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Microbiome dynamics modeling and analysis in relation to spatio-temporal changes in physicochemical conditions of the water ecosystem

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Abstract: In this paper, we consider the development of reliable tools to assess the water quality and state of aquatic ecosystems in dynamic conditions a crucial need to address. One of such tools could be devised by monitoring the taxonomic structure of reservoirs' microbiomes. Microbial taxa's ecological and metabolic characteristics suggest their essential roles in maintaining the water ecosystem's environmental equilibrium. The study aimed to explain the role of diversity and seasonal variability of the microbial communities in the ecosystem stability on the example of Goczałkowice Reservoir (Poland). The structure of the reservoir microbiome was studied using bioinformatics and modeling techniques. Water was sampled periodically in July & November 2010, and April 2011 at four representative sites. The abundance and relative fraction of the limnetic taxonomic units were determined in respect to the physicochemical indices. Significant seasonal variations in the number of operational taxonomic units (OTU) were observed within the reservoir basin's main body but not at the main tributary's mouth. The highest values of the correlation coefficients between OTU and physicochemical variables were obtained for Burkholderiales, Pseudoanabenales, Rickettsiales, Roseiflexales, Methylophilales, Actinomycetales, and Cryptophyta. These microorganisms are proposed as indicators of environmental conditions and water quality. Metataxonomic analyses of the freshwater microbiome in the reservoir, showed that microorganisms constitute conservative communities that undergo seasonal and local changes regarding the relative participation of the identified taxa. Therefore, we propose that monitoring those variations could provide a reliable measure of the state of aquatic ecosystems.

Introduction

Anthropogenic pressures and hydrogeologic processes result in the aging of dam water reservoirs, which decreases their functional and ecological potential. The Water Framework Directive (WFD – Directive 2000/60/EC of the European Parliament and the Council of Europe 23 October 2000) requires that reservoir managers obtain and maintain high water quality and good environmental conditions, respecting its economic functions.

Dam reservoirs are anthropogenic ecosystems of strategic roles, such as floods preventing water retention, drinking water supply, or hydroelectricity purposes. Moreover, dam reservoirs have contributed to climate change mitigation, including water management in the threat of potable water shortage. Therefore, long-term and farsighted protection of their homeostasis, ensuring their longevity and reliability, is crucial and may be maintained only by providing their ecological sustainability. To ensure this, the recognition and monitoring of dam reservoirs at every level of their biological organization and trophic level, beginning with a basal link crucial in biogeochemical cycles, are necessary.

In the presented paper, we focused on the sensitivity of microbial assemblages to changes in physical and/or chemical water characteristics and the prospect of using them as a bioindication or as a monitoring tool.

Maintaining the strategical roles of dam reservoirs requires the introduction of modern and effective environmental analysis methods and monitoring corresponding to the complexity and dynamics of the processes within a reservoir and its catchment. The ongoing progress in studying the biodiversity of water microbial communities as well as modern methods and

standards of environmental analyses have provided tools for the management of the environment. Despite cyanobacteria, nosogenic bacteria, and eukaryotic algae, the role of microorganisms in water and their impact on the quality of the environment is yet poorly recognized. However, their role in the circulation of nutrients and the processing of organic matter is unquestionable (Thatoi et al., 2013).

Conventional analyses of the microbial diversity in the environment so far have been based on laboratory cultures, which allowed no more than 1% of the microorganisms present in the material to be identified and characterized. Modern analytical techniques such as metataxonomics (MT), phospholipid fatty acid (PLFA) profiles reveal the complexities of a microbiome (microbial assemblages) and its spatial and temporal variability, as well as the ecological functions of the identified taxa (Stanimirova et al., 2016). The enormous diversity and complexity of these assemblages demonstrate that the structure, ecology, and biochemical functions of microbiomes need further investigation that should be dedicated to each particular biota (Haas et al., 2011). However, to date, MT analyses have only allowed for a crude determination of the population density (number and biomass) of particular taxa in the analyzed environmental material. Such meticulous and multidimensional research of the reservoir (molecular and modeling techniques) is unique for this kind of multifunctional dam reservoir.

In this study, we applied knowledge about microbial diversity and implemented effective microbiological analyses methods into the processes used to recognize, predict and manage the status of water reservoirs.

Materials and Methods

Goczałkowice Dam Reservoir

Goczałkowice Reservoir (GR), is located in the southern part of Upper Silesia (Poland) (Figure 1). The reservoir, built in 1956, is the fourth largest (maximal volume of 161.3 hm^3, surface at a maximum of damming of 32 km^2, maximum depth of 13 m) and one of the shallower in Poland. Its main function is protection against flooding and it serves as a water resource for three million people. In 2004, the reservoir was included in the European Network of Important Bird and Biodiversity Areas (IBAs), as it constitutes an important habitat for over 200 nesting and migratory bird species (Gwiazda et al., 2014).

Land cover analysis of the Goczałkowice Reservoir catchment area, prepared on the basis of *CORINE Land Cover 2012* data, shows domination of non-irrigated arable lands together with pastures, complex cultivation patterns and agriculture with natural vegetation (in total up to 40%), forests (37%) and discontinuous urban fabric (ca. 13%; Figure 2).

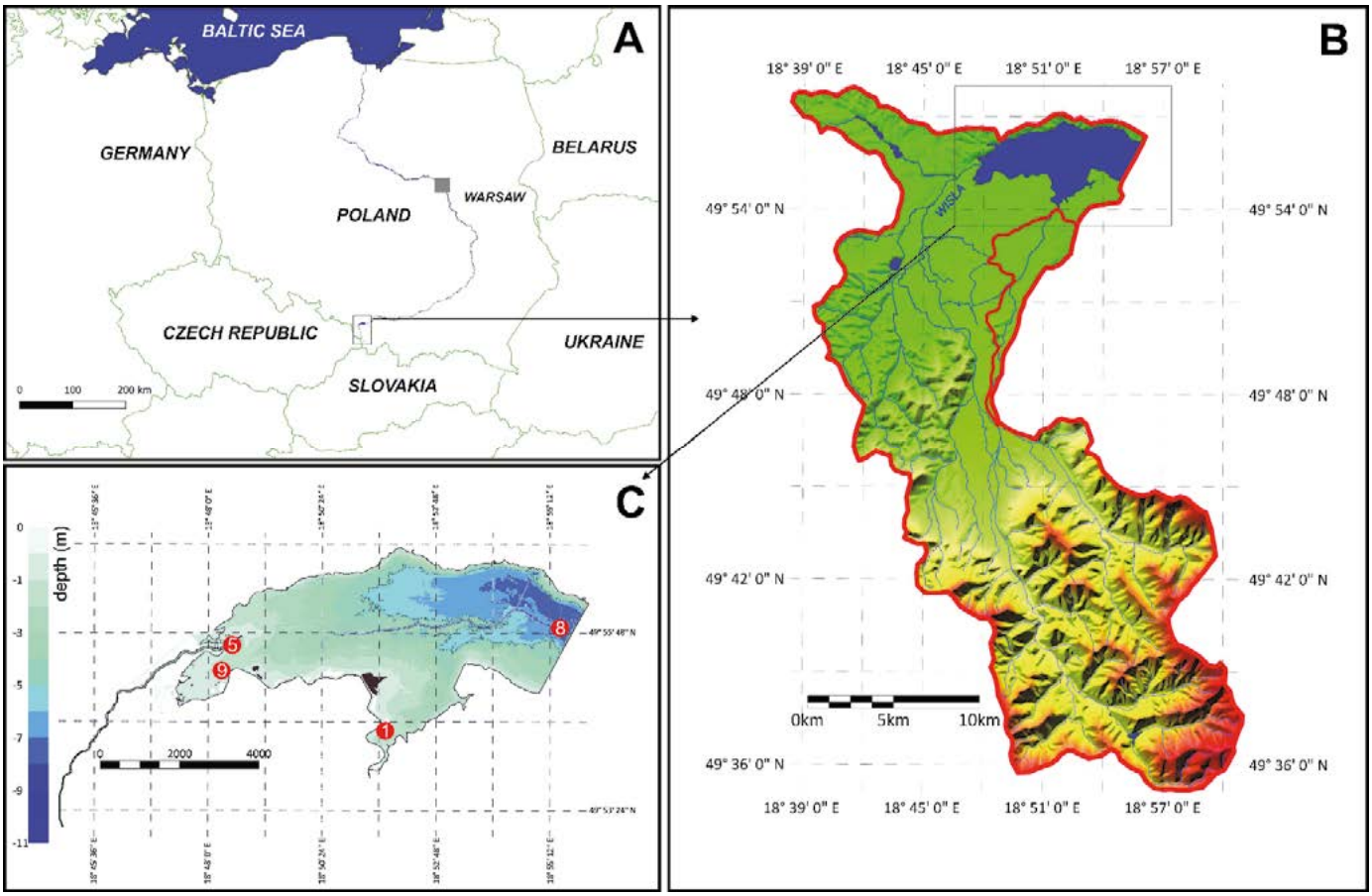

Fig. 1. Location of the Goczałkowice Reservoir: A – Map of Poland with the location of the catchment of the Upper Vistula indicated (PL: Mała Wisła); B – Catchment area of Upper Vistula with the location of the Goczałkowice Reservoir indicated; C – Bathymetric map of the Goczałkowice Reservoir: blue line shows the amount of water at a damming level of 255.5 m; red circles numbered as 1, 5, 8 & 9, represent the sampling sites Z01, Z05, Z08 & Z09 for the metataxonomic, biochemical and physicochemical analyses.

Water sampling and physicochemical analyses

The four sampling sites (Figure 1) were selected based on the modeling conducted within the water body of the reservoir. The results (including meteorological data) permitted the properties and flows of the water masses in the reservoir to be modeled using the ELCOM-CAEDYM model (current name: AEM3D – Aquatic Ecosystem Model 3D) (Hodges & Dallimore, 2006) Lake and Coastal Ocean Model.

Sampling sites Z01, Z05, Z08, and Z09 were selected, as the most characteristic for the GR, representing various local impacts and water flow dynamics basing on the ELCOM--CAEDYM model. The linear velocity of the water flow did not exceed 0.04 $m \cdot s^{-1}$. Site Z01 is located at the inlet of the Bajerka River – the secondary tributary of the reservoir. Site Z08 is located in the deepest area of the reservoir in the old riverbed of the Vistula near the lower outlet of the reservoir (Figure 1). Site Z05 is located at the mouth of the Vistula River at its outlet into the reservoir, and site Z09 is located in a shallow stagnant cove (backwater) to the south near the mouth of the Vistula where preliminary research showed potamic water transport into the reservoir. Site Z09 is topographically close to the site Z05. It is the main nesting site of birds (mainly cormorants – Natura 2000 Sites – Birds Directive). Thus, this site may potentially, differ from the Z07 site. Therefore, metataxonomic analyses were conducted just once there, just for comparison's sake.

Five liters of water were collected from all four sites at a depth of 1.5–2 m using five L Limnos Water Samplers on: 19 July 2010, 8 November 2010, and 5 April 2011. The oxygen concentration in the deeper layers of the reservoir was analyzed at the deepest site Z08 at a depth of about 10 m.

Water samples were analyzed for their physicochemical properties (ammonium, solutes, inorganic carbon, volatile and non-volatile substances in water, orthophosphate, calcium, nitrate, nitrite, magnesium, sulphate, BOD, total suspended solids, potassium, polyphosphate, dissolved organic phosphorous, pH, DO, water temperature, organic carbon, chlorophyll) in the certified laboratory with the ISO standard accreditation (Accreditation Certificate PCA Nr AB 950) at the Institute of Environmental Engineering Polish Academy of Sciences in Zabrze (Poland), according to the Polish Standards (PN). Analytical methods used for the measurements were in accordance with reference methods indicated in Annex 5 to the Regulation of the Minister of the Environment (May 13, 2009) on the forms and methods of monitoring surface and groundwater bodies (Dz. U. Nr 81, poz. 685) and certified research procedures. Secchi depth was measured under *in situ* conditions.

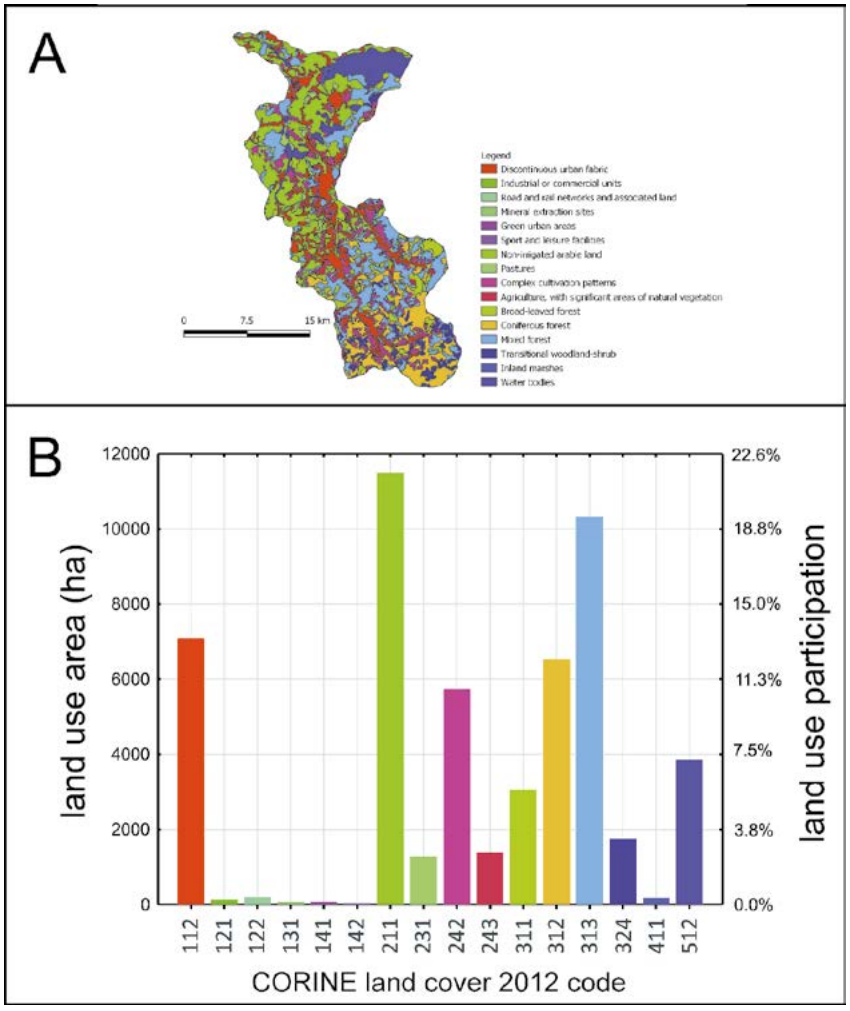

Fig. 2. Land cover of the Goczałkowice reservoir catchment area developed on the CORINE Land Cover 2012 basis. Bars colors on the charts are related to the color on the maps.

DNA extraction, PCR and 16S rDNA sequencing

Metataxonomics analyses were conducted in 0.5 L of the sample, which was filtered on an ultrafiltration system (Sartorius Funnel 500 ml, stainless) using 0.45 µm pore-size polycarbonate filters. The filters were stored frozen at -20°C until DNA isolation. DNA was extracted directly from the filters using a PowerWater DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA USA) as described by the manufacturer's instructions, except that the DNA elution was performed in 70 µL of PowerWater 6 solution no 6 (PW6). DNA concentration was measured using NanoDrop (Thermo Scientific, Wilmington, MA USA) and its integrity was evaluated electrophoretically on 1.0% agarose gel. The isolated bacterial 16S rRNA genes were amplified in PCR using the broadly conserved bacterial primer. Inner PCR was performed using forward oligonucleotide PR primer A 5′-CGTATCGCCTCCCTCGCGCCA-TCAG-MID-ATCACTCTTTGGCARCGACC-3′ and reverse oligonucleotide PR primer B 5′-CTATGCGCCTTGCCAGCCCGC-TCAG-MID-CCTGGCTTTAATTTTACTGGTACAG-3′ including A and B sequences at the 5′-ends necessary for the 454 Roche FLX/Titanium pyrosequencing method (underlined), respectively, different multiplex identifiers (MID) and HIV-1 specific parts (Bibby et al., 2010; Stanimirova et al., 2016; Woźnica et al., 2013).

The PCR mixtures for each sample were prepared in quadruplicate in 20 µL volumes and included a 1x Phusion HF Buffer (Thermo Scientific, Wilmington, MA USA), 0.2 mM deoxyribonucleoside triphosphates, 0.1 µM each of forward and reverse primers, 0.4 U Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Wilmington, MA USA) and a 20 ng DNA template. PCR was performed in a TProfessional thermal cycler (Biometra) under the following conditions: initial denaturation at 98°C for 30 s; 35 cycles at 98°C for 10 s, 56°C for 15 s, 72°C for 10 s and a final extension at 72°C for 5 min. Amplicons were separated electrophoretically on 1.5% agarose gel and slices corresponding to 400–700 bp length amplicons were cut out. Two gel slices were pooled together and DNA was extracted from them using a QIAquick Gel Extraction Kit (QIAGEN, Venlo, Netherlands) according to the manufacturer's protocol. Elution was performed in 30 µL of an EB solution. All replicates were pooled together and used for sequencing in a GS Junior system (454/Roche). Immediately prior to library preparation, the amplicons were additionally purified using Agencourt AMPure XP (Beckman Coulter Inc, Mississauga, ON Canada) and quantified using a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Burlington, ON Canada) using a TBS-380 Fluorometer (Turner Biosystems, CA, USA) following the "Amplicon Library Preparation Method Manual" of the 454 GS Junior Titanium System (454 Life Sciences/Roche, Branford, CT USA). Emulsion PCR was performed according to the 'em-PCR Amplification Method Manual – Lib A' and sequencing was performed in a single run of a 454 GS Junior Titanium System following the "Sequencing Method Manual" (454 Life Sciences/Roche Branford, CT USA).

Bioinformatics

The sequences were processed and analyzed using the Quantitative Insights Into Microbial Ecology pipeline (QIIME version 1.6.0; http://qiime.org/) (Zwart et al., 2002)) with the default settings. Multiplexed reads were assigned to the samples based on their nucleotide barcode. During quality filtering, any low quality (< Q25) or ambiguous reads were removed. Chimeras were removed using ChimeraSlayer (DeSantis et al., 2006; Haas et al., 2011).

Data calculation and statistical analysis

The resulting chimera-free reads were clustered into Operational Taxonomic Units (OTUs) based on their sequence similarity at a 97% pairwise identity using the seed-based Uclust algorithm. Representative sequences from each OTU were aligned to the Greengenes imputed core reference alignment (Greengenes version 12_10; (DeSantis et al., 2006); http://greengenes.lbl.gov) using PyNAST. Taxonomy assignments were made using the Ribosomal Database Project (RDP) classifier ver. 2.5 (Wang et al., 2007). Abundance measures such as the Shannon-Wiener H index, Chao1 index of richness, observed species and phylogenetic distance, PD_whole_tree (Chao et al., 2010), were calculated using QIIME. The relationship among the samples was determined using the unweighted pair group method with an arithmetic mean (UPGMA) within QIIME.

All data were collected and filtered using a Microsoft SQL Server and Microsoft Excel 2010. The following statistical analyses were performed using StatSoft, Inc. STATISTICA (data analysis software system), version 10: PCoA – Principal Coordinate Analysis (Multidimensional Scaling) for the metataxonomic data characterized by the coordinates of their unweighted UniFrac values (similarities were determined using the Euclidean distance method), correlation of the orders of microbes and water physicochemical indices, and Kruskal-Wallis ANOVA test for water physicochemical indices.

Results

Seasonal and spatial variation in microbial diversity

Because the number of OTU increased as a function of the total number of reads and the rarefaction curves showed no evidence of a plateau being reached at any of the analyzed points, the values that were used to compare the diversity and richness (Table 1) were adjusted to a common number of reads, which was 24,682 (the number in the smallest data set in Z01 in November).

The diversity of microbial communities, as shown by the value of Shannon-Wiener H Index (Table 1), was the highest at site Z05 (H ≈ 10,1–10,5), slightly lower at sites Z01 and Z08 in July 2010 (H > 9), lower (H ≈ 8) in April 2011 and November 2010 and still lower at the Z08 site in November 2010 (H = 7.39). The lowest microbial diversity was found at the Z09 site in April 2011 (H = 6.62).

The Chao1 richness index for microbial assemblages (Table 1) showed that site Z01 is characterized by the largest seasonal variation of richness, and was three times higher in July 2010 (31,437 – the maximum for all of the sampling sites) than in April 2011 (11,546). The Chao1 values at the Z05 site did not show significant temporal variability and reached 24,000–25,000. There was a slight variability in the richness index at site Z08 of between 13,227 and 19,220.

Significant seasonal variations in the number of OTU were revealed at sites Z01 and Z08, but not at site Z05 where only

minor variations were observed. The highest number of OTU (ca. 7,900) was found at site Z05 in November 2010 and April 2011, while the lowest was found at site Z08 in November 2010 and at sites Z01 and Z09 in April 2011 (ca. 4,000–4,500). At site Z01 the number of OTU decreased from July 2010 to November 2010 by 25% and by further 25% in April 2011 (7,739; 5,849; 4,449, respectively). At site Z08, a 30% decrease in the number of OTU was observed from July to November 2010 (5,891 and 4,272, respectively) with a subsequent increase in the number of OTU in April 2011 (5,437).

Spatial variability of microbial assemblages

PCoA demonstrated (Figure 3) a similarity of the microbial assemblages at site Z05 (characterized by three distinguishable coordinates: PC1, PC2, PC3 and represented as the distance between the points on the coordinate plane), which is independent of time (July 2010–April 2011). An overlapping cluster was also constituted by the microbial assemblages in November 2010 at three sites: Z01, Z05, Z08 characterized by coordinates PC1 and PC3.

Taxonomic structures of the microbial assemblages in the dam reservoir and their variability

The structures of the microbial assemblages in Goczałkowice Reservoir are dominated by *Betaproteobacteria*, *Alphaproteobacteria*, *Synechococcophycideae*, *Acidimicrobiia* and *Actinobacteria*. However, the participation of these classes varied among the analyzed sites (Figure 4 A). *Betaproteobacteria* was the most abundant class at site Z05 in all of the seasons and accounted for ca. 50–70% of the sequences. At the other sampling sites, *Betaproteobacteria* participated in 9–18% of the determined sequences. *Synechococcophycideae* (*Cyanobacteria*) dominated at site Z09 in April 2011 accounting for more than 60% of the obtained sequences, while in July it constituted 44% of the total rDNA sequences at site Z01 and about 11% of the rDNA sequences at sites Z05 and Z08. At other sampling sites and points, *Synechococcophycideae* constituted less than 1% of the sequences. Micro-algae chloroplast ribosomal DNA constituted an important part of the rDNA fragments that were investigated. rDNA from the chloroplast of eukaryotic micro-algae accounted for 4% in July

Table 1. Location (GPS) of the sampling sites, date of sampling, water flow in the reservoir, number of 16S rDNA sequences, DNA content, number of OTU, Chao1 index of richness and Shannon-Wiener H index of diversity calculated for the microbial assemblages that were distinguished.

Site ID	Position Lon °E	Position Lat °N	Date	Water Inflow-Outflow [m^3s^{-1}]	Water temp. [°C]	No. of raw sequences	DNA content [$\mu g\ L^{-1}$]	No. of OTU	Chao1 index of richness	H index of diversity
Z01	18.8637	49.9063	July 2010	2.40–2.50	20.4	84,835	1.652	7,739	31,437	9.71
			Nov 2010	3.62–2.00	8.1	66,389	0.462	5,849	19,601	8.34
			April 2011	5.50–5.00	14.2	75,511	0.630	4,448	11,546	8.30
Z05	18.8099	49.9277	July 2010	2.40–2.50	17.0	71,146	0.840	7,686	25,864	10.10
			Nov 2010	3.62–2.00	8.1	63,420	2.240	7,917	24,555	10.53
			April 2011	5.50–5.00	12.0	80,451	1.120	7,898	23,846	10.52
Z08	18.9234	49.9312	July 2010	2.40–2.50	19.7	81,999	0.812	5,891	19,221	9.14
			Nov 2010	3.62–2.00	8.1	65,666	1.736	4,273	13,228	7.39
			April 2011	5.50–5.00	13.2	87,517	1.162	5,438	17,178	8.64
Z09	18.8068	49.9236	April 2011	5.50–5.00	16.0	87,336	nd	4,091	13,635	6.62

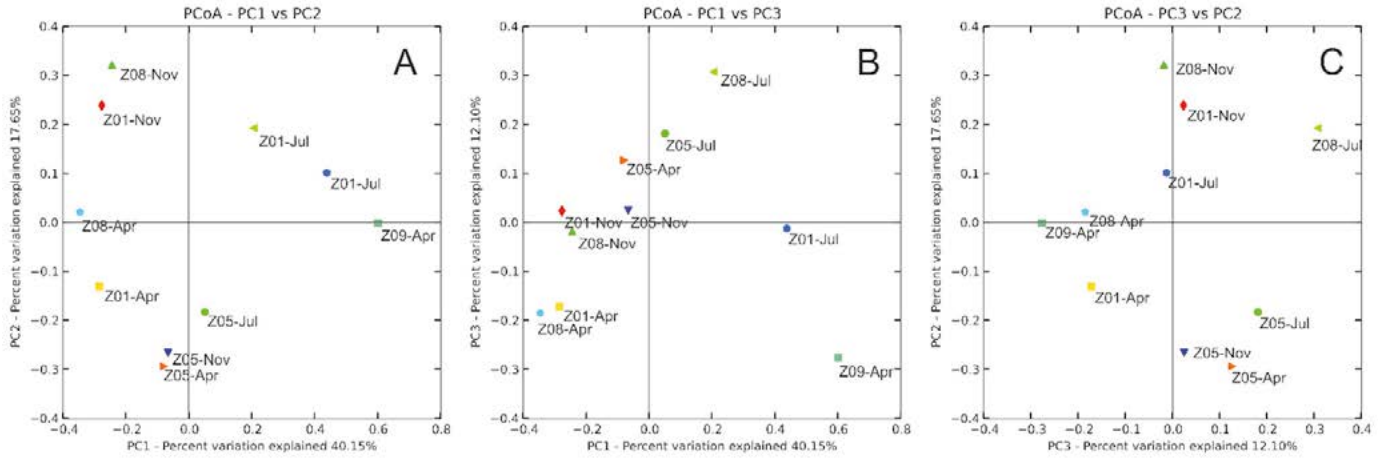

Fig. 3. Phylogenetic similarity of the metataxonomic data characterized by the coordinates of their unweighted UniFrac values. Results for the data obtained at sites Z01–Z09 in July 2010, November 2010 and April 2011 (P = 0.01).

2010 at all of the sampling sites and increased to 36% of the sequences in November 2010, and was the most abundant class at sites Z01 and Z08, but not at site Z05. In April 2011 the participation of the eukaryotic chloroplast rDNA declined to 6% at site Z01 and to 28% at site Z08, but was still the most abundant in this point. *Acidimicrobiia* accounted for 2.5–10% sequences and reached a peak of 33% at site Z01 and 20% at site Z08 in April 2011. *Actinobacteria* participation varied from a 2% minimum at site Z09 to 15% maximum at site Z01 across the analyzed samples in April 2011. The peak abundance of *Actinobacteria* at site Z05 was also observed in April 2011, while it occurred at site Z08 in November 2010. In July 2010 the participation of *Actinobacteria* was the lowest at all of the sites. The participation of the *Alphaproteobacteria* class was relatively stable and accounted for 10–18% of the rDNA that was detected at all of the sites and time points, except for site Z08, where *Alphaproteobacteria* constituted 27% of rDNA sequences in July 2010, and subsequently declined to 15% in November 2010 and to 4% in April 2011. The OTU identified as SL56, which belong to the phylum *Chloroflexi* were detected at a higher level at sites Z01 and Z08 (from 2.5% to 11%) than at sites Z05 and Z09 (less than 1%) and reached the highest values in November 2010. Other representatives of *Chloroflexi* accounted for less than 1% and reached 2% to 6% participation at all of the sites in April 2011 and at site Z08 in July 2010. *Phycisphaerae* comprised 7% at site Z08 in April 2011 and ca. 2% at site Z01 in July 2010 and April 2011, whereas at other sampling points it accounted for less than 0.5%. The *Sphingobacteriia* class was the most abundant at all points at site Z05 in April (5.5%) and at site Z08 in July (3.5%). In the remaining samples, *Sphingobacteriia* made up 0.5–1.5% of all of the sequences.

The class *Alphaproteobacteria* was represented by three orders: *Rhizobiales*, *Rhodospirillales* and *Rhodobacterales* (Figure 4 B). *Rhizobiales* were detected at all points and comprised from 0.33% to 6.85% of the community with the highest values being observed in July 2010. *Rhodospirillales* accounted for less than 1% of the total identified microbes, except for a few percent peak in July 2010 at all sites and in April 2011 at site Z09. The order *Rhodobacterales* was detected at site Z05 where it constituted up to 7% in all of the seasons, as well as at sites Z01 and Z09 in April 2011. In the remaining cases it accounted for less than 1%. The order *Burkholderiales* dominated within the *Betaproteobacteria* and represented more than 50% of all of the sequences at site Z05 in July 2010 and April 2011 and up to 68% in November 2010. At sites Z01, Z08 and Z09 *Burkholderiales* comprised 10–15% of the detected sequences with a decrease to 7% in November 2010 at site Z08. *Methylophilales* was the second most abundant order within the class *Betaproteobacteria*, but accounted for only 0.3% (Z05 site – April 2010) to 2% (Z01, November 2010) of the sequences. Orders *Synechococcales* and *Pseudanabaenales* (class *Synechococcophycideae*, *Cyanobacteria*), which were usually detected at a level of less than 1% (or not detected at all), participated with *Synechococcales* in seasonal blooms and represented 11%, 11% and 34% of the determined OTUs at sites Z05, Z08 and Z01 in July 2010, respectively and as much as 61% at site Z09 in April 2011. At the same time, the next most abundant order, *Pseudanabaenales*, reached a maximum of 8% of relative abundance at site Z01 during the bloom of July 2010. *Actinomycetales* was the most abundant order within the class *Actinobacteria* and accounted for 1% to 11% of the determined OTUs with the highest values at site Z01 in April 2011 and at site Z08 in July and November 2010, while the lowest values were observed at site Z09 in April 2011. Among the class *Acidimicrobiia*, the most abundant order, *Acidimicrobiales*, constituted from 1% at site Z09 to 32% at sites Z01 and Z08 in April 2011, whereas at the other time points and sites, *Acidimicrobiales* participated in 2.5–9% of the 16S rDNA sequences (sites Z01, Z08 in July and November 2010 and site Z05 during the entire observation period). *Cryptophyta* and *Stramenopiles* were the most abundant eukaryotic orders that were detected based on the presence of their chloroplast 16S rDNA sequences. The peaks of the abundance of *Cryptophyta* were observed at sites Z01 and Z08 in November 2010 and accounted for ca. 35% of the discovered taxa and for 26% at site Z08 in April 2011. In the remaining cases, *Cryptophyta* represented 1% to 5% of the OTUs. *Stramenopiles* accounted for 0.5–2% of the OTUs, except for site Z05 in April 2011, where a peak of 5.8% of its abundance was observed. *Phycisphearales* (class *Phycisphaerae*) comprised 7% at site Z08 in April 2011 and 2% of the OTUs at site Z01 in July 2010 and April 2011, but did not exceed the level of 0.5% participation in the remaining sites and seasons. Order *Gemmatales*, a representative of the class *Planctomycetes*, participated in 1.5–4% of OTUs at site Z05 in November 2010 and at sites Z01 and Z08 in April 2011. *Sphingobacteriales* (*Sphingobacteria*) and *Roseiflexales* (*Chloroflexi*) were at a similar level at all points (0.5–3%), except at sites Z01 and Z05 in April 2011, where *Roseiflexales* accounted for ca. 6% of the sequences. Among the class *Thermoleophilia*, the most abundant order was *Solirubrobacterales*, which was detected at a level lower than 0.5% with an exceptional 3% peak at site Z08 in July 2010.

Selected indices of water quality

The variability in the water concentrations of ammonium, dissolved oxygen (DO) and water temperature did not differ significantly ($P > 0.05$) at all sampling sites (Figure 5 A, G, I). The lowest DO value in the water (6.02 mg O_2 L^{-1}, 48% saturation) was observed in November 2010 at site Z05. The highest DO was observed in November 2011 at site Z09 (18.92 mg O_2 L^{-1}, 192% saturation). During the analyzed period, 34% of samples were characterized by supersaturation with oxygen, in 66% of cases the oxygen saturation exceeded 70%. The oxygen concentration in the deeper layers of the reservoir was only analyzed at site Z08 (the deepest area of the reservoir with thermal stratification) and no statistically significant differences were observed between the layers – above and below the thermocline ($P > 0.05$).

The Kruskal-Wallis ANOVA revealed a significant spatial variability in the hydrochemical indices that determine the trophy of the reservoir, with the lowest values (median value close to 0) at site Z09 for the content of nitrate ($P < 0.00001$) in relation to Z08 and Z05 (the highest median ≈7 mg L^{-1}), Secchi depth (median ≈ 0.50 m; $P < 0.00001$) in relation to all other sites (max value 2.9 m on Z08), while they were the highest for chlorophyll (median ≈ 58 mg L^{-1}; $P < 0.0004$) in relation to Z05 and Z08 (Figure 5 B, D, E).

Cluster analysis of the structure of the microbial assemblages and their relationships to environmental conditions

Analysis of the relationships among 22 physicochemical indices that characterize the water environment of the reservoir (Figure 6 A) and the abundance of 18 microbial orders that constituted more than 2% of the assemblages (Spearman's Rank Correlation Coefficient $P < 0.05$) revealed that the environmental indices were grouped into three sets/clusters (Figure 6 A).

The relationships among the environmental indices allowed three clusters to be determined: the first cluster, containing the branches a–c, the second one – branch d, and the third one – branch e (Figure 6 A).

These clusters of the physicochemical indices caused the grouping of microbial taxa into three main corresponding clusters (Figure 6 A):

i) branch 1: *Sphingobacteriales*, *Actinomycetales*, *Rickettsiales* (class *Alphaproteobacteria*), *Methylophilales*, and unclassified taxa – determined based on the correlation with the first cluster of environmental indices (mainly potassium polyphosphates, and orthophosphates),

ii) branch 2 and 3 (without the last separate branch of the cluster 3): *Acidimicrobiales Sphingomonadales, Rhodospirillales, Rhizobiales, Synechoccocales (Cyanobacteria), Rhodobacterales, Solirubrobacterales, Gemmatales, Burkholderiales,*

A

	Betaproteobacteria	Alphaproteobacteria	Synechococcophycideae	Acidimicrobiia	Actinobacteria	SL56	Sphingobacteriia	Phycisphaerae	Chloroflexi	Other (lower than 5%)
Z01.Jul.2010	13,5%	18,4%	45,5%	6,1%	4,1%	3,0%	0,7%	2,5%	0,9%	5,2%
Z05.Jul.2010	58,2%	13,2%	12,4%	3,4%	4,5%	0,7%	1,3%	0,4%	0,9%	5,1%
Z08.Jul.2010	13,6%	27,8%	11,4%	6,2%	12,5%	9,3%	3,6%	0,6%	2,6%	12,4%
Z01.Nov.2010	29,2%	26,4%	0,1%	11,9%	14,0%	11,8%	2,7%	0,0%	0,4%	3,4%
Z05.Nov.2010	73,1%	10,3%	0,3%	2,7%	6,6%	0,4%	1,6%	0,0%	0,5%	4,6%
Z08.Nov.2010	15,0%	23,0%	0,0%	15,5%	21,6%	17,5%	2,2%	0,0%	0,7%	4,5%
Z01.Apr.2011	18,3%	9,2%	0,9%	35,0%	16,3%	5,4%	0,9%	1,9%	6,8%	5,4%
Z05.Apr.2011	56,1%	17,1%	0,5%	2,4%	9,9%	0,2%	6,0%	0,0%	1,9%	5,9%
Z08.Apr.2011	25,5%	5,3%	1,2%	28,3%	8,2%	3,9%	1,1%	10,4%	4,5%	11,6%
Z09.Apr.2011	13,6%	10,9%	68,4%	1,6%	2,1%	0,2%	0,6%	0,0%	0,7%	1,9%
total participation	31,6%	16,2%	14,1%	11,3%	10,0%	5,2%	2,1%	1,6%	2,0%	6,0%

B

	Burkholderiales	Synechococcales	Acidimicrobiales	Actinomycetales	Rhizobiales	Rhodobacterales	Roseiflexales	Sphingobacteriales	Rhodospirillales	Phycisphaerales	Gemmatales	Pseudanabaenales	Methylophilales	Solirubrobacterales	other (lower than 2%)	Procaryota not classified to order
Z01.Jul.2010	11,9%	35,5%	5,9%	3,4%	5,4%	0,7%	0,9%	0,7%	4,9%	2,4%	0,0%	8,1%	0,7%	0,6%	4,1%	14,9%
Z05.Jul.2010	57,1%	12,3%	3,4%	4,0%	7,1%	1,7%	0,9%	1,3%	2,0%	0,4%	0,1%	0,0%	0,4%	0,3%	5,6%	3,5%
Z08.Jul.2010	12,1%	11,3%	6,1%	11,1%	3,9%	0,5%	2,6%	3,6%	4,0%	0,6%	0,0%	0,0%	1,0%	3,3%	9,8%	30,0%
Z01.Nov.2010	23,3%	0,1%	11,6%	12,4%	0,5%	0,3%	0,4%	2,7%	0,1%	0,0%	0,0%	0,0%	3,5%	0,0%	6,6%	38,5%
Z05.Nov.2010	71,2%	0,3%	2,6%	6,1%	2,6%	3,2%	0,5%	1,6%	0,7%	0,0%	1,7%	0,0%	0,1%	0,0%	6,6%	2,7%
Z08.Nov.2010	10,9%	0,0%	15,2%	17,0%	0,9%	0,1%	0,7%	2,1%	0,1%	0,0%	0,0%	0,0%	3,5%	0,1%	6,7%	42,5%
Z01.Apr.2011	17,6%	0,9%	34,5%	9,7%	3,2%	3,0%	6,6%	0,8%	0,6%	1,8%	2,3%	0,0%	0,1%	0,1%	4,2%	14,4%
Z05.Apr.2011	53,8%	0,5%	2,4%	6,9%	4,0%	6,1%	1,9%	6,0%	1,1%	0,0%	0,6%	0,0%	0,0%	0,0%	9,8%	6,7%
Z08.Apr.2011	24,5%	1,2%	28,0%	5,5%	1,2%	1,0%	4,4%	1,1%	0,4%	10,3%	5,4%	0,0%	0,3%	0,3%	7,7%	8,8%
Z09.Apr.2011	13,0%	66,3%	1,6%	1,5%	1,7%	4,3%	0,7%	0,6%	3,1%	0,0%	0,1%	0,4%	0,0%	0,0%	2,9%	3,7%
total participation	29,5%	12,8%	11,1%	7,8%	3,1%	2,1%	2,0%	2,0%	1,7%	1,6%	1,0%	0,9%	1,0%	0,5%	6,4%	16,6%

Fig. 4. Participation matrix of the main microbe orders (A) and classes (B) in the water samples from sites Z01, Z05, Z08 and Z09 in July 2010, November 2010 and April 2011 (estimated based on metataxonomical analysis (based on 16S rDNA using QIIME)). The resolution threshold for the orders was 2% and for classes it was 5% of participation.

Phycisphaerales, Roseiflexales and other orders that participated in less than 2% of the assemblages and corresponding with the second set of environmental conditions (mainly water temperature),

iii) the last separate branch of the cluster 3: *Pseudoanabaenales* (*Cyanobacteria*) and *Cryptophyta* (eukaryotic chloroplast markers) corresponded with organic carbon and chlorophyll-a (corresponding to the branch e)).

Analysis of the correlation among the abundance of specified microbial taxa (Figure 6 B) revealed four groups of associated orders:

i) *Sphingobacterales, Acidimicrobiales, Actinomycetales, Rickettsiales* (class *Alphaproteobacteria), Methylophilales* and unclassified taxa,

ii) *Sphingomonadales, Rhodobacterales, Stramenopiles, Synechoccocales, Rhizobiales, Burkholderiales, Gemmatales*,

iii) *Solirubrobacterales, Phycisphaerales, Roseiflexales*, other (< 2%);

iv) *Pseudoanabaenales* (*Cyanobacteria*), *Cryptophyta* (eukaryotic chloroplast markers).

It should be noted that *Pseudoanabaenales* were characterized by positive correlations with the concentration of chlorophyll-a (0.72) and organic carbon (0.69), but negatively correlated with the calcium (-0.74) and nitrite (-0.72) concentrations. Moreover, an abundance of *Pseudoanabaenales* was negatively correlated with the presence of *Cryptophyta* but positively correlated with *Acidimicrobiales*). The Euclidean distances were large enough to consider *Acidimicrobiales*, *Cryptophyta* and *Pseudoannabelales* as stand-alone orders.

An abundance of *Rickettsiales* (*Alphaproteobacteria*) was correlated with water silicate, polyphosphate and potassium concentrations (0.74, 0.88, 0.72, respectively), while an abundance of *Methylophillales* (*Betaproteobacteria*) was positively correlated with the concentrations of polyphosphates (0.88), orthophospates (0.63), potassium (0.64), sulphates (0.64) and BOD (0.75).

Discussion

In the present study, at least 34 clusters of 16S rRNA sequences representing typical freshwater bacteria were identified from among 689 sequences from different freshwater clone libraries

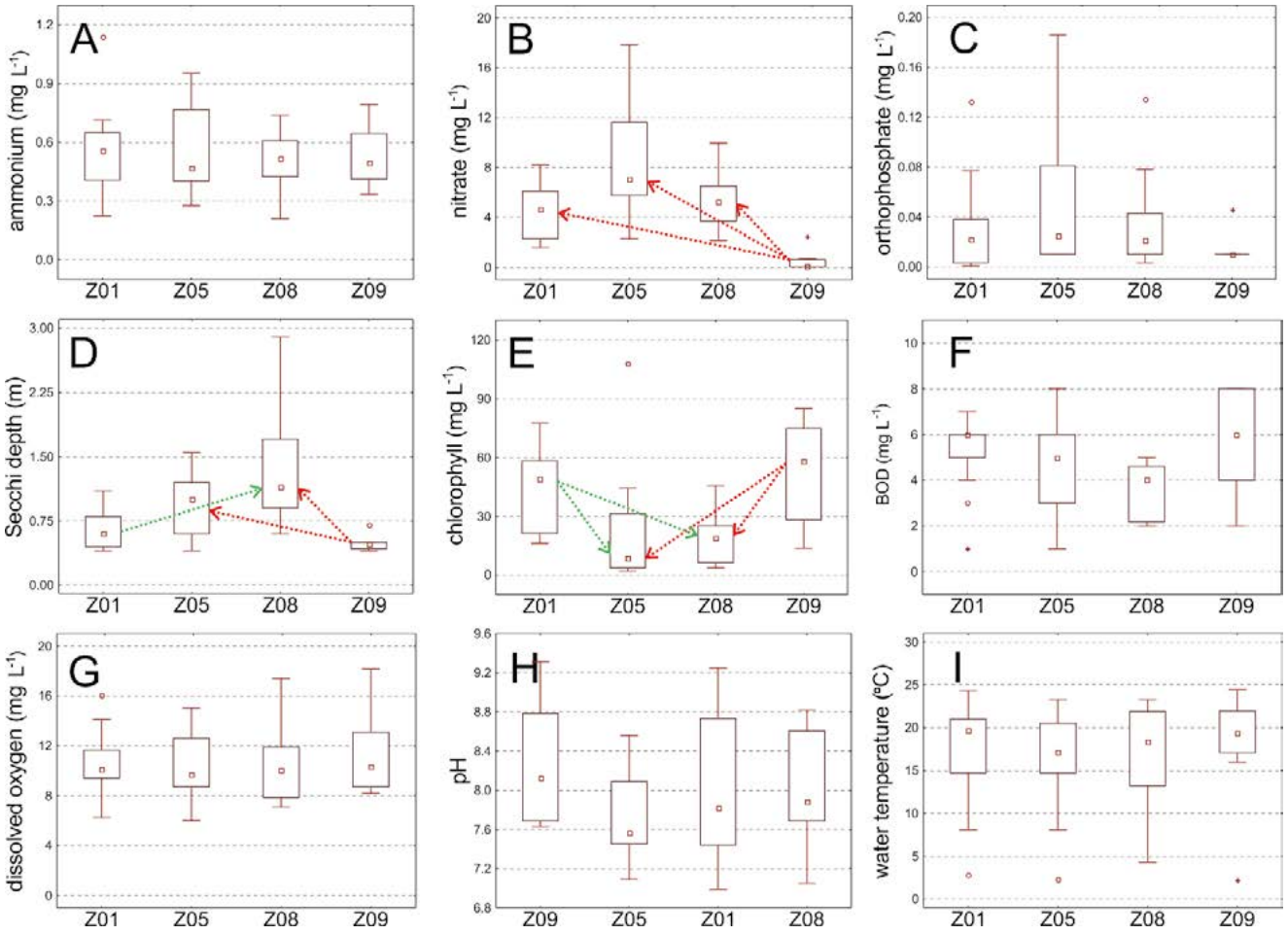

Fig. 5. Range of physicochemical indices of water quality in the Goczałkowice Reservoir on Z01–Z09 sites (data cumulated from all seasons): A – Ammonium; B – Nitrate; C – Orthophosphate; D – Secchi depth (visibility); E – chlorophyll; F – Biological Oxygen Demand (BOD); G – Dissolved Oxygen (DO); H – pH; I – Water surface temperature. The median is marked as a small square inside the box, which corresponds to a range between the 1st and 3rd quartile, while a whisker indicates the minimal and maximal value. The rings on the outside stand for outliers. Significant differences between the sites are indicated with arrows (Kruskal-Wallis Test, statistically significant differences P < 0,05).

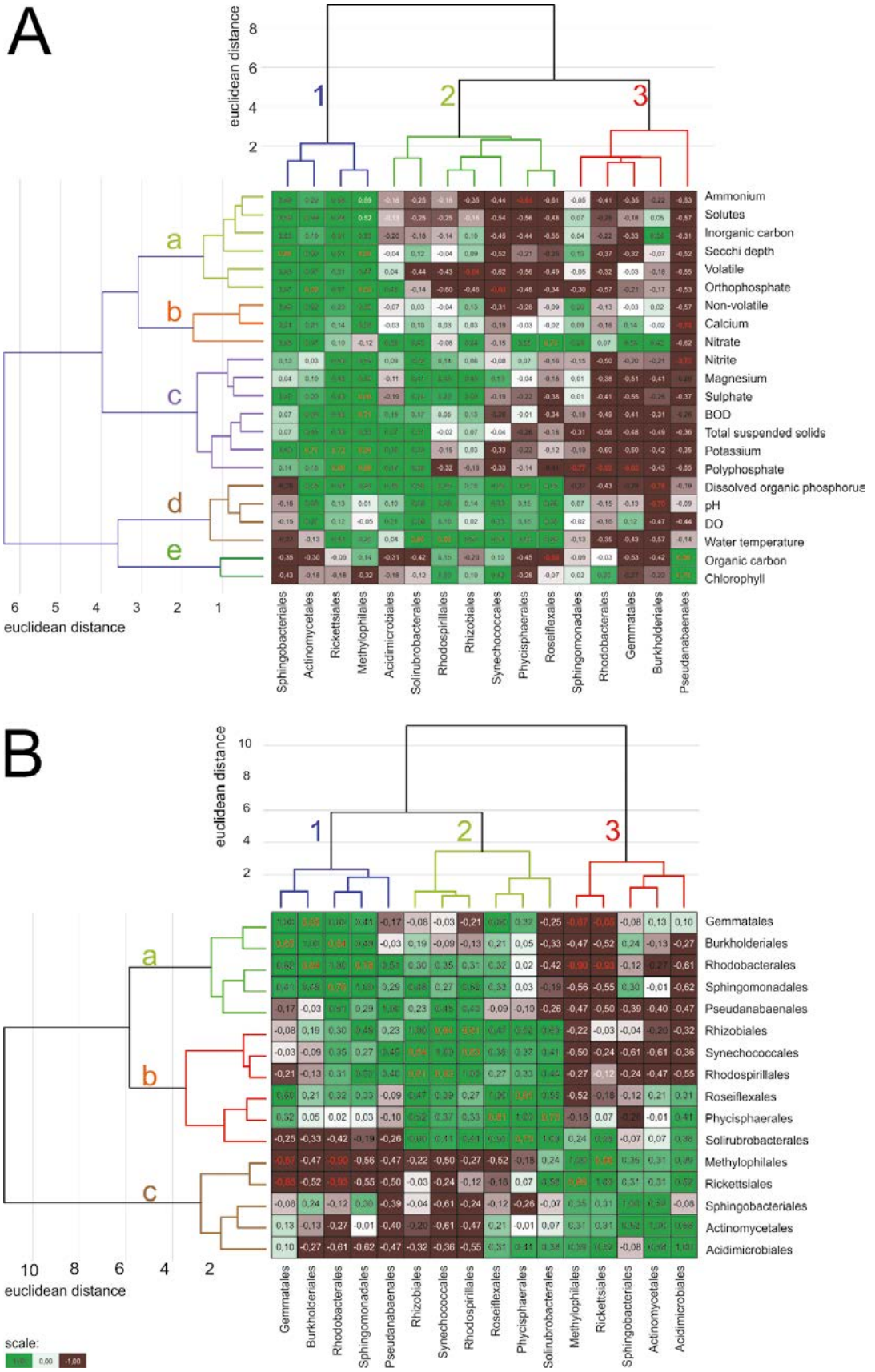

Fig. 6. Ecological correlations of the selected orders of microbes: A – relations among physicochemical indices and orders in assemblages/communities; B – inter-taxa relations among the same orders in assemblages/communities. Dendrograms based on agglomerative hierarchical clustering, similarities were determined using the Euclidean distance method. Correlations based on Spearman's rank correlation. Positive correlations are shown in green, negative correlations are shown in brown; increasing color intensity indicates increasing correlation strength. Statistically significant correlation values are marked in red ($P < 0.05$).

that were included (Zwart et al., 2002) we analyzed the available database of 16S rDNA sequences from freshwater plankton, including 24 new sequences from Parker River (Massachusetts, USA. Most of these freshwater clusters were affiliated with *Cyanobacteria*, *Bacteroidetes*, α, β and γ-*Proteobacteria*, *Actinobacteria* and *Verrucomicrobia* (Eiler & Bertilsson, 2004). In our study, the taxa that dominate in the GR were represented by *Alphaproteobacteria – Pelagibacter*; *Betaproteobacteria – Commamonadaceae*, and *Cyanobacteria – Synechococcus*.

Correlation of dam reservoir microbiome with physicochemical and land use data

Relations between the state of the reservoir water and the land cover are postulated. Statistical analysis of reservoirs in Poland showed a significant positive correlation between artificial surfaces and total phosphorus variability (Absalon et al., 2020). However, the concentrations of 'biogenic' nitrogen and phosphorus compounds in the GR did not show noticeable values that could stimulate primary production within the reservoir and thus alter the structure of the trophic chains and promote heavy algal blooms. The high concentration of nitrogen and phosphorus in water is considered a typical cause of changes in the structure of phytoplankton communities and harmful blooms of either cyanobacteria or dinoflagellates (Smith et al., 2006). However, the conditions for the primary production of cyanobacteria, although mainly attributed to the availability of biogenic elements as well as temperature, appear to be much more complex. As it was experimentally proved, it also depends on the presence of microbes other than cyanobacteria (Jankowiak et al., 2019). The balance between primary production of phytoplankton and the depletion of available biogenic compounds is illustrated in the case of the lowest levels of nitrogen and phosphorus, which was observed at site Z09 where nitrate concentration was significantly different from other sites, $P < 0.0001$, while the same site was characterized by the lowest Secchi depth (about 50 cm) and the highest concentration of chlorophyll-a and organic substances in the water (for which BOD is an estimate). Based on the obtained results, the GR can be classified, due to their long water retention time (182 days), between mesotrophic and eutrophic lakes.

Ecological value and conservativeness of microbial community structure

Our study confirmed the results presented in other papers (Glockner et al., 2000; Percent et al., 2008) that the bacterial communities of freshwater limnetic ecosystems are dominated by *Actinobacteria*, followed by *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*. The general structure of the bacterial communities, which were isolated from various significantly diversified aquatic systems, appears to be conservative in contrast with the results obtained for other natural bacterial communities such as rivers (Beier et al., 2008). However, our study revealed an important seasonal variability in the relative participation of the microbial taxa in the overall structure of the local microbiome. Interestingly, this seasonal variability concerns the occurrence of *Actinobacteria*, *Betaproteobacteria* (*Commomonadaceae*), and eukaryotic *Cryptophyta* (detected as plastid 16S rDNA) and may reflect interactions among the various components of bacteria- and phytoplankton, including signs of various types of blooms.

Despite the mechanisms of changes in the structure of microbial populations, the first signs of environmental changes will be recorded at the level of the abundance and density of microbes. The physical and chemical properties of a tributary and, as a result, the waters in the main basin are going to have a decisive impact on the microbial communities in a dam reservoir. Although the analyzed microbial communities demonstrate a relatively high conservativeness in the taxonomic structure, simultaneously, the participation of particular taxa is variable, reflecting the temporal changes in environmental conditions. These changes, which were correlated with the detection of highly specific marker species, may be the basis for the effective monitoring of the condition of dam reservoirs.

Distribution and ecological role of the identified microbial taxa, markers of water quality

The rapid lifecycle and metabolism of bacteria may result in a higher ecological significance of a microbiome in a water basin than expected based on the microbial biomass. One possible way to investigate the structural relationships of microbial communities in water basins and their ecophysiological functions is the analysis of the correlations of the abundance and density of microorganism species and the hydrochemical indices and quality markers of water. The results obtained by Miller in hot springs (Miller et al., 2009) suggest that particular microbe species are distributed along isotherms and their abundance is unrelated to the concentration of nutrients or trace elements. These observations demonstrate a simple relationship to the primary limiting factor (according to Liebig's law of minimum or the Shelford tolerance law), but does not exclude more complex processes. Particular microbial species are characterized by various ecological requirements because they are either autotrophs and chemoautotrophs or heterotrophs (Arora-Williams et al., 2018) with devastating economic and ecological consequences. Microorganisms deplete oxygen during biomass decomposition, degrading the habitat of many economically important aquatic animals. Microbes then turn to alternative electron acceptors, which alter nutrient cycling and generate potent greenhouse gases. As oxygen depletion is expected to worsen with altered land use and climate change, understanding how chemical and microbial dynamics impact dead zones will aid modeling efforts to guide remediation strategies. More work is needed to understand the complex interplay between microbial genes, populations, and biogeochemistry during oxygen depletion. Results: Here, we used 16S rRNA gene surveys, shotgun metagenomic sequencing, and a previously developed biogeochemical model to identify genes and microbial populations implicated in major biogeochemical transformations in a model lake ecosystem. Shotgun metagenomic sequencing was done for one time point in Aug., 2013, and 16S rRNA gene sequencing was done for a 5-month time series (Mar.-Aug., 2013 and produce a wide range of characteristic secondary metabolites that are then consumed by organisms from higher trophic levels. Unique metabolic processes such as the light-driven proteorhodopsin proton pump-based ATP synthesis are features of particular taxa such as marine *Pelagibacter*, *Vibrio* and *Flavobacteria* (DeLong & Béjà, 2010). The changes in the

microbiome of the reservoir may disrupt the homeostasis and, in result, may change the character of the reservoir. In addition, the instantaneous state of microbial communities and the entire environment of a water basin should be reflected by an abundance of several marker taxa that are the most sensitive to particular environmental conditions. These taxa are candidates to be fast and effective biomarkers of the condition of a water reservoir that can be used as early warning indicators.

Correlation analysis revealed a representative group of microorganisms that may be potential bioindicators of the environmental state. The order *Methylophilales*, whose abundance correlated with six physicochemical parameters (polyphosphate, BOD, Secchi depth, potassium, sulphate, orthophosphate) presented crucial importance (Figure 6 A).

Metataxonomic analyses of the water samples allowed for the identification of the chloroplast 16S rDNA of eukaryotic algae from the phylum *Cryptophyta*. *Cryptophyta* form a symbiosis with bacteria and thus are considered to be mixotrophs. Their abundance and importance in an aquatic environment mean that they can be readily detected and quantified.

Based on the obtained results, bioindicator features can be also attributed to the abundance of orders dependent on at least two physicochemical parameters: *Burkholderiales, Pseudoanabenales, Rickettsiales, Roseiflexales, Methylophilales, Actinomycetales,* and the phylum *Cryptophyta*. Microbes representing these orders, which are typical representatives of the GR microbiome, are sensitive to the environmental changes that were studied.

The genus *Synechococcus*, which is a representative of oxygenic, photosynthesizing cyanobacteria and which was extremely abundant in the GR, may serve as a candidate for a convenient bioindicator species since their cells contain phycoerythrin, a fluorescent pigment. This feature allows them to be distinguished from other picophytoplanktonic species under epifluorescence microscopy or flow cytometry (Chorus & Bartram, 1999).

The distribution of *Cyanobacteria* and both green and red *Chloroflexi* that was observed in the GR suggests an important role for biotic interactions in community organization although not in the manner that was predicted by the coadaptation hypothesis (Miller et al., 2009). Some interspecies interactions among *Cyanobacteria* were also observed between the population density of *Synechococcus* and *Mastigocladus*, which was much lesser than the one determined by the thermal performance curves of strains that had been grown separately in laboratory (Miller et al., 2009).

The order *Burkholderiales* (*Betaproteobacteria*) was the most prevalent group of bacteria in the GR with *Comamonadaceae* and genus *Limnohabitans* being the dominant family. The notable metabolic versatility of these bacteria that has been described (Hahn et al., 2010; Kasalicki et al., 2010; Zeng et al., 2012) demonstrates that they are responsible for autotrophic carbon fixation, nitrogen cycle and sulphur oxidation. The relatively large genome of *Limnohabitans*, which consists of 3,500 kbp, was shown to encode the genes responsible for photosynthesis, CO_2 uptake, as well as the oxidation of sulphur and ammonia (Hahn et al., 2010; Zeng et al., 2012).

Rickettsiales from the group LD12, which is a freshwater counterpart of the marine-specific group SAR 11 (constituting more than 25% of marine picoplankton (Logares et al., 2010) were observed in the GR at levels higher than 10% at sites Z08 and Z01 in 2010 (class *Alphaproteobacteria*). *Rickettsiales* were regarded as parasitic or mutualistic organisms (Fredricks, 2006), but recent reports have shown that some of them such as maritime *Rickettsia* SAR 11 (*Pelagibacter*), which are found in the Sargasso Sea as well the freshwater group LD12, are free-living (Ghai et al., 2011) and may constitute a major part (0.25 or even 0.5) of an entire microbiome. Several differences at the genome level among groups SAR11 and LD12 are primarily related to adaptive changes (Logares et al., 2010). These bacteria contain a rhodopsin-based phototrophic system, which is responsible for their photoheterotrophy (Fuhrman et al., 2008), however, they are frequently treated as auxotrophs. The growth of such microorganisms is characterized by periods of carbon starvation (Fuhrman et al., 2008). Due to these metabolic features, *Rickettsiales* are characterized by an oligotrophic lifestyle with an efficient but slow uptake of dissolved organic carbon (Salcher et al., 2011). These microorganisms appear to contribute substantially to secondary production (as measured by the rate of leucine incorporation) and participate in the assimilation of dissolved organic matter substrates such as amino acids, protein, dimethylsulfoniopropionate and glucose (Malmstrom et al., 2005; Vila-Costa et al., 2006). Their notable abundance in the selected sites of the GR is related to the thin euphotic zone.

The order *Sphingobacteriales* (Phylum *Bacteroidetes*), which is capable of producing sphingolipids, was poorly represented (< 10%) in the GR with a maximum at sites Z05 and Z08 (in April 2011 and July 2010, respectively). The abundance of *Sphingobacteriales* was correlated with the abundance of *Burkholderiales*, *Rhizobiales*, *Rhodobacterales* and *Stramenopiles* and also with low concentrations of ammonia, nitrates, orthophosphates, potassium and inorganic carbon. The observed abundance of *Sphingobacteriales* was different than that reported in the metataxonomic analyses of water from lakes Limmaren, Ekoln, Erken and Vallentunasjön, where this order participated to about one third of the bacterial clones in the 16S rDNA libraries (Cottrell & Kirchman, 2000; Eiler & Bertilsson, 2004; Glockner et al., 2000).

The approach to utilize metataxonomic analyses for the assessment of microbial assemblages in aquatic ecosystems is not common in the literature. However, it is the only method to determine the structure of populations of various groups of microorganisms, including uncultivable ones. Metataxonomic gives the unique ability to identify the taxa with no necessity to cultivate them. Therefore, this way it is possible to indicate the taxa of a specific character, e.g., sensitivity to external conditions. The knowledge about the microbiome, combined with the data on the type of metabolism of individual groups of bacteria, provides information about the potential of the reservoir to process nutrients by this (often overlooked) trophic level.

Concluding, metagenomic analyses of the freshwater microbiome in a model dam reservoir that is classified as limnetic and eutrophic/mesotrophic enabled us to confirm the hypothesis that microorganisms constitute a conservative community that undergoes seasonal and local changes in respect to the relative participation of the identified taxa. The taxonomic structure, relative participation of identified taxa and abundance of specific

bioindicators can be regarded as additional reliable tools for the monitoring of water dam reservoirs and ecological balance. This monitoring may be based on the detection of individual elements/ions/chemicals, giving the image of potential pressing factors, or identifying specific microorganismal groups – bioindicators. The use of bacterial assemblages refers to the second option, i.e., a taxon's occurrence or not. The method can be used with other tools to monitor ecological stability (e.g., Babczyńska et al., 2001) or to reservoirs that are transformed by anthropopressure (e.g., Kostecki, 2021). Moreover, knowing the taxons' metabolic activity, it is possible to assess the pathways predominating in the reservoirs and thus – the substances and/or nutrients that are currently processed.

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