

APPLICATION OF BIOLOG ECOPLATE™ TECHNIQUE
FOR ASSESSING THE EFFECTS OF MACROPHYTE
HARVESTING ON AQUATIC BACTERIAL COMMUNITIES

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Abstract

Macrophyte overgrowth in freshwater ecosystems is a worldwide problem, and a common management practice for these ecosystems is to remove the aquatic vegetation. In the study, the short-term effects of macrophyte harvesting in a mountain lake (Trevnoto Lake, Bulgaria) on both bacterial metabolic activity and fingerprints were assessed using Biolog Ecoplate™ assay. Bacterial responses to human activity were manifested by: 1) increased bacterial metabolic activity by 15%, and 2) shifts in bacterial metabolic fingerprints. The most affected carbon sources were those with low availability for aquatic bacteria – 85% of them were influenced and the rates of their utilizations changed from a decrease of 96% to an increase of 681%. In general, a clear trend was formed that the greater the bioavailability of the carbon sources, the less susceptible is their utilization to the environmental change. This preliminary study indicates an immediate impact of macrophyte harvesting on lake bacteria. In this aspect, Biolog Ecoplate™ assay could be useful in lake monitoring programmes in order to develop better sustainable management practices for restoration.

Key words: macrophyte harvesting, Biolog Ecoplate™, AWCD, CLPP

Introduction. Like many ecosystems, mountain lakes are confronted with the effects of climate change [1], land-use in the catchment [2], and human recreational activities [3]. Shallow lakes are more vulnerable to anthropogenic stress,

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combined with climate change [4]. Increased water temperature and nutrient catchment discharge induce lakes' primary productivity and one of the most visible effects of this is the macrophyte overgrowth [5], which has a considerable impact on ecosystem processes [6] by increasing the area of sediments with anoxic conditions, changing biochemical cycling by increasing methane generation, denitrification and phosphorus release [7]. Appropriate management of macrophyte biomass via harvesting is crucial for maintaining inland waters. However, considerable knowledge gaps exist regarding the effects of macrophyte harvesting on lake bacterial communities. Heterotrophic bacteria are one of the most abundant biotic components in aquatic ecosystems, being the major mineralizers of aquatic organic carbon [8]. As bacteria are at the bottom of the food webs, changes in their communities are often a precursor to changes in the ecosystem health in general. In order to assess the effects of macrophyte harvesting on bacteria we applied Biolog Ecoplate™ test as a valuable and inexpensive technique to elucidate changes in bacterial metabolic capacity after macrophyte harvesting.

Material and methods. Object. Trevnoto Lake is located at 1 419 m a.s.l. in the Smolyan region of the Rhodope Mountains (41°36'36.54" N, 24°40'08.59" E). The lake is 163 m long and 135 m in width with a surface area of 1.63 ha and a depth of 4 m [9]. Plant communities belong to the union Sphagno-Utricularion with dominant species *Carex rostrata*, *Utricularia vulgaris*, *U. minor*, *Ceratophyllum demersum*, *Sparganium minimum*, *S. ramosum*, *Drepanocladus* spp., *Calliergon* spp., *Sphagnum* spp., *Phragmites australis*, *Lemna minor*, *Potamogeton* spp., and *Typha* spp. Approximately 30% of the lake surface is taken up by up to a 1 m-thick layer of peat, and around 2% (personal surveillance) of the littoral area is seasonally overgrown by macrophytes. The macrophyte coverage was much higher in the past, but due to regular harvesting procedures it decreased to 2% (on average). *Potamogeton* spp. is harvested by hand, once per year, as a routine management practice.

Sampling. Water sampling was conducted before (August 6, 2022) and after (August 7, 2022) macrophyte harvesting at three points from the deepest part of the lake throughout the whole water column. Water samples for bacterial analysis were taken by and kept in sterile equipment/containers at 4 °C.

Physico-chemical analysis. Dissolved organic carbon (DOC; mg C L⁻¹) concentrations, and DOC spectral properties SUVA₂₅₄ (L mg C⁻¹ m⁻¹) [10], and E₂/E₃ [11] were analyzed using TOC analyzer (TOC-5000, Shimadzu, Japan), and UV-VIS spectrophotometer (CECIL CE 3021), respectively.

Bacterial metabolism. Biolog EcoPlate™ (Biolog Inc., Hayward CA, USA) was used for evaluation of bacterial metabolism before and after macrophyte harvesting. The microplates were inoculated with 120 µl lake water, incubated at 22 ± 1 °C in the dark, and the wells' optical density was measured every 12 h for five days at 590 nm (Microplate Reader LKB 5060-006 with software package DV990 "Win 6"). Biolog Ecoplate™ contains 31 different carbon sources

(CSs) and a control in three replicates. The CSs are noted as: Pyruvic acid methyl ester (B1), Tween 40 (C1), Tween 80 (D1), α -Cyclodextrin (E1), Glycogen (F1), D-Cellobiose (G1), α -D-Lactose (H1), β -Methyl-D-glucoside (A2), D-Xylose (B2), i-Erythritol (C2), D-Mannitol (D2), N-Acetyl-D-Glucosamine (E2), D-Glucosaminic acid (F2), Glucose-1-phosphate (G2), D,L- α -Glycerol-phosphate (H2), D-Galactonic acid γ -lactone (A3), D-Galacturonic acid (B3), 2-Hydroxy benzoic acid (C3), 4-Hydroxy benzoic acid (D3), γ -Amino butyric acid (E3), Itaconic acid (F3), α -Keto butyric acid (G3), D-Malic acid (H3), L-Arginine (A4), L-Asparagine (B4), L-Phenylalanine (C4), L-Serine (D4), L-Threonine (E4), Glycyl-L-Glutamic acid (F4), Phenylethylamine (G4) and Putrescine (H4). Since colour development in the microplate wells typically follows a sigmoidal trend with time, an area under curve technique (AUC) [12] was used for calculation of bacterial metabolic metrics: average well colour development (AWCD) as a proxy of total bacterial activity and community level physiological profile (CLPP) as a bacterial metabolic fingerprint [13].

Statistical analyses. Each data point in the paper represented the mean value of the respective parameter $\pm$ standard error ($n = 3$ for water and $n = 9$ for bacterial analysis). ANOVA was performed to examine the differences in the means of the parameters. One to one technique and Pearson correlation analysis were also applied. The above statistics were performed with the packages PAST [14] at a significance level of $p < 0.05$.

Results. DOC concentrations and properties. DOC greatly impacts bacterial metabolism depending on its concentration and spectral properties. Data on DOC before and after macrophyte harvesting are presented in Fig. 1.

Human activity in the lake changed insignificantly the concentrations of DOC and its aromatic fraction (SUVA_{254}), and noticeably increased the fraction of high

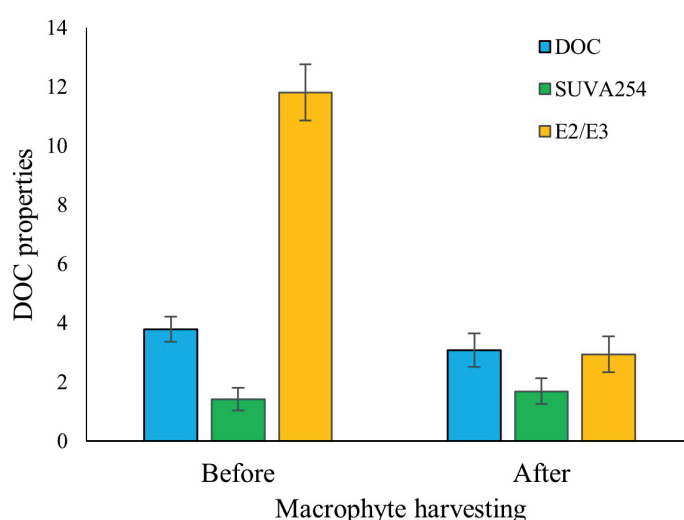


Fig. 1. Dissolved organic carbon (DOC) concentration and spectral characteristics (SUVA_{254} , E_2/E_3) before and after macrophyte harvesting, expressed as mean ($n = 3$); bars are standard errors

molecular-weight constituents (E_2/E_3). Pearson correlation analysis indicated insignificant correlation between DOC and $SUVA_{254}$ ($p = 0.07$) and significant negative correlation between DOC and E_2/E_3 ($p < 0.001$). Additionally, changes in $SUVA_{254}$ correlated significantly and positively with changes in E_2/E_3 ($p = 0.03$). These relationships indicated that macrophyte harvesting caused changes not so much in DOC concentration as in its chemical composition.

Bacterial metabolic characteristics. Metabolic activity of aquatic bacteria, expressed as AWCD, increased after macrophyte harvesting by 15%, i.e. from AUC value of 5.73 ± 0.27 to 6.78 ± 0.31 . In general, bacteria were not able to utilize 2-Hydroxy benzoic acid, whereas the utilization rates of the other CSs were classified as low ($< 30\%$ of AWCD; B2, H2, E3, F3, C4, F4 and H4), medium (31–109% of AWCD; D1, E1, H1, C2, G2, D3, G3, A2, H3, E4 and G4) and high ($> 110\%$ of AWCD; B1, C1, F1, G1, D2, E2, F2, A3, B3, A4, B4 and D4) with means (AUC $\pm$ SD) of 0.87 ± 0.53 , 4.80 ± 1.23 and 9.42 ± 1.11 , respectively.

The shift in AWCD after macrophyte harvesting was significant ($F = 5.92$; $p = 0.014$), affecting mainly the utilization rates of 18 CSs (Fig. 2A).

To evaluate the shifts into the utilization rates after macrophyte harvesting, a ‘one to one’ analysis was conducted (Fig. 2B–D). Highly affected by the human activity was the group of CSs with low availability to bacteria – 85.7% of the members of the group were affected by macrophyte harvesting (Fig. 2AB). The utilization rates of B2 (43%) and E3 (84%) decreased, whereas the utilization rates of the other CSs increased from 386% (H2) to 680% (F3) in comparison to those before the macrophyte harvesting. The groups of medium (Fig. 2AC) and highly (Fig. 2AD) available CSs were less affected – 54.5% (medium) and 50.0% (high) of the CSs in the respective groups were affected. Two CSs (E4 and G4) from the group with medium availability were affected negatively, and the utilization of the other members of the group increased from 164% (G2) through 261% (D1) to 366% (H1). The utilization of the CSs with high availability was much more stable than the other CSs shifting in narrow limits after macrophyte harvesting (from -35% to $+147\%$), except A4, which utilization rate decreased by 64.6%.

Pearson correlation analysis was conducted to clarify the influence of DOC characteristics on the utilization of CSs (Table 1).

Most of the CSs (24 of 30) correlated significantly with DOC and its spectral characteristics, and the most influenced CSs (100%) were amino acids (A4–F4), amines (G4–H4) and polymers (C1–F1), followed by carbohydrates (G1–H1, A2–E2 and G2–H2; 78%) and carboxylic acids (B1, F2 and A3–H3; 50%).

Discussion. Macrophyte harvesting had a little effect on DOC concentration (a slight decrease) but increased its aromaticity ($SUVA_{254}$), which corresponded with an increase in the fraction of high-molecular organic constituents (E_2/E_3). Probably, changes in DOC resulted from the sediment disturbance during the human activity, causing increased flux of the sediment detritus (high-molecular and water insoluble organic fraction), including aromatic compounds, to the overlying

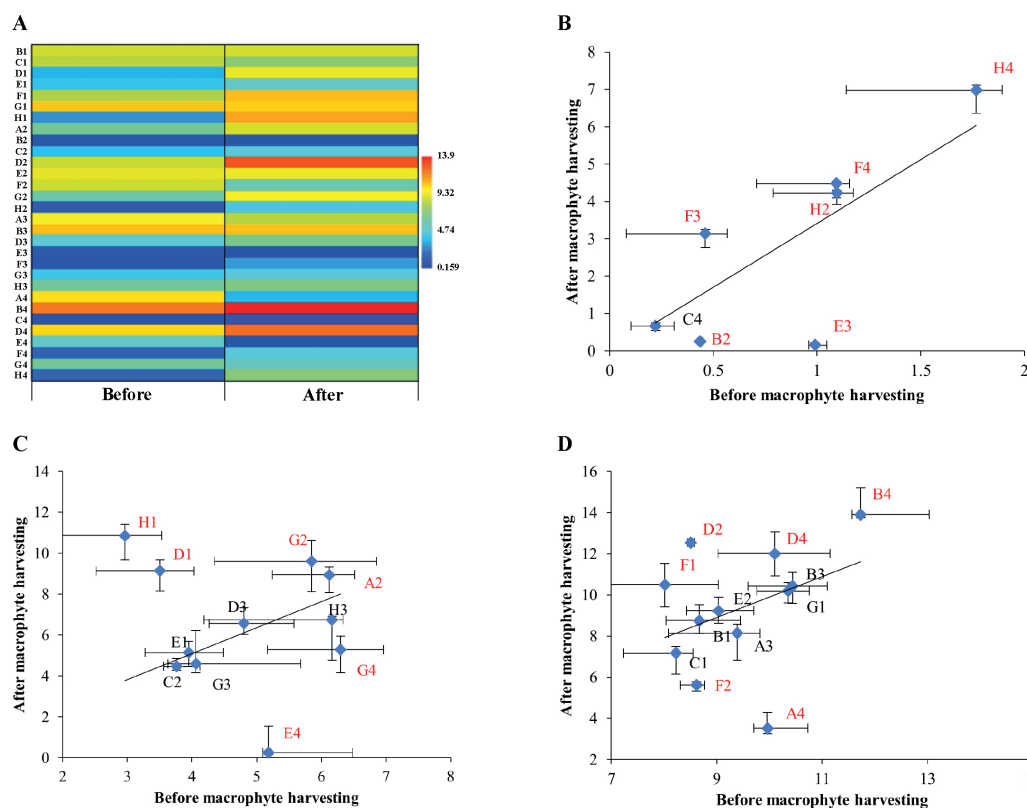


Fig. 2. Community level physiological profiles of aquatic bacteria (A), and 'one to one' comparison of the utilization rates of the low- (B), medium- (C) and high- (D) bioavailable CSs before and after macrophyte harvesting. Diamond symbols illustrate the mean ($n = 9$) utilization rate of the respective carbon source, and the red coloured letter indicates significant differences ($p < 0.05$); bars are standard errors

water column. The sediment flux changed the DOC pool, inducing adaptation of bacterial metabolism to newly created environment. It is well known that heterotrophic bacteria are capable of utilizing more than one type of carbon sources, being metabolically adaptive to environmental changes, including pulses in the size and composition of nutrient pools [15]. In this case, the total bacterial metabolic activity increased after macrophyte harvesting by 15% due to significant changes into the utilization rates of 18 of 30 CSs. A very interesting result was the linear trend formed between the CS bioavailability and the share of the impacted CSs. The CSs with low availability were more strongly impacted in comparison to the CSs with medium and high availability. Such phenomenon of very high stimulation of the utilization of the CSs with low availability was also detected in our earlier study [16] of soil treated with fungicide Quadris^R, assuming common bacterial responses to anthropogenic stress independently of its origin. Additionally, in this case the recorded impact was expressed both to the share of the affected carbon

T a b l e 1

Relationships between CS utilization rates and DOC properties. Only significant ($p < 0.05$) relationships are shown

Carbon source	DOC	SUVA ₂₅₄	E ₂ /E ₃	Carbon source	DOC	SUVA ₂₅₄	E ₂ /E ₃
C1	0.69		−0.67	A3		−0.75	
D1	−0.91	0.81	0.93	D3	−0.66	0.55	0.68
E1	−0.62		0.58	E3	0.98	−0.83	−0.99
F1		0.70	0.64	F3	−0.94	0.84	0.96
H1	−0.92	0.87	0.95	A4	0.96	−0.78	−0.97
A2		0.74	0.65	B4	−0.61	0.68	0.64
B2	0.99	−0.81	−0.99	C4	−0.87	0.51	0.84
C2	−0.72		0.67	D4		0.75	0.52
D2	−0.98	0.86	0.99	E4	0.86	−0.84	−0.88
F2	0.98	−0.80	−0.98	F4	−0.98	0.74	0.97
G2	−0.62	0.96	0.71	G4		−0.54	
H2	−0.78	0.93	0.85	H4	−0.98	0.76	0.97

sources from the respective group of bioavailability and to the level of the impact. The average rate of changes into the utilization of CSs with low availability was $321 \pm 227\%$, whereas that of the groups of medium and high bioavailable CSs was $149 \pm 94\%$ and $99 \pm 30\%$, respectively. The most affected were the utilization rates of F4 ($\uparrow 681\%$), H4 ($\uparrow 395\%$), H2 ($\uparrow 386\%$), H1 ($\uparrow 366\%$), D1 ($\uparrow 261\%$), E4 ($\downarrow 95\%$), E3 ($\downarrow 84\%$) and A4 ($\downarrow 65\%$).

In our study, Pearson correlation analysis clearly indicated the importance of DOC for bacterial metabolism, showing significant relationships between most of the CSs and DOC metrics. The different directions of these correlations reveal complex bacterial responses to changes occurring in lake nutrient pool.

Conclusion. The results show, that the application of Biolog EcoPlate™ technique can be used successfully in the assessment of the effects of macrophyte harvesting on the lake bacterial community. This new approach may lead to a development of bacterial functional indicators (metabolic activity, carbon utilization fingerprints and capacity for utilizing chemically different carbon sources) for better assessment of the vegetation management practices in lakes.

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