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Research Article

Restoration of high-mountain lakes: effects on nutrient concentrations and biological communities

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Abstract

Lakes worldwide are under severe pressure from increasing anthropogenic impacts and global warming, creating a growing demand for restoration efforts and sustainable lake management strategies. However, there has been limited practice in restoring high-altitude lakes, particularly those under protected status. This paper focuses on passed lake restoration with special emphasis on the effects of restoration on lake ecosystems. Restoration efforts in four lakes within Rila National Park, Bulgaria, were carried out in two steps: macrophyte harvesting followed by sediment removal. These were immediately followed by a monitoring programme to evaluate the effects of human activities. The most significant effects on nutrient concentrations (PO_4-P, NO_3-N, TP and TN) and chlorophyll-a levels were observed one week after the restoration, with these effects diminishing one year later. Changes in bacterial metabolism were characterised by a reduction in metabolic activity, a shift from preferential utilisation of carbohydrates and polymers to an increased use of carboxylic acids and a decrease in functional richness and diversity, followed by partial recovery. Some of the changes observed in lake environments and biotic components were attributed to seasonal shifts, while others were linked to the implementation of human activities. We conclude that the initial steps of lake restoration were effective, with minimal to negligible environmental impact.

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However, the long-term effectiveness of restoration remains uncertain, so the monitoring programme will continue to address still unresolved questions, particularly to distinguish between seasonal and anthropogenic effects on ecosystems.

Keywords

lake restoration, macrophyte harvesting, sediment removal, nutrients, chlorophyll-a, Biolog Ecoplate, high-mountain lakes

Introduction

Eutrophication is a natural process that occurs as aquatic ecosystems age, but human activities can greatly accelerate its progression (Schindler 2006). The primary environmental impacts of eutrophication include increased suspended particles from extensive algal blooms, reduced water clarity and higher rates of organic sediment precipitation and accumulation. Other notable effects are bottom-water hypoxia, elevated CO_2 production from the intense decomposition of organic matter, intensified water acidification and disruptions to nutrient cycling (Dorgham 2014).

Mountain lakes are often regarded as some of the least disturbed aquatic ecosystems due to their remoteness. However, recent international studies reveal that high-mountain lakes are increasingly impacted by human activities on both local and global scales, making eutrophication a growing concern. Key anthropogenic drivers include global warming (Hantel and Hirtl-Wielke 2007, Gobiet et al. 2014, Pastorino and Prearo 2020), long-range transport of pollutants (Bogdal et al. 2010, Pastorino and Prearo 2020), watershed alterations (Schmidt et al. 2002) and tourism activities (Nyaupane et al. 2014, Nikolova et al. 2018, Mitova 2025).

Mountain lakes are important for their biodiversity and the ecosystem services they provide. They also hold significant cultural and socioeconomic value, serving as hubs for various human activities. This creates a dilemma for lake managers, who must balance preserving the lakes' natural state with accommodating traditional human uses. Human activities, whether in the short or long term, often lead to substantial ecological changes (Knapp et al. 2001, Schindler 2001), making mountain lakes particularly sensitive to environmental disturbances.

The Rila Mountains are a popular tourist destination in Bulgaria. The most visited area is the cirque of the Seven Rila Lakes, located within Rila National Park at elevations between 2,095 m and 2,535 m. The lakes are situated one above the other, interconnected by small streams. Their accessibility attracts over 2,000 tourists daily during the summer season (https://biodiversity.bg/bg/Rilska-misiya-prebroyavane-na-turisti-na-Sedemte-rilski-ezera.p1707). Tourist-induced soil erosion (Buchwał et al. 2009, Mitova 2020), increasing air (and consequently water) temperatures over time (Nikolova et al. 2018) and artificial fish introduction/stocking (e.g. in Ribnoto Lake) have caused

changes in the lakes, especially those situated at lower elevations. Numerous publications in the national and social media have focused on the high level of eutrophication in the lakes and the increasing spread of macrophytes, particularly *Ranunculus aquatilis* and *Sparganium angustifolium*, in the lakes (Zafirov 2021, Mitova 2025). Under the project “Sustainable Management of Rila National Park, Phase II” (BG16M1OP002-3.007-0002), the Rila Park Authority conducted extensive restoration work on Bliznaka (Bliz), Trilistnika (Tri), Ribnoto (Rib) and Dolnoto (Dol) lakes. The activities included harvesting aquatic vegetation (*Ranunculus aquatilis*) and removing sediment deposits which were purely experimental in nature, as there is no evidence they have ever been applied before in high-mountain lakes. Briefly, the removal of both macrophytes and sediments from the lakes was carried out on three small scale areas in the littoral of each lake. The areas represent squares with a side of 20 m^2 (400 m^2 each) and are located in areas with increased sediment loads/macrophyte densities. Each of the areas was divided into four smaller squares with a side of 10 m, with sediments/ macrophytes removed in two of them, diagonally located to each other. This allows a comparison of the effects within the areas after the removal procedures were completed. The macrophyte and sediment removal was carried out in two consecutive summers (July – September 2022 and July – August 2023) from different sets of areas. The macrophyte harvesting was carried out by manually removing the entire plants. An inflatable boat was used to transfer the removed biomass to the lake shore. The removed plants were dried onsite on polyethylene sheets. After drying, they were deposited at predetermined locations outside the park. On average, 80.2 ± 13.7 g fresh weight of *R. aquatilis* were removed per square metre from the lakes. Sediment removal was carried out in such a way that it did not lead to disruption of the profile of the lake shores, as well as additional deepening of the lake bottom. For this purpose, only the fine loose sediment was removed by hand-held water lift (a dredging device that combines a water pump and flow divider). The removed sediments contained macrophyte fragments and seeds, thus increasing the efficiency of the macrophyte and dead organic matter removal. The dredged material was collected in dewatering filter bags that traps sediment particles as water seeped out. The bags were positioned onshore on a permeable layer and the water that seeped was channelled back to the lakes. On average 1.16 ± 0.32 kg drained sediment was retained in the bags per square metre from the lakes. Additionally, in order to reduce soil erosion, hiking trails in the watershed were gravelled and demarcated (personal communication).

This study aimed to assess the effects of restoration activities on the lakes' physical environments and the more rapidly responsive biological components, specifically phytoplankton and heterotrophic bacteria. Phytoplankton responds to increased nutrient levels by increasing its biomass, while bacteria adjust their metabolism, based on the quantity and quality of available organic matter. Phytoplankton productivity supports aquatic life, while microorganisms serve as the primary pathway for transferring organic matter to higher trophic levels (Sommaruga and Casamayor 2009). These processes are crucial for maintaining the ecological integrity of lake ecosystems.

In the study, chlorophyll-a (Chl-a) was used as an indicator of phytoplankton biomass, while the Biolog Ecoplate™ (Biolog Inc., Hayward, CA, USA) was employed to assess the ability of heterotrophic bacterial communities to utilise 31 different carbon sources before and after lake restoration. The use of Biolog EcoPlates for the functional analysis of microbial communities through the inoculation of natural microbial samples was first described by Garland and Mills (1991), with the carbon set later optimised by Insam (1997). This culture-based physiological approach has proven to be an effective tool for studying the range and diversity of bacterial metabolism, as well as for distinguishing spatial and temporal functional changes within microbial communities (Hill et al. 2000).

Material and methods

Sampling site and sampling procedure

The study focused on four (Bliz, Tri, Rib and Dol) of the Seven Rila Lakes, a high-mountain group of glacial origin located in the northwest Rila Mountains, Bulgaria (Fig. 1).

Figure 1.

Map of the Seven Rila Lakes cirque, northwest Rila Mountains, Bulgaria (https://osmap.org/#map=15/2596309.12/5191591.09/0).

The restored lakes are situated in the lower part of the watershed and exhibit different hydro-morphological characteristics, which are presented in Table 1.

Table 1.

Hydro-morphometric characteristics of the studied Rila Lakes (Nikolova et al. 2021).

Lake	Altitude (m)	Area (ha)	Volume (m^3)	Max. depth (m)	Watershed area (ha)
Bliznaka (Bliz) 42°12′02′′N, 23°18′57′′E	2,243	9.1	590	27.5	205
Trilistnika (Tri) 42°12′22′′N, 23°19′06′′E	2,216	2.6	54	6.5	223
Ribnoto (Rib) 42°12′24′′N, 23°19′24′′E	2,184	2.3	38	2.5	251
Dolnoto (Dol) 42°12′39′′N, 23°19′32′′E	2,095	5.9	240	11.0	281

Sampling surveys were conducted in August 2015, October 2021 and in July 2022 (before restoration activities) to calculate the mean biotic and abiotic values typical of the pre-restoration period. The post-restoration effects were assessed through surveys conducted in August 2023 (approximately one week after restoration), October 2023 (one month after restoration) and October 2024 (one year after restoration). Sampling was done at three points in the area of the treated areas in the littoral zone of the lakes. Water samples for chemical and microbiological analyses were kept and transported at 4°C. Samples for microbiological analysis were taken and kept in sterile containers.

Water physico-chemical properties

Dissolved oxygen (DO; mg O_2/l), water temperature (T; ˚C), pH and electrical conductivity (EC; µS/cm) were measured in situ using hand-held meters (WTW and Hanna), following Bulgarian State Standards BDS EN 25814 (DO), BDS ISO 10523 (pH) and BDS EN 27888 (EC), respectively. In the laboratory, a part of water samples was filtered through glass fibre filters (Whatman GF/F; 0.7 µm) before analysing ammonium nitrogen (NH_4-N in µg/l; ISO 7150/1), nitrate nitrogen (NO_3-N in µg/l; 1.14773.0001) and phosphate phosphorus (PO_4-P in µg/l; EN ISO 6878). Unfiltered samples were used for total nitrogen (TN in µg/l; 1.14773.0001) and total phosphorus (TP in µg/l; EN ISO 6878). Chlorophyll-a (Chl-a; µg/l) concentration was determined according to ISO 10260. All colourimetric analyses were carried out on the CECIL CE 3021 spectrophotometer.

Inorganic nitrogen (IN) was calculated as the sum of NO_3-N and NH_4-N concentrations. Organic phosphorus (OP) and organic nitrogen (ON) concentrations were determined by subtracting the respective inorganic forms from the TP and TN concentrations, respectively.

The trophic state classification was based on chlorophyll-a as a better predictor of algal biomass than either of the two indices and the trophy scale of Carlson (2007) was used.

Bacterial metabolic profiles

Biolog Ecoplate™ set (Biolog Inc., Hayward CA, USA; https://www.biolog.com/wp-content/uploads/2023/08/00A-012-Rev-F-EcoPlate-IFU.pdf) was used to assess bacterial metabolic capacity, utilising 31 Ecoplate carbon sources (B1 – H4), with each microplate run in triplicate. Ecoplate™ carbon sources (CSs) were categorised into five carbon guilds (CGs), including carbohydrates (CH), polymers (Polym), carboxylic acids (CA), amino acids (AA) and amines (Amin) (Weber and Legge 2009). The microplates were inoculated with 120 µl of lake water, incubated at 20 ± 1°C in the dark and the optical densities (ODs) of the wells were measured every 12 h for five days at 590 nm (Microplate Reader LKB 5060-006 with software package DV990 “Win 6”). Kinetic data were used to calculate the area under the curve (AUC; square units - SU), applied to evaluate the following endpoints:

1. bacterial metabolic capacity calculated as average well colour development (AWCD);
2. community level physiological profiles (CLPPs) used as a bacterial community fingerprint of the utilisation rates of CSs and CGs;
3. functional richness (R) and Shannon-Weaver diversity (H′) indices, expressing the number of utilisable Ecoplate CSs (R) and functional diversity (H′) of lake bacterial communities. The applied equations were:

$$AUC = \sum \frac{OD_n + OD_{n+1}}{2 * (t_{n+1} - t_n)}$$

where OD_n and OD_{n+1} represent the optical densities at two consecutive measurements at times t_n and t_{n+1} (Guckert et al. 1996).

AWCD was calculated as:

$$AWCD = \sum \frac{AUC_i}{N}$$

where AUC_i is the area under the curve of the i^{-th} CS and N is the number of CSs (N = 31) in the Ecoplate™ set (Garland and Mills 1991).

The CS richness (R) and Shannon-Weaver diversity (H′) indices were calculated as:

R = number of utilisable Ecoplate CSs

$$H' = \sum p_i * ln(p_i)$$

where p_i is the ratio between the AUC of the i^{-th} CS to the sum of AUCs of all Ecoplate™ CSs (Magurran 1988). Before the calculation of AWCD, CGs, CLPPs, R and H′, the control AUC was subtracted from the AUC of each CS.

Statistical analyses

Each data point in the paper represents the mean value of the respective parameter ± standard deviation. Repeated-measures ANOVA, followed by Tukey's test, was performed to examine the significance of the differences in lake parameters (abiotic and biotic) between pre- and post- restoration times. Pearson correlation analysis was applied to assess the relationships between the studied metrics. Redundancy analysis (similarity index: Euclidean) was used to ordinate the lakes according to their physical environments and CLPPs. A two-way PERMANOVA (perm.: 9999, similarity index: Euclidean) was performed on CLPP data to determine whether significant effects occurred by 'lake identity' and 'sampling time' as explanatory factors and if there were any interactive effects between them. The Similarities Percentages Procedure (SIMPER) of CLPPs was used to determine the CSs that contributed the most to the Bray-Curtis dissimilarity amongst the lakes between the pre- and post- restoration periods, as well as between each pair of sampling occasions. The above statistics were performed with the PAST package (Hammer et al. 2001) at a significance level of $p < 0.05$.

Results

The physico-chemical parameters of the lakes are summarised in Suppl. material 1. Water temperature ranged from 6.7°C to 18.2°C and the water was generally well-oxygenated (DO: 8.7 - 10.7 mg/l), except for Lake Dol in October 2023, where the DO was 6.3 mg/l. The lakes exhibited neutral to alkaline pH values, ranging from 6.98 to 8.42. Dissolved ion concentrations were low, as reflected in EC values of 18 µS/cm to 32 µS/cm. Nutrient concentrations in the lakes were generally low, with NH_4–N ranging from 20 µg/l to 90 µg/l, PO_4–P from 17 µg/l to 40 µg/l and TP from 21 µg/l to 90 µg/l. Concentrations of NO_3–N and TN ranged from 420 µg/l to 1000 µg/l and 950 µg/l to 2000 µg/l, respectively. The proportion of PO_4–P to TP generally ranged between 33% and 50%, except in October 2024, when it increased to 83%. The dominant form of inorganic nitrogen (IN) in the lakes was NO_3–N, while NH_4–N accounted for 1.6% to 17.35% of it (Suppl. material 1). IN constituted approximately 82% of TN (on average for all lakes) during the pre-restoration period, declining to 33% in October 2023, before partial recovering to 52% in October 2024.

Overall, one year after the end of the restoration activities, various nutrient changes were observed. At that time most of the nutrient concentrations have returned to the pre-restoration levels except PO_4-P in Bliz and Tri. (Fig. 2).

In order to evaluate the overall changes in lake environments, non-metric multidimensional scaling (NMDS) was conducted. The pre-restoration lake environments clustered closely together along the right side of Axis 1, except for Dol. The primary factors contributing to the variability along this axis were NO_3-N and T (Fig. 3). Restoration activities shifted the positions of the lakes counterclockwise on the ordination plot. The movement pattern was consistent for the lakes in August and October 2023, with a transition from the fourth to the third quadrant (excluding Bliz and Dol) and, in October

2024, from the third to the second quadrant. The factors with the greatest influence on the respective localisation of the lakes were PO_4-P, EC and T (August and October 2023), as well as T and NH_4-N (Bliz) in October 2024. In general, Dol did not follow this pattern of changes and the alterations observed in its environment, after the restoration, were the smallest. The significant segregation of Bliz in August 2023 from the other lakes was associated with the sequence of intervention in the lakes, which proceeded from Dol to Bliz. Overall, the lake environments in October 2024, although showing a trend of recovery, have not yet returned to their pre-restoration levels. Additionally, the restoration activities caused a divergence in the characteristics of the lake environments, lasting for more than a year.

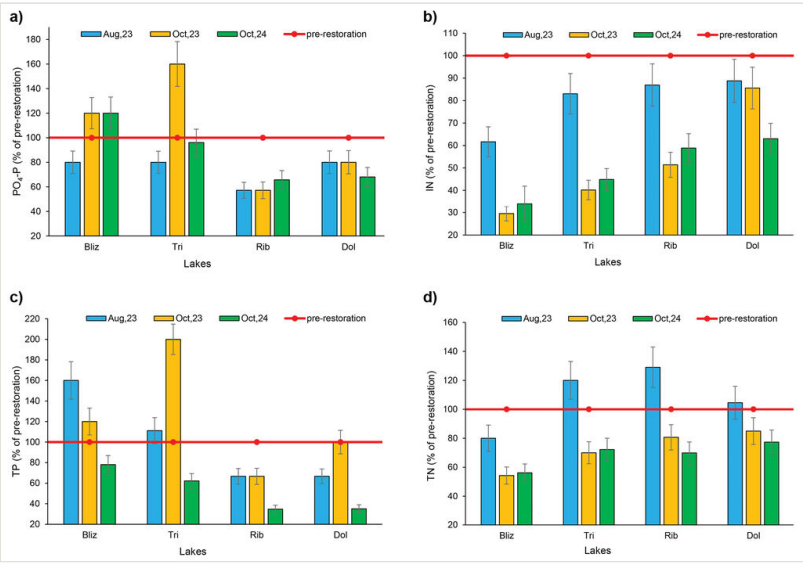

Figure 2.

Water concentrations of a) phosphate phosphorus (PO_4-P), b) inorganic nitrogen (IN), c) total phosphorus (TP) and d) total nitrogen (TN) for lakes during the post-restoration period (August 2023, October 2023 and October 2024) expressed as percentage of the respective pre-restoration value. Bars represent the standard deviations of the means.

Biotic responses

Lake biotic responses to restoration activities were evaluated by measuring Chl-a concentrations and bacterial metabolic activity and functional profiles. Chl-a served as a proxy for phytoplankton biomass, while bacterial metabolic activity reflected the microbiome ability to adapt and tolerate environmental changes.

a. Changes in chlorophyll-a content

The mean Chl-a concentrations during the pre-restoration period were 2.71 ± 0.27 µg/l (Bliz), 2.08 ± 0.21 µg/l (Tri), 1.71 ± 0.17 µg/l (Rib) and 6.58 ± 0.66 µg/l (Dol). These values classified the lakes as oligotrophic (Bliz, Tri and Rib) and mesotrophic (Dol).

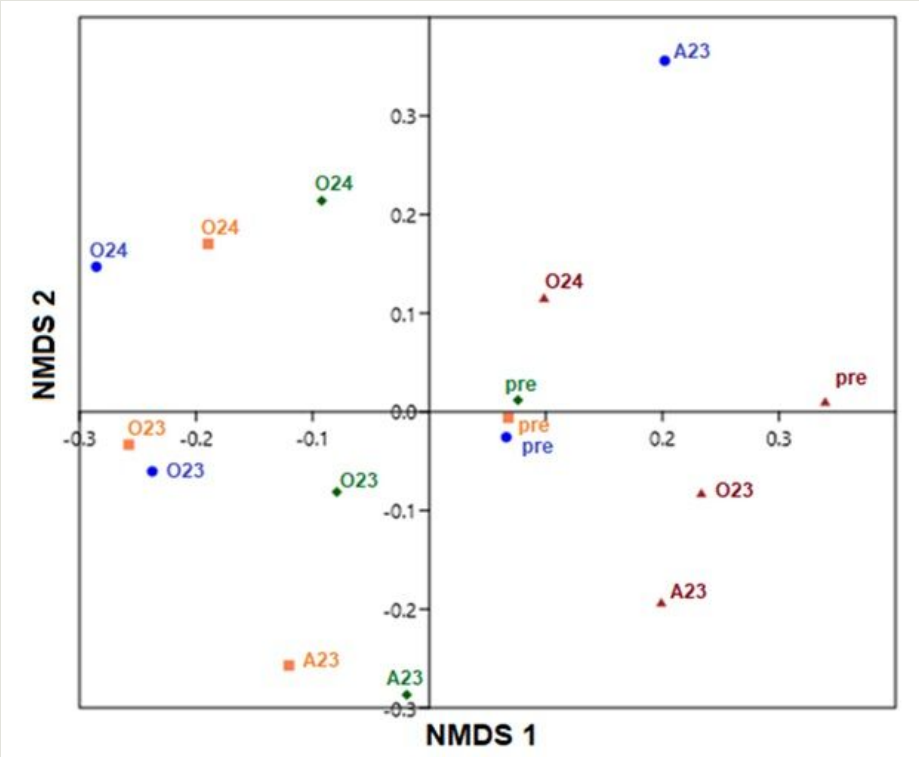

Figure 3.

Non-metric multidimensional scaling (NMDS) ordination, based on a Euclidean similarity matrix of lake physico-chemical parameters. Symbols represent the water environment of Bliz (blue dot), Tri (coral quadrate), Rib (green diamond) and Dol (brown triangle) in pre-restoration period (pre), August (A23) and October (O23) 2023 and October 2024 (O24). Two-dimensional stress = 0.11.

Different trends were recorded in the lakes after restoration activities compared to the Chl-a status before their restoration (Fig. 4): 1) in August 2023, dramatic decrease (by 32% for Bliz and 48% for Tri) or increase (by 170% for Rib and 26% for Dol) in Chl-a concentrations, being significantly different from that of the pre-restoration time ($p < 0.05$); 2) in October 2023, the stimulation effects on Chl-a persisted, even in Tri, where the trend of Chl-a changes reversed compared to August 2023; and 3) in October 2024, Chl-a concentrations returned to pre-restoration level (Tri) or below it (Bliz, Rib and Dol). In this aspect, an improvement in lake trophic status was observed, especially in Dol, where the trophic state shifted from mesotrophy (pre-restoration) to the boundary between oligotrophy and mesotrophy.

Pearson correlation analyses showed that Chl-a concentrations correlated significantly with different numbers of environmental factors across sampling occasions (Suppl. material 2) and the more stable in time were the relationships with T ($r > 0.79$; $p < 0.002$) and EC ($r > -0.66$; $p < 0.02$).

b. Changes in bacterial functional profiles

Changes in bacterial metabolism were expressed by set of parameters such as average metabolic activity (AWCD), functional profile (CLPP) and indices of functional richness (R) and diversity (H′).

- ***AWCD***

During the pre-restoration period, the AWCD was recorded as 1.97 ± 0.14 SU (Bliz), 1.80 ± 0.07 SU (Tri), 2.47 ± 0.13 SU (Rib) and 2.19 ± 0.10 SU (Dol). Restoration activities generally reduced AWCD, except for Tri and Dol in August 2023, where bacterial metabolic activity increased by 66% (Tri) and 30% (Dol) (Fig. 5a). The trend of reduced AWCD was long-lasting over time, with the average AWCD remaining at 87 ± 1.3% of pre-restoration levels one year later. The exception in October 2024 was Tri, where AWCD approached pre-restoration values.

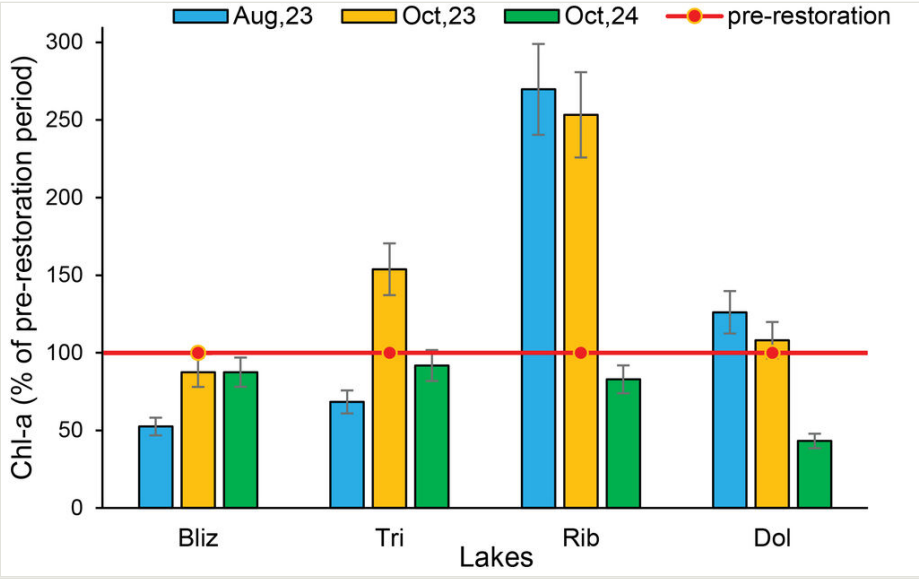

Figure 4.

Water concentrations of chlorophyll-a (Chl-a) during the post-restoration period (August and October 2023 and October 2024) expressed as percentage of the respective pre-restoration Chl-a concentration. Bars represent the standard deviations of the means.

Pearson correlation coefficients revealed a relatively low number of significant relationships between AWCD and water metrics, with these relationships being temporally dependent (Suppl. material 2). During the pre-restoration, phosphorus revealed significant a positive relationship with AWCD ($p < 0.05$), whereas in August, it became insignificant (PO_4-P) or negative (TP).

- **CLPPs**

AWCD was generated by utilisation of 30 CSs (C3; carboxylic acid 2-Hydroxy benzoic acid was not utilisable at all) divided into five chemically distinct CGs: CH (9 CSs), Polym (4 CSs), CA (9 CSs), AA (6 CSs) and Amin (2 CSs). The utilisation of the CGs is shown in Fig. 5b. During the pre-restoration period, the most preferred CGs were Polym and CH, with utilisation rates (on average) approximately 23% higher than the AWCD. After restoration, the importance of CH remained high, but decreased in October 2023 and 2024. Polym use declined in August and October 2023, while the utilisation of CA increased after restoration compared to pre-restoration period. Utilisation of AA and, especially, Amin was lower compared to other CGs and relatively stable over the monitoring period.

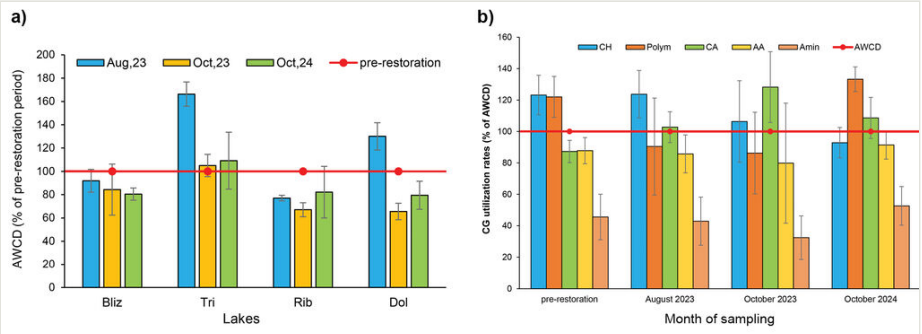

Figure 5.

Bacterial a) metabolic activity (AWCD) and b) carbon guild (CG) utilisation rates. AWCD of post-restoration period (August and October 2023 and October 2024) was expressed as percentage of the respective pre-restoration AWCD. CG utilisation was expressed as percentage of the respective AWCD. Bars represent the standard deviations of the means.

Within CGs, aquatic bacteria utilised CSs at varying rates (0.11 – 7.89 SU) and these abilities changed over time, creating different functional profiles (CLPPs) of bacterial communities. Some CSs were unutilisable (C3), while the utilisation of others was dependent on both time and lake (G4, G3, B1, C1, D1, A3, E3, A4 and D4). In contrast, certain CSs (A2, D2, E2, B3 and B4) were the most preferred throughout the monitoring period (Suppl. material 3). In the context of great variability of CSs utilisation, NMDS was conducted to assess the ordination of lakes' metabolic activity, based on bacterial functional profiles (CLPPs) (Fig. 6).

The ordination plot revealed closely-clustered CLPPs from the pre-restoration period and October 2024, indicating a high degree of similarity both amongst the lakes and between these two sampling occasions. In contrast, the CLPPs from August and October 2023 were more dispersed, with those from October 2023 forming two distinct groups (Bliz + Tri and Rib + Dol) located far apart from each other. The counterclockwise shift in CLPP locations on the ordination plot was associated with changes in the utilisation of CGs, transitioning from CH and Polym in the pre-restoration period to CA, Polym and AA in the post-restoration period.

PERMANOVA followed by SIMPER was conducted to compare the main factors influencing on CLPPs and to identify the most variable CSs. PERMANOVA revealed that sampling time (F = 3.34; p = 0.0011) and lake identity (F = 2.06; p = 0.0016), but not the interaction between the two factors (F = 0.99; p = 0.48) significantly influenced on CLPP dissimilarity, assessed between pre- and post- restoration periods (Suppl. material 4). The overall average CLPP dissimilarity (SIMPER) between the two sampling periods was 32.97% and the most variable CSs (10 of 30 CSs), contributing approx. 50% to the CLPP variability were E3, A2, G1, D2, D1, G2, D4, B4, E2 and C1 (Suppl. material 5). Amongst these CSs were CH (56% of CH in Ecoplate set), Polym (50%), AA (33%) and CA (11%). SIMPER analyses confirmed the results from NMDS for the greatest dissimilarity in CLPPs between August 2023 and October 2023 (40.73%) and the smallest between pre-restoration CLPPs and that from October 2024 (29.07%).

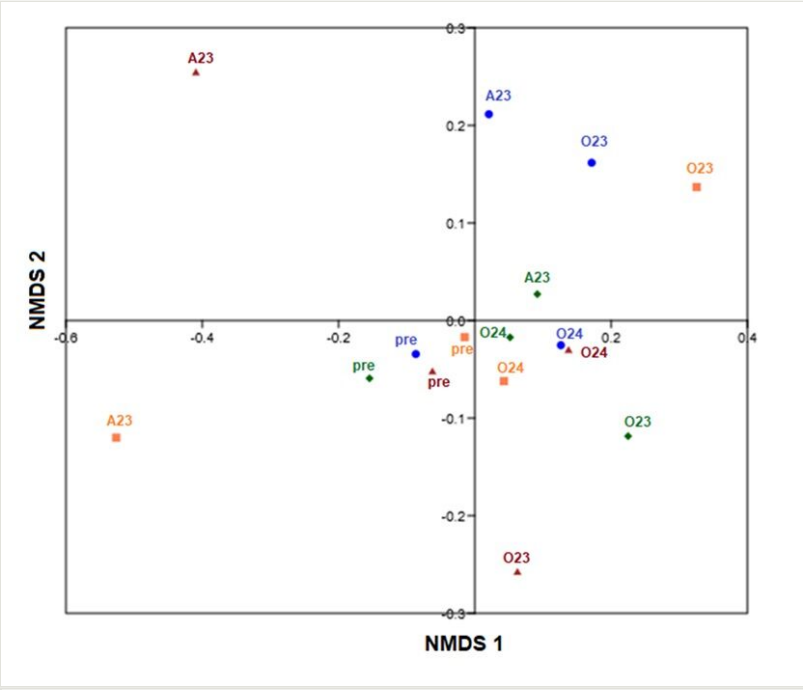

Figure 6.

Non-metric multidimensional scaling (NMDS) ordination, based on a Euclidean resemblance matrix, calculated from the functional profiles (CLPPs) of lake bacteria. Symbols represent the CLPPs of Bliz (blue dot), Tri (coral quadrate), Rib (green diamond) and Dol (brown triangle) in pre-restoration period (pre), August (A23) and October (O23) 2023 and October 2024 (O24). Two-dimensional stress = 0.17.

The rate of changes in bacterial metabolism was evaluated also by indices of functional richness (R) and diversity (H′) (Suppl. material 6). The number of utilisable CSs (R) decreased after restoration activities (by 21% compared to the pre-restoration), with a trend of partial recovery observed in October 2024. H′ followed a similar pattern of

change as R, with a strong positive correlation between the two indices ($r = 0.89$; $p < 0.0001$).

Discussion

The restoration strategy for Bliz, Tri, Rib and Dol lakes was aligned with their protection status and included a series of actions such as gravelling hiking trails, macrophyte harvesting and sediment removal. These measures aimed to achieve specific targets for reduction of the macrophyte biomass in the lakes without increasing nutrient concentrations or deteriorating water quality. This study assessed the success of lake restoration efforts and their impact on ecosystem functions by addressing three primary objectives:

1. maintaining stable or reduced nutrient concentrations (PO_4-P, TP, NO_3-N and TN);
2. achieving stable or reduced phytoplankton growth (measured as Chl-a);
3. maintaining the metabolic activity (rate and range) of heterotrophic bacteria close to pre-restoration levels.

Changes in nutrient concentrations

Many studies have reported that macrophyte harvesting and/or sediment removal lead to increased nutrient concentrations in the water, attributing this effect to sediment resuspension (Søndergaard et al. 2007, Sayer et al. 2010). In this study, some resuspension was recorded, but, due to the water lift design, the effects were temporary and only in certain parts of the lakes. Overall, nutrient concentrations decreased during the post-restoration period, on average by 14% for both PO_4-P and TP, 12% for TN and 58% for IN. The lowest nutrient concentrations (approximately 50% lower than pre-restoration values) were observed in October 2023 (IN) and 2024 (IN and TP). These reductions were attributed to a seasonal shift, likely reflecting a collapse in phytoplankton growth (Grover and Chrzanowski 2000) alongside sustained high grazing activities (Kaushal and Lewis 2005). Climatic factors (e.g. warmer and drier growing seasons) are also reducing nutrient levels in surface waters through a longer and increasingly steep thermal stratification (Sánchez-España et al. 2017). The seasonality of nutrient transitions was further evidenced by:

1. elevated levels of NH_4-N, particularly in October 2024, when its concentrations were 268% higher compared to pre-restoration values;
2. changes in the nutrient composition ratios, with the share of IN in TN decreasing from 82% to 49.5% (October 2023 and 2024) and that of PO_4-P in TP increasing from 48% to 83% (October 2024) compared to the pre-restoration period.

Time dynamics of nutrients and high NO_3-N content did not influence on the trophic states of the lakes, assessed by Chl-a content, classifying them as oligotrophic (Bliz), oligo-mesotrophic (Tri and Rib, depending on sampling time) and mesotrophic (Dol). The low primary productivity of high-mountain lakes is well-documented, attributed to strong phosphorus control (Rigosi et al. 2014, Mamun and An 2017, Atique and An 2019) and

restricting climatic conditions (Huang et al. 2019). In this study, restoration activities did not significantly alter nutrient concentrations.

The application of an ordination technique (NMDS) indicated overall shifts in lake environments, driven by changes in some primary determining factors. These factors shifted from T, NO_3-N and COD (pre-restoration period) to T (August and October 2023), PO_4-P and NH_4-N (August 2023 – October 2024) and COD (October 2024). While variations in some factors, such as water T, can be attributed solely to seasonal changes, others result from the complex interplay of seasonal and human impacts. A general trend of changes in lakes' environment was observed, except in Dol, where environmental shifts were minimal and the lake's recovery in October 2024 was closest (compared to the other lakes) to its pre-restoration state. Overall, macrophyte and sediment removal did not dramatically alter lakes' nutrient and trophic states, although some shifts in the water parameters were recorded, likely due to seasonal variability and, to a lesser degree, human activities.

Biological responses

Biological variables are direct indicators of environmental quality, offering a clear representation of how changes in physical environments affect ecosystem life. This study evaluated restoration success and its effects on lake ecosystem by analysing two components of food webs that respond rapidly to environmental changes: phytoplankton biomass expressed as Chl-a and bacterial metabolic activity.

Chl-a response to restoration

Macrophyte harvesting and sediment removal have been widely used in lake management to expand clear water areas and remove decaying plant materials, along with sources of nutrients and oxygen demand. However, previous studies have identified an increased risk of algal blooms following macrophyte harvesting (Scheffer 1998). The extent of this impact, as noted by some researchers, depends on the intensity of macrophyte harvesting (Zhu et al. 2022, Lin et al. 2024). While low-intensity harvesting has minimal effects on water quality (Lin et al. 2024), excessive harvesting can promote phytoplankton growth (Zhu et al. 2022). Determining the optimal intensity of macrophyte harvesting in this restoration programme was challenging, as insufficient control risked a shift to a phytoplankton-dominated state. This outcome would contradict the lakes' protected status and efforts to maintain their historical conditions. Immediately following the restoration, Chl-a concentrations increased by an average of 56% in August 2023 (in Rib and Dol) and 41% in October 2023 (in Tri, Rib and Dol), indicating stimulated phytoplankton growth. This effect on phytoplankton productivity was anticipated, due to nutrients (mainly phosphates) release from the sediments and was classified as modest and short-termed. By October 2024, Chl-a concentrations had returned to pre-restoration levels in Bliz, Tri and Rib, while significantly lower levels were observed in Dol. In this context, it can be argued that the goal of the restoration was achieved with minimal negative impact on water quality, at least in the short term. We have established that the restoration activities neither drastically increase the nutrient content in the water nor

remove the limiting control of phosphorus (TN/TP: 17.5 – 56.33) on phytoplankton growth. Other factor with limiting control on phytoplankton proliferation, especially in October, was low water temperature (on average, 11.62 ± 1.9°C in October 2023 and 7.25 ± 1.0°C in October 2024). A linear relationship was established between water temperature and Chl-a content, making it plausible to assume that the higher levels of phytoplankton observed after the restoration are more likely a seasonal peak rather than a negative effect of the restoration. Overall, although the Chl-a content was slightly increased in 2023, a trend of reduction, particularly in Dol, was observed when comparing the results from October 2023 and October 2024.

Bacterial response to restoration

Heterotrophic bacterial communities play a crucial role in sustaining life in high-altitude lakes (Gurung and Urabe 2011) by transforming organic carbon directly to higher trophic levels within food webs. Understanding bacterial responses to restoration efforts can shed light on the impacts of possible adverse effects on lake ecosystem functioning. These efforts include also elucidating trophic interactions between phytoplankton and bacterioplankton. In general, phytoplankton and benthic algae are recognised as the primary sources of dissolved organic carbon (DOC) for lake bacteria (Baron et al. 1991).

AWCD and CLPP

Bacterial functioning changed after restoration, as evidenced by: 1) changes in AWCD; 2) shifts in CLPPs, manifested by: i) preferential utilisation of CA over Polym and CH and ii) a counterclockwise relocation of bacterial functional profiles in the NMDS ordination plot; and 3) a decrease in metabolic richness and diversity. We assumed that some of these changes reflected seasonal variations, others resulted from human activities and, most likely, a combination of both factors was responsible.

The AWCD increase in August 2023 was anticipated, coinciding with the peak of phytoplankton proliferation during the mid-summer (Boteva et al. 2010, Taş 2016) and possible increase in sediment resuspended organic carbon (Søndergaard et al. 2007) after human activities. In fact, at that time in two of the lakes (Tri and Dol), an increase in AWCD was recorded (on average, by 48%), whereas in the other lakes (Bliz and Dol), an opposite change was observed. The reason for this controversy remained unclear, but we assumed local effects of restoration on lake environments, i.e. on lake organic carbon pool. In October 2023 and 2024, the reduction in AWCD was 16% (on average) and might be interpreted as a seasonal effect, aligning with lower water temperatures (on average, 9.62 ± 2.62°C), reduced levels of organic carbon expressed as the sum of organic phosphorus and organic nitrogen (on average, 546 ± 79 mg/l vs. 795 ± 196 mg/l for pre-restoration and August 2023) and the cessation of phytoplankton growth, coupled with zooplankton grazing activities outweighing primary production (Carrillo et al. 2002, Descy 2002).

During the monitoring period, the relationships between AWCD and Chl-a (used as a proxy for phytoplankton biomass - a source of organic carbon for heterotrophic bacteria)

were insignificant and shifted from positive (pre-restoration period and August 2023) to negative (October 2023 and 2024). The absence of a significant relationship was expected, as many authors highlight, on one hand, the importance of algal exudates and cell lysates, rather than algal biomass, as the primary sources of organic carbon for bacteria (Medina-Sánchez et al. 2004, González-Olalla et al. 2018) and, on the other hand, the non-linear relationship between phytoplankton biomass and rate of exudation (Descy 2002, Panzenböck 2007). In this context, the positive correlations observed in August 2023 might indicate bacterial utilisation of exudates, which are predominantly excreted during the exponential or stationary phases of algal proliferation (Nagata 2000). In contrast, the negative correlations observed in October suggested the potential use of cell lysates rather than exudates as a source of organic carbon for bacteria or the utilisation of more recalcitrant allochthonous organic carbon from autumn watershed inflows (Grasset et al. 2018, McCullough et al. 2018).

The changes in bacterial functioning were more clearly reflected by alterations in their CLPPs than by AWCDs. Bacterial metabolism is known to mirror the nutrient pool, accounting not only for the total organic carbon quantity, but also the chemical composition of this nutrient pool (Benner 2003). Before restoration of the lakes, CH and Polym (complex carbohydrates) were used preferentially, which were aligned both with their bioavailability as algal exudates/lysates (Biddanda and Benner 1997, Karl et al. 2000, Thornton 2014, Boteva et al. 2024) and their high energy value for microorganisms (Görke and Stülke 2008). After the restoration, bacteria were found to shift their preference from CH (October 2023 and 2024) and Polym (August and October 2023) to CA utilisation, indicating seasonal influences (CH) and restoration effects (Polym and CA) on the lake organic carbon dynamics. The greatest variability in CH and Polym dynamics was also confirmed by SIMPER analysis, which showed that 56% (CH) and 50% (Polym) of the respective Biolog Ecoplate CSs contributed more than 50% to the CLPP dissimilarity between the pre- and post-restoration periods. The utilisation of CH was closely linked by authors (Karl et al. 2000, Seiter et al. 2005, Thornton 2014) to seasonal availability driven by algal excretion activities. In contrast, CA utilisation was observed to increase in lakes experiencing stress (environmental or anthropogenic), during cooler months (Grover and Chrzanowski 2000) or coinciding with a peak in zooplankton populations (Höfle et al. 1999). In this context, a decreased share of CH utilisation in AWCD in October might reflect seasonal depletion of these CSs due to termination of algal growth (Karl et al. 2000, Seiter et al. 2005, Thornton 2014). In our case, CA utilisation increased from August 2023 (the warmest month of the study) to October 2024 (the coldest month of the study), suggesting an anthropogenic impact rather than a seasonal shift. This assumption was supported by the Pearson correlation analysis where utilisation of CA positively correlated with water temperature.

Additionally, the CLPP results indicated that bacteria prioritised specific CSs from each carbon group. Notably, all 10 of these CSs accounted for approximately 50% of the dissimilarity in CLPPs (SIMPER; 32.97%) between the pre- and post-restoration periods. Variations in their utilisation rates may reflect the stability of the organic carbon pool, influenced by both seasonal and human factors. Significant impacts on bacterial

functionality could disrupt biochemical cycling and trophic relationships in affected environments. However, this does not appear to be the case, as CLPP ordination and SIMPER analysis indicated functional convergence (functional recovery) between bacterial communities from the pre-restoration period and October 2024.

Conclusions

Macrophyte harvesting and sediment removal did not significantly deteriorate water quality for the monitoring period — stronger impacts were observed only one week after restoration. In the longer term (October 2024), the lakes showed reduced values in some abiotic (PO_4-P, TP, IN and TN) and biotic (Chl-a) parameters compared to pre-restoration conditions. Overall, most adverse effects appear to be short-lived and relatively minor when compared to the longer-term benefits of small-scale restoration activities even in the mountain lakes. This monitoring programme will continue in the future in order to identify the long-term effects of the restoration activities.

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All authors whose names appear on the submission: made substantial contributions to the conception, implementation and analysis of the work; revised the work critically for important intellectual content; approved the version of the manuscript to be published; agreed to be accountable for all aspects of the work in ensuring that questions related to

the accuracy or integrity of any part of the work are appropriately investigated and resolved. The authors confirm that this manuscript is original and has not been published or is currently under consideration for publication elsewhere.

Author contributions

This paper was initiated and conceptualised by Anelia Kenarova who also led the writing and statistical analysis process. The sampling and laboratory work was conducted by Silvena Boteva, Ivan Traykov and Boyanka Angelova. All authors jointly and equally contributed to its completion and final structure.

Conflicts of interest

The authors have declared that no competing interests exist.

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Supplementary materials

Suppl. material 1: Table 1S doi

Authors: Anelia Kenarova
Data type: physico-chemical
Brief description: Water physico-chemical characteristics of the Seven Rila Lakes at pre-restoration (mean of August 2015, October 2021 and July 2023) and post-restoration (August and October 2023 and October 2024) periods. Results are shown as mean and (standard deviation).
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Suppl. material 2: Table 2S doi

Authors: Anelia Kenarova
Data type: statistical
Brief description: Pearson correlation analysis per sampling occasion. Significant correlations are bolted.
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Suppl. material 3: Figure 1S doi

Authors: Anelia Kenarova
Data type: statistical
Brief description: Community level physiological profiles of aquatic bacteria. The colour scale of carbon utilisation rates is calculated on the respective AWCD. Red: utilisation rates below 30%, orange: rates between 31% and 100%, blue: rates between 101% and 130% and green: rates above 131%.
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Suppl. material 4: Table 3S doi

Authors: Anelia Kenarova
Data type: statistical
Brief description: Two-way PERM/test of bacterial metabolic capacity, based on Bray-Curtis dissimilarities of community level physiological profiles (CLPPs) between pre- and post-restoration periods.
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Suppl. material 5: Table 4S doi

Authors: Anelia Kenarova
Data type: statistical
Brief description: SIMPER analysis identifying the carbon sources that contributed most significantly to the overall average dissimilarity amongst the community level physiological profiles (CLPPs) between pre- and post-restoration periods.
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Suppl. material 6: Figure 2S doi

Authors: Anelia Kenarova
Data type: statistical
Brief description: Indices of bacterial (a) functional richness and (b) Shannon-Weaver diversity at post-restoration time (August and October 2023 and October 2024) calculated as percentages from the respective pre-restoration values.
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