Ciencias Marinas (2025), 51:e3564 **Research Article**

Spatiotemporal dynamics of the microbial community in the water column of Ojo de Liebre Lagoon, Baja California Sur, Mexico

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ARTICLE INFO

Article history:
Received 6 June 2025
Accepted 7 October 2025
Published 26 November 2025

LEER EN ESPAÑOL:
https://doi.org/10.7773/cm.y2025.3564

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ABSTRACT. Microbial communities in hypersaline environments play a key role in biogeochemical cycles and ecological productivity. This is the first study to characterize the composition, structure, and metabolic potential of the prokaryotic community in the water column of Ojo de Liebre Lagoon (Baja California Sur, Mexico), a hypersaline ecosystem of ecological and socioeconomic importance. From the information obtained from 4 sampling campaigns (2021–2022) conducted at 18 stations, we analyzed physicochemical variables (salinity, temperature, and density) and sequenced the V4–V5 region of the 16S rRNA gene. Data were processed using QIIME2, and metabolic potential was inferred via PICRUSt2. The community was dominated by Actinobacteriota, Bacteroidota, Cyanobacteriota, Pseudomonadota, and Verrucomicrobiota, with spatiotemporal differences linked to temperature (r^2 = 0.77, P = 0.001) and salinity (r^2 = 0.39, P = 0.014) gradients. Rare taxa (<1% abundance), such as Thermoanaerobaculales and Desulfobacterota, contributed to diversity, particularly in November. Metabolic inference revealed significant pathways ($P < 0.05$), including vitamin B6 biosynthesis, chitin degradation, and nitrate reduction, suggesting roles in biogeochemical cycles and adaptation to extreme conditions. The prokaryotic community structure in the lagoon responded to physical and chemical variations, with a stable taxonomic core and rare taxa enhancing diversity under specific conditions. The inferred metabolic potential highlights their participation in biogeochemical processes and their capacity to degrade complex organic compounds. These results establish a baseline for understanding microbial dynamics in hypersaline systems and their impact on ecosystem function.

Key words: hypersaline environments, prokaryotic diversity, 16S rRNA sequencing, metabolic inference, biogeochemical cycles, spatiotemporal variation, Ojo de Liebre Lagoon.

INTRODUCTION

Microbial communities are made up of microorganisms that interact and perform a variety of functions in a space defined by physicochemical factors, where they find the necessary conditions for their survival (Díaz and Wacher 2003, Begon et al. 2006, Callieri et al. 2018). Microbial communities are key to ecological dynamics in the biosphere due to the close relationship between their composition, structure, and metabolism and the biogeochemical cycles of the planet. For example, due to their phylogenetic and genomic diversity, microbial communities carry out metabolic functions that are a crucial component in the continuous production and recycling of organic matter and nutrients; these functions promote energy flows between different trophic levels and, in addition, regulate a variety of biogeochemical transformations (Rousk and Bengtson 2014, Kost et al. 2023). Thus, evaluating the variations in the composition and structure of microbial communities that result from physicochemical factors allows us to predict the metabolisms that contribute to the energy flow in an ecosystem from the base of the food chain and, therefore, to understand the capacity of an ecosystem to sustain life.

The metabolic versatility of prokaryotic microorganisms has allowed them to adapt to virtually any ecosystem, including marine environments with physical and chemical conditions

Open Access
Online ISSN: 2395-9053
Screened via Similarity Check powered by iThenticate
https://doi.org/10.7773/cm.y2025.3564

that exceed average salinity, temperature, and pressure and are therefore considered extreme environments (Oliart-Ros et al. 2016). Hypersaline water bodies are aquatic environments characterized by a salt concentration significantly higher than that of average seawater (35 PSU) (Karleskint et al. 2010). From an environmental and ecological perspective, hypersaline water bodies harbor a specialized biodiversity of microorganisms that are adapted to these high-salinity conditions, which enables them to survive and maintain ecosystem functionality (Oren 2002, 2010). Prokaryotic microbial communities in hypersaline environments show particular composition and structure characteristics; above all, these communities exhibit diverse adaptations (Kimbrel et al. 2018) that allow them to maintain essential metabolic activities, such as protein synthesis and DNA replication, under these conditions. Furthermore, these microorganisms exhibit specialized metabolic strategies, such as osmoregulation through the accumulation of compatible solutes and the production of exopolysaccharides, to maintain their osmotic balance (Oren 2002, 2008).

However, due to their sensitivity to environmental disturbances, hypersaline water bodies and the microorganisms that inhabit them can serve as indicators of environmental, climatic, and anthropogenic alterations, by reflecting these in changes in their composition and structure with effects on their metabolism that transcends as impacts on the function of the ecosystem (Ventosa et al. 1998). For example, many hypersaline bodies, such as coastal salt flats, are used for salt production, providing employment and important economic resources to local communities. At the same time, due to their unique enzymes and compounds, which could be applied in industries such as pharmaceuticals, cosmetics, and biotechnology, the microorganisms in these environments are of biotechnological interest (Ghosh et al. 2019). Therefore, culturally and historically, hypersaline ecosystems represent unique natural heritages that require conservation and protection to maintain their biodiversity and the ecosystem services they provide (Ventosa et al. 1998, Arahal et al. 2007). Thus, to better understand their ecological functions and maximize their benefits to society, it is essential to conserve and study these water bodies.

Ojo de Liebre Lagoon, located in the municipality of Mulegé, Baja California Sur, Mexico, is a shallow coastal lagoon (5–12 m) characterized by its hypersalinity (Villa-Guerrero 2019), which is mainly explained by the combination of high temperatures and winds that accelerate the evaporation process. In addition, the scarcity of rainfall for most of the year in the region results in a lack of freshwater input. Together, these factors create a salinity gradient, with values increasing towards the head of the lagoon, where salinity levels of up to 47 PSU have been recorded (Contreras 1985). This hypersaline lagoon is part of the El Vizcaíno Biosphere Reserve (REBIVI, for its acronym in Spanish) and is recognized as a World Heritage Site by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) due to its importance in terms of ecological interactions and socioeconomic activities. For example, it serves as a refuge for various bird species and the Gray Whale (*Eschrichtius robustus*) during its migration, and it provides habitat for endangered species, such as the Green Turtle (*Chelonia mydas*) (Bocanegra-Castillo 1998).

In addition to its ecological value, Ojo de Liebre Lagoon sustains a number of economic activities for local communities. An important economic activity in the region is the sustainable fishing of species, such as finfishes, the Maura Pen Shell (*Atrina maura*), and the Chocolata Clam (*Megapitaria squalida*) (Crespo-Guerrero and Jiménez-Pelcastre 2016). Tourism also boosts the local economy; specifically, the annual migration of the Gray Whale facilitates social interactions that attract visitors from around the world (Ponce-López 2002, Bermúdez-Almada 2003). Another crucial source of income is the presence of the largest solar saltworks in the world, with 303.51 km² of salt ponds; however, there is concern about brine spills and their potential to alter the physicochemical composition of the water (Ponce-López 2002).

Due to the above, prokaryotic microbial communities in the water column play a fundamental role in the functioning and health of ecosystems such as Ojo de Liebre Lagoon. These communities regulate energy flows, biogeochemical cycles, and organic matter remineralization, which enable diverse ecological and socioeconomic interactions. Nonetheless, the composition and structure of the prokaryotic microbial community in the water column of this ecosystem are still unknown. Therefore, the main objective of this study was to provide novel information on the composition, structure, and spatiotemporal changes of the prokaryotic microbial community to create baseline knowledge that facilitates assessment of the biogeochemical aspects of Ojo de Liebre Lagoon from the trophic base, which support its characteristic ecological and socioeconomic interactions.

Materials and methods

Sample collection

Four samplings were conducted in Ojo de Liebre Lagoon, during the months of August and November 2021 and March and June 2022. At each sampling event, water samples (4 L) were collected at 18 stations distributed along the lagoon (Fig. 1a), and data of physicochemical variables, such as salinity, temperature, density, and depth, were recorded using a CastAway YSI CTD (Yellow Springs Instruments, Yellow Springs, USA). Water samples were taken using a 5-L Niskin bottle at a maximum depth of 2 m from the surface. The water was then transferred to 5-L Nalgene dark bottles using a funnel covered with a 200-µm nylon mesh. The bottles were stored on ice until the water was filtered and processed to obtain biomass concentrations. To obtain the final biomass concentration, up to 2 L of water were filtered using 0.2-µm Track-end membrane filters (Nucleopore, Pleasanton, USA). The filters were stored at –20 °C until the subsequent DNA extraction in the laboratory.

Molecular analysis

To extract DNA, we used the DNeasy PowerWater® kit (Qiagen, Venlo, The Netherlands) and its Quick-Start protocol, which was modified with an additional incubation step at 65 °C for 60 min after the lysis buffer was added and prior to the mechanical lysis step. Subsequently, the total extracted DNA was quantified using a NanoDrop (Thermo Fisher Scientific, Waltham, USA). The total DNA was sent to the Integrated Microbiome Resource (IMR) center at Dalhousie University, Halifax, Canada, for library generation and 16S rRNA gene amplicon sequencing on the Illumina MiSeq platform (2 × 250 PE). According to the quality control protocols of IMR, samples with concentrations greater than or equal to 1 ng·µL^{-1} and a minimum value of 260/280 equal to 1.8 were considered. The libraries were generated using the V4–V5 region, with the universal primers 515FB (GTGYCAGCMGCCGCG-GTAA) and 926R (CCGYCAATTYMTTTRAGTTT) of the 16S rRNA gene (Parada et al. 2015, Walters 2015). According to IMR protocols, amplicon fragments were amplified by duplicate PCR from template DNA, using independent dilutions and the high-fidelity polymerase Phusion Plus (Sigma Aldrich, St. Louis, USA). A single round of PCR was performed using fusion primers containing Illumina adapters (San Diego, USA), indexes (identification codes), and V4–V5 universal primers, which enabled simultaneous amplification and library preparation for sequencing. PCR products were visually verified by electrophoresis on a high-throughput Nimbus Select workstation (Hamilton Company, Reno, USA) using Coastal Genomics analytical gels (Burnaby, Canada).

Bioinformatic analysis

An amplicon analysis of the 16S rRNA gene was performed using the QIIME2 platform to evaluate the composition and

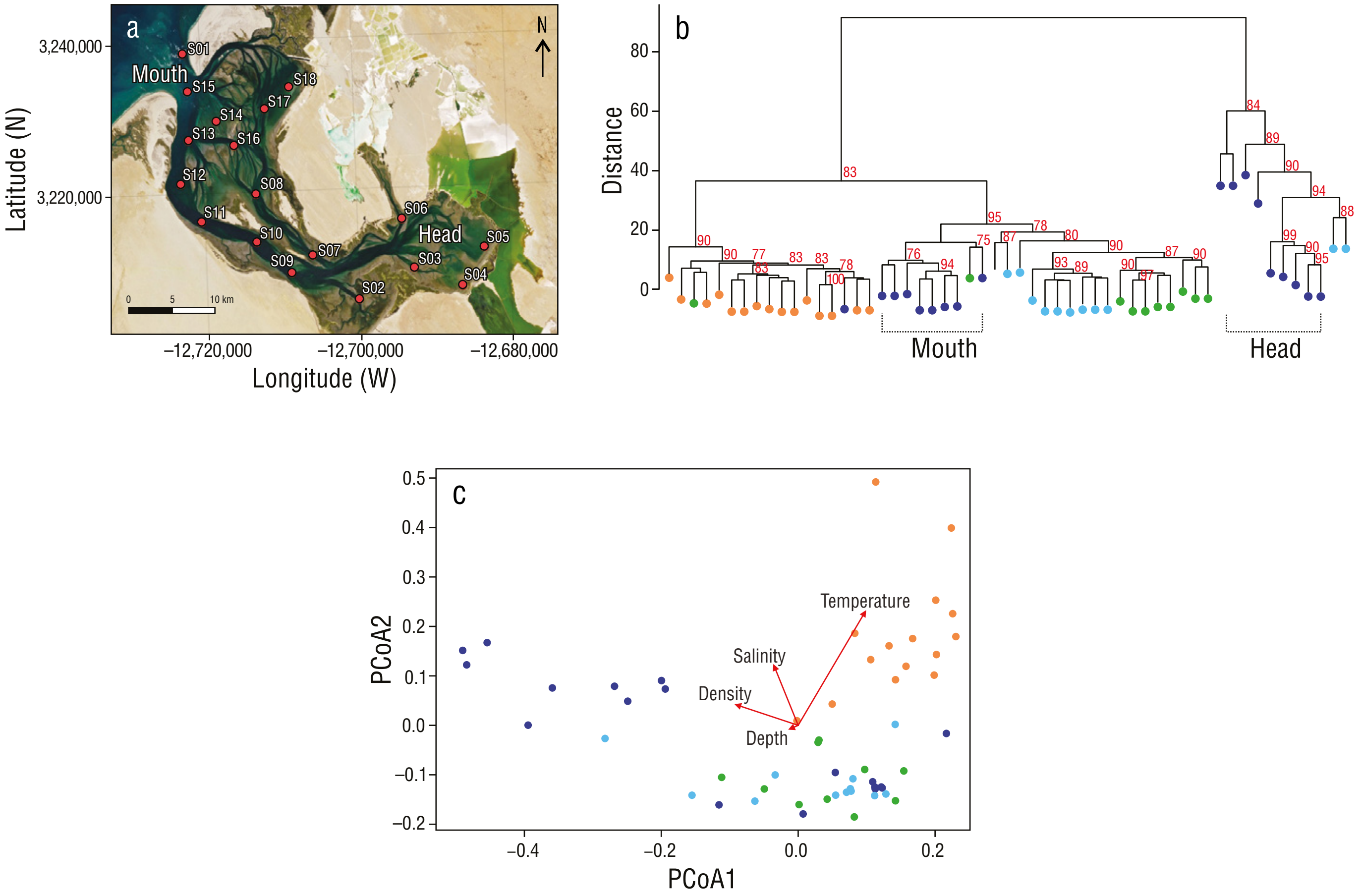

Figure 1. Analysis of the structure of the prokaryotic community in the water column of Ojo de Liebre Lagoon. Map of the location of sampling stations (S1–S18) (**a**). Hierarchical clustering dendrogram based on Manhattan distance, showing the similarity between samples from the 4 months of the study: August 2021 (orange), November 2021 (dark blue), March 2022 (green), and June 2022 (light blue). Values at the nodes indicate bootstrap support (>70%, based on 1,000 iterations) for significant clusterings ($P < 0.05$) (**b**). Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity. Vectors (red arrows) represent the direction and strength of the correlation between environmental variables (depth, temperature, salinity, and density) and community composition (**c**).

structure of the prokaryotic microbial community (Bolyen et al. 2019). As a result, a table was created with the taxonomic assignment of the amplicon sequence variants (ASVs) obtained using the Silva138 database (Yarza et al. 2014). After taxonomic assignment, ASVs that were likely contaminants or noise due to their low frequency (<0.5%) and their taxonomic label (i.e., mitochondria, chloroplasts, or unassigned) were removed. Based on this, a frequency table of the final ASVs was created using the 'feature-table filter-features' and 'taxa filter-table' commands in the QIIME2 environment. With this last ASV frequency table, the Shannon index was calculated as a metric of alpha diversity following the command 'diversity core-metrics-phylogenetic' in the QIIME2 environment. The relative abundance of ASVs was calculated using the 'dplyr' library in RStudio (RStudio Team 2020) by taking the absolute frequency of each ASV in a sample and dividing it by the total number of ASVs in the same.This process was performed independently for each sample, resulting in a proportional representation of each ASV.

The evaluation of differences in taxonomic composition and community structure in relation to physicochemical parameters was performed using the final ASV frequency table generated by the 'pvclust' library (Suzuki and Shimodaira 2006) and visualized with 'ggplot2' (Wickham 2016) in RStudio (RStudio Team 2020). The Manhattan distance with 1,000 replicates was used for the hierarchical clustering analysis, and the principal coordinate analysis (PCoA) was performed using a Bray–Curtis dissimilarity matrix with 1,000 replicates, including vectors corresponding to the correlation and significance of salinity, temperature, and density variables. The statistical analyses and visualization of this comparison were performed using the 'vegan' (Oksanen et al. 2019) and 'ggplot2' (Wickham 2016) libraries in the RStudio environment (RStudio Team 2020).

The PICRUSt2 tool was used as a module of QIIME2 to infer the microbial metabolism (Langille et al. 2013). A matrix of inferred metabolic pathways was obtained based on the presence and frequencies of representative taxonomic groups of different metabolic classes according to the databases of orthologous gene groups from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaCyc (Caspi et al. 2014). Finally, a temporal comparison of the genes and the resulting metabolic pathways was performed according to a differential abundance analysis with the package 'ggpicrust' (Chen et al. 2023), and the visualization of this comparison was performed with the library 'ggplot2' (Wickham 2016) in the RStudio environment (RStudio Team 2020).

RESULTS

Physicochemical parameters

At the 18 sampling stations, temperature, salinity, and density variables were measured to assess the effect of environmental physicochemical factors on the microbial community, (Fig. 1a; Supplementary Material Table S1). In general, an upward gradient of salinity, temperature, and density was observed from the mouth to the head of the lagoon. Specifically, the maximum monthly average temperature was observed in August (25.66 ± 0.48 °C), whereas the minimum monthly average was recorded in March (16.75 ± 0.34 °C). For the salinity, the maximum monthly average was observed in August with a value of 35.00 ± 4.5, whereas the minimum monthly average was observed in March with a value of 33.50 ± 4.1. Density values ranged from 1,023 to 1,030 kg·m^{-3}), whereas August had the lowest (∼1,023–1,024 kg·m^{-3}) throughout the lagoon. March had the highest density range (∼1,024–1,029 kg·m^{-3}), whereas August had the lowest (∼1,023–1,024 kg·m^{-3}).

Structure of the prokaryotic community and its relationship with environmental variables

Two approaches were considered to define the community structure and highlight its spatiotemporal distribution. First, a hierarchical clustering analysis was performed to identify temporal patterns based on the dissimilarity of the community composition along the lagoon and during the 4 months of sampling. The results showed that the samples were mainly grouped in relation to the sampling season (Fig. 1b). For example, a group that corresponded mainly to the August sampling stations was observed; this group was characterized by having the highest values of temperature (25–27 °C) and salinity (∼34–45 PSU). A second group corresponded to the stations sampled in November, and an intermediate group corresponded to the stations sampled in March and June, with wider temperature ranges (16–24 °C) and lower salinities (∼34–41 PSU). Regarding spatial patterns, the distinction between the stations at the head and mouth of the lagoon was particularly evident in November, showing a greater distance from those located at the head (Fig. 1b).

In addition, a PCoA was performed using correlation vectors with physicochemical variables to identify the relationship between the environmental variables of temperature, salinity, and density and the community structure (Fig. 1c). Alike in the hierarchical cluster analysis, 3 main groups were identified in the PCoA: the first corresponding to August, the second composed mainly of March and June, and the third corresponding to November in the region of the head of the lagoon. In general, temperature ($r^2 = 0.77$, $P = 0.001$) and salinity ($r^2 = 0.39$, $P < 0.05$) significantly explained the prokaryotic community structure (Table 1). Specifically, temperature showed a direct association with the community in August, and salinity had an inverse association with the groups of March and June. Density did not show a significant correlation ($r^2 = 0.31$, $P = 0.088$), but its vector in the PCoA space suggested an association with the prokaryotic community in the head region in November (Fig. 1c).

Taxonomic composition of the prokaryotic community

To determine the composition and taxonomic structure of the prokaryotic community in the water column, we

Table 1. Correlation values (r^2) and significance (P-value) of the physicochemical factors with the principal coordinates (PCoA).

	X1	X2	r^2	P
Depth	–0.86	–0.52	0.05	0.05
Temperature	0.39	0.92	0.77	0.77
Salinity	–0.28	0.96	0.39	0.39
Density	–0.91	0.42	0.31	0.31

performed an analysis of the 16S rRNA gene sequences. We analyzed a total of 58 samples and generated a total of 3,528,490 sequences; after processing these, we identified a total of 1,700 ASVs. The taxonomic assignment of the ASVs allowed us to identify 2 domains, 207 phyla, 198 classes, and 181 orders. As a first approach to evaluate the composition of the prokaryotic community and identify general and robust patterns in the community structure along an environmental gradient, we identified the taxa considered "core" based on a relative abundance value of ≥1% (Custer et al. 2023) for a maximum taxonomic level of order, considering that they were present in all sampling stations and months. The cutoff value of 1% relative abundance, applied up to the taxonomic level of order, allowed us to distinguish between the dominant or "core" groups and the subdominant or "rare" groups (<1%; Custer et al. 2023) in the context of our taxonomic resolution and independently of the stations along the lagoon where their presence was found (Fig. 2).

In the core composition of the community, the dominant phyla within the Bacteria domain were Actinobacteriota, Bacteroidota, Cyanobacteriota, Pseudomonadota, Planctomycetota, and Verrucomicrobiota (Fig. 2a). Regarding the spatial characterization of the core of the prokaryotic community, the taxonomic composition was observed with greater resolution, and we identified different orders such as Flavobacteriales, Synechococcales, Pirellulales, Rhodobacterales, SAR11, Burkholderiales, and Verrucomicrobiales (Fig. 2b); in addition, we identified Thermoplasmata, which belongs to Marine Group II, as the only dominant class within the Archaea domain (Fig. 2a). Furthermore, temporal differences were observed between the classes of the different phyla, including the classes Bacteroidia (37.07%) and Alpha-proteobacteria (30.96%), which showed greater abundance compared to the class Gamma-proteobacteria (9.68%) during the 4 months of sampling (Fig. 2a). In particular, temporally, the phylum Actinobacteriota was observed as the dominant group, with the most representative classes being Acidimicrobia (1.49%) and Actinobacteria (1.32%). The relative abundance of the phylum Cyanobacteria was also observed; this was higher in March (16.62%) than in August (15.67%) and June (8.24%) and decreased in November (5.65%) (Fig. 2a). In addition, the phylum Thermoplasmata showed a greater abundance in November (4.70%) (Fig. 2a).

Regarding rare taxa, they were observed in 4.92–35.32% of the total ASVs identified. However, temporal differences in their frequency were observed, with a direct effect on the total diversity of the sampling month. For example, November had the highest presence of rare taxa (35.32%), whereas August had the lowest (4.92%) (Fig. 2a). In addition, differences in community diversity were analyzed for the sampling months based on the Shannon diversity index (Fig. 2c). In particular, the distribution of the median, first quartile, and lower minimum limit varied temporally, with August and November marking the extremes of diversity. All communities temporally exhibited high diversity (values between 4.7 and 6.6), which is typical of environments considered complex; nonetheless, August showed the lowest value (4.7), whereas November showed the highest (6.6). Furthermore, it was possible to identify, within the sampling stations, those that showed a diversity value above or below the lower or upper limit (outliers) in a given season. For example, at station S3 (6.0), located at the head, the maximum value in August was an outlier; similarly, in June, stations S15 (6.6) and S5 (5.5), located at the mouth and head, respectively, also showed outliers (Fig. 2c).

Spatiotemporal variation in community composition

To assess specific changes in community composition related to spatiotemporal variation, we compared 6 stations distributed along the mouth (S12 and S13), middle part (S9 and S10), and head (S4 and S5) of the lagoon (Fig. 1a) during November and August. The stations and seasons were selected based on hierarchical clustering (Fig. 1b) and diversity (Fig. 2c) analyses. Differences in community composition were observed in relation to the regions of the lagoon, including the mouth region (S12 and S13). These differences were particularly evident during November, when an increase in Thermoplasmata from Marine Group II and an increase in Puniceispirillales were observed, along with the presence of Flavobacteriales and Synechococcales among the abundant orders. In the region of the islands (S9 and S10), a clear change was observed in the proportion of Thermoplasmata from Marine Group II and Verrucomicrobiales. Specifically, at station S10, there was a significant increase in the proportion of Pirellulales and Sphingomonadales in November, whereas Flavobateriales and Rhodobacteriales predominated in August. For the head region (S4 and S5), the proportion of SAR11 was significantly higher in November, whereas the proportion of Synechococcales was higher in August. Finally, at station S5, an increase in the Gammaproteobacteria class was observed in November, whereas Flavobacteriales and Rhodobacteriales had the highest abundances (Fig. 2b).

Furthermore, considering the contribution of rare groups to community diversity, we evaluated differences in their occurrence at the same selected stations (Supplementary Material:

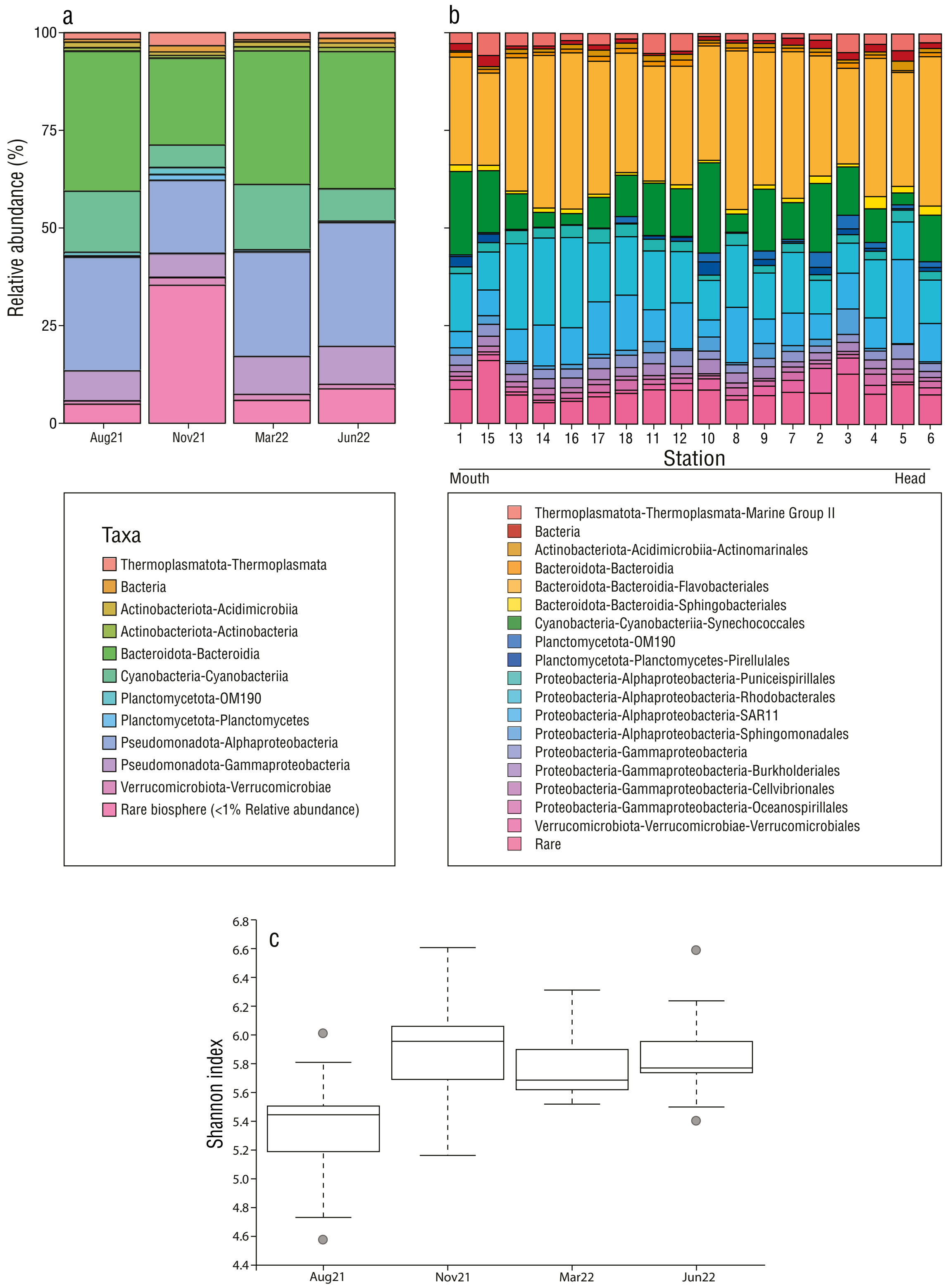

Figure 2. Composition and diversity of the prokaryotic community in the water column. Relative abundance (%) of the most representative taxonomic classes (>1% abundance) in the 4 sampling campaigns (August and November 2021; March and June 2022). Taxonomic assignment is indicated by the color code shown in the legend (**a**). Relative abundance (%) of the most representative taxonomic orders (>1% abundance) at the sampling stations in the lagoon (**b**). Alpha diversity of the community estimated using the Shannon index for each sampling season. The box represents the range between quartiles (lower = 25; upper = 75), the inner line represents the median, and the whiskers represent the range of the data. Individual points represent outliers (**c**).

Table S2 and Fig. S1). Primarily, previously unobserved taxa were identified, including the orders Thermoanaerobaculales, Microtrichales, Desulfobacterales, and Desulfobulbales. In addition, previously mentioned orders such as Corynebacteriales, PeM15, and Propionibacteriales of Actinobacteria, Chitinophagales of Bacteridia, and Vibrionales and Xanthomonadales of Gammaproteobacteria were observed. Distribution patterns were also identified in the different regions of the lagoon (Supplementary Material: Table S2 and Fig. S1). In the mouth region, at station S13, the order Vibrionales was observed. In the region of the islands, at station S9, Microtrichales and PeM15 were observed in August, whereas Chitinophagales was observed in November. At station S10, Corynebacteriales, Propionibacteriales, and Xanthomonadales were observed only in November. In the head region, station S4 only presented the order PeM15 in August; in November, no taxon was recorded. Finally, station S5 presented the archaeon Thermoanaerobaculia and the orders Desulfobacterales and Desulfobulbales, although only in November (Supplementary Material: Table S2 and Fig. S1).

Metabolism inference

To infer the metabolic potential in the water column, the sequences and taxonomic assignments of the ASVs that defined the taxonomic composition of the prokaryotic community were used. The differential analysis was performed using 2 main approaches, considering the seasonal difference in the community resolved with hierarchical clustering and diversity analyses: (1) gene inference and (2) metabolic pathway inference. Therefore, the inferences of genes and metabolic pathways present in August and November were analyzed and compared. The analysis of the seasonal differential abundance showed a total of 4,529 genes, of which 2,214 contributed significantly ($P < 0.05$) to this seasonal difference (Fig. 3a). Among the metabolic categories related to the inferred genes, the most prominent were the cofactor and vitamin metabolism (9%), xenobiotic degradation (2%), terpenoid and polyketide metabolism (2.7%), and secondary metabolite biosynthesis (0.5%), in addition to categories within the central metabolism such as carbohydrate (20%), lipid (4%), and amino acid (11%) metabolism, and energy generation (16%). In particular, genes within the categories of cofactor and vitamin metabolism, xenobiotic degradation, terpenoid and polyketide metabolism, and secondary metabolite biosynthesis were selected. The genes that stood out were those related to the metabolic pathways of vitamin B6, riboflavin (B2), and biotin (B7) (cofactor and vitamin metabolism); the biosynthesis of flavonoids and streptomycin (secondary metabolite biosynthesis); the biosynthesis of siderophores, carotenoids, and terpenoids (terpenoid and polyketide metabolism); and the degradation of ethylbenzene, styrene, nitrotoluene, benzoate, and chloroalkane (xenobiotic degradation) (Fig. 3a).

Regarding the inference of metabolic pathways, a total of 420 metabolic pathways were identified, of which 377 could be assigned to a category. Of the total assigned pathways, 86.47% were related to the biosynthesis of molecules, including amino acids; 11.93% were directly related to biogeochemical cycles, such as the carbon, nitrogen, and sulfur cycles; and 1.56% were related to hydrocarbon degradation. Furthermore, 130 metabolic pathways were found to contribute significantly ($P < 0.05$) to the seasonal differentiation of the inferred metabolism for the community (Fig. 3b). The nitrate reduction pathway, vitamin B6 biosynthesis, chitin degradation, and toluene 4-sulfonate degradation stood out among the significant categories observed.

Discussion

In this study, we analyzed the spatiotemporal variation in the composition and structure of the prokaryotic microbial community in the water column of Ojo de Liebre Lagoon. The analysis of the 16S rRNA gene sequences revealed a prokaryotic community dominated by phyla commonly found in aquatic systems where salinity gradients occur. Specifically, the community structure was found to be significantly influenced by physicochemical variables, such as temperature and salinity, primarily dividing the community into 3 groups according to the sampling month; spatially, the communities at the mouth and head of the lagoon were well differentiated. Moreover, primary metabolic pathways related to the biosynthesis of molecules, such as amino acids, and heterotrophic metabolism were inferred, in addition to pathways related to the biogeochemical cycles of carbon, nitrogen, and sulfur, and hydrocarbon degradation. This suggests that Ojo de Liebre Lagoon is a diverse system with respect to the microbial community in the water column, which has the potential to directly contribute to the biogeochemistry of the ecosystem.

Seasonality of physicochemical factors

A temperature and salinity gradient was observed in Laguna Ojo de Liebre with a significant relationship to the structure of the prokaryotic community, which is spatially and temporally divided into groups (Fig. 1a), similar to what has been reported for other hypersaline environments (Thompson and Lee 2020). The greatest difference in community composition occurred between August and November, months associated with a physicochemical gradient influenced by neap tide periods during the sampling days. These periods favor seasonal differentiation, unlike spring tides, which promote a strong exchange of water with Vizcaíno Bay, modifying the temperature, salinity, and density of the lagoon (Álvarez-Borrego and Granados-Guzmán 1992). Temperature showed the most significant correlation ($r^2 = 0.77$, $P = 0.001$) with community composition, which explained the clustering of samples from August, the warmest month; this coincides with previous reports on the direct effect of temperature on marine microbial communities (Zhou et al. 1996). Furthermore, according to the ordination analysis, the relationship between density and the clustering of the November samples and their separation between the mouth and the inner lagoon is consistent with the dynamics reported for other hypersaline coastal lagoons influenced by tides, evaporation, circulation, and morphology (Clementino et al. 2008, Carvalho et al. 2024).

The spatial differentiation between the mouth and the head of the lagoon can be attributed to the effect of the tide on water residence time, which influences the turnover and persistence of taxa (Álvarez-Borrego and Granados-Guzmán 1992). For example, the longest residence time (124 days)

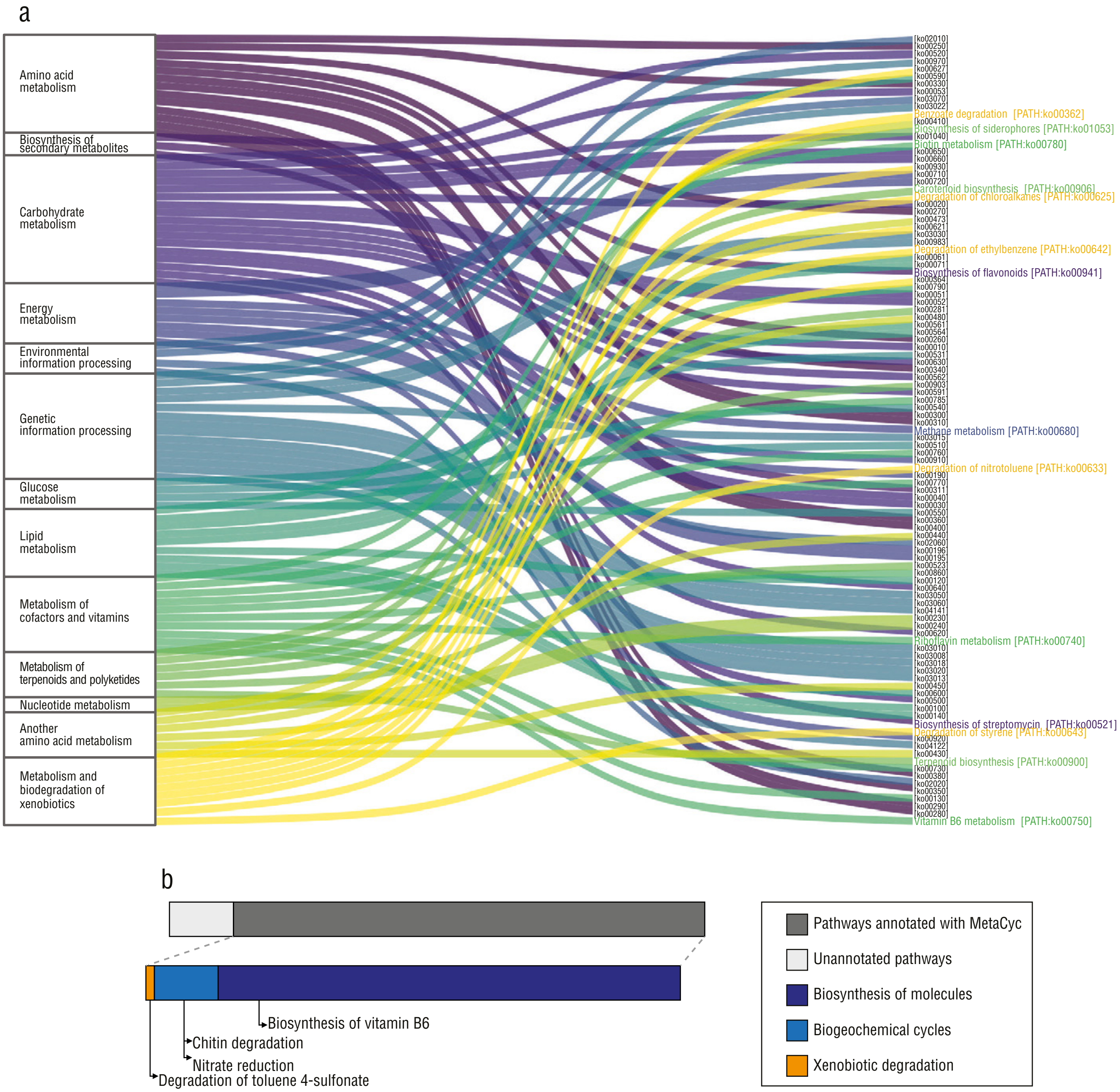

Figure 3. Inferred metabolic potential of the prokaryotic community using PICRUSt2. Abundance of genes associated with key metabolic pathways. Each horizontal bar represents a metabolic pathway (identified by its Kyoto Encyclopedia of Genes and Genomes Orthology [KO] ortholog), grouped by functional categories. Pathways involved in vitamin metabolism, xenobiotic degradation, and biosynthesis of secondary metabolites and terpenoids are highlighted (**a**). General functional profile of the community, showing the proportion of inferred metabolic pathways classified into MetaCyc categories. Relevant examples of biosynthesis (vitamin B6), biogeochemical cycles (chitin degradation and nitrate reduction), and biodegradation (toluene-4-sulfonate degradation) are highlighted (**b**).

recorded corresponds to the month of November (Soto-López 2024). Furthermore, during spring tides, the exchange of water masses is considerable in the mouth region, whereas residence time is longer in the head, depending on the circulation in Vizcaíno Bay and wind patterns (Soto-López 2024).

Composition and structure of the prokaryotic community

For the objectives of this study, which aimed to identify general patterns in the prokaryotic structure along an environmental gradient, the analysis at the order level proved pertinent and informative. It has been suggested that the definition of the microbial "core," including the taxonomic level and abundance cutoff, should be established according to the research questions and the ecological scale of the study (Custer et al. 2023). In this case, by working at the order level with a cutoff of >1% relative abundance, we sought to identify the main taxonomic groups that persistently formed the structural base of the community. This approach allowed us to discern the key taxa that responded to the environmental conditions of the time frame considered in this study. Although the time frame of this study only considered 1 annual cycle, the results obtained provide unprecedented information for Ojo de Liebre Lagoon regarding the influence of variations in physicochemical factors and dynamics in the water column on the composition of the microbial community. Thus, it was possible to differentiate a "core" formed by recurrent and abundant taxa, such as the phyla Pseudomonadota, Bacteroidota, Actinobacteriota, Cyanobacteria, and Verrucomicrobiota, which is similar to what has been reported in other hypersaline systems with comparable salinity and temperature ranges, such as Araruama Lagoon (salinity: 36–50; Clementino et al. 2008) and Thau Lagoon (salinity: 25–40; temperature: ~10–20 °C; Trombetta et al. 2022). This supports the consistency of our observations and establishes a baseline of knowledge for future interannual comparisons that will allow us to elucidate the influence of mesoscale climatic phenomena on the composition and structure of microbial communities in the lagoon.

Seasonal dynamics influenced the distribution and abundance of this core group (Fig. 2a). Cyanobacteria were most abundant in March and gradually decreased, whereas Planctomycetota and Verrucomicrobiota increased in November. These latter 2, typically classified as mesophiles (preferring ~20–35 °C) and associated with sediments rich in organic matter, often co-occur and participate in the degradation of complex compounds, including hydrocarbons (Schlesner and Stackebrandt 1987, Hedlund and Staley 2006, Lee et al. 2009). On the other hand, Actinobacteriota, with the class Acidimicrobia, has shown hypersalinity adaptations, with solute accumulation, spore formation, and metabolic versatility (Ventura et al. 2007). Furthermore, unlike in other hypersaline environments where Euryarchaeota, Thaumarchaeota, and Halobacteriota are common (Clementino et al. 2008, Trombetta et al. 2022), only the phylum Thermoplasmata (Marine Group II) was detected, which was reported previously in the microbial mats of Vizcaíno Bay (Robertson 2009, García-Maldonado et al. 2023). This group participates in the renewal of organic matter derived from phytoplankton (Hugoni et al. 2013), which is consistent with the high productivity of the lagoon.

Rare taxa (<1% relative abundance) also contributed to seasonal diversity (Supplementary Material Fig. S1), and these can influence biological interactions, food webs (Lyons et al. 2005), and the degradation of pollutants (Pester et al. 2010, Delgado-Baquerizo et al. 2016). The identification of rare groups such as Crenarchaeota, Acidobacteriota, Bdellovibrionota, Chloroflexota, Gemmatimonadota, Latescibacterota, Marinimicrobia, and Myxococcota suggests that the possible resuspension of sediments by environmental disturbances changes the composition of the sediment community, as has been observed in benthic microbial communities (Garsteki et al. 2002, Pusceddu et al. 2005). This is relevant in Ojo de Liebre Lagoon because the activity of boats and presence of the Gray Whale could favor mixing and sediment resuspension.

Diversity showed a spatiotemporal pattern in which November had the highest diversity and abundance of rare taxa, whereas August had the lowest values. In the head of the lagoon (S5), with a long residence time, Thermoanaerobaculia (Thermoanaerobaculales) and Desulfobacterota (Desulfobacterales and Desulfobulbales) were frequent. In the middle zone (S9 and S10), with greater water exchange, Actinobacteriota (e.g., Microtrichales and Corynebacteriales) predominated, which coincided with greater diversity. The variability in residence times, associated with the circulation in Vizcaíno Bay, affects water renewal and could explain the temporary presence of these rare groups in unique microhabitats during periods of low circulation (Mehrabi et al. 2014, Wu et al. 2024).

Inference of microbial metabolism

Metabolic inferences based on 16S rRNA data using PICRUSt2 provided a functional view of the metabolic potential of the prokaryotic community in the water column of the lagoon. However, it is crucial to consider the uncertainties inherent in this methodological approach. Agrawal et al. (2019) reported that PICRUSt can exhibit variations in the accuracy of its predictions compared to direct methods, such as shotgun metagenomics or real-time gene quantification (qPCR) (Agrawal et al. 2019). Therefore, we have interpreted the results obtained as a robust and qualitative indication of the potential presence of metabolic pathways in the ecosystem. In particular, the consistency in detecting enzymes related to the same pathway (i.e., associated with the nitrogen cycle or chitin degradation) strengthens the reliability of our inferences about the functional capabilities of the community, despite acknowledging the margin of error described for the tool.

The potential for vitamin B biosynthesis was identified. Among the key taxa were the orders Rhizobiales, Rhodobacterales, and Sphingomonadales, and the clade SAR11 (Pseudomonadota), as well as Flavobacteriales and Sphingomonadales (Bacteroidota), the latter potentially involved in biotin (B7) production (Sañudo-Wilhelmy et al. 2006). For example, vitamin B6 (such as PLP) facilitates the synthesis of osmoprotectants in microorganisms, such as SAR11 and cyanobacteria, in response to salinity fluctuations (Sañudo-Wilhelmy et al. 2012). Vitamin metabolism (B2, B6, and B7) is crucial for microbial survival in hypersaline environments. These vitamins, synthesized primarily by marine microorganisms, act as cofactors in essential processes, such as amino acid metabolism (B6), oxidation-reduction reactions (B2), and fatty acid synthesis (B7), directly affecting ecosystem productivity (Oren 2011, Sañudo-Wilhelmy et al. 2012, Sañudo-Wilhelmy et al. 2014, Wang et al. 2023). Their availability influences biogeochemical cycles and the activity of phytoplankton and bacterioplankton, as has been documented in coastal areas (Averianova et al. 2020).

Furthermore, metabolic inference suggests the production of secondary metabolites. The potential to synthesize antibiotics, such as streptomycin, was detected; this was supported by the previous isolation of the genus *Streptomyces* (phylum Actinobacteriota) in the lagoon (Zamora-Quintero et al. 2022). In addition, the biosynthesis of siderophores (i.e., deferrioxamine E, carotenoids, and terpenoids) was inferred; this is a key adaptive strategy in the face of oxidative stress, high UV radiation, and nutrient limitations, such as the generally reported absence of iron in the sediments (Gunde-Cimerman et al. 2018, Valdivieso-Ojeda et al. 2021, Zamora-Quintero et al. 2022). The environmental conditions during the study (salinity: 34–45; temperature: 19–27 °C; UV radiation: <134.4 kW·m^{-2}·d^{-1} in August) support the argument that these metabolisms are determinants in the structure of the microbial community (Alonso-Reyes et al. 2021, NASA POWER 2022) and regulate interactions by exclusion or dominance of taxa (Vraspir and Butler 2009, Oren 2014). An example of this environment-metabolism relationship is the seasonal fluctuation of the order Synechococcales. Its greater abundance at the head of the lagoon (station S4) in August, a period of higher salinity, temperature, and UV radiation, suggested a potential association with the production of flavonoids, a metabolic process that contributed significantly to the seasonal variation ($P < 0.05$). This is consistent with previous reports, which indicate that cyanobacteria, such as *Synechococcus*, synthesize these compounds for protection (Gunde-Cimerman et al. 2018).

Regarding the potential biodegradation of hydrocarbons, although halophilic archaea are known to degrade hydrocarbons in hypersaline environments (Oren 2014), their low diversity in this study (mainly Thermoplasmatota) suggests that the degradation of compounds such as toluene in the water column is limited. Potential sources of hydrocarbons in the lagoon include fuel spills associated with tourist boat activities, fishing, and salt transport. Despite the fact that the current regulatory actions of the lagoon management program promote that vessels entering the reserve avoid the dispersion of hydrocarbons in the water bodies (CONANP and SEMARNAT 2016), we suggest that subsequent studies quantify and characterize these compounds, which could be concentrated in the sediments and could be resuspended into the water column by the dynamics of the lagoon, and evaluate the activity of the hydrocarbonoclastic microbial community (Widdel and Rabus 2001).

Finally, the potential for chitin degradation, which is crucial for carbon and nitrogen recycling, is highlighted. Although the specialized orders Chitinophagales and Vibrionales were found in low abundance in November, their presence, along with the zooplankton activity reported in the lagoon (Sánchez-Cobarrubias et al. 2021), suggests that this process could be relevant after peaks in zooplankton abundance, connecting substrate availability with microbial metabolism and productivity (Keyhani and Roseman 1999, Souza et al. 2011).

CONCLUSIONS

This study is the first characterization of the prokaryotic community in the water column of Ojo de Liebre Lagoon. The results show that the prokaryotic community is primarily temperature-driven and composed of a microbial core that is characteristic of hypersaline environments. In addition, we identified potential ecological functions that the community could maintain, primarily the biogeochemical cycles fundamental to the ecosystem, which adds to the importance of our study. Furthermore, a fraction of rare taxa, which proliferate under conditions of low water renewal, have the potential to contribute specialized metabolic capabilities to ecosystem resilience. Thus, the combined results of this study suggest that the interaction between the stable core and the dynamic rare community is fundamental to sustaining the productivity and health of this vital coastal ecosystem.

English translation by Claudia Michel-Villalobos.

DECLARATIONS

Supplementary material

The supplementary material for this work can be downloaded from: https://cienciasmarinas.com.mx/index.php/cmarinas/article/view/3564/420421270.

Acknowledgments

We thank *Exportadora de Sal* S.A. for their general and logistical support in Guerrero Negro, Baja California Sur, for the collection of sediment samples. We also thank the student volunteers from the *Ecología del Fitoplancton* research group of the *Universidad Autónoma de Baja California* for their invaluable support in collecting the samples.

Funding

This study was funded by the *Universidad Autónoma de Baja California* with the project "*Indicadores ambientales del estado de salud del humedal Laguna Ojo de Liebre* (*Reserva de la Biósfera El Vizcaíno*)" (no. 401-1-C-35-22). Aileen Pimental received a scholarship for Master's studies from SECIHTI (CVU 1227483).

Conflict of interest

The authors declare they have no conflict of interest.

Author contributions

Conceptualization: APC, MTB, NMA; Data curation: APC, MTB; Formal analysis: APC, MTB; Funding acquisition: APC, JLC; Research: MTB, NMA, JLC, MNCC; Methodology: MTB, NMA; Project management: JLC; Resources: MTB, JLC; Software: APC, MTB; Supervision: MTB, NMA, JLC, MNCC; Validation: MTB, NMA, JLC, MNCC; Visualization: APC, MTB; Writing—original draft: APC; Writing—review and editing: MTB, NMA, JLC, MNCC.

Data availability

The sequencing data for this study are available from the National Center for Biotechnology Information (NCBI) (BioProject: PRJNA1274005). This study was conducted in accordance with current regulations and in compliance with the requirements of the El Vizcaíno Biosphere Reserve.

Use of AI tools

The authors did not employ any AI tools in this work.

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