, and phosphorus. This pipeline assembled reads using MEGAHIT 1.1.3 (LI et al., 2016) and predict open reading frames (ORFs) from all contigs using Prodigal 2.6.3 (HYATT et al., 2010).

The functional taxonomic profiling of each biogeochemical process was obtained using MetAnnotate pipeline 0.9.2 (PETRENKO et al., 2015). The input of this pipeline was composed of the ORFs generated by DiTing pipeline and the 'HMM' profile obtained for each KO using KofamKOLA database. The taxonomic database used was the Reference Genome Sequences (RefSeq) provided by MetAnnotate.

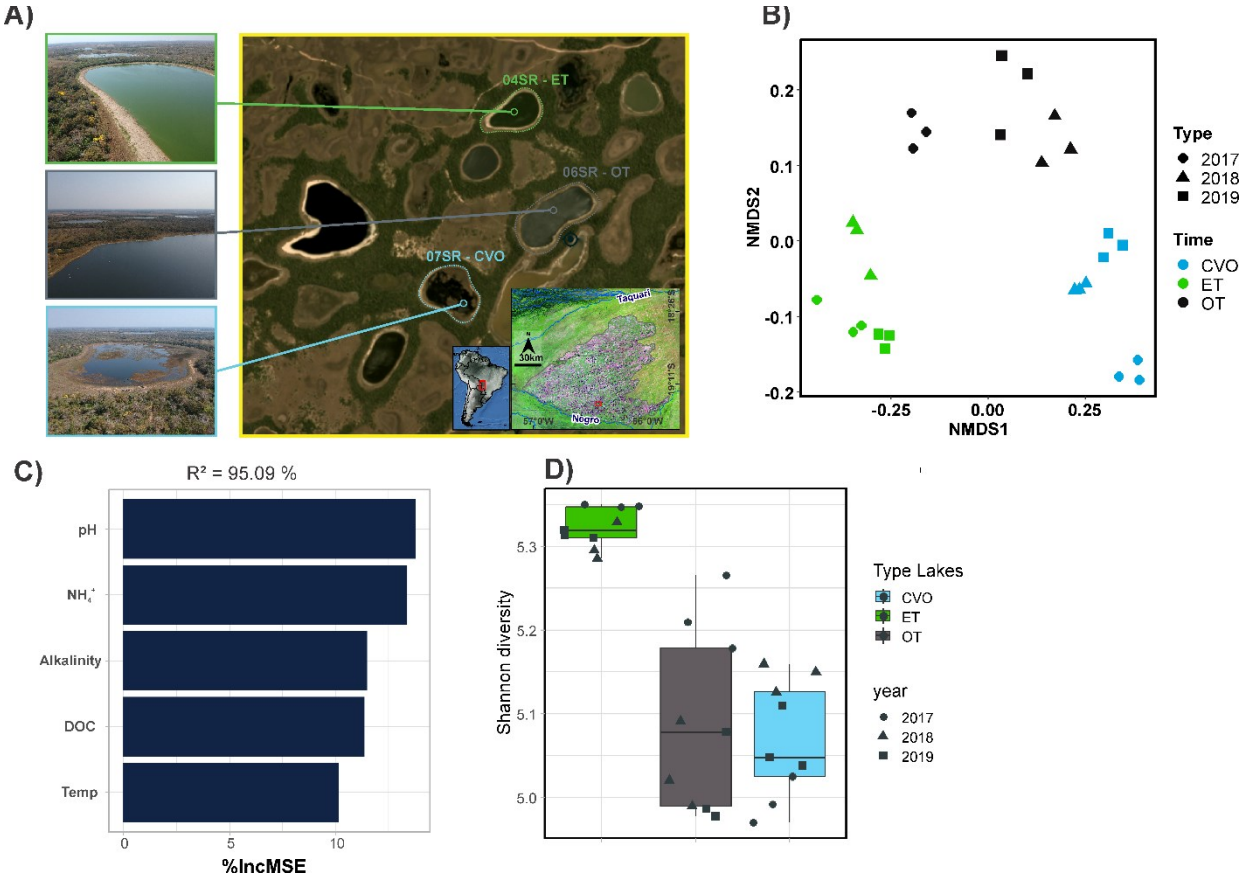
4.2.4 Statistical and Data Analysis

Functional and taxonomic data was analyzed in R language (TEAM, 2012). The analysis of variance (ANOVA) and post-hoc Tukey tests were used to determine statistical differences across lakes using ExpDes.pt package (FERREIRA et al., 2018). A non-metric multidimensional scaling (NMDS) based on the biogeochemical functional genes was performed to determine the similarity between samples using Vegan Package (OKSANEN, et al., 2013). Vegan package was also used to calculate Alpha diversity based on annotated functional genes between samples using as reference the Shannon index. A random forest model was applied to find the best environmental predictors that better explain the functional diversity. For this purpose, the "*randomForest*" package (LIAW; WIENER, 2002) was used to construct a first model using *NMDS1* and *NMDS2* from the *NMDS* distance matrix of each lake against the environmental variables table. We selected the optimal Mean Decrease Accuracy value (%incMSE = 5.5%) by reducing the %incMSE variable importance while analyzing the final model's R^2 until the best result was reached.

4.3 Results

The biogeochemical functional genes of the tropical soda lakes were analyzed in this study (Figure 1A). The evaluated lakes displayed remarkable differences, regardless of the sample year as observed in the NMDS ordination (p=0.001) (Figure 1B). The NMDS of functional biogeochemistry were best explained by five variables (r^2 = 95.09 %) such as pH, NH_4^+, alkalinity, dissolved organic carbon (DOC) and water temperature (Figure 1C). The ET lake showed a higher functional diversity compared to OT and CVO lakes ($p < 0.001$) (Figure 1D). The following sections of the results were organized according to the evaluated biogeochemical cycles (carbon, nitrogen, sulfur, and phosphorus) to improve the reading.

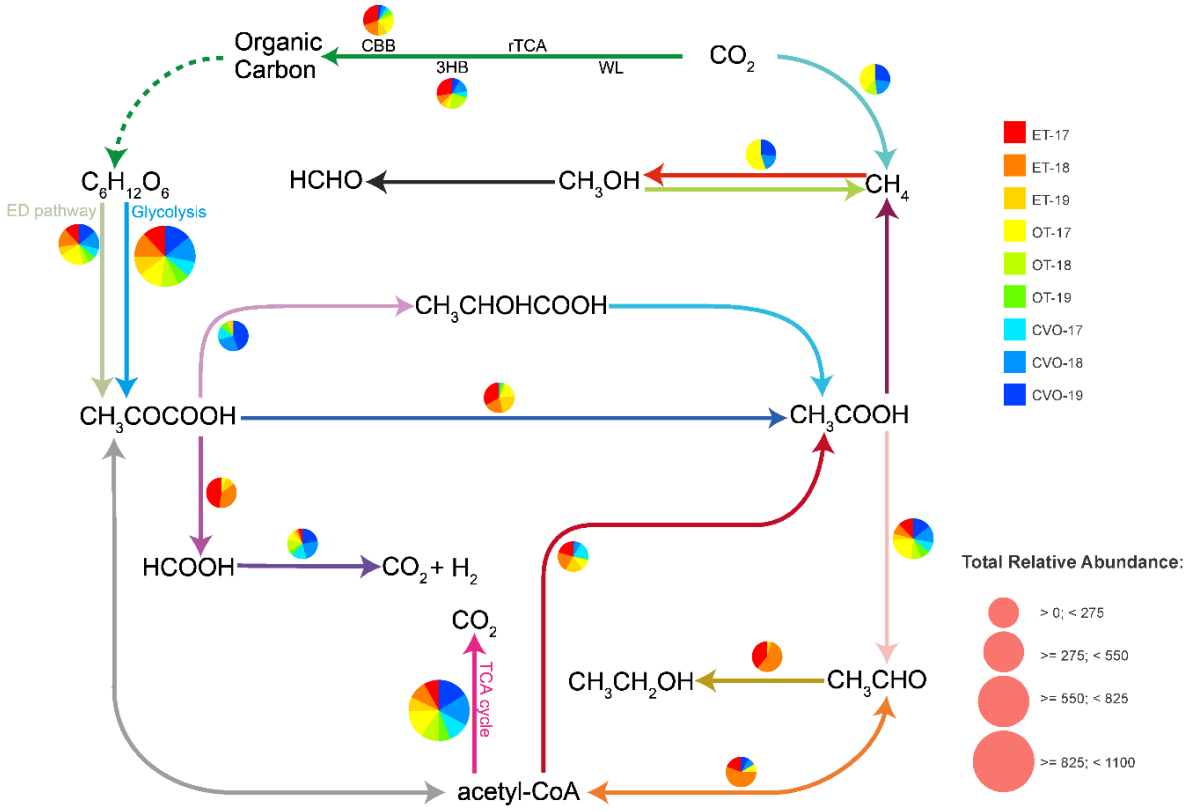
Figure 1. Geographic localization and aerial photographic of lake ET (04SR), OT (06SR) and CVO (07SR) (A); Non-metric distance scale of biogeochemical functions in lakes ET, OT and CVO, in distinct years (B); Alpha diversity (Shannon) of lakes ET, OT and CVO in the distinct year



4.3.1 Carbon cycle

The representative pathways of central carbon metabolism (glucogenesis, fermentation of succinate, glycolysis, and the tricarboxylic acid cycle) were identified in the evaluated soda lakes (Figure 2). In addition, the Entner-Doudoroff pathway (ED), other fermentative pathways (ethanol, acetate, and formate), and other mechanisms associated with carbon fixation as Calvin-Benhson-Bassham (CBB) and 3-hydroxypropionate bicycle (3HB) pathways were also identified in all lakes, albeit the lower frequency (Figure 2).

Figure 2. Schematic representation of functional genes and carbon metabolism in distinct soda lakes and sampled year



The pie charts represent the gene relative abundance of each step in each lake. The pie chart size is proportional to the number of genes per million – (GPM) of this process in the metagenome of each sample. rTCA: reversal citric acidcycle; CBB: Calvin–Benson–Basham; WL: Wood–Ljungdahl; ED: Entner-Doudoroff; 3HB: 3-hydroxypropionate bicycle.

In general photosynthesis (represented by the photosystems I and II) and CBB were the predominant pathways of the carbon fixation in ET lake as a result of cyanobacteria bloom (Supplementary Fig. S6). Still considering the ET lake, the fermentation of ethanol using acetaldehyde as a substrate and the fermentation of acetate were prevalent. For CVO lake,

it was observed a prevalence of TCA cycle, methanogenesis, fermentation of lactate and formate, and the anoxygenic photosystem II. OT lake had a prevalence of TCA cycle, fermentation of acetate, methanogenesis, and methane oxidation processes (Figure 2).

In relation to the seasonality the carbon fixation was abundant in ET lake in 2018 and in OT lake in 2017. Even at low abundance, genes linked with methanogenesis were twice as prevalent in OT lake compared to CVO lake, and six to eight times more prevalent than genes associated with methanotrophy, suggesting a potential for methane production. The fermentative processes were higher in 2017 for both ET and OT while for the CVO lake, it was observed an opposite trend whereas a higher abundance was observed in 2019.

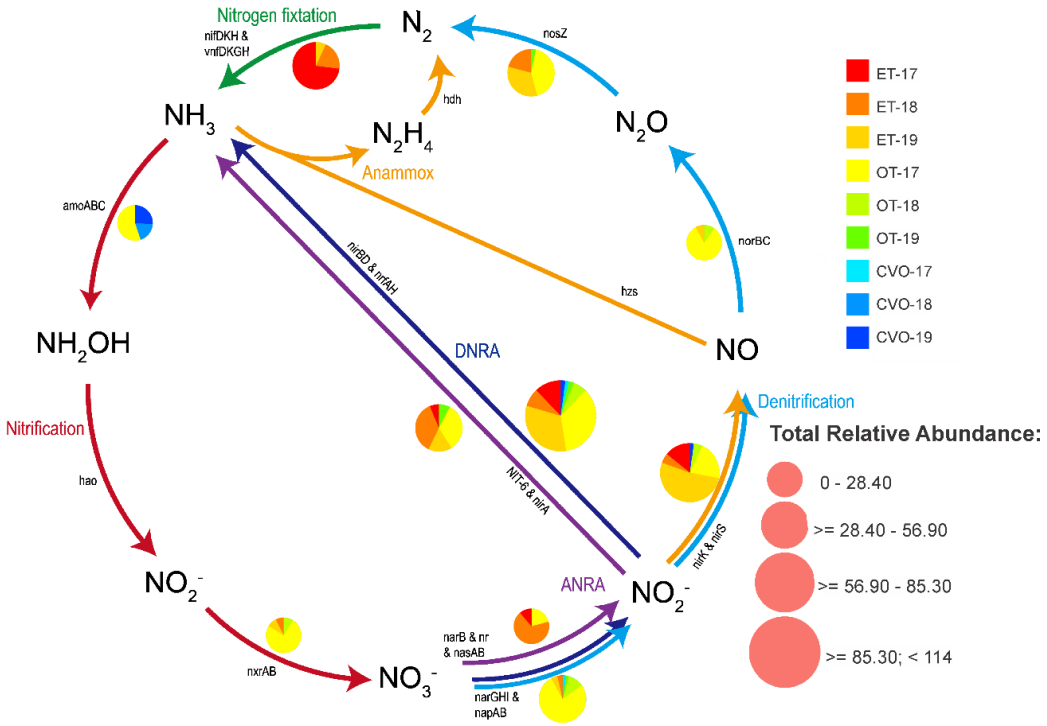
Considering the CO_2 fixation (CBB and 3HB), the ET lake had a more diverse associated bacterial community than OT and CVO lakes. In this lake, members belonging the Acidimicrobiia, Actinomycetia, Cytophagia, Flavobacteria, and Alpha-Gammaproteobacteria were associated with this process (Figure 5; Supplementary Figure S1). In OT and CVO lakes, Actinomycetia, Alpha- and Betaproteobacteria were prevalent. The methanogenic Archaea member of the Euryarchaeota (Methanomicrobia) was only found in OT lake, while the methanotrophic organisms (*pmo-amoABC*) were found in both OT and CVO lakes, predominant affiliated to Nitrospirae, Alphaproteobacteria, and Thaumarchaeota. The bacterial community associated to fermentative processes varied according to the substrate and the lakes. The fermentation of acetate, lactate and succinate were more taxonomic diverse than fermentation of ethanol and formate. In general, the ET lakes presented high abundance of Actinomycetia, Bacteroidetes (Cytophagia, Flavobacteria, Bacteroidia, Saprospira), Alpha-Gammaproteobacteria, Cyanobacteria (Oscillatoriales), while the OT and CVO lakes showed a prevalence of Actinomycetia, Planctomycetia and Alpha-Betaprotebacteria.

4.3.2 Nitrogen cycle

In general, the majority processes associated with nitrogen cycling were detected in almost all evaluated lakes, except for nitrogen fixation and nitrification (Figure 3). The remarkably transformations recovered in ET lake was the dissimilatory nitrite reduction to ammonia and denitrification (nitrite to nitric oxide and nitrous oxide to dinitrogen). Moreover, assimilatory nitrite reduction to ammonia and nitrogen fixation was also predominantly, albeit a lower proportion (Figure 3). Considering the OT lake, processes such as the dissimilatory nitrate reduction to ammonia (DNRA), assimilatory nitrite reduction to ammonia (ANRA) and the reduction of nitrous oxide to dinitrogen were abundant. Low abundance of nitrogen genes was found in CVO lake, within the dissimilatory nitrite reduction to ammonia and nitrite to

nitric oxide the prevalent functions found. For all lakes evaluated, ammonia oxidation was shown to be less prevalent than the reduction of nitrate, nitrite, and nitrogen gas forms (NO, N_2O).

Figure 3. Schematic representation of functional genes and nitrogen metabolism in distinct soda lakes and sampled year



The pie charts represent the gene relative abundance of each step in each lake. The pie chart size is roportional to the number of genes per million – (GPM) of this process in the metagenome of each sample. ANRA = Assimilatory nitrate reduction to ammonia; DNRA = Dissimilatory nitrate reduction to ammonia

When considering seasonality, we observed that the highest gene abundances in OT lake occurred during the dry season (2017), while in ET, the highest abundances occurred during the wet season (2018). Nitrogen fixation enhanced during the dry season in the ET lake, whereas the reduction of nitrate to nitrite (assimilatory and dissimilatory), nitrite to ammonia (assimilatory), and nitrous oxide to dinitrogen increased during the wet season (2018) (Figure 3). In 2019, dissimilatory nitrite reduction to ammonia and nitrite to nitric oxide were prevalent for this lake. In OT lake, an increase in nitrogen gene abundance was seen during the dry season, followed by the wet and the intermediary season (2019). During the dry season in CVO lake, dissimilatory nitrate reduction to ammonia (DNRA) was predominant, while nitrification (ammonia to hydroxylamine) was prevalent during the wet period. The reduction of nitrite to

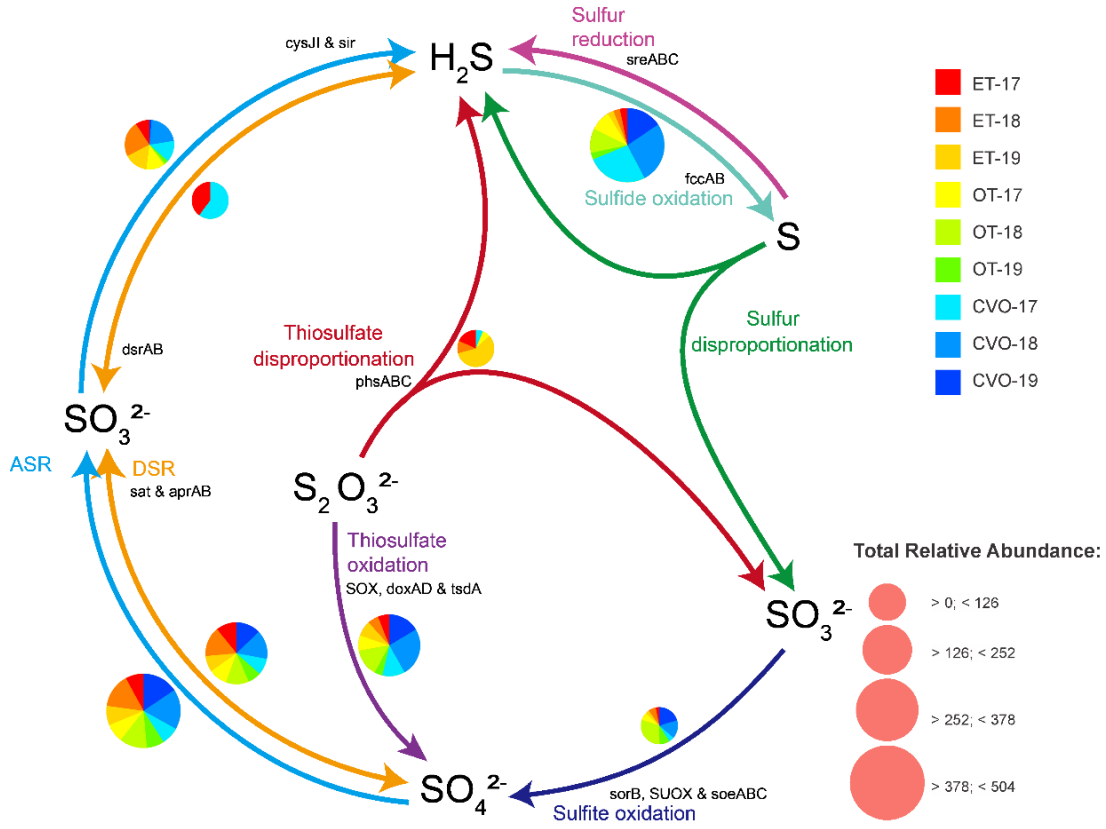
nitric oxide was identified as the primary pathway for CVO lake in 2019. It is supposed that nitric oxide was produced in ET and OT lakes due to the lower abundance of *norBC* genes than *nirKS*. In addition, given that the *nosZ* was higher than the *norBC* we may assume that these lakes could have low N_2O emissions (Figure 3).

In general, bacterial groups affiliated with the nitrogen fixation processes was Alpha-Beta-Gammaproteobacteria, Cyanobacteria (Nostocales), and Actinomycetia (Figure 5; Supplementary Figure S2). Nostocales was the primarily responsible for this step, whereas Betaproteobacteria and Alphaproteobacteria predominate in OT and CVO lakes (Figure 3). In OT and CVO lakes, Alphaproteobacteria, Nitrospirae and Thaumarchaeota (Archaea) was found associated to ammonia oxidation. Intriguingly, no taxa were affiliated to the conversion of nitrite to nitrate in any of the lakes or years examined. The conversion of nitrate to nitrite by assimilatory and dissimilatory transformation was carried out by members of Alpha-Beta-Gammaproteobacteria and Actinomycetia. However, Alpha and Betaproteobacteria were more assigned to this function in OT and CVO lakes, while ET lakes displayed a more diverse taxa distribution. The denitrification steps such as NO_2^- to NO, and NO to N_2O was performed mainly by members of Alpha-Beta-Gammaproteobacteria, Flavobacteria and Cytophagia while the N_2O to N_2 was associated with Cyanobacteria (Synechococcales), Cytophagia, Flavobacteria, Blastocatellia and Alphaproteobacteria.

4.3.3 Sulfur cycle

The sulfur cycle was prominent in the studied soda lakes, and a wide variety of functions were detected. For the soda lakes evaluated in this study, the most prevalent reactions included sulfide oxidation, assimilatory and dissimilatory sulfate reduction (ASR and DSR respectively), and thiosulfate oxidation (Figure 4). The abundance of genes related to reduction of SO_4^{2-} to SO_3^{2-} was similar in all three lakes. In ET lakes, the SO_3^{2-} and $S_2O_3^{2-}$ reduction were the dominant sources of H_2S production. In OT lake, the conversion of SO_3^{2-} to SO_4^{2-} predominated. Interestingly, the CVO lake concentrates the oxidative pathways of H_2S and $S_2O_3^{2-}$ (Figure 4).

Figure 4. Schematic representation of functional genes and sulfur metabolism in distinct soda lakes and sampled year



The pie charts represent the gene relative abundance of each step in each lake. The pie chart size is proportional to the number of genes per million – (GPM) of this process in the metagenome of each sample. ASR = Assimilatory sulfate reduction; DSR = Dissimilatory sulfate reduction.

In both lakes, the ASR and DSR to sulfite were predominant in oligotrophic conditions (wet season – 2018). The transformation of SO_3^{2-} was higher in ET and CVO lake, majority during the wet season. A similar pattern was found in OT and CVO lake to thiosulfate oxidation, which was abundant in 2018, while in ET lake was abundant in 2019. Sulfite oxidation was frequent in OT and CVO in 2018 and 2019, respectively, while sulfide oxidation was high in both lakes in 2018. We could observe a low potential to H_2S emission in these soda lakes, due to the sulfide oxidation to elemental sulfur (S), that was higher than assimilatory and dissimilatory sulfate reduction and thiosulfate disproportionation (Figure 4).

The dissimilatory and assimilatory sulfate reduction to sulfide were predominantly associated with Alpha-Gammaproteobacteria, Nostocales, Oscillatoriales and Cytophagia in ET lake. In contrast, OT and CVO lakes were affiliated with Actinomycetia, Betaproteobacteria, Planctomycetia and Synechococcales (Figure 5; Supplementary Figure S3). The sulfide oxidation was associated with Alphaproteobacteria and Gammaproteobacteria

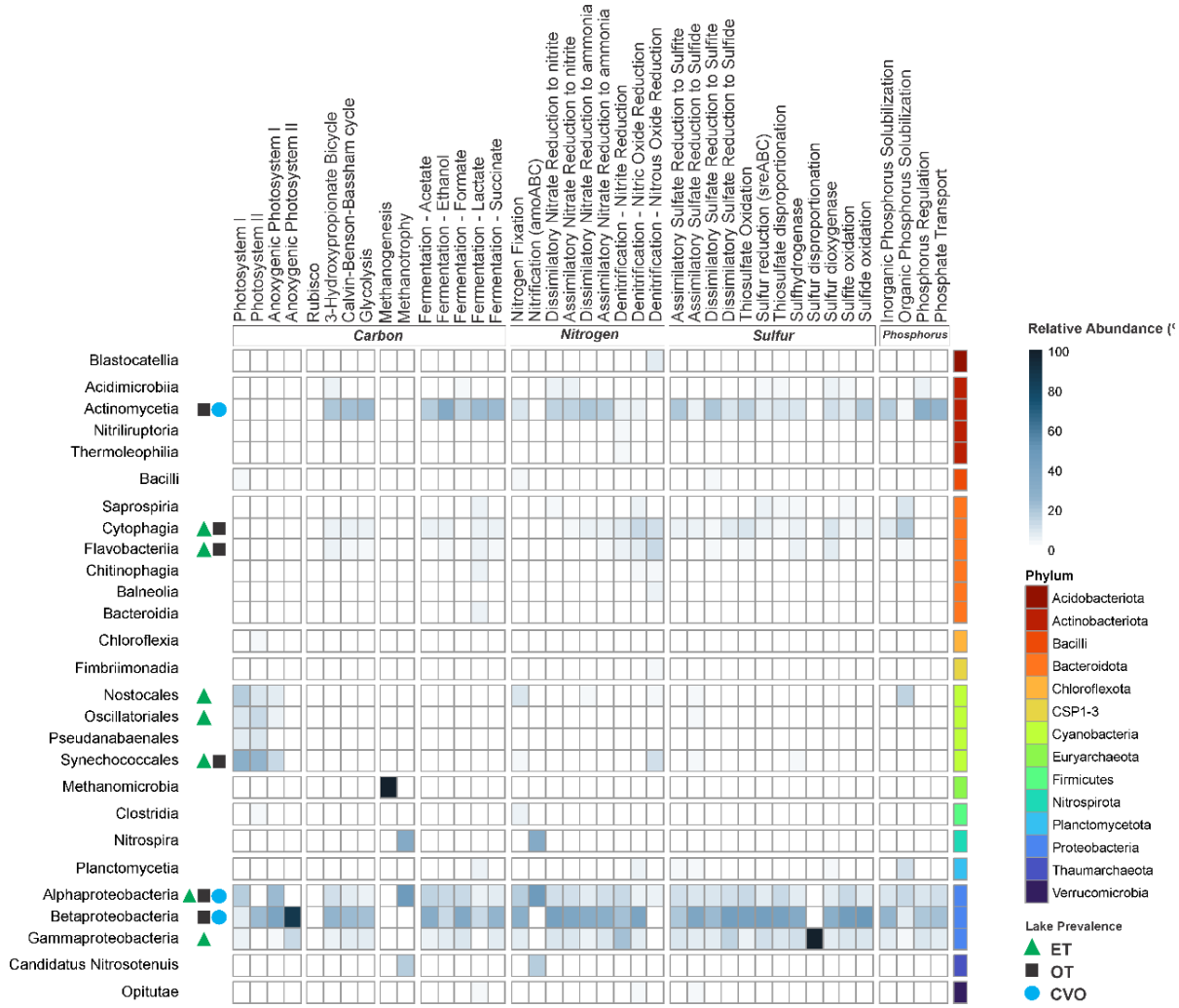
in ET and OT, Gammaproteobacteria in ET, and Actinomycetia and Betaproteobacteria in OT and CVO. The thiosulfate oxidation to sulfate were prevalent associated with Alpha-Gammaproteobacteria and Cytophagia in ET lake and Actinomycetia and Betaproteobacteria in OT and CVO.

4.3.4 Phosphorus transformations

A prevalence of genes associated with the phosphorus uptake and phosphate transporters (*pstSABC*) were found in evaluated lakes (Supplementary Figure S4). Phosphate transporters were abundant in CVO and OT lakes, where phosphorus availability was reported to be lower. Solubilization of inorganic phosphorus was the second most prevalent function (*ppa, ppk*). Regarding organic phosphorus mineralization, no evident trend was detected. In ET lake, organic phosphorus mineralization was predominant, with alkaline phosphatase (*phoA, phoD*) and C-P lyase of methylphosphonate (*phnJ*) playing the most important roles. Additionally, the *phnJ* gene was also abundant in CVO lake. High levels of inorganic phosphate transporter (TC.PIT) and sn-glycerol-3-phosphate transport was detected.

In 2018, when nutrients were more diluted, phosphorus functions in ET lake were higher, except for organic phosphorus mineralization, which was higher in 2019. An opposite pattern was found for OT lake, which had a high relative abundance in 2017, with the exception for phosphorus regulation, which was high in 2018. In CVO lake, no clear trend was observed, with phosphorus regulation higher in 2017, organic phosphorus mineralization and phosphorus transport in 2018, and inorganic phosphorus solubilization high in 2019.

Figure 5. Taxon-specific functional genes associated with biogeochemical cycles of carbon, nitrogen, sulfur and phosphorus from selected soda lakes



Actinomycetia, Alpha-Beta and Gammaproteobacteria were shown to be the most important taxa performing phosphorus transformation (Fig. 5; Supplementary Figure S5). In ET lake, Bacteroidia, Gammaproteobacteria, Oscillatoriales and Saprospiria were the predominant taxonomy associated to inorganic phosphorus solubilization, while for OT and CVO, Actinomycetia, Betaproteobacteria, Clostridia and Planctomycetia were predominant. Considering the organic phosphorus mineralization, members of Bacteroidetes (Cytophagia and Saprospira) and Gammaproteobacteria were prevalent in ET while Actinomycetia, Betaproteobacteria and Planctomycetia were prevalent in OT and CVO. Phosphorus transportations were affiliated with the Alphaproteobacteria, Gammaproteobacteria and Cyanobacteria (Nostocales, Oscillatoriales) in ET lake and Actinomycetia, Betaproteobacteria and Planctomycetia for OT and CVO lake. For phosphorus regulation were prevalent affiliated

to Acidimicrobia and Gammaproteobacteria in ET while for OT and CVO a prevalence of Actinomycetia and Betaproteobacteria were found.

4.4 Discussion

Several studies had attempted to unveil the microbial community composition and functional diversity in soda lakes worldwide. Its inhabitants have especial evolutionary adaptations that allowed them to thrive under high pH and salinity levels (SOROKIN et al., 2015). Moreover, these microorganisms have a rich metabolic diversity and versatility, which allows them to use distinct compounds as energy and nutritional sources, as observed in this study (SOROKIN et al., 2015; HOEFT et al., 2016; OREMLAND et al., 2017). These adaptations had found in soda lakes separated by large distances (Russia and Canada), demonstrating that common selection drives community composition in alkaline conditions (ZORZ et al., 2019). However, in the Nhecolândia, nearby soda lakes exhibit remarkable metabolic and taxonomic diversity even at short distances (lakes separated by less than 1 km). This functional composition was correlated with variables such as pH, NH_4^+, alkalinity, DOC and water temperature. These factors showed to vary spatially and temporally among Nhecolândia soda lakes, ranging from oligotrophic to eutrophic, oligohaline to saline, and varying according to geomorphological lake characteristics (BARBIERO et al., 2018; GUERREIRO et al., 2019; PELLEGRINETTI et al., 2022).

In the ET lake, a higher functional diversity was found independently of the season. A slight fluctuation in the functional diversity over the seasons allowed to suggest that this lake has a functional robustness and resilience. This assumption corroborated with those found in Cariboo Plateau soda lakes, which attributed this functional robustness to the Cyanobacteria abundance and diversity (ZORZ et al., 2018). Cyanobacteria improve the organic profile of aquatic systems by performing carbon and nitrogen fixation, but also inorganic compounds solubilization. A high relative abundance of genes linked to CO_2 and N_2 fixation, nitrate and nitrite reduction, alkaline phosphatases and sulfate reduction was positively correlated with the nutrient concentrations (C, N, S and P) and relative abundance of autotrophic organisms (mainly Cyanobacteria). The Cyanobacterial blooms in ET lake had strong effects on the microbial community since they may improve the nutrient availability. Photosynthetic organisms are key players in aquatic environments due to the release of carbon and nitrogen, stimulating heterotroph organisms' growth (MORANA et al., 2014; LINZ et al., 2020). During the extreme drought (2017) metabolic versatility was pronounced, where multiple processes of carbon, nitrogen, phosphorus and sulfur were enriched. These harsh conditions impose to

microorganisms to invest in distinct mechanisms to nutrient acquisition and energy sources (MALIK et al., 2020; COTTA et al., 2022). In contrast, when stress was alleviated (during the wet season), the community invested in biomass growth, prevailing nutrient assimilation mechanisms such as reduction of nitrate (ANRA and DNRA), sulfate (ASR and DSR), and photosynthesis.

A distinct biogeochemical functioning was found in OT lake, potentially attributed to its reduced salinity, nutrient concentration and absence of Cyanobacterial blooms, as observed in recent studies (ANDREOTE et al., 2018; BARBIERO et al., 2018; PELLEGRINETTI et al., 2022). During the extreme drought (2017) the OT lake displayed a higher functional diversity. In this season, low frequent processes were more abundant, including methanogenesis, methane oxidation and nitrification. Moreover, other functions including ED pathway, DNRA, denitrification, carbon fixation and photosynthesis were found. The competition for nutrient resources requires to microbial community the necessity to use a wide range of metabolic pathways. Even at high nutrient concentrations (observed in dry season due the high evaporation rate), the low nutrient quality and availability induces to microorganisms to mine resources and develop different nutrient uptake mechanisms (HICKS et al., 2021; COTTA et al., 2022). Similarly, to ET lake, the OT lake increased their functional diversity during harsh conditions (salinity and pH) as found in dry period (2017), as a strategy to overcome this difficulty. This pulse disturbance may have a severe change in community, stimulating mechanisms to resist in the face of disturbance (SHADE et al., 2012). When these conditions were alleviated, the OT lake returned to a stable state (after rainy periods), reducing functional diversity and possibly investing in biomass growth by consumption of labile nutrients. The stable state is considered as a condition that microbial community return to its original composition or function following a disturbance (community equilibrium) (SHADE et al., 2012). In OT lake (06SR), this state is composed by moderate salinity (0.7 to 1.27 g L^{-1}), pH levels (between 9.09 and 9.45) and nutrient availability (dissolved nitrogen below 15 mg L^{-1}) (PELLEGRINETTI et al., 2022).

In CVO lake, a lower abundance of biogeochemical functions was correlated with low nutrient concentrations. According to a study in Nhecolândia, lakes similar to CVO lake exhibited a lower quality and availability of dissolved organic matter (rich in fulvic and humic-type compounds), making it difficult to be assimilated (MARIOT et al., 2007). For this, the microbial community needs to adopt mechanisms for microbial growth efficiency (TAKRITI et al., 2018) and improve strategies to nutrient uptake (HICKS et al., 2021). In addition, the low autotrophic abundance limits the bacterial contribution to carbon input, thus reinforcing the

necessity to use these available resources. These results indicate that the microbial community of CVO lake allocate functional processes to nutrient acquisition for cellular maintenance, reducing then the biomass yield (COTTA et al., 2022). The CVO lake showed a remarkable abundance of genes associated with sulfur cycle, including sulfate and sulfite reduction, and thiosulfate and sulfide oxidation. Although CVO lake showed a considerably lower salinity, the enrichment of sulfur's transformation was similar to those observed in soda lakes in Russia (VAVOURAKIS et al., 2019). In the wet season, these processes have been performed by lower bacterial diversity (PELLEGRINETTI et al., 2022). This lower bacterial diversity in the wet season was also observed in freshwater lakes. It is known that in freshwater lakes the nutrient availability shaped the microbial community, presenting reduced taxonomic diversity in wet seasons due to the dilution of nutrients (AGUILAR et al., 2018).

The function-specific taxonomy revealed a clearly different lake composition that also fluctuates among the seasons. However, these changes in the relative taxonomy abundance did not follow a similar trend among the evaluated lakes. The ET lake displayed a more diverse taxonomy affiliated with the biogeochemical functions compared to OT and CVO lake. This taxonomy was well distributed and slightly fluctuate between the seasons. This pattern suggests a high taxonomic resilience in face of disturbances, also indicating that multiple organisms performing distinct metabolic pathways resulted in functional stability (SHADE et al., 2012). In contrast, environmental changes negatively impacted the taxonomy composition of functional genes in OT and CVO lakes. This indicated that microbial communities in these lakes are more sensitive to environmental changes promoted by seasonality, presenting a lower resilience (SHADE et al., 2012). We could observe that microorganisms in OT and CVO lake was more affected by nutrient limitation than to salinity and pH stress. Actinobacteria and Alpha-Betaproteobacteria were the prevalent taxon associated to functional genes. These two classes are considered versatile taxa with an intensive ability to exploit a wide variety of carbon and nitrogen sources (KALTENPOTH, 2009; BHOWAL; CHAKRABORTY, 2011).

Considering the biogeochemical cycles in the soda lakes of Nhecolândia, we can suggest some patterns based on the functional gene abundances. According to the proportion of autotroph/heterotroph organisms, we may expect that a larger production of CO_2 may occur in OT and CVO lakes. Moreover, OT and CVO lakes exhibited a high relative abundance of the fermentative process of formate fermentation (producing CO_2). A study of soda lakes of Nhecolândia indicated that oligotrophic lakes (black water lakes) are CO_2 and N_2O sources, corroborating our work (BARBIERO et al., 2018). Barbiero et al (2018) also described that eutrophic lakes (green water lake) are CO_2 and N_2O sinks but CH_4 sources. The methanogenesis

genes were found in a low relative abundance only in OT and CVO lakes. However, this CH_4 could be produced in sediment zones, where methanogenic archaea could be prevalent (SOROKIN et al., 2015; DENG et al., 2017). The denitrification step of NO reduction to N_2O was low abundant in our study, indicating a low contribution of surface water to N_2O emissions. As observed for the methanogenesis process denitrification and N_2O production only occur in anoxic local conditions and probably would occur in the sediment of these lakes (ANDREOTE et al., 2018). It is interesting to notice that the abundance of these genes increased in stressful conditions observed in OT lake (2017) suggesting that the emission of N_2O could increase in an extremely dry season, as expected to occur in a climate change scenario.

4.5 Conclusion

In conclusion, we observed that nearby lakes (less than 1 km of distance) had distinct metabolism highlighting the metabolic diversity of this region. Moreover, seasonality has a substantial effect on the lake functional diversity. The taxonomic profiling of the functional genes revealed a diverse taxonomy in ET lake while a less diverse taxonomy in OT and CVO lakes was observed. Compared to OT and CVO lakes, ET lakes displayed a higher functional resilience under harsh conditions (high salinity, pH and low nutrient availability). In face to recent hydrology instability in Nhecolândia, the extreme droughts and rainfalls could result in drastic changes in microbial community and functional composition but also in the biogeochemical cycles. The dataset presented in this study may provide guidelines for management practices and conservation of the Pantanal's biodiversity.

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5. FINAL REMARKS

The results reported in this thesis achieve the goals proposed for the project. This doctoral research revealed the bacterial and functional composition of Nhecolândia soda lakes in contrasted seasons and environmental factors.

Using a combination of techniques, we were able to characterize the water chemistry as well as the functional and taxonomic composition of six distinct Nhecolândia. A robust dataset generated in this study allowed for the classification of Nhecolândia soda lakes based on their microbial composition and water chemistry. Seasonality was found to be an important factor in the dynamics of the bacterial community.

In addition to the first study, we were able to disentangle the lifestyle of bacterial communities and the strategies adopted by microorganisms to survive in these harsh conditions. A distinct lifestyle was discovered to differ depending on lake type and environmental hostility.

This doctoral thesis also investigated the biogeochemical cycles and its associated taxonomy in distinct soda lakes. A wide range of metabolic functions were discovered, and they were found to vary significantly between lakes that were only a short distance located. This metabolism and the way as microbes respond to environmental changes different among lake types and over the seasons.

APPENDICES

Appendix A: Supplementary material of chapter 2

Supplementary Materials includes: Supplementary Table: Table S1~S4 and Supplementary Figures: Fig S1~S6

Supplementary Table S1. Metagenomic sequence data information of each sample with sequence statistics

Lake	Replicate	Year	Condition	MG-RAST ID	Name	bp count	seq. count	Sample	Library
01SR	1	2018	Wet	mgm4838795.3	01SR_1	1,438,154,106	13,496,390	mgs862344	mgl862346
01SR	2	2018	Wet	mgm4838800.3	01SR_2	1,119,117,208	10,507,992	mgs862347	mgl862349
01SR	3	2018	Wet	mgm4838794.3	01SR_3	1,223,520,246	11,483,539	mgs862350	mgl862352
04SR	1	2018	Wet	mgm4838799.3	04SR_1	1,345,319,774	12,713,054	mgs862353	mgl862355
04SR	2	2018	Wet	mgm4838805.3	04SR_2	1,357,083,462	12,797,969	mgs862356	mgl862358
04SR	3	2018	Wet	mgm4838802.3	04SR_3	1,495,629,850	14,161,839	mgs862359	mgl862361
05SR	1	2018	Wet	mgm4838797.3	05SR_1	1,442,708,291	13,530,912	mgs862362	mgl862364
05SR	2	2018	Wet	mgm4838796.3	05SR_2	1,751,796,625	16,463,547	mgs862365	mgl862367
05SR	3	2018	Wet	mgm4838806.3	05SR_3	1,563,910,936	14,842,719	mgs862368	mgl862370
06SR	1	2018	Wet	mgm4838804.3	06SR_1	1,432,503,479	13,582,769	mgs862371	mgl862373
06SR	2	2018	Wet	mgm4838807.3	06SR_2	1,423,123,405	13,439,888	mgs862374	mgl862376
06SR	3	2018	Wet	mgm4838798.3	06SR_3	1,361,132,122	12,805,567	mgs862377	mgl862379
07SR	1	2018	Wet	mgm4838793.3	07SR_1	1,518,537,577	14,351,314	mgs862380	mgl862382
07SR	2	2018	Wet	mgm4838808.3	07SR_2	1,472,471,573	13,932,318	mgs862383	mgl862385
07SR	3	2018	Wet	mgm4838810.3	07SR_3	1,369,696,269	12,929,134	mgs862386	mgl862388
08SR	1	2018	Wet	mgm4838809.3	08SR_1	1,243,543,929	11,688,268	mgs862389	mgl862391
08SR	2	2018	Wet	mgm4838801.3	08SR_2	1,222,161,465	11,465,538	mgs862392	mgl862394
08SR	3	2018	Wet	mgm4838803.3	08SR_3	1,227,478,239	11,540,390	mgs862395	mgl862397
01SR	1	2019	Dry	mgm4875744.3	01SR-2_S104	1,873,166,936	18,124,853	mgs862260	mgl862262
01SR	2	2019	Dry	mgm4875754.3	01SR-3_S105	1,593,282,817	15,441,618	mgs862263	mgl862265
01SR	3	2019	Dry	mgm4875748.3	01SR-4_S106	1,832,510,569	17,719,328	mgs862266	mgl862268
04SR	1	2019	Dry	mgm4875743.3	04SR-5_S107	1,948,809,237	19,003,652	mgs862269	mgl862271
04SR	2	2019	Dry	mgm4875750.3	04SR-6_S108	2,318,515,061	22,569,810	mgs862272	mgl862274

04SR	3	2019	Dry	mgm4875752.3	04SR-8_S109	1,876,662,955	18,298,168	mgs862275	mgl862277
05SR	1	2019	Dry	mgm4875756.3	05SR-10_S111	1,983,805,401	19,177,046	mgs862278	mgl862280
05SR	2	2019	Dry	mgm4875753.3	05SR-12_S112	2,093,555,759	20,259,424	mgs862281	mgl862283
05SR	3	2019	Dry	mgm4875747.3	05SR-9_S110	2,388,611,076	23,154,899	mgs862284	mgl862286
06SR	1	2019	Dry	mgm4875751.3	06SR-13_S113	2,106,079,813	20,444,036	mgs862287	mgl862289
06SR	2	2019	Dry	mgm4875736.3	06SR-15_S114	2,438,756,825	23,850,354	mgs862290	mgl862292
06SR	3	2019	Dry	mgm4875742.3	06SR-16_S115	1,639,688,611	15,913,272	mgs862293	mgl862295
07SR	1	2019	Dry	mgm4875740.3	07SR-18_S116	1,736,602,590	16,848,446	mgs862296	mgl862298
07SR	2	2019	Dry	mgm4875746.3	07SR-19_S117	1,952,426,491	19,031,279	mgs862299	mgl862301
07SR	3	2019	Dry	mgm4875745.3	07SR-20_S118	2,175,750,060	21,204,102	mgs862302	mgl862304
08SR	1	2019	Dry	mgm4875749.3	08SR-22_S119	2,099,867,859	20,442,511	mgs862305	mgl862307
08SR	2	2019	Dry	mgm4875738.3	08SR-23_S120	2,235,100,505	21,819,562	mgs862308	mgl862310
08SR	3	2019	Dry	mgm4875741.3	08SR-24_S121	1,718,149,807	16,708,202	mgs862311	mgl862313

Supplementary Table S2. Synthesis of the physical and chemical variables of sampled lakes in, dry and wet periods

Variables	Units	01SR - OT		04SR – ET		05SR – ET		06SR – OT		07SR – CVO		08SR - ET		Mean	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
WT	°C	$25.5^{hi} \pm 0.14$	$33.26^{ab} \pm 0.32$	$26.12^{fgh} \pm 1.05$	$26.56^{ef} \pm 0.59$	$26.00^{ghi} \pm 0.77$	$35.31^{a} \pm 0.51$	$26.34^{fg} \pm 0.40$	$25.33^{i} \pm 0.13$	$27.11^{de} \pm 0.25$	$27.41^{cd} \pm 0.14$	$25.41^{i} \pm 0.17$	$27.87^{bc} \pm 0.42$	26.08^{b}	29.30^{a}
Secchi disc	(cm)	23.75 ± 3.50	4.00 ± 0.00	5.00 ± 0.00	4.00 ± 0.00	NA	12.00 ± 0.00	7.00 ± 0.82	7.00 ± 0	109.5 ± 7.37	80.00 ± 5.00	NA	5.00 ± 0.00	36.31	18.67
pH		$9.26^{g} \pm 0.10$	$9.67^{e} \pm 0.03$	$10.03^{b} \pm 0.06$	$10.26^{a} \pm 0.01$	$9.81^{d} \pm 0.01$	$9.97^{b} \pm 0.02$	$9.09^{h} \pm 0.01$	$9.45^{f} \pm 0.01$	$8.62^{i} \pm 0.00$	$9.05^{h} \pm 0.01$	$9.42^{f} \pm 0.05$	$9.91^{c} \pm 0.02$	9.37^{b}	9.72^{a}
DO	mg L^{-1}	5.67 ± 0.48	15.92 ± 0.16	11.71 ± 3.67	2.19 ± 4.39	0.00 ± 0.00	47.07 ± 1.06	6.30 ± 0.54	8.44 ± 2.94	6.60 ± 0.49	10.32 ± 1.31	0.00 ± 0.00	30.11 ± 4.05	5.04	19.01
EC	mS cm^{-1}	$1.12^{g} \pm 0.02$	$2.01^{f} \pm 0.01$	$1.73^{e} \pm 0.00$	$3.20^{a} \pm 0.06$	$1.01^{h} \pm 0.02$	$2.33^{c} \pm 0.05$	$0.68^{j} \pm 0.01$	$1.27^{f} \pm 0.03$	$0.56^{k} \pm 0.00$	$0.69^{i} \pm 0.00$	$1.00^{h} \pm 0.01$	$2.55^{b} \pm 0.02$	$1,014.96^{b}$	$2,011.21^{a}$
Salinity	g L^{-1}	$0.95^{DE} \pm 0.04$	$1.58^{BC} \pm 0.02$	$1.63^{B} \pm 0.18$	$2.42^{A} \pm 0.11$	$0.77^{EF} \pm 0.12$	$1.12^{CD} \pm 0.20$	$0.83^{EF} \pm 0.01$	$0.90^{DE} \pm 0.11$	$0.53^{FG} \pm 0.00$	$0.41^{G} \pm 0.06$	$0.97^{DE} \pm 0.02$	$1.62^{BC} \pm 0.11$	0.95^{B}	1.34^{A}
Alkalinity	mmol L^{-1}	$5.34^{EF} \pm 0.47$	$9.78^{C} \pm 0.15$	$8.94^{C} \pm 1.34$	$16.46^{A} \pm 1.19$	$6.08^{D} \pm 0.64$	$12.03^{B} \pm 0.54$	$3.72^{G} \pm 0.29$	$5.70^{DE} \pm 0.18$	$3.60^{G} \pm 0.47$	$3.45^{G} \pm 0.10$	$5.21^{F} \pm 0.29$	$11.73^{B} \pm 0.25$	5.48^{B}	9.86^{A}
DOC	mg L^{-1}	$31.23^{f} \pm 0.73$	$74.36^{d} \pm 8.89$	$79.02^{cd} \pm 2.96$	$251.90^{a} \pm 11.62$	$34.76^{f} \pm 1.82$	$103.18^{bc} \pm 16.62$	$12.08^{g} \pm 0.34$	$35.99^{ef} \pm 8.20$	$15.95^{g} \pm 1.12$	$33.96^{f} \pm 14.72$	$40.35^{e} \pm 0.86$	$138.42^{ab} \pm 15.68$	35.56^{b}	106.30^{a}
DIC		$107.27^{DE} \pm 0.66$	$164.74^{CD} \pm 38.10$	$171.01^{CD} \pm 1.81$	$406.62^{A} \pm 50.98$	$91.74^{EF} \pm 1.26$	$194.20^{BC} \pm 54.50$	$54.87^{G} \pm 0.94$	$96.03^{E} \pm 3.86$	$48.23^{H} \pm 1.62$	$83.41^{FG} \pm 2.70$	$91.83^{EF} \pm 0.40$	$296.76^{B} \pm 37.00$	94.16^{B}	206.96^{A}
TN		$6.01^{d} \pm 1.24$	$25.39^{c} \pm 9.26$	$17.49^{c} \pm 2.79$	$90.43^{a} \pm 4.90$	$5.09^{d} \pm 1.16$	$27.77^{c} \pm 10.83$	$1.99^{e} \pm 0.08$	$18.23^{c} \pm 4.90$	$2.22^{e} \pm 0.01$	$10.55^{d} \pm 13.74$	$5.94^{d} \pm 0.70$	$49.82^{ab} \pm 14.06$	6.46^{b}	37.04^{a}
TDN		$4.35^{cd} \pm 0.08$	$23.25^{a} \pm 9.30$	$10.94^{bc} \pm 0.98$	$22.64^{a} \pm 1.58$	$3.59^{de} \pm 0.08$	$18.32^{ab} \pm 9.72$	$1.01^{f} \pm 0.05$	$16.58^{ab} \pm 4.82$	$1.27^{ef} \pm 0.07$	$9.17^{cd} \pm 13.81$	$4.28^{cd} \pm 0.09$	$23.85^{ab} \pm 15.27$	4.24^{b}	18.97^{a}
TP		$0.18^{h} \pm 0.10$	$0.47^{f} \pm 0.05$	$3.35^{d} \pm 0.3$	$22.81^{a} \pm 1.88$	$1.28^{g} \pm 0.33$	$2.85^{d} \pm 0.48$	$1.28^{d} \pm 0.34$	$15.03^{b} \pm 1.22$	$0.07^{h} \pm 0.04$	$0.02^{i} \pm 0.00$	$0.43^{fg} \pm 0.05$	$6.28^{c} \pm 0.46$	0.96^{b}	7.91^{a}
NH_4^+		$0.35^{GH} \pm 0.11$	$0.040^{DF} \pm 0.00$	$0.69^{B} \pm 0.18$	$1.14^{A} \pm 0.03$	$0.09^{DE} \pm 0.034$	$0.26^{BC} \pm 0.10$	$0.04^{HI} \pm 0.00$	$0.04^{EFG} \pm 0.01$	$0.04^{J} \pm 0.01$	$0.02^{I} \pm 0.03$	$0.07^{FG} \pm 0.01$	$0.47^{CD} \pm 0.08$	0.21^{B}	0.33^{A}
NO_2^-		$0.20^{ab} \pm 0.05$	$0.04^{d} \pm 0.00$	$0.03^{de} \pm 0.03$	$0.06^{cd} \pm 0.03$	$0.04^{ef} \pm 0.08$	$0.00^{f} \pm 0.00$	$0.09^{bc} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.00^{f} \pm 0.00$	$0.00^{f} \pm 0.00$	$0.00^{f} \pm 0.00$	$0.04^{d} \pm 0.01$	0.06^{a}	0.06^{a}
NO_3^-		$0.24^{bc} \pm 0.01$	$0.11^{d} \pm 0.02$	$0.12^{d} \pm 0.02$	$0.32^{ab} \pm 0.13$	$0.19^{cd} \pm 0.15$	$0.04^{e} \pm 0.06$	$0.25^{bc} \pm 0.06$	$0.90^{a} \pm 0.07$	$0.01^{e} \pm 0.02$	$0.01^{e} \pm 0.015$	$0.03^{e} \pm 0.04$	$0.16^{d} \pm 0.04$	0.14^{b}	0.26^{a}
PO_4^{3-}		$0.18^{g} \pm 0.10$	$0.23^{g} \pm 0.07$	$2.38^{d} \pm 0.38$	$15.3^{a} \pm 0.00$	$0.06^{h} \pm 0.03$	$1.05^{e} \pm 0.17$	$1.28^{e} \pm 0.34$	$12.40^{b} \pm 0.90$	$0.07^{h} \pm 0.04$	$0.02^{i} \pm 0.01$	$0.43^{f} \pm 0.05$	$4.73^{c} \pm 0.54$	0.73^{b}	5.62^{a}
TN:TP		13.05 ± 10.65	54.15 ± 21.96	2.00 ± 0.94	3.98 ± 0.38	8.01 ± 10.96	10.22 ± 4.82	0.83 ± 0.32	1.22 ± 0.37	8.13 ± 7.25	497 ± 708.31	3.94 ± 1.81	8.00 ± 2.48	5.99	95.76
Chl-*a*	µg L^{-1}	$27.78^{h} \pm 0.35$	$112.78^{e} \pm 5.33$	$122.11^{d} \pm 6.15$	$4123^{a} \pm 361.49$	$26.63^{h} \pm 3.50$	$498.34^{c} \pm 32.76$	$9.83^{j} \pm 0.28$	$22.93^{i} \pm 0.83$	$5.89^{k} \pm 0.93$	$60.86^{f} \pm 3.30$	$36.98^{g} \pm 2.90$	$821^{b} \pm 193.30$	38.20^{b}	939.82^{a}
PPP		$3.28x10^{5\ EG}$	$6.04x10^{6\ B}$	$3.99x10^{6\ BC}$	$1.49x10^{6\ A}$	$2.86x10^{5\ FG}$	$1.17x10^{6\ CD}$	$6.13x10^{3\ I}$	$4.26x10^{5\ DEF}$	$1.03x10^{5\ H}$	$2.70x10^{4\ GH}$	$5.05x10^{5\ CE}$	$5.91x10^{6\ B}$	$8.53x10\text{^}5^{B}$	$2.71x10^{7\ A}$
HP		$2.02x10^{7\ CE}$	$1.60x10^{7\ DE}$	$9.04x10^{7\ B}$	$4.91x10^{8\ A}$	$1.00x10^{6\ FG}$	$4.98x10^{7\ BCD}$	$6.97x10^{3\ H}$	$8.92x105^{\ FG}$	$2.33x10^{6\ EF}$	$5.19x10^{5\ G}$	$1.21x10^{7\ E}$	$6.04x10^{7\ BC}$	$2.10x10\text{^}7^{B}$	$1.03x10^{8\ A}$

The average data was presented with standard deviation (values after ±). Superscript letters indicate statistically significant differences between samples. The lowercase was the parametric test and capital letters non-parametric test of variance. WT: Water temperature; DO = Dissolved oxygen; EC = electric conductivity; Alkalinity: as (HCO_3^- + CO_3^-); DOC: Dissolved organic carbon; DIC: Dissolved inorganic carbon; TN: Total nitrogen; TDN: Total dissolved nitrogen; TP: Total phosphorus; Chl-*a*: Chlorophyll-*a*; PPP: Phototrophic Picoplankton; HP: Heterotrophic Prokaryotes; N.A.: Not available data.

Supplementary Table S3. Synthesis of major ions and trace elements dissolved in water of sampled lakes in dry and wet periods

Variables	Units	01SR - OT		04SR – ET		05SR – ET		06SR – OT		07SR – CVO		08SR - ET		Mean	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Cl^-	mg L^{-1}	$55.59^{bc} \pm 3.54$	$115.40^{a} \pm 5.07$	$40.00^{cd} \pm 8.03$	$79.30^{ab} \pm 3.49$	$28.51^{ef} \pm 18.31$	$31.95^{de} \pm 7.75$	$20.23^{f} \pm 0.94$	$26.54^{ef} \pm 6.23$	$7.00^{g} \pm 0.38$	$11.83^{g} \pm 0.84$	$26.67^{ef} \pm 11.23$	$57.31^{bc} \pm 8.73$	29,67^{b}	53,72^{a}
SO_4^{2-}		$6.33^{e} \pm 0.46$	$16.23^{cd} \pm 2.32$	$0.00^{g} \pm 0.00$	$5.92^{e} \pm 1.95$	$5.39^{e} \pm 3.26$	$2.70^{f} \pm 1.48$	$22.73^{bc} \pm 3.37$	$35.34^{a} \pm 8.20$	$0.47^{g} \pm 0.94$	$0.00^{h} \pm 0.00$	$12.21^{d} \pm 7.07$	$31.50^{a} \pm 14.51$	7,86^{b}	15,28^{a}
Na^+		$347.83^{d} \pm 8.84$	$762.35^{b} \pm 16.43$	$563.95^{c} \pm 134.13$	$1085.62^{a} \pm 47.68$	$344.92^{d} \pm 44.25$	$605.91^{bc} \pm 131.95$	$222.95^{e} \pm 15.03$	$350.98^{d} \pm 72.93$	$84.17^{f} \pm 11.97$	$173.81^{f} \pm 4.67$	$232.49^{e} \pm 22.07$	$675.31^{bc} \pm 96.66$	299,38^{b}	609,00^{a}
K^+		$38.98^{F} \pm 5.09$	$84.83^{CD} \pm 19.47$	$150.56^{B} \pm 39.46$	$335.09^{A} \pm 16.99$	$68.89^{DE} \pm 17.54$	$143.64^{B} \pm 35.37$	$49.13^{EF} \pm 11.18$	$77.34^{CD} \pm 6.19$	$46.71^{EF} \pm 5.00$	$95.33^{BD} \pm 8.78$	$48.28^{EF} \pm 10.05$	$115.15^{BC} \pm 23.51$	67,09^{B}	141,90^{A}
Mg^{2+}		$11.13^{d} \pm 4.68$	$8.59^{de} \pm 8.04$	$24.07^{a} \pm 1.39$	$22.67^{ab} \pm 7.31$	$11.25^{d} \pm 4.76$	$12.39^{cd} \pm 11.30$	$14.81^{bcd} \pm 15.02$	$9.01^{de} \pm 6.48$	$20.65^{abc} \pm 3.22$	$53.10^{a} \pm 6.33$	$9.27^{de} \pm 5.39$	$1.27^{e} \pm 1.56$	15,20^{b}	17,84^{a}
Ca^{2+}		$57.97^{abc} \pm 7.96$	$43.07^{def} \pm 11.44$	$66.92^{ab} \pm 7.07$	$58.14^{abcd} \pm 17.63$	$68.58^{abc} \pm 19.08$	$38.86^{ef} \pm 10.70$	$78.51^{abc} \pm 39.69$	$50.17^{cde} \pm 16.57$	$41.46^{def} \pm 6.83$	$67.95^{a} \pm 9.34$	$53.32^{bcde} \pm 9.18$	$8.60^{f} \pm 0.60$	61,13^{a}	44,47^{ba}
Al	µg L^{-1}	$2.31^{d} \pm 0.06$	$4.33^{c} \pm 0.59$	$0.01^{hi} \pm 0.01$	$0.04^{f} \pm 0.01$	$0.55^{e} \pm 0.03$	$0.04^{f} \pm 0.01$	$9.40^{b} \pm 0.14$	$17.02^{a} \pm 0.70$	$0.01^{i} \pm 0.00$	$0.01^{h} \pm 0.00$	$0.05^{f} \pm 0.01$	$0.02^{g} \pm 0.00$	2,06^{a}	3,58^{b}
B		$273.91^{f} \pm 3.16$	$380.36^{d} \pm 13.80$	$510.00^{c} \pm 11.11$	$901.65^{a} \pm 24.39$	$127.70^{i} \pm 2.85$	$211.50^{g} \pm 19.01$	$97.38^{k} \pm 1.99$	$145.58^{h} \pm 1.49$	$94.86^{l} \pm 0.01$	$120.00^{j} \pm 0.17$	$293.28^{e} \pm 0.29$	$530.11^{b} \pm 3.21$	0,23^{a}	0,38^{b}
Cu		$0.73^{c} \pm 0.16$	$4.01^{b} \pm 0.77$	$0.15^{d} \pm 0.10$	$4.57^{b} \pm 2.33$	$0.27^{d} \pm 0.11$	$1.62^{c} \pm 0.80$	$2.97^{b} \pm 0.24$	$11.23^{a} \pm 0.79$	$0.95^{c} \pm 0.08$	$0.87^{c} \pm 0.54$	$0.37^{d} \pm 0.27$	$3.83^{b} \pm 2.25$	0,00^{a}	0,00^{b}
Fe	mg L^{-1}	$8.11^{d} \pm 0.21$	$12.39^{c} \pm 1.52$	$0.14^{h} \pm 0.01$	$0.17^{g} \pm 0.02$	$2.12^{d} \pm 0.11$	$0.10^{e} \pm 0.02$	$41.64^{b} \pm 0.60$	$77.72^{a} \pm 4.50$	$0.07^{j} \pm 0.01$	$0.03^{k} \pm 0.00$	$0.27^{f} \pm 0.02$	$0.08^{i} \pm 0.03$	8,72^{b}	15,08^{a}
Mn		$0.41^{d} \pm 0.01$	$0.73^{c} \pm 0.09$	$0.01^{i} \pm 0.00$	$0.02^{h} \pm 0.00$	$0.10^{e} \pm 0.00$	$0.01^{ij} \pm 0.00$	$1.60^{b} \pm 0.03$	$2.74^{a} \pm 0.10$	$0.07^{f} \pm 0.00$	$0.03^{h} \pm 0.00$	$0.03^{g} \pm 0.00$	$0.01^{j} \pm 0.00$	0,37^{a}	0,59^{a}
Ni	µg L^{-1}	$8.34^{d} \pm 0.24$	$9.97^{c} \pm 0.95$	$4.66^{f} \pm 0.17$	$8.10^{d} \pm 0.36$	$5.12^{ef} \pm 0.10$	$5.93^{e} \pm 1.10$	$20.16^{b} \pm 0.28$	$29.47^{a} \pm 0.83$	$2.61^{h} \pm 0.05$	$2.97^{h} \pm 0.14$	$3.90^{g} \pm 0.11$	$4.68^{f} \pm 0.55$	0,01^{b}	0,01^{a}
Si	mg L^{-1}	$48.75^{cde} \pm 0.68$	$106.74^{ab} \pm 60.42$	$35.53^{efg} \pm 0.79$	$122.21^{a} \pm 4.72$	$39.06^{def} \pm 0.98$	$117.92^{a} \pm 18.12$	$91.01^{abc} \pm 2.16$	$274.28^{a} \pm 156.70$	$27.51^{g} \pm 0.20$	$63.08^{bcd} \pm 0.79$	$31.99^{fg} \pm 0.45$	$91.70^{abc} \pm 12.03$	45,64^{b}	129,33^{a}
Zn	µg L^{-1}	$10.41^{bc} \pm 4.51$	$18.10^{ab} \pm 3.19$	$6.43^{c} \pm 3.78$	$12.24^{bc} \pm 2.02$	$29.32^{ab} \pm 21.95$	$5.90^{cd} \pm 4.63$	$60.32^{a} \pm 57.53$	$37.77^{a} \pm 0.93$	$11.74^{bc} \pm 11.06$	$0.95^{d} \pm 0.37$	$8.22^{c} \pm 8.86$	$7.14^{c} \pm 4.10$	0,02^{a}	0,01^{b}

The average data was presented with standard deviation (values after ±). Superscript letters indicate statistically significant differences between samples, which lowercase was the parametric test and capital letters non-parametric test of variance.

Supplementary Table S4. Flux cytometric population abundance and inferred cytometric diversity.

Lakes	**01SR - OT**		**SR - ET**		**05SR – ET**		**06SR – OT**		**07SR - CVO**		**08SR - ET**	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
PPP	327514	6041310	3987042	1.49E+08	285657	1171940	61289	426048	10318	26990	499646	5909383
HP	20214149	15919750	90263249	4.91E+08	1005165	49764000	6970	892375	2329909	518375	12071674	60390000
PcyPC	102907	5677412	910287	1.47E+08	112847	927465	1313	411620	3975	20409	101843	195250
Peuk	142525	155723	8250	3667	61439	2548	2930	11697	2836	4558	13409	41067
Perec	17576	110	1686868	227333	10336	17563	93	18	97	194	66192	2494800
PcyPE_2	150	18	37	1833	0	0	5	0	1276	282	5	183
PcyPE_1	64297	208028	1381545	1881000	100331	224363	1489	2713.33	2027.67	1483	222414	3173867
Neuk	59	18	55	0	704	0	299	0	106	64	95781	4217
AlphaHP	5.85	5.87	5.75	5.61	5.81	5.81	4.54	4.52	4.42	5.87	5.69	5.82
PielouHP	0.88	0.92	0.87	0.88	0.90	0.90	0.99	0.98	0.86	0.99	0.87	0.95
AlphaPhyto	8.95	7.89	8.12	6.85	8.44	7.51	7.27	7.25	7.15	7.73	8.77	7.78
PielouPhyto	0.94	0.87	0.87	0.88	0.94	0.90	0.99	0.94	0.99	0.97	0.91	0.95

Figure S1. Monthly accumulated rainfall and land surface temperature registration in Nhecolândia during studied period between 2017 and 2019. The blue triangle represents the first sampling, while the pink circle represents the second.

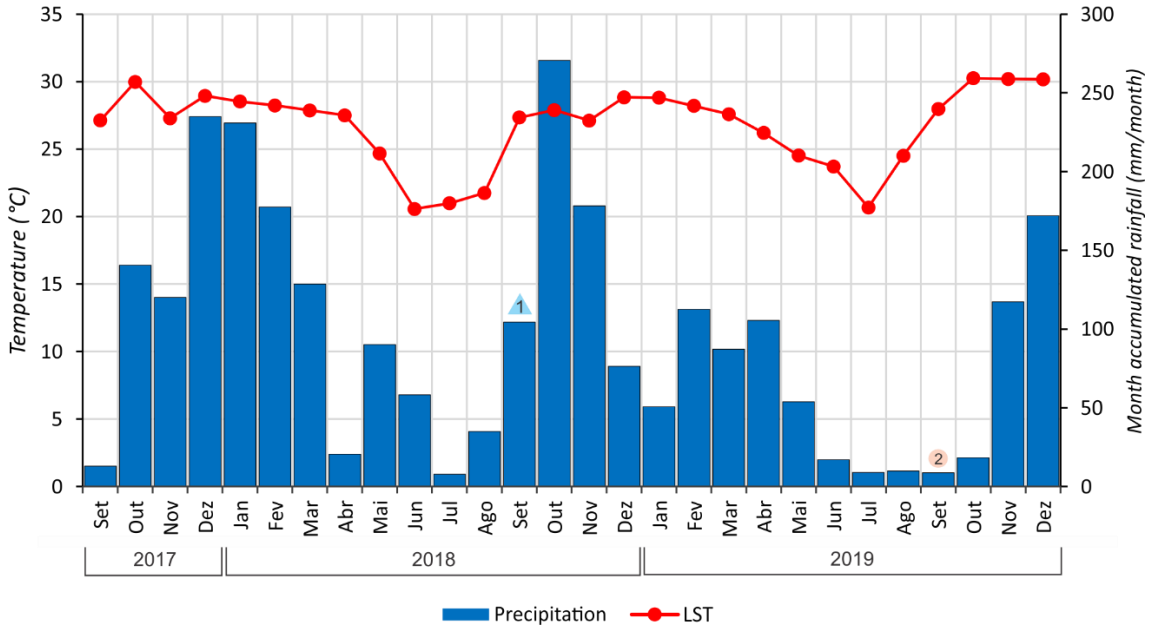


Figure S2. Chao1 and Shannon diversity indices of lake types (ET, OT and CVO) and seasonal conditions (dry and wet) (A). Chao1 and Shannon diversity indices for separated samples under dry and wet conditions.

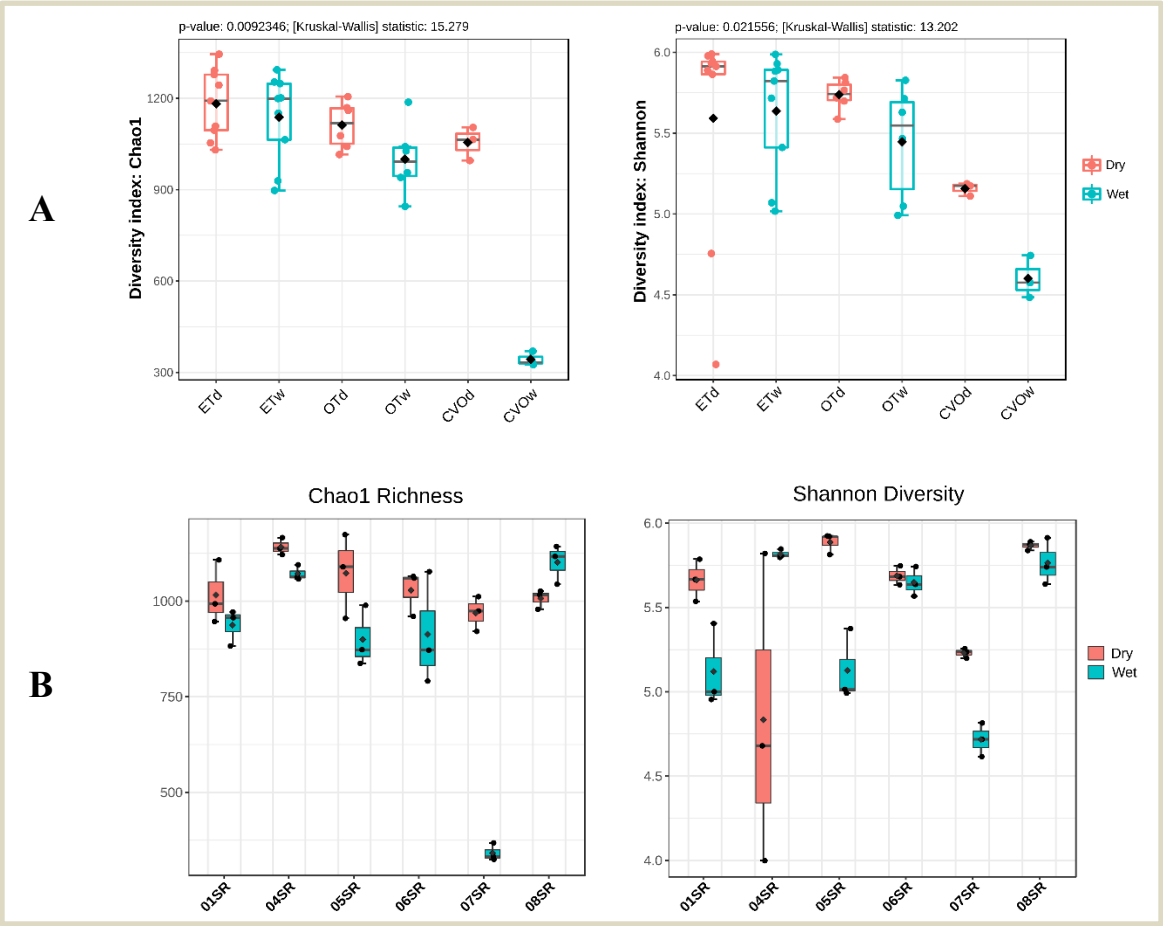


Figure S3. Bar plot of bacterial relative abundance at the class level for each lake under dry and wet condition.

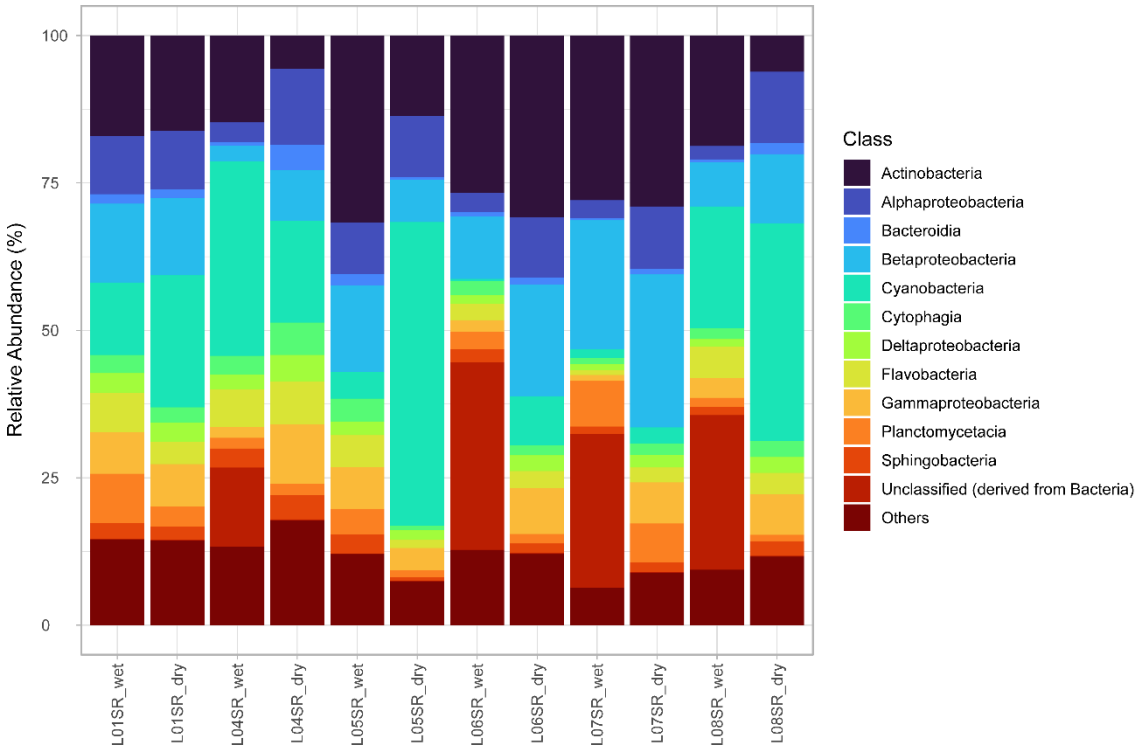


Figure S4. Heatmap of functional genes at level 1 of the SEED subsystem in each lake under dry and wet conditions.

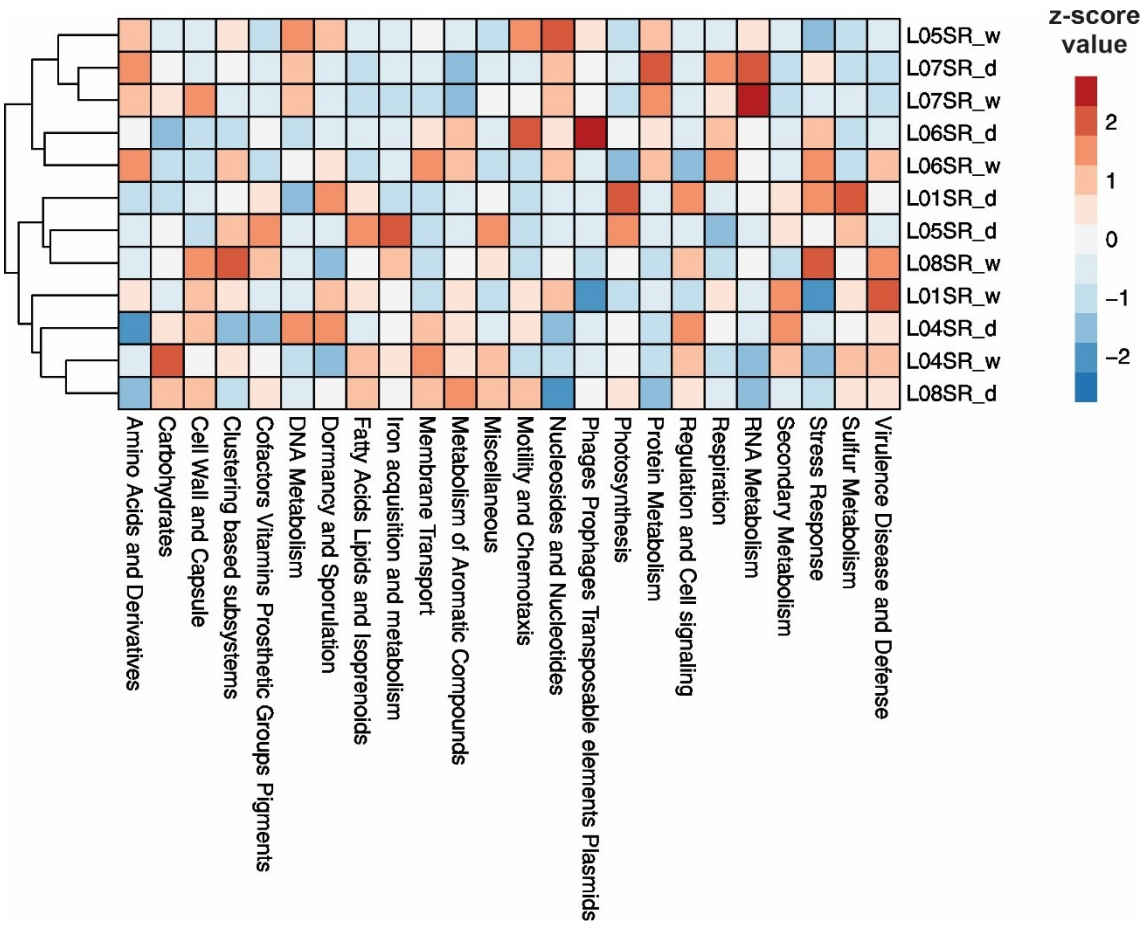


Figure S5. Principal Component Analysis of the SEED level 1 and soda lakes samples sites.

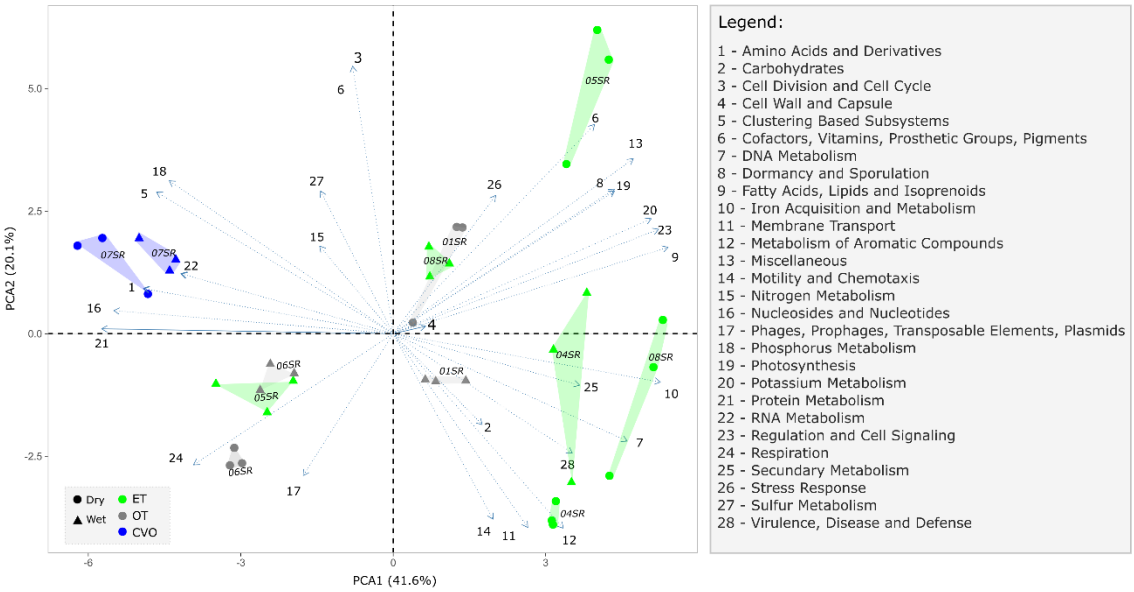
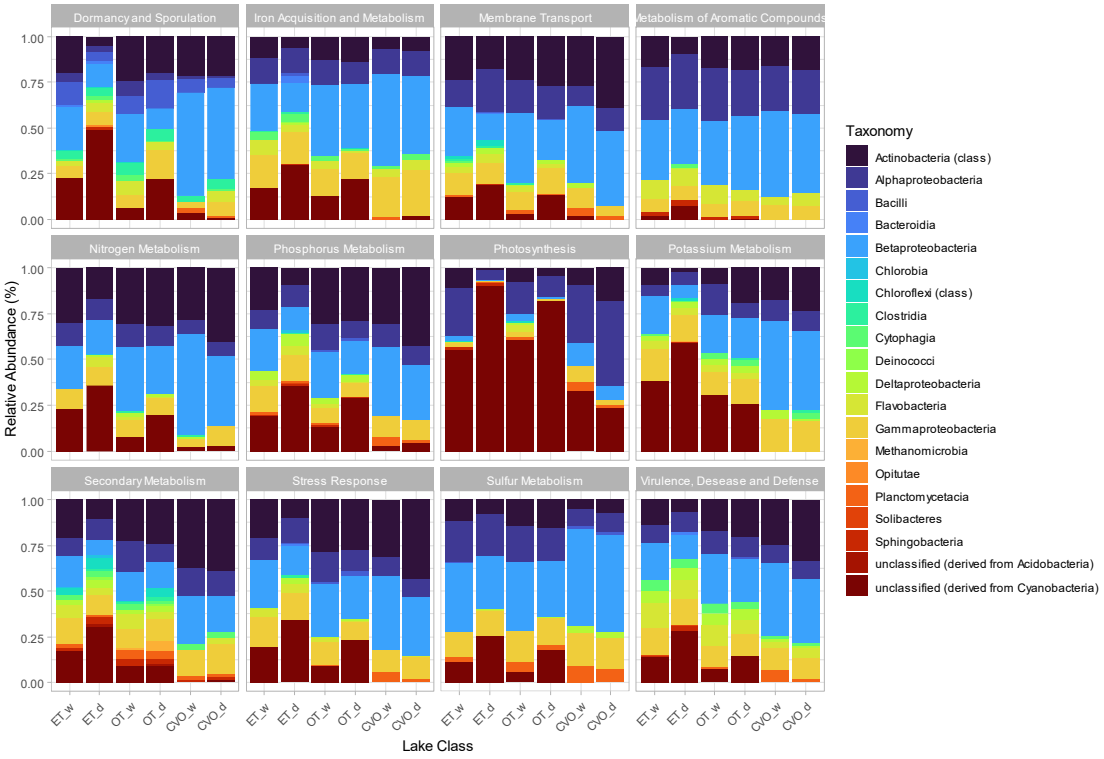


Figure S6. Taxonomy of relevant selected functions of the SEED level 1 of each lake types of lakes under dry and wet conditions.



Appendix B: Supplementary material of chapter 3

Supplementary Materials includes: Supplementary Table: Table S1~S2 and Supplementary Figures: Figs. S1~S5

Supplementary Table 1: Nutrient content of shallow alkaline lakes

	04SR_D	06SR_D	07SR_D	08SR_D	04SR_W	06SR_W	07SR_W	08SR_W
K (mg L-1)	$1{,}739 \pm 99^{Aa}$	959.4 ± 161^{Ab}	188.4 ± 15^{Ac}	$1{,}680 \pm 75^{Aa}$	157.75 ± 45^{Ba}	50.69 ± 13^{Bb}	46.2 ± 5.99^{Bb}	45.55 ± 10^{Bb}
Al (mg L-1)	0.31 ± 0.04^{Aab}	1.16 ± 0.81^{Aa}	0.009 ± 0.002^{Ab}	0.15 ± 0.01^{Aab}	0.009 ± 0.003^{Bb}	9.38 ± 0.16^{Ba}	0.007 ± 0.004^{Bb}	0.04 ± 0.007^{Bb}
B (mg L-1)	6.03 ± 0.09^{Ab}	2.02 ± 0.20^{Ac}	0.29 ± 0.01^{Ad}	10.87 ± 0.21^{Aa}	0.51 ± 0.007^{Ba}	0.09 ± 0.002^{Bc}	0.094 ± 0.002^{Bc}	0.29 ± 0.003^{Bb}
Cr (mg L-1)	0.01 ± 0.0003^{Aa}	0.01 ± 0.0009^{Ab}	0.011 ± 0.0004^{Ab}	0.013 ± 0.001^{Ab}	0.001 ± 0.00001^{B}	0.006 ± 0^{B}	0.001 ± 0^{B}	0.001 ± 0^{B}
Cu (mg L-1)	0.04 ± 0.008^{Ab}	0.088 ± 0.01^{Aa}	0.01 ± 0.0008^{Ac}	0.04 ± 0.004^{Ab}	0.0001 ± 0^{Bc}	0.002 ± 0^{Ba}	0.009 ± 0^{Bb}	0.0002 ± 0.0001^{Bc}
Fe (mg L-1)	0.27 ± 0.03^{Aab}	0.93 ± 0.58^{Aa}	0.06 ± 0.001^{Ab}	0.17 ± 0.01^{Ab}	0.14 ± 0.01^{Bb}	41.66 ± 0.72^{Ba}	0.07 ± 0.009^{Bb}	0.27 ± 0.021^{Bb}
Mg (mg L-1)	138.2 ± 55^{Bc}	192.7 ± 160^{Bd}	43.52 ± 6^{Ba}	203.1 ± 95^{Bb}	23.65 ± 1.36^{A}	18.95 ± 15^{A}	20.22 ± 3.80^{A}	9.47 ± 6.5^{A}
Mn (mg L-1)	0.12 ± 0.009^{Ab}	0.22 ± 0.08^{Ab}	0.87 ± 0.07^{Aa}	0.21 ± 0.003^{Ab}	0.009 ± 0.0004^{Bd}	1.60 ± 0.04^{Ba}	0.07 ± 0.006^{Bb}	0.03 ± 0.003^{Bc}
Ni (mg L-1)	0.09 ± 0.001^{Aa}	0.06 ± 0.008^{Ac}	0.02 ± 0.001^{Ad}	0.08 ± 0.002^{Ab}	0.004 ± 0.0002^{Bb}	0.02 ± 0.0003^{Ba}	0.002 ± 0^{Bd}	0.003 ± 0^{Bc}
Si (mg L-1)	207.3 ± 10^{Aa}	100.2 ± 7^{Ac}	64.84 ± 4^{Ad}	173.42 ± 6^{Ab}	35.82 ± 0.64^{Bb}	90.79 ± 2.59^{Ba}	27.46 ± 0.21^{Bd}	31.97 ± 0.54^{Bc}
Zn (mg L-1)	0.07 ± 0.01^{Aa}	0.03 ± 0.002^{Ab}	0.015 ± 0.006^{Ab}	0.06 ± 0.007^{Aa}	0.004 ± 0.001^{Bb}	0.068 ± 0.067^{Ba}	0.013 ± 0.012^{Bab}	0.01 ± 0.009^{Bb}

The letter D correspond to the dry season while the letter W correspond to the wet season. The uppercase letters compare the seasons (dry and wet), while lowercase letters compare the lakes ($P < 0.05$).

Supplementary Table 2: The relative abundance of bacterial functions used in trait-based framework

Functions (level_2)	Strategies	**04SR_W**	**06SR_W**	**07SR_W**	**08SR_W**
ABC transporters	A	0,96112%	1,07783%	0,89794%	0,96231%
Chemotaxis, response regulators	A	0,00756%	0,00270%	0,00275%	0,00243%
Flagella protein?	A	0,00763%	0,00598%	0,00372%	0,00499%
Flagellar motility in Prokaryota	A	0,43384%	0,43459%	0,47299%	0,46463%
Glycoside hydrolases	A	0,05197%	0,01715%	0,01844%	0,03559%
Protein degradation	A	1,49750%	1,54603%	1,48674%	1,48713%
Protein secretion system, Type I	A	0,02826%	0,02027%	0,01466%	0,01815%
Protein secretion system, Type II	A	0,25779%	0,22238%	0,14678%	0,18302%
Protein secretion system, Type VI	A	0,12443%	0,10431%	0,09385%	0,10053%
Protein secretion system, Type VII (Chaperone/Usher pathway, CU)	A	0,00272%	0,00064%	0,00022%	0,00007%
Protein secretion system, Type VIII (Extracellular nucleation/precipitation pathway, ENP)	A	0,02028%	0,01963%	0,01304%	0,01479%
Protein translocation across cytoplasmic membrane	A	0,56705%	0,69843%	0,80048%	0,64979%
Selenoproteins	A	0,20651%	0,18314%	0,21250%	0,19839%
Siderophores	A	0,02959%	0,02234%	0,01607%	0,03144%
Sugar Phosphotransferase Systems, PTS	A	0,02754%	0,03709%	0,07216%	0,04032%
Social motility and nonflagellar swimming in bacteria	A	0,00130%	0,00170%	0,00060%	0,00039%
Sulfatases and sulfatase modifying factor 1 (and a hypothetical)	A	0,06045%	0,06443%	0,02805%	0,05416%

Two related proteases	A	0,08744%	0,09291%	0,07671%	0,07900%
Uni- Sym- and Antiporters	A	0,27323%	0,14784%	0,04179%	0,17868%
proteosome related	A	0,12110%	0,16224%	0,09667%	0,14615%
Alanine, serine, and glycine	Y	0,77354%	0,82245%	0,76680%	0,76311%
Aminosugars	Y	0,12016%	0,09153%	0,08394%	0,09632%
Arginine; urea cycle, polyamines	Y	1,64515%	1,62220%	1,55899%	1,64002%
Aromatic amino acids and derivatives	Y	1,19506%	1,35399%	1,40759%	1,30283%
Branched-chain amino acids	Y	1,84284%	2,18258%	1,92727%	1,90348%
CO2 fixation	Y	0,99864%	0,79580%	0,81217%	0,97911%
Carbohydrates	Y	0,14117%	0,08830%	0,07230%	0,10156%
Central carbohydrate metabolism	Y	4,68262%	4,66847%	4,81967%	4,56826%
Di- and oligosaccharides	Y	0,79279%	0,50201%	0,41891%	0,52569%
Fatty acids	Y	0,99648%	0,99746%	0,87356%	0,94028%
Fermentation	Y	0,79497%	0,73299%	0,75746%	0,78237%
Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	Y	0,88286%	0,99474%	1,00091%	0,90916%
Lysine Biosynthesis	Y	0,00512%	0,01267%	0,01600%	0,00989%
Monosaccharides	Y	0,88125%	0,87562%	0,93990%	0,77586%
One-carbon Metabolism	Y	1,22250%	1,41392%	1,18962%	1,27644%
Organic acids	Y	0,63920%	0,72346%	0,84469%	0,64958%
Phospholipids	Y	0,35651%	0,33456%	0,34538%	0,34989%
Proline and 4-hydroxyproline	Y	0,15546%	0,13162%	0,13526%	0,13668%
Protein biosynthesis	Y	6,56495%	7,73691%	7,98019%	7,03897%
Purines	Y	1,87679%	2,07413%	2,05920%	1,96454%
Pyrimidines	Y	1,02893%	1,07342%	1,07920%	1,07258%

Sugar alcohols	Y	0,37328%	0,42103%	0,34419%	0,31201%
Capsular and extracellular polysacchrides	S	1,45248%	1,25259%	1,37154%	1,35963%
Cell wall of Mycobacteria	S	0,13308%	0,12587%	0,11714%	0,12261%
DNA recombination	S	0,09583%	0,11606%	0,13084%	0,11293%
DNA repair	S	2,55365%	2,66030%	2,60237%	2,55469%
Gram-Negative cell wall components	S	1,03300%	1,16187%	1,41878%	1,32808%
Gram-Positive cell wall components	S	0,07845%	0,06231%	0,05418%	0,06635%
Osmotic stress	S	0,14110%	0,16784%	0,12469%	0,15239%
Oxidative stress	S	0,93982%	0,90574%	0,77174%	0,94541%
Periplasmic Stress	S	0,04570%	0,05827%	0,05924%	0,05156%
Spore DNA protection	S	0,00002%	0,00015%	0,00008%	0,00007%

Complementation of Supplementary Table 02

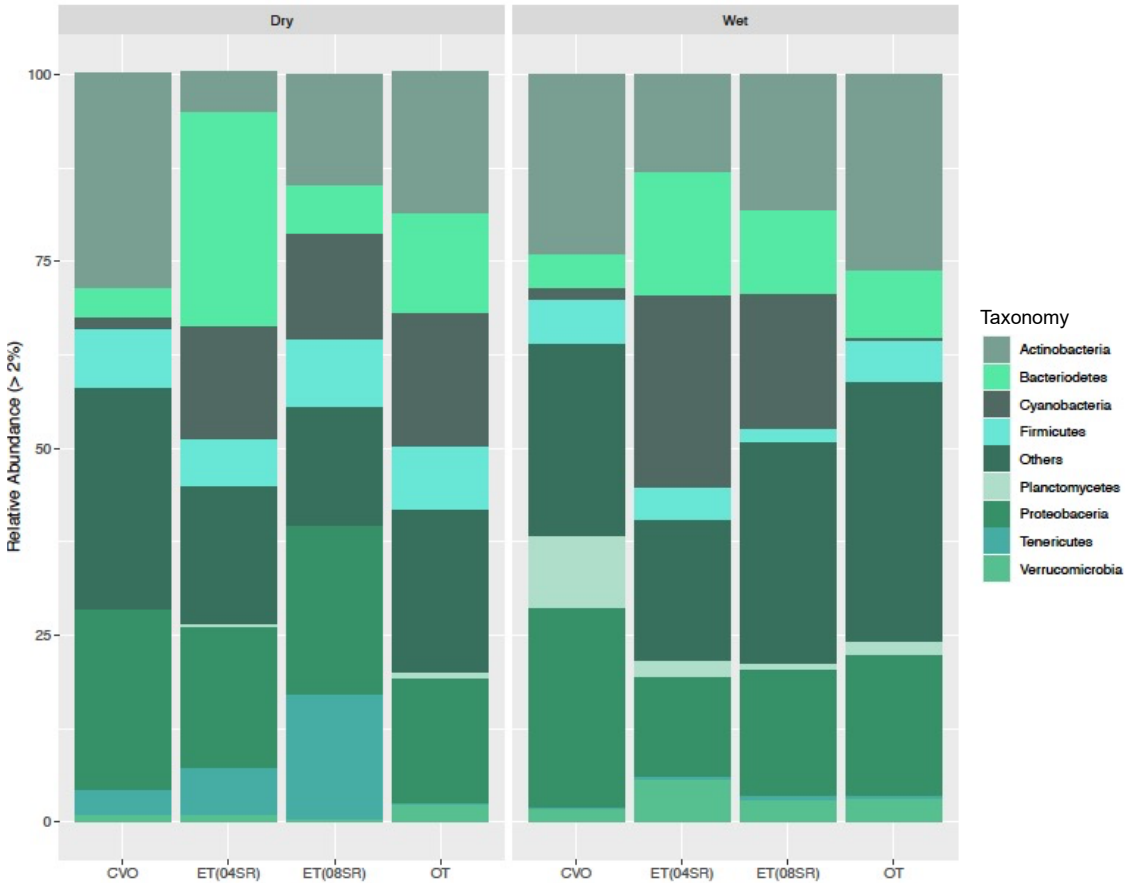
Functions (level_2)	Strategies	**04SR_D**	**06SR_D**	**07SR_D**	**08SR_D**
ABC transporters	A	0,85403%	0,98740%	1,13964%	1,05031%
Chemotaxis, response regulators	A	0,01610%	0,00543%	0,00400%	0,01680%
Flagella protein?	A	0,01134%	0,00834%	0,00352%	0,01024%
Flagellar motility in Prokaryota	A	0,48978%	0,51010%	0,42647%	0,86871%
Glycoside hydrolases	A	0,05557%	0,02838%	0,01709%	0,04120%
Protein degradation	A	1,51058%	1,60484%	1,49557%	1,39779%
Protein secretion system, Type I	A	0,01654%	0,01855%	0,01571%	0,02826%
Protein secretion system, Type II	A	0,21799%	0,32864%	0,15559%	0,18557%
Protein secretion system, Type VI	A	0,09505%	0,13435%	0,09674%	0,07521%

Protein secretion system, Type VII (Chaperone/Usher pathway, CU)	A	0,00052%	0,00033%	0,00047%	0,00142%
Protein secretion system, Type VIII (Extracellular nucleation/precipitation pathway, ENP)	A	0,03129%	0,02278%	0,02262%	0,01901%
Protein translocation across cytoplasmic membrane	A	0,57434%	0,63160%	0,75811%	0,55188%
Selenoproteins	A	0,23813%	0,21945%	0,20882%	0,21127%
Siderophores	A	0,02815%	0,02437%	0,02690%	0,02100%
Sugar Phosphotransferase Systems, PTS	A	0,03556%	0,03443%	0,07076%	0,04847%
Social motility and nonflagellar swimming in bacteria	A	0,00116%	0,00094%	0,00061%	0,00122%
Sulfatases and sulfatase modifying factor 1 (and a hypothetical)	A	0,03110%	0,05767%	0,03077%	0,01336%
Two related proteases	A	0,08909%	0,09285%	0,12534%	0,12579%
Uni- Sym- and Antiporters	A	0,32184%	0,27457%	0,09686%	0,34468%
proteosome related	A	0,07984%	0,16878%	0,07633%	0,06475%
Alanine, serine, and glycine	Y	0,78665%	0,81430%	0,78760%	0,69098%
Aminosugars	Y	0,16188%	0,09347%	0,08069%	0,17617%
Arginine; urea cycle, polyamines	Y	1,65692%	1,60326%	1,53643%	1,96168%
Aromatic amino acids and derivatives	Y	1,20983%	1,30463%	1,29586%	1,22505%
Branched-chain amino acids	Y	1,77924%	2,00840%	1,56767%	1,30903%
CO2 fixation	Y	0,93271%	0,91472%	0,83122%	0,99754%
Carbohydrates	Y	0,15265%	0,09882%	0,10194%	0,15965%
Central carbohydrate metabolism	Y	4,39897%	4,74127%	4,68432%	4,01332%
Di- and oligosaccharides	Y	0,97609%	0,76662%	0,69008%	0,91631%
Fatty acids	Y	1,00877%	1,02947%	0,92117%	0,87048%

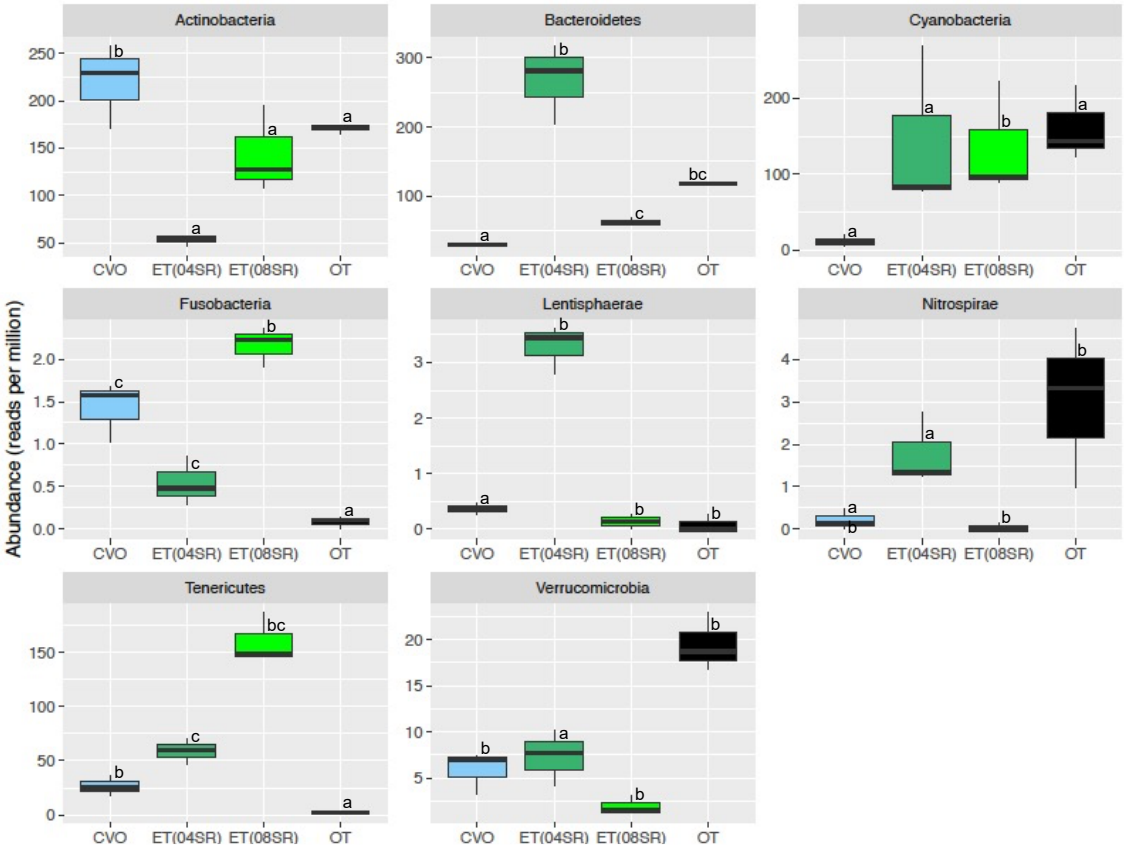
Fermentation	Y	0,80113%	0,77138%	0,71230%	0,72539%
Glutamine, glutamate, aspartate, asparagine; ammonia assimilation	Y	0,88620%	0,86412%	0,89040%	0,78785%
Lysine Biosynthesis	Y	0,00614%	0,00771%	0,02186%	0,00828%
Monosaccharides	Y	0,73403%	0,82917%	0,77543%	0,77543%
One-carbon Metabolism	Y	1,14219%	1,26617%	1,03815%	0,85448%
Organic acids	Y	0,61931%	0,64390%	0,59017%	0,56268%
Phospholipids	Y	0,33825%	0,38604%	0,38177%	0,35039%
Proline and 4-hydroxyproline	Y	0,13044%	0,20999%	0,09096%	0,11483%
Protein biosynthesis	Y	6,62489%	7,02775%	8,34375%	6,88505%
Purines	Y	1,82383%	1,97983%	1,98987%	1,71585%
Pyrimidines	Y	1,01186%	1,02363%	1,09710%	1,00425%
Sugar alcohols	Y	0,35666%	0,60021%	0,33133%	0,52924%
Capsular and extracellular polysacchrides	S	1,52941%	1,21912%	1,35995%	1,20095%
Cell wall of Mycobacteria	S	0,12200%	0,13321%	0,12123%	0,09034%
DNA recombination	S	0,10114%	0,10166%	0,12912%	0,10941%
DNA repair	S	2,65976%	2,65847%	2,82956%	2,67760%
Gram-Negative cell wall components	S	0,98011%	0,98003%	1,36749%	0,98178%
Gram-Positive cell wall components	S	0,08524%	0,08127%	0,05908%	0,08005%
Osmotic stress	S	0,19193%	0,18386%	0,10109%	0,21633%
Oxidative stress	S	0,95621%	0,92672%	0,79168%	0,91717%
Periplasmic Stress	S	0,04523%	0,05915%	0,05756%	0,05301%
Spore DNA protection	S	0,00067%	0,00038%	0,00128%	0,00219%

All the significant functions used in the trait-based framework were represented here.

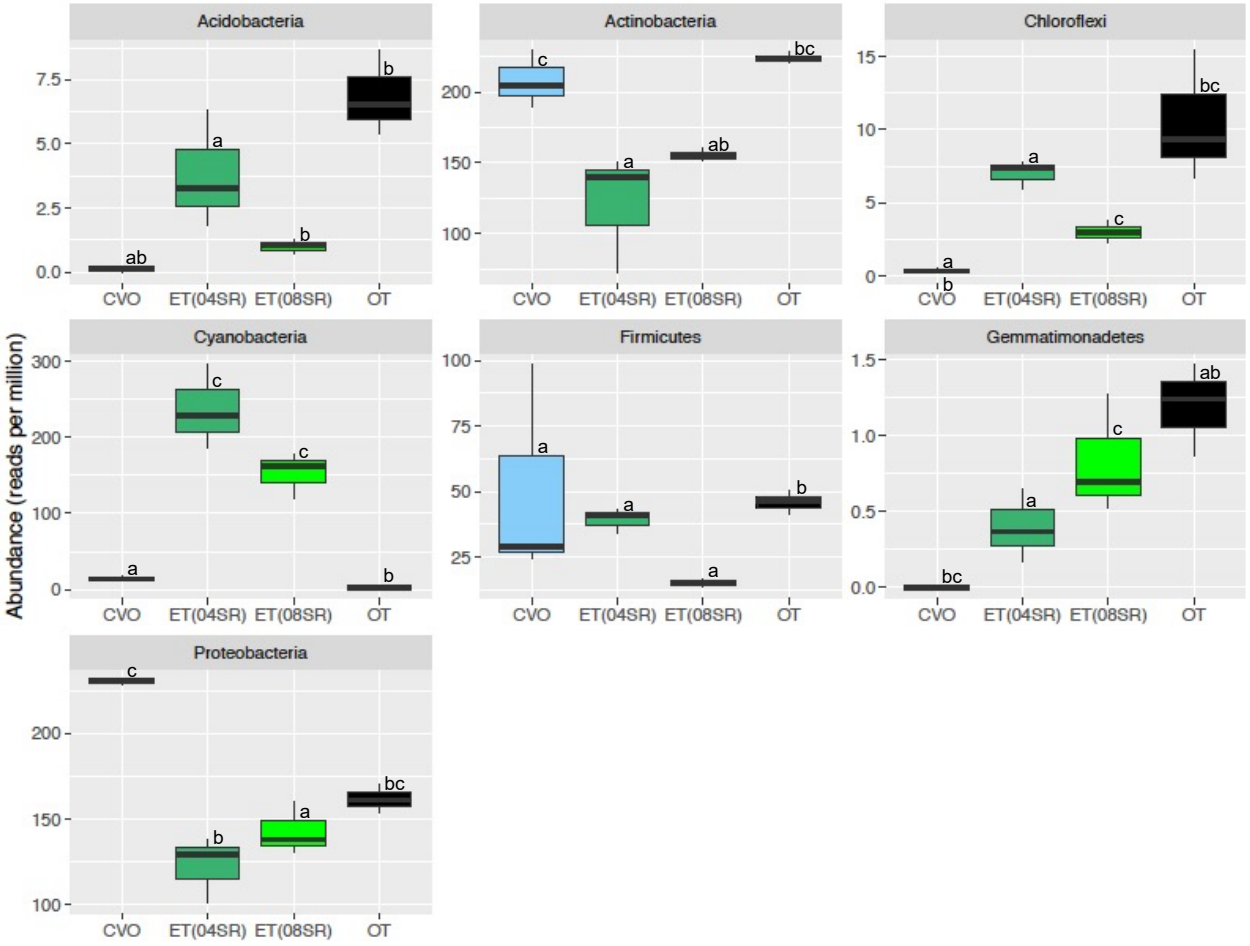
Supplemetary Figure 1: The relative abundance of the main bacterial phyla founded on shallow alkaline lakes.



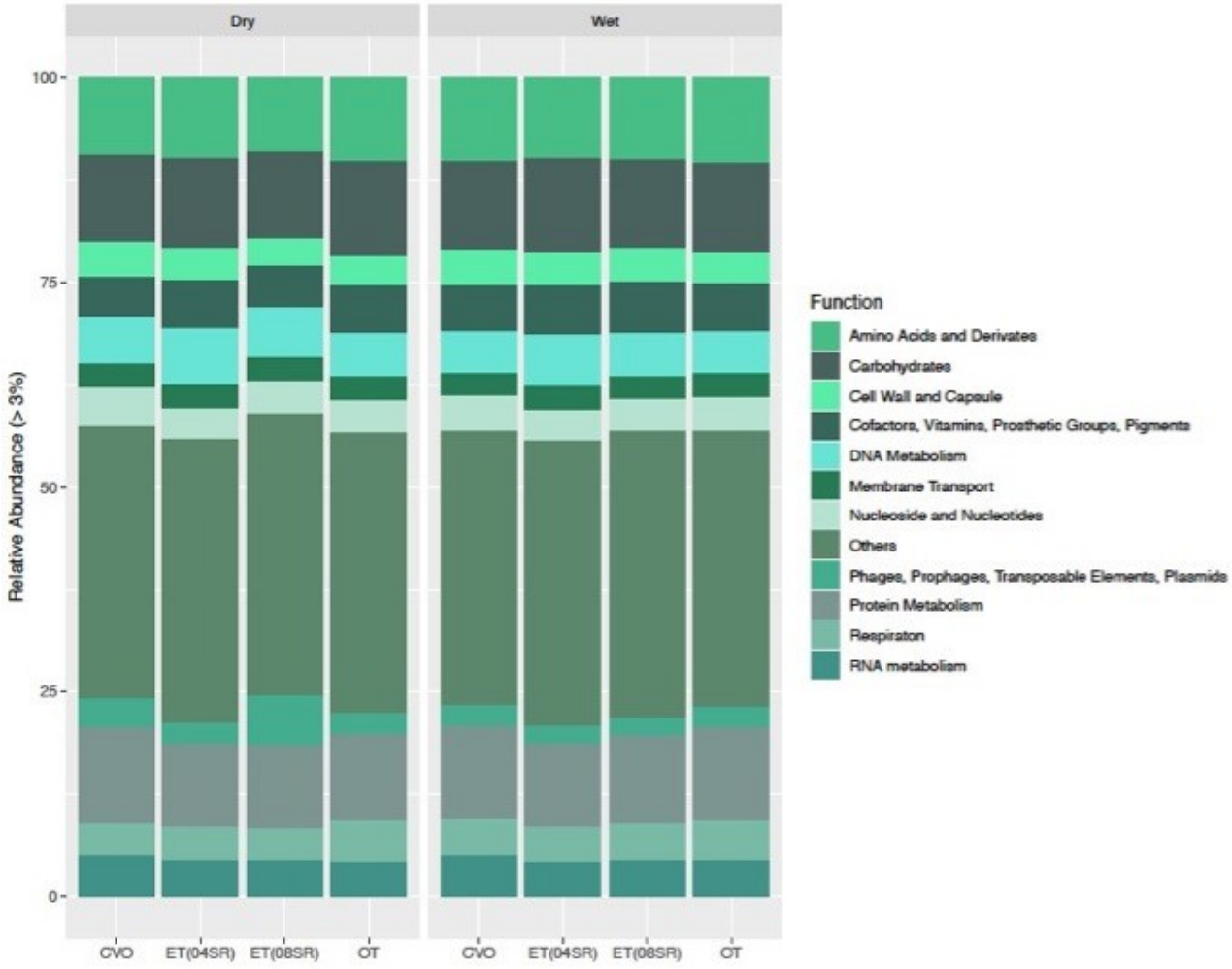
Supplementary Figure 2. The differential abundance of bacterial groups during the dry season ($p < 0.05$).



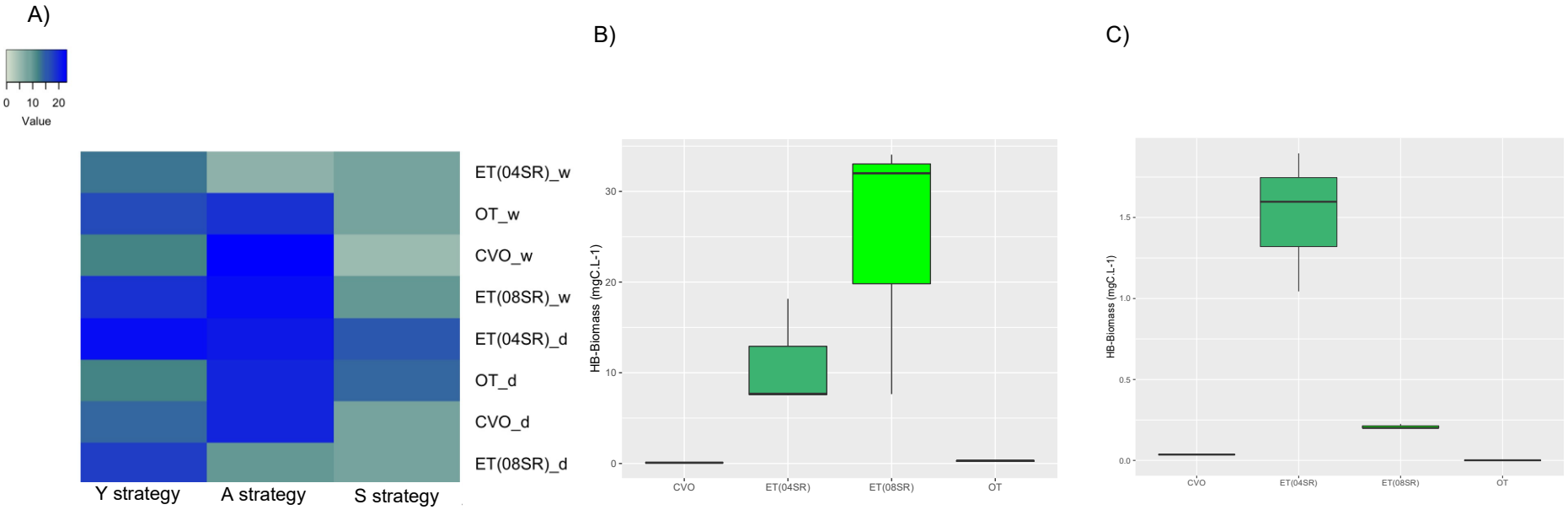
Supplementary Figure 3



Supplementary Figure 4. The differential abundance of bacterial groups during the wet season ($p < 0.05$).



Supplementary Figure 5. The bacterial community traits-based analysis excluding the Cyanobacteria phylum. The heatmaps (letters A and C) represent the number of traits affiliated with each life strategy, while the boxplot represents the biomass of heterotrophic bacteria (letters B and D). The letters A and B represent the dry season, while the letters C and D represent the wet season.



Appendix C: Supplementary material of chapter 4

Supplementary Materials includes: Supplementary Table: Table S1~S2 and Supplementary Figures: Figs. S1~S5

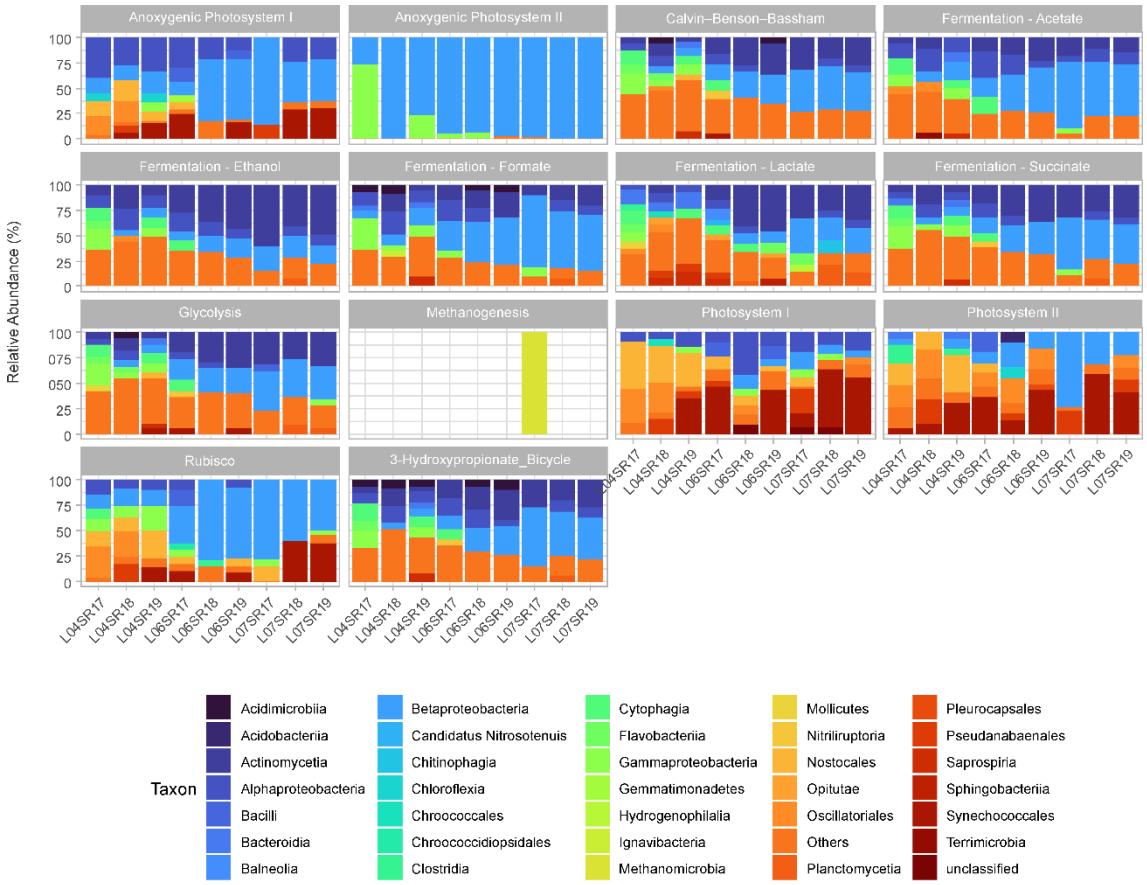
Supplementary Table 1. Table of environmental conditions including in situ analyzes and macro and micronutrients in Nhecolândia soda lakes during thre distinct years (2017,2018 and 2019).

Samples	WT	EC	pH	Sal	Alk	NH_4^+	NO_2^-	NO_3^-	Cl^-	PO_4^{3-}	Na	K	SO_4^{2-}	DOC	DIC	TDN	TP	TN	Al	B	Ca	Cu	Fe	Mg	Si'	Zn
	ºC	uS cm^{-1}		g L^{-1}	mmol L^{-1} $CaCO_3$																					
ET17_1	26.50	15660.00	10.00	13.09	98.33	3.03	0.14	0.30	712.00	84.71	4160.00	1200.00	70.76	1076.00	1180.00	1467.54	178.43	715.16	0.27	6.01	11.76	0.04	0.24	0.15	199.77	0.06
ET17_2	26.50	16705.00	10.02	10.39	93.33	5.92	0.23	0.28	612.00	87.80	3840.00	1310.00	74.08	731.10	1211.00	1337.21	179.40	1525.23	0.36	5.95	12.16	0.05	0.32	0.16	203.15	0.09
ET17_3	26.30	16520.00	10.02	9.84	100.83	5.10	0.16	0.28	698.20	79.76	4180.00	1400.00	76.74	961.30	1218.00	1364.35	179.56	1496.95	0.33	6.13	11.46	0.03	0.26	0.18	219.24	0.07
ET18_1	24.70	1727.00	9.95	1.55	6.95	0.78	0.02	0.14	36.81	2.10	485.43	129.73	0.01	77.61	171.29	10.59	3.20	19.15	0.01	0.51	75.28	0.00	0.15	22.29	35.27	0.01
ET18_2	26.16	1719.00	10.00	1.78	9.93	0.79	0.02	0.12	51.95	2.40	764.50	209.66	0.01	77.39	173.41	11.00	3.80	14.95	0.01	0.51	69.76	0.00	0.14	23.65	35.68	0.01
ET18_3	26.37	1725.00	10.06	1.77	9.43	0.77	0.07	0.13	36.57	2.10	494.44	133.87	0.01	77.61	170.09	9.92	3.20	14.27	0.01	0.52	63.46	0.00	0.13	25.01	36.53	0.00
ET19_1	25.94	3130.00	10.28	2.46	18.12	1.12	0.06	0.27	75.93	15.30	1020.12	311.72	6.05	238.50	395.30	21.36	21.75	97.08	0.06	0.93	56.89	0.00	0.19	24.08	128.28	0.01
ET19_2	26.25	3170.00	10.26	2.44	15.81	1.13	0.10	0.52	82.23	15.30	1127.59	350.17	6.36	253.70	345.20	22.96	25.50	90.16	0.03	0.87	47.75	0.00	0.14	32.31	118.00	0.01
ET19_3	26.77	3250.00	10.25	2.27	15.41	1.13	0.04	0.25	82.39	15.30	1113.05	344.63	3.29	266.50	418.00	24.76	21.30	85.29	0.04	0.90	44.52	0.00	0.16	15.64	119.01	0.01
OT17_1	37.48	17915.00	9.66	9.17	66.09	0.16	0.32	0.29	649.00	81.81	3630.00	770.00	830.69	333.00	1055.00	153.73	144.14	298.51	0.67	2.18	11.28	0.10	0.59	-0.01	108.02	0.03
OT17_2	37.80	18022.00	9.71	9.59	64.77	0.49	0.36	0.30	467.90	99.86	3170.00	520.00	788.98	286.20	1074.00	164.29	155.27	269.91	0.72	2.11	9.62	0.09	0.61	0.00	98.97	0.03
OT17_3	37.70	18034.00	9.72	10.40	70.00	0.23	0.31	0.28	550.50	79.08	3540.00	530.00	765.60	299.40	1098.00	213.53	166.40	305.99	2.10	1.79	8.92	0.07	1.61	0.00	93.76	0.03
OT18_1	26.59	672.00	9.16	0.82	3.97	0.04	0.09	0.24	20.59	1.30	213.40	61.47	21.19	12.50	54.41	1.02	1.30	2.02	9.39	0.10	135.72	0.00	41.54	13.24	92.34	0.03
OT18_2	26.58	678.00	9.12	0.84	3.48	0.04	0.09	0.22	20.78	1.60	244.32	44.43	25.18	11.95	56.08	1.09	1.60	2.08	9.45	0.10	74.88	0.00	41.54	2.38	91.63	0.04
OT18_3	25.74	678.00	9.01	0.82	3.97	0.04	0.09	0.34	20.73	1.40	222.54	54.55	25.84	11.70	55.07	0.97	1.40	1.83	9.55	0.10	50.20	0.00	42.45	36.34	92.25	0.03
OT19_1	25.32	1260.00	9.44	1.04	5.60	0.05	0.20	0.83	33.73	11.25	441.34	81.68	46.62	38.43	91.95	19.57	13.40	21.68	16.60	0.15	42.04	0.01	76.92	13.95	350.71	0.04
OT19_2	25.16	1240.00	9.44	0.77	5.50	0.05	0.20	0.98	18.54	12.25	263.13	68.16	27.23	42.26	100.96	16.94	14.93	18.27	16.28	0.14	30.75	0.01	71.88	0.00	39.24	0.04
OT19_3	25.44	1290.00	9.46	0.88	5.91	0.03	0.21	0.85	27.33	13.38	343.75	80.03	35.11	39.34	94.32	20.17	16.30	21.70	17.42	0.15	63.52	0.01	82.46	13.50	354.19	0.04
CVO17_1	27.24	1777.00	8.55	0.82	7.69	0.20	0.02	0.05	69.00	0.47	3290.00	200.00	4.08	82.90	173.00	383.50	8.86	345.31	0.01	0.27	9.54	0.01	0.06	0.64	68.80	0.01
CVO17_2	27.25	1777.00	8.55	1.13	8.40	0.47	0.02	0.05	84.00	0.36	3400.00	190.00	5.88	74.32	176.80	422.01	10.01	364.81	0.01	0.31	12.63	0.01	0.06	0.70	65.15	0.01
CVO17_3	27.26	1777.00	8.56	1.03	8.00	0.27	0.02	0.05	70.60	0.42	3390.00	190.00	4.73	71.50	176.80	454.35	9.44	365.95	0.01	0.30	11.46	0.01	0.06	0.77	60.58	0.02
CVO18_1	26.94	556.00	8.58	0.54	3.97	0.03	0.00	0.01	6.46	0.12	66.41	40.30	1.89	16.44	49.09	1.29	0.12	2.35	0.01	0.09	37.57	0.00	0.08	15.89	27.28	0.00
CVO18_2	26.85	554.00	8.58	0.53	2.98	0.03	0.00	0.04	7.33	0.04	90.82	48.23	0.01	15.97	49.30	1.33	0.04	2.20	0.00	0.10	44.96	0.00	0.05	21.91	27.67	0.01
CVO18_3	27.29	557.00	8.68	0.54	3.97	0.03	0.00	0.01	7.15	0.06	87.65	52.28	0.01	17.00	48.70	1.31	0.06	2.19	0.01	0.10	34.15	0.00	0.07	21.76	27.70	0.03
CVO19_1	27.33	696.00	9.04	0.37	3.50	0.02	0.00	0.01	12.46	0.02	177.10	97.95	0.00	26.20	83.10	2.05	0.02	3.61	0.01	0.12	71.77	0.00	0.03	50.26	62.31	0.00
CVO19_2	27.61	695.00	9.05	0.50	3.50	0.03	0.00	0.03	10.59	0.03	167.37	83.93	0.00	27.38	85.03	2.59	0.03	3.95	0.01	0.12	54.40	0.00	0.02	48.79	63.59	0.00
CVO19_3	27.44	694.00	9.04	0.39	3.50	0.02	0.00	0.00	12.20	0.02	173.35	105.00	0.00	56.03	79.75	29.89	0.02	31.17	0.01	0.12	70.02	0.00	0.02	62.51	63.92	0.00

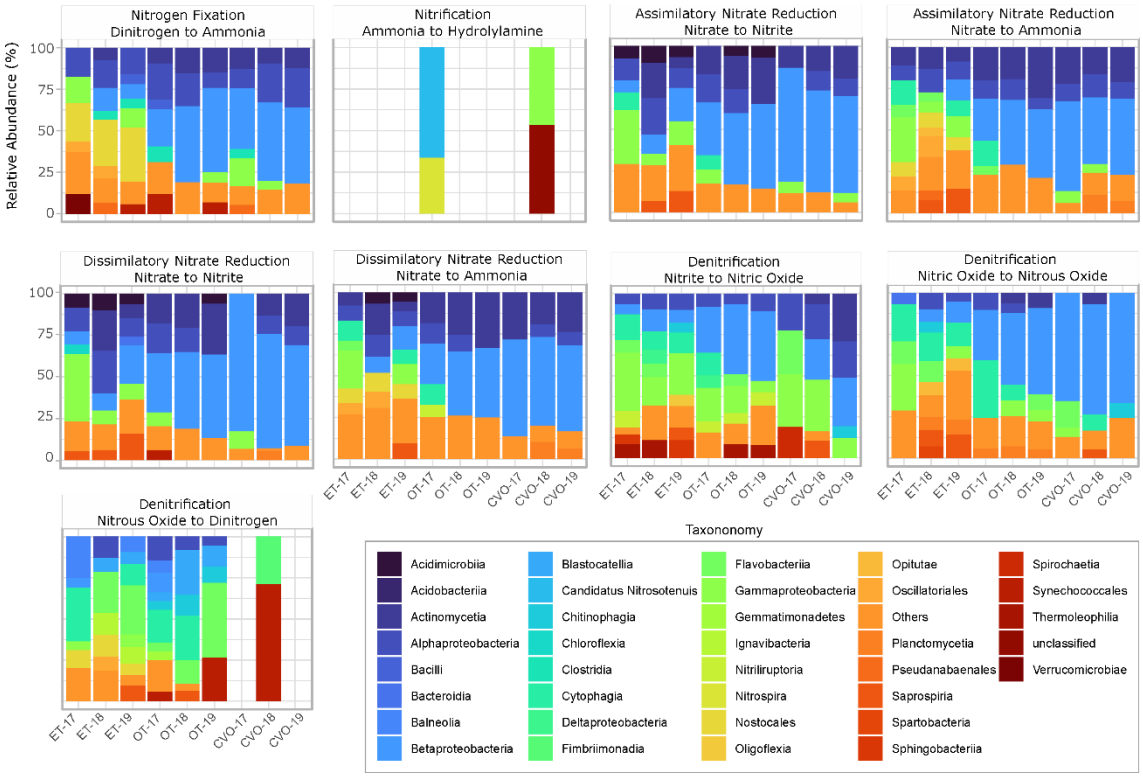
ET = Eutrophic turbid lake; OT = Oligotrophic turbid lake; CVO = Clear vegetated oligotrophic lake.WT = Water temperature in Celsius degree; EC = Water electrical conductivity (uS cm^{-1}); Sal = Salinity (g L^{-1}); Alk = Alkalinity (mmol CaCO3); DOC = Dissolved organic carbon;

DIC = Dissolved inorganic carbon; TDN = Total dissolved nitrogen; TP = Total phosphorus; TN = total nitrogen.

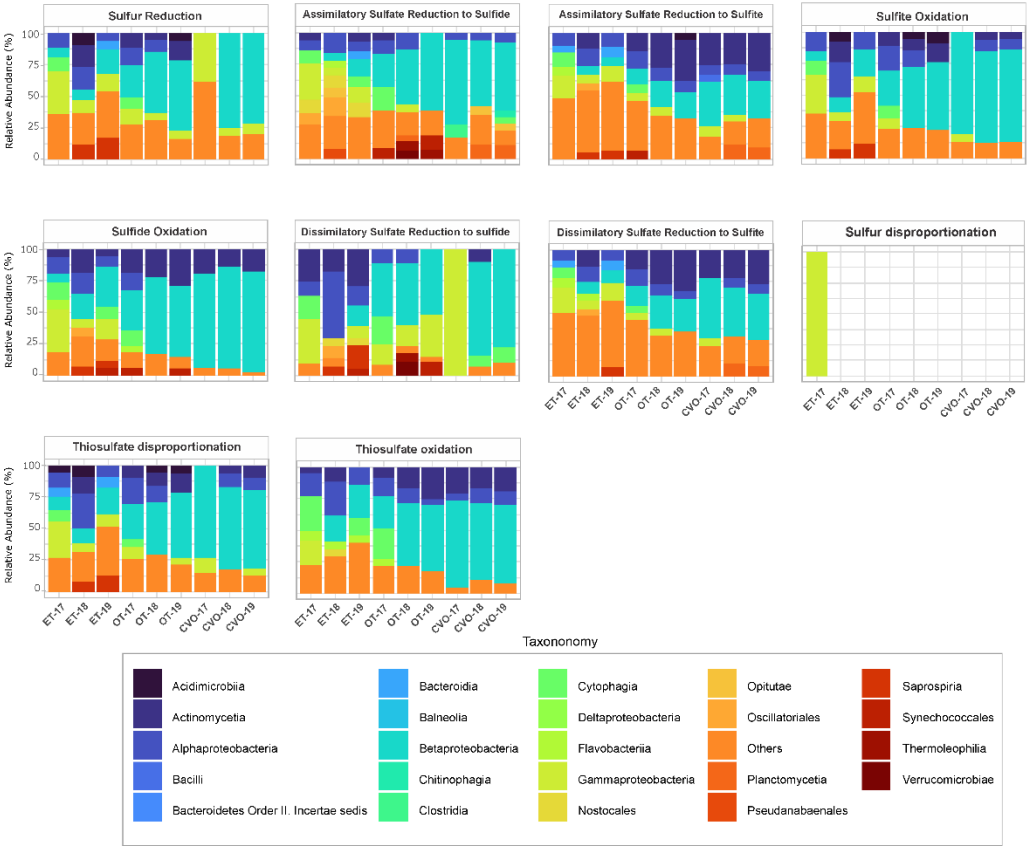
Supplementary Figure S1. The taxon-specific relative abundance of carbon cycle represented by bar plot in all lakes and sampled years.



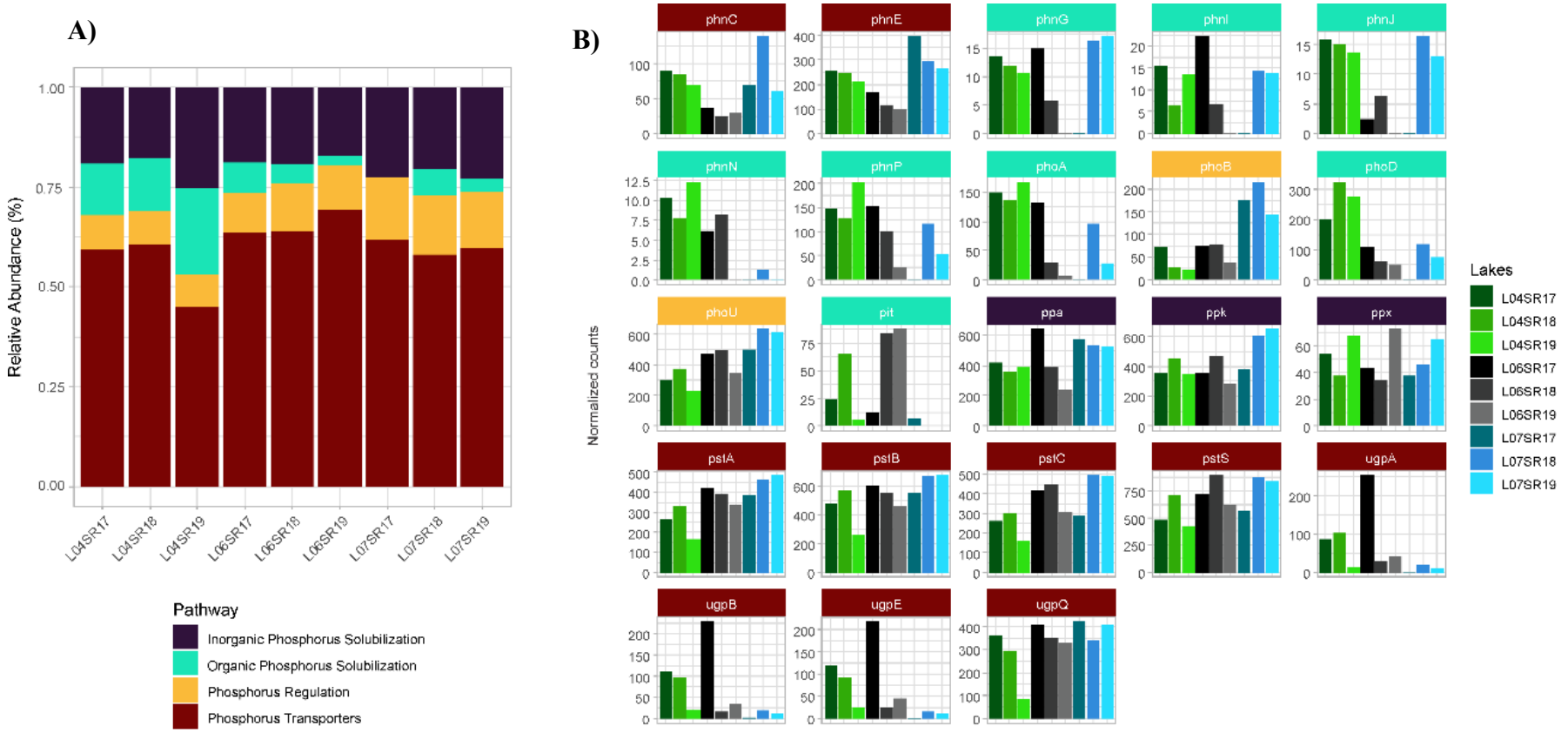
Supplementary Figure S2. The taxon-specific relative abundance of nitrogen cycle represented by bar plot in all lakes and sampled years.



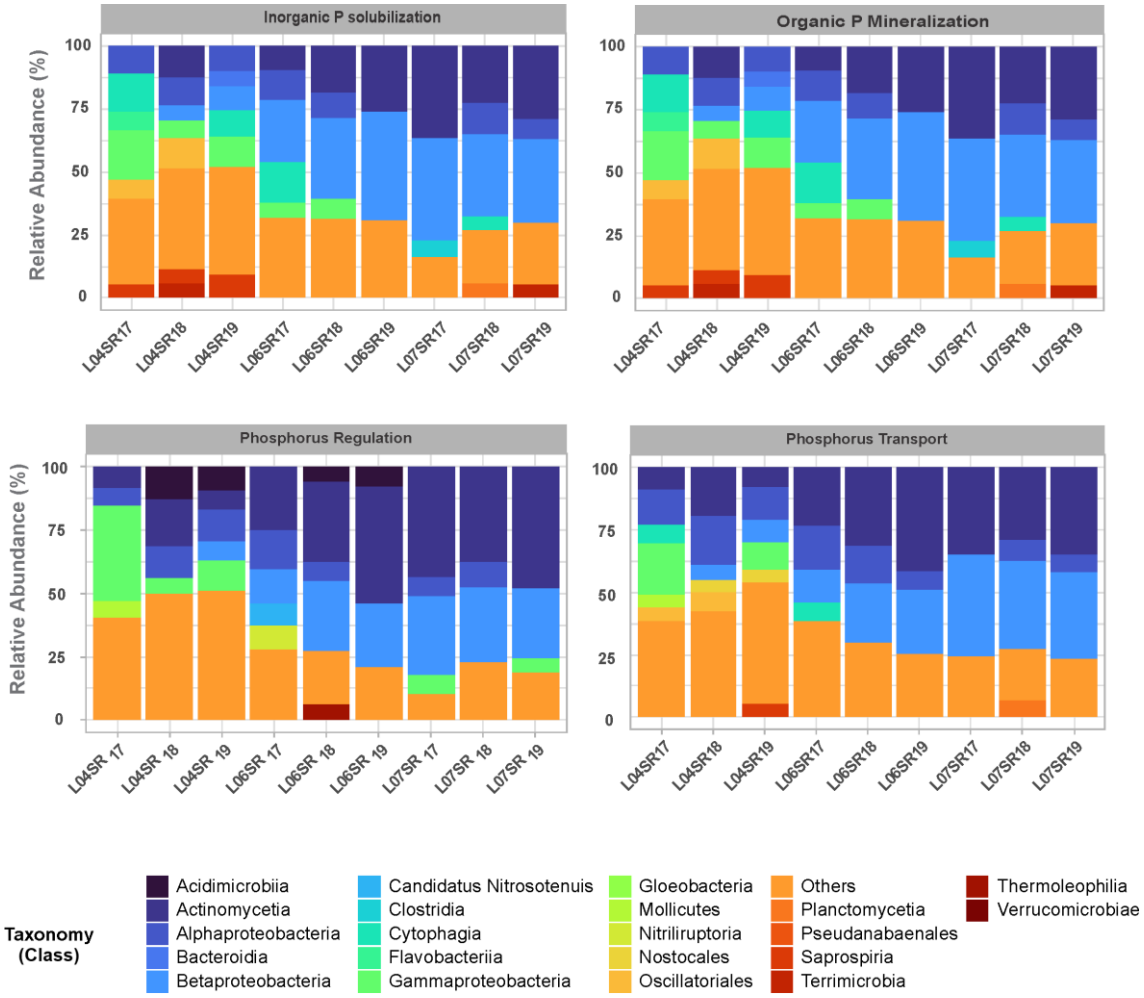
Supplementary Figure S3. The taxon-specific relative abundance of sulfur cycle represented by bar plot in all lakes and sampled years.



Supplementary Figure S4. Relative abundance of phosphorus cycle genes along different lake types and sampled years (A); Normalized count of each phosphorus gene along different lake types and sampled years (B). Distinct color represents each phosphorus cycling process.



Supplementary Figure S5. The taxon-specific relative abundance of phosphorus cycle represented by bar plot in all lakes and sampled years.



Supplementary Figure S6. Heatmap of biogeochemical functional gene abundance among the soda lakes of Nhecolândia in three distinct years.