

Differences in planktonic microbial communities associated with three types of macrophyte stands in a shallow lake

Anikó Mentés¹, Attila Szabó¹, Boglárka Somogyi², Balázs Vajna¹, Nóra Tugyi², Bianka Csitári¹, Lajos Vörös², Tamás Felföldi^{1*}

¹ Department of Microbiology, ELTE Eötvös Loránd University, Pázmány Péter stny. 1/c., H-1117 Budapest, Hungary

² Balaton Limnological Institute, MTA Centre for Ecological Research, Klebelsberg Kuno u. 3., H-8237 Tihany, Hungary

*Corresponding author. E-mail: tamas.felfoldi@gmail.com; Tel.: +36-1-372-2500/8384; Fax: +36-1-381-2178

Acknowledgments

The authors are thankful to Laura Jurecska for her useful advices regarding chemical measurements, to Tímea Szabó and Balázs Németh for their help during sampling.

Funding

This work was financially supported by the National Research, Development and Innovation Office (grant no. K116275 and PD112449). Purchase of equipment was financed by the National Development Agency (grant no. KMOP-4.2.1/B-10-2011-0002 and TÁMOP-4.2.2/B-10/1-2010-0030). The work of A. M. was supported through the New National Excellence Program of the Ministry of Human Capacities (Hungary). B. S and T. F. were supported by the Bolyai János Research Grant (Hungarian Academy of Sciences).

Conflict of interest. None declared.

KEYWORDS: shallow lake, bacterioplankton, phytoplankton, community composition, aquatic macrophytes

Abstract

Little is known about how various substances from living and decomposing aquatic macrophytes affect the horizontal patterns of planktonic bacterial communities. Study sites were located within Lake Kolon, which is a freshwater marsh and can be characterized by open water sites and small ponds with different macrovegetation (*Phragmites australis*, *Nymphaea alba* and *Utricularia vulgaris*). Our aim was to reveal the impact of these macrophytes on the composition of the planktonic microbial communities using comparative analysis of environmental parameters, microscopy and pyrosequencing data. Bacterial 16S rRNA gene sequences were dominated by members of phyla *Proteobacteria* (36-72%), *Bacteroidetes* (12-33%) and *Actinobacteria* (5-26%), but in the anoxic sample the ratio of *Chlorobi* (54%) was also remarkable. In the phytoplankton community, *Cryptomonas* sp., *Dinobryon divergens*, *Euglena acus* and chrysoflagellates had the highest proportion. Despite the similarities in most of the measured environmental parameters, the inner ponds had different bacterial and algal communities, suggesting that the presence and quality of macrophytes directly and indirectly controlled the composition of microbial plankton.

1. Introduction

Planktonic bacterial communities in aquatic ecosystems play a fundamental role in nutrient cycling and energy flow. The composition of the bacterioplankton within a lake can be different due to environmental heterogeneity (e.g. different depths of a stratified water column, littoral versus pelagic zone, inner pond in a lake covered with macrophytes) (Wetzel 2001). The most intensively studied factors which affect the composition of bacterial communities are pH, temperature, nutrients, salinity, UV radiation and trophic status (Sigeo 2005), although little is known about how various substances from living and decomposing macrophytes affect the composition of bacterial communities. Vertical changes in bacterioplankton composition was described in many lakes (e.g. Salcher *et al.* 2008, Máthé *et al.* 2014). However, only few publications (Wu *et al.* 2007, Ng *et al.* 2010, Zeng *et al.* 2012) provided detailed data about the within-lake horizontal heterogeneity of the bacterial communities, despite the fact that the effect of inhabiting aquatic macrophytes may result in a comparable horizontal pattern as detected on the vertical scale in the case of deep lakes.

Plant decomposition is modulated by internal (e.g. plant nutrient content, C/N and C/P ratio; Enriquez *et al.* 1993) and external factors (e.g. water physical and chemical characteristics, composition and abundance of microbial and other decomposer communities; Sollins *et al.* 1996). During the decay of algal and plant material, dissolved organic matter is formed, a significant part of which is refractory and gives a yellow-brown colour to natural

waters (Wetzel 2001). These macrophyte-derived coloured substances (coloured dissolved organic matter, CDOM) are N-containing organic acids (Schulten 1995).

CDOM plays a significant role in freshwater and marine ecosystems, its contribution to the DOC (dissolved organic carbon) pool could be as high as 40-60% in the majority of aquatic environments (Thurman 1985). CDOM modifies the optical properties of the water by absorbing both visible and ultraviolet radiation. Thus, aquatic macrophyte cover may cause light limitation in lakes due to the shading effect of macrophytes and to the light absorption of macrophyte-derived CDOM. As a result, light limitation may decrease oxygen production of the phytoplankton. In addition, photochemical and biological degradation of CDOM consumes oxygen. Therefore, aquatic vegetation has dual influence on planktonic bacterial communities: an indirect one, by suppressing algal growth (which results lower oxygen concentration and lower algal extracellular release), and a direct one, by supplying carbon and nutrient source for the microbes (Sigeo 2005). Furthermore, the presence of the higher plants reduces the wind-induced sediment resuspension in shallow lakes.

Over the last decade, the understanding of microbial ecology and diversity has significantly increased due to next-generation DNA sequencing (NGS) methods, like pyrosequencing, as it has been used widely to analyse the bacterial community composition of various environmental samples.

In this paper, we studied the impact of different macrophyte stands on the community composition of planktonic microorganisms. In this regard, Lake Kolon (Hungary) represented a suitable model system, since the lake is a reed-covered marsh consisting artificially created inner ponds with bladderwort and water-lily and a relative large open-water area.

2. Materials and methods

2.1. Study site and sampling

Lake Kolon is a freshwater marsh located in the Kiskunság National Park, Hungary, Central Europe (Fig. 1). The lake is mesotrophic and has a neutral or slightly alkaline pH (~7.0-8.5) and Ca^{2+} - Mg^{2+} - HCO_3^- ion dominance (Mádl-Szőnyi & Tóth 2009). Lake surface (29 km²) is almost entirely covered by reed (*Phragmites australis*), in which man-made smaller and larger inner ponds can be found (with a total area of 11 km²) with a minimum depth of 1.0 m and a maximum depth of 2.6 m. Some inner ponds are open water areas, while others are colonized

by aquatic macrophytes such as the leaf-floating water-lily (*Nymphaea alba*) or the submerged bladderwort (*Utricularia vulgaris*).

Samples were collected after the vegetation period, on 18th November 2014 from four different sites of Lake Kolon (Fig. 1, Table 1): site O (46°45'38"N, 19°20'25"E; an open-water site without macrophyte cover), site R (46°46'23"N, 19°20'20"E; a sampling site within the reed belt, close to site L), site B (46°48'06"N, 19°20'10"E; bladderwort-dominated inner pond) and site L (46°46'23"N, 19°20'24"E, water-lily-dominated inner pond). Water samples from different depths were collected with Meyer bottle, were transferred to the laboratory at 4 °C in a thermo box, and processing started within 8 hours after sampling.

2.2. Physical and chemical analyses

Temperature, pH and conductivity values were measured with a WTW pH 315i and a Hanna HI9033 portable field meter, while dissolved oxygen (DO) concentration was measured with a Hach HQ30 portable meter on site. Additional chemical analyses were performed in the laboratory: concentration of total nitrogen (TN) according to Eaton *et al.* (2005); total phosphorus (TP) and orthophosphate (SRP) according to Murphy & Riley (1962) and Mackereth *et al.* (1989), respectively; total organic carbon (TOC), DOC and CDOM according to V.-Balogh *et al.* (2009); chlorophyll *a* (Chl) according to Wellburn (1994) and bacteriochlorophyll *a* (Bchl) according to Biel (1986).

2.3. Microscopy

The abundance and composition of autotrophic picoplankton (APP) was determined according to MacIsaac & Stockner (1993) with an Olympus BX51 epifluorescence microscope at 1,000× magnification using blue–violet (U-MWBV2) and green (U-MWG2) excitation light. This allowed to distinguish between two (phycoerythrin- and phycocyanin-rich) types of picocyanobacteria (PCya) and pico-sized eukaryotic algae (PEuk). 20 fields (~400 cells) were photographed with an Olympus DP71 colour camera and cells were counted on the images to avoid fluorescence fading. Abundance of infrared-fluorescent, bacteriochlorophyll-containing cells (BC cells) was determined on the same fields photographed with an Olympus XM10 infrared camera using blue (350-550 nm) excitation light according to Jiao *et al.* (2006). Nano- and microplankton (abbreviated simply with NP) samples were fixed with Lugol's solution, their abundance and composition (based on morphospecies identification) was determined with an inverted microscope (Utermöhl

1958). Total biovolume of the nano-, micro- and picoplankton was calculated on the basis of cell volume and abundance values. Biomass (wet weight) was estimated from the total biovolume of the fractions assuming a specific gravity of 1.0 g/cm³.

Heterotrophic bacteria were enumerated following staining with DAPI according to Hobbie *et al.* (1977) with an Olympus BX51 epifluorescence microscope at 1,000× magnification using ultraviolet (UV-MNU2) excitation light. 20 fields (~400 cells) were photographed with an Olympus DP71 colour camera and bacterial cells were counted on the images.

2.4. Next-generation DNA sequencing (NGS)

From each water sample, 200 ml was filtered through a 0.22 µm pore-size filter (Millipore, Billerica, USA), and the filters were stored at -20°C until the community DNA isolation started. Total genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing were performed as described in detail by Szabó *et al.* (2017). Briefly, the 16S rRNA gene was amplified with universal bacterial primers S-D-Bact-0341-b-S-17 forward (5'-CCT ACG GGN GGC WGC AG-3') and S-D-Bact-0785-a-A-21 reverse (5'-GAC TAC HVG GGT ATC TAA TCC-3') according to Klindworth *et al.* (2013). Quality control of the amplicons was carried out using a model 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Emulsion PCR, amplicon library processing and pyrosequencing were performed on a GS Junior sequencing platform according to the Lib-L protocol of the manufacturer (Roche/454 Life Sciences). Raw sequence data have been deposited to the NCBI Sequence Read Archive and accessible through the BioProject accession number PRJNA385391.

2.5 Bioinformatic and statistical analyses

Bioinformatic analysis of the resulting sequence reads were carried out with mothur (Schloss *et al.* 2009) as described in detail by Szabó *et al.* (2017). Cluster analysis was performed based on operation taxonomic units (OTUs) using the PAST software (Hammer *et al.* 2001). OTUs were generated using 97% 16S rRNA gene sequence similarity, corresponding to the prokaryotic species-level threshold according to Tindall *et al.* (2010). Alpha diversity [estimated using the Shannon-Wiener and Inverse Simpsons's (1/D) diversity indices] and species richness values (using the Chao1 and the ACE richness metrics) of the bacterioplankton communities were calculated using mothur, while diversity indices and

species richness of algal communities were calculated based on phytoplankton biomass data using the PAST software.

Relationships between environmental variables and bacterial or algal community composition were revealed by principal component analysis (PCA) ordination combined with vector-fitting. Environmental variables were fitted as vectors with 'envfit' function (package *vegan*, Oksanen *et al.* 2016) onto the PCA ordination of bacterial OTUs or algal biomass data, and the significance of fittings was tested with random permutations in program R (R Development Core Team 2016; <http://www.r-project.org/>). Similarity percentage (SIMPER) method (Clarke 1993) was used in the PAST software to identify which bacterial or algal community members have the largest effect on sample separation.

3. Results

3.1. Physical and chemical characteristics of Lake Kolon water

Sampling sites had similar physical and chemical characteristics regarding most of the measured parameters. However, open water samples (OS and OM) were characterized by higher DO content (Fig. 2), and the sample BB had lower pH, DO concentration and higher concentration of bacteriochlorophyll *a* and inorganic nutrients, namely TP and SRP (Table 2). The temperature of the water body varied between 8.9 °C and 9.9 °C (therefore it was omitted from the subsequent statistical analysis), and the pH was around neutral. The water colour value (Pt units) ranged between 124 and 273 mg l⁻¹. Algal biomass was moderately low with chlorophyll *a* concentration between 5 and 10 µg l⁻¹ excepting the reed sampling site (RS), where higher value was found (Table 2).

3.2. Phytoplankton community composition of Lake Kolon

Phytoplankton biomass (wet weight) varied between 200 and 1500 µg l⁻¹ (Table 3, Fig. 3B). The highest values were detected at the reed- and at one of the bladderwort-dominated sampling sites (sample RS, BM), while the lowest biomass values were found at the bottom of the bladderwort and water-lily dominated sampling sites (samples BB and LB). Vertical decrease of the phytoplankton biomass corresponded well to the DO concentration pattern, particularly at the bladderwort-dominated sampling site. Biomass of APP was negligible in all samples (Table 2). At the majority of the sampling sites, phytoplankton was dominated by small cryptophytes (*Cryptomonas* sp.), however significant contribution of heterokont flagellates (*Dinobryon divergens*) was observed at the open-water and the water-lily

dominated sampling sites (Table 3, Fig. 3). Euglenophytes (*Euglena acus*, *Phacus pyrum* and *Strombomonas* sp.) had also a significant contribution at the surface and middle layers of the bladderwort-dominated sampling sites as well as that of the water-lily dominated sampling site. Oxygen-depleted, bottom layer of the bladderwort dominated sampling site (sample BB) differed significantly from the other sites showing almost exclusive dominance of small-sized chrysoflagellates (*Heterokontophyta*).

3.3. Bacterioplankton community composition of Lake Kolon

A total of 51,757 high-quality bacterial 16S rRNA gene sequences were obtained from the samples by NGS. Good's coverage values and rarefaction curves (Table 4, Suppl. Fig. 1) showed that sequencing depth was sufficient to recover all major taxa.

At phylum level, dissimilar bacterial communities were found at the sampling sites (Fig. 4A). All samples were dominated by members of phyla *Proteobacteria* (36-72%), *Bacteroidetes* (12-33%) and *Actinobacteria* (5-26%), but in sample BB the ratio of *Chlorobi* (54%) was also considerable. Within *Proteobacteria*, higher percentages of taxa affiliated with classes *Beta*- and *Gammaproteobacteria* were present in the open-water samples and in those that were collected from the bladderwort-dominated inner lake, while in addition to these classes, *Deltaproteobacteria* were also found in high abundance at the water-lily-dominated site, whereas classes *Alpha*- and *Betaproteobacteria* were the most numerous in the samples collected from the reed-belt.

Table 5 shows the 10 most abundant cultivated genera in the nine samples analysed by NGS, while in Fig. 4A all major detected genera are depicted. *Rhodoluna*, *Polynucleobacter*, *Limnohabitans*, *Flavobacterium* and 'unclassified *Actinobacteria* (hgcl clade)', were abundant in all samples. Genera *Bacteriovorax*, *Methylobacter* and *Limnohabitans* were detected as characteristic taxa of the water-lily-dominated inner lake. Genera *Crenothrix* and *Methylocaldum* were only identified in the open-water samples. 'Unclassified *Methylococcales* (CABC2E06)' were abundant in the upper layers (samples BS and BM) of the bladderwort-dominated inner lake and the water-lily-dominated inner lake. Amount of 'unclassified *Rhizobiales*' was significant in the sample taken from the surface layer of the reed belt (sample RS). Different community was found at the deep region of the bladderwort-dominated sampling sites (sample BB) with representatives of genera *Chlorobium*, 'unclassified *Chlorobiaceae*', 'unclassified *Desulfomonadales*', 'unclassified *Parcubacteria* (OD1)' and 'unclassified *Bacteriodetes*'.

3.4. Microbial diversity in Lake Kolon

Number of observed species and species richness estimators (Chao1 and ACE) showed the highest bacterial and algal α -diversity in sample LM (water-lily, medium depth) and the lowest in sample BM (bladderwort, medium depth). Bacterial diversity indices had the highest values in samples collected from the open water site (OS and OM), while the highest algal diversity was observed in sample LM. Algal and bacterial communities had the lowest diversity values in sample BB (taken from the anoxic deeper region of the bladderwort-dominated inner lake) (Table 4).

3.5. Exploratory data analysis

Similarity among the bacterioplankton and the phytoplankton communities at different sampling sites was revealed by cluster analysis (CA) (Fig. 5). CA clearly showed horizontal shifts in the bacterial community composition within the lake, since samples characterized by the same type of macrophyte stand clustered into four major group (45% similarity). Lower dissimilarities were observed by different water depth (Fig. 5A). Furthermore, the composition of algal communities was also determined by the macrophytes (Fig. 5B). The anoxic bladderwort-dominated sample (sample BB) was remarkably distinct from others in the case of both the bacterio- and phytoplankton.

Principal component analysis (PCA; Fig. 6) confirmed the results of CA. Significantly fitted environmental variables were plotted onto the PCA ordinations of the bacterial and algal communities. Fig. 6A shows that the most unique community was found in sample BB, which was separated from the other samples along axis PC1. This axis correlated positively with the changes in DAPI, CDOM and SRP. The other bacterioplankton communities were separated along axis PC2 according to their sampling sites. Fig. 6B shows that biomass of the three dominant phytoplankton species correlated with the distribution of limnological variables. Algal communities in waterlily-dominated and open-water sites separated from the other samples.

Discussion

To the best of our knowledge, this is the first work comparing total bacterial and algal communities inhabiting the inner ponds dominated by three different macrophyte stands of the same freshwater lake using high-throughput methods. Based on the observed environmental parameters and obtained microscopy and pyrosequencing data, our results

clearly supported that the absence, presence and the type of the macrovegetation determined the planktonic microbial communities.

Differences of the bacterial communities within a lake might be explained by several environmental factors, and one of those could be the compounds originating from the macrovegetation (Enriquez *et al.* 1993). Our sampling sites were characterized by three different macrophyte species, which have different nutrient content and C/N, C/P ratios (Table 6). The high nutrient content of plant detritus could decay rapidly because of the associated heterotrophic microbial populations (Enriquez *et al.* 1993). This could contribute significantly to the divergent microbial communities observed at different macrophyte-dominated sites of Lake Kolon. In addition, some of the compounds deriving from vascular plants could change the environmental factors, such as light (chromophoric substances), and affect others, e.g. oxygen conditions (by suppressing algae or stimulating aerobic heterotrophs) (Wetzel 2001). On the other hand, some plant species could release allelochemicals (Wetzel 2001) against microorganisms (Fossen *et al.* 1998, Cisowska *et al.* 2007).

Average amount of nutrients (TP and TN) and chlorophyll *a* content of the samples referred to a meso-eutrophic water type (Wetzel 2001). Chromophoric dissolved organic matter (CDOM) concentration (Pt units) ranged between 124 and 273 mg l⁻¹ at the sites, based on these values, Lake Kolon had highly coloured water (>100 Pt units) according to the classification of Hessen & Tranvik (1998). CDOM most probably originated from decomposing macrophytes, since samples were collected after the vegetation period (however it should be noted that standing stocks of reed are continuously present in the lake). Additionally, Lake Kolon has no inflow, like brooks, therefore DOC present in lake water has almost exclusively autochthonous origin. V-Balogh *et al.* (2006) studied a similar environment (Kis-Balaton reservoir) in the same biogeographical region, which had shallow water (1-1.5 m depth) and was covered with reed. Their experiments, using a stable carbon isotopic technique, showed that 1 g of reed leaf produced at least 20 mg DOC and 200 mg Pt-colour under aerobic conditions, while anaerobic decomposition resulted in 30 mg DOC and 200 mg Pt-colour. Since moderate algal biomass was present in the samples collected from Lake Kolon (chlorophyll *a* concentration ranged from 5 to 10 µg l⁻¹), humic substances (and other compounds from decaying plant material) could be available as the major energy and carbon source for planktonic bacteria (Moran & Hodson 1990).

Pyrosequencing results (Fig. 4) showed that samples contained several bacterial taxa which are able to utilize plant degradation products or humic substances. Representatives of

phylum *Bacteroidetes* are mainly chemoorganotrophic bacteria that consume various forms of organic matter (Krieg *et al.* 2010), while freshwater pelagic *Actinobacteria* were selectively favoured by the addition of allochthonous DOC or humic material (Rosenberg 2013).

Common planktonic freshwater genera, such as *Rhodoluna*, *Polynucleobacter*, *Limnohabitans* and ‘unclassified *Actinobacteria* (hgcl clade)’, were abundant in all samples. *Flavobacterium* is a typical genus which is capable to degrade macromolecules (Krieg *et al.* 2010). Members of genus *Polynucleobacter* might utilize photodegradation products of humic substances in aerobic humic-rich habitats (Jezberová *et al.* 2010), but some free-living *Polynucleobacter* similarly to *Limnohabitans* might rely on substrates derived from algal primary production. *Limnohabitans* prefer monosaccharides (like fructose, glucose and mannose) and certain amino acids (like L-alanine) contrary to some members of *Polynucleobacter* (Jezbera *et al.* 2012).

Differences in the taxonomic composition of microbial communities among sampling sites were unequivocally observed, which were more conspicuous at the genus level compared to phylum-level differences (Fig. 4). The highest dissimilarities of bacterioplankton communities were detected in the case of comparing the bladderwort-dominated site with the others. The deep region of the bladderwort-dominated sampling site (BB) was found to be different having lower pH and DO concentration, higher concentration of bacteriochlorophyll *a* and higher nutrient content, namely TP and orthophosphate (Table 3). This latter could be explained with the observation that under experimental conditions *Utricularia* release its phosphorus content completely within 30 days, and even at low temperature (8 °C) 25% of total phosphorus from the decomposing plant could be released into the water (Kovács & Istvánovics 1994). Additionally, 60-80% of its phosphorus content is orthophosphate (Kovács & Istvánovics 1994) which is easily consumed by aquatic microorganisms (Sigeo 2005). Furthermore, its relative phosphorus content is the highest among the three major plant species present in Lake Kolon (Table 6). The low oxygen content of sample BB could be explained with the low light intensity (virtually no phytoplanktonic oxygen release, Fig. 2), with the large amount of macrophyte-derived dissolved organic matter (high rate of microbial respiration, Vörös 1994) and possibly with the internal trap structures of *Utricularia* which are able to consume the O₂ rapidly and therefore may cause anoxia (Adamec 2011). The different physical and chemical parameters and the higher P content of *Utricularia* may explain that the most unique community was found at the deep region of the bladderwort-dominated sampling site (sample BB) with representatives of genera *Chlorobium*, ‘unclassified *Chlorobiaceae*’, ‘unclassified *Desulfomonadales*’,

‘unclassified *Parcubacteria* (OD1)’ and ‘unclassified *Bacteroidetes*’. Sulfate-reducing *Desulforomonadales* (Brenner *et al.* 2005), BChl-a-containing, green sulfur *Chlorobiaceae* and *Chlorobium* grow under anoxic conditions (Rosenberg 2013). *Parcubacteria* and *Bacteroidetes* are mostly anaerobic; *Parcubacteria* has small, reduced genomes and possibly a symbiotic lifestyle (Nelson & Stegen 2015), while *Bacteroidetes* (as mentioned above) may utilize proteins and other substrates (Krieg *et al.* 2010).

Methylotrophs (including methanotrophs), like *Crenothrix*, *Methylocaldum* and *Methylobacter*, represented approximately 10% of the bacterioplankton community in almost all samples (Table 5), however they were practically absent in the bladderwort-dominated inner pond. A possible explanation for this observation is that the large amount of humic compounds derived from decomposing aquatic macrophytes could be used as terminal electron acceptors in the process of anaerobic microbial respiration which may competitively suppress methane formation (Klöpffel *et al.* 2014).

The relative abundance of many genera was below 6% in each sample, implying high bacterial diversity in the samples. Diversity of microbial communities was corresponded with the presence and the type of dominant macrophytes. The availability of carbon sources could have a profound effect on the microbial community structure and biodiversity in humic shallow lakes (Sigeo 2005). Sample BB with the lowest bacterial species number had highest level of orthophosphate, and therefore the growth of some bacterial species sensitive to anoxic condition and high levels of orthophosphate might be restrained, which could reduce the bacterial diversity in the samples (López & Margalef 1958). Similarly, fluctuating environmental conditions (e.g. wind-driven turbulence) and low nutrient content might result an increased diversity in the open water site (Wu *et al.* 2007). In macrophyte-absent areas of shallow lakes, wind-driven turbulence is more intense than in macrophyte-covered sites (Jeppesen *et al.* 2012) since aquatic higher plants have a calming influence on sediment resuspension and on other wind-driven process. Sediment particles in the water column usually lead to spatial and chemical heterogeneity in the open-water area (Simon *et al.* 2002), which create further ecological niches for planktonic bacteria (Wu *et al.* 2007), and as a result increasing the observed species richness and diversity.

The relationship between bacterial communities and aquatic plants has been studied previously, although we found only few detailed research studies, which were performed with mostly low throughput analyses or were based on microcosm experiments, and focused on other macrophyte species. Huss & Wehr (2004) conducted micro- and mesocosm experiments to study the possible effects of *Vallisneria americana* (water-celery) on bacterial growth and

water chemistry in the mesotrophic Calder Lake (USA). They concluded that the submersed macrophyte exerts a strong, indirect effect on the bacterial community by changing nutrient status and/or suppressing algal communities. The other site studied in this regard was the eutrophic, shallow Lake Taihu (China), where different macrophytes cover distinct parts of the lake, and it has been observed that aquatic plants can influence the total bacterioplankton community (Wu *et al.* 2008, Zeng *et al.* 2012) and the denitrifiers within the bacterioplankton and epiphyton (Fan *et al.* 2016). Zhao *et al.* (2013) reported from the same lake that submerged macrophytes (*Ceratophyllum demersum*, *Potamogeton crispus* and *Vallisneria spiralis*) can modify also the bacterial community composition in the sediment. Hempel *et al.* (2008) compared the composition of epiphytic bacteria on two common aquatic macrophytes (the macroalga *Chara aspera* and the angiosperm *Myriophyllum spicatum*) from two habitats, the freshwater Lake Constance (Central Europe) and the brackish Schaproder Bodden (Baltic Sea). Their results showed that the plant (in this case a substrate of bacterial biofilm) and habitat had a combined impact on the bacterial community composition which might be also effected by the polyphenol content of the plant.

Nevertheless, macrovegetation could also strongly influence the composition and biomass of phytoplankton (Wetzel 2001), which presumably affect the bacterial communities (Grossart & Simon 2007). In the open-water and the water-lily-dominated samples, *Dinobryon*, a typical planktonic alga, was the most abundant. Since the majority of water-lily leaves had been decayed until the time of sampling, for algae similar conditions were present there as in the open-water site. At almost all sites, *Cryptomonas* was a dominant member of the phytoplankton community, this alga prefers habitats with high organic matter content that might derive even from the decomposing macrophytes in our case.

Our phytoplankton results could be compared with the microscopy data obtained from Lake Fertő (Lake Neusiedl, Austria/Hungary) (Somogyi *et al.* 2010). Lake Fertő is a large, shallow, alkaline, meso-eutrophic lake with a turbid open-water area, and similarly to Lake Kolon, it also has reed-belt-enclosed brown-water inner ponds. Comparing the taxonomic composition of phytoplankton inhabiting the two lakes, Heterokontophyta and Cryptophyta were characteristic members of the communities, and genus *Cryptomonas* was also frequent in Lake Fertő. In the case of Lake Fertő, based on the analysis of Somogyi *et al.* (2010), samples taken from the reed belt (artificial canal) clearly separated from the open water sites, similarly to the results obtained from Lake Kolon. Unfortunately, no further comparison is possible, since planktonic algal communities in Lake Fertő were dominated by picophytoplankton (representing up to 80% of total phytoplankton biomass) which was

negligible in Lake Kolon, and the taxonomic composition of this group could be revealed only with DNA sequencing. Furthermore, the only data available on the composition of Lake Fertő bacterioplankton was obtained by cultivation-based methods (Borsodi *et al.* 1998), which also hinders a detailed comparison. However, it was confirmed that macrophytes are the main carbon source of bacterioplankton in that ecosystem (Reitner *et al.* 1999).

Conclusion

This is the first comprehensive study, which demonstrated that the composition of algal and bacterial communities in a meso-eutrophic, shallow freshwater lake was significantly altered by the macrophyte cover. Since most of the measured environmental parameters of the samples were similar, the main difference among sampling sites was the species of the dominant macrophyte stands. We observed that freshwater microbial communities (including both bacteria and algae) are not only determined by the presence or absence of aquatic higher plants, but the type of macrophyte also controls their composition. A higher biogenic nutrient concentration present in macrophytes is associated with higher decomposition rate (Enriquez *et al.* 1993). Bladderwort has higher phosphorus content and lower C/N ratio, therefore it may be decomposed more quickly than water-lily or reed. The resulted large amount of organic matter caused anoxia in the waterbody at the bladderwort-dominated area, which impacted negatively the diversity of bacterioplankton. Additionally, some compounds derived from aquatic plant species may act as antimicrobial agents against bacteria, e.g. water-lily produces anthocyanins (Fossen *et al.* 1998), and due to their selective action (Cisowska *et al.* 2011) such compounds could also have structuring effect on the bacterial and algal community in aquatic ecosystems. In spite of the low water temperature, these differences were also clearly observable in Lake Kolon, possibly because of the fact that samples were collected after the vegetation period, i.e. huge amount of decaying plant biomass was available in the water.

References

- Adamec L. Mineral Nutrition of Carnivorous Plants: A Review. *Ekol Pol* 1992;**40**(2): 147–192.
- Adamec L. The smallest but fastest. Ecophysiological characteristics of traps of aquatic carnivorous *Utricularia*. *Plant Sign Behav* 2011;**6**(5):640–646.
- Biel AJ. Control of bacteriochlorophyll accumulation by light in *Rhodobacter capsulatus*. *J Bacteriol* 1986;**168**:655–659.
- Borsodi AK, Farkas I, Kurdi P. Numerical analysis of planktonic and reed biofilm bacterial communities of Lake Fertő (Neusiedlersee, Hungary/Austria). *Water Res* 1998;**32**(6):1831–1840.

- Brenner DJ, Krieg NR, Staley JT. The Proteobacteria. In: George M. Garrity (ed.). *Bergey's manual of systematic bacteriology* 2nd ed. New York, USA: Springer, 2005.
- Cisowska A, Wojnicz D, Hendrich AB. Anthocyanins as antimicrobial agents of natural plant origin. *Nat Prod Commun* 2011;**6**:149–156.
- Clarke KR. Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* 1993;**18**:117–143.
- Eaton AD, Clesceri LS, Rice EW *et al.* *Standard methods for the examination of water and wastewater* (21st ed). Washington, DC, USA: APHA, 2005.
- Enriquez S, Duarte CM, Sand-Jensen K. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia* 1993;**94**:457–471.
- Fan Z, Han RM, Ma J *et al.* Submerged macrophytes shape the abundance and diversity of bacterial denitrifiers in bacterioplankton and epiphyton in the shallow fresh Lake Taihu, China. *Env Sci Pollut Res* 2016; **23**(14):14102–14114.
- Fossen T, Larsen Å, Andersen ØM. Anthocyanins from flowers and leaves of *Nymphaea × marliacea* cultivars. *Phytochemistry* 1998;**48**:823–827.
- Grossart HP, Simon M. Interactions of planktonic algae and bacteria: effects on algal growth and organic matter dynamics. *Aquat Microb Ecol* 2007;**47**(2):163–176.
- Hahn MW, Lang E, Brandt U *et al.* Emended description of the genus *Polynucleobacter* and the species *P. necessarius* and proposal of two subspecies, *P. necessarius* subspecies *necessarius* subsp. nov. and *P. necessarius* subsp. *a symbioticus* subsp. nov. *Int J Syst Evol Microbiol* 2009;**59**(8):2002–2009.
- Hahn MW, Schmidt J, Taipale SJ *et al.* *Rhodoluna ladicola* gen. nov., sp. nov., a planktonic freshwater bacterium with stream-lined genome. *Int J Syst Evol Microbiol* 2014;**64**:3254–3263.
- Hammer Ř, Harper DAT, Ryan PD. Past paleontological statistics software package for education and data analysis. *Palaeontol Electron* 2001;**4**(1):9.
- Hempel M, Blume M, Blindow I *et al.* Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. *BMC Microbiol* 2008;**8**(1):58.
- Hessen DO, Tranvik LJ. *Aquatic Humic Substances: Ecology and Biogeochemistry*. Berlin: Springer, 1998.
- Hobbie JE, Daley RJ, Jasper S. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Env Microbiol* 1977;**33**:1225–1228.
- Hornibrook ERC, Longstaffe FJ, Fyfe WS & Bloom Y. Carbon-isotope ratios and carbon, nitrogen and sulfur abundances in flora and soil organic matter from a temperate-zone bog and marsh. *Geochem J* 2000;**34**:237–245.

- Huss AA, Wehr JD. Strong indirect effects of a submersed aquatic macrophyte, *Vallisneria americana*, on bacterioplankton densities in a mesotrophic lake. *Microb Ecol* 2004;**47**(4):305–315.
- Jiao N, Zhang Y, Chen Y. Time series observation based InfraRed Epifluorescence Microscopic (TIREM) approach for accurate enumeration of bacteriochlorophyll-containing microbes in marine environments. *J Microbiol Meth* 2006;**65**: 442–452.
- Jeppesen E, Sondergaard M, Sondergaard M & Christofferson K. (Eds.). Nutrient Dynamics, Sedimentation, and Resuspension. In *The structuring role of submerged macrophytes in lakes* (Vol. 131). USA, Germany: Springer Science & Business Media, 2012.
- Jezbera J, Jezberová J, Koll U *et al.* Contrasting trends in distribution of four major planktonic betaproteobacterial groups along a pH gradient of epilimnion of 72 freshwater habitats. *FEMS Microbiol Ecol* 2012;**81**:467–479.
- Jezberová J, Jezbera J, Brandt U *et al.* Ubiquity of *Polynucleobacter necessarius* subsp *asymbioticus* in lentic freshwater habitats of a heterogenous 2000 km² area. *Env Microbiol* 2010;**12**: 658–669.
- Kämpfer P, Busse HJ, Longaric I *et al.* *Pseudarcicella hirudinis* gen. nov., sp. nov., isolated from the skin of the medical leech *Hirudo medicinalis*. *Int J Syst Evol Microbiol* 2012;**62**: 2247–2251
- Klindworth A, Pruesse E, Schweer T *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;**41**:e1, DOI: 10.1093/nar/gks808.
- Klüpfel L, Piepenbrock A, Kappler A *et al.* Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. *Nat Geosci* 2014;**7**:195–200.
- Kovács A és Istvánovics V: A víz alá merült makrofitonok szerepe a Kis-Balaton II. ütemének foszfor forgalmában [The role of submerged macrophytes in the phosphorous cycle of Kis-Balaton II.]. MHT XII. Országos Vándorgyűlése I. kötet (1994, Siófok) (in Hungarian)
- Krieg NR, Staley JT, Brown DR *et al.* The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. In: *Bergey's manual of systematic bacteriology* 2nd ed, New York, USA: Springer, 2010.
- MacIsaac EA, Stockner JG. Enumeration of phototrophic picoplankton by autofluorescence. In Kemp, P.F., B. F. Sherr, E. B. Sherr & J. J. Cole (eds), *Handbook of methods in aquatic microbial ecology*. Boca Raton, Ann Arbor, London, Tokyo: Lewis Publishers, 1993, 187–197.
- Mackereth FJH, Heron J, Talling JF. Water analysis: some revised methods for limnologists. *FBA Sci Pub*, 1989, 84–90.
- Mackie EAV, Leng MJ, Lloyd JM *et al.* Bulk organic $\delta^{13}\text{C}$ and C/N ratios as palaeosalinity indicators within a Scottish isolation basin. *J Quat Sci* 2005;**20**(4):303–312.

- Mádl-Szőnyi J, Tóth J. A hydrogeological type section for the Duna-Tisza Interfluve, Hungary. *Hydrogeol J* 2009;**17**(4):961–980.
- López I, Margalef R. *Temporal succession and spatial heterogeneity in phytoplankton*. University of California press, 1958.
- Máthé I, Borsodi AK, Tóth EM *et al*. Vertical physico-chemical gradients with distinct microbial communities in the hypersaline and heliothermal Lake Ursu (Sovata, Romania). *Extremophiles* 2014;**18**:501–514.
- Moran MA, Hodson RE. Bacterial production on humic and nonhumic components of dissolved organic carbon. *Limnol Oceanogr* 1990;**35**:1744–1756.
- Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 1962;**27**: 31–36.
- Nelson WC, Stegen JC. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Front Microbiol* 2015, **6**:713.
- Newman S, McCormick PV, Miao SL *et al*. The effect of phosphorus enrichment on the nutrient status of a northern Everglades slough. *Wetl Ecol Manag* 2004;**12**: 63–79.
- Ng HT, Marques DM, Jeppesen E *et al*. Bacterioplankton in the littoral and pelagic zones of subtropical shallow lakes. *Hydrobiologia* 2010;**646**: 311–326.
- Oksanen J, Blanchet FG, Friendly M *et al*. vegan: Community Ecology Package. R package version 2.4-0. 2016, <https://CRAN.R-project.org/package=vegan>.
- Paganin P, Chiarini L, Bevivino A *et al*. Vertical distribution of bacterioplankton in Lake Averno in relation to water chemistry. *FEMS Microbiol Ecol* 2013;**84**: 176–188.
- R Core Team. R. *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2016. URL <https://www.R-project.org>
- Reitner B, Herzig A, Herndl GJ. Dynamics in bacterioplankton production in a shallow, temperate lake (Lake Neusiedl, Austria): evidence for dependence on macrophyte production rather than on phytoplankton. *Aquat Microb Ecol* 1999;**19**(3):245–254.
- Rosenberg E. *The prokaryotes* (4th ed., DeLong EF, Lory S, Stackebrandt E, Thompson F (Eds.)). New York, USA: Springer, 2013.
- Schloss PD, Westcott SL, Ryabin T *et al*. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Env Microbiol* 2009;**75**:7537–7541.
- Schulten HR. The three-dimensional structure of humic substances and soil organic matter studied by computational analytical chemistry. *Fres J Anal Chem*, 1995;**351**(1): 62–73.
- Sigeo DC. *References, in Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. Chichester, UK: John Wiley & Sons, Ltd, 2004.

- Simon M, Grossart HP, Schweitzer B & Ploug H. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* 2002;**28**:175–211.
- Sollins P, Homann P, Caldwell BA. Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* 1996;**74**:65–105.
- Somogyi B, Felföldi T, Dinka M *et al.* Periodic picophytoplankton predominance in a large, shallow alkaline lake (Lake Fertő/Neusiedlersee). *Ann Limnol - Int J Lim* 2010;**46**:9–19.
- Stoecker K, Bendinger B, Schöning B *et al.* Cohn's *Crenothrix* is a filamentous methane oxidizer with an unusual methane monooxygenase. *Proc Natl Acad Sci USA* 2006;**103**(7): 2363–2367.
- Szabó A, Korponai K, Kerepesi Cs *et al.* Soda pans of the Pannonian steppe harbor unique bacterial communities adapted to multiple extreme conditions. *Extremophiles* 2017;**21**(3): 639–649.
- Thurman EM. Aquatic humic substances. In *Organic geochemistry of natural waters*. Netherlands: Springer, 1985.
- Tindall BJ, Rossello-Mora R, Busse H-J *et al.* Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;**60**: 249–266.
- Utermöhl H. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitt Int Ver Theor Angew Limnol* 1958;**9**: 1–38.
- Van der Valk AG. Flooding and the decomposition of litter of four emergent plant species in a prairie wetland. *Wetlands* 1991;**11**(1):1–16.
- V.-Balogh K, Présing M, Vörös L, Tóth N. A study of the decomposition of reed (*Phragmites australis*) as a possible source of aquatic humic substances by measuring the natural abundance of stable carbon isotopes. *Internat Rev Hydrobiol* 2006;**91**:15–28.
- V.-Balogh K, Németh B, Vörös L. Specific attenuation coefficients of optically active substances and their contribution to the underwater ultraviolet and visible light climate in shallow lakes and ponds. *Hydrobiology* 2009;**632**: 91–105.
- Vörös L. A fitoplankton és a hínárnövényzet elsődleges termelése a Kis-Balatonban [Primary production of phytoplankton and tangles in Kis-Balaton], MHT: XII: Országos Vándorgyűlés kiadványa, 1994. (in Hungarian)
- Wellburn AR. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 1994;**144**:307–313.
- Wetzel RG. *Limnology: Lake and River Ecosystems* (3rd ed.). San Diego, CA: Academic Press. 2, 2001.
- Wu QL, Zwart G, Wu J *et al.* Submersed macrophytes play a key role in structuring bacterioplankton community composition in the large, shallow, subtropical Taihu Lake, China. *Env Microbiol* 2007;**9**:2765–2774.

Zeng J, Bian Y, Xing P *et al.* Macrophyte species drive the variation of bacterioplankton community composition in a shallow freshwater lake. *Appl Env Microbiol* 2012;**78**(1):177–184.

Zhao DY, Liu P, Fang C *et al.* Submerged macrophytes modify bacterial community composition in sediments in a large, shallow, freshwater lake. *Can J Microbiol* 2013;**59**(4):237–244.

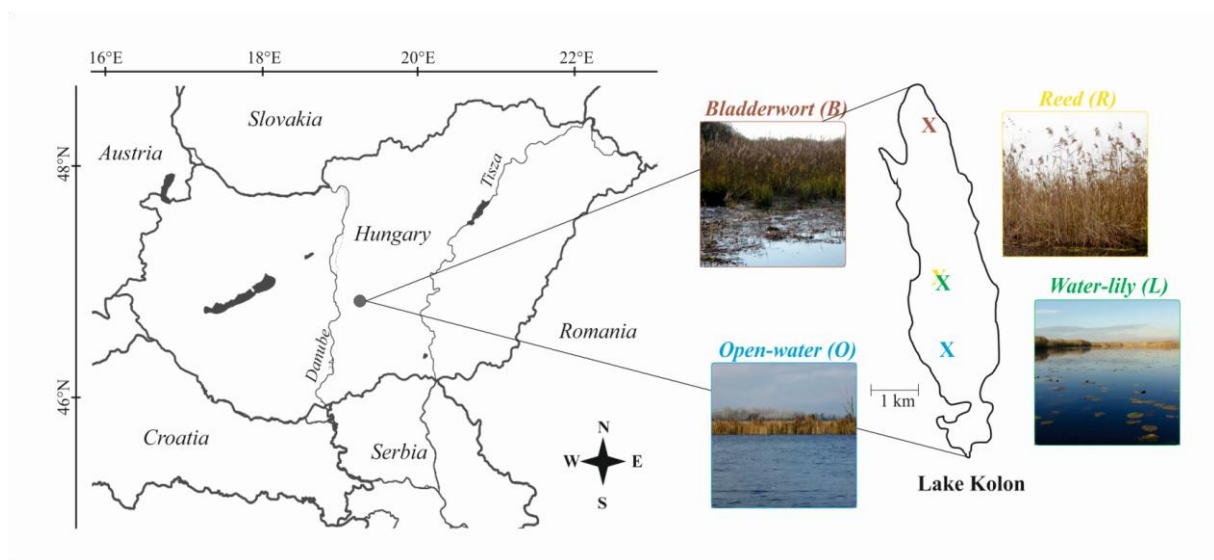


Fig. 1. Geographic location and the view of sampling sites.

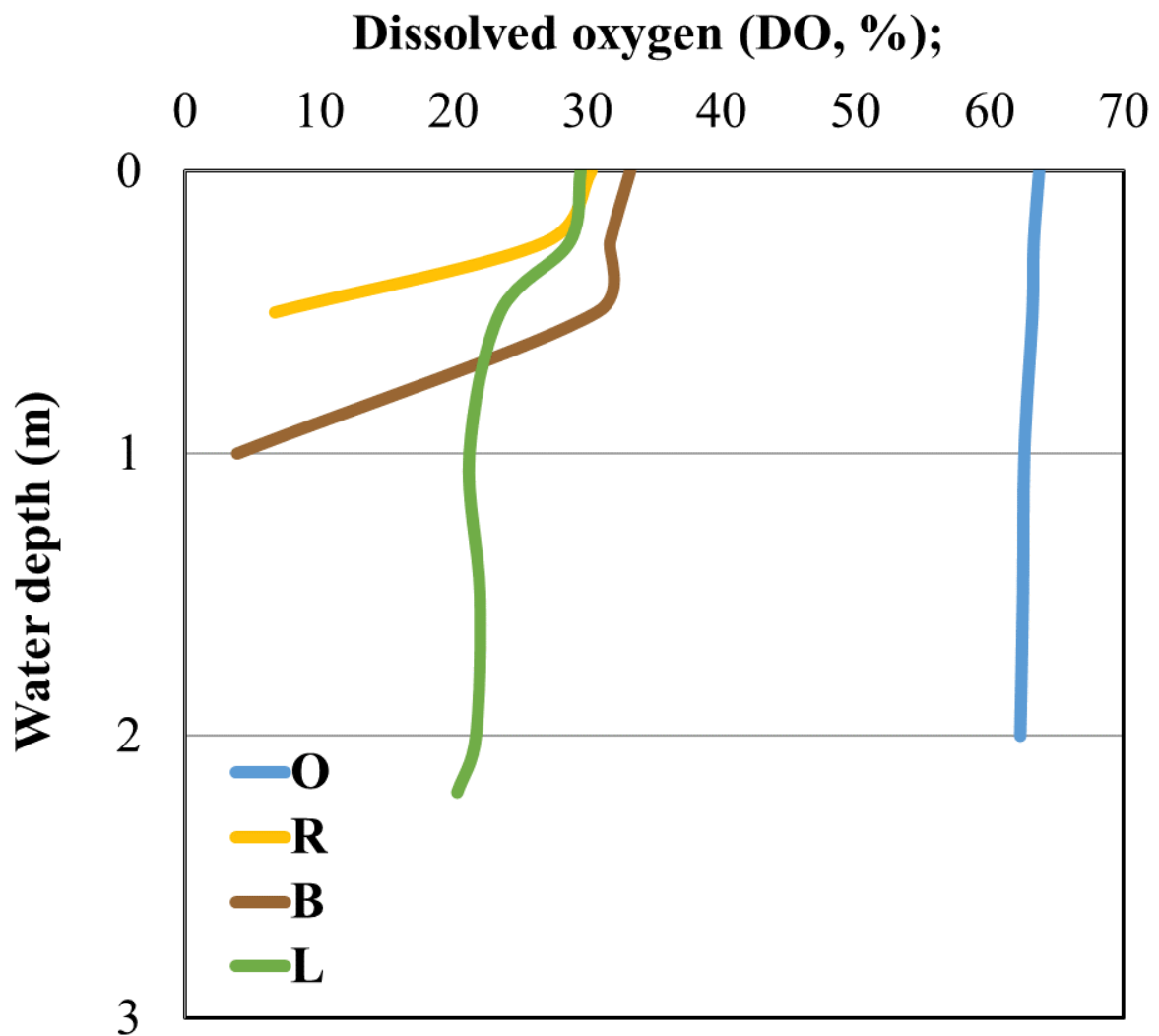


Fig. 2. Depth-specific changes of selected physicochemical parameters at the sampling sites of Lake Kolon.

For site codes, see Table 2.

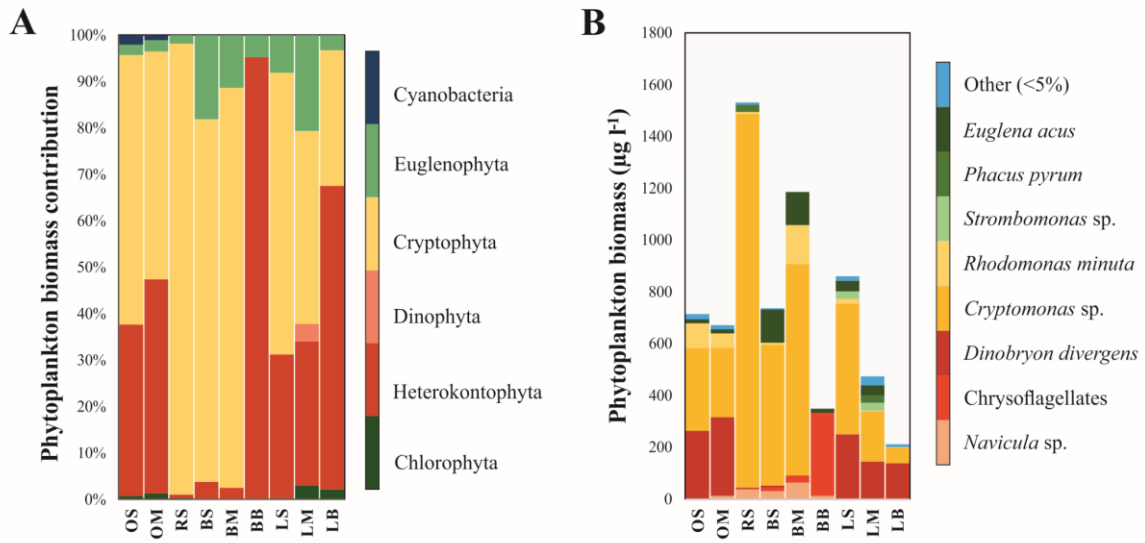


Fig. 3. Taxonomic composition of phytoplankton communities in Lake Kolon at the phylum (A) and species level (B) based on microscopy data.

For sample codes, see Table 1.

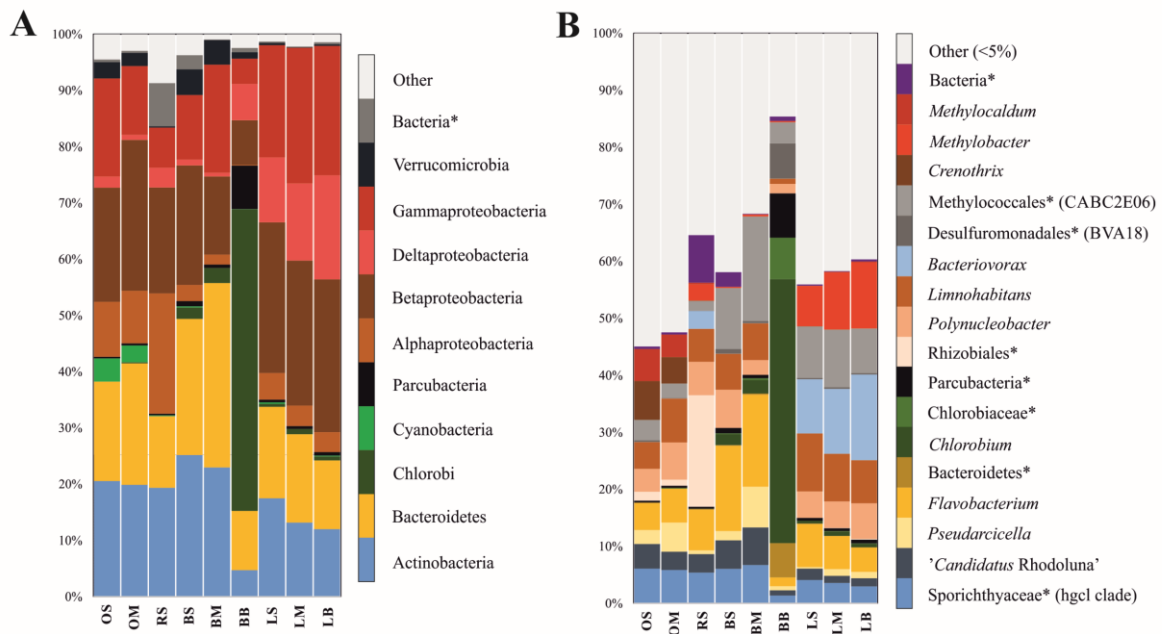


Fig. 4. Taxonomic composition of bacterioplankton communities in Lake Kolon at the phylum (A) and genus level (B) based on NGS data.

Chloroplast reads were excluded from the dataset. Note that, in the case of sample BB, DAPI cell counts were much higher than in the case of other samples (see Table 2) due to the high abundance of genus *Chlorobium*. In Fig. 4B., class level distribution is shown for phylum

Proteobacteria. Taxa not identified at the genus level are identified by an asterisk and their highest taxonomic identification. For sample codes, see Table 1.

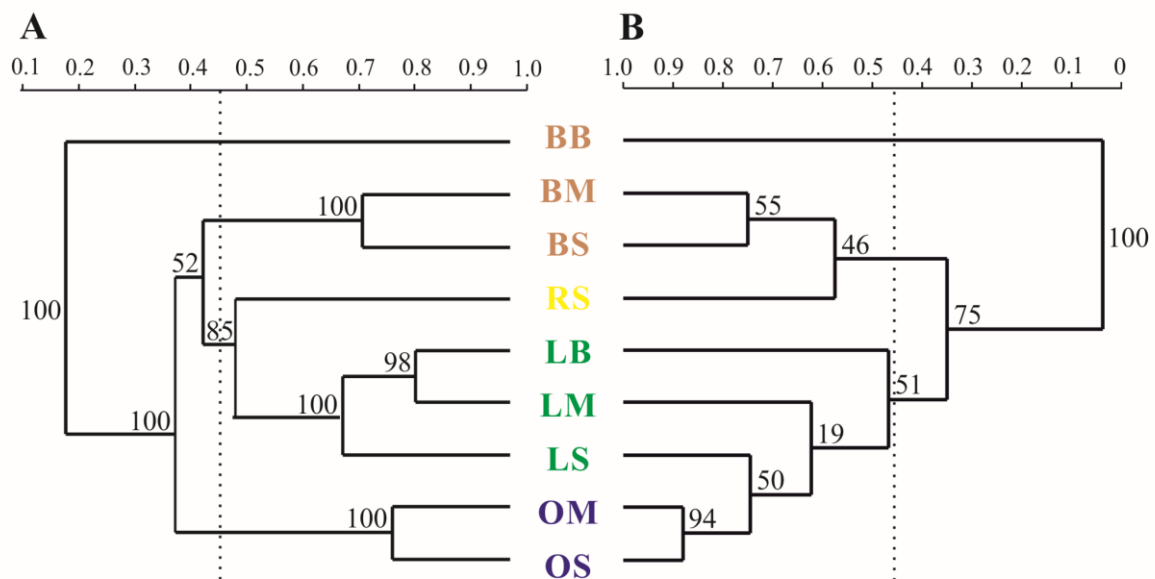


Fig. 5. Comparison of Lake Kolon bacterioplankton (A) and phytoplankton (B) communities with cluster analysis based on NGS and microscopy data.

Cluster analysis was calculated using the unweighted-pair group mean averages and the Bray–Curtis similarity index. In the case of NGS data, OTUs used for the analysis were generated with 97% 16S rRNA gene sequence similarity level. Bootstrap values are given at the nodes. For sample codes, see Table 1.

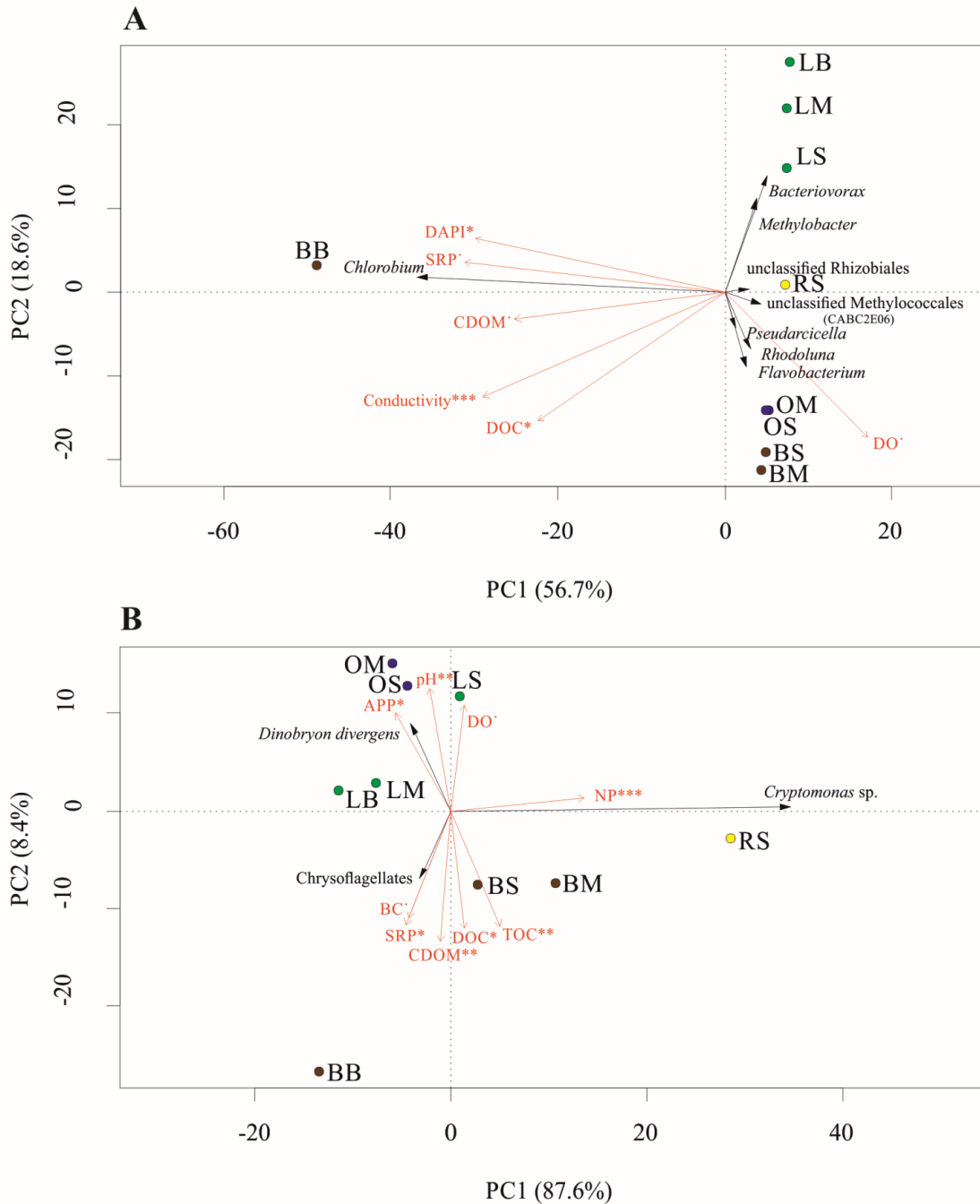


Fig. 6. PCA ordination of the (A) bacterial and (B) algal communities of Lake Kolon based on NGS and microscopy data. Community members, contributing at least 2% or 10% (in case of bacteria or algae respectively) to sample separation based on SIMPER analysis, are shown with black arrows on the biplot. Fitted environmental variables appear as red arrows, where asterisks denote the significance of fitting (‘***’ $p < 0.001$, ‘**’ $p < 0.01$, ‘*’ $p < 0.05$, ‘.’ $p < 0.1$). For sample codes, see Table 1.

Table 1. Coding of water samples collected from Lake Kolon

Depth values are given in meters.

Site	Open-water (O)		Reed (R)		Bladderwort (B)		Water-lily (L)	
	Depth	Code	Depth	Code	Depth	Code	Depth	Code
Surface (S)	0.1	OS	0.1	RS	0.1	BS	0.1	LS
Middle (M)	1.0	OM			0.5	BM	1.0	LM
Bottom (B)					1.0	BB	2.0	LB

Table 2. Limnological characteristics of the water samples collected from Lake Kolon

Abbreviations: DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus; SRP, soluble reactive phosphorus (orthophosphate); TOC, total organic carbon; DOC, dissolved organic carbon; CDOM, coloured dissolved organic matter as color; Chl, chlorophyll *a* concentration; Bchl, bacteriochlorophyll *a*; NP, nano- and microphytoplankton biomass; PCya, abundance of picocyanobacteria; PEuk, abundance of picoplanktonic eukaryotic algae; BC, abundance of bacteriochlorophyll-containing bacteria; DAPI, abundance of heterotrophic bacteria. Asterisk marks sample where the majority of the measured Chl was bacteriochlorophyll *c*. For sample codes, see Table 1.

Sampling code	OS	OM	RS	BS	BM	BB	LS	LM	LB
Temperature (°C)	9.8	9.7	9.9	9.0	8.9	9.0	9.8	9.0	8.9
pH	7.48	7.62	7.11	7.10	7.26	6.88	7.81	7.55	7.29
Conductivity ($\mu\text{S cm}^{-1}$)	482	494	388	510	523	769	423	382	363
DO (mg l^{-1})	7.16	7.05	3.39	3.79	3.53	0.44	3.31	2.43	2.49
TN (mg l^{-1})	1.17	1.19	1.78	1.33	1.24	1.78	1.75	1.11	1.32
TP ($\mu\text{g l}^{-1}$)	29.5	20.7	40.2	14.6	30.5	80.8	27.1	25.9	25.3
SRP ($\mu\text{g l}^{-1}$)	2.97	3.19	4.44	4.21	3.16	13.1	2.96	4.28	3.76
TOC (mg l^{-1})	20.8	22.5	29.4	28.3	28.0	32.6	25.4	21.3	22.4
DOC (mg l^{-1})	20.4	21.5	23.2	27.8	25.8	30.1	20.9	19.2	19.8
CDOM (mg l^{-1})	124	126	163	210	204	272	163	158	154
Chl ($\mu\text{g l}^{-1}$)	5.96	7.09	21.7	6.67	7.15	67.7*	9.93	5.96	5.10
Bchl ($\mu\text{g l}^{-1}$)	<0.01	<0.01	<0.01	<0.01	<0.01	8.4	<0.01	<0.01	<0.01
NP ($\mu\text{g l}^{-1}$)	700	647	1493	706	1122	336	859	467	208
APP ($\mu\text{g l}^{-1}$)	15	12	<0.01	<0.01	<0.01	<0.01	2	8	4
PCya (10^4 cells ml^{-1})	2.86	1.43	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PEuk (10^4 cells ml^{-1})	<0.01	0.22	0.11	<0.01	<0.01	<0.01	0.09	0.37	0.22
BC (10^4 cells ml^{-1})	53.1	58.2	85.3	46.1	41.2	278.7	<0.01	74.3	51.1
DAPI (10^6 cells ml^{-1})	1.91	2.86	2.21	1.11	2.06	6.82	2.67	2.65	2.11

Table 3. Percentage distribution of major phytoplankton species detected by microscopy in different habitats of Lake Kolon.

For site codes, see Table 1.

Phylum	Species	O	R	B	L	Characteristic habitat (Reynolds <i>et al.</i> 2002)
Euglenophyta	<i>Euglena acus</i>	2.3%	0%	10.9%	4.4%	small organic ponds (W1)
Euglenophyta	<i>Phacus pyrum</i>	0%	1.8%	0%	2.0%	small organic ponds (W1)
Euglenophyta	<i>Strombomonas</i> sp.	0%	0%	0%	3.3%	small organic ponds (W1)
Cryptophyta	<i>Rhodomonas minuta</i>	10.8%	0.5%	4.6%	1.2%	small, enriched lakes (Y)
Cryptophyta	<i>Cryptomonas</i> sp.	42.3%	94.1%	47.5%	42.6%	small, enriched lakes (Y)
Heterokontophyta	<i>Dinobryon divergens</i>	41.1%	0.4%	0.4%	41.7%	small, oligotrophic, base poor lakes or heterotrophic ponds (E)
Heterokontophyta	chrysoflagellates	0%	0%	31.9%	0%	shallow, clear, mixed layers (X3)
Heterokontophyta	<i>Navicula</i> sp.	1.0%	2.5%	4.5%	0%	benthic diatom

Table 4. Bacterial species richness (ACE and Chao1) and diversity indices [Inverse Simpson's (1/D) and Shannon-Wiener] calculated from NGS data, and algal diversity indices [Simpson (1-D), Shannon (H)] calculated from algal biomass data of Lake Kolon water. For sample codes, see Table 1.

	Sample	OS	OM	RS	BS	BM	BB	LS	LM	LB
bacterial community	No. of sequences*	4476 (4476)	4476 (5128)	4476 (5301)	4476 (6001)	4476 (4852)	4476 (5831)	4476 (5253)	4476 (8097)	4476 (6818)
	Coverage (%)	99.60	99.28	98.79	99.20	98.88	99.35	98.94	99.14	98.64
	ACE**	221 (216; 233)	231 (221; 252)	230 (218; 251)	243 (223; 277)	176 (163; 200)	227 (209; 257)	225 (212; 250)	270 (245; 311)	256 (235; 290)
	Chao 1**	214 (213; 221)	218 (212; 232)	214 (208; 227)	216 (205; 239)	159 (153; 173)	204 (194; 224)	210 (202; 229)	246 (227; 283)	235 (221; 264)
	Inv. Simpson's (1/D)**	39.6 (37.8; 41.6)	39.2 (37.6; 40.9)	15.6 (14.7; 16.6)	18.9 (18.1; 19.8)	15.1 (14.4; 16.0)	4.6 (4.4; 4.9)	24.3 (23.3; 25.4)	20.2 (19.3; 21.2)	17.4 (16.5; 18.4)
	Shannon-Wiener**	4.21 (4.17; 4.25)	4.16 (4.12; 4.19)	3.57 (3.52; 3.62)	3.59 (3.54; 3.63)	3.37 (3.33; 3.41)	2.71 (2.64; 2.77)	3.77 (3.73; 3.82)	3.67 (3.62; 3.71)	3.60 (3.55; 3.65)
algal community	No. of species	6	8	8	7	5	3	8	11	5
	Simpson (1-D)	0.65	0.63	0.11	0.43	0.50	0.16	0.57	0.72	0.49
	Shannon (H)	1.20	1.21	0.31	0.88	1.01	0.34	1.11	1.59	0.87

*numbers in parentheses stand for the total number of high-quality sequences obtained with NGS; for calculating richness estimators and diversity indices, read numbers were subsampled to the read number of the sample having the lowest sequence count

**numbers in parentheses stand for the lower and upper limits of 95% confidence intervals

Table 5. Percentage distribution of major bacterial genera having cultured representatives which were detected by NGS in different habitats of Lake Kolon.

Metabolic type: CO, chemoorganotrophic; R, respiratory type of metabolism; PR, strong proteolytic activity; M, degradation of macromolecules; LA, lithoautotrophic; ME, methanotrophic/methylotrophic; Relation to oxygen: (s)AN, (strictly) anaerobic; (s)AE, (strictly) aerobic; fAN, facultatively anaerobic. For site codes, see Table 1.

Higher rank	Genus	Metabolic type	Relation to oxygen	O	R	B	L	References
Actinobacteria								
Microbacteriaceae	<i>Rhodoluna</i>	CO	AE	3.8%	3.2%	4.2%	1.6%	Hahn <i>et al.</i> 2014
Bacteroidetes								
Cytophagaceae	<i>Pseudarcicella</i>	CO	AE	3.8%	0.7%	3.2%	0.8%	Kämpfer <i>et al.</i> 2012
Flavobacteriaceae	<i>Flavobacterium</i>	CO, R, PR, M	sAE	5.3%	7.1%	10.9%	5.9%	Krieg <i>et al.</i> 2010
Chlorobi								
Chlorobiaceae	<i>Chlorobium</i>	LA	sAN	0%	0%	16.8%	0.6%	Rosenberg 2013
β-Proteobacteria								
Burkholderiaceae	<i>Polynucleobacter</i>	CO	fAN	5.3%	5.9%	3.7%	5.2%	Hahn <i>et al.</i> 2009
Comamonadaceae	<i>Limnohabitans</i>	CO	fAN	6.2%	5.8%	4.5%	8.7%	Jezbera <i>et al.</i> 2012.
δ-proteobacteria								
Bacteriovoracaceae	<i>Bacteriovorax</i>	CO, R	AE	0%	3.2%	0%	12.0%	Brenner <i>et al.</i> 2005
γ-proteobacteria								
Crenotrichaceae	<i>Crenothrix</i>	ME	sAE	5.7%	0.1%	0%	0%	Stoecker <i>et al.</i> 2006
Methylococcaceae	<i>Methylobacter</i>	ME, R	sAE	0%	2.8%	0.2%	9.6%	Brenner <i>et al.</i> 2005
Methylococcaceae	<i>Methylocaldum</i>	ME		4.8%	0.3%	0%	0.1%	Brenner <i>et al.</i> 2005

Table 6. Average elemental composition of aquatic plants characteristic in Lake Kolon (based on literature data).

All data are expressed on a dry weight basis.

	C%	N%	P%	C:N	C:P	Organ	Reference
<i>Utricularia vulgaris</i>	41.8%	2.5%	0.19%	17	220	shoot	Adamec 1992; Hornibrook <i>et al.</i> 2000
<i>Nymphaea alba</i>	44.5%	2.5%	0.09%	18	494	leaf	Mackie <i>et al.</i> 2005; Newman <i>et al.</i> 2004
<i>Phragmites australis</i>	47.5%	0.3%	0.03%	158	1583	shoot	Van der Valk 1991