



## Article

# Bacterial Community Dynamics along a River-Wetland-Lake System

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**Abstract:** Balaton is the largest shallow lake in Central Europe. It is one of the few lakes where eutrophication processes have been successfully reversed. The creation of a wetland area on the lower part of the main inflow river played a major role in the re-oligotrophication processes. After several decades of operation and multiple transformations, an attempt was made to explore the microbial community of the water reservoir and its relationship with algal groups and environmental parameters. In the main inflow river, floods had the greatest effect on the microbial community composition, while in the open water areas of the wetland system, the algae production that develops due to external nutrient load was the main driver of the bacterial community. In the high-macrophyte-covered areas, the decomposition processes of the dying algae biomass were the most decisive. Finally, in the large open water areas of Lake Balaton, the external and internal nutrient loads together determined the algal production and thus the bacterial community composition.

**Keywords:** wetland; shallow lake; bacterial community; algal community; trophic gradient



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## 1. Introduction

Lake Balaton in Hungary, with a surface area of 594 km<sup>2</sup> and a water volume of 1.9 km<sup>3</sup>, is the largest Central European shallow lake. The calcareous lake sediment is frequently suspended by the high wind-induced turbulence intensity [1]. The lake is fed by numerous water courses, 20 of which have a permanent discharge. The largest of these, the Zala River, contributes nearly half of the lake's total phosphorus and nitrogen load and drains half of the total catchment into the western part of the lake. The sole outlet is the highly regulated Sió channel on the eastern side, thus creating a west–east trophic gradient along the 77 km long lake. As a result of the large external phosphorus load, the lake eutrophicated in the 1970s and algal blooms were frequent, mainly caused by N-fixing cyanobacteria. After that, strict water protection regulations were introduced.

To retain external nutrient loads, the implementation of the Kis–Balaton Water Protection System (hereinafter referred to as KBWPS) was an essential element of the water-quality-protecting measures [2]. The construction of the KBWPS was planned in two phases. The first phase of the system, with an area of 18 km<sup>2</sup>, was inundated in 1985. However, instead of becoming a wetland, thanks to the light conditions and the decreased water

velocity, it became an open lake dominated by planktonic algae. The second phase was initially planned for a size of 51 km<sup>2</sup>; however, due to the lack of financial resources, only the northern part of Ingói-berek, with an area of 16 km<sup>2</sup>, was flooded in 1992 [3,4]. During the operation, the area did not live up to expectations. Due to dense vegetation (mainly reed) cover, the algal biomass of the hypertrophic water body from the first phase perished during the passage. As a result of high organic matter biodegradation, the area quickly became anoxic and the phosphorus balance became slightly positive. At the same time, due to the increased water level, reed started to vanish [5]. The construction of the second phase of the KBWPS was finally completed in 2015 with the inundation of Fenéki Pond. Unlike the first phase, this area is dominated by macrophyte vegetation. Overall, Lake Balaton became one of the few lakes where water quality improved after the 1990s, thanks to the measures put in place to reduce the external load of both P and N. In the last decade, the lake has returned to its meso-eutrophic character with summer chlorophyll at a maximum of 30–40 µg/L.

The reconstructions carried out in the area of the KBWPS, as well as the weather anomalies and extremes caused by global warming, have had a great impact on the lake and its watershed ecosystem. During the restoration of the KBWPS second phase, higher chlorophyll a and phytoplankton biomass values were observed in the KBWPS outflow, which decreased to the previously detected values after the return to normal operation [6]. Additionally, the average water level of Lake Balaton was raised by 20 cm. The lake's natural water balance has turned negative seven times since 2000, and this phenomenon had not been observed for 80 years. The synergistic effect of these changes caused a transition in the structure of the phytoplankton, which after a few years culminated in the record-breaking algal bloom of 2019. According to Istvánovics [6], the estimated minimum P requirement of the record bloom must have been primarily supported by the internal load, since the external load was far too low to support this magnitude of algae blooming.

The aim of this project was to explore the spatial and temporal microbial diversity of the river–wetland–lake system and an attempt was made to find a link between the level of the major nutrients and the members of the planktonic algal and bacterial communities. We presumed that microbial communities of the main parts of the cascade system would differ from each other and that there would be an observable difference across the trophic gradient. We hope these findings can provide insight into urgent issues impacting sensitive shallow lakes facing challenges like climate change and also support actions for maintaining the oligotrophic state of the lake and ensuring good water quality.

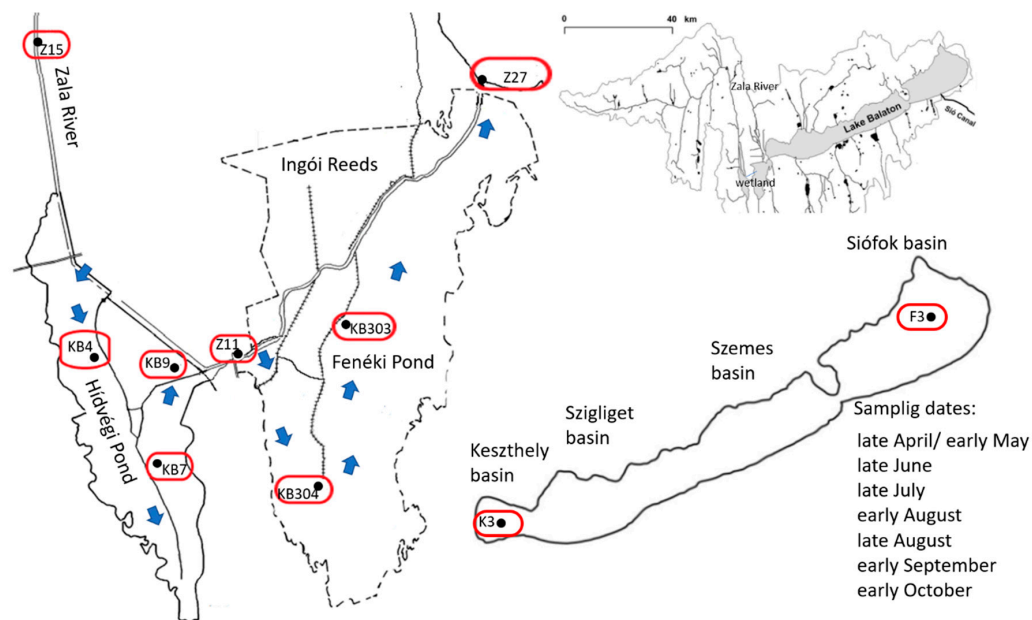
To the best of our knowledge, the present study provides the first comprehensive analysis of the planktonic bacterial communities of the KBWPS.

## 2. Materials and Methods

### 2.1. Sampling Methods and Measurement of Field Parameters

To reveal the spatial and temporal heterogeneity of planktonic bacterial communities, seven sampling periods were conducted along a river–wetland–lake system at nine sampling areas. Composite water samples were collected into 1 L sterile bottles at Z15, KB4, KB7, KB9, Z11, KB303, KB304, Z27, K3 and F3 sampling sites at seven different time points from April to October in 2019. Among the ten sampling sites, one was located at the Zala River, three at the KBWPS Phase I site, one at the interface between the first and the second phase, three at the KBWPS Phase II site, and two at Lake Balaton. The area of the survey, the sampling points and dates are displayed in Figure 1. The physical and chemical parameters at the Zala River and KBWPS sampling areas were measured by the West-Transdanubian Water Directorate, while for Lake Balaton data was partially determined by the Central Transdanubian Water Authority. The following standards were used: MSZ ISO 5813:1992 (dissolved oxygen–DO), MSZ EN 1484:1998 (total organic carbon–TOC), MSZ EN 1899–2:2000 (biological oxygen demand–BOD), MSZ ISO 7150–1:1992 (ammonium–nitrogen–NH<sub>4</sub>–N), MSZ 1484–13:2009 (nitrite– and nitrate–nitrogen NO<sub>2</sub>–N, NO<sub>3</sub>–N), MSZ EN 12260:2004 (total nitrogen–TN), MSZ EN 12260:2004 (total

phosphorus–TP, orthophosphate–phosphorus,  $\text{PO}_4\text{-P}$ ), MSZ 12750–6:1971 (total suspended solids–TSS, total dissolved organic matter–TDOM), MSZ ISO 10260:1993 (chlorophyll a). At Lake Balaton, the TN, TP, TSS, SRP, and chlorophyll a values were measured by the Balaton Limnological Research Institute as previously described [7].



**Figure 1.** Location of the sampling areas on the main inflow Zala River, wetland area of Kis–Balaton Water Protection System and Lake Balaton. The sampling points are highlighted by red circles. The blue arrows indicate the flow direction in the wetland area.

## 2.2. Investigation of Algal Community Composition

Phytoplankton samples were fixed with Lugol’s solution. Algal cells in the Lugol-solution-fixed algal samples were counted with an inverted microscope and at least 400 cells were counted [8]. The biovolume of the different taxa was calculated according to their size and shape [9]. The specific gravity of the algae was assumed to be 1.0.

## 2.3. DNA Isolation, Illumina 16S rDNA Amplicon Sequencing and Bioinformatics Analysis

Total community DNA samples were extracted from the filtrates of 500 mL water samples using the DNeasy PowerSoil Kit (QIAGEN, Venlo, The Netherlands), according to the manufacturer’s instructions. The spatial heterogeneity of nine different sites were investigated at two time points in April and at the end of August. Four of the sampling areas were investigated in more detail during the sampling period, since according to the results of Hatvani et al. [10] these points represent the cascade system inlets and outlets. The Z15 sampling point was the Zala River inflow to the first phase of the KBWPS, which characterizes the Zala River and its upstream catchment. The Z11 area was the interface between the first and the second phase, which represents the Phase I ecosystem. The Z27 sampling point was the outflow of the KBWPS, which typifies the macrophyte-dominated Phase II. Finally, the K3 at the middle of the Keszthely basin of Lake Balaton characterizes the nutrient-dense western part of the lake.

The bacterial community composition was determined by Illumina 16S rRNA gene amplicon sequencing as previously described [7]. Briefly, for paired-end 16S rDNA amplicon sequencing, the variable V3 and V4 regions of the 16S rRNA gene were amplified [11]. Amplicons were analyzed by agarose gel electrophoresis. Illumina MiSeq sequencer was used to generate paired-end fragment reads. Read numbers ranged between 43,987 and 224,698. Primary data analysis was performed with Bcl2fastq software (v2.17.1.14, Illumina, San Diego, United States). Quality- and length-trimming of the sequences were carried out in CLC Genomics Workbench Tool 9.5.1 using an error probability of 0.05 (Q13) and a

minimum length of 50 nucleotides as the threshold. Trimmed sequences were processed using mothur v 1.41.1 [12] as recommended by the MiSeq SOP page [13]. The sequence assortment based on the alignment was performed with the SILVA 132 SSURef NR99 database [14]. Chimera detection was performed with the mothur's uchime command [15]. The 'split.abund' command was used to remove singleton reads [16]. The standard 97% similarity threshold was used to determine operational taxonomic units (OTUs) as was suggested for prokaryotic species delineation [17]. According to the microscopic algal identification results, we observed that the standard 97% similarity threshold is not enough to determine the main cyanobacterial OTUs; therefore, a 99% threshold was used for cyanobacterial sequences. Raw sequence reads were deposited into the NCBI SRA under BioProject ID PRJNA877813. Rarefaction curves showed high sequencing coverage in all samples (Figure S1). The most abundant OTUs were identified using the EzBioCloud 16S rDNA database [18]. Correlation between the dominant OTUs, algal groups and environmental parameters was calculated with PAST 3.26 [19] software using Spearman analysis with a permutation test. PAST 3.26 software was used to compare the microbial community composition by UPGMA hierarchical clustering of mothur-generated OTUs, based on Bray–Curtis distance analysis, and for the non-metric multidimensional scaling plot of the different water samples computed on the OTU abundance.

### 3. Results

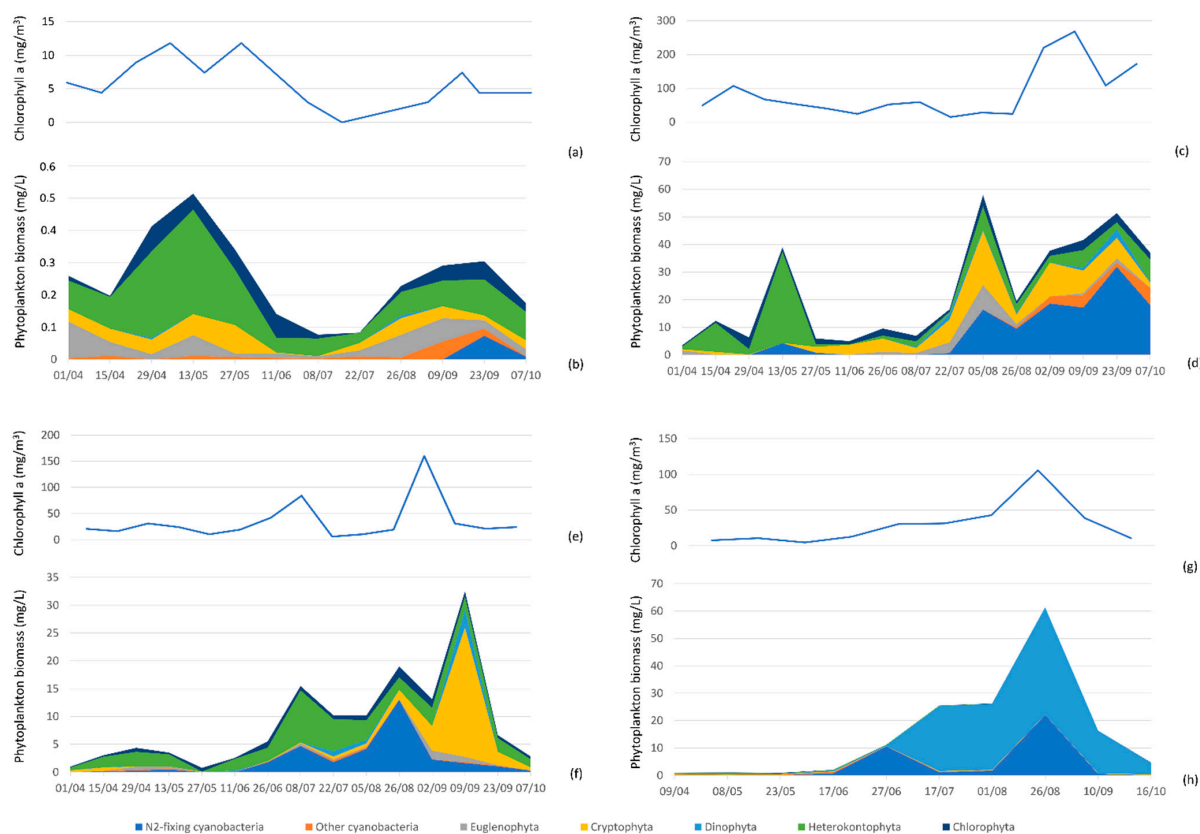
#### 3.1. Physical and Chemical Parameters of the Water Reservoir

Physical and chemical parameters of the Z15, Z11, Z27 and K3 areas are summarized in the Supplementary Materials (Tables S1–S4). The colder water delivered by the Zala River (11.6 and 21.2 °C from April to October) warms by 2–7 °C in the first stage of the water reservoir. Only minor fluctuations in temperature were observed at the sampling areas of the wetland and Lake Balaton. However, we have to note that the water temperature of the second phase of the wetland was always lower than the first. This phenomenon is due to the fact that the first phase is an open water region, while the second is an area highly covered with reed stands. On the river and open water regions of the water system, dissolved oxygen values were usually between 6–12 mg/L, which corresponds to the values expected for good ecological conditions. At the second phase of the wetland, the DO content values differed greatly from those previously experienced, with the highest value being detected in April (6.5 mg/L), while hypoxic conditions (below 2 mg/L) typically prevailed during the summer. In autumn, the concentration of oxygen rose slightly, varying between 2 and 5 mg/L. The monthly discharge of Zala River was 29–69% of the Lake Balaton total inflow. At the sampling period, higher flow rates of 3.1–4.6 m<sup>3</sup>/s were typical in spring and early summer, while from July to October the monthly averages were between 1.3 and 2.2 m<sup>3</sup>/s. At the wetland area, higher average monthly hydraulic regimes with 2.9–7.1 m<sup>3</sup>/s values were found from April to June, while during the rest of the sampling period the monthly average was between 1–3.6 m<sup>3</sup>/s. The total suspended solids content was usually between 12 and 49 mg/L at Zala River, but during floods this value increased to 124–252 mg/L. The amount of TSS continuously decreased through the wetland system, and at the Z27 outlet area only 3–20 mg/L was found. In Lake Balaton, TSS values increased (13 and 52 mg/L) due to the shallow water and large surface area exposed to the wind-induced turbulence. At the river, higher TOC values of 16–18 mg/L were also detected at higher water regimes, while lower values of 6–11 mg/L were observed at low water levels. At the wetland area, the TOC values were two to four times higher than in the riverine samples. The total phosphorous (0.10 and 0.26 mg/L) was quite similar at the Zala River and the wetland area, while at Lake Balaton much lower (0.04–0.09 mg/L) values were measured. A significant decline was observed in the PO<sub>4</sub>-P values, since they were usually many times less (0.005–0.11 mg/L) at the interface than at the inlet. At Phase II, the values were increased, but they remained still lower than at the Z15 inlet, while the lowest values were detected at Lake Balaton (0.01–0.04 mg/L). At the wetland area, the TN, NO<sub>2</sub>-N and NO<sub>3</sub>-N values were lower than in the riverine samples. The NH<sub>4</sub>-N values were slightly lower

at the interface area, but a three-times-higher average (0.14 mg/L) was found at the outflow of the wetland system. The lowest TN values (typically between 0.70–1.46 mg/L) were detected at Lake Balaton, although at the end of August a spike (3.05 g/L) was registered. The values of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  were many times lower than at the previous points of the cascade system.

### 3.2. Algal Community Composition of the Water Reservoir

The chlorophyll a values were the lowest at the Zala River, varying between 0–10.4  $\text{mg}/\text{m}^3$ , and the total algal biomass was only 0.08–0.41  $\text{mg}/\text{L}$  (Figure 2a,b). High ratios of Heterokontophyta species were observed in the riverine sample (27.4–71.6%), and they were dominant during mid-spring and in autumn. Euglenophyta and Chlorophyta species were also found to be abundant in the river. During two floods, at the end of June and the beginning of August, the algal biomass values were not determined due to the high sediment content. At the open surface area of the wetland system, the chlorophyll a content reached the 268  $\text{mg}/\text{m}^3$  maximum and the algae biomass (up to 75  $\text{mg}/\text{L}$ ) was the highest in this area of the water reservoir. The dominant algae in spring were the Heterokontophytes (84.7%), while in summer and autumn the Cryptophyta and nitrogen-fixing Cyanobacteria were the most abundant. At the macrophyte-covered second part of the wetland, the chlorophyll a values (5.9 and 42.0  $\text{mg}/\text{m}^3$ ) and the total algae biomass (2.8–32.0  $\text{mg}/\text{L}$ ) decreased (Figure 2e,f). From spring to July, the dominant algae were Heterokontophyta (49.2–83.3%), while in August the N-fixing Cyanobacteria became dominant (41.3–68.6%). In autumn, an increased proportion of Cryptophytes was observable.



**Figure 2.** (a) Chlorophyll a values and (b) phytoplankton biomass measured at Zala River (Z15); (c,d) at wetland interface (Z11); (e,f) at wetland outflow (Z27); (g,h) at Lake Balaton (K3) in 2019.

At Lake Balaton, both chlorophyll a and total algae biomass values continuously increased from the end of June until the algae peak at the end of August. Dinophyta and N-fixing Cyanobacteria dominated the growing algal biomass. The Dinophyta phylum was

observed in high abundance from July to October, while Cyanobacteria had two dominant periods, one at the end of June and another at the end of August. Based on microscopic observations, *Ceratium furcoides* accounted for most of the Dinophyta, while *Aphanizomenon flos-aquae* was the most dominant nitrogen-fixing Cyanobacteria.

### 3.3. Bacterial Community Composition of the Water Reservoir

According to the 16S rRNA gene amplicon sequencing results, the highest Shannon Wiener Diversity Index was found at the river, while the lowest values were observed at the lake (Table 1).

**Table 1.** Shannon Wiener Diversity Index of the different samples of the water system in 2019.

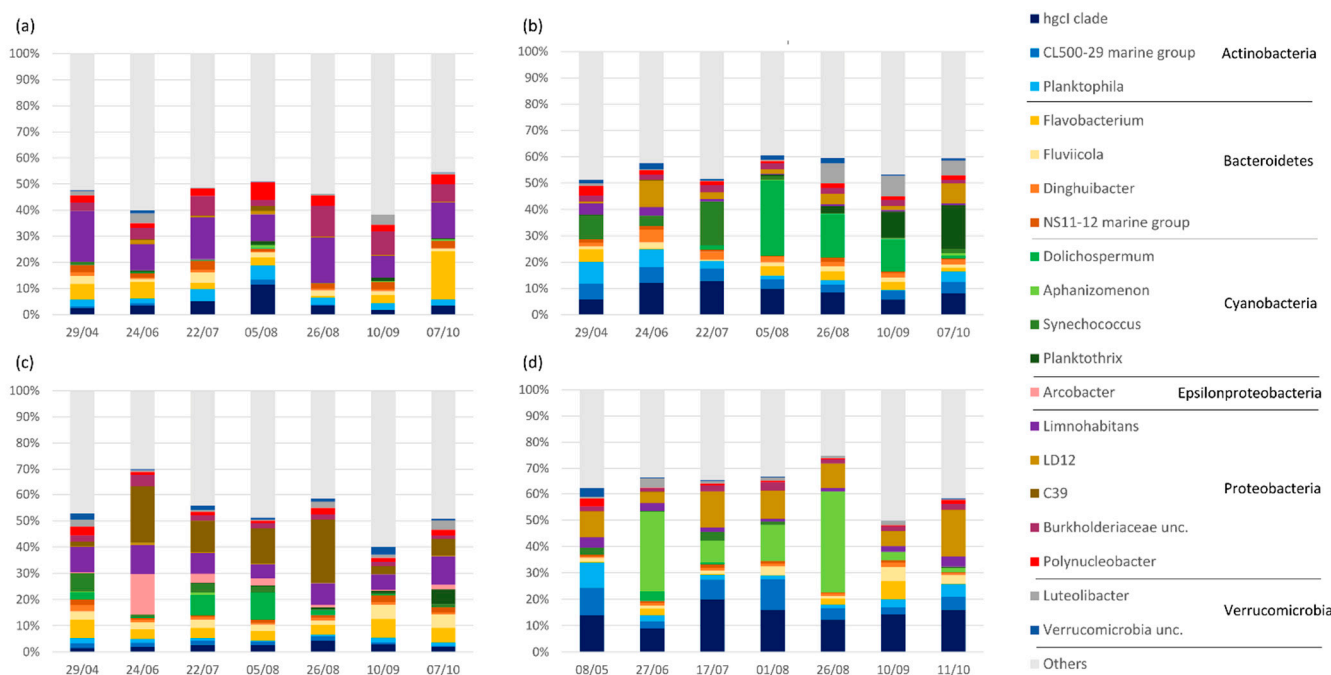
	April/May	June	July	Early August	Late August	September	October
Zala River	4.64	5.75	4.42	4.56	4.47	5.08	4.13
Wetland Phase I	4.68	4.70	4.74	4.05	4.63	4.52	4.58
Wetland Phase II	4.81	3.96	4.68	4.59	4.48	5.11	4.54
Lake Balaton	3.94	3.43	4.05	4.09	3.06	4.35	4.15

Proteobacteria (19.2–55.5%), Bacterioidetes (12.6–33.7%), Actinobacteria (6.2–37.2%), Cyanobacteria (0.7–39.4%) and Verrucomicrobia (1.4–12.6%) were dominant between April to October at all sampling sites. In addition, Patescibacteria were also detected with notable abundance in the riverine samples, while Epsilonproteobacteria was found in notable amounts at the outflow area of the wetland system.

Within the phylum Proteobacteria, the dominance of the family Burkholderiaceae was observed, in which the genus *Limnohabitans* was the most abundant at the river and the second part of the wetland system (5.3–19.3%) (Figure 3). The ratio of genus *Limnohabitans* decreased when the TSS increased in the water column. However, the correlation was not significant for each member of the genus according to Spearman analysis (Figure S2). On the other hand, a very strong inverse correlation was detected with the amount of total algae biomass and, some genus members also showed moderate positive correlation with the concentration of PO<sub>4</sub>-P and NO<sub>2</sub>-N, NO<sub>3</sub>-N, respectively. *Polynucleobacter* sequences were usually detected in a high ratio in spring at the water reservoir; however, they were observed in a notable amount (6.8%) at the beginning of August in the riverine sample. A moderate negative correlation was observed with total algae biomass and the presence of the genus.

Members of the genus *Rhodoferrax* were detected in large numbers (8%) only in the spring riverine sample. A strong inverse correlation was found between the presence of *Rhodoferrax*, the water temperature and the total biomass of the algae. Members of genera *Pseudorhodobacter* and *Rhodobacter* in the *Rhodobacteraceae* family were present at 1–4% in the riverine samples, while they were less abundant in other parts of the water reservoir. The presence of the genus *Pseudorhodobacter* showed a strong positive correlation with NO<sub>2</sub>-N and NO<sub>3</sub>-N and a strong negative correlation with the amount of total algae biomass. Additionally, we observed a moderate negative correlation with water temperature. The C39 microorganism was the most dominant at the second phase of the wetland system, occurring in high ratio (10–24%) under oxygen-deficient conditions. This bacterium showed a strong negative correlation with dissolved oxygen and TSS, and a moderate positive correlation was found with PO<sub>4</sub>-P. Several members of the methane-oxidizing *Methylococcaceae* (0.4–8.4%) and *Methylomonaceae* (0.3–8.1%) families were detected at a high proportion in this part of the system. The presence of these bacteria either strongly or moderately negatively correlated with the oxygen concentration. The *Methylophilaceae* family showed wider distribution, since it was found in a notable amount in every part of the cascade system (0.5–4.3%), with *Candidatus Methylopumilus* being the most abundant family member. The abundance of LD12 was the highest at Lake Balaton (4.3–20.3%) and

at the KB9 area of the wetland system (16–18.5%). The members of this lineage showed a moderate correlation with the amount of dissolved oxygen and TSS.



**Figure 3.** The temporal distribution of the main 20 bacterial taxa at (a) Zala River (Z15); (b) wetland interface (Z11); (c) wetland outflow (Z27); (d) Lake Balaton, Keszthely basin (K3) in 2019.

Within the *Bacteroidetes* phylum, the genera *Emticicia* and *Aquirufa* were found in significant amounts in the riverine samples. The presence of both genera showed a positive correlation with  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ . Members of *Aquirufa* showed moderate positive correlation with the  $\text{PO}_4\text{-P}$  parameter, and strong negative correlation with the amount of total algae. The NS11–12 marine group was detected in the Zala River at 1–3.5%, and slightly lower abundance was observed in the other parts of the water system (0.5–2.0%). The presence of these genera showed a positive correlation with nitrite and nitrate. Members of the genus *Dinghuibacter* were the most abundant at the wetland interface (1.5–4.8%). The genera *Flavobacterium* and *Fluviicola* were present in significant proportion in almost all sampling areas. Both genera were dominant (up to 22.3% and 5.2%) in the Zala River and the second part of the wetland area, and the latter was also found in a higher ratio (1.2–5.5%) in the Keszthely basin of Lake Balaton. The presence of genera *Sediminibacterium*, *Fluviicola* and *Flavobacterium* increased significantly (4.4–6.7%) at the western part of the lake in early September following the peak of the algal bloom. OPB56 bacteria was abundant at the interface of the wetland system, while *Algoriphagus* was detected at the Keszthely basin in significant number.

Within the *Actinobacteria*, the hgcl clade was the most dominant in all sampling areas; its abundance was highest at Lake Balaton (8.9–20.1%) and the interface area of the wetland system (5.7–13%), while the lowest values (1.5–4.3%) were observed at the reed-covered second phase. Members of this lineage exhibited a moderate negative correlation with  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and a positive correlation was found with the water temperature. *Candidatus* Planktophilia were most common in Lake Balaton and the interface area of the water system. The CL500–29 marine group was detected at a highest proportion (2.7–14.7%) in the lake and it was also abundant at the wetland interface. The CL500–29 marine group showed an inverse relationship with the amount of nitrite and nitrate, and moderate positive correlation with the total algae.

*Arcobacter* members of the phylum Epsilonproteobacteria were found to be highly abundant in the reed-covered second part of the wetland system. These bacteria showed a strong negative correlation with dissolved oxygen and a moderate negative correlation with TSS.

Cyanobacteria were found to be highly abundant at the wetland area and Lake Balaton, whereas their abundance typically remained below 6.7% in the riverine samples.

The *Synechococcus* species were found to be dominant at the wetland system and the lake generally in spring (up to 8.8%), although we were able to detect them in a high proportion in some summer samples (e.g., interface of KBWPS in July 17.3%, or Siófok basin of the lake in August 9.8%) as well. At the wetland area, the *Dolichospermum* species of the phylum Cyanobacteria was dominant (up to 28.5%) in summer and September, while the *Planktothrix* genus became abundant during autumn. *Sphaerospermopsis* and *Cylindrospermopsis* species became abundant (5.5–5.6%) only for a short period (August and September), and otherwise they were detectable at a very low rate. At Lake Balaton, the dominance of *Aphanizomenon flos-aquae* was observed in June (30.2%) and August (38.3%).

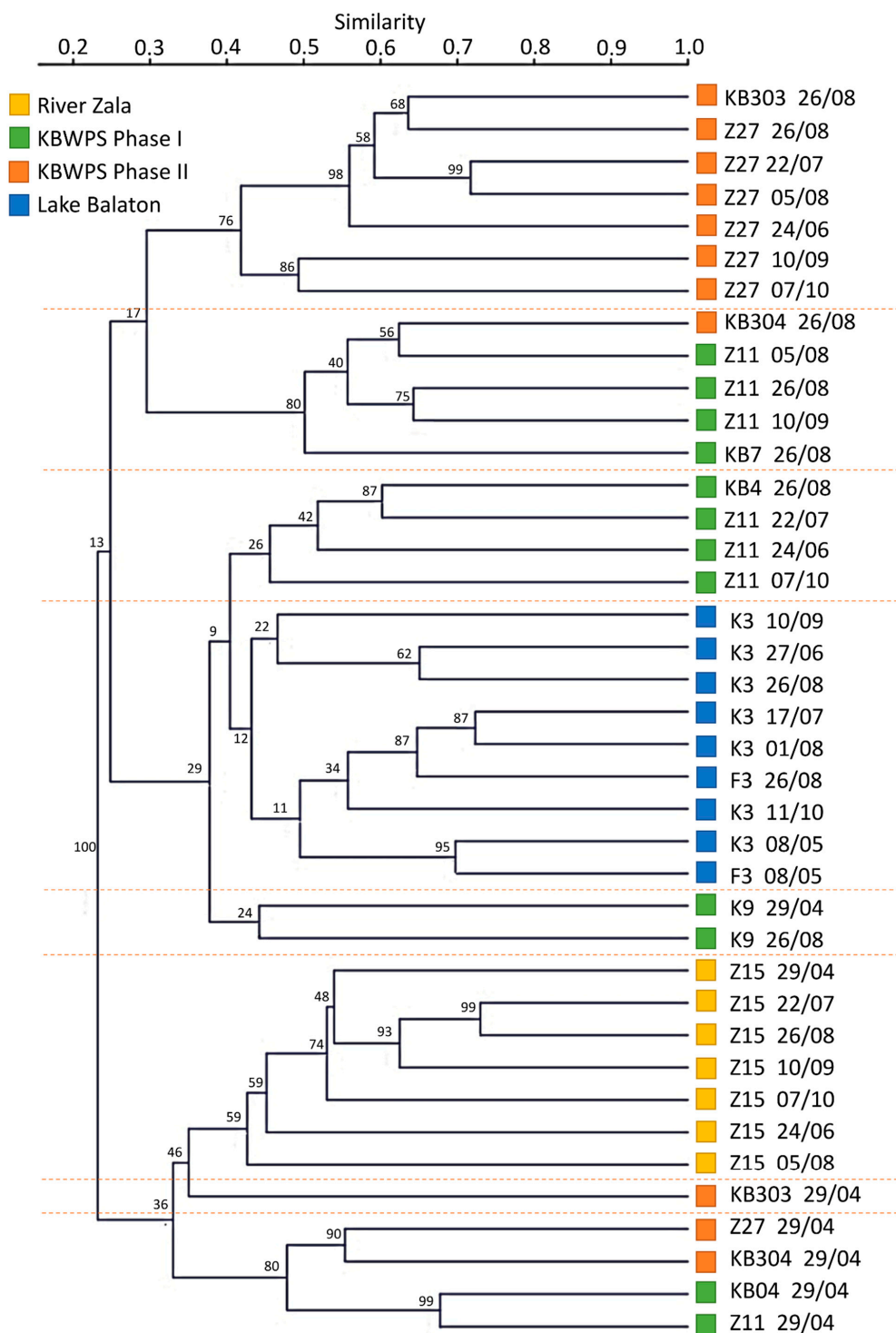
From the *Verrucomicrobia* phylum, representatives of genera *Luteolibacter* were the most abundant. The genus was the most dominant in the interface area of the wetland system in late summer and autumn (5.7–7.8%). *Terrimicrobium* (6%) was found in a high proportion in spring at the western part of the lake, while LD29 (4.2%) bacteria were abundant in the interface at this time.

### 3.4. Bacterial Community Dynamics along the River–Wetland–Lake System

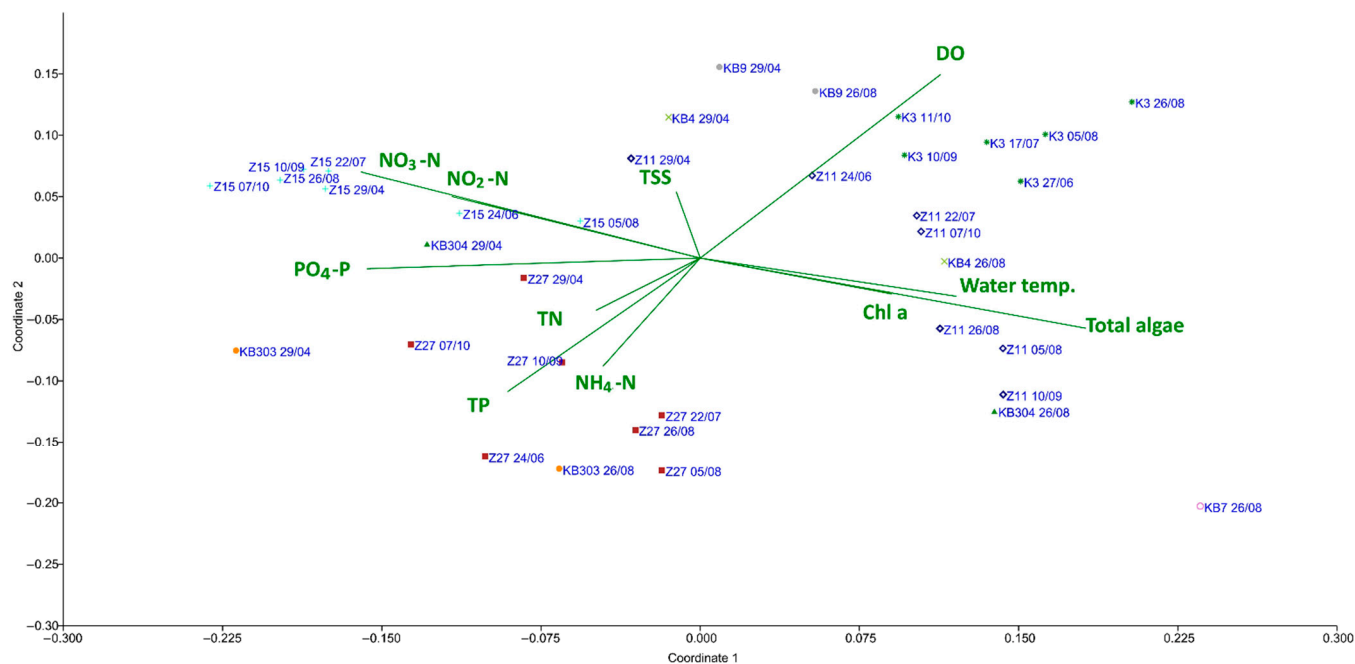
According to the Bray–Curtis similarity index of 16S rRNA gene amplicon sequencing results (Figure 4), samples from different parts of the river–wetland–lake system formed eight significantly distinct clusters. The Zala River samples had the most unique profiles and typically differed from the wetland and lake samples. According to nonmetric multi-dimensional scaling (Figure 5), the high  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and high  $\text{PO}_4\text{-P}$  concentrations were the most decisive factors in the microbial community of the river. Due to the more balanced temperature, and the variable water regime of the river, the seasonal effect was found to be marginal on the microbial community. Higher differences were observed in the bacterial composition during the floods, at the end of June and the beginning of August. The spring samples originating from the KBWPS grouped together and the cluster showed more similarity with the riverine samples than the others. In spring, lower algae production was observable, and the dissolved oxygen concentrations were more similar through the KBWPS. During spring and autumn, water is released from Kis–Balaton to Lake Balaton, while in summer the water of the Zala River is retained in the wetland system. The different operational practices in water management result in a seasonal difference in retention time, flow rate and oxygen supply. The Z11 interface samples formed two different clusters, with samples from August to September and additionally the late August samples from areas KB7 and KB304 forming one group. Early summer and late autumn samples with the summer sample from the KB4 area form another group. The main difference between the clusters was caused by the different proportion of cyanobacteria, since while these bacteria were dominant in the first cluster, they were less abundant in the second one. The Z27 samples with the late August sample of KB303 also grouped together. We detected very different oxygen concentrations in the K303 and K304 areas; in the former, the amount of oxygen drastically decreased and from late spring to October hypoxic values detected were similar to the Z27 area. The microbial communities of the Lake Balaton samples form a large common cluster. However, the summer and early autumn samples, which were dominated by cyanobacteria, showed a higher similarity to each other, while the spring samples were also slightly different from the other time points. It can be observed that the samples from the KB9 area of the KBWPS are also well separated from the other areas. The separation of the KB9 samples is not surprising, since during normal operation this area (called cassette) is not an integral part of the wetland area, and therefore the waterflow here



is minimal. However, it provides an opportunity to mitigate flooding and to store and treat any contaminated water that may arrive from the Zala River.



**Figure 4.** UPGMA dendrogram of 16S rDNA Illumina amplicon sequencing results of planktonic bacterial communities based on Bray–Curtis similarity index.



**Figure 5.** Non-metric multidimensional scaling (NMDS) plot of the different water samples computed on the OTU abundance.

#### 4. Discussion

By the time of writing, only a few studies had investigated the planktonic bacterial community of the largest Central European shallow lake and its main inflow. Moreover, none of them covered the area of the Kis–Balaton wetland area. To the best of our knowledge, the present study provides the first comprehensive analysis of spatial–temporal bacterial and algal diversity for the whole water reservoir.

The Zala River carries nutrients-rich but phytoplankton-poor water to the wetland. The microbial community of the river was dominated by *Proteobacteria* and *Bacteroidetes* species, such as the members of *Limnohabitans*, *Polynucleobacter*, *Pseudarcicella* and *Aquirufa* genera which were typically abundant in this area. The genus *Limnohabitans* plays an important role in the carbon cycle of freshwater ecosystems. *Limnohabitans* contains heterotrophic and photoheterotrophic species grouped into five lineages according to Kasalický et al. [20,21]. Within this genus, we were able to differentiate three OTUs. The 16S rRNA gene amplicon sequences showed 100% homology with strains of heterotrophic Rim8 (LimA), and with *L. parvus* II–B4 (LimC) and photoheterotrophic Rim 11 (LimB). All OTUs could be found in the Zala River (Z15); however, the heterotrophic LimA-related sequences were mainly detectable in the light-limited second part of the wetland; therefore, a strong negative correlation was found between the oxygen level and the presence of this bacterium. This partially contradicts the results of Jezberová et al. [22] that lineage members are positively correlated with the amount of oxygen and TP. In our case, all OTUs showed a strong negative correlation with algal biomass, and consequently these bacteria were not abundant at the open water area of the wetland, nor in the lake. The different *Limnohabitans*-related OTUs showed negative correlation with several algae groups (Figure S2). The strongest inverse correlation was demonstrated between the LimB, LimC OTUs and the nitrogen-fixing Cyanobacteria. The negative effect of the Cyanobacteria on these *Limnohabitans* lineages is supported by several studies [22,23]. However, in the case of the LimA OTU, this negative correlation cannot be clearly stated since it became dominant in that area of the wetland where the decaying mass of algae can provide these bacteria with nutrients. Furthermore, the *L. parvus* II–B4 (LimC) genotype was present at a high proportion in April, which is in agreement with the results observed in the Řimov reservoir

(Czech Republic), where this group of bacteria was also found to be abundant in the spring and showed a strong correlation with the SRP [22].

Along with *Limnohabitans*, *Polynucleobacter* is one of the best-studied genera. This genus was previously divided into five subclusters [24,25]. The main OTU detected in our study belongs to the best known PnecC (*P. necessarius*) subcluster. Recent studies have suggested that the *P. necessarius* cluster has an opportunistic life strategy and is specialized in the degradation of photodegradates from DOM (humic substances) [26–28]. The genus was observed at the highest ratio in the Zala River, which is in agreement with the results of Jezbera et al. [29], who found that the PnecC lineage markedly dominated the allochthonous-loaded river station of Římov reservoir. However, the distribution of the genus may be mostly influenced by temperature, since we found moderate negative correlation with the temperature and the genus was also detected in a high ratio in spring at the other parts of water reservoir.

*Aquirufa* sequences showed more than 99% homology with *Aquirufa antheringensis* described by Pitt et al. [30]. This ubiquitous bacterium was found in various inland water systems like rivers, lakes, ponds, groundwater and lake sediments. We found a moderate positive correlation of the genus with nitrite and nitrate. The previous finding is in agreement with the fact that a gene cluster necessary for the uptake and assimilation of nitrate and nitrite was detected in the majority of the *A. antheringensis* strains examined by Pitt et al. [30]. It is surprising that, despite its widespread distribution, together with the genus *Emticia*, we were only able to detect a high abundance of these microorganisms in river water samples.

The water slows down and warms up in the open water area of the wetland, which favors the photosynthetic oxygen-producing algae species. The orthophosphate, nitrite and nitrate content of the water decreased to a great extent, for which the high algae production probably played a key role. Due to frequent and long-lasting algal blooms, the average chlorophyll a content was highest in this area of the water reservoir. In late summer and autumn, the dominance of nitrogen-fixing cyanobacterial *Dolichospermum* genus was observed. The algae *D. flos-aquae* was also detected in the area of the KBWPS in the previous year by Marinović et al. [31], although in that year *A. flos-aquae* and *D. spiroides* were the dominant Cyanobacteria. Both *D. flos-aquae* and the *A. flos-aquae* presence are also common at Lake Balaton; however, clarifying the role of algae and their akinetes originating from the wetland on the Balaton algal communities requires further research.

The largest proportion of the *Verrucomicrobia* phylum was found in the interface of the wetland system; however, their abundance was also high at the other parts of the water reservoir. Although they are very common community members in both terrestrial and freshwater environments, their ecological appearance is poorly understood [32]. Various studies indicate that *Verrucomicrobia* may play an important role in the carbon cycle [33]. The verrucomicrobial *Luteolibacter algae* H18 and '*Lentimonas*' sp. CC4 strains were found to be highly specialized degraders of algae fucoidans and other complex polysaccharides [34,35]. According to Lindh et al. [36], the abundance of *Verrucomicrobia* in the Baltic Sea increased considerably just prior to and during the cyanobacterial summer bloom and decreased again about a month after. We were able to find links between several OTUs of the phylum and the algae community composition, which suggests that the group could play a key role in the utilization of algae-derived organic matter. The elevated abundance of an unknown *Luteolibacter* (5.7–7.8%) was observed at the interface area from August to October, corresponding to when the N-fixing Cyanobacteria became dominant in that area. The main OTU, an unknown *Luteolibacter*, showed strong positive correlation with the N-fixing Cyanobacteria and total algae. An unknown *Terrimicrobium*, which was the most abundant at Keszthely basin in the spring, showed a strong positive correlation with the presence of the phyla Heterokontophyta and Chlorophyta and the value of total algae. Additionally, the abundance of LD29 was positively correlated with the Heterokontophyta algae.

The actinobacterial *hgcl* was also represented in high proportion at the open surface water areas of the water reservoir; these bacteria have small cell sizes and highly streamlined genomes. Ghylin et al. [37] investigated 11 acI single-amplified genomes (SAGs) from four different lakes. The presence of actinorhodopsins was detected in most genomes, which may enable the ability to grow photoheterotrophically, thus helping survival during nutrient starvation periods. The ability to consume N-rich organic compounds including cyanophycin was also demonstrated. Therefore, a potential interaction between cyanobacteria or other cyanophycin-producing microbes can be assumed. A Pst complex was found in most of the genomes, which ensures phosphorus uptake for the bacteria in freshwater lakes with pure phosphorus limitations like Lake Balaton. The *hgcl* clade was represented by two OTUs in the water reservoir, one of which showed more than 99% 16S rRNA gene similarity with *Nanopelagicus abundans*, while the other was an as yet uncultured bacterium belonging to the genus *Nanopelagicus*. Both OTUs showed almost the same distribution as the genus itself, and a positive correlation was observed in the case of water temperature. In contrast, a negative correlation was observed with nitrite and nitrate. The unknown *Nanopelagicus* OTU showed a strong positive correlation with the dominant N-fixing cyanobacteria species and total algae biomass. Meanwhile, the *Nanopelagicus abundans*-related OTU correlated moderately with the presence of many algal groups. This result may indicate the ability of the *Nanopelagicus* strains to degrade cyanophycin.

At the interface of the KBWPS and at the lake, the second most abundant actinobacterial group was the CL500–29 marine group. Although this group has been retrieved from a marine estuarine and delta ecosystem [38], it is also often detected in freshwater systems [39,40], where it is the main component of bacterioplankton together with the *hgcl* clade [41]. Despite the wide distribution of this bacterial group, we have limited knowledge about its ecological role. Within the group, we were able to distinguish two main OTUs; one could only be detected in Lake Balaton, while the other could be found also at the interface of the wetland. Both OTUs showed a moderately negative correlation with  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ .

Interestingly, several microbial community members that prefer nutrient-poor conditions (e.g., LD12 subclade, *Synechococcus* sp.) were also found in a relatively high proportion in the eutrophic waters of Phase I; however, their abundance typically declined under the dominance of *Cyanobacteria* species.

At the second part of the wetland system, typically lower chlorophyll values were measured due to the shading effect of the macrophyte cover, which is not favorable for the photosynthetic oxygen-producing algae. The breakdown of the dead algae biomass consumes a lot of oxygen, creating hypoxic or anoxic conditions in the system. Parallel with the oxygen reduction elevated levels of TP,  $\text{PO}_4\text{-P}$  and  $\text{NH}_4\text{-N}$  were observed at Z27, and it can be assumed that these nutrients were probably released during the decomposition of large amounts of dead algal biomass. Zhu et al. [42] investigated the influence of algal bloom degradation on nutrient release at the sediment–water interface in Lake Taihu, China. According to their results, during algal decay the levels of TP, TN,  $\text{PO}_4\text{-P}$  and  $\text{NH}_4\text{-N}$  in the overlying water showed a dramatic increase.

Compared to the open water area, we experienced an increased proportion of *Proteobacteria* in the reed-covered area. Within the phylum, in addition to the previously mentioned *Limnohabitans*, the C39 bacterium was dominant. The corresponding OTU showed 98.3% 16S rRNA gene homology with *Fluviibacter phosphoraccumulans* SHINM1<sup>T</sup>, which was isolated from a Japanese river, and the cells contained intracellular polyphosphate granules [43]. In our case, the C39-related OTU showed a strong negative correlation with oxygen and TSS, and a moderate positive correlation with TP. Under aerobic conditions, polyphosphate-accumulating bacteria such as *F. phosphoraccumulans* utilize poly-B-hydroxyalkanoate stores as nutrient and energy sources while accumulating polyphosphate in their granulomas. Under anaerobic conditions, polyphosphate is used as an energy source and polyhydroxyalkanoate reserves are formed from short-carbon metabolic products (e.g., fatty acids, sugars, alcohols). During the process, orthophosphate is released

from poly-P degradation [44,45]. The advantages of this process are often used in wastewater treatment plants, with the successive application of anaerobic and aerobic phases; however, in our case, the direction of the process appears to be reversed, and thus the amount of orthophosphate entering Lake Balaton not only decreases, but also increases in the reed-covered part of the second phase.

The hypoxia experienced in the upper layers of the second part of the wetland could have caused anoxia in the hypolimnion and in the sediment. The seasonally anaerobic conditions promoted the enrichment of methane in the sediment. Accordingly, we observed a high proportion of *Methyloparacoccus* and an unknown *Methylococcaceae* genus. The methane-oxidizing *Methylococcaceae* family was already detected in our previous study [7] at a similar order of magnitude at the outlet of the wetland.

On the basis of several decades of observations, from spring to autumn the dominance of picocyanobacteria, in addition to nitrogen-fixing cyanobacteria, can be observed at Lake Balaton. It seems that in warming waters, filamentous diazotrophic cyanobacteria have an advantage over picocyanobacteria due to their ability to fix nitrogen, and therefore filamentous cyanobacteria are usually dominant in the nutrient-rich western half of the lake, while the proportion of picocyanobacteria is higher in the eastern half [46]. We found the picocyanobacteria genus, *Synechococcus*, in a high proportion in the water system from spring to summer. Different OTUs were found at different time points. The dominant OTU in spring showed a close relationship with *S. rubescens* SAG 3.81, which is a phycoerythrin-rich, subalpine cluster I member of *Synechococcus*. Members of the subalpine cluster I often show a negative correlation with water temperature [47,48]. In summer, an unknown *Synechococcus* species became dominant, and interestingly it was the most abundant in July at the Z11 interface area; however, a high ratio was also found in the eastern part of the lake at the end of August. The decrease in the proportion of *Synechococcus* in the wetland area from August is likely due to the rise of *Dolichospermum* species.

In Lake Balaton, we detected an unusually high algal peak in summer and early autumn, which can be linked to the nitrogen fixing *Aphanizomenon flosaquae* (Cyanobacteria) and *Ceratium furcoides* (Dinophyta) species. The dominance of *Ceratium* species in Lake Balaton is not new; however, *C. furcoides*, which was first recorded from the lake in 1996 by Grigorszky et al. [49], had previously not caused large-scale algal blooms. In the summers of 2013 and 2014, *Ceratium hirundinella* dominance was observable at the western part of the lake; however, typically in summertime, filamentous nitrogen-fixing blue-green algae (*Aphanizomenon flos-aquae*, *Aphanizomenon issatschenkoi* and *Cylindrospermopsis raciborskii*) are dominant in this area [50]. The ecological needs of *C. hirundinella* and *C. furcoides* are very similar, although winter, spring and early summer dominance was described only for the former, while the latter species were mainly dominant in the warmer waters of summer and early autumn [51,52]. The higher temperature preference seems to be supported by the rapid invasion of the species *C. furcoides* in tropical and subtropical areas [53]. Surprisingly, the mass presence of *Ceratium* species has not been detected at the wetland area until now, although they are often detected in large proportions in the surrounding fishponds. The possible explanation for this difference could be that the water retention time is typically shorter in the wetland system, which might not favor the spread of these species. Both *Aphanizomenon* and *Ceratium* are able to use resources unavailable for other species; N<sub>2</sub> fixation by *A. flos aquae* and mixotrophy by *Ceratium* benefits from P pulses due to their high storage capacity. In addition, both species, due to their relatively large size and toxin production, are resistant to grazing and able to form dormant *Aphanizomenon akinetes* and *Ceratium* cysts [6,54]. According to Istvánovics [6], the estimated minimum P requirement of the record algae bloom must have been primarily supported by the internal load, since the external load was far too low to induce this magnitude of algae blooming. Their statistical model indicated that bottom-hypoxia-causing anoxic P pulses were likely to develop at the western part of Lake Balaton before the blooming.

LD12-*Fonsibacter*, which belongs to the phylum *Proteobacteria*, was detected at the highest ratio (up to 20%) in the open water areas of the water reservoir (K3, F3, KB9

and Z11). The LD12 bacteria play an important role in the microbial assimilation of low-molecular-weight organic compounds with slow but efficient uptake in freshwater oligotrophic ecosystems. Salcher et al. [55] found three distinct abundance maxima of the genus at Lake Zurich. During their research, they found that LD12 cells were most active when phosphorus concentrations were seasonally low, and a significant negative relationship between cell densities and nitrate and ammonia concentrations was found. We found a moderate negative relationship between LD12 abundance and  $\text{NO}_2\text{-N}$  concentrations, and a positive correlation with TSS and the oxygen level of the water. Similar to Salcher et al. [55], we were able to observe three abundance peaks of these bacteria in the K3 area: one in spring, another from July to August, and the last in October.

The phylum *Bacteroidetes* was also represented at a high proportion in all sampling areas. Unlike other common freshwater groups, the phylum typically does not show seasonal dynamics or habitat-specific distribution. The increased abundance of *Bacteroidetes* bacteria is usually the result of high organic matter input, or can be observed after phytoplankton blooms [56,57]. In the Z11 area, the chlorophyll *a* values were high from the end of August to October; even so, members of the phylum *Bacteroidetes* showed an even distribution during the investigated period. Nevertheless, at the KBWPS outflow (Z27), the abundance of the genera *Flavobacterium* and *Fluviicola* was the highest during early September when the maximum chlorophyll *a* value was detected. At the Keszthely Basin, an elevated ratio of *Cyanobacteria* resulted in an increase in the abundance of *Flavobacterium* and *Sediminibacterium*, and the highest ratio of both genera was observed following the algae peak. Members of the genus *Sediminibacterium* are often detected in algae-dominated natural ecosystems and laboratory algae-enrichment cultures. According to the results, these bacteria use organic substances produced by algae or released after their death [58,59].

## 5. Conclusions

The microbial communities of the Zala River had the most unique profile; the composition was shaped greatly by the high nutrient composition and the floods, while the seasonal effect was less significant. The microbial community of the river was dominated by *Proteobacteria* and *Bacteroidetes* species, such as *Limnohabitans*, *Pseudarcicella* and *Aquirufa* species, which were abundant typically in this area. The microbial community composition of the wetland showed a more uniform picture in the spring period; however, as the temperature and algae production increased, differences between the sampling areas emerged. In the open water area of the wetland in the summer and autumn periods, the dominance of the cyanobacterial *Dolichospermum* species was observed. In addition, we were also able to identify species related to the verrucomicrobial *Luteolibacter* genus and the actinobacterial hgcI clade, which presumably play a key role in the breakdown of the organic matter produced by the algae. Interestingly, several microbial community members that prefer nutrient-poor conditions (e.g., LD12 subclade, *Synechococcus* sp.) were also found in a relatively high proportion in the eutrophic waters of Phase I; however, their abundance typically declined under the dominance of *Cyanobacteria* species. In the reed-stands-dominated part of the wetland, the bacterial decomposition of dead algal biomass took over the key role. The conditions were favorable for the presumably polyphosphate-accumulating C39 bacteria. However, it seems that these bacteria are only able to reduce the  $\text{PO}_4\text{-P}$  content of the water to a small extent due to the low oxygen concentration. The seasonally occurring oxygen-limited conditions also help the enrichment of methanotrophs such as *Methyloparacoccus* or an unknown *Methylococcaceae* bacteria in the KBWPS outflow.

The microbial community of Lake Balaton reflects an open water community in which microorganisms adapted to nutrient-poor oligotrophic conditions were typically dominant. The actinobacterial hgcI clade and CI500–29 members, and the proteobacterial LD12 subclade members, were found in high proportion in early and late summer. The cyanobacterium *Aphanizomenon flos-aquae*-dominated microbial communities were observable in the nutrient-dense western part of the lake. In addition to the west–east trophic gradient, the internal load also played a major role in triggering the algal bloom. The fact that the

*Dolichospermum* species which were dominant in the KBWPS area were much less abundant in the Keszthely Basin is presumably due the dense macrophyte vegetation of the wetland area, which acts as a natural boundary between the two open water areas.

Our study confirmed that: (i) Cyanobacteria have a negative effect on the abundance of the *Limnohabitans* genus; (ii) positive links were found between several *Verrucomicrobia* lineages and the algae community composition, which suggests that this group could play a key role in the utilization of algae-derived organic matter; (iii), *Synechococcus* LD12, hgcl clade members showed various distribution, indicating that higher organic matter concentration does not necessarily have a negative influence on their abundance; and (iv) the invasive *Ceratium furcoides* caused a large-scale aquatic bloom for the first time at the largest Central European shallow lake.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14213519/s1>, Figure S1: Rarefaction curve of Illumina MiSeq 16S amplicon sequencing dataset; Figure S2: Spearman's rank correlation between the top 20 bacterial OTUs, main algal phyla and environmental parameters. The red and blue dots represent the negative and positive Spearman's rank correlation coefficient ( $r$ ), considering  $p$  value  $< 0.01$ , respectively; Table S1: Physical and chemical parameters of water samples from Zala River (Z15) in 2019; Table S2: Physical and chemical parameters of water samples from KBWPS interface (Z11) in 2019; Table S3: Physical and chemical parameters of water samples from KBWPS outflow (Z27) in 2019; Table S4: Physical and chemical parameters of water samples from Lake Balaton, Keszthely basin (K3) in 2019.

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## References

1. G.-Tóth, L.; Párpala, L.; Balogh, C.; Tátrai, I.; Baranyai, E. Zooplankton community response to enhanced turbulence generated by water-level decrease in Lake Balaton, the largest shallow lake in Central. *Limnol. J. Oceanogr.* **2011**, *56*, 2211–2222. [[CrossRef](#)]
2. Pomogyi, P. Nutrient retention by the Kis-Balaton Water Protection System. *Hydrobiologia* **1993**, *251*, 309–320. [[CrossRef](#)]
3. Tátrai, I.; Kálmán, M.; Korponai, J.; Paulovits, G.; Pomogyi, P. The role of the Kis-Balaton Water Protection System in the control of water quality of Lake Balaton. *Ecol. Eng.* **2000**, *16*, 73–78. [[CrossRef](#)]
4. Hatvani, I.G. Application of State-of-the-Art Geomathematical Methods in Water Protection—On the Example of the Data Series of the Kis-Balaton Water Protection System. Ph.D. Dissertation, Eötvös Loránd University, Budapest, Hungary, 2014.
5. Honti, M.; Gao, C.; Istvánovics, V.; Clement, A. Lessons Learnt from the Long-Term Management of a Large (Re)constructed Wetland, the Kis-Balaton Protection System (Hungary). *Water* **2020**, *12*, 659. [[CrossRef](#)]

6. Istvánovics, V.; Honti, M.; Torma, P.; Kousal, J. Record—Setting algal bloom in polymictic Lake Balaton (Hungary): A synergistic impact of climate change and (mis)management. *Freshw. Biol.* **2022**, *67*, 1091–1106. [[CrossRef](#)]
7. Farkas, M.; Kaszab, E.; Radó, J.; Háhn, J.; Tóth, G.; Harkai, P.; Ferincz, Á.; Lovász, Z.; Táncsics, A.; Vörös, L.; et al. Planktonic and Benthic Bacterial Communities of the Largest Central European Shallow Lake, Lake Balaton and Its Main Inflow Zala River. *Curr. Microbiol.* **2020**, *77*, 4016–4028. [[CrossRef](#)]
8. Wetzel, R.G.; Likens, G. *Limnological Analyses*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2000; p. 429.
9. Hillebrand, H.; Dürselen, C.D.; Kirschtel, D.; Pollinger, U.; Zohary, T. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* **1999**, *35*, 403–424. [[CrossRef](#)]
10. Hatvani, I.G.; Clement, A.; Kovács, J.; Kovács, I.S.; Korponai, J. Assessing water–quality data: The relationship between the water quality amelioration of Lake Balaton and the construction of its mitigation wetland. *J. Great Lakes Res.* **2014**, *40*, 115–125. [[CrossRef](#)]
11. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next–generation sequencingbased diversity studies. *Nucleic Acids Res.* **2013**, *41*, e1. [[CrossRef](#)]
12. 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.; et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [[CrossRef](#)]
13. Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a dual–index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **2013**, *79*, 5112–5120. [[CrossRef](#)]
14. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web–based tools. *Nucleic Acids Res.* **2012**, *41*, D590–D596. [[CrossRef](#)] [[PubMed](#)]
15. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**, *27*, 2194–2200. [[CrossRef](#)] [[PubMed](#)]
16. Kunin, V.; Engelbrekton, A.; Ochman, H.; Hugenholtz, P. Wrinkles in the rare biosphere: Pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ. Microbiol.* **2010**, *12*, 118–123. [[CrossRef](#)] [[PubMed](#)]
17. Tindall, B.J.; Rosselló–Móra, R.; Busse, H.J.; Ludwig, W.; Kämpfer, P. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 249–266. [[CrossRef](#)] [[PubMed](#)]
18. Yoon, S.H.; Ha, S.M.; Kwon, S.; Lim, J.; Kim, Y.; Seo, H.; Chun, J. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 1613–1617. [[CrossRef](#)]
19. Hammer, Ř.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
20. Kasalický, V.; Jezbera, J.; Šimek, K.; Hahn, M.W. *Limnohabitans planktonicus* sp. nov. and *Limnohabitans parvus* sp. nov., planktonic betaproteobacteria isolated from a freshwater reservoir, and emended description of the genus *Limnohabitans*. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2710–2714. [[CrossRef](#)]
21. Kasalický, V.; Zeng, Y.; Piwosz, K.; Šimek, K.; Kratochvilová, H.; Koblížek, M. Aerobic Anoxygenic Photosynthesis Is Commonly Present within the Genus *Limnohabitans*. *Appl. Environ. Microbiol.* **2017**, *84*, e02116–17. [[CrossRef](#)]
22. Jezberová, J.; Jezbera, J.; Znachor, P.; Nedoma, J.; Kasalický, V.; Šimek, K. The *Limnohabitans* genus harbors generalistic and opportunistic subtypes: Evidence from spatiotemporal succession in a canyon–shaped reservoir. *Appl. Environ. Microbiol.* **2017**, *83*, e01530–17. [[CrossRef](#)]
23. Šimek, K.; Kasalický, V.; Zapomělová, E.; Horňák, K. Alga–derived substrates select for distinct betaproteobacterial lineages and contribute to niche separation in *Limnohabitans* strains. *Appl. Environ. Microbiol.* **2011**, *77*, 7307–7315. [[CrossRef](#)] [[PubMed](#)]
24. Hahn, M.W. Isolation of strains belonging to the cosmopolitan *Polynucleobacter necessarius* cluster from freshwater habitats located in three climatic zones. *Appl. Environ. Microbiol.* **2003**, *69*, 5248–5254. [[CrossRef](#)] [[PubMed](#)]
25. Wu, Q.L.; Hahn, M.W. Differences in structure and dynamics of *Polynucleobacter* communities in a temperate and a subtropical lake revealed at three phylogenetic levels. *FEMS Microb. Ecol.* **2006**, *57*, 67–79. [[CrossRef](#)] [[PubMed](#)]
26. Buck, U.; Grossart, H.P.; Amann, R.; Pernthaler, J. Substrate incorporation patterns of bacterioplankton populations in stratified and mixed waters of a humic lake. *Environ. Microbiol.* **2009**, *11*, 1854–1865. [[CrossRef](#)] [[PubMed](#)]
27. Watanabe, K.; Komatsu, N.; Ishii, Y.; Negishi, M. Effective isolation of bacterioplankton genus *Polynucleobacter* from freshwater environments grown on photochemically degraded dissolved organic matter. *FEMS Microbiol. Ecol.* **2009**, *67*, 57–68. [[CrossRef](#)]
28. Jezberová, J.; Jezbera, J.; Brandt, U.; Lindström, E.S.; Langenheder, S.; Hahn, M.W. Ubiquity of *Polynucleobacter necessarius* ssp. *asymbioticus* in lentic freshwater habitats of a heterogeneous 2000 km area. *Environ. Microbiol.* **2010**, *12*, 658–669. [[CrossRef](#)]
29. Jezbera, J.; Jezberova, J.; Koll, U.; Hornak, K.; Šimek, K.; Hahn, M.W. Contrasting trends in distribution of four major planktonic betaproteobacterial groups along a pH gradient of epilimnia of 72 freshwater habitats. *FEMS Microb. Ecol.* **2012**, *81*, 467–479. [[CrossRef](#)]
30. Pitt, A.; Schmidt, J.; Koll, U.; Hahn, M.W. *Aquirufa antheringensis* gen. nov., sp. nov. and *Aquirufa nivalisilvae* sp. nov., representing a new genus of widespread freshwater bacteria. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, 2739–2749. [[CrossRef](#)]
31. Marinović, Z.; Tokodi, N.; Backović, D.D.; Ščekić, I.; Kitanović, N.; Simić, S.B.; Đorđević, N.B.; Ferincz, Á.; Staszny, Á.; Dulić, T.; et al. Does the Kis–Balaton Water Protection System (KBWPS) Effectively Safeguard Lake Balaton from Toxic Cyanobacterial Blooms? *Microorganisms* **2021**, *9*, 960. [[CrossRef](#)]



32. Chiang, E.; Schmidt, M.L.; Berry, M.A.; Biddanda, B.A.; Burtner, A.; Johengen, T.H.; Palladino, D.; Deneff, V.J. Verrucomicrobia are prevalent in north-temperate freshwater lakes and display class-level preferences between lake habitats. *PLoS ONE* **2018**, *13*, e0195112.
33. He, S.; Stevens, S.L.; Chan, L.K.; Bertilsson, S.; del Rio, T.G.; Tringe, S.G.; Malmstrom, R.R.; McMahon, K.D. Ecophysiology of freshwater Verrucomicrobia inferred from metagenome-assembled genomes. *MSphere* **2017**, *2*, e00277-17. [[CrossRef](#)] [[PubMed](#)]
34. Ohshiro, T.; Harada, N.; Kobayashi, Y.; Miki, Y.; Kawamoto, H. Microbial fucoidan degradation by *Luteolibacter* algae H18 with deacetylation. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 620–623. [[CrossRef](#)] [[PubMed](#)]
35. Sichert, A.; Corzett, C.H.; Schechter, M.S.; Unfried, F.; Markert, S.; Becher, D.; Fernandez-Guerra, A.; Liebeke, M.; Schweder, T.; Polz, M.F.; et al. Verrucomicrobia use hundreds of enzymes to digest the algal polysaccharide fucoidan. *Nat. Microbiol.* **2020**, *5*, 1026–1039. [[CrossRef](#)] [[PubMed](#)]
36. Lindh, M.V.; Sjöstedt, J.; Andersson, A.F.; Baltar, F.; Hugerth, L.W.; Lundin, D.; Muthusamy, S.; Legrand, C.; Pinhassi, J. Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. *Environ. Microbiol.* **2015**, *17*, 2459–2476. [[CrossRef](#)]
37. Ghylin, T.W.; Garcia, S.L.; Moya, F.; Oyserman, B.O.; Schwientek, P.; Forest, K.T.; Mutschler, J.; Dwulit-Smith, J.; Chan, L.K.; Martinez-Garcia, M.; et al. Comparative single-cell genomics reveals potential ecological niches for the freshwater acl Actinobacteria lineage. *ISME J.* **2014**, *8*, 2503. [[CrossRef](#)]
38. Stepanauskas, R.; Moran, M.A.; Bergamaschi, B.A.; Hollibaugh, J.T. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. *Aquat. Microb. Ecol.* **2003**, *31*, 85–98. [[CrossRef](#)]
39. Hugoni, M.; Vellet, A.; Debroas, D. Unique and highly variable bacterial communities inhabiting the surface microlayer of an oligotrophic lake. *Aquat. Microb. Ecol.* **2017**, *79*, 115–125. [[CrossRef](#)]
40. Papale, M.; Rappazzo, A.C.; Mikkonen, A.; Rizzo, C.; Moscheo, F.; Conte, A.; Michaud, L.; Lo Giudice, A. Bacterial Diversity in a Dynamic and Extreme Sub-Arctic Watercourse (Pasvik River, Norwegian Arctic). *Water* **2020**, *12*, 3098. [[CrossRef](#)]
41. Samad, M.S.; Lee, H.J.; Cerbin, S.; Meima-Franke, M.; Bodelier, P. Niche Differentiation of Host-Associated Pelagic Microbes and Their Potential Contribution to Biogeochemical Cycling in Artificially Warmed Lakes. *Front. Microbiol.* **2020**, *11*, 582. [[CrossRef](#)]
42. Zhu, M.; Zhu, G.; Zhao, L.; Yao, X.; Zhang, Y.; Gao, G.; Qin, B. Influence of algal bloom degradation on nutrient release at the sediment-water interface in Lake Taihu, China. *Environ. Sci. Pollut. Res.* **2013**, *20*, 1803–1811. [[CrossRef](#)]
43. Watanabe, K.; Morohoshi, S.; Kunihiro, T.; Ishii, Y.; Takayasu, L.; Ogata, Y.; Shindo, C.; Suda, W. *Fluviibacter* phosphoraccumulans gen. nov., sp. nov., a polyphosphate-accumulating bacterium of *Fluviibacteraceae* fam. nov., isolated from surface river water. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 5551–5560. [[CrossRef](#)] [[PubMed](#)]
44. Satoh, H.; Mino, T.; Matsuo, T. Anaerobic uptake of glutamate and aspartate by enhanced biological phosphorus removal activated sludge. *Water Sci. Technol.* **1998**, *37*, 579–582. [[CrossRef](#)]
45. Seviour, R.J.; Mino, T.; Onuki, M. The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiol. Rev.* **2003**, *27*, 99–127. [[CrossRef](#)]
46. Somogyi, B.; Felföldi, T.; Tóth, L.G.; Bernát, G.; Vörös, L. Photoautotrophic picoplankton—A review on their occurrence, role and diversity in Lake Balaton. *Biol. Futur.* **2020**, *71*, 371–382. [[CrossRef](#)] [[PubMed](#)]
47. Felföldi, T.; Duleba, M.; Somogyi, B.; Vajna, B.; Nikolausz, M.; Présing, M.; Márialigeti, K.; Vörös, L. Diversity and seasonal dynamics of the photoautotrophic picoplankton in Lake Balaton (Hungary). *Aquat. Microb. Ecol.* **2011**, *63*, 273–287. [[CrossRef](#)]
48. Becker, S.; Richl, P.; Ernst, A. Seasonal and habitat—Related distribution pattern of *Synechococcus* genotypes in Lake Constance. *FEMS Microbiol. Ecol.* **2007**, *62*, 64–77. [[CrossRef](#)]
49. Grigorszky, I.; Borics, G.; Nagy, S.; Vasas, G.; Padisák, J.; Varga, S.; M-Hamvas, M.; Molnár, E.; Dévai, G.; Borbély, G. Adatok Magyarország Fajainak Ismeretéhez I. *Hidr. Közl.* **1999**, *78*, 348–350.
50. Somogyi, B.; Tugyi, N.; Vörös, L. A fitoplankton szezonális dinamikája a Balatonban 2016-ban. *Ecol. Lake Bal.* **2016**, *3*, 16–26.
51. Lindström, K. *Ceratium* in Lake Erken: Vertical distribution, migration and form variation. *Nord. J. Bot.* **1992**, *12*, 541–556. [[CrossRef](#)]
52. Pérez-Martínez, C.; Sánchez-Castillo, P. Winter dominance of *Ceratium hirundinella* in a southern north-temperate reservoir. *J. Plankton Res.* **2002**, *24*, 89–96. [[CrossRef](#)]
53. Macêdo, R.L.; Russo, P.; Corrêa, R.F.; Rocha, O.; dos Santos, L.N.; Branco, C.W.C. The drifting dinoflagellate *Ceratium furcoides* (Levander) Langhans 1925: Fundamental niche shift during global invasion. *Hydrobiologia* **2021**, *848*, 2105–2117. [[CrossRef](#)]
54. Reynolds, C.S. *Vegetative Processes in the Pelagic: A Model for Ecosystem Theory*; Ecology Institute: Oldendorf/Luhe, Germany, 1997; 371p.
55. Salcher, M.M.; Pernthaler, J.; Posch, T. Seasonal bloom dynamics and ecophysiology of the freshwater sister clade of SAR11 bacteria ‘that rule the waves’ (LD12). *ISME J.* **2011**, *5*, 1242–1252. [[CrossRef](#)] [[PubMed](#)]
56. Zeder, M.; Peter, S.; Shabarova, T.; Pernthaler, J. A small population of planktonic Flavobacteria with disproportionately high growth during the spring phytoplankton bloom in a prealpine lake. *Environ. Microbiol.* **2009**, *11*, 2676–2686. [[CrossRef](#)]
57. Parulekar, N.N.; Kolekar, P.; Jenkins, A.; Kleiven, S.; Utkilen, H.; Johansen, A.; Sawant, S.; Kulkarni-Kale, U.; Kale, M.; Sæbø, M. Characterization of bacterial community associated with phytoplankton bloom in a eutrophic lake in South Norway using 16S rRNA gene amplicon sequence analysis. *PLoS ONE* **2017**, *12*, e0173408. [[CrossRef](#)]

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58. Seymour, J.R.; Amin, S.A.; Raina, J.B.; Stocker, R. Zooming in on the phycosphere: The ecological interface for phytoplankton–bacteria relationships. *Nat. Microbiol.* **2017**, *2*, 17065. [[CrossRef](#)]
  59. Sethuraman, A.; Stancheva, R.; Sanders, C.; Caceres, L.; Castro, D.; Hausknecht–Buss, H.; Henry, S.; Johansen, H.; Kasler, A.; Lastor, S.; et al. Genome of a novel Sediminibacterium discovered in association with two species of freshwater cyanobacteria from streams in Southern California. *G3 Genes Genomes Genet.* **2022**, *12*, jkac123. [[CrossRef](#)]