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Exploring Microbial Diversity in the Isla Po'i Lagoon: Nanopore Metagenomic Sequencing Data Analysis

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ABSTRACT

The Paraguayan Chaco, situated in western Paraguay, is characterized by an arid ecosystem and a limited human population. The Isla Po'i Lagoon, situated in the Department of Boquerón, is a key ecological feature that serves as one of the few perennial water sources in times of drought and provides a vital habitat for a diverse range of species. Microorganisms are integral components of aquatic ecosystems, and their abundance varies based on ecological niches. Traditional culture-based methods are limited by their ability to explore microbial diversity. High-throughput sequencing has significantly transformed environmental microbiology, facilitating direct study of microbial communities in their natural habitats. This study employed long-read sequencing using MinION technology to investigate the microbial diversity in the Isla Po'i Lagoon. To determine taxonomic composition, analyses were conducted based on sequencing reads. We observed an average diverse community composition comprising bacteria (86.2%), eukaryota (11.6%), viruses (2%), and archaea (0.2%). The dominant phylum identified across all classifiers was Proteobacteria (44.3-94.0%), which underscores its importance in various aquatic environments. Nanopore sequencing provided a detailed characterization of the microbial community, offering insights into the diversity of the Isla Po'i Lagoon.

INTRODUCTION

The Paraguayan Chaco is a vast area located in the western region of Paraguay, and is known for its dry ecosystem, terrain, and scarce human population **(Andrade-Díaz *et***

***al.*, 2023)**. The Isla Po'i Lagoon, in the Department of Boquerón, is a brackish ecosystem that provides habitats for diverse organisms **(Salinas Romero *et al.*, 2023)**.

Microorganisms—particularly bacteria and fungi—act as crucial agents in nutrient cycling, and are vital components of the food chain **(Kuehn *et al.*, 2014)**. In brackish environments such as the Isla Po'i Lagoon, understanding the diversity of microorganisms adapted to such conditions will be key in future studies seeking to comprehend the structure and functioning of the ecosystem, which has not yet been well-studied. These microorganisms can serve as indicators of environmental health, and have potential biotechnological applications **(Chakraborty *et al.*, 2021)**.

Exploring microbial biodiversity is challenging due to the limitations of conventional culture-based methods. High-throughput sequencing has been used to address this issue, enabling researchers to directly study microbial communities in natural environments. Long-read sequencing technologies, such as PacBio and Oxford Nanopore Technology, have been instrumental in profiling microbial communities at species-level resolution by sequencing entire marker gene amplicons (e.g., 16S, 18S rRNA, and ITS) **(Mishra *et al.*, 2022; Stevens *et al.*, 2023)**.

Metagenomics allows the examination of genetic material extracted directly from water samples. By sequencing the DNA or RNA of various organisms inhabiting aquatic systems, researchers can obtain detailed information regarding their diversity and distribution patterns, including those that are difficult to culture using traditional methods **(Zhou & Ning, 2017; Akaçin *et al.*, 2022)**.

Nanopore sequencing offers advantages, as it requires minimal preparation time and can effectively analyze long DNA fragments for genome assembly and the analysis of complex genomic regions **(Simpson *et al.*, 2017; Pavlović *et al.*, 2022, Ciuffreda *et al.*, 2023)**.

The Isla Po'i Lagoon was the primary focus of our research, chosen for its ecological uniqueness and yet unexplored characteristics in metagenomic studies. Metagenomic analysis not only sheds light on the adaptability of microorganisms under extreme conditions, but also provides information about their composition, which could be useful in the field of microbial ecology and future actions aimed at conserving the ecosystem. Therefore, our objective was to investigate the microbial diversity in environmental samples collected from the Isla Po'i Lagoon in the Paraguayan Chaco, using MinION as a cost-effective and efficient tool.

MATERIALS AND METHODS

1. Sampling site

In December 2021, samples were obtained from the Loma Plata district (22°30'01.9"S; 59°44'04.0"W), located 425km from Asunción. The lagoon has a length of around 2.5 kilometers and is positioned 3.5km from the bioceanic route connecting Brazil, Paraguay, and Argentina (Fig. 1).

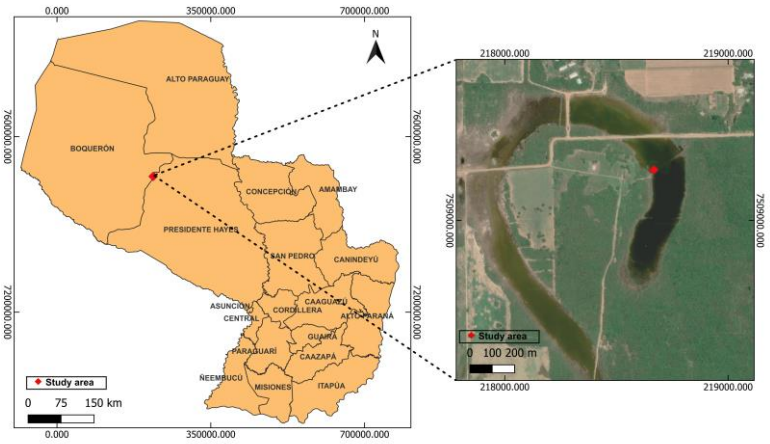

Fig. 1. Map displaying the location of the Isla Po'i Lagoon area in Paraguay. The left side shows a general map of Paraguay with outlined departmental boundaries. The red dot indicates the sampling site situated within the Boquerón Department. On the right side, a high-resolution satellite image highlights Isla Po'i Lagoon and specifies the exact sampling location.

2. Sampling collection

Surface water samples were collected and pooled to obtain a total volume of 500ml **(Gao *et al.*, 2024)**. The sample was transported to the Center for Multidisciplinary Technological Research (CEMIT) laboratories and refrigerated at 4°C. Then, it was filtered through a 0.22um nitrocellulose membrane **(Gu *et al.*, 2023)**. The procedure was performed under sterile conditions. *In situ* parameters such as water temperature, pH, conductivity, and dissolved oxygen were measured using a WTW MULTI 350 instrument.

3. DNA extraction and purification

DNA was extracted using a modified protocol based on the Wizard® genomic DNA purification kit. The extracted DNA was purified using ProNex® size-selective purification system intended for next-generation sequencing libraries by Promega.

4. Library preparation

A library was prepared using the SQK-RAD004 rapid sequencing kit from Oxford Nanopore. The kit components were thawed at room temperature, briefly centrifuged, and mixed by pipetting. After thawing, all components were kept on ice for further use.

To prepare the DNA, approximately 400ng of genomic DNA was transferred into a DNA LoBind tube and diluted with nuclease-free water to a final volume of 7.5µL. The mixture was gently mixed by flicking the tube and briefly spun down in a microfuge to avoid shearing. In a thin-walled PCR tube, a 10µL mixture was created by combining

400ng of template DNA with 7.5µL of fragmentation mix (FRA). This mixture was also gently mixed and spun down.

The tube was then incubated at 30°C for one minute, followed by a one-minute incubation at 80°C before being briefly cooled on ice. Next, 400ng of fragmented DNA in a volume of 10µL was prepared. To attach the adapter, 1.0µL of rapid adapter (RAP) was mixed gently with the tube by flicking it and then spun down. The reaction mixture was incubated at room temperature for 5 minutes **(Rapid Sequencing gDNA SQK-RAD004, 2023)**.

5. Data analyses

Sequencing was conducted using a MinION DNA sequencer and MinKNOW software. A flow cell (R9.4.1) FLO-MIN106D was used. The conversion from fast5 to fastq was done with the ont-guppy-cpu basecaller. Sequencing data were filtered to retain reads with quality scores higher than Q7. Subsequently, we utilized several classification software packages, including EPI2ME **(https://epi2me.nanoporetech.com/)**, Kaiju **(Menzel *et al.*, 2016)**, MEGAN-LR **(Huson *et al.*, 2018)**, MetaMaps **(Dilthey *et al.*, 2019)**, Kraken 2 **(Wood *et al.*, 2019)**, and BugSeq **(Fan *et al.*, 2021)** to process the data and assign taxonomy to the long reads.

Graphical representation was conducted using RStudio **(Posit Team, 2023)** and R **(R Core Team, 2023)**. Data were organized and filtered using dplyr **(Wickham *et al.*, 2023)**. The domain bar chart was created with ggplot2 **(Wickham, 2011)**, employing the geom_bar function, while the phylum heatmap was generated with ampvis2 using the amp_heatmap function **(Jiang *et al.*, 2021)**. Both graphs were combined into a single figure using the plot_grid function from the cowplot package **(Wilke, 2023)**.

RESULTS

1. The relative composition of domains as identified by various classifiers

In domain classification analysis, a marked predominance of the bacteria domain was observed across all bioinformatics tools employed. EPI2ME exhibited 100% classification in the bacteria domain, followed by Kraken2 with 99.5%, BugSeq with 99.1%, Kaiju with 97.9%, and MEGAN-LR with 96.8%. Metamaps, while also showing a predominance of bacteria, had a significantly lower percentage (57.4%), indicating greater diversity in domain classification compared to the other tools (Fig. 2A).

The Eukaryota domain presented 6.6% classification in Metamaps; considerably higher than BugSeq (0.9%), Kraken2 (0.5%), and MEGAN-LR (0.3%). Viruses also showed variability among the classification tools. Metamaps reported 34.4% classification, followed by MEGAN-LR (2.9%) and Kaiju (1.1%). The high proportion of viruses detected by Metamaps may reflect the greater capacity of this tool to identify viral sequences in samples. Finally, the archaea domain was detected in small proportions in Metamaps (1.6%) and Kaiju (1.1%). These percentages, although low, are significant

because they indicate the presence of archaea in the samples, which may have important implications for understanding the microbial ecology and biogeochemical cycles in the studied environment (Fig. 2A).

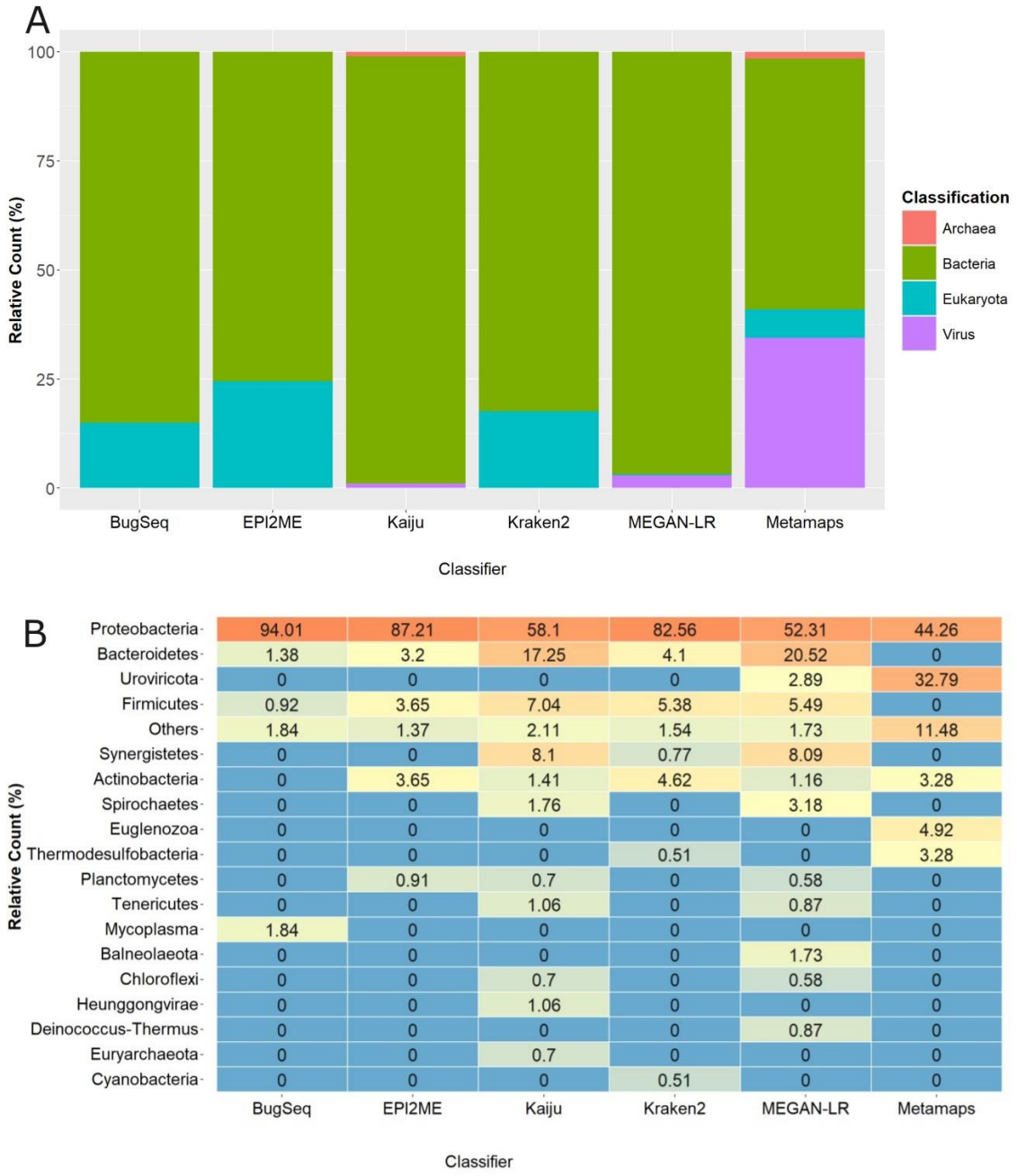

Fig. 2. Taxonomic classification at the domain and phylum levels. **A)** Relative composition of domains identified by various classifiers. **B)** Detailed breakdown of phylum-level composition for each classifier. The data illustrate the predominance of Proteobacteria across all classifiers, with variations in the detection of other phyla using different classification tools

2. Detailed breakdown of phylum-level composition for each classifier

The BugSeq classifier demonstrated a notable predominance of the phylum Proteobacteria, with a relative abundance of 94.01%. Other phyla identified included Mycoplasma (1.84%), Bacteroidetes (1.38%), and Firmicutes (0.92%). Additionally, the "others" group represented 1.84%, suggesting the presence of a variety of less-abundant phyla.

The MEGAN-LE classifier exhibited broader phylogenetic diversity, with Proteobacteria predominating at 52.31%. Bacteroidetes accounted for 20.52%, followed by Synergistetes (8.09%), Firmicutes (5.49%), and Spirochaetes (3.18%). Other phyla detected included Uroviricota (2.89%), Balneolaeota (1.73%), Actinobacteria (1.16%), Deinococcus-Thermus (0.87%), Tenericutes (0.87%), Planctomycetes (0.58%), and Chloroflexi (0.58%). The "others" group accounted for 1.74%.

EPI2ME showed a strong predominance of Proteobacteria (87.21%). Other significant phyla were Actinobacteria, Firmicutes (3.65%), and Bacteroidetes (3.20%). Planctomycetes was present at 0.91%, while the "others" group totaled 1.38%.

Kaiju revealed a predominance of Proteobacteria at 58.10%. Bacteroidetes was the second most abundant phylum (17.25%), followed by Synergistetes (8.10%), Firmicutes (7.04%) and Spirochaetes (1.76%). Other phyla detected included Actinobacteria (1.41%), Tenericutes (1.06%), Heunggongvirae (1.06%), Chloroflexi (0.70%), Planctomycetes (0.70%), and Euryarchaeota (0.70%). The "others" group accounted for a total of 2.10%.

The Metamaps classifier reported considerable diversity, with Proteobacteria at 44.26% and Uroviricota at 32.79%. Other phyla were Euglenozoa (4.92%), Actinobacteria (3.28%), and Thermodesulfobacteria (3.28%). The "others" group represented 11.48%, indicating a high diversity of less represented phyla.

Kraken2 showed a predominance of Proteobacteria at 82.56%, followed by Firmicutes (5.38%), Actinobacteria (4.62%), and Bacteroidetes (4.10%). Other phyla recorded included Synergistetes (0.77%), Cyanobacteria (0.51%), and Thermodesulfobacteria (0.51%). The "others" group accounted for 1.56%.

Proteobacteria was the dominant phylum across all classification tools, although the relative abundance varied among the classifiers, ranging from 44.26% in Metamaps to 94.01% in BugSeq. Bacteroidetes, Firmicutes, and Actinobacteria were consistently detected, albeit in lower proportions. The variability in the detection of other phyla (such as Synergistetes, Spirochaetes, and Uroviricota) between classifiers underscores the importance of using multiple classification tools to obtain a comprehensive view of microbial diversity. These results reflect the complexity and diversity of the microbiome in the Isla Po'i Lagoon and highlight the ecological relevance of these phyla in the biogeochemical cycles of this aquatic ecosystem.

DISCUSSION

In our analysis of the relative abundance (Fig. 2A), we observed a community composition made up mostly of bacteria (86.2%), followed by Eukaryota (11.6%), viruses (2%), and archaea (0.2%). These findings align with previous studies, emphasizing the representativeness of our results **(Elshafey *et al*., 2023; ZeinEldin *et al*., 2023)**. The predominance of bacteria indicates their significant contribution to microbial biomass, which is typical in brackish surface water samples **(Cisneros-Martínez *et al*., 2023)**.

Regarding the bacterial classifiers, Kaiju identified 97.9%, MEGAN-LR 96.8%, BugSeq 85.0%, Kraken 82.4%, EPI2ME 75.5%, and Metamaps 57.4%. This pattern supports the essential role of bacteria in the biogeochemical cycles of aquatic ecosystems, including carbon and nitrogen cycles **(Feng *et al*., 2023; Lanclos *et al*., 2023; Ren *et al*., 2023; Wang *et al*., 2023)**.

The presence of Eukaryota (EPI2ME=24.5%, Kraken2=17.6%, BugSeq=15.0%, Metamaps=6.6%, and MEGAN-LR=0.3%) and viruses in lesser proportions suggests a complex trophic network with interactions between viruses and different biological domains. These interactions might include predation, symbiosis, or competition, as well as associations with physicochemical processes **(Feng *et al*., 2023)**. The detection of archaea, though in lower quantities, is significant indicating specific niches within the lagoon that favor their growth and their contribution to the carbon, nitrogen, and sulfur cycles in aquatic ecosystems **(Jifiriya *et al*., 2023)**.

Proteobacteria consistently dominated across all samples, ranging from 44.3 to 80.6% (Fig. 2B), regardless of the classification method used. This pattern indicates the widespread presence and the significant ecological role of this phylum in the Isla Po'i Lagoon. BugSeq recorded the highest relative abundance of Proteobacteria at 81%, followed by Kraken2 at 68%, EPI2ME at 66%, and Kaiju at 58%, whereas MEGAN-LR and Metamaps showed the lowest relative values, with 52% and 44%, respectively.

Discrepancies among the classifiers at the domain and phylum levels can be attributed to differences in classification algorithms or reference databases, which may favor the identification of certain groups by specific software **(Fu *et al*., 2023; Geli-Cruz *et al*., 2023)**. This variability highlights the necessity of employing multiple classifiers to achieve a comprehensive and accurate depiction of the microbial diversity **(Portik *et al*., 2022)**.

Proteobacteria are recognized for their prevalence in most brackish waters **(Mohapatra *et al*., 2020; Rojas-Jimenez *et al*., 2021; Cisneros-Martínez *et al*., 2023; Zhang *et al*., 2023; Lin *et al*., 2024)**. These findings further underscore the significance of Proteobacteria in our study environment and reinforce the concept of their substantial presence across various aquatic habitats **(Mohapatra *et al*., 2020)**.

Other phyla, such as Bacteroidetes, Firmicutes, and Actinobacteria, were recorded in smaller proportions across almost all classifiers (Fig, 2B). These phyla have also been reported in other brackish ecosystems **(Feng *et al.*, 2023)**. However, in the Isla Po'i Lagoon, Bacteroidetes, and Actinobacteria were found to be less abundant than in other similar ecosystems **(Mohapatra *et al.*, 2020)**. This variation could be due to different *in-situ* physicochemical conditions at our study site, such as pH (9.01), conductivity ($87ms.cm^{-1}$), and dissolved oxygen ($2mg.L^{-1}$), which could affect the development of these phyla **(Feng *et al.*, 2023; Ren *et al.*, 2023; Wang *et al.*, 2023)**.

CONCLUSION

In the analysis of microbial diversity in the Isla Po'i Lagoon, microorganisms belonging to the domains bacteria, archaea, Eukaryota were identified, as well as viruses. Bacteria were the group with the highest relative abundance, standing out as the predominant domain in all classifiers used. Proteobacteria was the dominant phylum in all classifiers, although the relative abundance varied significantly: BugSeq recorded 94.01%, while Metamaps showed 44.26%. Other phyla consistently detected included Bacteroidetes, Firmicutes, and Actinobacteria, although in smaller proportions. The variability in the detection of other phyla such as Synergistetes, Spirochaetes and Uroviricota among classifiers underscores the complexity and diversity of the microbiome in the Isla Po'i Lagoon. This study provides valuable insights into the microbial diversity of Isla Po'i Lagoon, using NGS (Next-Generation Sequencing). It offers data on microorganisms adapted to extreme conditions, fundamental to the local ecology. These findings are crucial for future research aimed at assessing the environmental health of this ecosystem and paving the way for innovative biotechnological applications based on microorganisms thriving under extreme conditions.

REFERENCES

Akaçin, İ.; Ersoy, Ş.; Doluca, O. and Güngörmüşler, M. (2022). Comparing the significance of the utilization of next generation and third generation sequencing technologies in microbial metagenomics. Microbiological Research, 264, 127154. https://doi.org/10.1016/j.micres.2022.127154

Andrade-Díaz, M.S.; Piquer-Rodríguez, M. and Baldi, G. (2023). Conservation opportunities for threatened paleochannel grasslands in the South American Dry Chaco. Journal for Nature Conservation, 71, 126306. https://doi.org/10.1016/j.jnc.2022.126306

Chakraborty, J.; Rajput, V.; Sapkale, V.; Kamble, S. and Dharne, M. (2021). Spatio-temporal resolution of taxonomic and functional microbiome of Lonar Soda Lake of India reveals metabolic potential for bioremediation. Chemosphere, 264, 128574. https://doi.org/10.1016/j.chemosphere.2020.128574

Cisneros-Martínez, A.M.; Eguiarte, L.E. and Souza, V. (2023). Metagenomic comparisons reveal a highly diverse and unique viral community in a seasonally fluctuating hypersaline microbial mat. Microbial Genomics, 9(7). https://doi.org/10.1099/mgen.0.001063

Ciuffreda, L.; Rodríguez-Pérez, H. and Flores, C. (2021). Nanopore sequencing and its application to the study of microbial communities. Computational and Structural Biotechnology Journal, 19, 1497–1511. https://doi.org/10.1016/j.csbj.2021.02.020

Dilthey, A.T.; Jain, C.; Koren, S. and Phillippy, A.M. (2019). Strain-level metagenomic assignment and compositional estimation for long reads with MetaMaps. Nature Communications, 10(1), Article 1. https://doi.org/10.1038/s41467-019-10934-2

Elshafey, N.; Mansour, M.A.I.; Hamedo, H. A.; Elnosary, M.E.; Hagagy, N.; Ahmed Al-Ghamdi, A. and Martínez-Espinosa, R. (2023). Phylogeny and functional diversity of halophilic microbial communities from a thalasso environment. Saudi Journal of Biological Sciences, 30(12), 103841. https://doi.org/10.1016/j.sjbs.2023.103841

Fan, J.; Huang, S. and Chorlton, S.D. (2021). BugSeq: A highly accurate cloud platform for long-read metagenomic analyses. BMC Bioinformatics, 22(1), 160. https://doi.org/10.1186/s12859-021-04089-5

Feng, L.; Zhang, Z.; Yang, G.; Wu, G.; Yang, Q. and Chen, Q. (2023). Microbial communities and sediment nitrogen cycle in a coastal eutrophic lake with salinity and nutrients shifted by seawater intrusion. Environmental Research, 225, 115590. https://doi.org/10.1016/j.envres.2023.115590

Fu, S.; Zhang, Y.; Wang, R.; Qiu, Z.; Song, W.; Yang, Q. and Shen, L. (2023). A novel culture-enriched metagenomic sequencing strategy effectively guarantee the microbial safety of drinking water by uncovering the low abundance pathogens. Journal of Environmental Management, 345, 118737. https://doi.org/10.1016/j.jenvman.2023.118737

Gao, M.; Tan, F.; Shen, Y. and Peng, Y. (2024). Rapid detection method of bacterial pathogens in surface waters and a new risk indicator for water pathogenic pollution, Sci. Rep. 14 1614. https://doi.org/10.1038/s41598-023-49774-y

Geli-Cruz, O.J.; Santos-Flores, C.J.; Cafaro, M.J.; Ropelewski, A. and Van Dam, A.R. (2023). Benchmarking assembly free nanopore read mappers to classify complex millipede gut microbiota via Oxford Nanopore Sequencing Technology. Journal of Biological Methods, 10, e99010003. https://doi.org/10.14440/jbm.2023.376

Gu, Y.; Li, Z.; Lei, P.; Wang, R.; Xu, H. and Friman, V.P. (2023). Phylogenetic distance–decay patterns are not explained by local community assembly processes in freshwater lake microbial communities, Environ. Microbiol. 25,1940–1954. https://doi.org/10.1111/1462-2920.16437

Huson, D.H.; Albrecht, B.; Bağcı, C.; Bessarab, I.; Górska, A.; Jolic, D. and Williams, R.B.H. (2018). MEGAN-LR: New algorithms allow accurate binning and easy interactive exploration of metagenomic long reads and contigs. Biology Direct, 13(1), Article 1. https://doi.org/10.1186/s13062-018-0208-7

Jiang, C.; Peces, M.; Andersen, M.H.; Kucheryavskiy, S.; Nierychlo, M.; Yashiro, E.; Andersen, K.S.; Kirkegaard, R.H.; Hao, L.; Høgh, J.; Hansen, A.A.; Dueholm, M.S. and Nielsen, P.H. (2021). Characterizing the growing microorganisms at species level in 46 anaerobic digesters at Danish wastewater treatment plants: A six-year survey on microbial community structure and key drivers. Water Research, 193, 116871. https://doi.org/10.1016/j.watres.2021.116871

Jifiriya, M.J.; Preena, P.G.; Rejish Kumar, V.J.; Nair, A.J. and Joseph, V. (2023). Role of archaea in aquaculture: Prospects and challenges. Aquaculture International. https://doi.org/10.1007/s10499-023-01317-y

Klair, D.; Dobhal, S.; Ahmad, A.; Hassan, Z.U.; Uyeda, J.: Silva, J.; Wang, K.H.; Kim, S.; Alvarez, A.M. and Arif, M. (2023). Exploring taxonomic and functional microbiome of Hawaiian stream and spring irrigation water systems using Illumina and Oxford Nanopore sequencing platforms. Frontiers in Microbiology, 14. https://www.frontiersin.org/articles/10.3389/fmicb.2023.1039292

Kuehn, K.A.; Francoeur, S.N.; Findlay, R.H. and Neely, R.K. (2014). Priming in the microbial landscape: Periphytic algal stimulation of litter-associated microbial decomposers. Ecology, 95(3), 749–762. https://doi.org/10.1890/13-0430.1

Lanclos, V.C.; Rasmussen, A.N.; Kojima, C.Y.; Cheng, C.; Henson, M.W.; Faircloth, B.C.; Francis, C.A. and Thrash, J.C. (2023). Ecophysiology and genomics of the brackish water adapted SAR11 subclade IIIa. The ISME Journal, 17(4), 620–629. https://doi.org/10.1038/s41396-023-01376-2

Lin, L.; Xiong, J.; Liu, L.; Wang, F.; Cao, W. and Xu, W. (2024). Microbial interactions strengthen deterministic processes during community assembly in a subtropical estuary. Science of the Total Environment, 906. Scopus. https://doi.org/10.1016/j.scitotenv.2023.167499

Mishra, A. K., Sudalaimuthuasari, N., Hazzouri, K. M., Saeed, E. E., Shah, I. and Amiri, K. M. A. (2022). Tapping into Plant–Microbiome Interactions through the Lens of Multi-Omics Techniques. Cells, 11(20), Article 20. https://doi.org/10.3390/cells11203254

Menzel, P.; Ng, K.L. and Krogh, A. (2016). Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nature Communications, 7(1), Article 1. https://doi.org/10.1038/ncomms11257

Méric, G.; Wick, R. R.; Watts, S. C.; Holt, K. E. and Inouye, M. (2019). Correcting index databases improves metagenomic studies (p. 712166). bioRxiv. https://doi.org/10.1101/712166

Mohapatra, M.; Behera, P.; Kim, J. Y. and Rastogi, G. (2020). Seasonal and spatial dynamics of bacterioplankton communities in a brackish water coastal lagoon. Science of The Total Environment, 705, 134729. https://doi.org/10.1016/j.scitotenv.2019.134729

Pavlović, J.; Bosch-Roig, P.; Rusková, M.; Planý, M.; Pangallo, D. and Sanmartín, P. (2022). Long-amplicon MinION-based sequencing study in a salt-contaminated twelfth century granite-built chapel. Applied Microbiology and Biotechnology, 106(11), 4297–4314. https://doi.org/10.1007/s00253-022-11961-8

Portik, D. M.; Brown, C. T. and Pierce-Ward, N. T. (2022). Evaluation of taxonomic classification and profiling methods for long-read shotgun metagenomic

sequencing datasets. BMC Bioinformatics, 23(1), 541. https://doi.org/10.1186/s12859-022-05103-0

Posit Team. (2023). Posit. Posit. https://www.posit.co/

R Core Team. (2023). R: The R Project for Statistical Computing. https://www.r-project.org/

Rapid Sequencing gDNA (SQK-RAD004). (n.d.). Oxford Nanopore Technologies. Retrieved December 24, 2023, from https://community.nanoporetech.com/protocols/rapid-sequencing-sqk-rad004/v/rse_9046_v1_revad_14aug2019

Ren, Z.; Ma, K.; Jia, X.; Wang, Q.; Zhang, C. and Li, X. (2023). Metagenomics Unveils Microbial Diversity and Their Biogeochemical Roles in Water and Sediment of Thermokarst Lakes in the Yellow River Source Area. Microbial Ecology, 85(3), 904–915. https://doi.org/10.1007/s00248-022-02053-1

Rojas-Jimenez, K.; Araya-Lobo, A.; Quesada-Perez, F.; Akerman-Sanchez, J.; Delgado-Duran, B.; Ganzert, L.; Zavialov, P.O.; Alymkulov, S.; Kirillin, G. and Grossart, H.P. (2021). Variation of bacterial communities along the vertical gradient in Lake Issyk Kul, Kyrgyzstan. Environmental Microbiology Reports, 13(3), 337–347. https://doi.org/10.1111/1758-2229.12935

Salinas Romero, S.G.; Casaccia-Ibarrola, C.; García-Calabrese, M.; Cabral Antúnez, N.; Eufemia, L.; Kacic, P. and Da Ponte, E. (2023). Impacts of national governments on the forest cover loss in Paraguayan Chaco between 1999 and 2021. Journal for Nature Conservation, 75, 126472. https://doi.org/10.1016/j.jnc.2023.126472

Simpson, J.T.; Workman, R.E.; Zuzarte, P.C.; David, M., Dursi, L.J. and Timp, W. (2017). Detecting DNA cytosine methylation using nanopore sequencing. Nature Methods, 14(4), Article 4. https://doi.org/10.1038/nmeth.4184

Stevens, B.M.; Creed, T.B.; Reardon, C.L. and Manter, D. K. (2023). Comparison of Oxford Nanopore Technologies and Illumina MiSeq sequencing with mock communities and agricultural soil. Scientific Reports, 13(1), Article 1. https://doi.org/10.1038/s41598-023-36101-8

Wang, L.; Lian, C.; Wan, W.; Qiu, Z.; Luo, X.; Huang, Q.; Deng, Y.; Zhang, T. and Yu, K. (2023). Salinity-triggered homogeneous selection constrains the microbial function and stability in lakes. Applied Microbiology and

Biotechnology, 107(21), 6591–6605. https://doi.org/10.1007/s00253-023-12696-w

Wickham, H. (2011). Ggplot2. WIREs Computational Statistics, 3(2), 180–185. https://doi.org/10.1002/wics.147

Wickham, H.; François, R.; Henry, L. and Müller, K. (2023). dplyr: A grammar of data manipulation. R Package Version 1.1.4, 3. https://github.com/tidyverse/dplyr, https://dplyr.tidyverse.org.

Wilke, C.O. (2023). cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2" (1.1.2) [Computer software]. https://cran.r-project.org/web/packages/cowplot/index.html

Wood, D.E.; Lu, J. and Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

ZeinEldin, R.A.; Ahmed, M. M.; Hassanein, W. S.; Elshafey, N.; Sofy, A.R.; Hamedo, H.A. and Elnosary, M.E. (2023). Diversity and Distribution Characteristics of Viruses from Soda Lakes. Genes, 14(2), Article 2. https://doi.org/10.3390/genes14020323

Zhang, H.; Cai, W.; Guo, F.; Bian, C.; Liu, F.; Zhang, L.; Liu, J. and Zhao, M. (2023). Microbial community composition and environmental response characteristics of typical brackish groundwater in the North China Plain. China Geology, 6(3), 383–394. https://doi.org/10.31035/cg2022073

Zhou, J. and Ning, D. (2017). Stochastic Community Assembly: Does It Matter in Microbial Ecology? Microbiology and Molecular Biology Reviews, 81(4), 10.1128/mmbr.00002-17. https://doi.org/10.1128/mmbr.00002-17.