UNRAVELING THE PUZZLES OF BACTERIOPLANKTON FUNCTIONING AND DIVERSITY IN SHALLOW LAKES WITH DIFFERENT MACROPHYTE COVER

ONE STEP CLOSER TO UNDERSTANDING THE ROLE OF THE LITTORAL ZONE

- F I N A L R E P O R T -

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Project participants:

Department of Microbiology, Eötvös Loránd University

Károly Márialigeti – professor, principal investigator
Erika Tóth – head of department, associate professor, senior researcher
Tamás Felföldi – assistant professor
Attila Szabó – assistant research fellow
Csaba Romsics – assistant research fellow
Sára Szuróczki – PhD student
Kristóf Korponai – PhD student

Balaton Limnological Institute, Centre for Ecological Research

Viktor Tóth – senior research fellow, principal investigator (consortional assoc.)
Boglárka Somogyi – research fellow, senior researcher
Emil Boros – senior research fellow
Károly Pálffy – research fellow
Attila Kovács – research fellow
Nóra Tugyi – PhD student

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EXTENSIVE MONTHLY STUDIES ON THE ROLE AND DIVERSITY OF BACTERIOPLANKTON IN SHALLOW LAKES

YEAR 1-2

Lake Fertő

Accumulation of submerged and emergent macrophyte biomass, phytoplankton primary production and the role and diversity of the bacterioplankton was studied monthly in different areas of Lake Fertő/Neusiedlersee between October 2015 and September 2016. Our aim was to determine how the variation in the dissolved organic carbon (DOC) source (phytoplankton, macrophytes) influence bacterial abundance, production and diversity. Considering the above, three sampling stations were chosen, representing different macrophyte influence: an open water sampling station (B0) with no macrophyte cover, an inner lake sampling station (Kis-Herlakni, abbreviated as KH) within the reed belt with significant cover of submerged macrophytes and a sampling station within the reed (*Phragmites australis (Cav.) Trin. ex Steud.*) belt (referred later as 'reed stand', abbreviated as N).

Phytoplankton biomass was considerably higher in the open water, with 12 µg/L yearly average chlorophyll *a* concentration, than in the inner lake and the reed stand, where the yearly average was 4 and 6 µg/L, respectively. On the basis of the ^{14}C uptake measurements, phytoplankton primary production (PP) was 82 g C/m^2/year in the open water, 21 g C/m^2/year in the inner lake and 35 g C/m^2/year in the reed stand. Maximum biomass of the green plants was 585 g dry weight(DW)/m^2 in the inner lake and 1030 g DW/m^2 in the reed stand. As a result of the plant decay, the amount of coloured dissolved organic matter (CDOM) in the water was much higher in the inner lake and in the reed stand (120-175 mg/L and 90-260 mg/L, respectively) than in the open water (between 14 and 40 mg/L).

The abundance of heterotrophic bacteria was similar at the different sampling stations, however, there were large differences between the bacterial biomass and production (BP). Opposite to the phytoplankton, yearly gross bacterial production was much less (58 g C/m^2/year) in the open water than in the inner lake (106 g C/m^2/year) and in the reed stand (155 g C/m^2/year) (**Fig. 1**). Such differences were also recognized in the community composition and diversity of bacterioplankton: the culture based bacterial counts (most probable number, MPN) of heterotrophic bacteria was lower in the open water than in the reed-associated areas, higher archaeal diversity values were detected at the reed-associated sampling sites, the bacterial and archaeal community composition of the open water differed from those of the other two sampling stations, and the planktonic prokaryotic communities of the inner pond and the reed-covered area showed significant similarities to each other (see later, **Fig. 5**).

This suggested that CDOM (with other marcophyte-derived organic compounds) was an appropriate carbon source for heterotrophic bacteria in the macrophyte-dominated littoral regions. This was experimentally confirmed in a test using more than 200 bacterial strains (selected from 559 strains isolated in this project), since the vast majority (83%) of bacterial cultures isolated from the reed-covered area were able to grow on a medium containing reed extract as a sole source of carbon.

In contrast, BP in the open water depended mainly on phytoplankton-related carbon sources. As a result, the role of bacterioplankton was more important in the studied littoral aquatic habitats than in the open water.

One strain from the culture collection isolated from Lake Fertő was described as a new genus with one representative species (*Phragmitibacter flavus* gen. nov., sp. nov.).

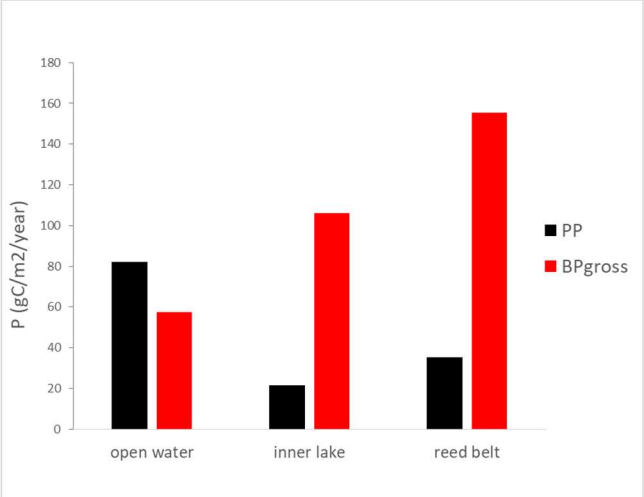

Fig. 1. Yearly planktonic primary production (PP) and gross bacterial production (BPgross) in Lake Fertő/Neusiedlersee

Lake Kolon

Accumulation of macrophyte biomass, phytoplankton primary production and the role and diversity of the bacterioplankton was studied monthly in different areas of Lake Kolon between October 2016 and September 2017 and in a more detailed spatial sample set collected previously in Nov 2014. Our aim was to determine how different DOC forms (i.e. the origin of DOC) influence the bacterial community and compare the obtained results with those obtained in Lake Fertő. Considering the above, three sampling stations were chosen, which were different in terms of macrophyte cover. The 'open water' sampling station (abbreviated as KR) had no macrophyte in its close vicinity, but this site was also highly influenced by the vegetation as a result of the surrounding emergent macrophytes. As a result, concentration of CDOM was relatively high (87.9±15.9 mg/L) comparing to other freshwater lakes. The inner lake ('Nymphea', abbreviated as TR) sampling station was covered mainly by floating macrophytes (*Nymphea alba*) with a maximum biomass of 318±127 g DW/m^2 and a higher CDOM content (118.9±27.9 mg/L). The third sampling station ('Utricularia', abbreviated as RC) was characterized with strong presence of submerged macrophytes *(Utricularia vulgaris* and *Ceratophyllum demersum*) with a maximum biomass of 841±287 g DW/m^2 and even higher CDOM content (202.8±31.9 mg/L). This variability of CDOM at the studied water bodies coincided with the increase of chlorophyll-a content of the water, while parallel to this, decrease of the O_2 content of the water was detected.

Phytoplankton primary production was similar at the sampling sites: on a yearly basis, the PP was 55 gC/m^2/year in the open water, 69 gC/m^2/year at the 'Nymphea' station and 55 gC/m^2/year at the

'Utricularia' station. In contrast, large differences were detected in the bacterial production (BP) among the study sites: the gross bacterial production increased with the increasing macrophyte cover and CDOM content (**Fig. 2**) from 96 g C/m^2/year to 246 g C/m^2/year. This suggests that similarly to Lake Fertő, marcophyte-derived organic compounds were appropriate carbon sources for heterotrophic bacteria. However, in contrast to Lake Fertő, BP in the open water also depended on macrophyte-related carbon sources instead of phytoplankton-related carbon sources.

Comparing samples collected at the same date, despite the similarities in most of the measured physicochemical parameters, sites had different bacterial and algal communities, suggesting that the presence and quality of macrophytes directly and indirectly controlled the composition of microbial plankton (**Fig. 3**). Such determining factors could be: (1) the higher phosphorus content and lower C/N ratio of *Utricularia*, therefore it may be decomposed more quickly than *Nymphea* or reed; (2) some compounds derived from aquatic plants may act as antimicrobial agents against bacteria (e.g. *Nymphea* species produce anthocyanins), and due to their selective action such compounds could have structuring effect on the planktonic bacterial and algal communities.

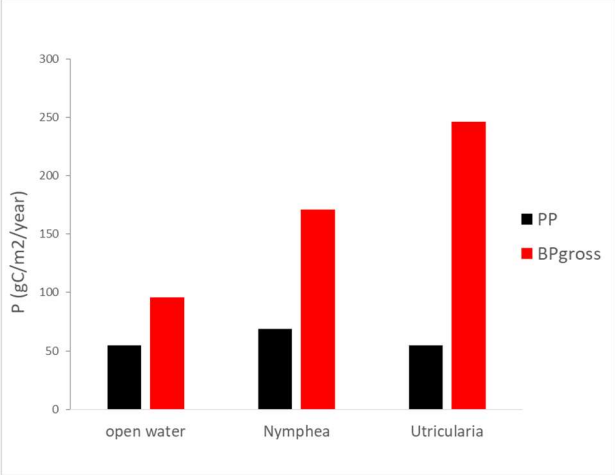

Fig. 2. Yearly planktonic primary production (PP) and gross bacterial production (BPgross) in Lake Kolon

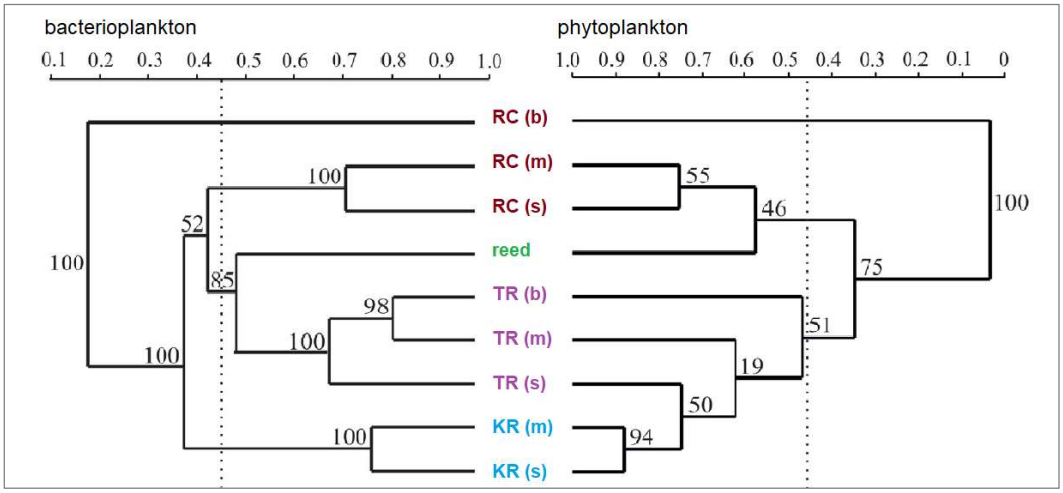

Fig. 3. Comparison of Lake Kolon bacterioplankton and phytoplankton communities of different sites with cluster analysis based on amplicon sequencing and microscopy data. (Cluster analysis was calculated using the unweighted-pair group mean averages and the Bray–Curtis similarity index. Bootstrap values are given at the nodes. Samples were taken from three different depths: s – surface, m – middle, b – bottom layer of the water column)

Comparison of shallow lakes

Summarizing the results, macrophytes had a strong impact on bacterioplankton. In case of Lake Fertő, higher CDOM resulted in higher bacterial production in macrophyte-covered areas than in the open water, where the bacterioplankton depended mainly on the primary production of the phytoplankton (**Figs 2 and 4**). As Lake Kolon is a swamp lake, the impact of macrophytes was much higher at all studied stations and smaller differences were observed among the sampling sites than in case of Lake Fertő (**Fig. 4**). The 'open water' of this swamp lake was similar in CDOM and BP to the macrophyte-covered parts of Lake Fertő and the yearly gross production of the bacterioplankton exceeded that of the phytoplankton (**Fig. 3**). However, the increasing macrophyte impact and CDOM could led to even higher bacterial production as was seen in 'Nymphea' and 'Utricularia' sampling stations of Lake Kolon.

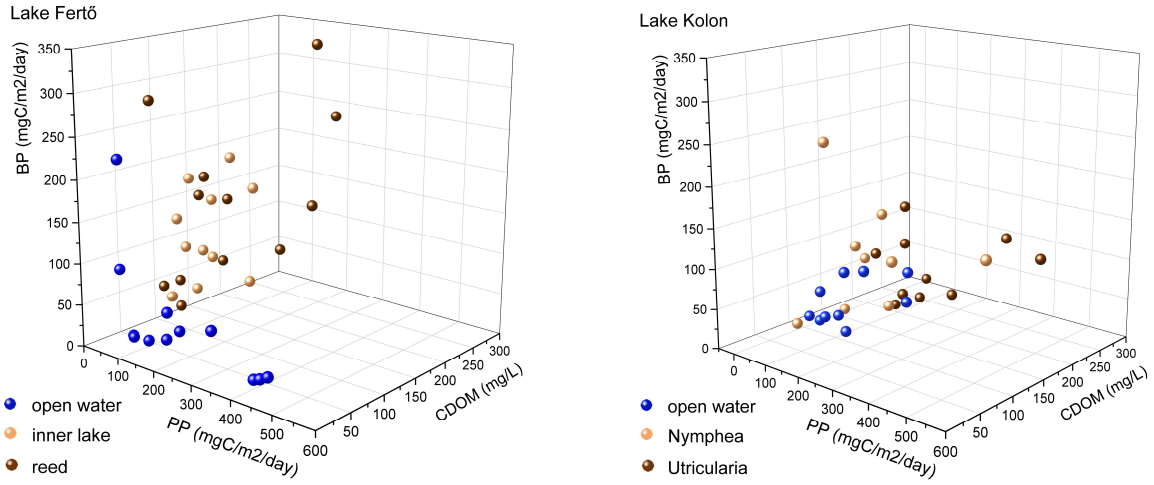

Fig 4. Bacterial production (BP) as a function of planktonic primary production (PP) and CDOM in Lake Fertő and Lake Kolon

Both in the water samples of Lake Fertő and Lake Kolon the bacterioplankton community was dominated by members of the phyla Proteobacteria, Bacteroidetes and Actinobacteria. The ratio of Archaea in the prokaryotic plankton was negligible, their relative abundance in the sediment of Lake Fertő and Lake Kolon was around 5-10% on average (therefore the diversity of Archaea was also significantly lower than that of Bacteria). All these corresponded well with results obtained in the case of lakes used as comparison from this region (Lake Velence, soda pans) and also with other freshwater lakes worldwide (Newton et al. 2011). Not surprisingly the sediment samples had higher prokaryotic diversity than the water samples and their community composition also showed remarkable differences both in the case of bacteria (**Fig. 5**) and archaea (**Fig. 6**).

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 (and higher CDOM content) was observed (**Fig. 5**). Additionally, the two studied lakes also differed remarkably in terms of bacterioplankton composition. Similarly to these, the bacterioplankton

communities of soda pans from Hungary which had different CDOM content (partially due to the differences of macrophyte cover) also showed remarkable differences in their composition.

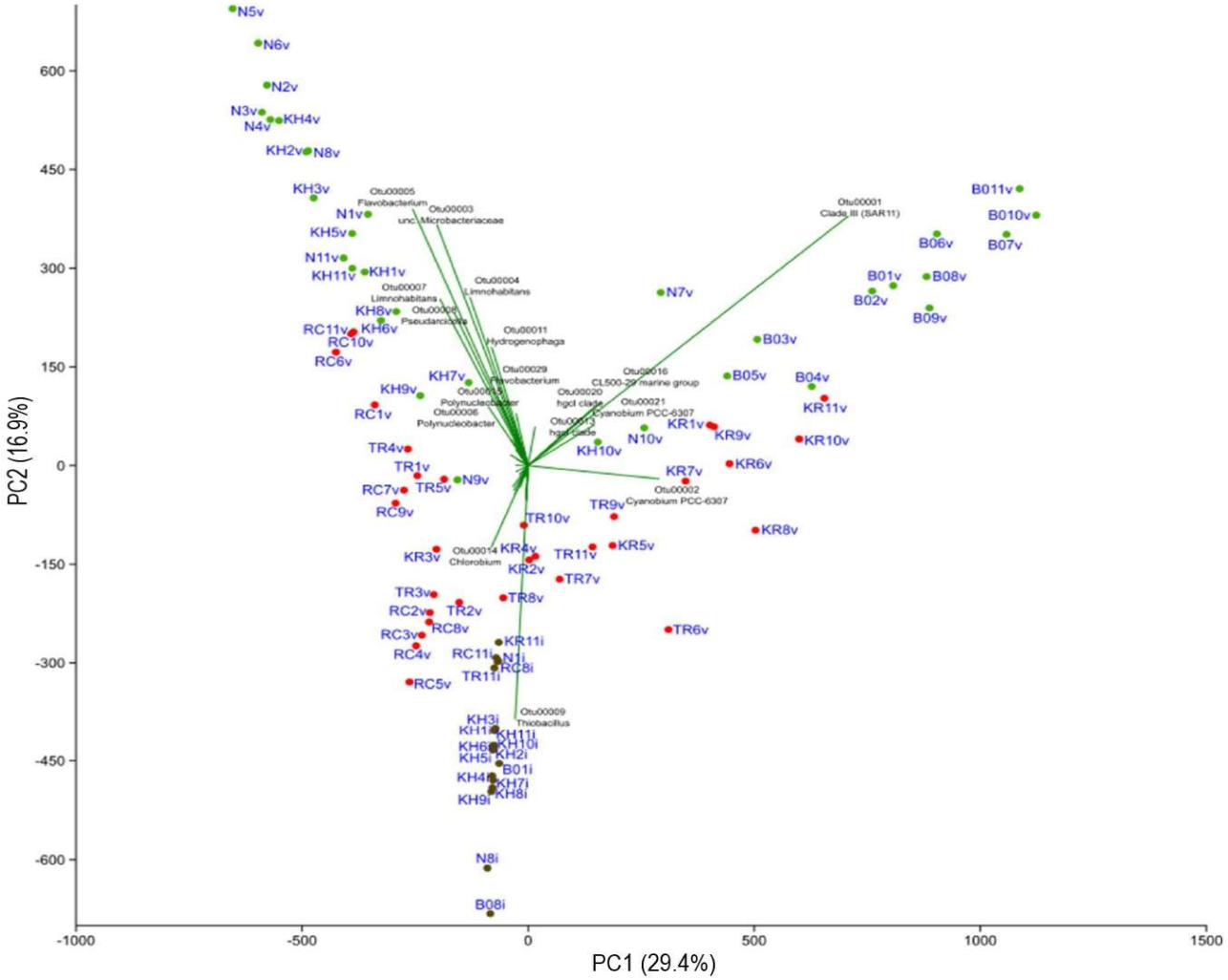

Fig. 5. Principal component analysis of the bacterial communities of samples from Lake Kolon and Lake Fertő. (Colour codes: green – water samples from Lake Fertő, red – water samples from Lake Kolon, brown – sediment samples)

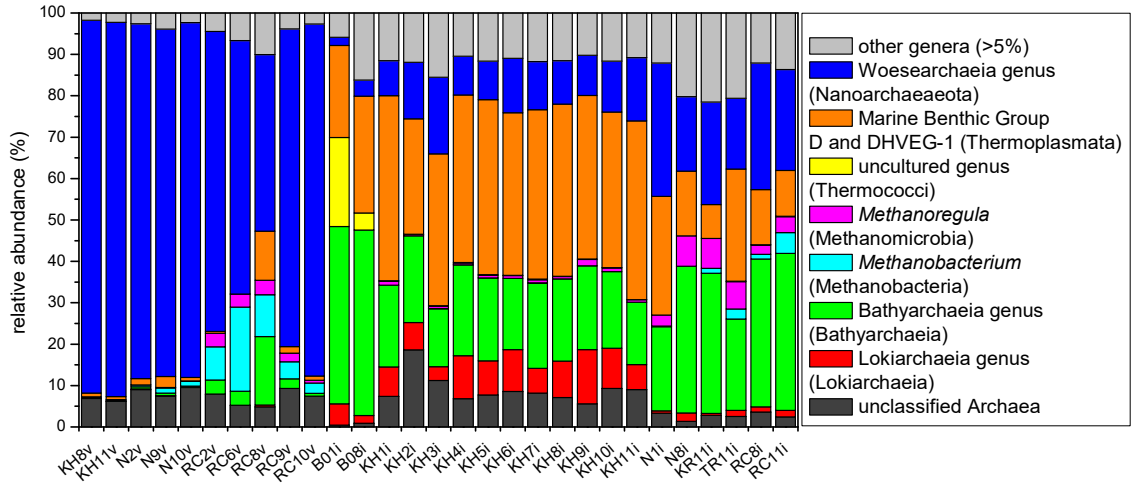

Fig. 6. Relative abundances of the archaeal genera in the samples from Lake Kolon and Lake Fertő (due to their low abundance, comparative analysis of the archaeal communities was possible only in the case of some samples; v – water samples, i – sediment samples)

Seasonal changes were also observable in the composition of bacterioplankton, but our results showed that the effect of marcophytes in several shallow lakes of the temperate zone could be higher on bacterioplankton than that of the seasonally changing physicochemical parameters.

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ASSESSING THE ROLE OF BACTERIOPLANKTON IN SHALLOW LAKES IN THE OPEN WATER AND IN AREAS COVERED WITH MACROPHYTE COMMUNITIES OF VARIOUS COMPOSITION AND EXTENT

YEAR 3

The effect of submerged and emergent macrophytes on the production, abundance and diversity of bacterioplankton and phytoplankton was studied on 24 sites in 12 freshwater water bodies of Hungary. A wide variety of water bodies of different phytoplankton content and level of dystrophy were chosen: lakes, a reservoir and oxbows of River Danube and River Tisza were included in the sampling in 2018 (**Fig. 7**). The lakes were examined from limnological (both biotic and abiotic factors), algological (primary production and content) and bacteriological (production, composition and diversity) point of view. The studied parameters were compared as a relation to macrophyte cover accumulation.

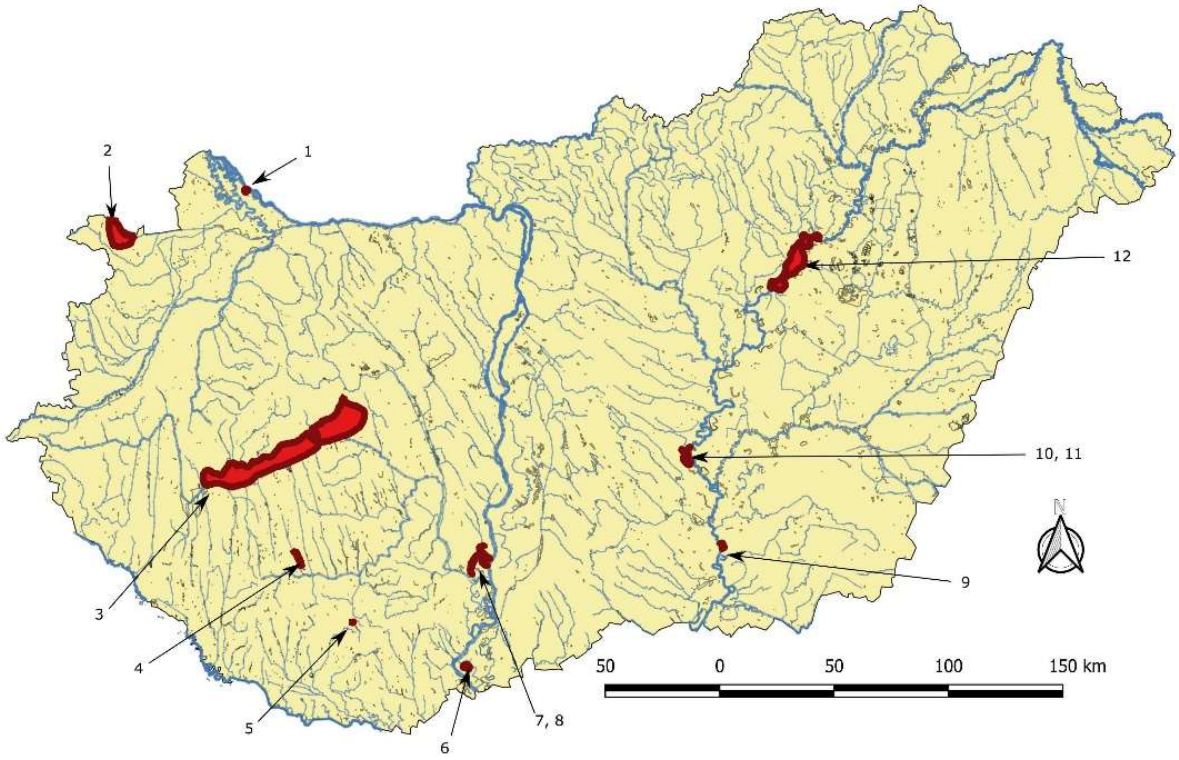

Fig. 7. Location of sampling sites in Hungary (1 – Lake Morotva in Lipót [LK, LN], 2 – Lake Fertő [B0, KH, N, NH], 3 – Lake Balaton [K, Ff, Z], 4 – Reservoir Deseda [Ds], 5 – Lake Kovácsszénája [Kn], 6 – Oxbow Riha [R], 7 – Oxbow Tolnai [TLO], 8 – Oxbow Fadd-Dombori [FDN, FDO], 9 – Oxbow Mártély [F, E], 10 – Oxbow Tiszaapáti [A, B], 11 – Oxbow Lakiteleki [C, D], 12 – Lake Tisza [TO, TS, TNB]; sample abbreviations are given in brackets)

Open water sites differed from water bodies with macrophytes in some limnological parameters, though differences were not significant (Kruskal-Wallis One Way Analysis of Variance on Ranks), differences were observed primarily in the pH, O_2 and CDOM content of the water (**Table 1**). Although the measured differences were not significant (Kruskal-Wallis One Way Analysis of Variance on Ranks),

the chlorophyll-a content of the water and the maximum potential photosynthetic rate of algal photosynthesis was higher in the open water samples with slightly lower bacterial production (**Table 1**). These consequently resulted in difference of the studied macrophyte covered and open waters (**Fig. 8**). These results were in agreement with the findings of studies performed in the previous two years.

Table 1. Limnological and algological parameters measured during the field campaign of 2018 grouped to show the open water areas, and the water bodies dominated by submerged and emergent macrophytes

	open water	**submerged**	**emergent**
temperature (°C)	28.2±0.5	27.4±0.8	29.1±0.9
K_d (1/m)	3.2±0.4	9.1±1.9	5.7±2.2
depth (cm)	272.1±44.4	131.9±42.3	72.5±17.9
pH	8.53±0.14	8.06±0.18	7.97±0.31
conductivity (µS/cm)	783±184	1093±385	1114±630
Secchi (cm)	54.6±11.9	54.1±10.6	44.8±7.4
TSM (mg/L)	18.3±3.0	12.6±3.7	18.0±3.6
CDOM (mg/L)	35.4±10.2	87.1±20.4	72.4±51.9
turbidity (FNU)	22.5±4.5	7.8±2.6	7.3±3.2
O_2 (mg/L)	8.4±1.3	4.3±1.1	4.8±2.0
chlorophyll-a (µg/L)	64.4±17.5	35.9±13.1	20.2±5.1
P_{max} (µg C/L/h)	486.3±174.4	155.4±47.3	99.8±38.2
bacterial productivity (µg C/L/h)	12.3±2.6	21.5±8.1	16.2±2.8

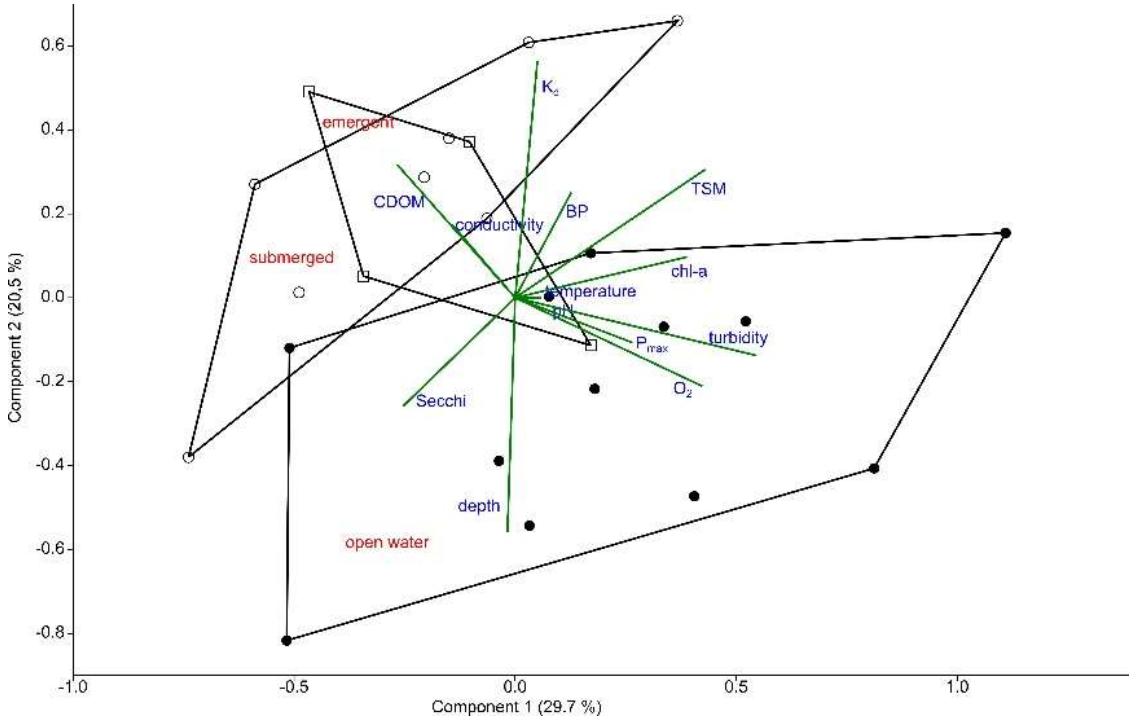

Fig. 8. Principal components analysis of the limnological, algological and bacteriological data of the studied water bodies

Bacterial production did not have correlation with water colour (CDOM) (Spearman rank-order correlation: r=0.252, P=0.233), but do correlated with the water chlorophyll-a content (Spearman rank-

order correlation: r=0.490, P=0.016). Moreover, macrophyte biomass accumulation on its own had no relation neither to the chlorophyll-a content of the water (R=-0.08, P=0.81), nor to the maximum potential photosynthetic rate of algal photosynthesis (R=-0.24, P=0.26) or bacterial production (R=0.04, P=0.83).

Chlorophyll-a content of the water correlated with the ORP conditions at the sediment-water level, and the redox conditions at 20 cm and 40 cm deep into the sediment (R=0.53, R=0.53 and R=0.78, P<0.05, respectively). Maximum potential photosynthetic rate of algal photosynthesis also correlated with ORP at 0 (water-sediment level) and 10 cm deep sediment (R=0.68, P=0.006 and R=0.52, P=0.04, respectively). Bacterial production also correlated with ORP at 10 cm deep into the sediment (R=0.53, P=0.04), showing the important regulatory role of ORP on the biological processes within the water column.

The difference among the studied water body types (lakes, reservoir and oxbows) was not prominent, although oxbows and lakes differed in some of the parameters (**Fig 9**). Generally, the oxbows contained slightly more algae (68±16 vs. 23±10 µg/L; Mann-Whitney Rank Sum Test, P<0.01) and total suspended matter (18±3 vs. 14±3 mg/L; not significant ANOVA). The maximum potential photosynthetic rate measured in oxbows was nearly 3 time higher as compared that of the lakes (327±77 vs. 124±45 µg C/L/h, Mann-Whitney Rank Sum Test, P<0.05). Nevertheless, not only algae, but also bacterial production was higher in oxbows (21±5 vs. 11±2 µg C/L/h), though the difference was not significant.

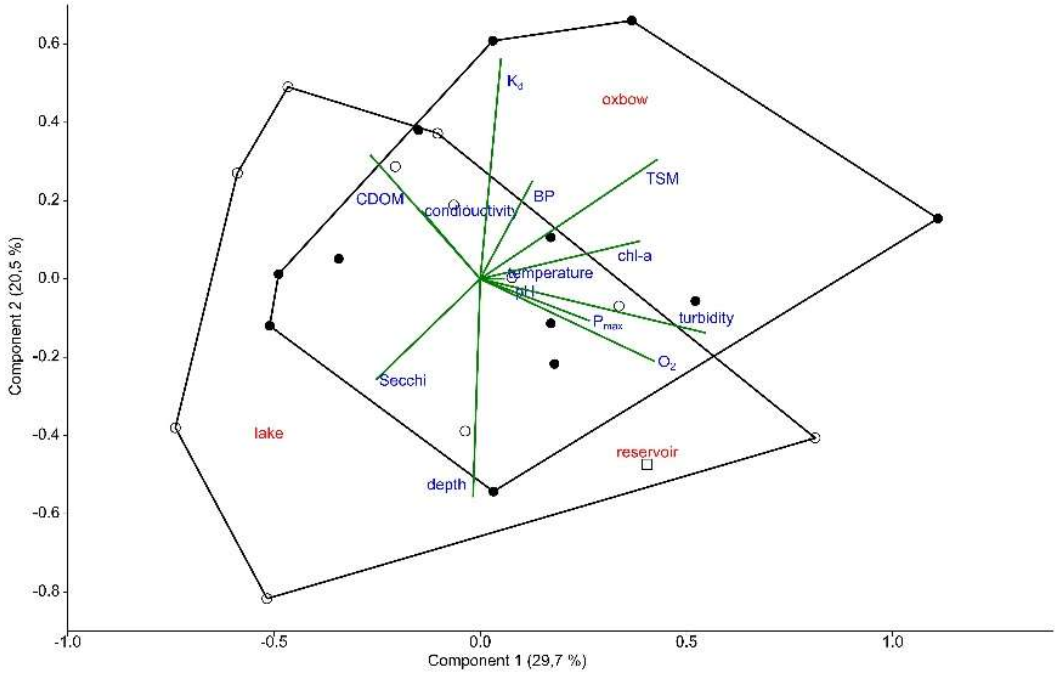

Fig. 9. Principal components analysis of the limnological, algological and bacteriological data of the studied water bodies

Macrophyte biomass did not correlate with bacterial production in the oxbows, while had a very strong (Pearson product moment correlation: R=0.914, P=0.03) relationship in lakes, suggesting significant differences between these two water bodies. This could be connected to the larger shoreline development indices of the oxbows (8.7±1.4 vs 3.8±0.2), i.e. larger exposure to terrestrial factors.

Similarly, no significant structuring effect of CDOM on the bacterial community composition and diversity was observed and the role of environmental factors (pH, conductivity and dissolved oxygen content) was the strongest ($P<10^{-4}$) on the composition and diversity of bacterioplankton, while effect of phytoplankton and available light (K_d) was also remarkable (**Fig. 10**).

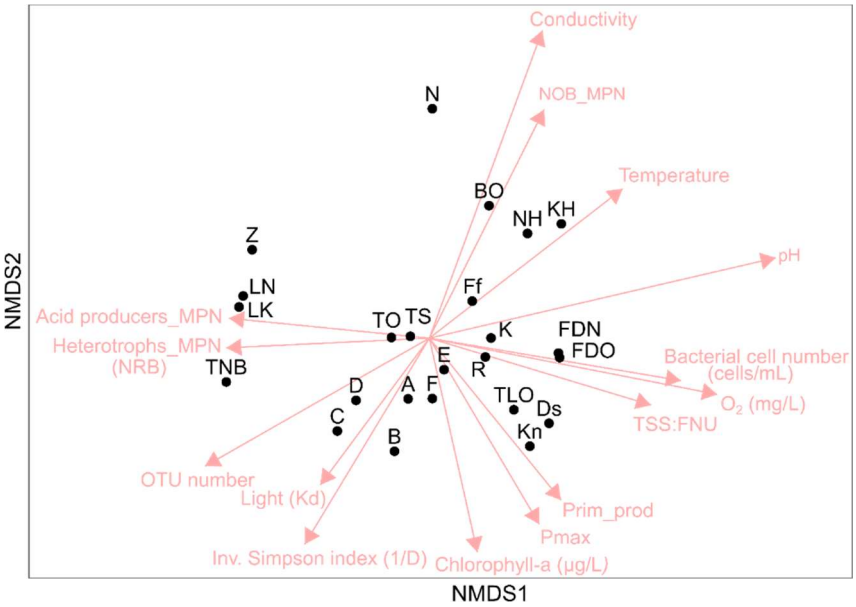

Fig. 10. NMDS ordination of samples based on the Bray-Curtis distance of the bacterial OTUs with fitted environmental variables ($P<0.05$ in all cases, except for light and heterotrphs-MPN, where $P<0.1$)

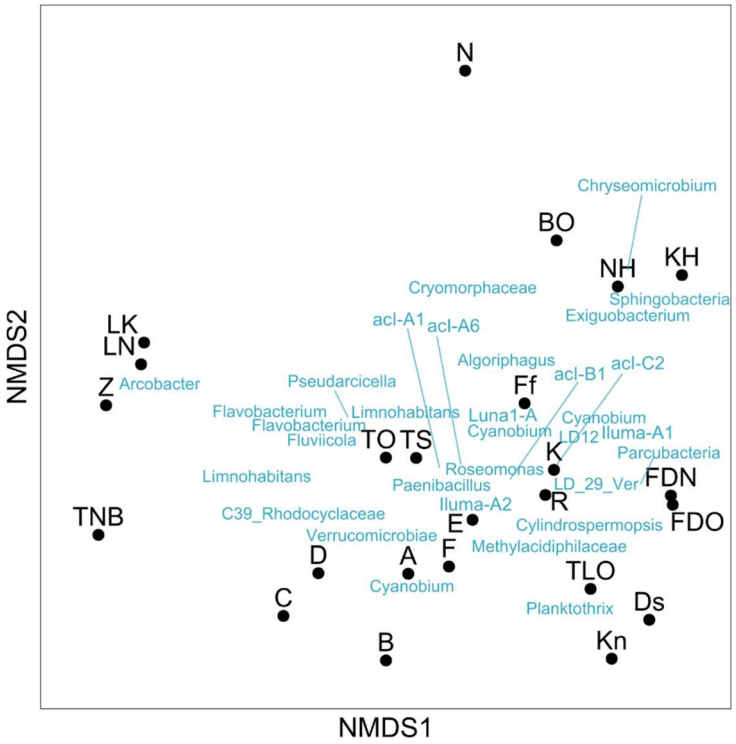

Fig. 11. NMDS ordination of samples based on the Bray-Curtis distance of the bacterial OTUs with bacterial taxa responsible for the separation of samples

In summary, no trend-like relationship between the algal and macrophyte biomass, as well as the bacterial productivity was discovered due to the high heterogeneity of the studied water bodies. The effect of the heterogeneity of the sites on bacterioplankton was reflected in the diverse taxon composition of the samples (**Fig. 11**). Yet, important knowledge was gained, as now we can better understand the background of these differences. The geomorphological specificities of oxbows that make them so specific, also can be perceived as an indicator showing the exposure of the given water body to anthropogenic stressors and also have ecological implications within the given ecosystem. Different land uses of the terrain directly adjacent to a water body could drive different changes in aquatic ecosystems and these different changes may not add up to a single general conceptual trend in the entire topographic area. Nevertheless, further study of these relations should be promoted as research on the relations between land use and ecological status of lakes is scarce.

MICROCOSM EXPERIMENTS FOR STUDYING THE AEROBIC DECOMPOSITION OF VARIOUS MACROPHYTES BY NATURAL BACTERIAL COMMUNITIES

YEAR 4

CDOM in natural waters is consisted of autochthonous and allochthonous aromatics, carbohydrates, and humic, fulvic and protein-like compounds. It affects physical, chemical and biological properties of the water (Fellman et al. 2010; Spencer et al. 2008), thus, it is crucial to gather more knowledge on the its formation, abundances, temporal and spatial dynamics in lakes for water quality management and ecological purposes. To study this, microcosm experiments were performed to test the decomposition of emergent (*Phragmites australis*) and submerged (*Utricularia vulgaris, Myriophyllum spicatum* - 50-50% *wt%*) macrophytes, sterile cotton and an algal culture (*Raphidocelis subcapitata, Chlorophyceae*) (see detailed method description in Appendix).

Despite performing the experiment for several months, between April and September of 2019, at different temperature regimes (10.4 → 25.2 ˚C), certain typical trends were observed. First of all, the addition of macrophytes significantly increased the CDOM amount in the jars by 4, 2200, 1120 and 10% for algae, submerged, reed and cotton samples, respectively. The change happened nearly instantly with the addition of the materials and was observed from the 1st day till the last (28th) day of the experiment. The addition of the macrophytes change not only the CDOM of the water, but also the pH (8.45±0.09, 7.01±0.33, 7.45±0.26 and 8.19±0.14 for algae, submerged, reed and cotton samples, respectively), conductance (772.5±14.2, 1051.9±109.5, 855.3±35.6 and 778.6±10.2 µS/cm for algae, submerged, reed and cotton samples, respectively) and O_2 content of the water (6.88±1.19, 0.16±0.24, 0.56±0.81 and 4.48±1.61 mg/L for algae, submerged, reed and cotton samples, respectively) as sampled on the 7th day of experiment.

Not only the quantity of the CDOM in the case of the submerged and emergent samples differed from the algae and cotton, but also their quality differed significantly (**Figs 12 and 13**). Both fluorometric and spectrophotometric categorisation distinctively separated the CDOM composition of submerged and reed samples from the algae and cotton samples.

Spectrophotometric characterisation of CDOM showed that submerged macrophytes affected the water CDOM content the most: the data suggested a high aromatic content ($SUVA_{254}$ ~ 0.75-0.85) and low molecular size (E_2/E_3 ~ 3.1-3.5) of wetland-terrestrial origin ($S_{275–295}$ ~ 0.010–0.013 nm^{-1}) (Helms et al. 2008; Spencer et al. 2012) (**Fig. 12**). The reed samples also quite specifically affected the CDOM content of the water, but they were also represented with high aromatic content ($SUVA_{254}$ ~ 0.31-0.66) with diverse molecular size (at some aspects sufficiently low - E_2/E_3 ~ 4.4-8.7; at other aspects slightly higher than in the case of the submerged samples E_4/E_6 ~ 3.9-5.3 vs 2.8-3.2 in submerged) and of wetland-terrestrial origin ($S_{275–295}$ ~ 0.010–0.017 nm^{-1}). The higher variability of absorbance data from reed was also conspicuous (**Fig. 12**).

On the other hand, the algal and cotton samples were very similar in spectrophotometric characteristics dominated by mostly high molecular size and lower aromatic content (**Fig. 12**). The temporal variability of the submerged macrophyte and reed samples was higher than that of the algal

and cotton samples. i.e. substantial shifts and reorganisation were recorded in submerged and reed samples, while the algal and cotton samples stayed nearly similar during the whole experiment.

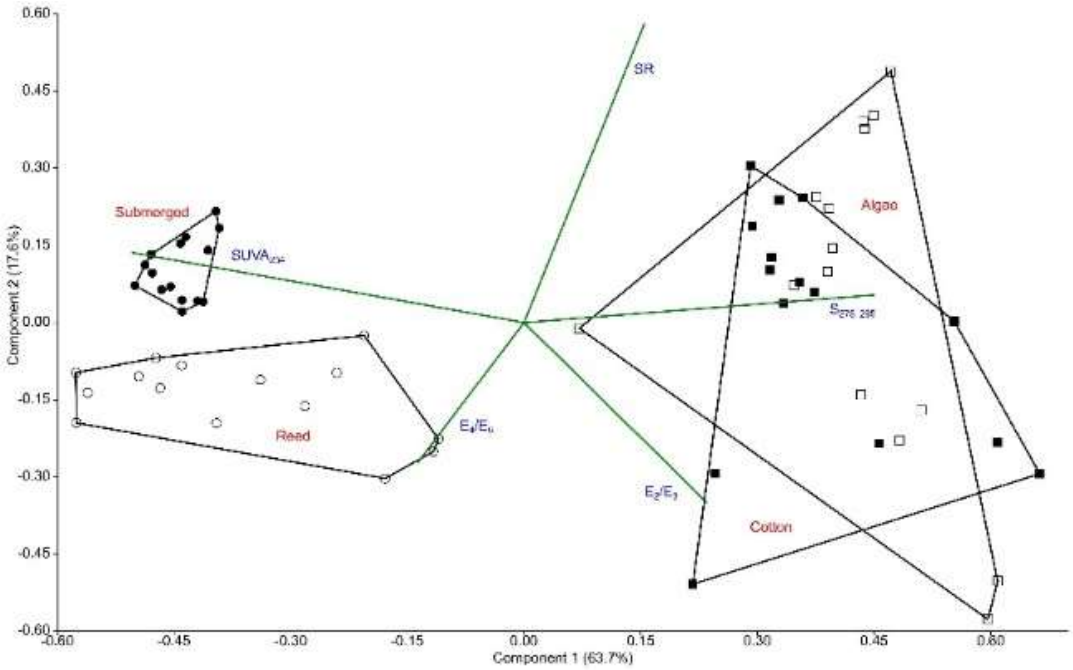

Fig. 12. Principal component analysis of the spectrophotometric data of algal, submerged macrophytes, reed and cotton samples on the 7th day of all experiments

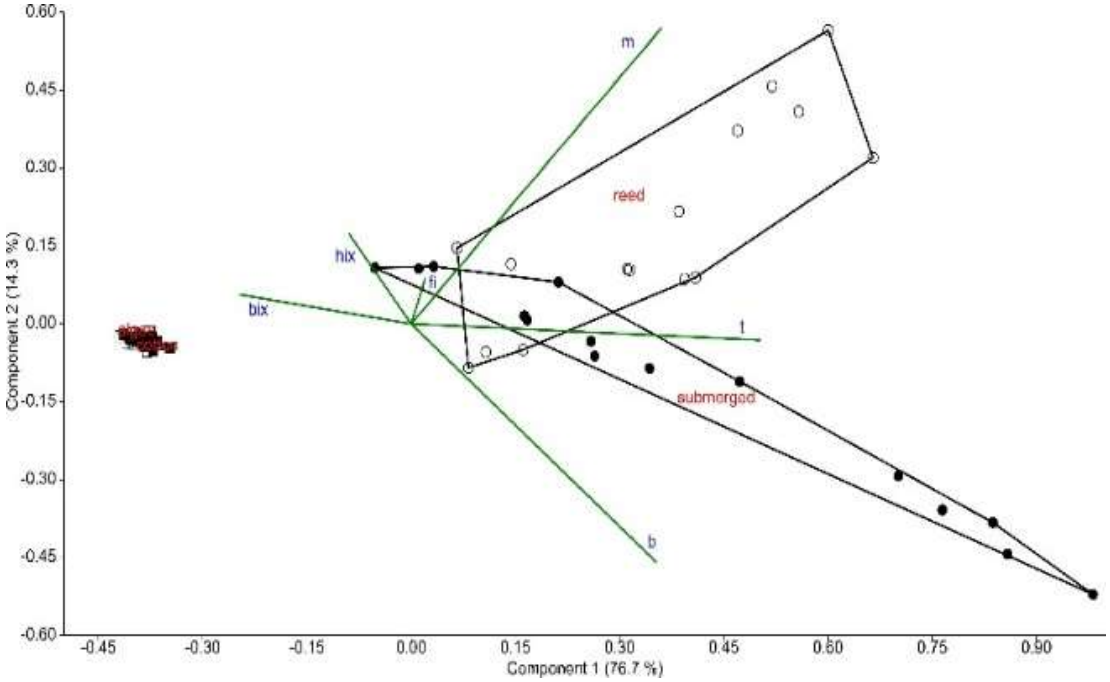

Fig. 13. Principal component analysis of fluorescence indices of algal, submerged macrophytes, reed and cotton samples on the 7th day of all experiments

The analysis of the fluorescence data (**Fig. 13**) echoed the spectrophotometric results. All in all, the fluorescence data well differentiated between the samples grouping submerged macrophyte samples

with reed samples, while algal and cotton samples were effectively indistinguishable (**Fig. 13**). While all samples were identified by the fluorescence data as organic matter of low to intermediate autochthonous component of aquatic bacterial origin, the submerged and reed samples had indication of increasing degree of humification (higher aromaticity, presence of complex molecules – higher fluorescence index *fi*). Nevertheless, the main difference between these (submerged, reed and the algal-cotton) groups was caused by the high protein-like (tyrosine-like, and tryptophan-like) content of submerged and reed samples (*b*>200, *t*>200) as compared to the algal and cotton samples (*b*<100, *t*<100) (**Fig. 13**).

Not only the differences of the fluorescence data were similar to the spectrophotometric data, but also the temporal dynamic of the changes during the run of the experiment was very similar. Generally speaking, the submerged and reed samples were very reactive showing large changes along the 28 day of the experiment: a sharp, 100-200% increase or decrease was measured in the first 7-14 days (*hix, fi, b, t*), with slow fall back to the original values by the end of the experiment.

Later, these CDOM specific parameters were correlated with the bacterial production measured along the experiments (**Table 2**). While nearly all of the parameters correlated with the bacterial production (no correlation with *hix* and *fi*), there were some parameters that had high impact (supposing causality) on bacterial production like spectrophotometric parameter E_2/E_3 predicting molecular size or spectrophotometric parameter $S_{275–295}$ showing potential origin of the organic matter, or the protein-like parameters derived from fluorescence data (b, t) (**Table 2**).

Table 2. Correlation of the studied spectrophotometric and fluorescence parameters of the sample's CDOM with the bacterial production of the same sample

	rs	P		rs	P
$SUVA_{254}$	0.510	$5.54*10^{-3}$	**bix**	-0.731	$1.01*10^{-5}$
A_{254}	0.897	$1.02*10^{-10}$	**hix**	-0.320	0.0973
A_{300}	0.916	$8.53*10^{-12}$	**fi**	0.014	0.9427
E_2/E_3	-0.918	$5.64*10^{-12}$	**b**	0.846	$1.47*10^{-8}$
E_4/E_6	0.402	$3.38*10^{-2}$	**t**	0.814	$1.38*10^{-7}$
S_{275_295}	-0.890	$2.34*10^{-10}$	**a**	0.596	$8.17*10^{-4}$
S_{350_400}	-0.785	$7.39*10^{-7}$	**m**	0.614	$5.16*10^{-4}$
S_{300_700}	-0.817	$1.12*10^{-7}$	**c**	0.550	$2.42*10^{-3}$
SR	-0.442	$1.83*10^{-2}$			

In this study, the CDOM originating from different sources, varying between phytoplankton, submerged, emergent macrophyte samples and sterile cotton were degraded in an *ex-situ* experiment in the laboratory. The produced CDOM had very specific absorption and fluorescence spectra dividing the studied substrates into two groups with submerged macrophytes and reed samples in one, and algal and cotton samples in the other. The used parameters were useful for CDOM characterisation that could describe the origin and rough content of the studied organic matter. We also found that some of the studied parameters correlated with the bacterial production, thus with further research these parameters could be used either to predict, or at least easily determine bacterial productivity.

Unfortunately, due to the COVID-19 pandemic we were not able to fully process the DNA-based analysis of the samples (sequencing will be completed by Jan 2021).

SUMMARY

In this project, we studied how aquatic marcophytes could affect the function and diversity of bacterioplankton in shallow lakes. Our results suggested that CDOM (with other marcophyte-derived organic compounds) was an appropriate carbon source for heterotrophic bacteria in the macrophyte-dominated littoral regions, and contrary to this, bacterial production in the open water areas depended mainly on phytoplankton-related carbon sources. As a result, the role of macrophytes on bacterioplankton was more important in the studied littoral aquatic habitats than in the open water. This was also reflected in the composition of the bacterioplankton community, since the clear separation of the open water samples from those with high macrophyte cover (and higher CDOM content) was observed. Laboratory experiments confirmed that this could be explained with the differences in the CDOM released from macrophytes and phytoplankton during bacterial decomposition. Seasonal changes were also observable in the composition of bacterioplankton, but our results showed that the effect of marcophytes (via presence or absence, differences in nutrient content and decomposition rate, production of antimicrobial compounds) in several shallow lakes of the temperate zone could be higher on the bacterioplankton than that of the seasonally changing physicochemical parameters. Our project also revealed that bacterial production correlates with macrophyte biomass in lakes, but the large heterogeneity of the water bodies (e.g. differences in geomorphological features and the importance of terrestrial factors) may blur the effects of marcophyte vegetation on bacterioplankton.

During this project we published two review papers summarizing the results of the project in a broader context:
[1] the occurrence, role and diversity of bacterial and eukaryotic picophytoplankton in the shallow Lake Balaton,
[2] microbial communities of shallow soda lakes and pans of the Carpathian Basin.

Project publications

Felföldi T. 2020. Microbial communities of soda lakes and pans in the Carpathian Basin: a review. *Biologia Futura (formerly Acta Biologica Hungarica)* **IF: 0.585 Q3**
https://doi.org/10.1007/s42977-020-00034-4

Somogyi B, Felföldi T, G-Tóth L, Bernát G, Vörös L. 2020. Photoautotrophic picoplankton – a review on their occurrence, role and diversity in Lake Balaton. *Biologia Futura (formerly Acta Biologica Hungarica)* **IF: 0.585 Q3**
https://doi.org/10.1007/s42977-020-00030-8

A P P E N D I X

M E T H O D S

Methods used in this project could be found in the corresponding project publications (see full references above):

sampling, physicochemical measurements, determination the composition of algae: Mentes et al. 2018

determination of bacterial abundance, production, primary production, macrophyte cover: Szabó-Tugyi & Tóth, 2020

cultivation-based studies of bacteria: Szuróczki et al. 2020b

quantitative PCR: Szabó et al. 2020

next-generation DNA sequencing, bioinformatic analyses, exploratory data analysis: Szabó et al. 2020 (Roche Junior), Szuróczki et al. 2020b (Illumina Miseq)

Description of unpublished methods (Year 4)

Experiment description

Set amount (10 g) of air-dry emergent (*Phragmites australis*), submerged (*Utricularia vulgaris, Myriophyllum spicatum* - 50-50% *wt%*) macrophytes, sterile cotton and algal cultures (*Raphidocelis subcapitata, Chlorophyceae* - 0.8 mg/L chl-a content) filtered into GFC filters were added into 4 litres jars (3 jars per material – 12 in total). All the materials were shredded to 1 cm pieces with scissors before adding them to the jars. Lake Balaton water was collected in the close vicinity of the Balaton Limnological Institute (Tihany, Hungary). The experiment was started with the addition of previously (for 12h) precipitated lake water. The experiments were performed at outside temperatures, in dark, without additional aeration.

Automatic oxidation-reduction potential (ORP) probes were inserted into the jars that recorded the water ORP every 30 minutes and 10 cm from the bottom of the jar. Basic limnological parameters (pH, conductivity, temperature, O_2 content) were determined, and 3 mL water samples were collected on day 0, 1, 2, 4, 7, 14, 21 and 28 of the experiment for bacterial activity determination, spectrophotometric and fluorescence measurements. After each sampling, the water in the jars was gently stirred. On the last day, the sample materials were collected from the jars, dried and weighted. The experiment was repeated 4 more times in different temperature regimes.

CDOM categorisation

In order to obtain the full characteristics of the collected water samples, spectrophotometric measurements in the UV–VIS range were performed with the Hitachi UV–VIS (U-2900) spectrophotometer. Based on the obtained absorption spectra, the following coefficients were calculated:

- absorbance at 254 nm (A_{254}) (Dobbs et al. 1972),
- absorbance at 300 nm (A_{300}) (Molot et al. 2005),
- ratio of absorbance at 250 to 365 nm (E_2/E_3) (De Haan & De Boer 1987),
- ratio of absorbance at 465 to 665 nm (E_4/E_6) (Summers et al. 1987),
- spectral slope within log-transformed absorption spectra range ($S_{275-295}$, $S_{350-400}$, $S_{300-700}$) and the ratio of $S_{275-295}$ to $S_{350-400}$ (SR) (Helms et al. 2008),
- absorbance at 254 nm per unit of carbon (C_{DOC}=4.95 + 0.3*m*$A_{275.5}$ - 1.1*m*A_{380} + 1,4*m*A_{730} + 0.2*m*$A_{292.5}$) ($SUVA_{254}$) (Weishaar et al. 2003).

Additionally, 3D fluorescence of water samples was measured using fluorometer (F-7000; Hitachi, Japan). Fluorescence emission spectra were recorded at excitation light between λ 250 and 450 nm, while emission between λ 280 and 600 nm. On the basis of fluorescence emission spectra, the following were determined:

- the humification index (*hix* defined as the integrated intensity of emission intensity in 435–480 nm divided by that in 300–345 nm at excitation 254 nm (Zsolnay et al. 1999),
- the biological activity input index (*bix*) calculated by emission intensity at 380 nm divided by430 nm at λex= 310 nm (Huguet et al. 2009),
- the fluorescence index (*fi* - f450/f500 calculated as a ratio at λ_{em} 450 nm to that at λ_{em}500 nm, given that λ_{ex}= 370 nm). This strongly correlates with the degree of structure complexity and aromaticity (McKnight et al. 2001),
- classical peaks based on manual peak picking (b, t, a, m, c) (Coble 1996);

The evaluation of recorded fluorescence and absorbance spectra of CDOM was performed using the "staRdom" package (Pucher et al. 2019) of the R-software 3.5.0 (Team R 2019). All data were graphed and statistically analysed using SigmaPlot 14.0, Past and R (Hammer et al. 2001; Team R 2019)

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