

## FAMILY-LEVEL NICHE DIFFERENTIATION IN BACTERIAL COMMUNITIES INHABITING GLACIAL LAKES IN RILA MOUNTAIN, BULGARIA

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### Abstract

Glacial lakes are harsh ecosystems harbouring unique microbiota. In this study, we investigated the distribution of bacterial families in the water and sediment of three glacial lakes located in Rila Mountain, Bulgaria. Our results revealed high bacterial diversity and pronounced habitat preferences, as evidenced by the presence of 310 bacterial families with distinct distribution patterns between lake water and sediments. Taxonomic dissimilarities between water and sediment bacterial communities ranged from 74% to 100%. In general, water communities exhibited a common set of bacterial families with relatively consistent abundances across lakes, whereas sediment communities were more lake specific. Dominant families in sediments included Bacteroidaceae, Veillonellaceae, and Nitrosomonadaceae, whereas Comamonadaceae, Mycobacteriaceae, and Sporichthyaceae were prevalent in water samples. These findings highlight a clear bacterial partitioning between lake habitats and provide new insights for glacial lake conservation in a changing environment, particularly in the context of global warming.

**Key words:** bacterial diversity, water and sediment bacteria, NGS, high-mountain microbiome, bacterial family taxa

**Introduction.** Glacial lakes are remote ecosystems characterized by extreme environmental conditions, including high solar irradiance, low temperatures, prolonged ice cover, a short growing season, and low nutrient availability [1]. In these

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environments, the microbial loop plays a crucial role in sustaining life by facilitating nutrient recycling and transferring energy to higher trophic levels [2]. Since the ecological significance of microorganisms in these systems has become increasingly recognized, research efforts have grown to better understand this component of the food web. Studies have revealed that bacterial communities in glacial lakes consist of core taxa, well-adapted to the harsh alpine conditions, and rare taxa, whose presence appears to be influenced by local factors [3]. Many of these studies reported bacterial diversity at the phylum or class level, often focusing on the inhabitants of a single lake habitat.

The Seven Rila Lakes are of great interest due to their unique abiotic characteristics, unique biotic communities, and their role as a major tourist attraction. In contrast to the well-documented physicochemical properties and lake biota, information on their bacterial communities remains scarce. Although several studies have focused on bacterial abundance and activity [4,5], the composition of bacterial communities remains poorly understood.

Advances in molecular biology now provide tools to assess the diversity and composition of lake bacterial communities, enabling, for the first time, efforts to identify the dominant bacteria in these ecosystems and to gain insights into their spatial distribution. In this context, the aim of this study was to evaluate the bacterial diversity of three glacial lakes from the cirque of the Seven Rila Lakes and to reveal the patterns of bacterial family distribution between the water and sediment habitats of the littoral zone.

**Materials and methods. Study lakes and sampling.** Bubreka (Bub), Okoto (Oko) and Sulzata (Sul) are glacial-origin lakes located in the Northwestern region of the Rila Mountain, Bulgaria, at elevations of 2282 m a.s.l. (Bub; 42°12'19.6"N 23°18'26.4"E), 2440 m a.s.l. (Oko; 42°11'57.5"N 23°18'23.1"E) and 2535 m a.s.l. (Sul; 42°11'50.0"N 23°18'38.9"E) without interconnection among them. Sampling was conducted in October 2023 due to the relatively stable lake conditions, expressed by progression of basin drying and a significant decline in watershed connectivity, as well as ending of growth season and decline in metabolite exudation. Water and sediment samples were collected from three points along the lakes' littoral zones using sterile equipment for sampling, storage and transport. Samples from each substrate type were combined into composite aliquots to create representative sample for subsequent analyses.

**DNA extraction.** Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit (Zymo Research Corp., USA) was used to extract metagenomic DNA from water filtrate and sediment samples according to the manufacturer's protocol.

**Next-generation sequencing (NGS).** NGS of the metagenomic DNA and the primary bioinformatic analysis were performed by Novogene Co. Ltd., UK, using an Illumina HiSeq platform. For bacterial identification, the V3–V4 region of the 16S rRNA gene was amplified using the 341F/806R primer pair. Effective tags were analyzed using software Uparse v7.0.1001 [6] and sequences with  $\geq 97\%$

similarity were assigned to the same operational taxonomic units (OTUs). The Silva database was used to annotate the taxonomic information based on Mothur software V1.48.0. [7, 8].

**Statistical analysis.** The alpha diversity indices (Shannon diversity, Chao 1 and ACE) were calculated. The Bray–Curtis similarities of bacterial communities between water and sediment were examined by cluster analysis (UPGMA; Bray–Curtis similarity index) using PAST software [9].

**Results. Bacterial diversity.** Each sample achieved over 95% coverage, with the exception of the sediments from Sul, indicating that the sequencing depth was generally sufficient (Table 1). In the sediments of Sul, 12% of the reads originated from OTUs that appeared only once in this sample. The number of OTUs, along with ACE, Chao1, and Shannon indices, revealed: a) greater variability between lake sediment compared to water samples; and b) higher bacterial diversity and richness in lake sediments than in the water, particularly in Sul. This trend in diversity distribution was not observed for Oko Lake.

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Richness and diversity indices of bacterial communities for sediment (S) and water (W) samples from Bubreka (Bub), Okoto (Oko) and Sulzata (Sul) lakes in Rila Mountain, Bulgaria.

| Sample | OTUs | Shannon | Chao1 | ACE  | Coverage |
|--------|------|---------|-------|------|----------|
| BubS   | 708  | 8.37    | 849   | 864  | 0.949    |
| OkoS   | 30   | 2.75    | 30    | 30   | 0.999    |
| SulS   | 1002 | 8.74    | 1573  | 1710 | 0.876    |
| BubW   | 177  | 5.70    | 282   | 317  | 0.983    |
| OkoW   | 128  | 4.82    | 180   | 168  | 0.991    |
| SulW   | 149  | 5.26    | 216   | 240  | 0.986    |

**Bacterial community composition and similarity analyses.** A total of 310 bacterial families were identified in the waters and sediments of the Rila lakes (Fig. 1). However, the analysis focused on the top 20 families, which accounted for 63% – 90% of the total relative abundance, except in the sediments of Bub (29.4%) and Sul (15.5%) lakes.

Distinct patterns of bacterial distribution were observed across lakes and substrates: a) Lake-specific high occurrences of certain families were noted, including Comamonadaceae (17.2%) and Mycobacteriaceae (12.4%) in Bub Lake; Bacteroidaceae (33.8%), Veillonellaceae (34.9%), Fusobacteriaceae (12.7%), and Crocinitomicaceae (12.1%) in Oko Lake; and Sporichthyaceae (33.6%) in Sul Lake; b) Typical sediment-dwelling bacteria included Nitrosomonadaceae, SC-I-84, and Xanthobacteraceae, with relative abundances ranging from 3% to 8.4%; c) Typical water-dwelling bacteria were represented by Burkholderiaceae, Crocinitomicaceae, Flavobacteriaceae, NS11-12\_marine\_group, Spirosomaceae, Armatimonadaceae,

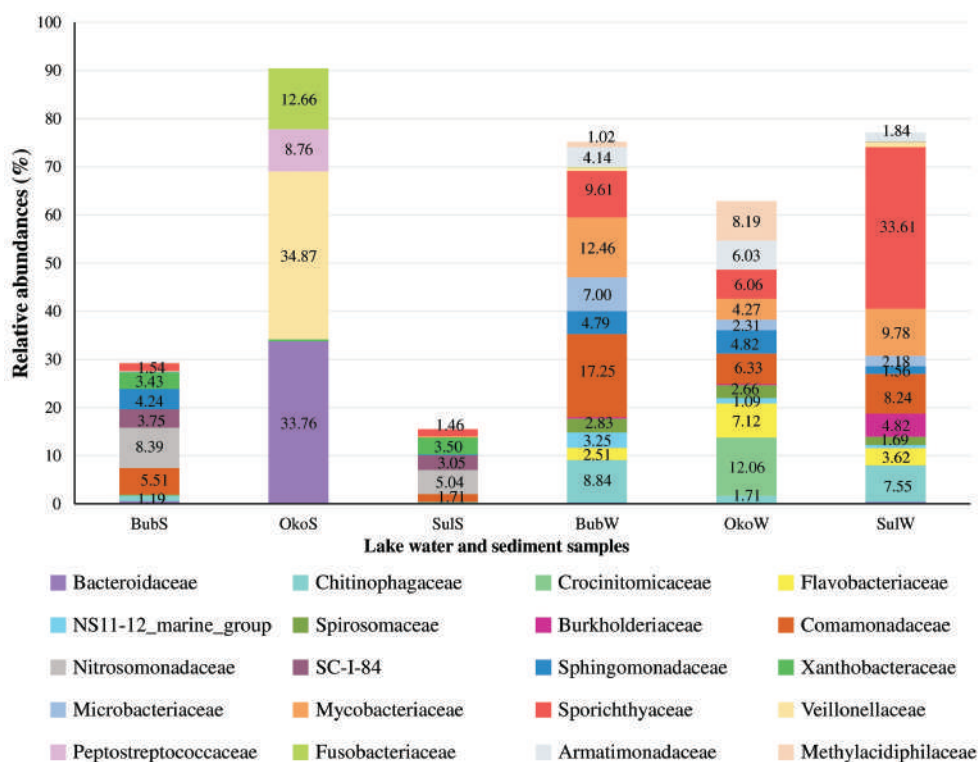


Fig. 1. Relative abundance of bacterial families in sediment (S) and water (W) samples of Bubreka (Bub), Okoto (Oko) and Sulzata (Sul) lakes. Only the relative abundances of families with over 1% have been indicated

Microbacteriaceae, and Methylacidiphilaceae, with abundances varying from 1% to 12.1%; d) Some bacteria showed a preference for lake water (1.7–33.6%) but were also detected in sediments at lower abundances (0.2–4.2%), including Mycobacteriaceae, Chitinophagaceae, Comamonadaceae, and Sporichthyaceae.

Taxonomic similarity of lakes was assessed using cluster analysis (CA) (Fig. 2). CA identified three main groups: Cluster A included the sediments from Sul and Bub lakes; Cluster B comprised all water samples, with a higher similarity observed between Sul and Bub; and Cluster C was defined by the sediment bacterial diversity of Oko Lake. Within-group Bray–Curtis similarities were 68% for Cluster A and 53% for Cluster B (on average). The dissimilarity between Clusters A and C was 98%. Within individual lakes, the taxonomic similarities between waters and sediments were 26% for Bub, 8% for Sul, and 0% for Oko lakes.

Additionally, CA grouped the bacterial families into four distinct clusters. The first cluster included Bacteroidaceae, Veillonellaceae, Peptostreptococceae, and Fusobacteriaceae, which were dominant in the sediments of Oko Lake, present at low abundances in Bub sediments, and absent in Sul sediments. The second

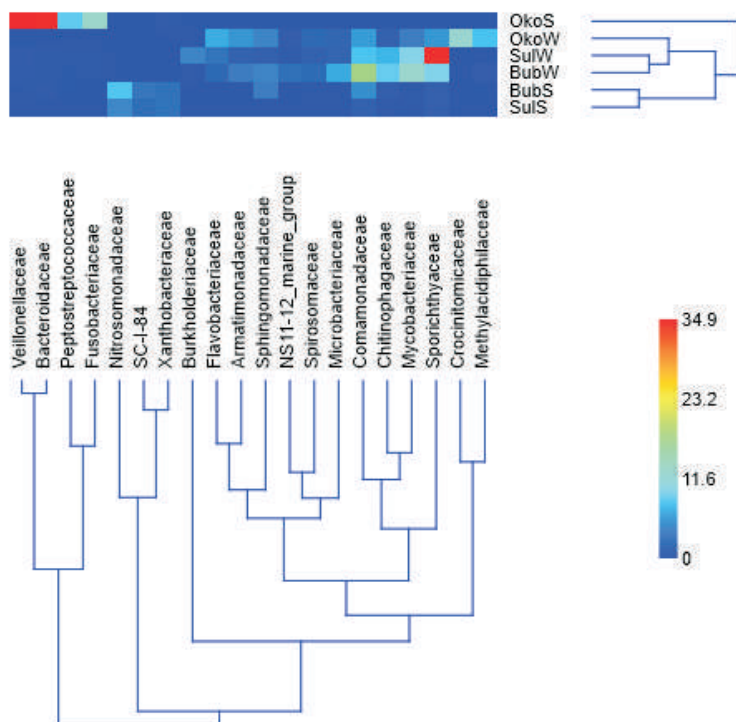


Fig. 2. Dendrogram of the clustering analysis illustrating the grouping of Bubreka (Bub), Okoto (Oko) and Sulzata (Sul) lake environments of waters (W) and sediments (S) based on bacterial taxonomic similarity

cluster comprised families present in Bub and Sul sediments, but absent from lake waters, such as Nitrosomonadaceae, SC-I-84, and Xanthobacteraceae. The third cluster consisted solely of Burkholderiaceae, which was detected in Sul lake water. All remaining families were grouped together in a fourth cluster, represented by water bacterial families.

**Discussion.** Glacial lakes play a crucial role in preserving the biological diversity of organisms and ecosystems worldwide, owing to their unique environments and inhabitants. This study, for the first time, identified bacterial families inhabiting three (Bubreka, Okoto, and Sulzata) of the Seven Rila Lakes, which were considered a part of the Bulgarian natural heritage. A total of 310 bacterial families were identified, most of which were present in low relative abundances, except Bacteroidaceae, Veillonellaceae, Sporichthyaceae, Comamonadaceae, Mycobacteriaceae, Fusobacteriaceae and Crocinitomicaceae, and exhibited local distribution by lake or substratum. Both bacterial diversity (except in the case of Oko Lake) and its variability were higher in lake sediments than in lake waters, highlighting the crucial role of sediments as reservoirs for lake-specific bacterial representatives. The pattern of higher bacterial diversity in lake sediments, along

with distinct differences between water and sediment bacterial communities, has also been reported for other glacial lakes [10,11], suggesting distinct environmental properties of the two substrata, and different sources of bacterial enrichment. According to some authors [10,12], bacterial water communities originate from rainfalls, and catchment inflows, whereas sediment bacterial communities develop through long-term adaptations, and processes of accumulation and deposition [10,11]. In this context, we expect that sediment bacteria reflect the unique characteristics of each lake, whereas water bacteria reveal the biogeographic connectivity among lakes.

CA revealed that the sediment bacterial community of Oko Lake was distinct from those of Bub and Sul lakes, primarily due to the high abundance of Veillonellaceae (34.9%), Bacteroidaceae (33.8%), and Fusobacteriaceae (12.7%), whose members are typically obligate anaerobes and chemoorganotrophs that strongly depend on organic matter load, and play a crucial role in its degradation [13–15]. In contrast, sediments of Bub and Sul lakes were dominated by Nitrosomonadaceae (5–8%), SC-I-84 (3–3.8%), and Xanthobacteraceae (3.4–3.5%), which are recognized as chemolithoautotrophs, with some groups involved in nitrogen fixation (Xanthobacteraceae), ammonia oxidation (Xanthobacteraceae), and denitrification (SC-I-84) [16,17].

The identified water families belonged to the phyla Bacteroidota (Chitinophagaceae, Flavobacteriaceae, NS11-12 marine group, and Spirosomaceae), Actinobacteriota (Microbacteriaceae, Mycobacteriaceae, and Sporichthyaceae), Pseudomonadota (Comamonadaceae and Sphingomonadaceae), and Armatimonadota (Armatimonadaceae). A localized distribution was observed for the families Crocinitomicaceae (phylum Bacteroidota) and Methylocaldiphilaceae (phylum Verrucomicrobiota) in Oko Lake, and Burkholderiaceae (phylum Pseudomonadota) in Sul Lake. Members of these families are freshwater cosmopolitans with preference to open waters, UV resistant, competitive under conditions of low nutrient availability, have negligible seasonal dynamics, and are capable to avoid grazing under different forms of self-protection [18].

**Conclusion.** The studied glacial lakes exhibit high bacterial diversity, harbouring members of 310 bacterial families. A clear pattern of bacterial distribution was observed, identifying typical sediment- and water-associated bacterial families. Sediment bacterial families are predominantly anaerobes known for their copiotrophic lifestyle, while water bacterial families are cosmopolitan and exhibit strong resistance to both abiotic and biotic pressures. Additionally, lake-specific distributions of certain bacterial families were recorded, and the causes of this segregation pattern will be the focus of future studies.

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