Science of the Total Environment 947 (2024) 174646

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# The role of microbial communities in biogeochemical cycles and greenhouse gas emissions within tropical soda lakes

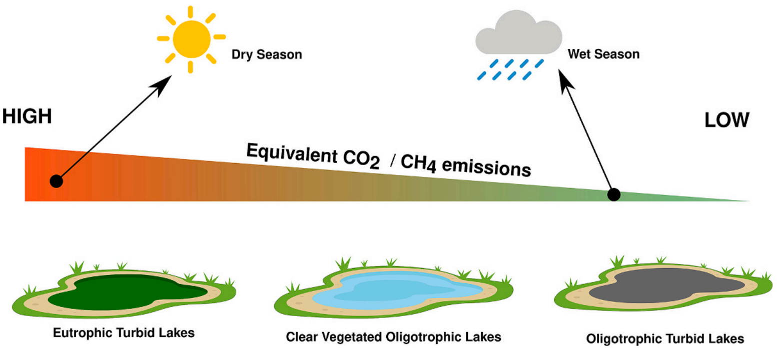
Thierry A. Pellegrinetti [a], Simone R. Cotta [a], Yara B. Feitosa [a], Paul L.A. Melo [a], Wanderlei Bieluczyk [a], Antonio M.M. Silva [b], Lucas W. Mendes [a], Hugo Sarmento [c], Plinio B. Camargo [a], Siu M. Tsai [a], Marli F. Fiore [a,*]

[a] University of São Paulo (USP), Center for Nuclear Energy in Agriculture, Avenida Centenário 303, Piracicaba, São Paulo 13416-000, Brazil
[b] University of São Paulo (USP), "Luiz de Queiroz" College of Agriculture, Soil Science Department, Piracicaba, São Paulo 13418-900, Brazil
[c] Federal University of São Carlos (UFSCar), Department of Hydrobiology, São Carlos, São Paulo 13565-905, Brazil

## HIGHLIGHTS

- Brazil's Pantanal wetlands are key sources of methane, especially from soda lakes.
- Eutrophic lakes emit more methane, driven by cyanobacterial blooms.
- Microbial communities play a crucial role in soda lake GHG emissions.
- Research and conservation in habitats like the Pantanal are essential to manage GHG emissions.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: JV Cruz

*Keywords:*
Warming climate
Microbial communities
Biogeochemical cycling
Metagenomics
Greenhouse gas emissions

## ABSTRACT

Although anthropogenic activities are the primary drivers of increased greenhouse gas (GHG) emissions, it is crucial to acknowledge that wetlands are a significant source of these gases. Brazil's Pantanal, the largest tropical inland wetland, includes numerous lacustrine systems with freshwater and soda lakes. This study focuses on soda lakes to explore potential biogeochemical cycling and the contribution of biogenic GHG emissions from the water column, particularly methane. Both seasonal variations and the eutrophic status of each examined lake significantly influenced GHG emissions. Eutrophic turbid lakes (ET) showed remarkable methane emissions, likely due to cyanobacterial blooms. The decomposition of cyanobacterial cells, along with the influx of organic carbon through photosynthesis, accelerated the degradation of high organic matter content in the water column by the heterotrophic community. This process released byproducts that were subsequently metabolized in the sediment leading to methane production, more pronounced during periods of increased drought. In contrast, oligotrophic turbid lakes (OT) avoided methane emissions due to high sulfate levels in the water, though they did emit $CO_2$ and $N_2O$. Clear vegetated oligotrophic turbid lakes (CVO) also emitted methane, possibly from organic matter input during plant detritus decomposition, albeit at lower levels than ET. Over the years, a concerning trend has

---

* Corresponding author.
*E-mail addresses:* tpellegrinetti@usp.br (T.A. Pellegrinetti), fiore@cena.usp.br (M.F. Fiore).

https://doi.org/10.1016/j.scitotenv.2024.174646
Received 4 March 2024; Received in revised form 2 July 2024; Accepted 7 July 2024
Available online 8 July 2024

emerged in the Nhecolândia subregion of Brazil's Pantanal, where the prevalence of lakes with cyanobacterial blooms is increasing. This indicates the potential for these areas to become significant GHG emitters in the future. The study highlights the critical role of microbial communities in regulating GHG emissions in soda lakes, emphasizing their broader implications for global GHG inventories. Thus, it advocates for sustained research efforts and conservation initiatives in this environmentally critical habitat.

## 1. Introduction

Greenhouse gas (GHG) concentrations in the atmosphere, including methane ($CH_4$), carbon dioxide ($CO_2$), and nitrous oxide ($N_2O$), have risen over the past 150 years due to human activities (Dijkstra et al., 2013). These anthropogenic emissions have triggered climate change, resulting in increasing temperatures, changes in precipitation patterns, and a high frequency of extreme weather events (Voigt et al., 2017). Simultaneously, these human-induced climate changes persist as one of the most urgent sustainability challenges confronting humanity today, threatening the health and functionality of ecosystems (Tian et al., 2015).

Additionally, there are concerns that climate change is threatening the ecosystem functioning of the Pantanal biome (Coelho-Junior et al., 2022). For example, deforestation in the Amazon rainforest alters precipitation patterns and directly impacts the hydrological dynamics of the entire Pantanal (Marengo et al., 2021). Climate change could trigger a chain effect on biogeochemical processes in the soda lakes in Pantanal, culminating in not yet well-known modifications in the regulation of GHG fluxes by the microorganisms. Therefore, investigating the effects of drying and rewetting dynamics of these lakes, as well as unveiling possible climate change impacts on these microbial communities in the vast area of the Pantanal, provide indispensable information to predict GHG trajectories under future global warming scenarios.

Although natural wetlands represent only about 5–8 % of the terrestrial landscape, they store approximately 20 %–30 % of the soil carbon on Earth (Mitsch et al., 2013) playing an important role in regulating the atmospheric $CO_2$ concentration and thus affecting the climate (Lu et al., 2017). Notably, the majority of this carbon retention is observed in tropical and subtropical regions (Mitsch et al., 2013). Wetlands play a significant role in global GHG emissions, acting both as sources and sinks. They emit large amounts of $CH_4$ and $N_2O$, but sequester $CO_2$, thus having a complex impact on the global carbon cycle (Li et al., 2022b; Mitsch et al., 2013; Rosentreter et al., 2021).

The Pantanal biome, the world's largest tropical wetland (153,000 $km^2$), is a region of immense ecological value, serving as a sanctuary for numerous wildlife species in South America (Guerreiro et al., 2019; Pott et al., 2011). Its biodiversity comprises >2000 species of plants, 580 birds, and at least 174 species of mammals (Nunes, 2011; Pott et al., 2011). The diverse avifauna population in Pantanal includes many endangered and migratory species, due to the abundant plankton biomass (Nunes, 2011). In the expansive Pantanal, the Nhecolândia subregion harbors around 900 soda lakes distinguished by their elevated pH levels (reaching up to 11), high salinity, shallow depths, and, in certain instances, the occurrence of cyanobacterial blooms (Andreote et al., 2018; Barbiero et al., 2018; Pellegrinetti et al., 2023). These soda lakes can be broadly categorized into three primary types, with each type being shaped by factors such as pH, salinity, and microbial composition (Pellegrinetti et al., 2023). There is a gradient of salinity, pH, and eutrophic conditions found in lakes classified as Eutrophic Turbid (ET), Oligotrophic Turbid (OT), and Clear Vegetated Oligotrophic (CVO). Nevertheless, this region confronts substantial environmental transformations owing to the dual impacts of global warming and anthropogenic activities. These activities include advances in agriculture, recurrent and intense episodes of fires, as well as extreme flooding and droughts, culminating in the complete desiccation of some lakes (Marques et al., 2021; Pellegrinetti et al., 2023; Tomas et al., 2021).

Despite their ecological significance, there is a lack of research on greenhouse gas (GHG) emissions in these habitats. This is a substantial knowledge gap, as these lakes have the capacity to release large amounts of methane and carbon dioxide, resulting in potential areas of intensive emissions. A recent study conducted in soda lakes within the Pantanal region has revealed significant methane emissions, reaching up to 500 mmol $m^{-2}$ $d^{-1}$ (Barbiero et al., 2018). These findings have also indicated that OT lakes are likely sources of both $CO_2$ and $CH_4$, while ET lakes act as sinks for $CO_2$ and $N_2O$ but as sources of $CH_4$. Although preliminary GHG emissions in a few Pantanal soda lakes have been provided (Barbiero et al., 2018), the knowledge of the mechanisms that regulate these emissions is still lacking, particularly regarding the roles of microbiota.

Microorganisms play a pivotal role in sustaining aquatic food webs. However, the biogeochemical cycles under high pH and near-saturation salinity environments are not yet fully comprehended. These unique conditions foster a diverse range of microorganisms, particularly bacteria and archaea, including some representatives of Alphaproteobacteria, Gammaproteobacteria, Cyanobacteriota, and sulfur-reducing bacteria (Sorokin et al., 2014; Zorz et al., 2019). Previous research has highlighted key biogeochemical processes in soda lakes, including nitrogen fixation, denitrification, photosynthesis, and organic matter decomposition (Antony et al., 2012; Sorokin et al., 2015; Zorz et al., 2019). Notably, the sulfur cycle is found to be particularly prevalent and active in these lakes (Vavourakis et al., 2019). However, saline-alkaline conditions can disrupt certain pathways, such as nitrification and organic matter decomposition, while simultaneously impacting microbial diversity. These changes in microbial composition and diversity also influence other processes like sulfate reduction and phosphorus availability, thus shaping the nutrient cycles and GHG emissions (Sorokin et al., 2015; Vavourakis et al., 2019).

Considering the information regarding biogeochemical processes in soda lakes and the role of microbial communities in these environments, we hypothesize that the planktonic microbial communities inhabiting the water columns of different tropical soda lake types would affect GHG emissions differently. This differentiation could arise from variations in microbial composition, metabolic pathways, and chemical compound balances. To test this hypothesis, we: i) quantified the GHG fluxes and water storage under two contrasting hydrological conditions; ii) identified the relative abundance of functional genes and the taxa associated with these genes across different lakes and environmental conditions; iii) associated the predicted compounds flux and carbohydrates enzymes influencing chemical interactivity; iv) assessing the potential future changes in GHG emission through the biogeochemical characteristics of the soda lakes using remote sensing tools. Together, our results could help to improve our ability to predict and understand the ecological implications of planktonic microbial communities and their role in GHG flux, with far-reaching implications for environmental management and conservation.

## 2. Material and methods

### 2.1. The description of sampling sites

Three soda lakes located in the São Roque Reserve (19° 23′28.61″S, 56° 19′53.37″W) in the Nhecolândia sub-region of Mato Grosso do Sul State, Brazil, were investigated. The geographic locations of the lakes are described by the following coordinates: 19°22′53.0″S, 56°19′33.3″W for ET lake, 19°23′24.1″S, 56°19′20.0″W for OT lake, and 19°23′54.4″S, 56°19′50.4″W for CVO lake. A detailed table with the geographic location of each sampling sites is described in Suppl. Table 1. The remote site has limited access *via* small dirt easement roads, which requires an approximately 8-hour journey in a four-wheel drive big truck. Nhecolândia is significantly influenced by annual weather patterns, with prolonged rainy seasons and subsequent months of flooding, often hindering terrestrial access. Therefore, sampling was strategically scheduled during months when weather conditions were conducive to vehicular access. One representative lake from each soda lake group was selected based on the previous classification described by Pellegrinetti et al. (2023). ET lakes have high pH (>9.5) and salinity, with frequent cyanobacterial blooms, particularly species such as *Arthrospira platensis* or *Anabaenopsis elenkinii*. OT lakes feature moderate pH (8.5–9.5) and salinity, and their water appears black due to the presence of mineral-associated organic matter. The third type, CVO lakes, is characterized by lower salinity and pH (8–8.5), very low particulate matter in the water, and a notable presence of aquatic macrophytes. Sampling expeditions were conducted in three distinct years (Oct 2017, Sep 2018, and Sep 2019) to cover the different hydrological conditions in Pantanal: extremely dry (2017), wet (2018), and dry (2019).

In the extremely dry season (Oct-2017), the water depth in the deepest points of the lakes ranged from 20 to 56 cm, depending on the specific lake assessed. In the wet season (Sep-2018), the water column was higher, ranging from 86 to 130 cm. Lastly, in the dry season (Sep-2019), we sampled an intermediary hydrogeochemical condition, with water levels ranging from 60 to 67 cm deep. Further details about the physical and chemical characteristics of each sampled lake are provided in the Suppl. Table 2. In all these field expeditions, we collected surface water samples (between 0 and 10 cm deep) in triplicate, with each sample being separated by at least 100 m.

### 2.2. Water surface area, chlorophyll index, and proportions of lake types in the Nhecolândia region

We analyzed satellite imagery to assess the temporal series of water area and chlorophyll among studied sites. These patterns were investigated using indices such as the Normalized Difference Water Index (NDWI) and Normalized Difference Chlorophyll Index (NDCI) over the last 20 years using the Google Earth Engine platform and Landsat Imagery. The NDWI was computed according to the formula: (Green - NIR) / (Green + NIR), where Green and NIR denote reflectance in the green and near-infrared bands, respectively. On the other hand, the NDCI was calculated as follows: (Red Edge - NIR) / (Red Edge + NIR), where Red Edge and NIR represent reflectance in the red edge and near-infrared bands, respectively. These indices were applied specifically to the water area of soda lakes, which was previously delimited (Pereira et al., 2020), which provided a shapefile delineating the boundaries of all lakes within the region, each accompanied by specific pH details.

Additionally, we expanded our remote sensing examination on the distribution and proportions of the distinct types of lakes (ET, OT, and CVO) present in the entire Nhecolândia region. This innovative analysis could be instrumental for future GHG emissions estimations in the Pantanal region. The lakes were first classified into two primary categories: freshwater lakes with pH levels below 8 and soda lakes with pH levels above 8. A secondary re-classification further differentiated the soda lakes according to pH and patterns provided in Pellegrinetti et al. (2023): ET lakes with pH above 9.5, OT lakes with pH between 8.5 and 9.4, and CVO lakes with pH between 8 and 8.5.

### 2.3. GHG emissions and measurements

Dissolved and emitted GHG data were collected for the years 2018 and 2019. Unfortunately, data collection for the year 2017 was hindered by the extreme drought event, resulting in significantly reduced water depths, which were only found in very small areas at the center of the lakes. Chambers were obstructed from floating over the almost empty lakes. For measuring GHG fluxes, we adapted the floating chambers methodology described by Barbiero et al., 2018. Briefly, in 2018 and 2019, the gas fluxes at the air-water interface were sampled using five polyethylene floating chambers (volume: 32 L; base area: 0.195 $m^2$) spaced along the lake aiming to cover its spatial variability (Suppl. Fig. 1). During incubation, the chambers were slowly traversed from one lake shore to another, anchored with a lightweight line to not disturb the sediment. Chambers were carefully placed with a maximum penetration of 2 cm into the water and were pulled by hand from the opposite bank at a maximum rate of 5 m $min^{-1}$. Collections were conducted at similar moments of the day across the lakes, starting the floating at 8, 11, 14, and 17 o'clock. The gas was sampled into vials at two times: T0, at the departure of the chambers set (ambient atmospheric air), and T30, the accumulation of 30 min of incubation, after the passage of chambers along the entire transect. Duplicate gas samples were collected for each chamber using two 60 mL syringes equipped with check valves, and sequentially transferred into 30 mL vials with gas-tight caps previously evacuated at 0.75 kPa with a vacuum pump. Importantly, after each sampling at T30 (*i.e.*, 8:30, 11:30, 14:30, and 17:30 h), the chambers were removed from the water for ventilation and cleaning in preparation for the next crossing.

The concentration of gas in the liquid phase was estimated indirectly using the headspace displacement technique (Hamilton et al., 1995). This process entailed filling 140 mL polypropylene syringes with 105 mL of water, leaving 35 mL of atmospheric air. Immediately, the syringes were manually agitated for 1 min, and the headspace air was injected into 20 mL vials, which were previously evacuated and sealed. We also collected a local atmospheric sample free of water at each lake point. This enabled the quantification of $CH_4$, $CO_2$, and $N_2O$ already present in the air of the syringe headspace, to be later subtracted in the dissolved calculation procedure. Both dissolved and emitted GHG concentrations were measured using Gas Chromatography (SRIGC-110®, Torrance, USA) with a packaged HAYESP™ column (80–100 mesh) maintained at 82 °C. The $N_2O$ concentration was quantified using an electron capture detector (ECD), while $CO_2$ and $CH_4$ concentrations were obtained by flame ionization detector (FID).

Specifically for emitted gas, the GHG fluxes were calculated by linear variation in the amount of each gas in the chambers (obtained by the Clapeyron equation) as a function of the incubation time (30 min). Afterward, the dissolved gas concentrations in the water were estimated from the corrected concentrations using the Ideal Gas Law.

### 2.4. Metagenomic DNA extraction and sequencing

The total environmental DNA of water samples was extracted using the PowerLyzer PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) from lyophilized water (50 mL, 0.5 g). The integrity of the extracted DNA was assessed by agarose gel electrophoresis (1 % *w/v*), and the concentration was quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). To prepare the DNA libraries, the Illumina Nextera XT DNA Library Preparation kit (Illumina, Inc., San Diego, CA, USA) was used following the manufacturer's recommendations. Paired-end reads of 2 × 100 bp (200 cycles) were generated by sequencing 27 DNA samples (3 lakes × 3 samples × 3 sampling periods) on an Illumina HiSeq 2500 platform. The obtained sequences were deposited on the MG-RAST server and are publicly available under projects "mgp86324", "mgp88859" and "mgp92377" for respectively years (2017, 2018, and 2019). Additionally, these metagenome sequences are archived in the NCBI BioProject database under the identifier PRJNA1111019 and in Zenodo under the link https://doi.org/10.5281/zenodo.12609061.

### 2.5. Bioinformatic analyses

The raw sequences underwent adapter trimming using CutAdapt v1.18 (Martin, 2011) and quality assessment with FastQC v0.10.1. PEAR software v0.9.6 (Zhang et al., 2013) was used to merge the paired-end reads, and Seqyclean v1.3.12 (Zhbannikov et al., 2017) was employed to filter out sequences with Phred scores lower than 20 and shorter than 50 bp. To determine the relative abundance and occurrence of metabolic and biogeochemical functional pathways of carbon, nitrogen, sulfur, and phosphorus, the DiTing pipeline v0.9 (Xue et al., 2021) was used. Briefly, Diting pipeline assembled reads using Megahit v1.1.3 (Li et al., 2016) and predicted open reading frames (ORFs) from all contigs using Prodigal v2.6.3 (Hyatt et al., 2010). ORFs were annotated using the KEGG orthology database through Hmmsearch from HMMER3 (http://hmmer.org/). The genes associated with each biogeochemical function and their respective KEGG orthology number are specified in the Diting github repository (https://github.com/xuechunxu/DiTing/blob/master/table/KO_affilated_to_biogeochemical_cycle.xlsx). The taxonomic annotation of each interesting biogeochemical KO was performed at the class level using the MetAnnotate pipeline 0.9.2 (Petrenko et al., 2015), which utilized the ORFs generated by DiTing pipeline and the 'HMM' profile obtained for each KO using KofamKOLA database. The relative abundance of taxonomic annotation for each KO was then obtained by ORF mapping against metagenomic sequence, which was provided by DiTing Pipeline as "Transcript per Million – TPM".

The functional taxonomic profiling of each biogeochemical pathway was processed using R base language. Contigs were processed and analyzed using the dbCAN3 tool to identify carbohydrate-active enzymes (CAZymes) and ascertain their role in carbohydrate metabolism (Zheng et al., 2023; Cantarel et al., 2009). The relative abundance of each CAZymes detected were measured by TPM from DiTing pipeline results. The flux balance analysis were calculated using fbatools v2.1.1 available in Kbase (Arkin et al., 2018), which is performed with RASTtk v.1.0.73 annotation (Brettin et al., 2015) and ModelSEED to metabolic pathway inference (Seaver et al., 2021).

### 2.6. Statistical and data analysis

Statistical analyses were conducted in R language to analyze the functional and taxonomic data. Variance analysis (ANOVA) and post-hoc Tukey tests were used to determine statistical differences across lakes and seasons using ExpDes.pt package (Ferreira et al., 2018). A non-metric multidimensional scaling (NMDS) based on the biogeochemical functional genes was performed to assess the similarity between samples using Vegan Package (Oksanen et al., 2013). Additionally, the Vegan package was used to calculate the alpha-diversity based on annotated functional genes between samples using the Shannon index. A random forest model was applied to determine the environmental predictors that better explain the biogeochemical functional composition. The randomForest package (RColorBrewer and Liaw, 2018) was used to construct a model with 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 filtering the %incMSE variable importance while analyzing the final model's $R^2$ until the highest value was reached. The statistical analysis for CAZymes and FBA data were previously evaluated with Shapiro-Wilk normality test and Bartlett's homogeneity test. ANOVA of aligned rank-transformed data was used to investigate the effect of treatments on the variables (Wobbrock et al., 2011), followed by pairwise comparisons using Tukey's adjustment. Ecological thresholds in taxonomic and functional annotations varying in pH gradient were calculated using the "Threshold Indicator Taxa Analysis (TITAN2)" for potential community-level changes. This analysis was conducted in the "TITAN2" R package (Baker et al., 2015).

## 3. Results

### 3.1. A general description of Pantanal's soda lakes features

A significant difference ($p$ = 0.001) between the functional gene profile was observed among the lake types (Suppl. Fig. 2A), and the effects of seasonal variations on sample clustering within each lake type were clearly discernible ($p = 0.001$) (Suppl. Fig. 2B). Five variables best explained the distribution of functional genes in lakes sampled: pH, $NH_4^+$, alkalinity, dissolved organic carbon (DOC), and water temperature ($r^2 = 0.95$), with pH being the most important factor ($p = 0.001$) (Suppl. Fig. 2C). The oligotrophic turbid lakes (OT) and clear vegetated oligotrophic (CVO) lakes experienced more pronounced effects on function diversity among seasons compared to eutrophic turbid (ET) lake ($p < 0.001$) (Suppl. Fig. 2D). The pH reflected in considerable differences between lakes, which values were higher in ET lakes (9.95 to 10.28) than OT (9.01 to 9.72) and CVO (8.55 to 9.05) (Suppl. Table 2). The $NH_4^+$ and DOC also had higher concentrations in ET lake than in the OT and CVO lakes. Moreover, some bacterial parameters associated with environmental productivity, such as DOC, and Chl-*a*, were positive correlated with $NH_4^+$. The $NH_4^+$ concentration ranged between 0.77 and 5.92 mg $L^{-1}$ for ET lake, 0.03 and 0.49 mg $L^{-1}$ for OT lake, and 0.02 and 0.47 mg $L^{-1}$ for CVO lake.

Based on satellite imagery analysis and lake classification, it was detected a total of 12,158 lakes (Fig. 1A), out of which 896 (7.36 % of the total) are soda lakes with a pH >8 (Fig. 1B). Among these soda lakes, 246 (27.45 % of all soda lakes) exhibit a pH higher than 10, an indicative of cyanobacterial blooms (Fig. 1C). Furthermore, our analysis revealed an increase in lake chlorophyll-a content and a significant reduction in water area between the years 2000 and 2022 (Fig. 1D; E). This trend was confirmed by the NDCI with an $R^2$ value of 0.07 ($p < 2e{-}16$) and the NDWI with an $R^2$ value of 0.17 ($p < 2e{-}16$), both of which showed significant changes over this period. The index anomalies in time (Fig. 1D; E) suggest the occurrence of frequent extreme events in Pantanal soda lakes, including intense droughts resulting in severe water area reduction, intense rainfall leading to over-increased water areas, and episodes of uncommon dense chlorophyll content (probably associated to cyanobacterial blooms).

During the wet season (2018), $CH_4$ was lower in comparison to the dry season (2019), with the ET and CVO lakes showing the highest emission. $CH_4$ emissions ranged from 8.63 to 96.28 mmol $m^{-2}$ $day^{-1}$ for ET and 25.79 to 53.23 mmol $m^{-2}$ $day^{-1}$ for CVO (Fig. 2; Suppl. Table 3). In comparison, OT lake had the lowest $CH_4$ emission, which ranged between 0.08 and 0.39 mmol $m^{-2}$ $day^{-1}$. The patterns of $CO_2$ emissions showed a different trend, being higher in CVO and OT lakes. Fluctuations of this gas varied between 44.12 and 139.54 mmol $m^{-2}$ $day^{-1}$ for OT and 81.96 to 119.82 mmol $m^{-2}$ $day^{-1}$ for CVO. Intriguingly, the ET lake displayed a reduced or negative $CO_2$ flux, which varied among seasons. In the dry season, ET lake indicated a $CO_2$ fixation pattern (−33.11 mmol $m^{-2}$ $day^{-1}$), while in the wet season a low $CO_2$ emission was observed (19.32 mmol $m^{-2}$ $day^{-1}$). In terms of $N_2O$, considerably low values were found, varying between −12.76 and 11.17 μmol $m^{-2}$ $day^{-1}$. Moreover, no trend was observed among lakes and seasons. It was interesting to note that some samples showed a $N_2O$ consumption, for example, the OT and CVO in the dry season, and ET and CVO in the wet season. Overall, when evaluating the total carbon emission by the average equivalent $CO_2$ fluxes (considering the Global Warming Potential - GWP), we found that the major emissions occurred in ET lake, and the emissions also were higher in general during the dry season. The average $CO_2$ equivalent emissions for the wet and dry season varied from 283.69 to 2377.48 mmol $m^{-2}$ $day^{-1}$ for ET, 105.92 to 74.58 mmol $m^{-2}$ $day^{-1}$ for OT, and 878.68 to 1486.42 mmol $m^{-2}$ $day^{-1}$ for CVO lake.

### 3.2. The detected microbial genes associated with biogeochemical functions

Genes associated with the cycling of essential nutrients, including carbon, nitrogen, sulfur, and phosphorus, were detected in all evaluated soda lakes (Fig. 3A). The central pathways associated with carbon and nitrogen metabolism were present in all samples, with TCA

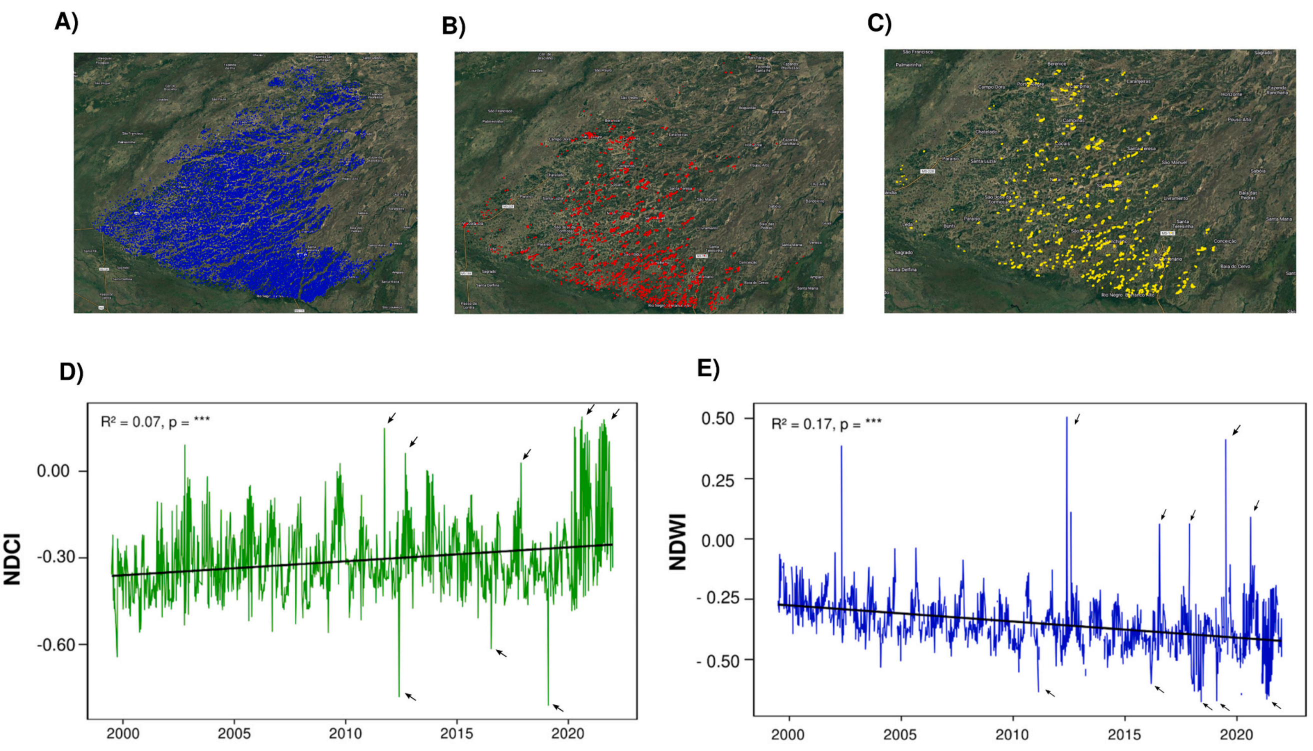


**Fig. 1.** A map portraying the delineations of all lakes within the Nhecolândia region (A), a map highlighting the specific locations of soda lakes (B), a map illustrating soda lakes experiencing cyanobacterial blooms (C), a temporal series of the Normalized Difference Chlorophyll Index (NDCI) between 2000 and 2022 in the soda lake region (D), and a temporal series of the Normalized Difference Water Index (NDWI) for the same period and region (E).

(tricarboxylic acid cycle) and Glycolysis being prevalent in oligotrophic lakes (OT and CVO), and photosynthesis and $CO_2$ fixation being enriched in ET lake, probably associated with the bloom of cyanobacteria. Methanogenesis and methanotrophy genes showed low relative frequency in the evaluated soda lakes, but they were more representative in OT and CVO lakes. Moreover, the *mcrABG* genes were not found in any sample of our evaluated period. The nitrification process, especially the ammonia oxidation step, appeared underrepresented in almost all lakes. The CVO lake exhibited a lower relative abundance of nitrogen cycling genes than the other lakes; however, the predominant functions were similar with the other lakes, including dissimilatory and assimilatory pathways of conversion of nitrite/nitrate to ammonia and denitrification. It is worth noting that nitrogen fixation seems to be prevalent in the ET lake.

Seasonality had varying impacts on the relative abundance of genes linked to the nitrogen cycle, with ET and CVO lakes exhibiting fluctuations across seasons, while the OT lake remained relatively constant. In ET lake, nitrogen fixation genes were enriched in the extremely dry season (2017), while genes associated with dissimilatory and assimilatory pathways of nitrite/nitrate to ammonia and denitrification, particularly the conversion of nitrite to nitric oxide, were prevalent in the wet (2018) and the intermediary (2019) season. The CVO lake experienced an enrichment of genes related to converting ammonia to hydroxylamine, which is the initial step of nitrification, during the wet season (2018).

As observed for nitrogen and carbon cycles, the central pathways associated with sulfur and phosphorus transformations were detected in all soda lakes. Sulfide oxidation, reduction of sulfate to sulfite, and assimilatory and dissimilatory pathways of conversion of sulfate transformations and thiosulfate oxidation were the most prevalent pathways associated with sulfur metabolism. The oxidative pathways of $H_2S$ and $S_2O_3^{-2}$ were concentrated in the CVO lake. Seasonality also impacted sulfur renewing, with the enrichment of assimilatory and dissimilatory sulfate reactions in the wet season (2018). Considering the dimethylsulfoniopropionate (DMSP) metabolism, the DMSP demethylation, which consists of the conversion of DMSP to methylmercaptopropionate and methanethiol, was overrepresented in lakes OT and CVO.

Regarding phosphorus metabolism, the CVO and OT lakes primarily exhibited mechanisms linked to phosphorus uptake, notably transporters, and the solubilization of inorganic forms of phosphorus. Conversely, the ET lake was enriched with genes associated with the metabolism of organic phosphorus sources, including genes associated with alkaline phosphatases, which were pronounced during the dry season. The presence of phosphorus regulation genes increased during the wet seasons in all lakes. The transport of phosphorus was more elevated in the OT and CVO lakes, with higher abundances observed during the dry season for OT and the wet season for CVO. In the ET lake, phosphorus transport was more pronounced during the wet period.

### 3.3. *The microbial community taxonomy associated with biogeochemical cycling genes*

In the unique environment of tropical soda lakes, a wide array of microorganisms plays a critical role in biogeochemical cycling, crucial for maintaining ecological balance. The Cyanobacteriota and Pseudomonadota phyla play a crucial role in photosynthesis, utilizing both oxygenic and anoxygenic pathways respectively (Fig. 3B). Genes

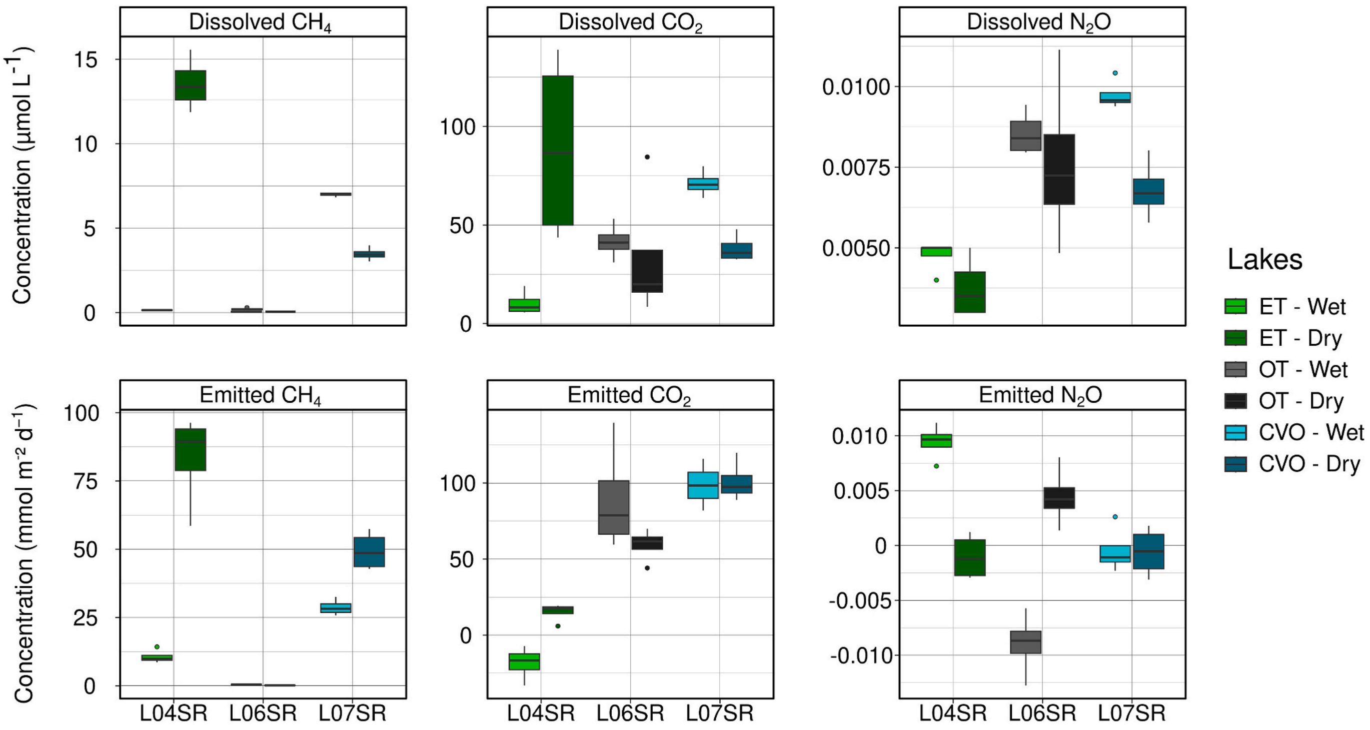


**Fig. 2.** Concentration of greenhouse gases ($CH_4$, $CO_2$, and $N_2O$) in the water column (as dissolved gas) and at the air-water interface (as emitted gas) in the three studied lakes (ET, OT, and CVO) (A). The box plot includes all data points representing gas concentration over the evaluation period for each lake type.

associated with the carbon cycle including the Calvin-Benson-Bassham (CBB) and the 3-Hidroxypropionate (THB) were found in some Cyanobacteriota members, but also to members of Cytophagia, Flavobacteriia, Acidimicrobiia, Actinomycetia, and Nitriliruptoria, showcasing a diverse array of organisms partaking in this process. Additionally, fermentation pathways have been linked to microorganisms from Pseudomonadota, Bacteroidota, and Actinomycetota, highlighting their role in organic matter decomposition and energy release.

The nitrogen cycle emerges as a dynamic and vital process in soda lakes, with a prominent contribution from Pseudomonadota, particularly the Betaproteobacteria class. Acidimicrobiia, Actinomycetia, and Nitriliruptoria also play significant roles in this cycle, particularly in the reduction of nitrate to nitrite, both in dissimilatory and assimilatory pathways. Bacteroidota and Cyanobacteriota, while less prominent, are still integral to this cycle. Nitrogen fixation, a crucial ecological process, is seen across various phyla including Pseudomonadota, Cyanobacteriota, Bacillota, and Actinomycetota. Notably, Nostocales, Synechococcales, Actinomycetia, and Betaproteobacteria stand out for their substantial contributions to this process.

Sulfur cycling, another pivotal ecological function, is influenced by a wide range of taxa, with Pseudomonadota (especially Betaproteobacteria) and Actinomycetia displaying a versatile array of genes related to this process. This indicates a broad capacity for both sulfur oxidation and reduction, essential for sulfur balance in these environments. Phosphorus solubilization, particularly of inorganic forms, is notably conducted by Pseudomonadota, with Betaproteobacteria being particularly active, along with Oscillatoriales and Synechococcales, Cytophagia, Acidimicrobiia, Actinomycetia, and Nitriliruptoria. Organic phosphorus mineralization, crucial for recycling this nutrient, involves classes such as Alphaproteobacteria, Planctomycetia, Nostocales, Cytophagia, and Saprospiria.

### 3.4. *The functional potential and taxonomic profile of the bacterial community along the pH gradient*

pH, $NH_4^+$, alkalinity, DOC, and water temperature were detected as prominent factors in modulating the distribution and occurrence of microbial functionality associated with biogeochemical cycling in Pantanal's soda lakes. The highest contribution was made by pH (Suppl. Fig. 2C), justifying the selection of this variable deep inside the microbial potential functionality and taxonomy dynamic. Although all evaluated lakes had high pH values (controlled by carbonate and bicarbonate concentrations), this variable differed for them, as explained previously (Supp. Table 1).

As the pH increases, genes related to GHG emissions such as "Fermentation to formate → $CO_2 + H_2$" and "Methanogenesis - $CO_2 \rightarrow CH_4$" decreased its relative abundance. In contrast, genes such as "Photosystem I and II", "Nitrogen Fixation", "Dissimilatory Nitrate Reduction", "Assimilatory Nitrate Reduction", "Denitrification", "Organic Phosphorus Mineralization", "Phosphorus Solubilization", "Sulfhydrogenase", "Alternative Thiosulfate Oxidation", "Thiosulfate Disproportionation" and "Sulfur Dioxygenase" showed increase their relative abundance under the same conditions (Suppl. Fig. 3). Interestingly, the conversion of nitric oxide to nitrous oxide (*norBC*) exhibits optimal occurrence at pH 9, while the conversion of nitrous oxide to dinitrogen exhibits optimal occurrence at pH 9.4.

### 3.5. *Predicted metabolites and enzymes in the water column*

Analyzing the predicted carbohydrate-active enzymes (CAZymes) and flux balance analysis (FBA) dataset we could observe a robust statistical difference in both lake types and during seasons (Figs. 4A–B, 5A–B). The ordination pattern of these two groups of dataset suggests that these lakes have a distinct metabolic profile in water column, which can impact or reflect differences in the microbial community.

In the ET lake during both the extremely dry and wet seasons, a significantly elevated PL (Polysaccharide Lyases) value was discerned when compared to other CAZymes categories (Fig. 4C). Interestingly, the dry season in the ET lake revealed an equitability among CAZymes categories, suggesting a steady-state microbial activity under such conditions. The OT lake during the extremely dry season displayed elevated AA (auxiliary activities) and GT (glycosyltransferases) values.

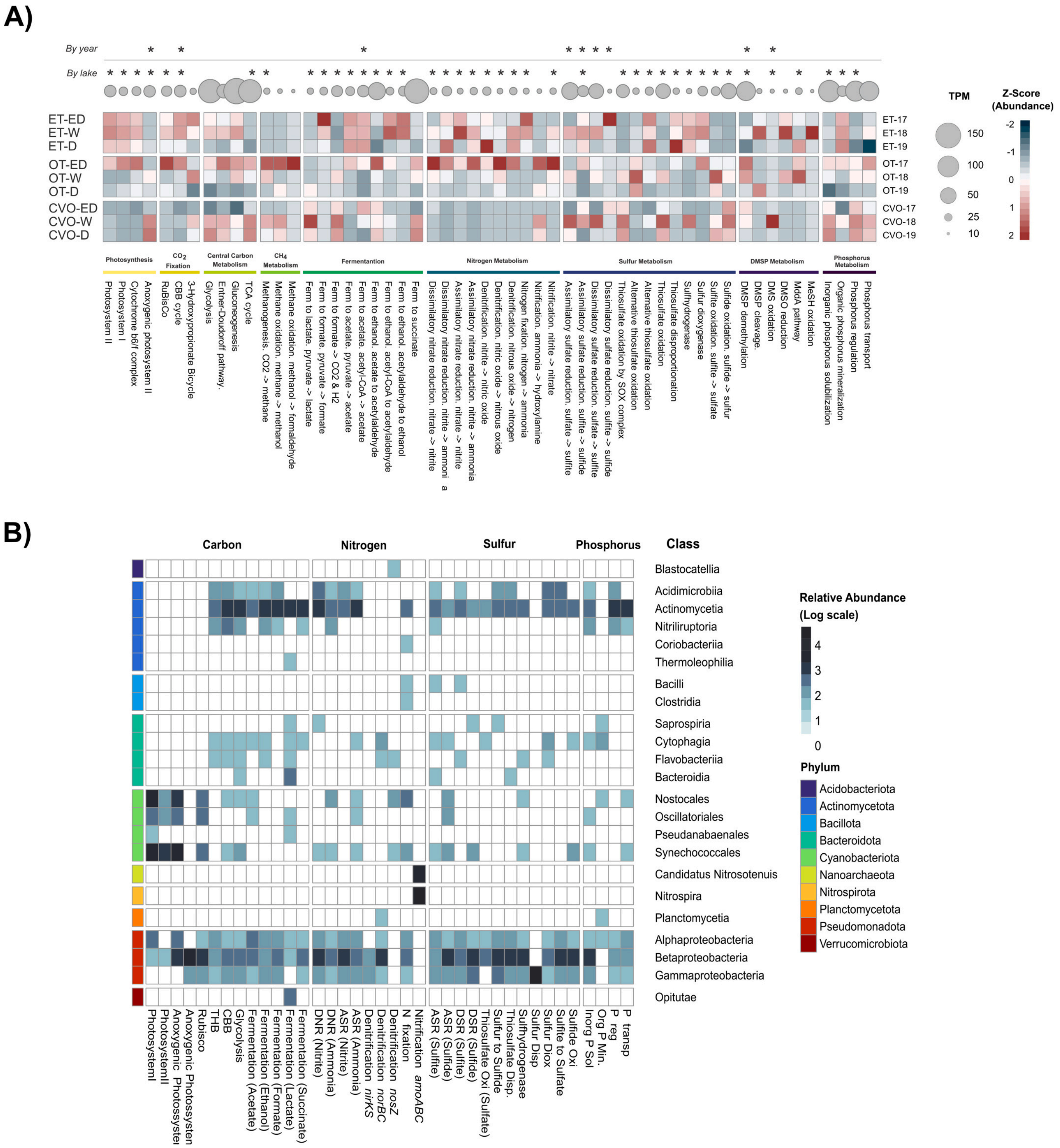


**Fig. 3.** Integrated Heatmaps of Gene Abundances in Biogeochemical Cycles of Soda Lakes. A) The heatmap delineates the relative abundances of genes involved in the cycling of carbon, nitrogen, sulfur, DMSP, and phosphorus by year and lake type, with color intensity from dark blue to dark red signifying a range from low to high abundance normalized by transcript per million (TPM). Average gene abundances are indicated by circle size, with asterisks denoting statistical significance. B) Taxon-specific gene abundances for the same biogeochemical cycles are shown, with color intensity on a log scale from white to dark blue representing relative abundance. The side bars classify genes by carbon, nitrogen, sulfur, and phosphorus functions and taxonomic class and phylum, providing a clear visualization of distribution patterns across different microbial classes and phyla.

However, the wet season in the OT lake witnessed a decline across all CAZymes categories. Notably, the dry season in OT lake was characterized by increased PL values, again pointing to an environmental adaptation requiring specific polysaccharide modifications. The CVO lake during the extremely dry season stands out with pronounced values across all CAZymes, particularly CBM (carbohydrate-binding modules). The CVO lake showed a stabilized CAZymes profile during the wet season, indicating a microbial community resilient to water column changes. Furthermore, the surge in AA and CE (carbohydrate esterases) values during the dry conditions of the CVO lake alludes to an

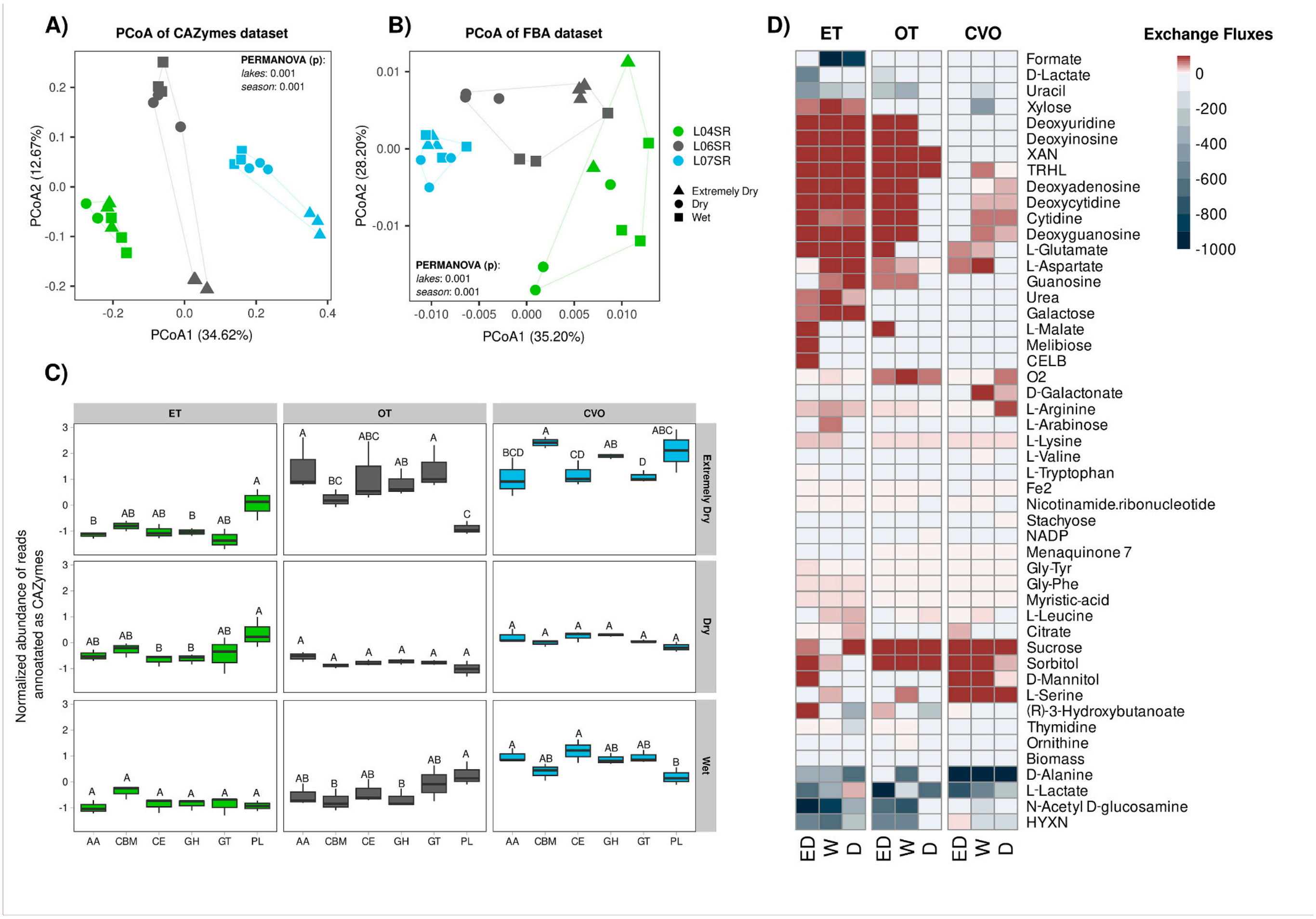


**Fig. 4.** Comparative insights into flux balance analysis (FBA) patterns and carbohydrate-active enzyme (CAZyme) profiles across distinct soda lake water columns. (A) Principal Coordinate Analysis (PCoA) visualization of the CAZymes dataset; (B) PCoA representation of the FBA dataset; (C) Boxplot illustrating the relative abundance of CAZymes across different lakes and seasons; (D) Heatmap showcasing the exchange fluxes for each compound in the respective lakes.

environment where de-acetylation processes and other ester bond cleavages are paramount, possibly in response to a more diverse carbohydrate substrate availability.

In analyzing predicted metabolites in water using flux balance analysis, distinct patterns of compound excretion and absorption were observed among the three different lake types, (Fig. 4D). Compounds excreted by the whole community were indicated by negative exchange values, whereas absorbed compounds were marked by positive exchange values. Regarding absorption, a consistent salinity gradient pattern was noted across the lakes, following the order ET → OT → CVO. Lakes with higher salinity levels exhibited increased absorption of various compounds. Specifically, ET lake showed a notable absorption of a diverse array of compounds throughout different seasons, including multiple amino acids and sugars such as xylose, xanthine, trehalose, galactose, urea, L-aspartate, L-glutamate, L-leucine, and L-lactate. In contrast, OT lake absorbed a narrower range of compounds compared to ET, with a noticeable decrease in absorption during the dry season. This lake absorbed compounds like xanthine, trehalose, L-glutamate, L-aspartate, and L-malate. Additionally, compounds such as sucrose, sorbitol, and L-serine were uniquely absorbed in OT lake, unlike in ET. CVO lake, while demonstrating a more limited absorption spectrum and lower absorption rates of xanthine, trehalose, and L-glutamate compared to ET and OT, showed a higher uptake of D-galactonate, sucrose, sorbitol, D-mannitol, and L-serine.

In terms of excreted compounds, certain trends were apparent. ET lake exhibited increased excretion of formate and N-Acetyl D-glucosamine. Other compounds, including D-Alanine, L-Lactate (notably in ET and W), and hypoxanthine, were also excreted, albeit at lower levels compared to OT and CVO. OT lake demonstrated significant excretion of these compounds in both ED and D conditions, while CVO lake showed a pronounced excretion of D-alanine, L-lactate, and xylose.

## 4. Discussion

Although soda lakes in the Pantanal are less prevalent compared to freshwater lakes, they exhibit significant diversity in their limnological parameters and microbial communities. At least three distinct types of soda lakes have been identified in Pantanal according to their water chemistry and microbial composition: Eutrophic Turbid (ET), Oligotrophic Turbid (OT) and Clear Vegetated Oligotrophic (CVO) lakes (Pellegrinetti et al., 2023). Previous studies have also highlighted distinctions in microbial structure and water chemistry composition among geographically close soda lakes (Lanzén et al., 2013; Simachew et al., 2016; Szabó et al., 2020; Zorz et al., 2019). The biogeochemical microbial gene profiles similarly adhere to this observed pattern. Nearest soda lakes, characterized by variable chemical and physical attributes, also manifest distinct functional profiles (Zorz et al., 2019). Specifically, in the case of Pantanal soda lakes, the preeminent biogeochemical functions associated with the microbial community were similar to those observed in other soda lakes worldwide. Processes such as photosynthesis, central carbon metabolism, and sulfur pathways exhibited potential prevalence, as indicated in previous studies (Sorokin et al., 2015; Zorz et al., 2019). Inversely, functions such as nitrification and nitrogen loss through denitrification appeared to be less widespread in

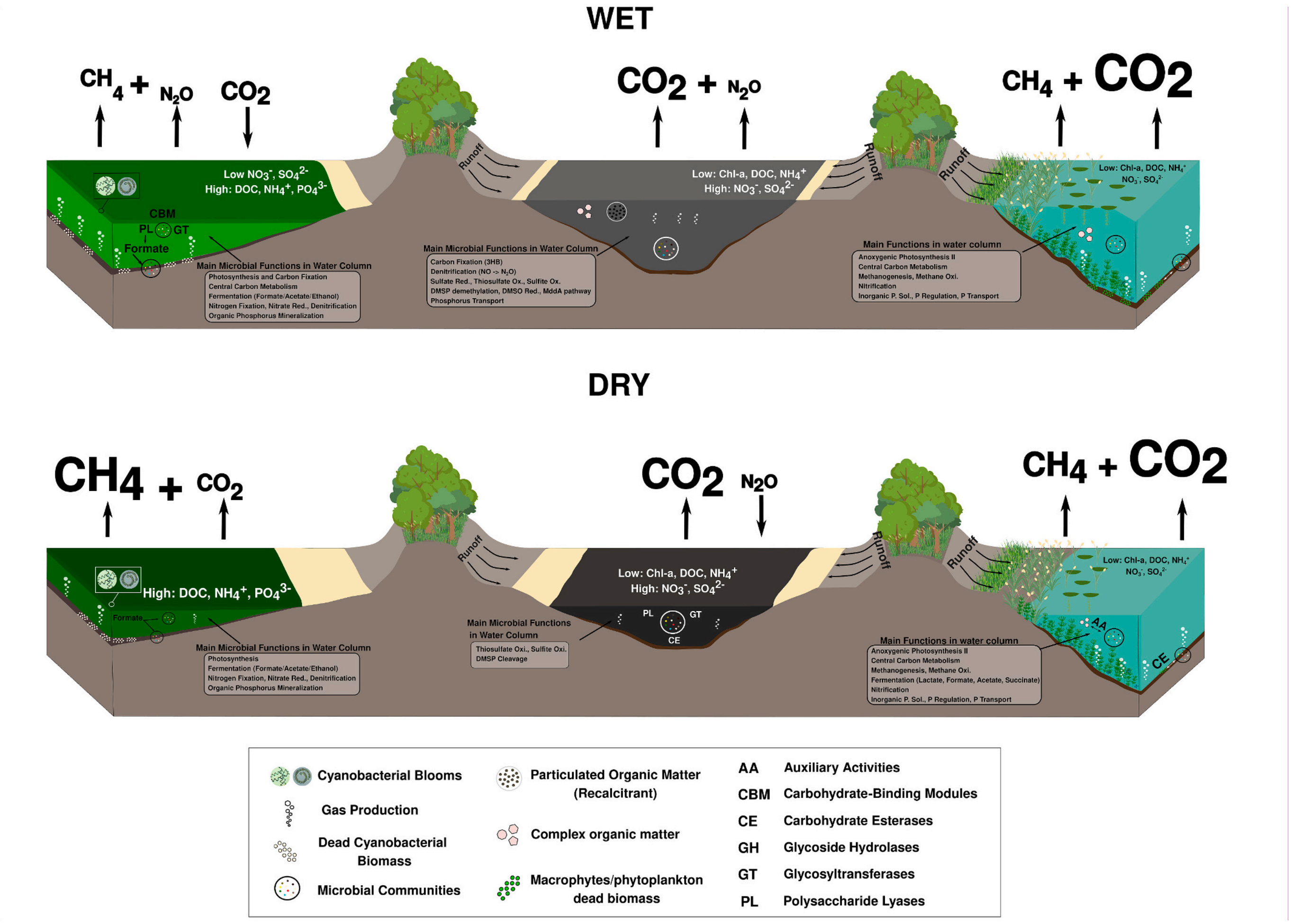


**Fig. 5.** Schematic overview of GHG dynamics and microbial functions in soda lake ecosystems across hydrological seasons. This diagram presents a comparative snapshot of the primary microbial functions, GHG balance, and CAZyme activity in lake ecosystems during wet (upper) and dry (lower) periods. Key indicators such as dissolved organic carbon (DOC), chlorophyll-a (Chl-a), and nutrient levels (ammonium, nitrate, phosphate, and sulfate ions) are contrasted between seasons. The arrows indicate changes in the GHG fluxes. Each symbol below correspond to specific elements and CAZymes involved in the carbon processing, linking environmental conditions to microbial metabolic activities.

comparison, as noted in the works of Sorokin et al. (2014) and Zorz et al. (2019).

Due to its eutrophicated status, the ET lake was enriched in functions associated with this characteristic, such as carbon and nitrogen fixation, nitrate reduction, organic phosphorus mineralization, and sulfur oxidation, while OT lake was enriched in functions linked to mineral and ions metabolization such as DMSP demethylation and phosphorus uptake (transporters). However, fluctuations in the relative abundance of functional genes are primarily attributable to seasonality. As environmental conditions shift, so do the microbial composition and functions capabilities within these ecosystems. As evidenced by the random forest analysis, parameters such as osmotic stress (salinity and alkalinity) and dissolved nutrients ($NH_4^+$ and DOC), beyond pH, emerged as robust predictors of biogeochemical profiles. Osmotic stress has been previously identified as a potent environmental factor influencing microbial behavior in soda lakes. The heterotrophic bacteria inhabiting the ET and OT lakes employ adaptive strategies linked to stress resistance, particularly during periods of extreme drought (Cotta et al., 2022). The nutritional status additionally undergoes seasonal variations due to the pronounced effect of intense evapotranspiration (Pellegrinetti et al., 2023). In the ET lake, an augmentation of biological nitrogen fixation is discernible during periods of extreme drought. Conversely, functions linked to denitrification, as well as nitrate/nitrite assimilative and dissimilative pathways, exhibit enrichment during both dry and rainy periods. Moreover, alterations in phosphorus metabolism are evident, with the prevalence of phosphorus transport mechanisms in the OT and CVO lakes during drought and rainfall, respectively. In response to these variables, the microbial communities inhabiting soda lakes reorganize their metabolic pathways to adapt their lifestyles and ensure survival in this highly dynamic environment. Moreover, it is noteworthy to emphasize that the behavior and lifestyles of microbial communities have repercussions on various environmental factors, including the gas dynamics in the atmosphere (Banda et al., 2020; Ersoy Omeroglu et al., 2021; Li et al., 2022a).

Greenhouse gas (GHG) fluxes exhibited notable variation across different lake types and seasons, influenced in part by taxonomy and biogeochemical gene composition of the microbial community. For example, $CO_2$ emissions in ET lake were exceptionally low, a phenomenon likely linked to the $CO_2$ assimilation into biomass by cyanobacteria and chemolithotroph bacteria. The high dissolved inorganic carbon concentrations from sodium carbonates in alkaline soda lakes contribute to the cyanobacteria massive biomass productivity (Burian et al., 2013; Zorz et al., 2019) mainly by mechanisms of carbon concentration and pH homeostasis (Kupriyanova et al., 2013; Schagerl and Burian, 2016). Additionally, these microorganisms exhibit diverse mechanisms for nutrient acquisition and resistance, including reductive sulfur compounds, nitrogen fixation, arsenic reduction, gas vesicles and others (Schagerl and Burian, 2016; Viana et al., 2023; Zhao et al., 2020). In the anoxic or low-oxygen zones of the lakes, fermentation by microorganisms, such as the conversion of formate to $CO_2$, contributes to the overall $CO_2$ emissions from these lakes. Microbial taxa like Acidimicrobiia, Actinomycetia, Nitriliruptoria, and Betaproteobacteria were assigned to these metabolic pathways. Conversely, in the ET lake, taxa such as Alpha- and Gammaproteobacteria, along with members of the Cytophagia, Flavobacteria, and Bacteroidia, were more prominently linked to these pathways. Regarding $N_2O$, we observed minimal fluxes, both positive and negative. This aligns with expectations given the low nitrogen concentration availability in these environments, leading to efficient nitrogen utilization and minimal loss due to the low abundance of genes related to nitrification and denitrification pathways. This finding was corroborated by the results from proteomics analyses of Cariboo Plateau soda lakes, which reported no detection of nitrification and denitrification proteins (Zorz et al., 2019).

Substantial $CH_4$, emissions were found in both ET and CVO lakes, with ET lake ranging from 8.63 to 96.28 mmol $m^{-2}$ $d^{-1}$ and CVO lake from 25.79 to 57.36 mmol $m^{-2}$ $d^{-1}$. These findings notably surpass those from other aquatic environments, such as alpine thermokarst lakes during ice-free periods (13.4 ± 1.5 mmol $m^{-2}$ $d^{-1}$) (Yang et al., 2023), the Baltic Sea (2.5 mmol $m^{-2}$ $d^{-1}$) and a range of freshwater ecosystems (between 250 nmol $m^{-2}$ $d^{-1}$ and 4.47 mmol $m^{-2}$ $d^{-1}$) (Gerardo-Nieto et al., 2017; Günthel et al., 2019; Humborg et al., 2019). In contrast, our study found lower dissolved methane concentrations, picking at 13.54 μmol $L^{-1}$. This contrast to the higher dissolved methane levels reported in soda lakes in Kenya and Russia, which varied between 156 and 700 μmol $L^{-1}$ (Fazi et al., 2021; Samylina et al., 2023). Although these studies did not directly measure methane fluxes, the elevated dissolved values imply that global methane emissions from soda lakes could be notably high.

Our analysis indicated that substantial methane emissions were also not linked to archaeal methanogenesis, as indicated by the lack of *mcrABG* genes in the water column. Instead, a potential correlation between methane emissions and the presence of Cyanobacteriota (*Arthrospira platensis* and *Anabaenopsis elenkinii*) in ET lake, and macrophytes in CVO lake were found. However, it is important to consider that we did not evaluate methanogenic genes in the sediment samples, which could be a likely source of methane. It is postulated therefore that biological, physical and chemical interactions in the water column are influencing methane production in the sediment layers. In these layers, $CH_4$ production may be stimulated by reduced oxygen levels and biomass decomposition in anoxic zones. An indicator of anoxic decomposition of organic matter is the description of a high abundance of recalcitrant organic matter (fulvic and phenolic acids) at the top of the sediments of soda lakes in the Pantanal region (Mariot et al., 2007). Organic matter from autotrophs, including cyanobacteria and macrophytes, could directly supply methanogens and fermenters with biomass, indirectly leading to hypoxia or anoxia (Rabalais et al., 2010; Welkie et al., 2019). This aligns with recent studies in freshwater lakes, where eutrophication has been shown to boost primary production, leading to increased methanogenic activity in sediments as phytoplankton biomass or macrophytes detritus accumulate (dos Santos Fonseca et al., 2017; Luo et al., 2020; Sun et al., 2021; Yang et al., 2015; Yvon-Durocher et al., 2011). Interestingly, as observed for nitrification and denitrification, the detection of methane oxidation genes such as *mmo*X and *pmo*A genes in the water column was low or absent in the analyzed samples. While this pattern could be linked to environmental factors such as high pH and salinity, the absence of such annotations may also originate from limitations in KO-based annotations and the lack of close representatives of microorganisms from extreme environments in sequence databases.

Predictions from metabolic modelling clarify some reactions and fluxes in the water column from the whole community. Compounds potentially being excreted such as formate and N-Acetyl D-Glucosamine in ET lake which could serve as crucial substrates for $CH_4$ production, further support our hypothesis. These substrates excreted by microbial community could serve as sources for methanogenesis and other metabolisms in Archaea as observed in other studies (Riemann and Azam, 2002; Schink et al., 2017; Vigneron et al., 2015; Wüst et al., 2009). Additionally, enzymes involved in the breakdown of polysaccharides (PL) and complex carbohydrates like lignin and cellulose (AA, CBM, CE) could support the degradation of macrophytes and recalcitrant materials in sediments. The decomposition of pectin from leaf litter in peatlands for example was shown to stimulate methane production rates (Corteselli et al., 2017; Yavitt et al., 2019). Moreover, a recent study in marine sediments suggests that *Bathyarchaeota* grows with lignin as an energy source, and bicarbonate as a carbon source (Yu et al., 2018). This degradation process, yet not well understood, likely contributes to the formation of substrates that are conducive to methane production in soda lakes.

In contrast we noted that some elements were shown to inhibit methanogenesis in the water column. For example, in OT lake, $CH_4$ levels were considerably low, and high levels of sulfate and nitrate, and low DOC values were found. This could be attributed to the presence of sulfate-and nitrate-reducing prokaryotes, which can compete with methanogens for organic substrates (Paulo et al., 2015; Yi et al., 2020). These prokaryotes use sulfate and nitrate, respectively, as electron acceptors in their metabolic processes, effectively outcompeting methanogens and thus suppressing methane production. In contrast, the ET and CVO lakes, which had lower concentrations of sulfate and nitrate, showed higher methane emissions. The reduced competition for substrates in these environments likely favors methanogenesis, as methanogenic archaea become the dominant microbial group utilizing the available organic matter for methane production.

Pantanal had passing by frequent extreme hydrological events, intensifying periods of both drought and heavy rainfall. Notably, the severity and frequency of these occurrences have amplified over the past five years. These episodes, marked by substantial hydrological shifts, as highlighted by our satellite analysis, have resulted in the desiccation of numerous lakes and recurrent loss of vegetation due to annual wildfires. These events primarily stem from climate shifts and extreme droughts, resulting from reduced transport of warm, humid summer air from the Amazon to the Pantanal (Bergier et al., 2018; Marengo et al., 2021). Moreover, our satellite data analysis from the past two decades reveals a marked increase in chlorophyll content, predominantly due to cyanobacterial blooms, which reflects a global pattern observed in both oceans and freshwater lakes, where ecosystems are being reshaped by rising temperatures and increased $CO_2$ levels (Paerl and Huisman, 2008; Visser et al., 2016). While the constraints inherent in satellite image analysis and pH-based delineation of soda lakes, including factors like salt composition, seasonal variations, daytime photosynthetic activity, and model precision, are recognized, this method provides crucial insights into the potential impacts of environmental changes and biogeochemical processes. Satellite imagery enables the acquisition of comprehensive data, including water depth, surface area, pigment concentration, turbidity, temperature, chlorophyll levels, cyanobacterial bloom dynamics, and alterations in land cover. These parameters are critical for elucidating both immediate and long-term effects on biogeochemical cycles and processes.

The extreme climate conditions in Nhecolândia lakes could significantly affect their functioning and GHG emissions. During prolonged and severe drought periods, this scenario might lead to heightened methane emissions and an increase in average $CO_2$ equivalent emissions. Elevated air temperatures, coupled with increased $pCO_2$ levels, could exacerbate the occurrence of cyanobacterial blooms, consequently accelerating organic matter decomposition and methane emissions in sediments (Bastviken et al., 2008). In contrast, extreme rainfall events in Nhecolândia resulting in increased water level can also bring different and indirect effects, including increase in methane emissions, by increasing water surface area and reducing sediment oxygen. For instance, heavy rains can load more terrestrial organic matter, input nutrients and metals and increase $CH_4$ and $CO_2$ emissions (Barel et al., 2021; Neumann et al., 2019). These episodes underscore the pressing need for concerted conservation efforts and proactive preservation measures to curb the intensification of GHG emissions. Despite the existing challenges, the importance of preserving the Pantanal, a biome that supports numerous global ecological processes, cannot be overstated. Soda lakes, due to their limitless inorganic carbon from the high concentration of dissolved carbonate and bicarbonate in water, act as significant natural carbon storage. Therefore, the protection and conservation of these habitats indirectly contribute to carbon sequestration efforts.

In conclusion, our study provides new insights into the complex biogenic factors influencing GHG emissions from tropical soda lakes. This underscores the significance of such ecosystems in global GHG dynamics and highlights the urgent need to further investigate these environments. These findings stress the importance of integrating microbial community composition and function into GHG emission models to provide a more holistic understanding of these ecosystems and their potential responses to future environmental changes.

## CRediT authorship contribution statement

**Thierry A. Pellegrinetti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simone R. Cotta:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yara B. Feitosa:** Writing – review & editing, Validation, Methodology, Investigation. **Paul L.A. Melo:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Wanderlei Bieluczyk:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Antonio M.M. Silva:** Writing – review & editing, Formal analysis. **Lucas W. Mendes:** Writing – review & editing, Validation, Formal analysis. **Hugo Sarmento:** Writing – review & editing, Validation, Methodology, Data curation, Conceptualization. **Plinio B. Camargo:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Siu M. Tsai:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition. **Marli F. Fiore:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The obtained sequences were deposited on the MG-RAST server and are publicly available under projects "mgp86324", "mgp88859" and "mgp92377" for respectively years (2017, 2018, and 2019).

## Acknowledgments

This work was funded by The São Paulo Research Foundation (FAPESP) (# 2016/14227-5). Thierry A. Pellegrinetti received a fellowship from FAPESP (Process 2017/12644-0). Simone R. Cotta received a fellowship from FAPESP (Process 2018/24049-2). We also acknowledge the São Roque Farm's staff for their support during the sampling collection period in Nhecolândia. We are thankful to Ary Tavares Rezende-Filho and Laurent Barbiero for helping us to reach and understand Nhecolândia soda lakes functioning. We also thanks to Juliana S. Costa, Luiz A. Joaquim and Moacir Tuzzin de Moraes for helping in sampling and analysis.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174646.

## References

Andreote, A.P.D., Dini-Andreote, F., Rigonato, J., Machineski, G.S., Souza, B. de C. e, Barbiero, L., Rezende-Filho, A.T., Fiore, M.F., 2018. Contrasting the genetic patterns of microbial communities in soda lakes with and without cyanobacterial bloom. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.00244.

Antony, C.P., Kumaresan, D., Hunger, S., Drake, H.L., Murrell, J.C., Shouche, Y.S., 2012. Microbiology of Lonar Lake and other soda lakes. ISME J. 7 (3) https://doi.org/10.1038/ismej.2012.137.

Arkin, A.P., Cottingham, R.W., Henry, C.S., Harris, N.L., Stevens, R.L., Maslov, S., Dehal, P., Ware, D., Perez, F., Canon, S., 2018. KBase: the United States department of energy systems biology knowledgebase. Nat. Biotechnol. 36 (7), 566–569.

Baker, M.E., King, R.S., Kahle, D., Kahle, M.D., 2015. Package 'TITAN2'.

Banda, J.F., Lu, Y., Hao, C., Pei, L., Du, Z., Zhang, Y., Wei, P., Dong, H., 2020. The effects of salinity and pH on microbial community diversity and distribution pattern in the brines of Soda Lakes in Badain Jaran Desert, China. Geomicrobiol. J. 37 (1), 1–12.

Barbiero, L., Siqueira Neto, M., Braz, R.R., Carmo, J.B. do, Rezende Filho, A.T., Mazzi, E., Fernandes, F.A., Damatto, S.R., Camargo, P.B. de, 2018. Biogeochemical diversity, O2-supersaturation and hot moments of GHG emissions from shallow alkaline lakes in the Pantanal of Nhecolândia, Brazil. Sci. Total Environ. 619–620, 1420–1430. https://doi.org/10.1016/j.scitotenv.2017.11.197.

Barel, J.M., Moulia, V., Hamard, S., Sytiuk, A., Jassey, V.E., 2021. Come rain, come shine: peatland carbon dynamics shift under extreme precipitation. Front. Environ. Sci. 233.

Bastviken, D., Cole, J.J., Pace, M.L., Van de Bogert, M.C., 2008. Fates of methane from different lake habitats: connecting whole-lake budgets and CH4 emissions. J. Geophys. Res. Biogeo. 113 (G2).

Bergier, I., Assine, M.L., McGlue, M.M., Alho, C.J., Silva, A., Guerreiro, R.L., Carvalho, J. C., 2018. Amazon rainforest modulation of water security in the Pantanal wetland. Sci. Total Environ. 619, 1116–1125.

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci. Rep. 5 (1), 8365.

Burian, A., Schagerl, M., Yasindi, A., 2013. Microzooplankton feeding behaviour: grazing on the microbial and the classical food web of African soda lakes. Hydrobiologia 710, 61–72.

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 37 (suppl_1), D233–D238.

Coelho-Junior, M.G., Diele-Viegas, L.M., Calheiros, D.F., Silva Neto, E.C., Fearnside, P. M., Ferrante, L., 2022. Pantanal port licence would threaten the world's largest tropical wetland. Nat. Ecol. Evol. 6 (5), 484–485.

Corteselli, E.M., Burtis, J.C., Heinz, A.K., Yavitt, J.B., 2017. Leaf litter fuels methanogenesis throughout decomposition in a forested peatland. Ecosystems 20 (6), 1217–1232. https://doi.org/10.1007/s10021-016-0105-9.

Cotta, S.R., Pellegrinetti, T.A., Andreote, A.P.D., Costa, J.S., Sarmento, H., Fiore, M.F., 2022. Disentangling the lifestyle of bacterial communities in tropical soda lakes. Sci. Rep. 12 (1), 1–10.

Dijkstra, F.A., Morgan, J.A., Follett, R.F., Lecain, D.R., 2013. Climate change reduces the net sink of CH4 and N2O in a semiarid grassland. Glob. Chang. Biol. 19 (6), 1816–1826.

dos Santos Fonseca, A.L., Marinho, C.C., de Assis Esteves, F., 2017. Potential methane production associated with aquatic macrophytes detritus in a tropical coastal lagoon. Wetlands 37 (4), 763–771. https://doi.org/10.1007/s13157-017-0912-6.

Ersoy Omeroglu, E., Sudagidan, M., Yurt, M.N.Z., Tasbasi, B.B., Acar, E.E., Ozalp, V.C., 2021. Microbial community of soda Lake Van as obtained from direct and enriched water, sediment and fish samples. Sci. Rep. 11 (1), 18364.

Fazi, S., Amalfitano, S., Venturi, S., Pacini, N., Vazquez, E., Olaka, L.A., Tassi, F., Crognale, S., Herzsprung, P., Lechtenfeld, O.J., 2021. High concentrations of dissolved biogenic methane associated with cyanobacterial blooms in East African lake surface water. Commun. Biol. 4 (1), 845.

Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., Ferreira, M.E.B., 2018. Package 'ExpDes. Pt'.

Gerardo-Nieto, O., Astorga-España, M.S., Mansilla, A., Thalasso, F., 2017. Initial report on methane and carbon dioxide emission dynamics from sub-Antarctic freshwater ecosystems: a seasonal study of a lake and a reservoir. Sci. Total Environ. 593, 144–154.

Guerreiro, R.L., Bergier, I., McGlue, M.M., Warren, L.V., Abreu, U.G.P. de, Abrahão, J., Assine, M.L., 2019. The soda lakes of Nhecolândia: a conservation opportunity for the Pantanal wetlands. Perspect. Ecol. Conserv. 17 (1), 9–18. https://doi.org/10.1016/j.pecon.2018.11.002.

Günthel, M., Donis, D., Kirillin, G., Ionescu, D., Bizic, M., McGinnis, D.F., Grossart, H.P., Tang, K.W., 2019. Contribution of oxic methane production to surface methane emission in lakes and its global importance. Nat. Commun. 10 (1), 5497.

Hamilton, S.K., Sippel, S.J., Melack, J.M., 1995. Oxygen depletion and carbon dioxide and methane production in waters of the Pantanal wetland of Brazil. Biogeochemistry 30 (2), 115–141. https://doi.org/10.1007/BF00002727.

Humborg, C., Geibel, M.C., Sun, X., McCrackin, M., Mörth, C.M., Stranne, C., Jakobsson, M., Gustafsson, B., Sokolov, A., Norkko, A., Norkko, J., 2019. High emissions of carbon dioxide and methane from the coastal Baltic Sea at the end of a summer heat wave. Front. Mar. Sci. 6, 493.

Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11 (1), 119. https://doi.org/10.1186/1471-2105-11-119.

Kupriyanova, E., Sinetova, M., Cho, S., Park, Y.-I., Los, D., Pronina, N., 2013. CO2-concentrating mechanism in cyanobacterial photosynthesis: organization, physiological role, and evolutionary origin. Photosynth. Res. 117, 133–146. https://doi.org/10.1007/s11120-013-9860-z.

Lanzén, A., Simachew, A., Gessesse, A., Chmolowska, D., Jonassen, I., Øvreås, L., 2013. Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. PloS One 8 (8). https://doi.org/10.1371/journal.pone.0072577.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., Lam, T.-W., 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102, 3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.

Li, X., Yang, M., Mu, T., Miao, D., Liu, J., Xing, J., 2022a. Composition and key-influencing factors of bacterial communities active in sulfur cycling of soda lake sediments. Arch. Microbiol. 204 (6), 317.

Li, W., Yuan, L., Lan, X., Cui, J., Chen, Y., Chen, H., Hu, B., Feng, D., 2022b. Methane and nitrous oxide production and their driving factors in Phragmites riparian wetlands of Dianchi Lake, China. Ecol. Indic. 145, 109696.

Lu, W., Xiao, J., Liu, F., Zhang, Y., Liu, C., Lin, G., 2017. Contrasting ecosystem CO2 fluxes of inland and coastal wetlands: a meta-analysis of eddy covariance data. Glob. Chang. Biol. 23 (3), 1180–1198.

Luo, P., Tong, X., Liu, F., Huang, M., Xu, J., Xiao, R., Wu, J., 2020. Nutrients release and greenhouse gas emission during decomposition of Myriophyllum aquaticum in a sediment-water system. Environ. Pollut. 260, 114015 https://doi.org/10.1016/j.envpol.2020.114015.

Marengo, J.A., Cunha, A.P., Cuartas, L.A., Deusdará Leal, K.R., Broedel, E., Seluchi, M.E., Michelin, C.M., De Praga Baião, C.F., Chuchón Ângulo, E., Almeida, E.K., 2021. Extreme drought in the Brazilian Pantanal in 2019–2020: characterization, causes, and impacts. Front. Water 3, 639204.

Mariot, M., Dudal, Y., Furian, S., Sakamoto, A., Vallès, V., Fort, M., Barbiero, L., 2007. Dissolved organic matter fluorescence as a water-flow tracer in the tropical wetland of Pantanal of Nhecolândia, Brazil. Sci. Total Environ. 388 (1–3), 184–193.

Marques, J.F., Alves, M.B., Silveira, C.F., Amaral e Silva, A., Silva, T.A., dos Santos, V.J., Calijuri, M.L., 2021. Fires dynamics in the Pantanal: impacts of anthropogenic activities and climate change. J. Environ. Manage. 299, 113586 https://doi.org/10.1016/j.jenvman.2021.113586.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal 17 (1). https://doi.org/10.14806/ej.17.1.200.

Mitsch, W.J., Bernal, B., Nahlik, A.M., Mander, Ü., Zhang, L., Anderson, C.J., Jørgensen, S.E., Brix, H., 2013. Wetlands, carbon, and climate change. Landsc. Ecol. 28, 583–597.

Neumann, R.B., Moorberg, C.J., Lundquist, J.D., Turner, J.C., Waldrop, M.P., McFarland, J.W., Euskirchen, E.S., Edgar, C.W., Turetsky, M.R., 2019. Warming effects of spring rainfall increase methane emissions from thawing permafrost. Geophys. Res. Lett. 46 (3), 1393–1401.

Nunes, A.P., 2011. Quantas espécies de aves ocorrem no Pantanal brasileiro. Atualidades Ornitológicas 160, 45–54.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community Ecology Package, Version, 2 (9), 1–295.

Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320 (5872), 57–58.

Paulo, L.M., Stams, A.J.M., Sousa, D.Z., 2015. Methanogens, sulphate and heavy metals: a complex system. Rev. Environ. Sci. Biotechnol. 14 (4), 537–553. https://doi.org/10.1007/s11157-015-9387-1.

Pellegrinetti, T.A., Cotta, S.R., Sarmento, H., Costa, J.S., Delbaje, E., Montes, C.R., Camargo, P.B., Barbiero, L., Rezende-Filho, A.T., Fiore, M.F., 2023. Bacterial communities along environmental gradients in tropical soda lakes. Microb. Ecol. 85 (3), 892–903. https://doi.org/10.1007/s00248-022-02086-6.

Pereira, O.J., Merino, E.R., Montes, C.R., Barbiero, L., Rezende-Filho, A.T., Lucas, Y., Melfi, A.J., 2020. Estimating water pH using cloud-based landsat images for a new classification of the Nhecolândia Lakes (Brazilian Pantanal). Remote Sens. 12 (7), 1090.

Petrenko, P., Lobb, B., Kurtz, D.A., Neufeld, J.D., Doxey, A.C., 2015. MetAnnotate: function-specific taxonomic profiling and comparison of metagenomes. BMC Biol. 13 (1), 92. https://doi.org/10.1186/s12915-015-0195-4.

Pott, V.J., Pott, A., Lima, L.C.P., Moreira, S.N., Oliveira, A.K., 2011. Aquatic macrophyte diversity of the Pantanal wetland and upper basin. Braz. J. Biol. 71, 255–263.

Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7 (2), 585–619.

RColorBrewer, S., Liaw, M.A., 2018. Package 'randomforest'. University of California, Berkeley, Berkeley, CA, USA.

Riemann, L., Azam, F., 2002. Widespread N-acetyl-d-glucosamine uptake among pelagic marine bacteria and its ecological implications. Appl. Environ. Microbiol. 68 (11), 5554–5562. https://doi.org/10.1128/AEM.68.11.5554-5562.2002.

Rosentreter, J.A., Al-Haj, A.N., Fulweiler, R.W., Williamson, P., 2021. Methane and nitrous oxide emissions complicate coastal blue carbon assessments. Global Biogeochem. Cycles 35 (2), e2020GB006858.

Samylina, O.S., Merkel, A.Y., Pimenov, N.V., 2023. Diurnal methane dynamics in the cyanobacterial community of soda lake bitter 1 (Kulunda Steppe, Altai Krai). Microbiology 92 (2), 293–299.

Schagerl, M., Burian, A., 2016. The Ecology of African Soda Lakes: Driven by Variable and Extreme Conditions, pp. 295–320. https://doi.org/10.1007/978-3-319-28622-8_12.

Schink, B., Montag, D., Keller, A., Müller, N., 2017. Hydrogen or formate: alternative key players in methanogenic degradation. Environ. Microbiol. Rep. 9 (3), 189–202. https://doi.org/10.1111/1758-2229.12524.

Seaver, S.M., Liu, F., Zhang, Q., Jeffryes, J., Faria, J.P., Edirisinghe, J.N., Mundy, M., Chia, N., Noor, E., Beber, M.E., 2021. The ModelSEED Biochemistry Database for the integration of metabolic annotations and the reconstruction, comparison and analysis of metabolic models for plants, fungi and microbes. Nucleic Acids Res. 49 (D1), D575–D588.

Simachew, A., Lanzén, A., Gessesse, A., Øvreås, L., 2016. Prokaryotic community diversity along an increasing salt gradient in a soda ash concentration pond. Microb. Ecol. 71 (2), 326–338. https://doi.org/10.1007/s00248-015-0675-7.

Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D., Muyzer, G., 2014. Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18, 791–809.

Sorokin, D.Y., Banciu, H.L., Muyzer, G., 2015. Functional microbiology of soda lakes. Curr. Opin. Microbiol. 25 https://doi.org/10.1016/j.mib.2015.05.004.

Sun, H., Lu, X., Yu, R., Yang, J., Liu, X., Cao, Z., Zhang, Z., Li, M., Geng, Y., 2021. Eutrophication decreased CO2 but increased CH4 emissions from lake: a case study of a shallow Lake Ulansuhai. Water Res. 201, 117363 https://doi.org/10.1016/j.watres.2021.117363.

Szabó, A., Korponai, K., Somogyi, B., Vajna, B., Vörös, L., Horváth, Z., Boros, E., Szabó-Tugyi, N., Márialigeti, K., Felföldi, T., 2020. Grazing pressure-induced shift in planktonic bacterial communities with the dominance of acIII-A1 actinobacterial lineage in soda pans. Sci. Rep. 10 (1), 1–12.

Tian, H., Chen, G., Lu, C., Xu, X., Ren, W., Zhang, B., Banger, K., Tao, B., Pan, S., Liu, M., 2015. Global methane and nitrous oxide emissions from terrestrial ecosystems due to multiple environmental changes. Ecosyst. Health Sustain. 1 (1), 1–20.

Tomas, W.M., Berlinck, C.N., Chiaravalloti, R.M., Faggioni, G.P., Strüssmann, C., Libonati, R., Abrahão, C.R., do Valle Alvarenga, G., de Faria Bacellar, A.E., de Queiroz Batista, F.R., Bornato, T.S., Camilo, A.R., Castedo, J., Fernando, A.M.E., de Freitas, G.O., Garcia, C.M., Gonçalves, H.S., de Freitas Guilherme, M.B., Layme, V.M.G., Morato, R., 2021. Distance sampling surveys reveal 17 million vertebrates directly killed by the 2020s wildfires in the Pantanal, Brazil. Sci. Rep. 11 (1), 1 https://doi.org/10.1038/s41598-021-02844-5.

Vavourakis, C.D., Mehrshad, M., Balkema, C., Van Hall, R., Andrei, A.-Ş., Ghai, R., Sorokin, D.Y., Muyzer, G., 2019. Metagenomes and metatranscriptomes shed new light on the microbial-mediated sulfur cycle in a Siberian soda lake. BMC Biol. 17, 1–20.

Viana, J.L.M., Steffler, D.A., Hernández, A.H., dos Santos Costa, J., Pellegrinetti, T.A., de Jesus, E.C.R., Cancian, M., Fiore, M.F., Rezende-Filho, A.T., Sussulini, A., Barbiero, L., Menegario, A.A., Fostier, A.H., 2023. Bioaccumulation and speciation of arsenic in plankton from tropical soda lakes along a salinity gradient. Sci. Total Environ. 895, 165189 https://doi.org/10.1016/j.scitotenv.2023.165189.

Vigneron, A., L'Haridon, S., Godfroy, A., Roussel, E.G., Cragg, B.A., Parkes, R.J., Toffin, L., 2015. Evidence of active methanogen communities in shallow sediments of the Sonora Margin cold seeps. Appl. Environ. Microbiol. 81 (10), 3451–3459.

Visser, P.M., Verspagen, J.M., Sandrini, G., Stal, L.J., Matthijs, H.C., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145–159.

Voigt, C., Lamprecht, R.E., Marushchak, M.E., Lind, S.E., Novakovskiy, A., Aurela, M., Martikainen, P.J., Biasi, C., 2017. Warming of subarctic tundra increases emissions of all three important greenhouse gases–carbon dioxide, methane, and nitrous oxide. Glob. Chang. Biol. 23 (8), 3121–3138.

Welkie, D.G., Rubin, B.E., Diamond, S., Hood, R.D., Savage, D.F., Golden, S.S., 2019. A hard day's night: Cyanobacteria in diel cycles. Trends Microbiol. 27 (3), 231–242.

Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The aligned rank transform for nonparametric factorial analyses using only anova procedures. Proc. SIGCHI Conf. Hum. Factors Comput. Syst. 143–146.

Wüst, P.K., Horn, M.A., Drake, H.L., 2009. Trophic links between fermenters and methanogens in a moderately acidic fen soil. Environ. Microbiol. 11 (6), 1395–1409.

Xue, C.-X., Lin, H., Zhu, X.-Y., Liu, J., Zhang, Y., Rowley, G., Todd, J.D., Li, M., Zhang, X.-H., 2021. DiTing: a pipeline to infer and compare biogeochemical pathways from metagenomic and metatranscriptomic data. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.698286.

Yang, H., Andersen, T., Dörsch, P., Tominaga, K., Thrane, J.-E., Hessen, D.O., 2015. Greenhouse gas metabolism in Nordic boreal lakes. Biogeochemistry 126, 211–225.

Yang, G., Zheng, Z., Abbott, B.W., Olefeldt, D., Knoblauch, C., Song, Y., Kang, L., Qin, S., Peng, Y., Yang, Y., 2023. Characteristics of methane emissions from alpine thermokarst lakes on the Tibetan Plateau. Nat. Commun. 14 (1), 3121.

Yavitt, J.B., Kryczka, A.K., Huber, M.E., Pipes, G.T., Rodriguez, A.M., 2019. Inferring methane production by decomposing tree, shrub, and grass leaf litter in bog and rich fen peatlands. Front. Environ. Sci. 7. https://www.frontiersin.org/articles/10.3389/fenvs.2019.00182.

Yi, X., Wu, R., Han, D., Li, Y., Li, X., Niu, G., Huang, M., Ying, G.-G., 2020. Adaptation of methane recovery, sludge characteristics and evolution of microbial community response to elevated nitrate under the methanogenic condition. J. Clean. Prod. 258, 120713 https://doi.org/10.1016/j.jclepro.2020.120713.

Yu, T., Wu, W., Liang, W., Lever, M.A., Hinrichs, K.-U., Wang, F., 2018. Growth of sedimentary Bathyarchaeota on lignin as an energy source. Proc. Natl. Acad. Sci. 115 (23), 6022–6027. https://doi.org/10.1073/pnas.1718854115.

Yvon-Durocher, G., Montoya, J.M., Woodward, G., Jones, J.I., Trimmer, M., 2011. Warming increases the proportion of primary production emitted as methane from freshwater mesocosms. Glob. Chang. Biol. 17 (2), 1225–1234. https://doi.org/10.1111/j.1365-2486.2010.02289.x.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2013. PEAR: a fast and accurate Illumina paired-end reAd mergeR. Bioinformatics 30 (5). https://doi.org/10.1093/bioinformatics/btt593.

Zhao, D., Zhang, S., Xue, Q., Chen, J., Zhou, J., Cheng, F., Li, M., Zhu, Y., Yu, H., Hu, S., Zheng, Y., Liu, S., Xiang, H., 2020. Abundant taxa and favorable pathways in the microbiome of soda-saline lakes in Inner Mongolia. Front. Microbiol. 11. https://www.frontiersin.org/articles/10.3389/fmicb.2020.01740.

Zhbannikov, I.Y., Hunter, S.T., Foster, J.A., Settles, M.L., 2017. SeqyClean. In: Proceedings of the 8th ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics. https://doi.org/10.1145/3107411.3107446.

Zheng, J., Ge, Q., Yan, Y., Zhang, X., Huang, L., Yin, Y., 2023. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. Nucleic Acids Res. 51 (W1), W115–W121.

Zorz, J.K., Sharp, C., Kleiner, M., Gordon, P.M.K., Pon, R.T., Dong, X., Strous, M., 2019. A shared core microbiome in soda lakes separated by large distances. Nat. Commun. 10 (1), 1 https://doi.org/10.1038/s41467-019-12195-5.