



Assessment of bacterioplankton community structure in relation to macrophyte cover in shallow lakes of the Pannonian Basin (Central Europe)

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ABSTRACT

Study region: The Pannonian Basin, Hungary.

Study focus: Macrophytes are essential components of freshwater ecosystems, influencing aquatic bacterial communities. This study investigated the impact of macrophytes on bacterioplankton abundance, metabolic activity and diversity across 12 shallow lakes in the Pannonian Basin. We assessed the interplay between macrophyte coverage and physicochemical parameters in structuring microbial communities using 16S rRNA gene metabarcoding and the most probable number (MPN) method.

New hydrological insights for the region: While macrophyte presence appeared to influence the distribution of certain bacterial groups, most operational taxonomic units (OTUs) exhibited site-specific patterns. Notably, Rhodocyclaceae and the genus *Limnohabitans* were more abundant in macrophyte- and reed-covered areas. 'Candidatus Patescibacteria' and Epsilonbacteraeota were found almost exclusively in certain samples, reaching 41.6% and 32.3% relative abundance, respectively. In contrast, freshwater SAR11 bacteria, particularly the LD12 clade, were present in nearly all sampled waters, with the highest observed relative abundance of 14.2% of total sequences.

MPN values of heterotrophic bacteria (10^3 – 10^6 MPN mL⁻¹) were highest in open water and submerged macrophyte samples; however, the abundance of other bacterial functional groups was linked with environmental factors such as dissolved oxygen levels. These findings suggest that beyond macrophyte coverage, other environmental parameters, such as hydrological conditions and surrounding land use, may exert a more deterministic effect on bacterioplankton in shallow lakes, even within a relatively small geographical area.

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1. Introduction

Aquatic plants play an important role in the life of shallow lakes. Both live and dead macrophyte biomass can directly and indirectly affect bacterioplankton by releasing organic compounds that serve as nutrients and by altering the chemical environment (Huss and Wehr, 2004). Different macrophyte species release substrates with varying nutrient contents and some of them even allelochemicals, therefore the dominant species in the macrophyte stand play a crucial role in structuring the bacterioplankton community composition (Wu et al., 2007, Hempel et al., 2009, Zeng et al., 2012, Mentés et al., 2018). While the metabolic significance of bacterioplankton is well-known in water bodies where algae are the dominant primary producers, relatively little is known about the bacterioplankton in water bodies covered with aquatic plants, which are important parts of shallow lakes and ponds and littoral zones of large lakes (Hahn, 2006, Wang et al., 2024, Fang et al., 2024, Barbosa et al., 2024).

The geographical location and climatic conditions of the Pannonian Basin contribute to its exceptional diversity of shallow freshwater bodies. Lake Balaton (the largest shallow lake in Central Europe) is located here, as well as slightly saline lakes such as Lake Velence and Fertő (the westernmost representatives of soda lakes found on the Eurasian Steppe) (Mezősi, 2017). In addition to all these, preserved wetlands, hundreds of oxbows, artificial reservoirs, pit lakes, and astatic soda pans can also be found in the region (Borics et al., 2016). In our previous works, the impact of different macrophyte stands on the community composition of planktonic microorganisms was studied in one of the protected freshwater marshes of Hungary (Lake Kolon) and in a shallow soda lake (Lake Fertő), both can be characterized by a mosaic of open waters, inner ponds and reed marshes (Mentés et al., 2018, Szuróczki et al., 2020). As a result, a strong impact of macrophytes on bacterioplankton was detected, and comparing the bacterioplankton community composition of different sites from Lake Fertő and Lake Kolon, a clear separation of the open water samples from those with high macrophyte cover, which also had a higher content of chromophoric dissolved organic matter (CDOM), was observed. Additionally, the two studied lakes also differed remarkably in terms of bacterioplankton composition, most probably determined by their different chemical character. Similarly to these, the bacterioplankton communities of soda pans from this region which had different CDOM content (partially due to the differences of macrophyte cover) also showed remarkable differences in their composition (Boros et al., 2020, Szabó et al., 2020, Márton et al., 2023).

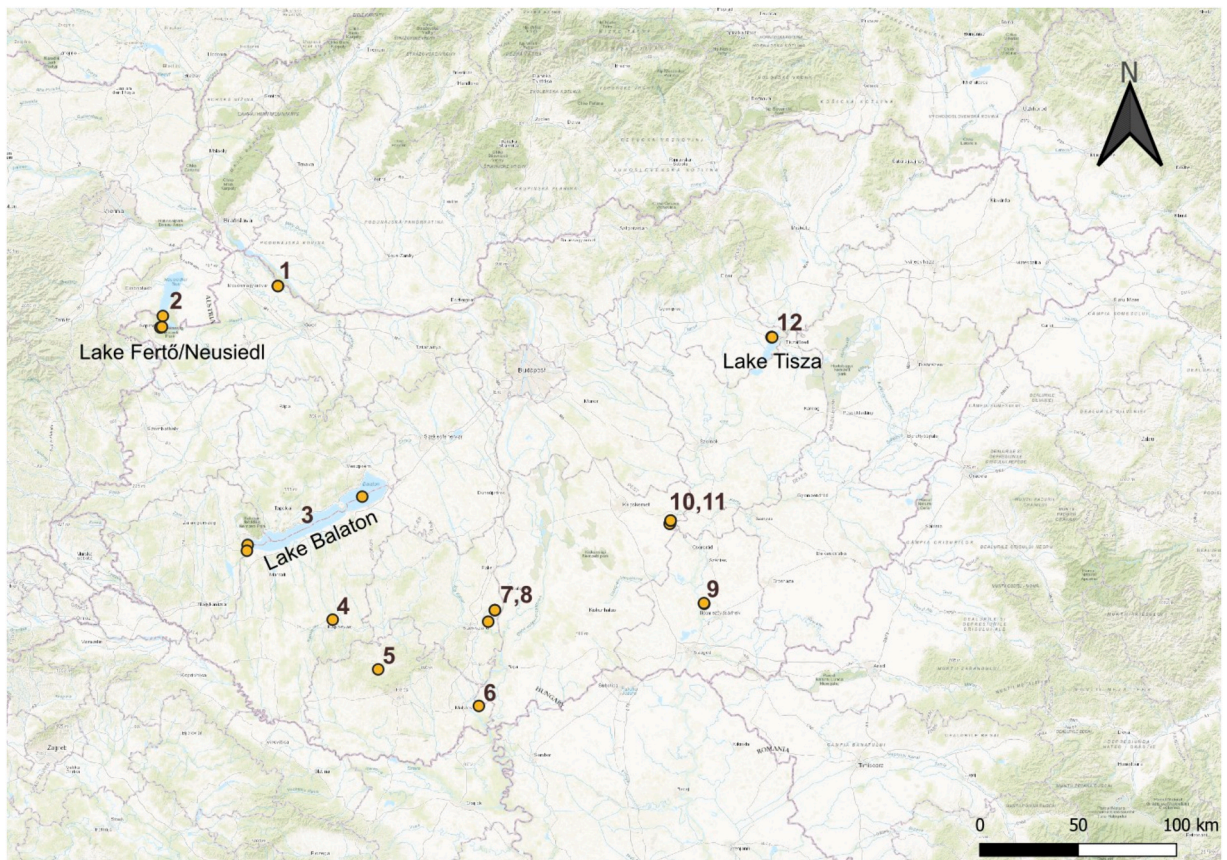


Fig. 1. Location of sampling sites in Hungary (1 – Lake Morotva in Lipót [LK, LN], 2 – Lake Fertő [BO, KH, N, NH], 3 – Lake Balaton [K, Ff, Z], 4 – Reservoir Deseda [Ds], 5 – Lake Kovácsszénája [Kn], 6 – Riha oxbow [R], 7 – Tolna oxbow [TLO], 8 – Fadd-Dombori oxbow [FDN, FDO], 9 – Mártély oxbow [F, E], 10 – Tiszaalpár oxbow [A, B], 11 – Lakitelek oxbow [C, D], 12 – Lake Tisza [TO, TS, TNB]; sample abbreviations are given in [Supplementary Table 1](#)).

This study expands upon previous research by comprehensively investigating the influence of submerged and emergent macrophytes on both bacterioplankton and phytoplankton across a diverse set of water bodies, encompassing a range of hydrological and ecological conditions characteristic of the Pannonian Basin. We assessed limnological (both biotic and abiotic factors), phycological (primary production and size distribution) and bacteriological (metabolic activity, composition and diversity) parameters in relation to macrophyte cover to advance understanding of the ecological dynamics of these shallow lakes under varying hydrological conditions.

2. Materials and methods

2.1. Description of the sampling sites

Samples were collected from 24 sites across 12 water bodies in Hungary, representing a diverse range of lacustrine systems with varying phytoplankton and macrophyte characteristics. The selected sites included lakes, oxbows and a reservoir (Fig. 1, Table 1). Sampling was conducted in July and August 2018. These shallow water bodies are located within the same geographic region of the Pannonian Basin with distances between sites ranging from 40 to 300 km. Their hydrological characteristics are given below.

Four lakes were selected for sampling represent distinct hydrological regimes and varying levels of anthropogenic influence. Lake Fertő (Neusiedler See) is the largest shallow, saline lake in Europe (Boros et al., 2014). It is a soda lake characterized by extensive reedbeds, primarily, composed of *Phragmites australis*, covering 55% of its total surface area (Pretzer et al., 2017). Sampling was performed from four different water bodies of Lake Fertő: from the open water, from two “inner” lakes within the reed belt and from a densely reed covered area. Lake Balaton is the largest shallow lake in Central Europe, with an area of 596 km² and an average water depth of 3.2 m (Istvánovics et al., 2007). Water samples were taken in the Eastern Basin (Siófok) and Western Basin (Keszthely) and from the main inflow of Lake Balaton, the Zala River. Additionally, two lakes from the Western Transdanubia region were also included in our research. Lake Kovácsszénája, part of the Orfű Lake System in southwestern Hungary, and Lake Deseda, a large flood retention reservoir, were selected. Both sites are characterized by remarkable recreational angling activity.

Four oxbows along the River Danube were involved in our sampling campaign. Lake Morotva, located in the northern region of the Danube, is one of the strictly protected oxbows of Hungary near the village of Lipót. In addition to the open water, there are also areas with extensive reed belts. Lake Riha in the southern region of the Hungarian Danube is also a strictly protected nature reserve area. In contrast, Tolna and Fadd-Dombori oxbows, located in the southern Danube region, are primarily used for angling. Additionally, the Fadd-Dombori oxbow functions as a reservoir for excess surface water and is actively utilized for both recreational and professional water sports. Due to their intensive use, the shorelines and vegetation of both oxbows have undergone significant degradation (Tóth et al., 2017).

For the other large river of this region, River Tisza, three oxbows (Mártély, Tiszaalpár and Lakitelek) and an artificial reservoir on the river, Lake Tisza were selected. Lake Tisza, which is located in the Middle-Tisza region, is the second largest water body in Hungary. A notable part of the lake is a nature conservation area (Jakab et al., 2002). Samples were collected from three different locations within the lake representing different habitat types. Several oxbows of the Lower-Tisza region are also protected nature reserve areas, yet they experience intensive angling activity. In these water bodies, both open-water sites and macrophyte-dominated areas were included in the sampling.

2.2. Estimation of macrophyte cover

Macrophytes were sampled from both emergent (e.g., *Phragmites australis*) and submerged (e.g., *Myriophyllum spicatum*, *Ceratophyllum demersum*) macrophyte-dominated sites. Due to the high plant heterogeneity, macrophytes were collected from 4 to 5 randomly selected 0.25 m² quadrants and samples were pooled. The processing of the collected macrophyte samples was performed as described in detail by Szabó-Tugyi and Tóth (2020).

Table 1

Sampling site codes, water characteristics, and mean water depth, with corresponding numbers on the map (Fig. 1).

Number on the map (see Fig. 1)	Name of the sampling site	Characteristic	Mean water depth during sampling (m)
1	Lake Morotva in Lipót	freshwater reservoir	0.49
2	Lake Fertő	slightly saline lake	1.01
3	Lake Balaton	freshwater lake	3.59
4	Reservoir Deseda	freshwater reservoir	4.05
5	Lake Kovácsszénája	fishpond	3.50
6	Riha oxbow	oxbow lake	0.75
7	Tolna oxbow	oxbow lake	1.60
8	Fadd-Dombori oxbow	oxbow lake	1.23
9	Mártély oxbow	oxbow lake	3.24
10	Tiszaalpár oxbow	oxbow lake	1.55
11	Lakitelek oxbow	oxbow lake	1.75
12	Lake Tisza	freshwater lake	1.28

2.3. Lake water physical and chemical analyses

The pH, temperature, conductivity and dissolved oxygen concentration were measured using WTW pH315i and Hanna HI9033 portable field meters, while the vertical attenuation coefficient (Kd) of photosynthetically active radiation was determined as described previously (Felföldi et al., 2011). The concentration of total suspended solids (TSS) and CDOM were measured in the laboratory as previously described by Szuróczki et al. (2020) and Szabó-Tugyi and Tóth (2020).

2.4. Phytoplankton measurements

Chlorophyll-a and pico-sized algae were measured using the methods described by Szabó et al. (2020). Photosynthesis-irradiance (P-I) curves were fitted, and photosynthetic parameters, including algal maximum photosynthetic activity (algal P_{max}), were determined as described by Somogyi et al. (2016).

2.5. Bacterial production and abundance determination

Bacterial production (BP) was measured using the radiolabeled leucine L-[4,5- 3H] incorporation method as described in Szabó-Tugyi and Tóth (2020).

To determine total bacterial cell counts, 10-mL aliquots from each water sample were fixed using 8% paraformaldehyde solution. After 12 h of incubation at 4 °C, fixed samples were filtered through 0.2 μm pore sized polycarbonate filters (Millipore, Billerica, MA, USA). The membrane filters containing the fixed cells were stained with 30 μL 1 $\mu g L^{-1}$ 4',6-diamidino-2-phenylindole (DAPI) solution, then washed with distilled water and dehydrated in 80% ethanol. The Vectashield mounting medium was applied for sample embedding. Detection of stained cells was carried out under ultraviolet (350–450 nm) excitation using an Olympus BX51 microscope. Heterotrophic bacterial abundance calculated following the methods described in detail by Szabó-Tugyi and Tóth (2020).

2.6. Determination of specific bacterial groups with the MPN method

The MPN method was applied to detect the abundance of aerobic heterotrophic, fermentative acidogenic, nitrate-reducing, denitrifying and nitrifying bacteria using different liquid growth media.

The R2A broth (Reasoner and Geldreich, 1985; pH 8.5) and a 'fermentative' broth (casein peptone, 3 g; yeast extract, 1.5 g; KH_2PO_4 , 1.2 g; K_2HPO_4 , 1.2 g; glucose, 6.5 g; Na_2CO_3 , 2.0 g; pH indicator bromothymol blue, 32 mg; completed with distilled water to 1000 mL; pH 8.5) were used for most probable number (MPN) counting of aerobic heterotrophic and fermentative acidogenic bacteria, respectively. Methodological details are provided in Szuróczki et al. (2020).

For the determination of the MPN of nitrate-reducing and denitrifying bacteria, 10-fold serial dilution in five replicates was applied in tubes containing inverted Durham tube (Smibert and Krieg, 1994). The liquid growth medium contained meat extract, 1 g; peptone, 2 g; $NaNO_3$, 1 g; Na_2CO_3 , 2 g; supplemented with 1000 mL distilled water. The pH was adjusted to 8.5 with 1 M NaOH before autoclaving at 121 °C for 20 min. After incubation (23°C, 1 week), the criterion for a positive result of denitrification was gas production in the inverted Durham tube. For the determination of the MPN of nitrate-reducing bacteria 40 μL of Griess-Ilosvay reagent (Alexander and Clark, 1965) was added to the tubes. The red color reaction indicated the presence of nitrite (positive reaction).

The determination of the MPN of the two groups of nitrifiers, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) was performed according to Lipponen et al. (2002).

2.7. Determination of bacterioplankton composition with Illumina amplicon sequencing

A volume of 250 mL water sample was filtered through a 0.22 μm pore-size polycarbonate filter (Millipore, Billerica, MA, USA). Total genomic DNA was extracted from the samples using the Ultra Clean Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions with the exception that cell disruption step was carried out by shaking at 30 Hz for 2 min in a Mixer Mill MM301 (Retsch, Haan, Germany). The PCR amplification using Bacteria-specific primers B341F (5'- CCT ACG GGN GGC WGC AG -3'; Herlemann et al., 2011) and 805NR (5'- GAC TAC NVG GGT ATC TAA TCC -3'; Apprill et al., 2015), and sequencing of the 16S rRNA gene were performed as described in detail by Szuróczki et al. (2020). Raw sequence data were processed using mothur v1.44.3 (Schloss et al., 2009) following the MiSeq SOP (https://www.mothur.org/wiki/miseq_sop/). Quality filtering included setting 'deltaq = 10' in the 'make.contigs' command, trimming primers, and removing chimeric sequences, with chimera detection carried out using the mothur-integrated version of VSEARCH. Taxonomic assignments were based on the Silva SSU 138 reference database (Quast et al., 2012) applying a minimum bootstrap confidence score of 80, and were further refined using the TaxAss tool with the FreshTrain database (2020 June 15 release) (Rohwer et al., 2018), which provided a reference set for freshwater-specific bacterial lineages. Sequences assigned to non-target groups (e.g., Archaea, Eukarya, Chloroplast, Mitochondria, unknown) were excluded from the analysis. Operational taxonomic units (OTUs) were clustered using the OptiClust algorithm (Westcott and Schloss, 2017) at a 99% similarity threshold. To standardize sequencing depth across samples, rarefaction was performed by randomly subsampling each sample to match the lowest read count observed.

The ratio of Archaea to Bacteria was determined using quantitative PCR (qPCR) targeting the 16S rRNA gene. Measurements were conducted in triplicate using the following primer pairs and TaqMan probes for Bacteria (BACT1369F: 5'-CGG TGA ATA CGT TCY CGG-3', PROK1492R: 5'-GGW TAC CTT GTT ACG ACT T-3', TM1389F: 5'-FAM-CTT GTA CAC ACC GCC CGT C-BHQ-3'; Suzuki et al., 2000)

and Archaea (Arch349F: 5'-GYG CAS CAG KCG MGA AW-3', Arch806R: 5'-GGA CTA CVS GGG TAT CTA AT-3', Arch516F: 5'-FAM-TGY CAG CCG CCG CGG TAA HAC CVG C-BHQ-3'; Takai and Horikoshi, 2000). The full protocol is described in detail by Korponai et al. (2019).

2.8. Statistical analyses

Environmental parameters were compared among habitat types with different macrophyte coverage using Kruskal–Wallis tests, followed by Dunn's post hoc tests with Benjamini–Hochberg correction, implemented via the `dunn.test` package in R v4.4.2 (R Core Team, 2024). Principal component analysis (PCA) was conducted on z-score standardized environmental variables using the `vegan` package v2.6.10 in R (Oksanen, 2025). Data arrangements were carried out with the `tidyverse` v2.0.0 (Wickham et al., 2019) and visualizations with the `ggplot2` v3.5.1 (Wickham, 2009) R packages. Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarities was performed in R using the `vegan` package to assess planktonic bacterial community composition. Environmental variables were fitted to the NMDS ordination using the `envfit` function. Percentage similarity analysis (SIMPER) was performed using the `Past3` program (Hammer et al., 2001). Permutational multivariate analyses of variance (PERMANOVA) tests were conducted using the `adonis2` function of the `vegan` package with 999 permutations to assess differences in bacterial community composition across macrophyte types and lakes. Pairwise comparisons were performed with `pairwise.adonis2` function of the `pairwiseAdonis` package (Martinez Arbizu, 2020).

2.9. Data availability

Raw sequence data can be accessed under the NCBI BioProject ID PRJNA1083048.

3. Results

3.1. Limnological parameters

In our study, we assessed bacterio- and phytoplankton communities of 24 sites from 12 shallow lakes with differing macrophyte cover (Supplementary Table S1). Water temperatures showed minimal variation among the lakes, ranging from 24.3 to 31.3 °C, while pH values spanned from 7.38 to 9.68. Lake Fertő, a shallow soda lake, was characterized by high pH (8.65 ± 0.1) and conductivity ($2825 \pm 200 \mu\text{S cm}^{-1}$) compared to the other lakes. Chlorophyll-a concentration was measured between 1.4 and $194.7 \mu\text{g L}^{-1}$. Lakes with high chlorophyll-a content were also characterized with elevated pH. In general, open-water sites had a higher DO content ($8.4 \pm 4.4 \text{ mg L}^{-1}$) than the macrophyte-dominated samples ($4.3 \pm 2.9 \text{ mg L}^{-1}$ for submerged macrophytes and $4.8 \pm 3.5 \text{ mg L}^{-1}$ for emergent macrophytes-dominated sites) (Table 2). The Secchi-disk transparency ranged from 20 to 178 cm in the open-water areas, 18–110 cm in the submerged macrophytes-dominated areas and 25–60 cm in the emergent macrophytes-dominated areas of the lakes. The abundance of picophytoplankton varied between 0 and $2.51 \times 10^6 \text{ cells mL}^{-1}$. In the open water areas, counts ranged from 0 to $2.07 \times 10^6 \text{ cells mL}^{-1}$. Areas dominated by submerged macrophytes showed a range of 7.49×10^3 to $2.23 \times 10^6 \text{ cells mL}^{-1}$, while emergent macrophyte areas exhibited counts between 1.80×10^4 and $2.51 \times 10^6 \text{ cells mL}^{-1}$. Cell counts determined by DAPI staining ranged from 10^6 to $10^7 \text{ cell mL}^{-1}$ among the samples. The highest planktonic cell counts were detected in the open water of the Lake Kovácsszénája. Macrophyte biomass (dry weight) varied between 0 and 2202 g m^{-2} . The average biomass values were higher in samples dominated by submerged macrophytes (1892.8 g m^{-2}) than in those dominated by emergent macrophytes (372.9 g m^{-2}).

Open-water sites were significantly different from macrophyte-dominated sites in certain limnological parameters, the former had lower macrophyte biomass, higher primary production, higher water depth and lower light attenuation coefficient (Kruskal–Wallis

Table 2

Limnological and algological parameters measured during the field campaign of 2018 grouped to show the open water and macrophyte dominated (submerged or emergent) sites.

	open water	submerged	emergent
temperature (°C)	28.2 ± 1.82	27.4 ± 2.1	29.1 ± 1.6
K_d (1/m)	3.2 ± 1.5	9.1 ± 5.0	5.7 ± 3.8
depth (cm)	272 ± 147	131 ± 111	73 ± 31
pH	8.5 ± 0.5	8.1 ± 0.5	8.0 ± 0.5
conductivity ($\mu\text{S/cm}$)	783 ± 583	1093 ± 1018	1114 ± 1092
Secchi (cm)	54.6 ± 39.5	54.1 ± 27.9	44.8 ± 12.8
TSS (mg/L)	18.3 ± 9.9	12.6 ± 9.7	18.0 ± 6.3
CDOM (mg/L)	35.4 ± 34.0	87.1 ± 54.0	72.4 ± 89.9
O ₂ (mg/L)	8.4 ± 4.4	4.3 ± 2.9	4.8 ± 3.5
chlorophyll-a ($\mu\text{g/L}$)	64.4 ± 57.9	35.9 ± 34.6	20.2 ± 8.8
algal P _{max} ($\mu\text{g C/L/h}$)	486.3 ± 578.3	155.4 ± 125.1	99.8 ± 66.2
bacterial productivity ($\mu\text{g C/L/h}$)	12.3 ± 8.7	21.5 ± 21.5	16.2 ± 4.9
macrophyte biomass (g DW/m ²)	0	372.9 ± 403.6	1892.8 ± 211.1

test, $p < 0.05$), other differences were not statistically significant. These differences contributed to the observed partial separation of habitat types based on macrophyte coverage (Fig. 2).

3.2. Diversity and composition of bacterioplankton communities

Archaea represented only a minor fraction (0.2% on average) of the prokaryotic community based on the qPCR results (Suppl. Fig. S1), with the highest average proportion observed in the sample from the Zala River inflow (25.8%). Therefore, we only assessed bacterioplankton composition by 16S rRNA gene amplicon sequencing using Bacteria-specific primers for amplification (Suppl. Fig. S2). In total, 548,106 high-quality bacterial 16S rRNA gene sequences were obtained from the 24 samples. Alphaproteobacteria, Gammaproteobacteria, Bacteroidota, Actinobacteria, Cyanobacteriota and Verrucomicrobiota represented the major bacterial groups across the sampled water bodies, however ‘*Candidatus* Patescibacteria’ and Epsilonbacteraeota ratios were also significant in the Fadd-Dombori oxbow and Zala River inflow samples. The most abundant OTU (OTU1) was assigned to the LD12 freshwater tribe within the SAR11 group of Alphaproteobacteria. Its relative abundances ranged from 0% to 14.2% of total sequences, with the highest proportion observed in the Nagy-Herlakni sample from Lake Fertő (Fig. 3). Representative sequences of OTU1 were detected in nearly all samples, though their presence was minimal in several samples and absent only from the Zala River inflow. Various lineages of Betaproteobacteria (Rhodocyclaceae, betI, betII, betIV) were also found with high relative abundance ranging from 4.5% to 32.6%. For example, OTU9, assigned to the unclassified genera ‘C39’ within the family Rhodocyclaceae, was identified with high relative abundance in the Zala River estuary of Lake Balaton. OTUs affiliated with the *Limnohabitans* genus were detected with the highest relative abundance in Lake Morotva samples (15.4% and 19.5%), reed-covered areas of Lake Tisza (8.3%) and in the macrophytes-covered area of the Zala River estuary (7.2%). The Lhab-A1 and Lhab-A3 tribes were represented among the most abundant OTUs (OTU10, OTU28, OTU29). OTUs classified within the phylum Bacteroidota also exhibited high relative abundances, ranging from 5.2% to 49.2% of the total bacterial community. These OTUs showed higher relative abundance in sites covered by macrophytes. For example, OTU4, OTU7 and OTU19, affiliated with taxa bacV, bacIII-A and Flavo-A3, dominated in the samples of Lake Morotva, while OTU18 and OTU22, members of the Algor tribe of the bacIII lineage and the family Cryomorphaeaceae were detected with high relative abundance in the emergent macrophytes dominated sample of Lake Fertő. Actinobacteria accounted for 3.9–30.2% of the planktonic bacterial community, with OTUs affiliated with the acI-B1, acI-C2, Iluma-A2 and Iluma-A1 tribes among the most abundant members. The dominant actinobacterial groups in the Mártély oxbow and Lake Balaton (Balatonfűzfő) were acI-B1 and Iluma-A2, while acI-C2 and Iluma-A1 were represented with high relative abundance in the open water of Lake Fertő and Reservoir Deseda. Cyanobacteria constituted 1.3–28.9% of the bacterial community, with five of the top 30 most abundant OTUs affiliated with the *Cyanobium*_PCC-6307, *Planktothrix* NIVA-CYA_15 and *Cylindrospermopsis*_CRJ1 clades. Cyanobacterial OTU15 (*Cyanobium*_PCC-6307) was abundant in the Lake Balaton (Balatonfűzfő), while other members of Cyanobacteria, such as OTU25 classified as *Planktothrix* NIVA-CYA_15 was characteristic in the open water of another site, Lake Kovácsszénája. *Cylindrospermopsis* affiliated OTUs (e.g., OTU24) were detected prevalent in the open water areas of Riha oxbow and Lake Balaton (Keszthely). The investigated water bodies were inhabited by other diverse planktonic microorganisms, including members of the phylum Verrucomicrobiota (0.3–21.7%). Among the most abundant OTUs, four were affiliated with Verrucomicrobiota. Two unclassified OTUs (OTU5 and OTU30) were prevalent in the Lakitelek oxbow samples, and OTU30 also exhibited high relative abundance in the Lake Morotva and Zala River estuary samples. Members of the LD29 lineage (e.g. OTU13) were present at high relative abundance in the planktonic bacterial communities of Riha oxbow and Reservoir Deseda. The relative abundance of ‘*Candidatus* Patescibacteria’ (also known as the Candidate Phyla Radiation, CPR) was notably high in the Fadd-Dombori oxbow (FDO: 35.3%; FDN: 41.6%). Representatives of OTU2 (GWA2–38–13b), affiliated with this phylum, were detected almost exclusively in these samples. The Zala River inflow was distinctly characterized by the dominance of Epsilonbacteraeota, which constituted 32.3% of the bacterial community, substantially higher than in all other sites, where this phylum was nearly absent (0–0.8%). This distinct composition was primarily due to the high relative abundance of *Arcobacter* (e.g., OTU8),

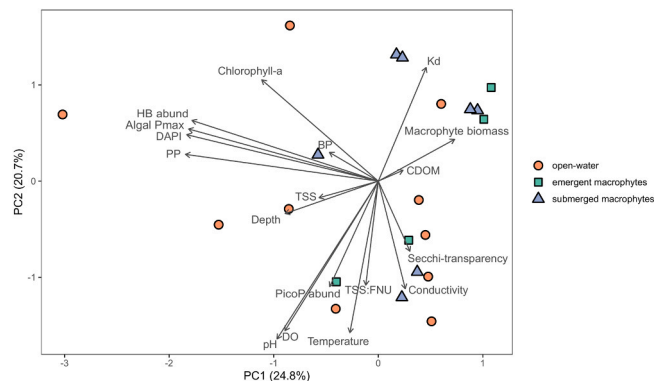


Fig. 2. Principal components analysis of the limnological, algological and bacteriological data of the studied water bodies. DO: dissolved oxygen, TSS: total suspended solids, CDOM: colored dissolved organic matter, BP: bacterial production, P_{max} : algal maximum rate of photosynthesis, PP: primary production, PicoP abund: picophytoplankton abundance, HB abund: heterotrophic bacteria abundance, DAPI: abundance of total bacteria.



Fig. 3. Heatmap of the relative abundances of the 30 most abundant bacterial OTUs across 24 lake sites. OTU labels include their taxonomy at the genus or closest classified rank. Color scale represents z-scores of standardized relative abundance per OTU across samples to highlight variation. OTUs are ordered by their overall abundance. Sample abbreviations are given in [Supplementary Table 1](#).

which accounted for 27.8% of the sequences in the Zala River estuary, compared to a maximum of 0.4% relative abundance elsewhere.

Differences in OTU-based community composition were visualized using nonmetric multi-dimensional scaling (NMDS) ordination based on a Bray–Curtis distance matrix ([Fig. 4](#)). The following environmental variables were significantly fitted ($p \leq 0.05$) onto the NMDS ordination: electric conductivity, temperature, pH, concentrations of dissolved oxygen (DO) and chlorophyll-a, picophytoplankton abundance (PicoP abund), algal maximum photosynthetic activity (algal P_{max}), and primary production (PP). Macrophyte biomass was fitted to the ordination with $p = 0.09$. Based on the SIMPER test, the following OTUs collectively accounted for at least 40% of the compositional dissimilarity among samples: OTU1 (LD12), OTU2 (GWA2–38–13b), OTU3 (acI-B1), OTU4 (bacV), OTU5 (Verrucomicrobiae), OTU6 (acI-C2), OTU7 (bacIII-A), OTU8 (*Arcobacter*), OTU9 (C39_Rhodocyclaceae), OTU10 (Lhab-A3), OTU11 (Iluma-A2), OTU12 (*Cyanobium* PCC-6307), OTU13 (LD29), OTU14 (Iluma-A1), OTU15 (*Cyanobium* PCC-6307), OTU16 (acI-A6), OTU17 (*Cyanobium* PCC-6307), OTU18 (Algor), OTU19 (Flavo-A3), OTU21 (*Chryseomicrobium*), OTU22 (Cryomorphaceae), OTU23 (*Paenibacillus*), OTU24 (*Cylindrospermopsis* CRJ-1), OTU25 (*Planktothrix* NIVA-CYA_15), OTU27 (*Exiguobacterium*), OTU36 (NS11–12_marine_group), OTU39 (*Cyanobium* PCC-6307). Based on the NMDS results, bacterial community composition appeared to be more strongly structured by site-specific conditions than by the macrophyte-dominated habitat types (open-water, submerged, or emergent macrophyte zones), as samples from the same site tended to cluster more closely in ordination space. PERMANOVA was conducted to evaluate whether bacterial community composition was significantly different across macrophyte coverage types (open water, emergent and submerged macrophytes) and among lakes of origin. PERMANOVA tests didn't show significant difference ($p < 0.05$) between macrophyte coverage types ($R^2 = 0.11$, $p = 0.11$) or among lakes ($R^2 = 0.48$, $p = 0.08$). However, a pairwise adonis test indicated a significant difference between open water and emergent macrophytes sites ($R^2 = 0.12$, $p = 0.01$).

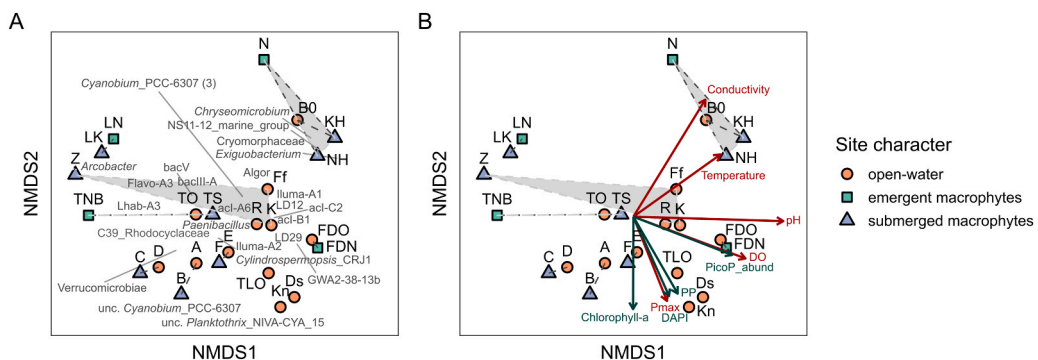


Fig. 4. Non-metric multidimensional scaling (NMDS) analysis of water samples from freshwater water bodies collected in 2018 based on the bacterial OTUs (stress: 0.13). A: Closest assigned taxa to OTUs contributing for 40% dissimilarity among samples based on SIMPER analysis, shown in gray. B: Significantly fitted ($p < 0.05$) environmental (red) and biological (green) variables are shown as vectors. Abbreviations: dissolved oxygen (DO), picophytoplankton abundance (PicoP abund), primary production (PP), algal maximum photosynthetic activity (P_{max}). Sample abbreviations are given in [Supplementary Table 1](#).

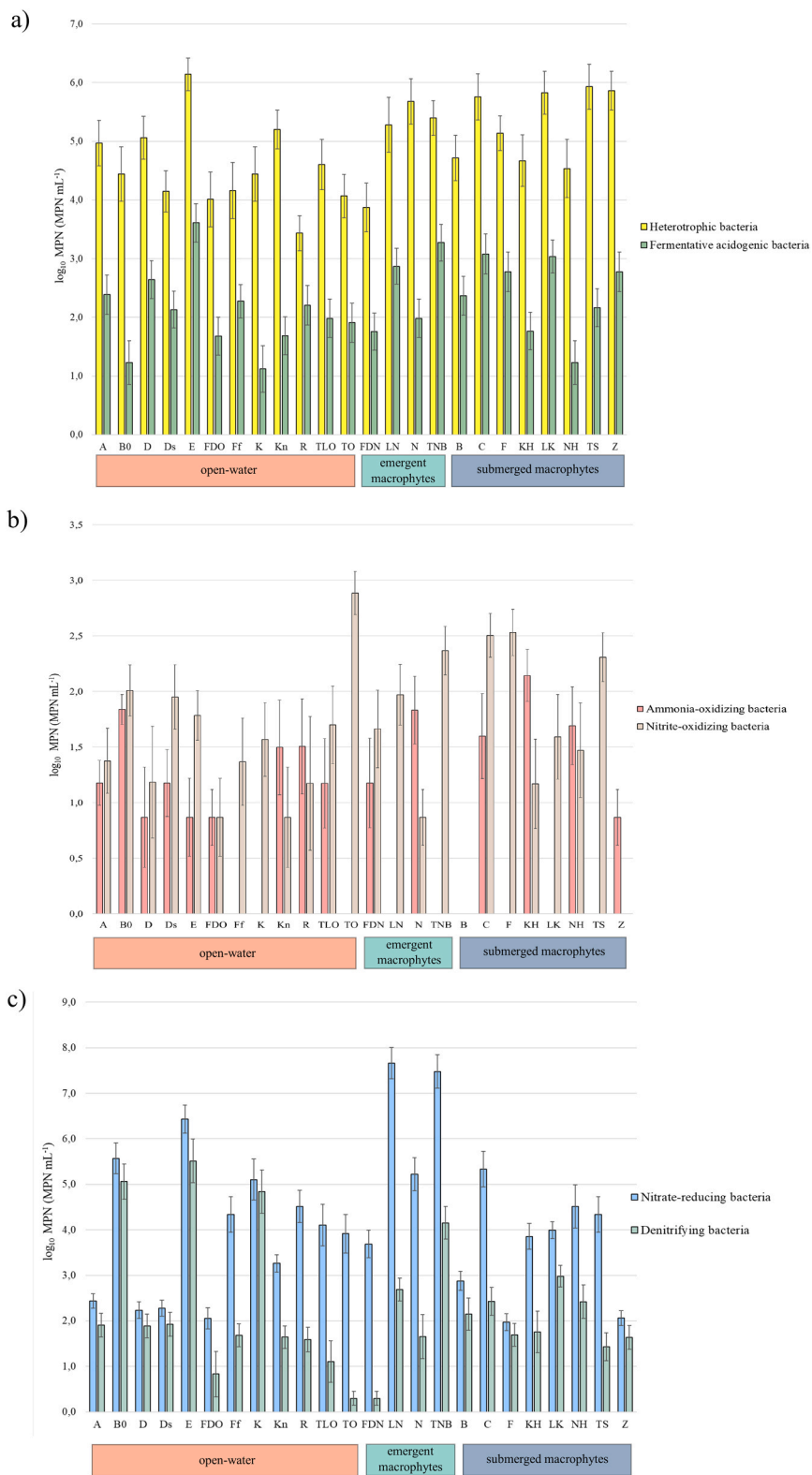


Fig. 5. Abundance of aerobic heterotrophic bacteria and fermentative acidogenic bacteria in the studied water bodies. MPN values are shown with 95% confidence intervals. MPN values not detected in the analyzed samples are not shown on the graph. Sample abbreviations are given in [Supplementary Table 1](#).

3.3. MPN counts

MPN counts of heterotrophic bacteria growing under aerobic conditions varied between 10^3 and 10^6 MPN mL⁻¹. The highest (1.39×10^6 mL⁻¹) value was detected in the open water sample of the Mártély oxbow, while the lowest (2.72×10^3 mL⁻¹) in the open water sample of Riha oxbow. In addition, high values were also measured for submerged macrophyte samples from the following sampling locations: Lakitelek oxbow (5.73×10^5 mL⁻¹), Lake Morotva in Lipót (6.71×10^5 mL⁻¹), Lake Tisza (8.52×10^5 mL⁻¹), Zala estuary of Lake Balaton (7.31×10^5 mL⁻¹).

Compared to the heterotrophic bacteria growing under aerobic conditions, fermentative acidogenic bacteria were present in remarkably lower numbers (10^1 - 10^3 MPN mL⁻¹) in the samples. The highest value (4.07×10^3 mL⁻¹) was also detected in the open water sample of the Mártély oxbow. MPN values around 10^3 mL⁻¹ were also measured in submerged macrophyte samples from Lakitelek oxbow (1.21×10^3 mL⁻¹) and Lake Morotva in Lipót (1.09×10^3 mL⁻¹).

Regarding the ammonia-oxidizing bacteria MPN results, most samples exhibited zero (TO, TS, TNB, LK, LN, Ff, K, B and F) or low values (<6.9 MPN mL⁻¹), except the Kis-Herlakni inner pond (Lake Fertő) sample which was characterized by the highest AOB-MPN value (14 MPN mL⁻¹) (Fig. 5). The NOB-MPN values were slightly higher than AOB-MPN values, except for the macrophytes-covered areas of Lake Fertő and open water areas of Lake Kovácsszénája and Riha oxbow. The highest MPN numbers (20.3 – 49.3 MPN mL⁻¹) were detected in the following samples: Lake Tisza (TO, TS, TNB), and the macrophytes-covered areas of the Lakitelek and Mártély oxbow.

Based on the MPN results, nitrate-reducing bacteria were present in all samples, but their MPN values showed notable differences (Fig. 5). In the reed-covered areas of Lake Tisza (TNB) and Lake Morotva in Lipót (LN) we obtained the highest nitrate-reducing MPN values (3.01×10^6 MPN mL⁻¹ and 4.58×10^6 MPN mL⁻¹), while we detected the lowest MPN values in the open water of the Fadd-Dombori oxbow (11.32 MPN mL⁻¹) and in the macrophytes-covered areas of the Zala estuary of Lake Balaton (11.49 MPN mL⁻¹) and Mártély oxbow (9.38 MPN mL⁻¹). Although denitrifying bacteria were also detected in all samples, their MPN values varied considerably between the samples (Fig. 5). Low values (<95.27 MPN mL⁻¹) were detected in several samples, in contrast to the high MPN counts observed for the open water areas of the Keszthely basin of Lake Balaton (6.91×10^3 MPN mL⁻¹), Lake Fertő (1.15×10^4 MPN mL⁻¹) and Mártély oxbow (3.26×10^4 MPN mL⁻¹), as well as in the reed-covered area of Lake Tisza (1.42×10^3 MPN mL⁻¹).

The PERMANOVA test didn't show significant difference between the MPN values of the examined sample types (open water, emergent and submerged macrophytes).

4. Discussion

Macrophytes play a fundamental role in freshwater ecosystems, and their effects on bacterial communities are diverse and not fully understood (Zeng et al., 2012, Kumar et al., 2023). Our previous research on an individual freshwater marsh (Lake Kolon) and on a shallow soda lake (Lake Fertő) within the region consistently demonstrated a clear local impact of macrophytes on bacterioplankton communities (Mentes et al., 2018, Szuróczi et al., 2020). This localized influence was also apparent in earlier studies of soda pans from the region (Boros et al., 2020, Szabó et al., 2020, Márton et al., 2023). These results are further supported by the work of Porcel et al. (2025), who investigated a broad set of 37 Patagonian shallow lakes and their findings also highlighted macrophyte cover as one of the crucial factors in shaping planktonic bacterial assemblages.

In the present study we found that while macrophyte cover clearly influenced bacterioplankton composition at the scale of individual lakes, at a wider regional scale, other environmental factors appeared to exert a stronger influence on community structure. The high heterogeneity among our study sites significantly limited the detection of broad-scale, overarching patterns between macrophyte or algal biomass and bacterial productivity or community structure. This observation aligns with the findings of Mateus-Barros et al. (2025), who demonstrated that on a broad geographical scale, both the spatial and environmental factors were important in shaping planktonic bacterial communities. For the studied lakes of the Pannonian Basin, we also showed the overriding importance of other site-specific factors (e.g., conductivity, temperature, pH, dissolved oxygen, chlorophyll-a) in structuring bacterial communities across this broader spectrum of water bodies (Fig. 4).

Our study revealed valuable insights into the bacterial communities inhabiting lentic ecosystems across Hungary. The observed diversity in bacterial taxa reflects the inherent complexity of these ecosystems and the influence of distinct hydrological regimes. The effect of site heterogeneity on bacterioplankton was demonstrated by the variable taxon composition observed across samples. Notably, some OTUs were consistently abundant and prevalent among most sites. Consistent with findings in other freshwater lakes (McMahon and Newton, 2024), the most abundant bacterial group in our study belonged to the LD12 freshwater tribe of SAR11 Alphaproteobacteria, comprising approximately 7% of the total sequenced reads. Previous research (Chiriac et al., 2023) has already indicated that LD12 populations can exhibit high abundances during summer. Heinrich et al. (2013) also reported that this tribe can constitute up to one-third of the total microbial community in upper water layers under specific conditions in freshwater lakes. The families Rhodocyclaceae, betI, betII, and betIV, were also highly abundant across all samples (ranging from 4.5% to 32.6% relative abundance). Specifically, Rhodocyclaceae (including OTU9, affiliated with the genus C39), appeared to be higher in areas with dense macrophyte cover, which suggests a potential link between the presence of macrophytes and the distribution of these bacterial groups. Genus *Limnohabitans* was notably abundant in Lake Morotva (19.5% and 15.4% in LK and LN samples, respectively), reed-covered areas of Lake Tisza (8.3%), and macrophyte-covered zones of the Zala estuary of Lake Balaton (7.2%). The prevalence of Lhab-A1 and Lhab-A3 tribes among the top 30 OTUs further emphasizes the ecological importance of *Limnohabitans* - a ubiquitous freshwater genus, typically comprising around 12% of the total bacterial population (Props and Deneff, 2020). Extensive marker gene surveys have shown that other *Limnohabitans* taxa, such as those in the LimA lineage, prefer surface waters but also occur at greater

depths. Our results, showing high abundance of *Limnohabitans* (including Lhab-A3) in macrophyte-rich zones, reed beds, and Lake Morotva (a lake impacted by terrestrial runoff), are consistent with previous studies linking this genus to environments often characterized by terrestrial organic matter (Shabarova et al., 2017). Furthermore, this variation underscores the importance of considering specific environmental characteristics, such as hydrological regime and nutrient availability, when investigating the distribution and ecological roles of this genus.

Interestingly, 'Candidatus Patescibacteria' and Epsilonbacteraeota were found almost exclusively in one site, indicating potentially unique ecological roles or niche adaptations. Despite the limited research, other studies (Chiriac et al., 2022, Haro-Moreno et al., 2023, Vigneron et al., 2020) have detected 'Candidatus Patescibacteria' (formerly CPR) in freshwater habitats with relative abundances ranging from 2.5% to 13%. These bacteria appear to be aerotolerant anaerobes, capable of fermenting certain molecules like acetate and pyruvate for energy, and depend on host cells for essential metabolic functions (Chiriac et al., 2022). While their exact role in the ecosystem remains unclear, the increasing number of studies detecting them suggests they may be more prevalent and ecologically important than previously thought. The high abundance of 'Candidatus Patescibacteria' (over 35%) observed in the Fadd-Dombori samples further support their potential importance in these specific freshwater habitats. Conversely, Epsilonbacteraeota distinctly characterized the Zala River inflow, being substantially more abundant there than in all other sites where this phylum was nearly absent. This distinct composition in the Zala River estuary was primarily due to the high relative abundance of *Arcobacter* (e.g., OTU8). The correlation between high *Arcobacter* abundance and the low dissolved oxygen concentration (0.28 mg L^{-1}) in this sample is remarkable, as *Arcobacter* species are well-established as microaerophilic organisms, capable of proliferating under oxygen-depleted conditions rich in organic matter (Collado and Figueras, 2011). Based on the investigations by Farkas et al. (2022), the microbial communities of the Zala River were greatly shaped by the high nutrient content of the water and by floods, while the seasonal effect was less significant. In the year studied, the *Arcobacter* genus was significant in the wetland outflow sample in June, as was the case for the sample we examined, and although it was present in several other months, its abundance was not as significant. This further reinforces the notion that hydrological events, such as floods or basin isolation, are decisive factors in shaping bacterial community composition (Macias et al., 2025; Porcel et al., 2025). At the same time, our results revealed that Cyanobacteria were more prevalent in open-water sites, while Bacteroidota dominated the macrophyte-covered environments, which is also consistent with the spatial distributions documented in a shallow subtropical lake, Lake Taihu (Wu et al., 2007) and Patagonian high-altitude lakes (Porcel et al., 2025). These findings highlight that local biotic influences and broader environmental gradients collectively, yet at different scales, determine the microbial diversity of shallow lakes.

While the PERMANOVA test did not reveal significant differences in MPN counts of functional bacterial groups between open water and macrophyte-dominated samples at a broad scale, the MPN data provided valuable insights in the context of individual sites and their specific environmental characteristics.

For instance, although the highest MPN values of heterotrophic bacteria growing under aerobic conditions were detected in the open water sample of Mártély oxbow, several macrophyte-dominated samples also exhibited elevated counts. Specifically, submerged macrophyte samples from Lakitelek oxbow and Lake Tisza showed higher heterotrophic bacterial abundance compared to open water samples within those same locations (Fig. 5). Previous studies (Teske et al., 2011, Cuevas-Lara et al., 2024) have underscored the heterogeneity of organic carbon substrates in aquatic environments, emphasizing the critical role of the C/N ratio in shaping site-specific microbial community composition. The availability of organic matter likely influences the adaptive strategies used by microorganisms to access and utilize resources. A similar, though less pronounced, trend was observed for fermentative acidogenic bacteria. While the highest count was detected in the open water of Mártély oxbow, macrophyte-dominated samples from Lakitelek oxbow and Lake Tisza also showed somewhat higher numbers compared to open water samples from those respective sites.

Ammonia-oxidizing bacteria (AOB) were detected across a broad DO range ($0.28\text{--}17.7 \text{ mg L}^{-1}$) in our study. Notably, the highest AOB-MPN values were observed in Lake Fertő ($3.04\text{--}7.44 \text{ mg L}^{-1}$ DO) where the presence of Nitrosococcales and Nitrosomonadaceae OTUs further supports the activity of this group. Members of Nitrosomonadaceae are well-known as dominant ammonia-oxidizing bacteria (AOB) in freshwater ecosystems globally (Prosser et al., 2014). While NOB populations generally outnumbered the AOB, this trend was not consistent across all sites. Among the typical nitrite-oxidizing bacteria, only OTUs belonging to the genus *Nitrospira* were identified in the TO and B0 samples, which are often the most widespread NOB in a variety of aquatic systems (Hovanec et al., 1998). Moreover, these OTUs were present in very low proportions. Although NOB populations were generally higher than AOB, the differences were not substantial.

Both nitrate-reducing and denitrifying bacteria were detected in all samples, however, as expected, the MPN values of the former exceeded those of the latter in all cases. The highest populations of nitrate-reducing bacteria were found in oxygen-depleted, reed-covered areas such as Lake Tisza (TNB, DO: 2.76 mg L^{-1}) and Lake Morotva (LN, DO: 2.53 mg L^{-1}), demonstrating their prevalence under these conditions.

Overall, the MPN data suggests potential localized influences of macrophytes on specific bacterial groups. The most compelling evidence for this localized influence comes from the elevated nitrate-reducing bacteria counts in the oxygen-depleted reed beds, highlighting the role of macrophytes in creating specific microenvironments that favor certain bacterial functional groups.

In conclusion, our study provides a regional-scale assessment of shallow lakes in the Pannonian Basin, revealing ecological patterns that extend beyond single-ecosystem observations. Despite the broad environmental gradient covered, no consistent relationships were identified between algal, macrophyte, and bacterial abundance, activity and composition across the investigated water bodies. This may be attributed to the combined influence of site-specific characteristics, subtle environmental variation, and unaccounted ecological or historical factors. Despite this complexity, the study provides important insights into the environmental factors influencing bacterial communities in freshwater ecosystems. The findings suggest that varying land use practices in the surrounding areas may significantly influence the ecological status of these water bodies, but further research is necessary to clarify the intricate interplay

between land use, water body characteristics, and microbial communities.

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CRediT authorship contribution statement

Dóra Anda: Writing – Original Draft, Visualization, Investigation, Formal Analysis, Data curation. **Tamás Felföldi:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Márton Zsuzsanna:** Writing – review & editing, Investigation. **Sára Szuróczi:** Writing – review & editing, Investigation. **Károly Márialigeti:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Viktor R. Tóth:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Attila Szabó:** Writing – review & editing, Visualization, Supervision, Formal Analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejrh.2026.103297](https://doi.org/10.1016/j.ejrh.2026.103297).

Data availability

Raw sequence data can be accessed under the NCBI BioProject ID PRJNA1083048.

References

- Alexander, M., Clark, F.E., 1965. Nitrifying bacteria. In: Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark, F.E. (Eds.), *Methods of Soil analysis, Part 2*. American Society of Agronomy, Madison, Wisconsin, pp. 1477–1483.
- Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137.
- Barbosa, M., Lefler, F.W., Berthold, D.E., Gettys, L.A., Leary, J.K., Laughinghouse IV, H.D., 2024. Macrophyte coverage drives microbial community structure and interactions in a shallow sub-tropical lake. *Sci. Total Environ.* 923, 171414.
- Borics, G., Ács, É., Boda, P., Boros, E., Erős, T., Grigorszky, I., Kiss, K.T., Lengyel, S., Reskóné, N.M., Somogyi, B., Vörös, L., 2016. Water bodies in Hungary—an overview of their management and present state. *Hung. J. Hydrol.* 96 (3), 57–67.
- Boros, E., Horváth, Z., Wolfram, G., Vörös, L., 2014. Salinity and ionic composition of the shallow astatic soda pans in the Carpathian Basin. *Ann. Limnol. Int. J. Limnol.* 50 (1), 59–69.
- Boros, E., V.-Balogh, K., Csitári, B., Vörös, L., Székely, A.J., 2020. Macrophytes and groundwater drive extremely high organic carbon concentration of soda pans. *Freshw. Biol.* 65 (9), 1555–1568.
- Chiriac, M.C., Bulzu, P.A., Andrei, A.S., Okazaki, Y., Nakano, S.I., Haber, M., Kavagutti, V.S., Layoun, P., Ghai, R., Salcher, M.M., 2022. Ecogenomics sheds light on diverse lifestyle strategies in freshwater CPR. *Microbiome* 10 (1), 84.
- Chiriac, M.C., Haber, M., Salcher, M.M., 2023. Adaptive genetic traits in pelagic freshwater microbes. *Environ. Microbiol.* 25 (3), 606–641.
- Collado, L., Figueras, M.J., 2011. Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clin. Microbiol. Rev.* 24 (1), 174–192.
- Cuevas-Lara, D., García-Oliva, F., Sánchez-Carrillo, S., Alcocer, J., 2024. Organic matter processing by heterotrophic bacterioplankton in a large tropical river: Relating elemental composition and potential carbon mineralization. *PLoS One* 19 (11), e0311750.
- Fang, H., Zhen, Z., Yang, F., Su, H., Wei, Y., 2024. Epiphytic bacterial community composition on four submerged macrophytes in different regions of Taihu Lake. *Front. Plant Sci.* 15, 1404718.
- Farkas, M., Szoboszlai, S., Vörös, L., Lovász, Z., Méhes, N., Mátyás, K., Sebök, R., Kaszab, E., Háhn, J., Tóth, G., Harkai, P., 2022. Bacterial community dynamics along a river-wetland-lake system. *Water* 14 (21), 3519.
- Felföldi, T., Duleba, M., Somogyi, B., Vajna, B., Nikolausz, M., Présing, M., Márialigeti, K., Vörös, L., 2011. Diversity and seasonal dynamics of the photoautotrophic picoplankton in Lake Balaton (Hungary). *Aquat. Microb. Ecol.* 63 (3), 273–287.
- Hahn, M.W., 2006. The microbial diversity of inland waters. *Curr. Opin. Biotechnol.* 17, 256–261.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4 (1), 1.

- Haro-Moreno, J.M., Cabello-Yeves, P.J., Garcillán-Barcia, M.P., Zakharenko, A., Zemska, T.I., Rodríguez-Valera, F., 2023. A novel and diverse group of *Candidatus* Patescibacteria from bathypelagic Lake Baikal revealed through long-read metagenomics. *Environ. Microbiome* 18 (1), 12.
- Heinrich, F., Eiler, A., Bertilsson, S., 2013. Seasonality and environmental control of freshwater SAR11 (LD12) in a temperate lake (Lake Erken, Sweden). *Aquat. Microb. Ecol.* 70 (1), 33–44.
- Hempel, M., Grossart, H.P., Gross, E.M., 2009. Community composition of bacterial biofilms on two submerged macrophytes and an artificial substrate in a pre-alpine lake. *Aquat. Microb. Ecol.* 58, 79–94.
- Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* 5, 1571–1579.
- Hovanec, T.A., Taylor, L.T., Blakis, A., Delong, E.F., 1998. Nitrospira-like bacteria associated with nitrite oxidation in freshwater aquaria. *Appl. Environ. Microbiol.* 64 (1), 258–264.
- Huss, A.A., Wehr, J.D., 2004. Strong indirect effects of a submersed aquatic macrophyte, *Vallisneria americana*, on bacterioplankton densities in a mesotrophic lake. *Microb. Ecol.* 47, 305–315.
- Istvánovics, V., Clement, A., Somlyódy, L., Speziár, A., G.-Tóth, L., Padišák, J., 2007. Updating water quality targets for shallow Lake Balaton (Hungary), recovering from eutrophication. In: Qin, B., Liu, Z., Havens, K. (Eds.), *Eutrophication of Shallow Lakes with Special Reference to Lake Taihu, China*. Springer, Dordrecht, pp. 305–318.
- Jakab, T., Müller, Z., Dévai, Gy., Tóthmérész, B., 2002. Dragonfly assemblages of a shallow lake type reservoir (Tisza-tó, Hungary) and its surroundings. *Acta Zool. Acad. Sci. Hung.* 48 (3), 161–171.
- Korponai, K., Szabó, A., Somogyi, B., Boros, E., Borsodi, A.K., Jurecska, L., Vörös, L., Felföldi, T., 2019. Dual bloom of green algae and purple bacteria in an extremely shallow soda pan. *Extremophiles* 23, 467–477.
- Kumar, S., Singh, R., Kumar, D., Baudh, K., Kumar, N., Kumar, R., 2023. An introduction to the functions and ecosystem services associated with aquatic macrophytes. In: Kumar, S., Baudh, K., Singh, R., Kumar, N., Kumar, R. (Eds.), *Aquatic Macrophytes: Ecology, Functions and Services*. Springer Nature, Singapore, pp. 1–20.
- Lipponen, M.T., Suutari, M.H., Martikainen, P.J., 2002. Occurrence of nitrifying bacteria and nitrification in Finnish drinking water distribution systems. *Water Res.* 36, 4319–4329.
- Macías, P.Y., Ferraro, M., Bustingorry, J.F., Escaray, R., de los Ángeles González Sagrario, M., Llamas, M.E., 2025. Flooding events reshape bacterioplankton assemblages and functional properties in a hypertrophic Shallow Lake (Laguna Chascomús, Argentina). *Hydrobiology* 1–21.
- Martínez Arbizu, P., 2020. *pairwiseAdonis: Pairwise multilevel comparison using adonis*. R package version 0.4.
- Márton, Z., Csitári, B., Felföldi, T., Hidas, A., Jordán, F., Szabó, A., Székely, A.J., 2023. Contrasting response of microeukaryotic and bacterial communities to the interplay of seasonality and local stressors in shallow soda lakes. *FEMS Microbiol. Ecol.* 99 (9), fiad095.
- Mateus-Barros, E., Caliman, A., da Silva, T.G., Sarmento, H., 2025. Beyond environmental selection: exploring the spatial structuring of a freshwater bacterial metacommunity on a broad geographical scale. *Aquat. Ecol.* 59, 1449–1464.
- McMahon, K.D., Newton, R.J., 2024. Chapter 23 - Pelagic Bacteria, Archaea and Viruses. In: Jones, I.D., Smol, J.P. (Eds.), *Wetzel's Limnology (Fourth Edition)*. Academic Press, pp. 705–757.
- Mentes, A., Szabó, A., Somogyi, B., Vajna, B., Tugyi, N., Csitári, B., Vörös, L., Felföldi, T., 2018. Differences in planktonic microbial communities associated with three types of macrophyte stands in a shallow lake. *FEMS Microbiol. Ecol.* 94, fix164.
- Mezősi, G., 2017. *Hydrography of Hungary*. In: *The Physical Geography of Hungary*. Springer International Publishing, Cham, pp. 121–164.
- Oksanen, J.F., 2025. *vegan: community ecology package*. R package version 2.6-10. (<https://CRAN.R-project.org/package=vegan>).
- Porcel, S., Sabio y García, C.A., Schiaffino, M.R., Chaparro, G., Diéguez, M.D.C., Lancelotti, J., Roesler, I., Izaguirre, I., 2025. How do environmental and spatial factors influence beta diversity and composition of abundant and rare Bacterioplankton communities in lakes from the Patagonian Plateaus? *Freshw. Biol.* 70 (5), e70047.
- Pretzer, C., Druzhinina, I.S., Amaro, C., Benediktsdóttir, E., Hedenström, I., Hervio-Heath, D., Huhulescu, S., Schets, F.M., Farnleitner, A.H., Kirschner, A.K., 2017. High genetic diversity of *Vibrio cholerae* in the European lake Neusiedler See is associated with intensive recombination in the reed habitat and the long-distance transfer of strains. *Environ. Microbiol.* 19 (1), 328–344.
- Props, R., Deneff, V.J., 2020. Temperature and nutrient levels correspond with lineage-specific microdiversification in the ubiquitous and abundant freshwater genus *Limnohabitans*. *Appl. Environ. Microbiol.* 86 (10), e00140-20.
- Prosser, J.I., Head, I.M., Stein, L.Y., 2014. The family Nitrosomonadaceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), *The Prokaryotes*. Springer, Berlin, Heidelberg, pp. 901–918.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1), D590–D596.
- R Core Team, R., 2024. *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. (<https://www.R-project.org/>) (URL).
- Reasoner, D.J., Geldreich, E., 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* 49 (1), 1–7.
- Rohwer, R.R., Hamilton, J.J., Newton, R.J., McMahon, K.D., 2018. TaxAss: leveraging a custom freshwater database achieves fine-scale taxonomic resolution. *mSphere* 3 e00327–e00318.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75 (23), 7537–7541.
- Shabarova, T., Kasalický, V., Šimek, K., Nedoma, J., Znachor, P., Posch, T., Pernthaler, J., Salcher, M.M., 2017. Distribution and ecological preferences of the freshwater lineage LimA (genus *Limnohabitans*) revealed by a new double hybridization approach. *Environ. Microbiol.* 19 (3), 1296–1309.
- Sibert, R.M., Krieg, N.R., 1994. Phenotypic characterization. In: Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R. (Eds.), *Methods for General and Molecular Bacteriology*. American Society for Microbiology, Washington, DC, pp. 607–654.
- Somogyi, B., Felföldi, T., Katalin, V., Boros, E., Pálffy, K., Vörös, L., 2016. The role and composition of winter picoeukaryotic assemblages in shallow Central European great lakes. *J. Gt. Lakes Res.* 42 (6), 1420–1431.
- Suzuki, M.T., Taylor, L.T., DeLong, E.F., 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl. Environ. Microbiol.* 66 (11), 4605–4614.
- Szabó, A., Korponai, K., Somogyi, B., Vajna, B., Vörös, L., Horváth, Z., Boros, E., Szabó-Tugyi, N., Máriaiget, K., Felföldi, T., 2020. Grazing pressure-induced shift in planktonic bacterial communities with the dominance of acIII-A1 actinobacterial lineage in soda pans. *Sci. Rep.* 10 (1), 19871.
- Szabó-Tugyi, N., Tóth, V.R., 2020. Interaction among bacterioplankton and macrophytes in shallow lakes with high macrophyte cover. *Aquat. Sci.* 82 (4), 1–9.
- Szuróczi, S., Szabó, A., Korponai, K., Felföldi, T., Somogyi, B., Máriaiget, K., Tóth, E., 2020. Prokaryotic community composition in a great shallow soda lake covered by large reed stands (Neusiedler See/Lake Fertő) as revealed by cultivation and DNA-based analyses. *FEMS Microbiol. Ecol.* 96, fiad159.
- Takai, K.E.N., Horikoshi, K., 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl. Environ. Microbiol.* 66 (11), 5066–5072.
- Teske, A., Durbin, A., Ziervogel, K., Cox, C., Arnosti, C., 2011. Microbial community composition and function in permanently cold seawater and sediments from an Arctic fjord of Svalbard. *Appl. Environ. Microbiol.* 77 (6), 2008–2018.
- Tóth, V.R., Endre, G., Kovács, S., Présing, M., Horváth, H., 2017. Morphological and genetic variability of *Myriophyllum spicatum* in different shallow water bodies of Hungary. *Wetlands* 37 (2), 351–362.
- Vigñeron, A., Craud, P., Langlois, V., Lovejoy, C., Culley, A.I., Vincent, W.F., 2020. Ultra-small and abundant: candidate phyla radiation bacteria are potential catalysts of carbon transformation in a thermokarst lake ecosystem. *Limnol. Oceanogr. Lett.* 5 (2), 212–220.

- Wang, X., Liu, Y., Qing, C., Zeng, J., Dong, J., Xia, P., 2024. Analysis of diversity and function of epiphytic bacterial communities associated with macrophytes using a metagenomic approach. *Microb. Ecol.* 87 (1), 37.
- Westcott, S.L., Schloss, P.D., 2017. OptiClust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. *mSphere* 2 (2), e00073-17.
- Wickham, H., 2009. *ggplot2: Elegant Graphics for Data Analysis*. Springer, Berlin.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. *J. Open Source Softw.* 4 (43), 1686.
- Wu, Q.L., Zwart, G., Wu, J., Kamst-van Agterveld, M.P., Liu, S., Hahn, M.W., 2007. Submersed macrophytes play a key role in structuring bacterioplankton community composition in the large, shallow, subtropical Taihu Lake, China. *Environ. Microbiol.* 9 (11), 2765–2774.
- Zeng, J., Bian, Y., Xing, P., Wu, Q.L., 2012. Macrophyte species drive the variation of bacterioplankton community composition in a shallow freshwater lake. *Appl. Environ. Microbiol.* 78 (1), 177.