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research area: Environmental studies

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ASSESSMENT OF THE EFFECTS OF CONSERVATION ACTIVITIES IMPLEMENTED IN THE CIRQUE OF THE SEVEN RILA LAKES

INTRODUCTION

Glacial lakes represent some of the most climate-sensitive aquatic ecosystems on Earth. Among the biotic components of glacial lakes, microbial communities play a central role in ecosystem functioning by driving carbon and nutrient cycling, organic matter transformation, and energy flow through pelagic and benthic food webs. Consequently, climate-driven alterations in microbial community structure and function may have far-reaching implications for high-mountain freshwater ecosystems. Recent warming trends directly affect microbial metabolism and indirectly influence microbial assemblages through shifts in primary production, nutrient availability, and terrestrial organic matter inputs. To evaluate climate-driven effects on sediment microbial communities in glacial lakes, we focused on the seasonal dynamics of bacterial and archaeal assemblages. Seasonal transitions in these systems represent natural temperature gradients that recurrently expose microbial communities to contrasting cold and warm conditions, thereby providing a valuable framework for assessing climate sensitivity. By using seasonal dynamics as a proxy for climate-driven change, this approach aims to improve predictions of how ongoing temperature increases may reshape sediment microbial communities and their associated ecosystem functions in glacial lake environments.

PROJECT GUIDELINES

Project lifespan: 1.03.2024 – 31.03.2026

Study site: littoral of lakes Bliznaka, Trilistnika, Ribnoto and Dolnoto from the Seven Rila Lakes cirque (Fig. 1).

Indicator for the investigation: sediment microbial communities.

Objective: assessing the effects of macrophytes and sediment debris removing in the Seven Rila Lakes and to gain new knowledge on the change of the sediment microbial communities after such conservation activities.



Figure 1. Map of the Seven Rila Lakes cirque, northwest Rila Mountains, Bulgaria with the Bliznaka, Trilistnika, Ribnoto and Dolnoto lakes.

METHODOLOGY

Sampling procedure: conducted in June, August, and October 2024; upper-core sediment samples were collected from two randomly selected sampling plots (1 m × 1 m), each with three subsampling points

Environmental Parameters: sediment temperature (T; °C) - measured in situ using handheld meter WTW (Oberbayern, Germany); porewaters were analysed for pH, dissolved carbon (DC; mg/L), dissolved inorganic carbon (DIC; mg/L), dissolved organic carbon (DOC; mg/L), and dissolved organic nitrogen (DON; mg/L); DC and DIC were analysed by high-temperature (850 °C) catalytic oxidation using Elementar Vario Select TOC analyser (Langensfeld, Germany). DOC aromaticity was expressed by $SUVA_{254} = A_{254}/DOC$.

Molecular Analysis: Total DNA extraction: Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit (Zymo Research Corp., Irvine, CA, USA). Next-Generation Sequencing: carried out by Novogene Co., Ltd. (Cambridge, UK) using the Illumina HiSeq platform. Softwares used for data processing and analysis of operational taxonomic units (OTUs): FLASH software (v. 1.2.7); FASTP (v. 0.23.1) software; VSEARCH package (v. 2.16.0); UPRASE (v. 7.0.1001); Mothur software (v. 1.48.0); sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1398596.

Statistical Analysis: non-metric multidimensional scaling (NMDS) ordination using the PAST (v. 4.03) software; MicrobiomeAnalyst software (v. 2.0); Mann–Whitney/Kruskal–Wallis; PERMANOVA for statistical significance of dissimilarity measures; Wilcoxon rank-sum test; Benjamini–Hochberg false discovery rate (FDR) correction.

RESULTS

Lake sediments were analyzed using ordination (NMDS; Bray–Curtis) to evaluate environmental similarities among different months (seasons), with the resulting clustering patterns shown in Figure 2. Pronounced temporal variability in sediment characteristics was observed, with the most distinct differences appearing at the beginning of the ice-free period (June) and during summer (August), while autumn (October) exhibited more moderate, lake-specific variation. The NMDS results demonstrated clear temporal and spatial organization of sediment environments across the lakes. Samples were mainly grouped according to sampling month, highlighting strong seasonal changes in sediment composition.

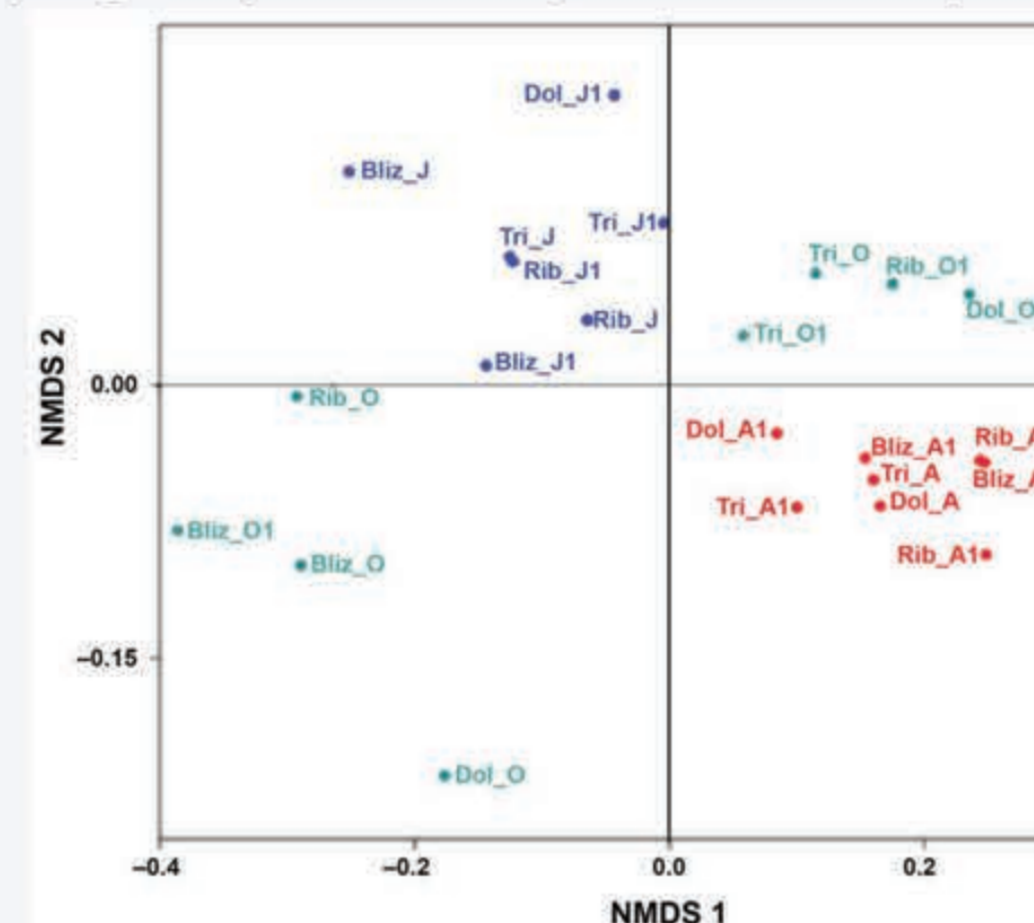


Figure 2. Non-metric multidimensional scaling plot (NMDS; stress: 0.095) of sediment environments of Bliznaka (Bliz), Trilistnika (Tri), Ribnoto (Rib), and Dolnoto (Dol) lakes in June (blue), August (red), and October (cyan) 2024.

Alpha-diversity indices (Shannon and Simpson) and richness estimators (observed species, ACE, and Chao1) were generally highest in August and lowest in June (for diversity) and October (for richness) (Figure 3). The mean values of these indices differed significantly between August and June/October ($p \leq 0.021$), while no significant differences were detected between June and October ($p \geq 0.57$). The Simpson index exhibited lower sensitivity, as it did not show significant variation among months ($p = 0.101$). Overall, the diversity metrics indicated marked temporal variability in sediment microbial communities, with August displaying the greatest divergence across sampling periods. The diversity metrics indicated pronounced temporal variability in sediment microbial communities, with August exhibiting the greatest divergence among sampling months.

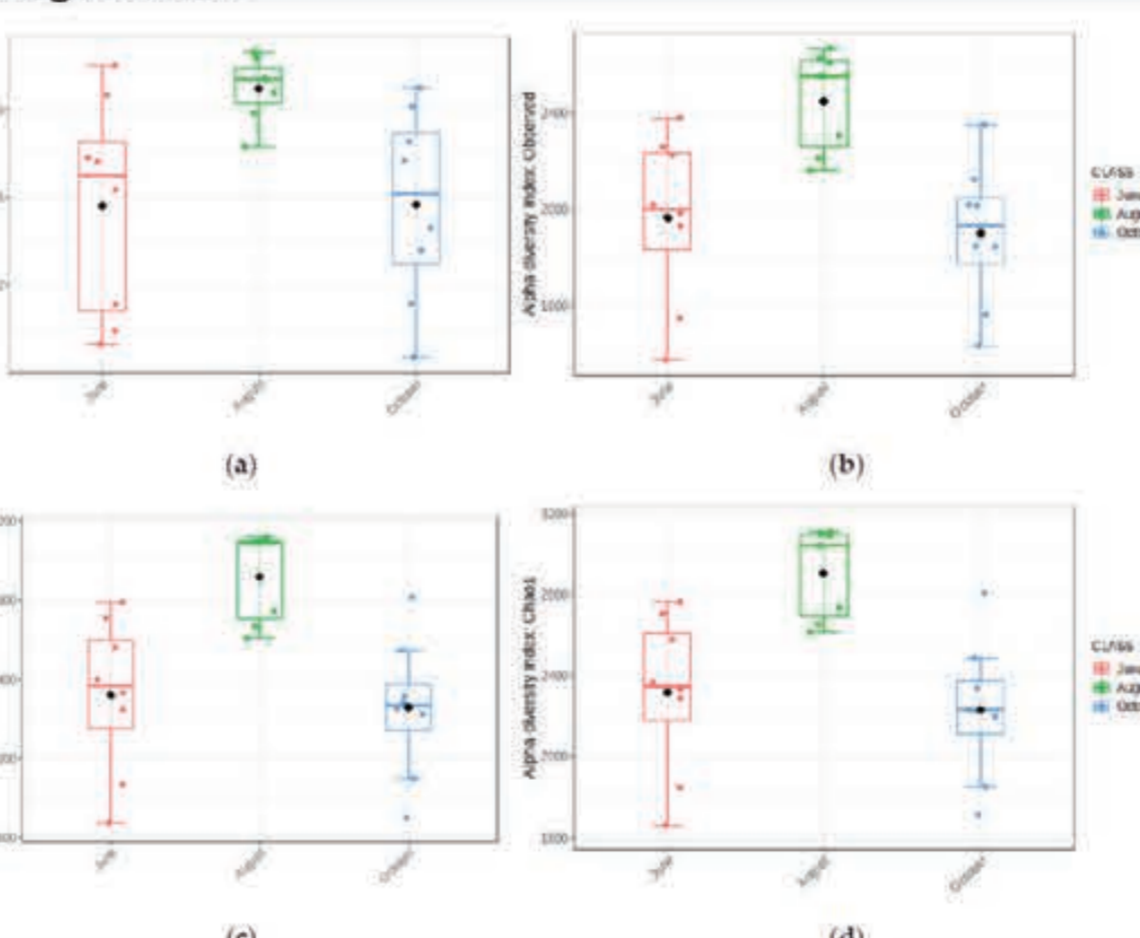


Figure 3. Alpha diversity (Shannon index) (a), richness indices expressed as observed OTUs (b), ACE (c), and Chao1 (d), illustrating the overall structure of the sediment microbiota.

Differences in archaeal and bacterial communities between months were explored across multiple taxonomic levels using heat tree analysis. Variations were visualized based on color-coded differences in the median relative abundance of each taxon. Statistical significance of pairwise monthly comparisons was assessed using the Wilcoxon rank-sum test, with p-values adjusted for multiple testing via the Benjamini–Hochberg false discovery rate (FDR) correction. Adjusted p-values < 0.05 were considered statistically significant. No archaeal taxa characteristic of the early growing season were identified in comparison to the other seasonal stages (Figure 4a). In contrast, August sediments showed enrichment of two lineages within the phylum Halobacteriota (families Methanotrachaceae and Methanospirillaceae) (a, b), while the order Group_1.1c (phylum Thermoproteota, class Nitrososphaeria) was reduced relative to June.

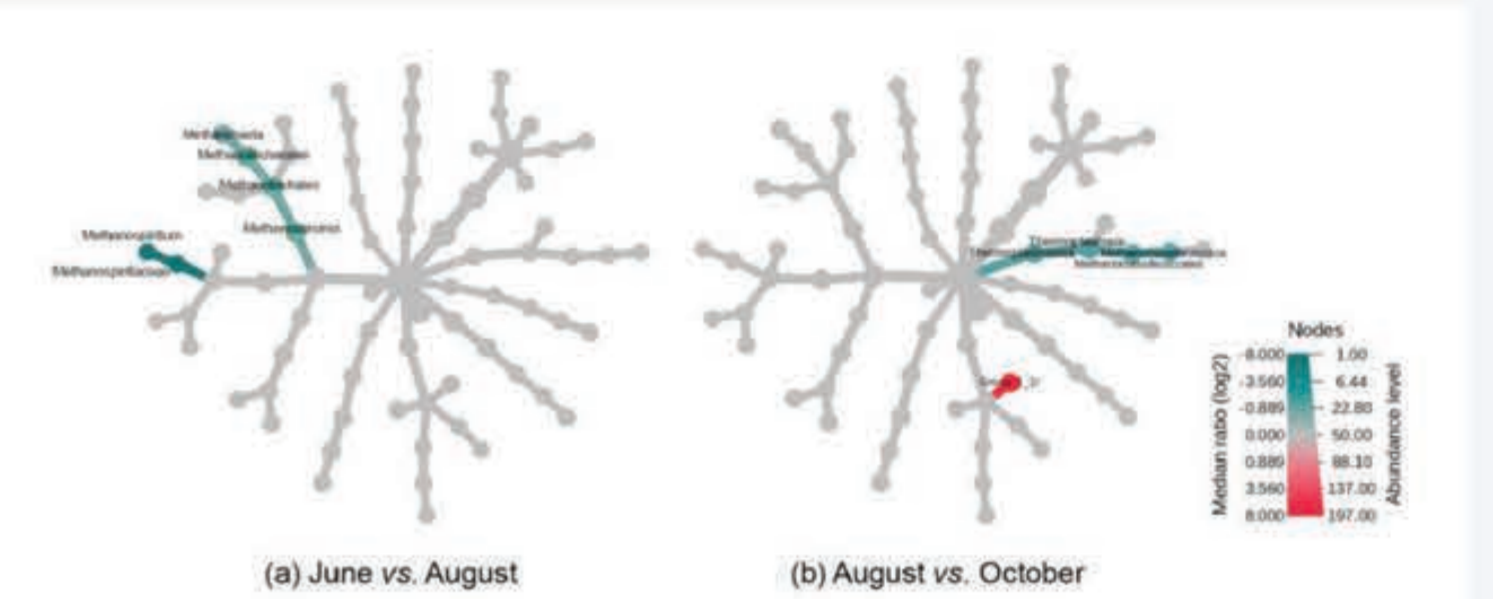


Figure 4. Between-month differences in archaeal communities visualized by heat tree analyses.

Significant differences between October and August sediments were observed in the abundance of Thermoplasmata across multiple taxonomic levels (Fig. 4b). In contrast, no significant differences in archaeal community composition were detected between June and October. Heat tree analysis of bacterial 16S rRNA gene OTUs showed that the most pronounced shifts in bacterial community structure occurred between June and August. During this period, bacterial communities shifted toward dominance by Pseudomonadota, with indicative taxa including the classes Alphaproteobacteria (Rhodobacterales), Betaproteobacteria (Burkholderiales, particularly the family Comamonadaceae), and Gammaproteobacteria (Methylococcales) (Fig. 5a, b).

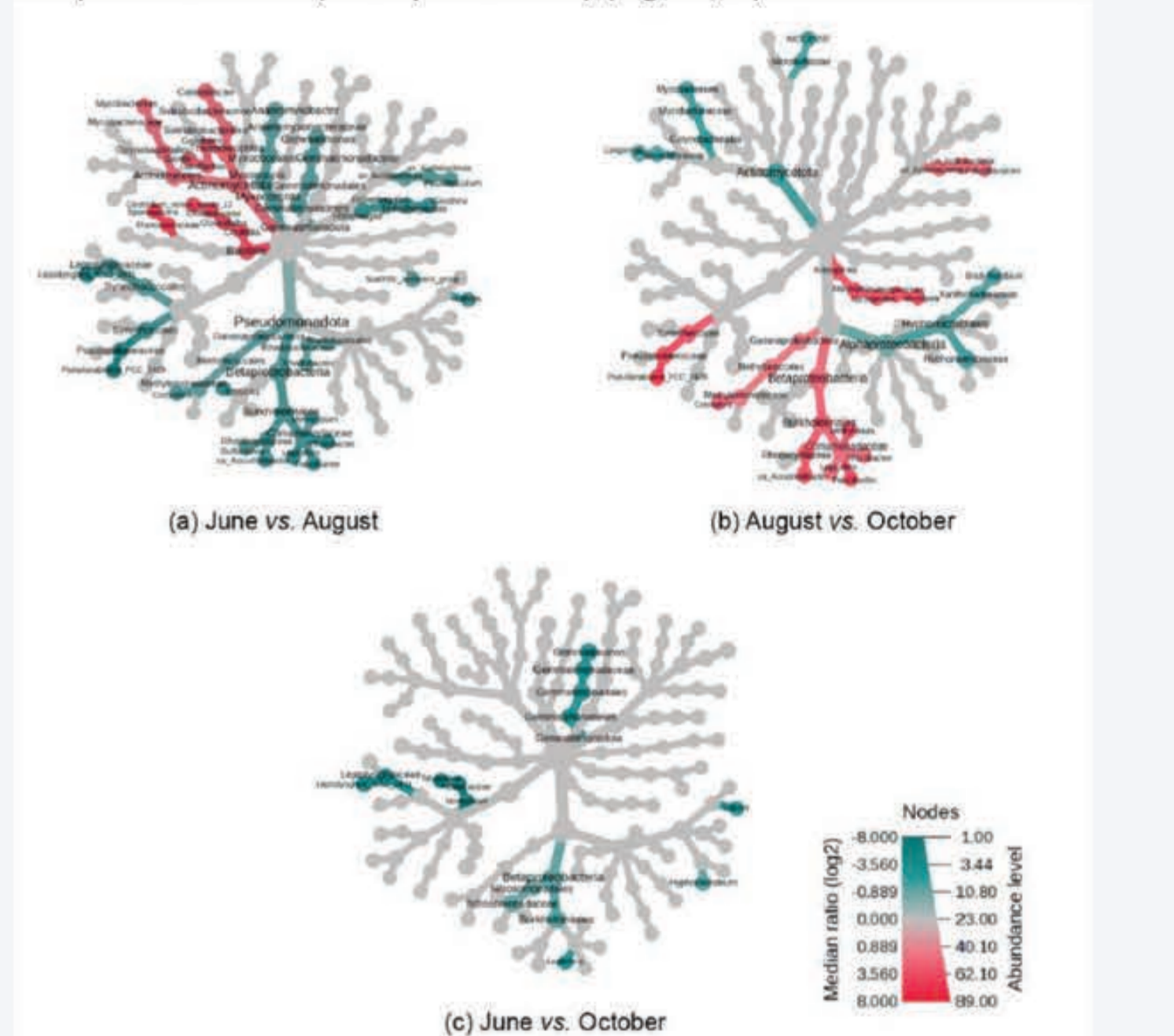


Figure 5. Between-month differences in bacterial communities visualized by heat tree analyses with indicative taxa.

Additional non-Pseudomonadota summer-indicative taxa included phyla Myxococcota (Anaeromyxobacteraceae) and Gemmatimonadota (Gemmatimonadaceae), as well as order Synechococcales (Leptolyngbyaceae) within the phylum Cyanobacteriota. Indicative of October were the order Nostocales (phylum Cyanobacteriota) with genus Tolypothrix, compared with August bacterial communities (b), and Betaproteobacteria (orders Nitrosomonadales and Burkholderiales) compared with June communities (c).

CONCLUSION

- Temperature is a primary driver of microbial community structuring in glacial lake sediments.
- Summer warming promoted temperature-tolerant and metabolically versatile taxa, particularly the methanogenic archaeon Methanoseta (phylum Halobacteriota), along with copiotrophic (*Paludibaculum*, *Paucibacter*, *Rhizobacter*) and methanotrophic (*Crenothrix*, *Methylibium*) bacterial genera, reflecting enhanced carbon processing during warm periods.
- Ongoing climate warming, extended ice-free periods, and increased organic matter availability are therefore expected to intensify methanogenesis and accelerate microbially driven carbon and nutrient cycling, potentially shifting glacial lakes from traditionally minor methane sources to increasingly important contributors to regional and global greenhouse gas emissions.

This research was funded by European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project SUMMIT BG-RRP-2.004-0008-C01. Project №70-123-193/12.02.2024. Head of the research group email: sbboteva@biofac.uni-sofia.bg