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Exploratory analysis of the relationship between the water quality and the microbiome in Santa Catarina reservoir, Querétaro, Mexico

Estudio exploratorio de la relación entre la calidad del agua y el microbioma de la presa Santa Catarina, Querétaro, México

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ABSTRACT

The Santa Catarina Dam (Querétaro, Mexico) is a freshwater reservoir supporting agriculture and aquaculture, but anthropogenic pressure has intensified nutrient enrichment and contamination. Water samples were collected from two points for physicochemical characterization and shotgun metagenomic profiling. DNA sequences were quality-filtered, assembled, and classified to quantify taxonomic composition and functional gene abundance. Processed data were statistically analyzed in Python 3.0 to compare physicochemical parameters, taxa, and gene abundance. Results showed that chemical oxygen demand (COD) and hydrogen potential (pH) exceeded recommended limits. Fecal coliform counts (FCC) were high at both points, while biochemical oxygen demand (BOD_5) and total nitrogen (TN) approached critical thresholds. Significant differences in water temperature (WT), dissolved oxygen (DO), and total

phosphorus (TP) were observed between sites. The microbial communities presented high diversity and a moderate dissimilarity among points. Besides, three cyanobacterial species were identified within the top 10 most abundant taxa. Functional annotation of the combined dataset revealed high gene abundance associated with energy production (35.40%), stress resistance (18.00%), and unknown functions (14.54%). These results indicate an eutrophic state of the water body and potential health risks. Differential statistical analysis revealed site-specific taxa and genes, several of which may have biotechnological applications.

Keywords: Artificial reservoir, metagenomics shotgun, microbiome, water quality.

RESUMEN

La Presa Santa Catarina (Querétaro, México) es un reservorio de agua dulce que sustenta actividades agrícolas y acuícolas; sin embargo, la presión antropogénica ha intensificado el enriquecimiento de nutrientes y la contaminación. Se recolectaron muestras de agua en dos puntos para su caracterización fisicoquímica y el perfil metagenómico shotgun. Las secuencias de ADN fueron sometidas a control de calidad, ensambladas y clasificadas para cuantificar la composición taxonómica y la abundancia de genes funcionales. Los datos procesados se analizaron estadísticamente en Python 3.0 para comparar los parámetros fisicoquímicos, los taxones y la abundancia génica. Los resultados mostraron que la demanda química de oxígeno (DQO) y el potencial de hidrógeno (pH) excedieron los límites recomendados. El conteo de coliformes fecales (CCF) fue elevado en ambos puntos, mientras que la demanda bioquímica de oxígeno (DBO_5) y el nitrógeno total (NT) se aproximaron a umbrales críticos. Se observaron diferencias significativas en la temperatura del agua (TA), el oxígeno disuelto (OD) y el fósforo total (PT) entre los sitios. Las comunidades microbianas presentaron alta diversidad y una disimilitud moderada entre puntos; además, se identificaron tres especies de cianobacterias dentro de los 10 taxones más abundantes. La anotación funcional del conjunto de datos combinado reveló alta abundancia de genes asociados con la producción de energía (35.40 %), la resistencia al estrés (18.00 %) y funciones desconocidas (14.54 %). Estos resultados indican un estado eutrófico del cuerpo de agua y posibles riesgos para la salud. El análisis estadístico diferencial reveló taxones y genes específicos de cada sitio, varios de los cuales podrían tener aplicaciones biotecnológicas.

Palabras clave: Calidad del agua, microbioma, metagenómica shotgun, reservorio artificial.

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1. INTRODUCTION

Water is an essential natural resource of high value, whose quality has been progressively impacted by anthropogenic factors. These factors represent significant threats to environmental conditions, highlighting the importance of monitoring both biotic and abiotic indicators. In Mexico, the Water Quality Management Department of the National Water Commission of Mexico (CONAGUA, by its Spanish acronym) monitors various physicochemical and microbiological parameters to classify surface water bodies using a color-coded system. Green indicates water of the highest quality, meeting all measured parameters; yellow represents cases where at least one non-critical parameter exceeds the permissible limits; and red denotes water with at least one critical parameter beyond acceptable thresholds (CONAGUA, 2007). In 2023, 450 surface water bodies were evaluated, of which 27.3% were classified as green, 21.8% as yellow, and 50.9% as red (CONAGUA, 2025), underscoring the importance of continuous monitoring and the implementation of effective strategies to preserve water resources.

The Santa Catarina reservoir, located in Querétaro, Mexico, has a storage capacity of 9.60 million m^3 and supports multiple uses, including fishing, agricultural irrigation, and recreation (Trejo-Sánchez, 2021). Despite its hydrological and socioeconomic importance, this reservoir lacks systematic monitoring for both water volume and quality. Previous studies have reported elevated concentrations of nutrients, like TN and TP, in the reservoir (López-Flores *et al*., 2025), which can lead to eutrophic states. These kinds of factors might substantially shape microbial communities, altering the presence and interdomain interactions among bacteria, archaea, and eukaryotes (Cheung *et al*., 2018). The microbiome in eutrophic and contaminated water bodies is commonly dominated by specific resistant microorganisms that are capable of thriving under these stressful conditions and actively participate in biogeochemical cycles.

Photosynthetic members, such as microalgae and cyanobacteria, proliferate under eutrophic conditions, leading to phytoplankton blooms (Anderson, 2001) and playing a central role in the dynamics of microbial interactions, besides being widely recognized as bioindicators of ecosystemic health (Fahmi *et al*., 2025). For example, *Chlorella* spp., *Nitzschia* spp., and *Scenedesmus* spp. are common microalgal genera found in contaminated waters, in addition to blue-green cyanobacteria, like *Microcystis* spp., *Anabaena* spp., and *Aphanizomenon* spp., which can produce toxins with health implications (Paerl *et al*., 2001). However, excessive anthropogenic nutrient loading from sources such as agricultural runoff and wastewater discharges has been shown to stimulate cyanobacterial dominance relative to other phytoplankton groups (as green algae or diatoms), particularly under conditions of high nitrogen and phosphorus availability (Gobler *et al*., 2024). Also, these environments present dominance of heterotrophic bacteria implicated in the breakdown of organic matter, including members of Proteobacteria, Bacteroidetes, and Firmicutes, while opportunistic and pathogenic bacteria increase, particularly when water is impacted by non-treated discharges, such as *Vibrio* spp., *Salmonella* spp., *Escherichia coli*, and other coliforms (de Obeso Fernández del Valle & Membrillo-Hernández, 2025). Additionally, microorganisms with bioremediation capabilities can be found, capable of removing contaminants such as organic compounds, metals, and emerging contaminants (Anderson, 2001). Altogether, these findings highlight the importance of frequent monitoring of microbial communities. Nevertheless, this task faces several challenges when applying traditional microbiological protocols: not all microorganisms are culturable, their characterization requires multiple laboratory tests over extended periods, and their wide

morphological variability at the microscopic level can lead to misidentification and inaccurate ecological interpretations.

Metagenomics is a powerful tool for exploring complex biological communities, enabling the identification of both cultured and uncultured organisms, their relative abundances, and their functional capabilities. This approach provides a more accurate representation of community composition, local biogeochemical dynamics, and microbial genetic adaptation. Specifically, shotgun metagenomics allows the analysis of the collective genome of a community, enabling the precise identification of taxa and genes (Sharpton, 2014). In this study, physicochemical and metagenomic analyses were integrated to (a) assess the water quality of an artificial reservoir, (b) characterize its microbial taxonomic and functional diversity, and (c) explore the relationships between physicochemical conditions, taxa abundance, and genetic functions. Therefore, this exploratory study aims to integrate the knowledge about water physicochemical parameters and microbiome profiles in a contaminated lentic water body, in order to visualize how its eutrophic and general contaminated state, and their variations between different sampling points, influence the structure of microbial communities, the potential environmental and health risks associated with this microbial profiles, and their biotechnological capabilities that may be further prospected.

2. MATERIALS AND METHODS

2.1. Sampling site

Samples were collected during the day in September 2024 at the Santa Catarina reservoir, a freshwater system located in central Mexico. Two sampling points were established to capture spatial variability within the system. The first point, P1, was located on the southern side of the reservoir (20°47′17.5″N, 100°27′10.4″W), near the dam curtain. The second point, P2, was established on the northern side, (20°48′07.4″N, 100°27′12.6″W) (Figure 1). These sampling points were positioned at opposite ends of the reservoir to represent contrasting environmental conditions. During sampling, the surrounding area was surveyed to characterize anthropogenic features. Rural communities can be observed surrounding the water body on the right, while a small industrial park was identified near P1, and a larger one near P2. Also, farmlands are near the second sampling point (P2). Additionally, an ecotourism center, a ranch, and several restaurants were identified on the site.

Sampling was conducted in accordance with the Mexican standard NMX-AA-003-1980. Water was collected at a depth of 25 cm below the surface. All samples were properly labeled and transported under refrigeration to the laboratory, where they were stored at 4 °C until further analysis.

2.2. Water physicochemical determinations

Water physicochemical parameters (WPP) were measured in situ and in the laboratory. Dissolved oxygen (DO), electrical conductivity (EC), hydrogen potential (pH), turbidity (T), and water temperature (WT) were measured in situ using an EZ-9909SP multiparameter meter. Mexican standard procedures were used to measure the biochemical oxygen demand (BOD_5), the chemical oxygen demand (COD), total coliform counts (TCC), fecal coliform counts (FCC), and total phosphorus (TP). Chlorophyll-a (Chl-a) and total nitrogen (TN) were quantified under

the methodology described by López-Flores *et al*. (2025) and Qiu *et al*. (2018), respectively (n=3 in all cases).

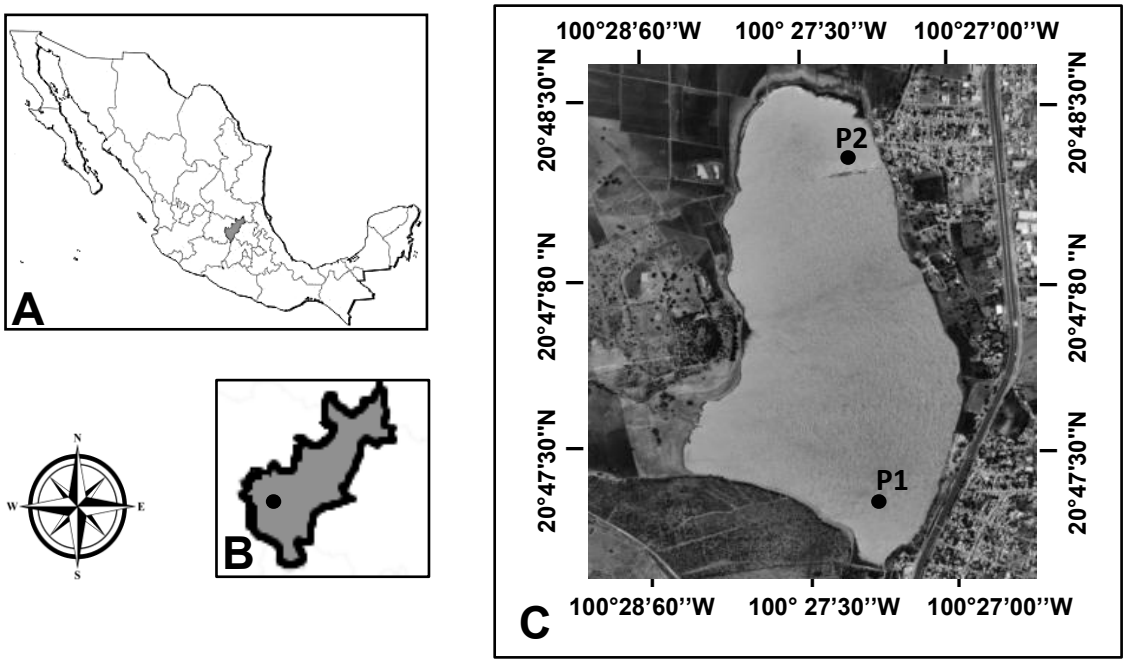

Fig. 1. Geographic location of the sampling site. The country (A), state (B), and specific site under study (C) are presented. P1 and P2 specify the sampling points. Satellite image source: Google Earth, 2025.

2.3. Preprocessing of sequences

Total DNA was extracted from each sample using the ZymoBIOMICS DNA miniprep kit (n=2, plus a negative control from the extraction process), following the protocol provided by the manufacturer. DNA quantification was performed (NanoDrop 2000c, Thermo Fisher Scientific) and shotgun sequencing was conducted by Zymo Research. The genetic libraries were provided by the company using the Illumina DNA prep kit (formerly Nextera Flex) and unique dual indexing (UDI) kits. The sequencing service was performed on the Illumina® NovaSeq 6000 platform with a depth of 10 million reads. Raw sequences were processed to check quality, remove adapters, low-quality regions, and cured from human DNA using FastQC v0.12.1 (quality control tool for high throughput sequence data by the Babraham Institute, Cambridge) (Andrews, 2010), Trimmomatic v0.39 (trimming tool by the Institute of Bio- and Geosciences, Germany) (Bolger *et al.*, 2014), and Bowtie2 v2.4.1 (published by the Institute for Advanced Computer Studies, University of Maryland, United States) (Langmead & Salzberg, 2012; Langmead *et al.*, 2018). The resulting quality-filtered reads were subsequently assembled with metaSPAdes v4.1.0 (published by the Center for Algorithmic Biotechnology, Institute for Translational Biomedicine, St. Petersburg State University, Russia) (Nurk *et al*., 2017) to generate contigs (~150 bp), which were used for downstream taxonomic and functional annotation. Results presented correspond to the averages of the two replicates processed in each case.

2.4. Taxonomic profiling

For taxonomic identification, custom databases were constructed with kraken2-build v2.1.0 tools (software developed by the Center for Computational Biology, Johns Hopkins University, United States) (Wood & Langmead, 2019) for the following taxonomic groups: bacteria (413 GB), archaea (49 GB), fungi (54 GB), and protozoa (47 GB). As custom databases for microalgae are not available, a specialized database (44 GB) was constructed by downloading sequences from the AlgaeBase website (Guiry & Guiry, 2025). Contigs were classified against the custom databases using Kraken2 v2.1.3, applying a confidence threshold of 0.3 for bacteria and 0.15 for the remaining taxonomic groups, following established recommendations (Wright *et al*., 2023). The resulting taxonomic assignments were then examined at the species level using the Pavian web platform v 2.1.3 (Breitwieser, 2021). Taxonomic abundances were estimated based on the number of times each assembled sequence was assigned to a given taxon within the reference databases (read counts). Relative abundance was calculated separately for each taxonomic group. Subsequently, all taxonomic databases were merged into a single dataset to determine the absolute abundance of each taxon, defined as the proportion that each taxon represents within the total microbial community (considering all groups). Alpha (Shannon index) and beta (Bray–Curtis dissimilarity) diversities were computed in Python v3.0 (published by the Python Software Foundation). Alpha diversity was used to estimate the diversity within each site (Willis, 2019), while beta diversity was applied to evaluate differences in taxonomic composition between sites (Sadeghi *et al.*, 2024).

2.5. Functional annotation

Functional annotation was performed as follows. MetaGeneMark v3.38 (published by the Georgia Institute of Technology, United States) was run using the program model file to predict open reading frames (ORFs) from both the prokaryotic and eukaryotic members (Gemayel *et al*., 2022). Subsequently, EggNOGMapper v1.0.1 (a tool developed in collaboration by the European Molecular Biology Laboratory, Germany, and the Swiss Institute of Bioinformatics, Switzerland), fed with MetaGenemark protein output, was used, running EggNOG Mapper with the command –itype protein, to allow the functional annotation of genes through the functions of the encoded proteins (Cantalapiedra *et al*., 2021). Finally, the obtained information was classified using the cluster of orthologous groups (COG) functional categories, also obtained in EggNOG Mapper. The data was grouped based on the COG classification to count the number of genes assigned to each category to determine the COG category frequency.

2.6. Statistical analyses

The significant differences in the WPP, taxa absolute abundances, and gene abundances between the two sampling points were estimated using Python v3.0. To this end, each variable (WPP, taxon, or gene) was first evaluated using Levene test to determine whether the variance between points was statistically significant (var=True) or not (var=False) (Yang *et al.*, 2023). Based on these results, a Student t-test was applied to the replicates of each variable to calculate both the p-value and the fold change ratio. The p-value was used to determine whether differences between points were statistically significant ($p \leq 0.05$). The magnitude of these differences was quantified by the fold change ratio, which was defined as the mean value of each variable at sampling point P1 (μ_{P1}) divided by the corresponding mean in P2 (μ_{P2}) (Dembélé

& Kastner, 2014). To improve interpretability, the logarithmic transformation of the fold change was applied to both values, the p-value and fold change ratio, presented as the log2PV and log2FC, respectively. In the case of the log2PV, the absolute value is used to maintain a positive scale. In the case of the log2FC value (Eq. 1), the use of both positive and negative scales is essential for proper data interpretation: positive values indicate higher abundances in P1, while negative values indicate higher abundances in P2. Finally, to prioritize the most significant and divergent observations in each variable (WPP, taxa, and genes), the absolute value of log2FC /log2PV ratio was calculated, with higher values denoting greater significance and larger differences between points.

$$log2FC = log_2(\frac{\mu_{P1}}{\mu_{P2}}) \quad \textbf{Eq. 1}$$

3. RESULTS

3.1. Water physicochemical determinations

WPP for P1 and P2 are presented in Table 1; maximum permissible limits (MPL) under Mexican regulation (NOM-001-SEMARNAT-2021) and by the U.S. Environmental Protection Agency (EPA) (Arizona Department of Environmental Quality) are presented for comparison purposes. Under Mexican regulation, two of the twelve parameters assessed were outside the MPL, both of which were abiotic: COD and pH. This number increases to five based on EPA recommendations, including biotic indicators: Chl-a, FCC, pH, TCC, and TN. Nutrient factors, such as COD, BOD_5, and TP, were significantly higher in P1 than in P2. Contrastingly, the significantly higher WPP in P2 were DO, EC, pH, and WT. Finally, no significant differences were found in Chl-a, FCC, T, TCC, and TN parameters between points.

3.2. Preprocessing of sequences

The total DNA concentration obtained from the samples ranged from 8 to 12 ng/µl. Sequencing quality was ≥ Q36, encompassing a range of 3.4 to 4.5 Gbp. After the cleaning and curing of sequences, 2.7 to 3.1 Gbp (~74%) were preserved in fragments between 99 and 150 bp, achieving a quality ≥ Q38. The number of final contigs ranged from 2’967,422 to 3’376,289, with N50 values > 919 bp and L50 > 75,816. The high base-call accuracy, substantial sequencing depth, and robust assembly metrics provide, together, an adequate coverage and contig length for reliable taxonomic profiling and functional annotation. Previous studies have demonstrated that sufficient sequencing depth and assembly quality are essential for accurate functional profiling in shotgun metagenomic analyses (Quince *et al*., 2017; Knight *et al*., 2018), thereby supporting the reliability of the functional inferences presented here.

3.3. Taxonomic profiling

A total of 2,607 different taxa were identified in the water samples. Analyzing each sampling point separately, 1,270 taxa were identified in P1 (52 archaea, 1,074 bacteria, 97 fungi, 15 microalgae, and 32 protozoa) and 1,243 taxa in P2 (53 archaea, 1,039 bacteria, 99 fungi, 19 microalgae, and 33 protozoa). The alpha-diversity indices were 4.81 for P1 and 4.95 for P2, indicating high taxonomic diversity at both sites, with slightly greater diversity in P2. The beta

diversity between samples (Bray–Curtis dissimilarity) was 0.31, indicating that the communities shared a large proportion of taxa and relative abundances, with moderate compositional differences, likely influenced by local environmental variation (Ochieng *et al*., 2024).

Table 1. Physicochemical characterization of the sampling points.

Parameter	Units	Mean value		Mexican MPL*	EPA limits
		P1	P2		
BOD_5	mg/L	75.33 ± 2.52[a**]	52.33 ± 3.21[b]	NE***	NE
Chl-a	mg/L	0.05 ± 0.04[a]	0.54 ± 0.32[a]	NE	0.04
COD	mg/L	201.33 ± 16.17[a]	159.67 ± 0.58[b]	140.00	NE
DO	mg/L	8.47 ± 0.31[a]	15.23 ± 0.68[b]	NE	>6.00
EC	µS/cm	574.00 ± 19.10[a]	649.00 ± 3.46[b]	NE	NE
FCC	MPN/100 mL	>2400[a]	>2400[a]	NE	14
pH	-	9.71 ± 0.03[a]	9.87 ± 0.03[b]	6.00-9.00	6.50-9.00
T	NTUs	40.05 ± 1.09[a]	40.68 ± 0.69[a]	NE	NE
TCC	NMP/100ml	>2400[a]	>2400[a]	NE	130
TN	mg/L	27.94 ± 1.80[a]	26.03 ± 1.55[a]	30.00	3.00
TP	mg/L	0.78 ± 0.00[a]	0.54 ± 0.00[b]	15.00	1.00
WT	°C	25.73 ± 0.91[a]	31.00 ± 0.20[b]	35.00	32.00

* MPL, maximum permissible limits under Mexican regulation (NOM-001-SEMARNAT-2021).
** Different lowercase letters indicate significant differences between sampling points, p≤0.05.
*** NE, not specified.

The ten most abundant taxa (based on relative abundances) within each microbial group, at each sampling point, are illustrated in Figure 2. The cumulative percentage of abundances (CP) in each case indicates the proportion of microbial groups represented by the ten most abundant taxa; considering this value, microbial groups can be segmented into three different categories: (a) strong dominance, evidenced by highly represented groups, whose ten most abundant members encompass more than 70% of the entire identified members (which is the case of

archaea, microalgae and protozoa); (b) moderate dominance, showing CP between 50-70% (bacteria); and, (c) low dominance, showing a representation below 50% of the entire identified group members (fungi).

The most abundant taxa within archaea, bacteria, and fungi were consistent between P1 and P2, corresponding to uncultured *Methanoregula* sp., *Acidovorax temperans*, and *Ustilaginoidea virens*, respectively. Uncultured *Methanoregula* spp., which was the most abundant archaea found in both sampling points, have been reported to be present in diverse aquatic environments as endosymbionts of ciliated protists (Bräuer *et al*., 2011). Members of the genus *Acidovorax* have been reported as have been reported as denitrifying bacteria that increase significantly under stress conditions in aquatic environments (Bunyoo *et al*., 2026; Fei *et al*., 2024, Liu *et al.,* 2025). *U. virens* is a fungal pathogen and the causal agent of rice false smut, capable of producing mycotoxins under favorable nitrogen and carbon sources, acidic pH conditions, and light exposure (Zhang, 2023).

Regarding eukaryotic microscopic communities, protozoa and microalgae differed markedly between samples. Among the ten most abundant microalgal taxa, only three were shared by both sites, with relative abundances higher in P1 in all cases. The detection of *Chlorella sorokiniana* and the site-exclusive *Tetradesmus obliquus* is consistent with eutrophic conditions, as both taxa are fast-growing chlorophytes commonly associated with elevated nutrient availability (Laabassi *et al*., 2026). *C. sorokiniana* has been reported as a microscope capable of reduce TN, TP and COD under high temperature rates (Rezzag *et al*., 2026). *T. obliquus* has widely been reported for several environmental purposes (Mungunkhuyag, 2026; Oliveira *et al*., 2021). Dominant protozoan taxa varied between sites, with *Plasmodium yoelii* prevailing in P1 and *Thalassiosira pseudonana* in P2. Thus, protozoan communities showed entirely distinct species compositions between sampling points: the ten most abundant species at each sample differed, indicating distinct community compositions.

To determine the overall taxonomic inter-domain abundances in the microbial communities of each sampling point, the absolute abundance percentages were assessed and are presented in Figure 3. The analysis of inter-domain abundances shows that bacteria clearly dominate the microbial communities at both sampling points, comprising the top ten taxa in absolute abundance. This reinforces the patterns observed in relative abundance, indicating that bacterial taxa are the primary drivers of community structure. In contrast, archaea and fungi, while present, contribute a smaller fraction to overall abundance, highlighting the dominant role of bacteria in shaping microbial ecosystem composition.

3.4. Functional annotation

A total of 22,592 genes were identified, of which 3,032 were exclusive to P1; 3,900 were exclusive to P2; and 15,660 were present at both sampling sites. The identified genes were classified into 23 different COG categories (Fig. 4), with 0.61% remaining unclassified. The largest fraction of genes belongs to the “S” category defined as “function unknown”, emphasizing the considerable proportion of genetic potential yet to be characterized. Among the annotated functions, genes related to metabolism, signal transduction, and energy production (E, C, T, P, and G categories) were the most abundant (with an accumulated percentage of 35.40%), reflecting the central roles of these processes in microbial community functioning. To further analyze the most abundant functions in the communities, the ten most abundant genes identified at each sampling point are described in Table 2.

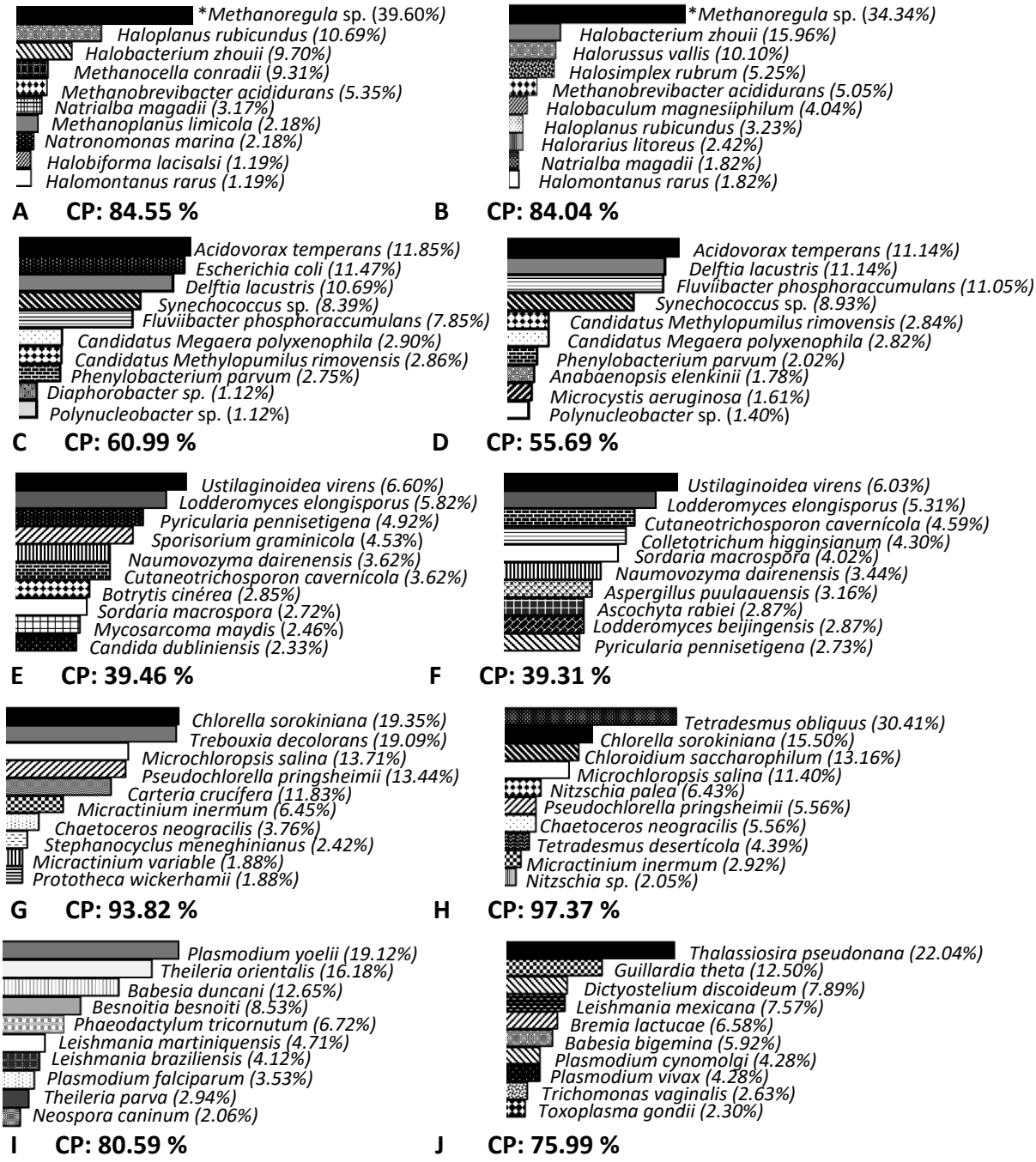

Fig. 2. Ten most abundant taxa for each microbial group in both sampling points. Archaea in P1 (A) and P2 (B); bacteria in P1 (C) and P2 (D); fungi in P1 (E) and (P2); microalgae in P1 (G) and P2 (H); and, protozoa in P1 (I) and P2 (J). Values in parentheses represent relative abundances. CP, Cumulative percentage of abundances. Uncultured species are marked (*).

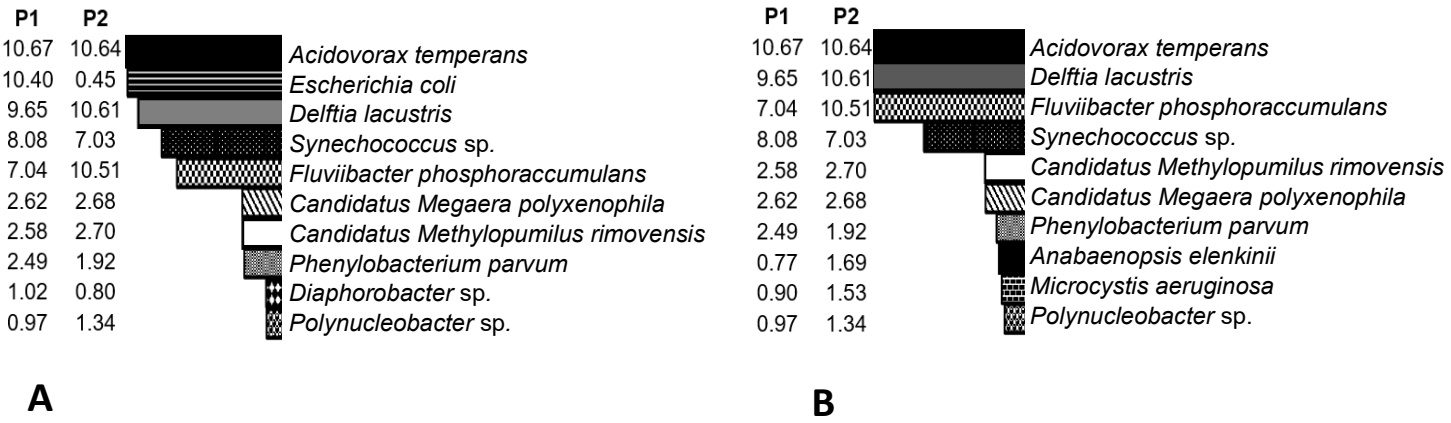

Fig. 3. Absolute abundance of the ten dominant taxa in the microbial communities for P1 (A) and P2 (B). Numbers at the left indicate the percentage values of abundances in each case.

As observed, gene *mhpA* was the most abundant in P1, while *atsA* was the most abundant in P2. Both genes are involved in the degradation of complex organic compounds; *mhpA* participates in the breakdown of lignin-derived aromatic intermediates, while *atsA* encodes an arylsulfatase, responsible for the hydrolysis of sulfur-containing ester compounds (Jo *et al*., 2000; Pollet *et al*., 2023). Besides *mhpA*, two additional genes were among the ten most abundant in both sampling sites: *MA20_05265* and *dnaE*; the first encodes for a protein of unknown function, whereas the second is involved in the DNA replication process.

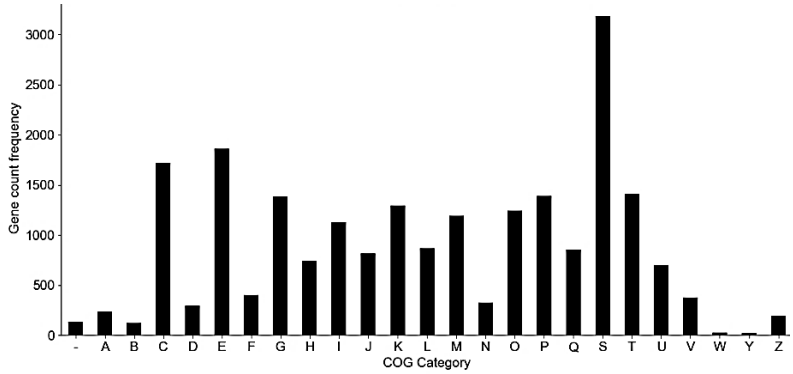

Fig. 4. Frequencies of COG categories in the metagenome, based on gene counts. COG category are defined as (-) Unclassified by the program, (A) RNA processing and modification, (B) Chromatin structure and dynamics, (C) Energy production and conversion, (D) Cell cycle control, cell division, chromosome partitioning, (E) Amino acid transport and metabolism, (F) Nucleotide transport and metabolism, (G) Carbohydrate transport and metabolism, (H) Coenzyme transport and metabolism, (I) Lipid transport and metabolism, (J) Translation, ribosomal structure and biogenesis, (K) Transcription, (L) Replication, recombination and repair, (M) Cell wall/membrane/envelope biogenesis, (N) Cell motility, (O) Post-translational modification, protein turnover, chaperons, (P) Inorganic ion transport and metabolism, (Q) Secondary metabolites biosynthesis, transport and catabolism, (R) General function prediction only, (S) Function unknown, (T) Signal transduction mechanisms, (U) Intracellular trafficking, secretion, and vesicular transport, (V) Defense mechanisms, (W) Extracellular structures, (Y) Nuclear structure, (Z) cytoskeleton.

Table 2. Ten most abundant genes identified in P1 and P2.

Gene	Counting mean P1	Counting mean P2	COG classification	Reported function	Reference
**mhpA*	2,141	1,694	C; PFAM monooxygenase FAD-binding	Metabolism; participates in the metabolism of lignin-derived phenylpropanoids.	(Xu & Zhou, 2020)
pehA	2,132	36	P; Arylsulfatase A and related enzymes	Metabolism; involved in the degradation of phthalate esters.	(Xu *et al*., 2018)
VPA0970	1,922	1	S; Protein of unknown function	Not reported function, hypothetical protein.	-
hsdR	1,901	1,228	L; Type I restriction enzyme R protein N terminus	Defense mechanism; part of the restriction–modification system that degrades foreign DNA.	(Obarska-Kosinska *et al*., 2008)
*MA20_05265	1,756	1,874	P; Protein of unknown function	Unknown function	-
livM	1,633	163	E; Belongs to the permease family	Metabolism; facilitates the ABC transporter for leucine, isoleucine, and valine.	(Wannawong *et al*., 2024)
natA	1,529	82	B; N(alpha)-acetyltransferase 16	Post-translational modification; regulates N-terminal acetylation of newly synthesized proteins.	(Zhang *et al*., 2025)
yapH	1,504	1,189	U; Autotransporter beta-domain	Substance secretion; encodes adhesins involved in microbial adhesion and surface colonization.	(Alvarez-Martinez *et al*., 2021)
exsH	1,476	894	Q; RTX toxins and related Ca^{+2} -binding proteins	Substance secretion; associated with infection or symbiotic interactions.	(York & Walker, 1997)
**dnaE*	1,462	1,579	L; DNA polymerase	Replication; participates in bacterial DNA replication.	(Vaisman *et al*., 2021)
atsA	252	2,100	P; Pfam Sulfatase	Metabolism; hydrolyzes aromatic sulfate esters in many bacteria.	(Beil *et al*., 1995)
MA20_23135	10	1,740	S; Protein of unknown function	Unknown function	-
fadD	472	1,625	I; AMP-dependent synthetase and ligase	Metabolism; binds free fatty acids to coenzyme A in order to produce energy.	(Pavoncello *et al*., 2022)
susC	594	1,458	P; PFAM TonB-dependent Receptor Plug	Metabolism; mediates oligosaccharide transport as part of the starch metabolism.	(Pollet *et al*., 2023)
addA	504	1,449	S; Protein of unknown function	DNA reparation; enables DNA repair, essential for survival under genotoxic stress.	(Amundsen & Smith, 2023)
vapC	1,436	1,435	G; Part of the toxin-antitoxin (TA) module	DNA replication; regulates gene expression under environmental stress	(Hollingshead *et al*., 2025)
tctC4	197	1,402	S; Protein conserved in bacteria	Unknown function	-

* Genes identified within the ten most abundant in both sampling points.

3.5. Differential statistical analyses

The statistical analysis contrasted P1 and P2 to quantify how different the WPP, taxon abundances, and gene abundances were between sampling points. Figure 5 illustrates the resulting associations, displaying WPP against taxonomic (Fig. 5A) and gene abundances (Fig. 5B). The black dots therein represent a specific taxon or gene with significant differences in abundance between samples; the farther a point appears from the significance threshold (horizontal dotted line), the greater its statistical relevance. In contrast, gray dots below the threshold indicate taxa or genes with no significant differences. The log2FC values, located on the X-axis, show the direction and magnitude of significant changes in abundances: positive values indicate higher abundance in P1, while negative ones indicate higher abundance in P2. Additionally, greater distance from the center indicates larger differences in abundance; therefore, the data are organized into three distinct clusters: elements (taxa or genes) enriched or exclusive in P1 (right group), shared elements with statistically significant differences (center group), and elements enriched or exclusive in P2 (left group). To highlight the ten most differentiated elements in each graph, these are indicated with lowercase letters in the figure and listed in the description. Some taxa shared identical log2FC/ log2PV ratio values, forcing them to be superimposed in Figure 5A; therefore, more than one species is indicated in the figure description (b, c, and f).

For the WPP, the analysis revealed two distinct clusters; the upper-left table in Figure 5A shows the cluster for P1 while the upper-left table in Figure 5B shows the cluster for P2. On the one hand, the nutrient-associated parameters (TP, BOD_5, and COD) showed significantly higher values at P1, whereas the physical parameters (DO, EC, pH, and WT) were significantly higher at P2. Among all variables, the most significant differential parameter is TP, followed by DO. Taxa with the highest log2FC/log2PV ratios were mostly exclusive to P2 (except *Methanosarcina barkeri*), with *Peribacillus frigoritolerans, Agromyces* sp., and *Aliiroseovarius crassostreae* ranking as the top three. *P. frigoritolerans* has been reported as a heterotrophic bacterium with plant-growth-promoting potential and tolerance to extreme conditions, including saline–alkaline stress, which can enhance plant nutrient uptake and stress resilience (Huang et al., 2026; Marik et al., 2023). Genera *Agromyces* has been frequently found in plant rhizosphere, and some strains have been reported to be alkaline resistant (Kim *et al.*, 2025; Xu *et al*., 2025). *A. crassostreae* is a bacteria known as the causative agent of roseovarius oyster disease in hatchery-raised juvenile oysters (Ulrich *et al*., 2026). These findings suggest that the microbial community in P2 is shaped by environmental stressors, including saline–alkaline conditions and other challenging site-specific factors. The presence of *A. crassostreae*, a known oyster pathogen, further indicates potential environmental risks, while the detection of taxa commonly associated with plants and soil reflects possible infiltration from surrounding landfill or terrestrial sources. These findings are consistent with other observations of P2, highlighting the influence of both environmental stress and anthropogenic inputs on microbial assemblages.

Similarly, the exploratory analysis of gene abundance revealed significant differences between sites. In this case, the genes clustered into two main groups: genes exclusive to P2 (left side of the graph) and genes present at both sites but with significantly different abundances (center of the graph). This latter group was shifted toward the left side, indicating higher overall abundance at P2, which might suggest microbial strategies associated with adaptation to punctual

physicochemical conditions. The three most significantly differential genes are *soxT*, *ptmF* and *MA20_26650*, which was detected at both sampling points, with a higher significant difference in P2. *soxT* is a sulfur-oxidation gene involved in biogeochemical cycling of sulfur, and is found in many sulfur-oxidizing bacteria (Lahiri *et al.*, 2006). Gene *ptmF* plays a key role in pactamycin biosynthesis, a secondary metabolite with antibacterial and antiprotozoal activity, whose production is highly sensitive to phosphate concentrations (Eida *et al.,* 2019; Lu *et al*., 2018). In contrast, *MA20_26650* is a gene of unknown function.

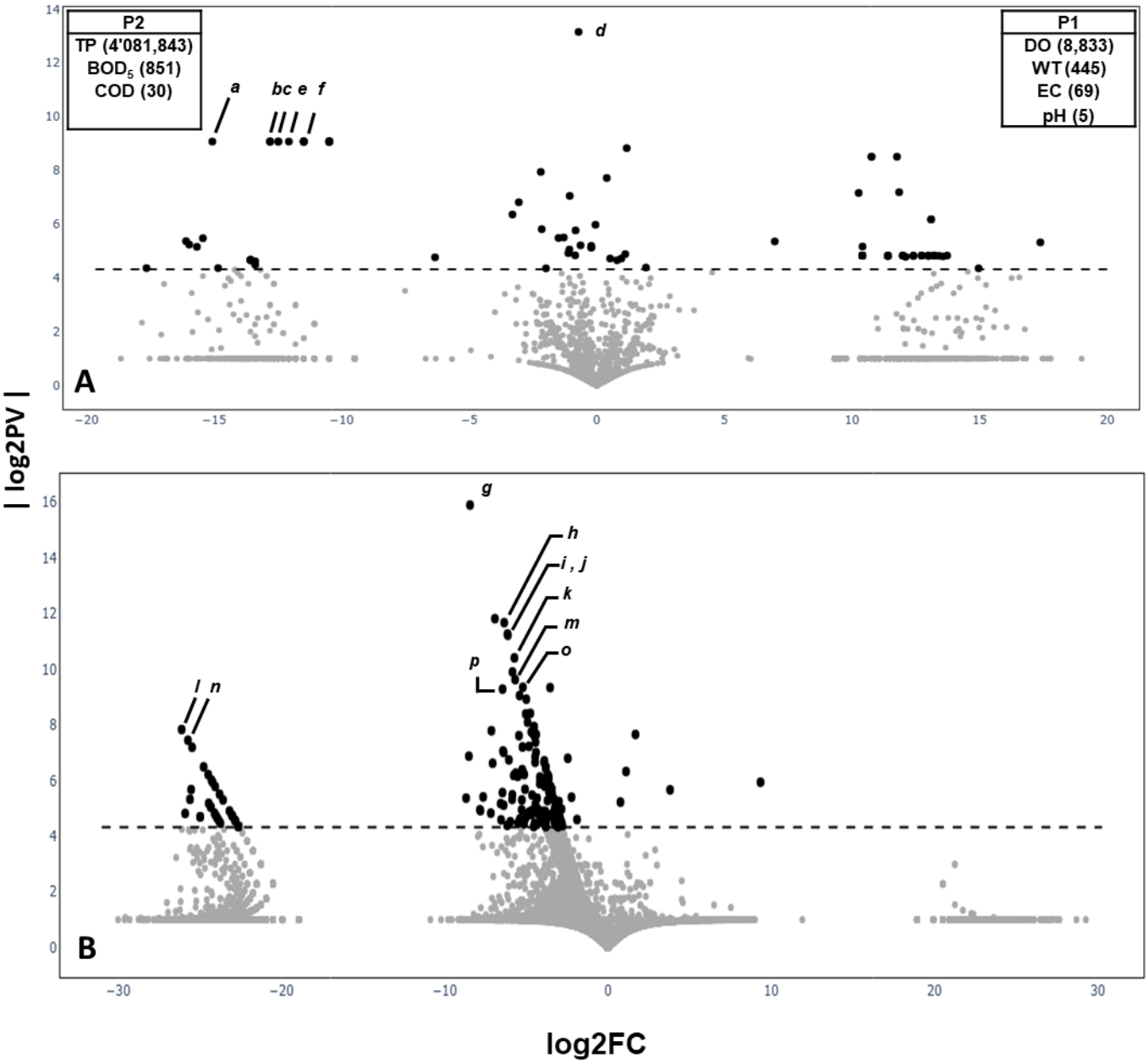

Fig. 5. Correlation analyses between (A) WPP-taxa and (B) WPP-genes. Each dot represents a taxon or a gene, respectively; significantly different values are shown in black (•) while non-

significantly different values are shown in grey (•). The log2FC/ log2PV ratio was used to rank all parameters, and the corresponding value is shown in parentheses. Arabic letters indicate the ten highest values, for taxa: (a) *Peribacillus frigoritolerans* , (b) *Agromyces* sp., *Aliiroseovarius crassostreae*, *Burkholderia contaminans*; (c) *Erwinia* sp., *Roseovarius* sp.; (d) *Methanosarcina barkeri*, (e) *Alteromonas macleodii*, (f) *Bradyrhizobium* sp., *Flavobacterium* sp.; for genes (g) *soxT*, (h) *ptmF*, (i) *MA20_26650*, (j) *bcr4*, (k) *yfiP*, (l) *higB*, (m) *araC*, (n) *MA20_09120*, (o) *CCT5*, (p) *mazF3*. The upper tables, labeled as P1 and P2, show the significantly higher WPP in each sampling point, also ranked by the log2FC/ log2PV ratio (in parentheses).

4. DISCUSSION

COD is an indicator of organic matter pollution. The values measured at both sampling sites exceeded the maximum permissible limits established by Mexican regulations, placing the Santa Catarina reservoir within the red category of the national color-coded water quality classification system. The ratio between BOD_5 and COD, known as the biodegradability index, reflects the proportion of biodegradable organic matter within the total organic load. The calculated biodegradability index ranged from 0.33 to 0.37, suggesting that the organic matter present is moderately biodegradable but approaches the threshold typically associated with low biodegradability (under 0.3) (Lingling *et al.*, 2025; Wei *et al.*, 2023). DO concentrations indicate supersaturation conditions, which are generally associated with active photosynthesis and may support aerobic heterotrophic metabolism. However, the observed BOD values did not increase proportionally. This apparent inconsistency could indicate that microbial degradation is constrained by factors other than oxygen availability or that a fraction of the organic matter is slowly degradable. DO is typically negatively correlated with WT due to solubility limitations. Nevertheless, both sampling points exhibited oxygen supersaturation under elevated temperature conditions. This pattern is consistent with intense photosynthetic activity and may explain the elevated Chl-a, DO, and pH values, particularly at P2, which are commonly reported in eutrophic aquatic systems (Díaz-Torres *et al.*,2021; García-Ramírez *et al*., 2025; Mamun *et al*., 2021; Sui *et al*., 2022; Wang *et al*., 2021; Zhang *et al*., 2019; Zepernick *et al*., 2021). Moreover, high rates of TCC and FCC were detected, indicating significant contamination by fecal matter, which represents serious environmental and public health risks, such as the enhancement of the mortality of sensitive species and the undermining of water use (Niculae *et al*., 2021).

The taxonomic profile confirmed the notorious presence of cyanobacteria (*A. elenkinii*, *M. aeruginosa*, and *Synechococcus* sp.). The high abundance of *Microcystis* and *Synechococcus* has also been reported in other eutrophic urban lakes in Mexico, characterized by similar WPP (Pineda-Mendoza *et al.*, 2020). It has been reported that *M. aeruginosa* proliferates under high-nutrient conditions (TN and TP) and generates elevated DO levels, which is used by specific aerobic heterotrophic bacteria (Coffin *et al*., 2018; Giomi *et al*., 2018; Kalra *et al*., 2025). In turn, these bacteria recycle nutrients and complement biochemical cycles necessary for *Microcystis* survival (Cai et al., 2024). The detection of cyanobacteria represents a health risk due to the possible toxin production. *M. aeruginosa* is capable of producing harmful toxins identified as

microcystins, which represent health and environmental risks, and are produced by the *mcy* gene cluster (Rajendran *et al*., 2025). The detection of the complete *mcy* gene cluster (*mcyA–J*) in this study (with high abundance of *mcyB* and *mcyH*) indicates the presence of potentially toxic *Microcystis* populations, which has been reported in other important Mexican water supplies (Tromas *et al*., 2025). It has been reported that microcystin production is influenced by environmental nutrient availability (Peck *et al.*, 2025), and that variations in microcystin levels can modify the composition of bacterial communities associated with *Microcystis* spp., favoring specific microbial taxa and driving community fluctuations (Große *et al*., 2025). Microcystins have been reported to negatively affect eukaryotic cells, including protozoa, microalgae, and fish. In particular, these toxins can inhibit microalgal growth and survival, inducing morphological alterations in exposed cells and underscoring their ecological relevance in aquatic systems (Teneva, 2023). Consequently, the elevated presence of pathogenic microorganisms and phytoplanktonic communities poses a serious threat to both the environment and local human populations that rely on the reservoir for fishing, agriculture, and recreational activities. Regarding microalgal and protozoan communities, previous studies have reported that protozoa are particularly sensitive to physicochemical conditions (Jiang *et al*., 2007). Therefore, the observed differences in the dominant protozoan taxa between sampling points may be associated with local environmental variability. Additionally, differences in the overall community composition could be influenced by localized inputs of contaminated water. This interpretation is consistent with the higher relative abundance of *E. coli* detected at P1 compared to P2 (10.40% and 0.45%, respectively), suggesting spatial heterogeneity in fecal contamination. However, further investigation would be required to establish a direct causal relationship.

Regarding taxonomic profiles, P1 exhibited a higher number of identified taxa (greater richness). Nevertheless, the alpha-diversity index was lower, indicating stronger dominance by specific taxa at this sampling point. Beta-diversity analysis showed that 69% of the community composition was shared between P1 and P2, reflecting a moderate level of similarity between the two sites (Baselga & Gómez-Rodríguez, 2019). Eutrophication has been reported to alter beta diversity by promoting species homogenization across freshwater ecosystems worldwide. This process is often reflected in the dominance of selective taxa, such as cyanobacteria, and is associated with an increased representation of genes related to stress response and core metabolic functions (Silva *et al*., 2025). In Mexico, several studies have documented the dominance of cyanobacteria, particularly *Microcystis*, across different geographic regions, highlighting the widespread nature of this environmental problem (Díaz-Torres *et al*., 2024; Pineda-Mendoza *et al*., 2020; Tromas *et al*., 2025; Yanez-Montalvo *et al*., 2022).

Regarding functional annotation, the most abundant genes were associated with the degradation of complex molecules, general metabolic pathways, and functions of unknown annotation. Several annotated pathways were related to the breakdown of structurally complex compounds, including lignin-derived molecules, starch, phthalates, esters, and aromatic compounds. The detection of genes involved in these pathways suggests microbial potential to transform recalcitrant organic substrates that may be present in the system; such substrates can originate from multiple natural or anthropogenic sources. The presence of genes linked to the degradation of complex compounds may also be consistent with the relatively low biodegradability index observed, as recalcitrant organic matter typically contributes to reduce the BOD/COD ratio (Man

& Simpson, 2025). Nevertheless, the involvement of cooperative metabolic interactions within the microbial community remains a plausible but untested hypothesis. Additionally, two biofilm formation genes were detected in P1 (*yapH* and *exsH*), which have been reported to confer protection to vulnerable species via a symbiotic resilience mechanism. Specifically, *Microsystis* spp. (found as one of the most abundant taxa) has been shown to form aggregated matrices with heterotrophic bacteria, creating symbiotic environments where protection for zooplankton is provided, while also allowing the community to share nutrients and complete metabolic pathways (Cai *et al*., 2024). *A. temperans* (the most abundant bacteria at both sampling points) is highly present in wastewater plants and significantly contributes to the resilience of microbial communities, as it has been reported to form biofilms that enhance the stability and protection of associated microorganisms, functioning as symbiotic resistance mechanisms (Lee, 2013).

Overall, complex microbial communities and their interactions might enable survival under the physicochemical stress in the reservoir. Importantly, the integration of WPP with functional gene profiles highlights the potential of combining environmental metrics and metagenomic data to identify functional bioindicators in eutrophic systems. Parameters such as elevated COD, TN, and TP may act as selective pressures, favoring microbial assemblages enriched in stress-response, degradation, and biofilm-associated genes. In this context, dominance-driven communities, although potentially less functionally diverse, may exhibit specialization toward stress-tolerant metabolic traits. Such stress-induced functional simplification can reduce ecological resilience while enhancing specific adaptive capacities (Fuggle *et al*., 2025). This integrative framework may be applied to other eutrophic freshwater systems to predict functional shifts under increasing anthropogenic pressure.

Differential statistical analysis revealed that taxa with the highest log2FC/log2PV ratios were predominantly exclusive to P2, except for *M. barkeri*, which was detected at both sampling points. The community identified at P2 was dominated by aerobic heterotrophic bacteria typically associated with organic-enriched environments, including *Roseovarius* sp., *A. crassostreae*, and *A. macleodii* (Rajeev & Cho, 2024). Members of the Roseobacter clade, such as *Roseovarius* sp., are frequently reported in biofilm-associated communities and are known to contribute to sulfur and carbon cycling. Metatranscriptomic evidence indicates that actively expressed *sox* genes in biofilms are largely affiliated with this clade (Ding *et al*., 2023). Accordingly, the detection of the *soxT* gene in P2 supports the presence of an aerobic sulfur oxidation system, suggesting functional potential for sulfur turnover under aerobic conditions (Lahiri *et al.,* 2006; *Li et al.,* 2024; Xu *et al*., 2024). Beyond ecological interpretation, the differential taxa detected may represent a functional genetic reservoir shaped by anthropogenic inputs. Several genera identified in P2, including *Peribacillus*, *Agromyces*, *Bradyrhizobium*, and *Erwinia*, are commonly associated with agricultural soils and plant–microbe interactions, supporting the hypothesis of runoff influence from nearby farmlands. Consistent with this pattern, previous metagenomic studies in hypertrophic systems such as Lake Cajititlán (Guadalajara, Mexico) have reported *Flavobacterium* and *Acidovorax* among the dominant genera across sampling periods (Díaz-Torres *et al.*, 2024). The abundance of *Flavobacterium* (exclusive present in P2) has been repeatedly linked to eutrophic and hypertrophic conditions, while *Acidovorax* (most abundant taxa in both sampling sites) species have been isolated from environments contaminated with herbicides and pesticides. Given that agricultural activity represents a major land use in the

surrounding basin, similar environmental pressures could be favoring the proliferation of these metabolically adaptable genera in our study sites.

Importantly, these taxa harbor traits with recognized biotechnological relevance. *P. frigoritolerans* produces industrially relevant amylases with applications in water treatment and biofertilization (Senchenkov et al., 2023). *Agromyces* spp. contribute to the degradation of complex organic matter and pollutants, supporting carbon cycling in contaminated environments (Zhao *et al*., 2016). Although *B. contaminans* is often described as an opportunistic pathogen, members of the order Burkholderiales are well known for carrying catabolic pathways for aromatic compounds and xenobiotics (Pérez-Pantoja *et al*., 2012; Morya *et al.,* 2020), highlighting their dual ecological and applied significance. Likewise, species within the genus *Erwinia* display diverse metabolic capacities, including roles in heavy metal transformation and environmental resilience (Ke *et al*., 2024; Wang & Xu, 2025). Taken together, the integration of differential taxonomic patterns, functional genes, and surrounding land use suggests that metabolically versatile microbial assemblage shaped by environmental and anthropogenic pressures (slightly more pronounced at P2). This assemblage may function not only as an indicator of nutrient and runoff influence but also as a reservoir of metabolic traits with potential applications in bioremediation, pollutant degradation, and environmental biotechnology.

The present study reveals that the Santa Catarina reservoir has been impacted by anthropogenic factors, resulting in complex ecological responses that could significantly affect economic activities and represent a threat to public health. High nutrient loads have resulted in eutrophication, alongside the presence of recalcitrant organic contaminants, which have overall promoted microbial species dominance and adaptation mechanisms, differentially present at the sampling points. Although this scenario might look environmentally dangerous, it may be taken as an example of the contamination status of a broad range of water reservoirs, and the metagenomic analysis has also revealed the potential that microbiomes might offer for biotechnological applications, like nutrient cycling and the degradation of complex molecules, such as pesticides and fertilizers, which are serious environmental problems. Although this study did not specifically investigate secondary metabolite biosynthetic pathways, eutrophic systems are widely recognized as reservoirs of metabolically specialized microorganisms, including taxa capable of producing bioactive compounds and bloom-associated metabolites. The functional profiles identified here provide a baseline for future targeted analyses of biosynthetic gene clusters and regulatory pathways that may be involved in sustainable approaches.

5. CONCLUSION

This study provides an integrated physicochemical and metagenomic characterization of the Santa Catarina reservoir, revealing clear associations between water quality parameters and microbial community structure and function. Significant differences in nutrient load and organic matter between sampling points were reflected in distinct taxonomic compositions and functional profiles, indicating that environmental changes directly shape microbial metabolic potential and, conversely, may reflect these conditions. The enrichment of nutrient-cycling, degradation-related, and stress-response genes highlights the adaptive capacity of the microbiome under anthropogenic pressure and supports the use of functional metagenomics as a complementary

tool for environmental monitoring. By linking water physicochemical parameters with taxonomic and functional profiles, this study contributes to a baseline framework for understanding microbial responses in eutrophic freshwater systems and provides insights that may promote future monitoring and remediation strategies.

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AUTHOR´S CONTRIBUTION

Michelle Tirado-Guerrero: Data analysis, Interpretation, Original draft preparation. Julia Verduzco-Feregrino: Experimentation, Data analysis. Maribel Quezada-Cruz: Physicochemical analysis. Norma G. Rojas-Avelizapa: Conceptualization, Methodology, Advisory. Jonathan H. Cordovillo-Armijo: Experimentation. Andrea M. Rivas-Castillo: Conceptualization, Methodology, Funding acquisition, Writing- Reviewing, and Editing.

CONFLICT OF INTEREST

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest in the conduct, analysis, or publication of this research.

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