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## Water quality and phytoplankton structure changes under the influence of effective microorganisms (EM) and barley straw – Lake restoration case study



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## HIGHLIGHTS

## GRAPHICAL ABSTRACT

- Shallow lake, restored by EM and barley straw, was under 5-year research.
- Phyto- and zooplankton structure changed in spring due to protective treatment.
- Ammonium N concentrations increased, while P content decreased during the research.
- Cyanobacteria were still dominants in phytoplankton afterwards the restoration.
- A shift from Oscillatoriacean to Nostocales in summer was noted.

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## ABSTRACT

Many lakes worldwide, especially shallow, experience great changes due to eutrophication, manifested in severe, usually toxic water blooms, disqualifying them from recreation. In order to improve water quality, restoration programs are implemented, including numerous methods. Intense nutrient cycling resulting from detrimental role of sediments impede obtaining of clear water state. One of the restoration methods proposed in recent years was Effective Microorganisms (EM), i.e. the set of microorganisms aiming at the inhibition of harmful bacteria through competitive exclusion. This approach was introduced in shallow Konin Lake (Western Poland), suffering from severe cyanobacterial water blooms. Prior to the treatment, protective action was conducted i.e. the elimination of external nutrient loads with backwater from the river. Changes in water chemistry, phytoplankton structure and macrophytes distribution were noted during the 5-year studies (2011–2015), covering the treatment (2013–2015) as well as two previous years. Oscillatoriacean cyanobacteria were most abundant in (2011–2012), while Nostocales in summer 2014–2015, as a result of decreased phosphorus but increased microgen concentrations. Slight increase in Cladoceran zooplankton was observed, but none in submerged macrophytes due to low water transparency. EM application initiated positive changes in the ecosystem by means of excessive organic matter decomposition and increased diversity of phytoplankton, nevertheless cyanobacteria blooms were still present due to high nutrient content.

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## 1. Introduction

Restoration is mostly conducted on lakes with severe cyanobacterial blooms, hampering ecological functioning of the ecosystem as well as human use of lake resources (fishing, angling, swimming, resting, sunbathing, etc.). Over the years numerous methods for the lake restoration, both technical, chemical and biological, have been proposed, aiming at obtaining a clear water state (Perrow and Davy, 2002, Klapper, 2003, O'Sullivan and Reynolds, 2005,). Phosphorus inactivation, hypolimnetic oxygenation and biomanipulation are among the most commonly used (Søndergaard et al., 2007; Gołdyn et al., 2014; Dondajewska et al., 2019). At first, applications were intense and drastically interfered with the ecosystem, consisting of e.g. sediment dredging or phosphorus precipitation with large quantities of coagulants (Klapper, 2003), but currently more eco-friendly applications, concerning the preservation of biodiversity, gained more attention of lake restoration managers (Angeler et al., 2014).

Effective microorganism (EM) application was proposed for in-lake restoration as an environmentally friendly and cost-effective (Zakaria et al., 2010). The concept of EM was primarily developed by Dr. Teruo Higa in Japan (Higa, 1998), who proposed a consortium of lactic acid bacteria, phototrophic bacteria, actinomycetes, yeasts and fermenting fungi, as a solution to a wide suite of waste and wastewater problems. This set of microorganisms is supposed to act synergistically to inhibit the growth of pathogenic and harmful bacteria through competitive exclusion, resulting in the dominance of beneficial species (Jóźwiakowski et al., 2009; Lürling et al., 2016). Initially, EM were used in agriculture to e.g. improve vegetable production (Daly and Stewart, 1999) or change the character of slurry manure (van Vliet et al., 2006). Simultaneously, EM were used in sewage management, mainly to reduce the volume of sludge and shorten the aeration time (Jin et al., 2005; Monica et al., 2011; Namsivayam et al., 2011; Grabas et al., 2016), and to diminish the odour emission from waste (Chen et al., 2003; Sekeran et al., 2005). Application during dairy wastewater purifying resulted in a reduction of ammonium N, total P and organic matter content (Rashid and West, 2007), while an EM addition to fish ponds led to an alteration of their bacteria composition in favour of more beneficial types, together with a reduction of mineral forms of nutrients (Padmavathi et al., 2012).

The successful improvement of water quality in the aforementioned cases encouraged EM application in lake restoration. A mixture of microorganisms is added to lake water as a liquid solution by spraying the water surface or in a form of mudballs. The latter are made of clay or soil, kneaded into a tennis sized ball, sprayed with liquid EM and dried for <del>ca</del> a week. Additionally, Bokashi, i.e. fermented organic matter, is sometimes added to this ball (Zakaria et al., 2010). The mud ball serves as a carrier for EM, allowing them to be directed into sediments. In the case of lake restoration, its composition and the diversity of the microbial community in the ball are crucial (Park et al., 2016).

Ganesh (2008) proposed EM application for small and mid-size waterbodies, reporting a reduction in nutrient concentrations in some reservoirs in Hungary and India, nevertheless, no inhibiting impact on cyanobacteria algae was noted. Zakaria et al. (2010) reported EM mudball success in water quality improvement in Malaysian rivers, however, scarce data are available to confirm this. Short term research of EM efficacy in shallow pond restoration was presented by Jóźwiakowski et al. (2009). Liquid EM produced by SCD Probiotics Technology was sprayed on the surface of a 0.11 ha pond fed by sewage partially purified by constructed wetland. As a result, the concentration of nutrients (ammonia N, total N, total P) decreased as well as chlorophyll-a, nevertheless, no detailed data on changes in phytoplankton structure were reported. A longer period was covered by the research of Sitarek et al. (2017) in the Muchawka Reservoir in Poland (40 ha), where liquid EM solution (16,000 L in two stages) was added to the surface water and to the water column by motor pump. Concentrations of mineral nutrients after the restoration were slightly lower, and the bacteriological state of the reservoir was stated as 'excellent'. Further results might have been hampered by the inflow of elevated nutrient content with water from the River Muchawka.

The inefficacy of EM in lake restoration was proved by Padisák (2014), who applied both mudballs and liquid material to a garden pond, observing an increase in orthophosphates and diminished transparency. The desired inhibiting impact of EM on cyanobacteria was analyzed in laboratory conditions, but no such effect was proved. Strains of *Microcystis aeruginosa* were hampered only by large EM mudbull doses as a result of increasing turbidity. Additionally, water enrichment with nutrients and metals (Al, Cd, Cu, La, Pb) was noted, which raised questions as to the effectiveness of this treatment as a restoration method (Lürling et al., 2009, 2010). Similar analyses were conducted by Dunalska et al. (2015), resulting in no changes in concentration of dissolved oxygen, conductivity, phosphates, total phosphorus or total nitrogen.

Considering all the above, conclusive data are lacking on water chemistry and phytoplankton structure reaction to EM restoration. Therefore, this multiannual research of a shallow lake, in which mudballs and liquid EM were applied, was undertaken in order to verify the following study hypothesis on its positive impact in lake water quality.

Additionally, barley straw floating bales were installed to inhibit cyanobacteria proliferation. It has been known for over 30 years that rotting barley straw can be used to prevent the development of cyanobacteria water blooms in freshwater bodies due to the liberation of a mixture of compounds as a result of lignin decomposition e.g. polyphenols (Iredale et al., 2012). Practical results of barley straw impact on phytoplankton have been observed i.a. in the UK (Everall and Lees, 1996; Barett et al., 1999) and in the USA (Brownlee et al., 2003). Apart from applying restoration methods to the studied lake, a protective measure was also used, i.e. the reduction of nutrient rich backwater from the downstream river. Thus, the aim of our research was to determine the changes in water chemistry and phytoplankton community structure as a result of these three factors. As expected, any changes in water transparency might influence macrophyte distribution, especially the case of submerged plants, so we also assessed changes in macrophyte cover throughout the analyzed period.

#### 2. Material and methods

## 2.1. Study site

Lake Konin, situated in the Lubuski Lake District (mid-western Poland) belongs to the group of small and shallow lakes with no thermal stratification throughout the year. It covers 88 ha and its maximum depth reaches 4 m, mean – 3.1 m. The lake is adjacent to the River Obra – a medium-size lowland river with a length of 164 km, being a left tributary of River Warta. The river catchment of 2758 km<sup>2</sup> was heavily modified by drainage treatment in the XIX<sup>th</sup> century, resulting in a complex water network, collecting surface and subsurface waters mainly from an agricultural area (Słowik, 2013). As a result river water is strongly eutrophic with severe cyanobacterial blooms in summer (Budzyńska and Dondajewska, 2017). Thus, despite feeding Lake Konin only as a backwater during high water level periods, mainly in the early spring, the River Obra has exerted a decisive impact on lake water quality. Consequently, protective measures were taken in 2013, creating a new dike with a closing device, due to which backwater was eliminated (Fig. 1). Additionally, the lake has a small, periodical tributary, flowing from east through a forest area (Brodzińska et al., 2010).

The lake catchment covers 259 ha; it consists of with moraine hills with large denivelations reaching up to 22.7 m. The main land use is coniferous forest (77%), while arable lands are situated at some distance from the lake (23% of catchment). There are no point sources of pollution as the holiday resort and lumber mill situated by the lake are equipped with septic tanks (Brodzińska et al., 2010).



Fig. 1. Location and bathymetry of the studied lake. Black dot indicates the sampling station (after: Brodzińska et al., 2010, changed).

Due to its low water depth and volume, increasing the impact of sediments on water quality, Konin Lake is very susceptible to degradation. Thus, protective and restoration measures are crucial to improve the water quality, especially in the light of its recreational use (swimming, angling). As a first step, the backwater of the Obra River was eliminated by reconstruction of the dike in 2013. Restoration treatment was conducted in 2014 by means of EM, applied as mudballs in spring (35 thousand), and a liquid solution of probiotics in summer and autumn (2–5.5 L ha<sup>-1</sup>). Additionally, barley-straw floating bales were distributed along the shore line in summer 2014 and removed in autumn.

#### 2.2. Field and laboratory methods

The study of the Lake Konin was conducted monthly from April to November for 5 years from 2011 to 2015. Water samples were taken in depth profile each meter (from the surface to 2 m) at the deepest place of the lake (Fig. 1). Physico-chemical and biological variables were assessed according to Polish standards (Elbanowska et al., 1999): water temperature, Secchi depth, electrolytic conductivity, pH, soluble oxygen concentration, water saturation with oxygen, nitrogen (ammonium, nitrite, nitrate, organic, and total), phosphorus (orthophosphates and total), chlorophyll *a* and suspended solid (TSS) concentrations.

Water samples for phytoplankton analyses were collected each meter in the studied depth profile, distributed to the dark bottles and immediately fixed with Lugol's solution. Qualitative and quantitative analyses of phytoplankton were done using a light microscope after sedimentation in phytoplankton chambers, according to Utermöhl's procedure (Wetzel and Likens, 1991). As a unit for the phytoplankton abundance, the number of organisms (cell, colony or trichomes with length of 100 µm) per mL were used. The participation of a minimum of 10% to the total phytoplankton biomass was chosen as dominating

species. Water samples for zooplankton were collected sieving 10 Liters of water through a plankton net of 40  $\mu$ m mesh size. The samples were preserved with Lugol's solution. Zooplankton abundance was assessed using a light microscope.

Macrophyte composition and distribution was analyzed in summer each year. Plant communities were studied with the use of mid-European phytosociological method by Braun-Blanquet (1964). The occurrence and size of patches of plant communities were marked using GPS. The presence of submerged associations was checked with a weed anchor. The surface of small patches of vegetation was estimated in situ, and larger patches were calculated using ArcGIS. The abundance of associations was determined based on the size of the area occupied by phytocoenoses in square meters following the method of Kolada and Ciecierska (2008). The obtained results were used to calculate the Ecological State Macrophyte Index (ESMI) according to the formula given by Kolada et al. (2014).

#### 2.3. Statistical methods

Basic statistical calculations were made using STATISTICA 10.0 software. Median and extreme values (min-max) were used as a descriptor of variables due to its resilience to outliers in a data set. The nonparametric Kruskall-Wallis test was used to determine the significance of value changes throughout the analyzed period as well as year to year with post hoc analysis.

To relate the species data set to the environmental variables a redundancy analysis was calculated using CANOCO 4.5 software. The decision to carry out a redundancy analysis was taken after a preliminary detrended correspondence analysis (DCA), which determined a gradient length of 4.981 for the first ordination axis. Such a gradient for the first ordination axis indicates CCA analysis as the most appropriate technique for the species-environmental parameter relationship (Lepš and Šmilauer, 2003).

## 3. Results

#### 3.1. Lake water chemistry

Water temperature varied from minimally <3 °C in autumn 2011 to maximally over 25 °C in summer 2013–15. Median values were slightly lower in 2011–13 in comparison to 2014–15 (Table 1). Water pH usually exceeded 7.5, reaching maximally over 9.0 in 2011–2012 and 2015. Conductivity increased up to 400–500  $\mu$ S cm<sup>-1</sup> in spring, decreasing in the following months below 300  $\mu$ S cm<sup>-1</sup>. Oxygen concentration medians were similar in all the analyzed years, varying from 8.7 to 10.5 mgO<sub>2</sub> L<sup>-1</sup>, resulting in oxygen saturation 95–108%. Minimum values – <1 mgO<sub>2</sub> L<sup>-1</sup> i.e. *ca* 10% – were noted periodically in summer near the bottom, and maximum – over 15 mgO<sub>2</sub> L<sup>-1</sup> (up to 193%) usually in spring and autumn at the surface.

Secchi depth was <1 m throughout the analyzed period. Lowest values were noted in 2012 - <40 cm. An increase appeared in April-June 2014 and 2015 up to 90 cm, however, in late summer it was diminished again to 30 cm (Fig. 2A). These changes were determined by phytoplankton proliferation influencing chlorophyll-a content in water. In 2012 phytoplankton abundance increased over 70,000 org mL<sup>-1</sup> (Fig. 2C), resulting in chlorophyll-a concentration over 100  $\rm mg~m^{-3}$ (Fig. 2B). The variability in chlorophyll-a concentrations was especially noticeable in 2015 – it decreased to ca 20 mg m<sup>-3</sup> in surface waters in May and June, but boosted again up to 297 mg m<sup>-3</sup> in August (Fig. 2B). Its changes were related to total phytoplankton abundance, ranging from <20,000 org mL<sup>-1</sup> in May to almost 60,000 org mL<sup>-1</sup> in July. TSS changed within wide limits in all analyzed years, but the highest variability was noted in 2011 and 2013, i.e. in the years when the inflow of water from the River Obra was the most significant, and in 2015, when a high variability of chlorophyll-a content was noted (Fig. 2D).

Among mineral N forms ammonium was dominating, varying from 0.65 to 2.02 mgN-NH<sub>4</sub> L<sup>-1</sup>. Both minimum and maximum values were noted in 2015, characterized by the highest variability of ammonium N content (Fig. 3A). Nitrite concentrations were low only in 2011 (<0.007 mgN-NO<sub>2</sub> L<sup>-1</sup>), increasing in the following years maximally up to 0.046 mgN-NO<sub>2</sub> L<sup>-1</sup>. Nitrate N content was low, varying from 0.048 to 0.437 mgN-NO<sub>3</sub> L<sup>-1</sup>. Thus, ammonium N exerted the greatest impact on mineral N concentrations, ranging from 0.70 to 2.41 mgN L<sup>-1</sup> (Fig. 3B).

Due to intense phytoplankton proliferation organic N concentration in Konin Lake water was high – the median ranged from *ca* 2.0 mgN  $L^{-1}$ in 2011 and 2014 to almost 3.0 mgN  $L^{-1}$  in 2012 (Fig. 3C). Each year the

Annual variability of basic la	ke water characteristics.
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Year	Value	Temperature [°C]	рН	Conductivity [µS cm <sup>-1</sup> ]	Oxygen concentration [mgO <sub>2</sub> L <sup>-1</sup> ]	Oxygen saturation [%]
2011	Median	16.6	-	395	10.2	105
	Min	2.7	7.54	314	0.9	10
	Max	22.3	9.33	597	22.7	193
2012	Median	18.7	-	337	10.4	108
	Min	10.1	7.98	280	1.6	18
	Max	22.4	9.46	395	15.9	183
2013	Median	18.8	-	432	8.8	101
	Min	5.1	7.10	392	0.5	6
	Max	25.3	8.74	559	18.7	175
2014	Median	20.4	-	312	10.5	106
	Min	10.6	7.59	262	1.3	15
	Max	25.5	8.92	395	13.3	133
2015	Median	20.3	-	311	8.7	95
	Min	8.1	7.92	245	0.3	4
	Max	25.2	9.51	411	15.1	178

greatest values were noted on the verge of summer and autumn (August–October), reaching maximally over 4.5 mgN  $L^{-1}$  in 2012 and 2015. Total N medians were over 3.0 mgN  $L^{-1}$  (Fig. 3D), exceeding 4.0 mgN  $L^{-1}$  in 2012 and 2015. The final year was characterized by high variability of organic and total N content.

The amounts of orthophosphates increased in 2012–2013 maximally up to 0.21 mgP  $L^{-1}$ , while the medians were 0.100–0.135 mgP  $L^{-1}$ . Orthophosphate content decreased below 0.12 mgP  $L^{-1}$ , with a median of <0.06 mgP  $L^{-1}$  in 2014–2015. In the case of total P, the highest values were noted in 2011 – up to 0.72 mgP  $L^{-1}$ , with a median of 0.21 mgP  $L^{-1}$  (Fig. 4B). The variability of concentrations decreased in 2012–2015, and the medians were diminishing from 0.186 to 0.147 mgP  $L^{-1}$ .

## 3.2. Phytoplankton

Cyanobacteria were the most abundant phytoplankton taxonomical group in 2011–2013. Diverse species composition was noted only in May 2011, but from June the abundance of cyanobacteria began to increase gradually, reaching almost 93,000 org mL<sup>-1</sup> in October. Throughout 2012 the number of individuals of cyanobacteria exceeded 69,000 org mL<sup>-1</sup> (Fig. 5), while total phytoplankton abundance exceeded 100,000 org mL<sup>-1</sup>. Planktothrix agardhii was the most abundant in both years, accompanied by Pseudanabaena limnetica and Limnothrix redekei (Table 2). Its abundance decreased by half in 2013, but this group still dominated over others throughout the year, and Planktothrix agardhii was most common. A change in phytoplankton composition was noted in 2014. Cyanobacteria abundance decreased to <10,000 org mL<sup>-1</sup> in April and June, and was even almost totally eliminated in June, shortly after the application of EM. Nevertheless, this alteration was of short-term character and from August till October 2014 cyanobacteria became dominant among phytoplankton, mainly representatives of Aphanizomenon gracile and Raphidiopsis raciborskii (syn. Cylindrospermopsis raciborskii) (Table 2). Similar changes in phytoplankton composition were noted in 2015, and cyanobacteria had already started to proliferate intensively by July, reaching maximally over 50,000 org mL $^{-1}$  in August.

Haptophyte and green algae abundance remained low in 2011–2013, with a maximum number of <5500 org mL<sup>-1</sup>. A significant increase was noted in spring/early summer 2014 and 2015 up to 63,750 org mL<sup>-1</sup>. in case of haptophyte and 19,000 org mL<sup>-1</sup> for Chlorophyta (Fig. 5). Abundancies decreased in late summer and autumn as the cyanobacteria proliferated, mostly due to *Desmodesmus communis*, *D. spinosus*, *Monoraphidium contortum*, *Oocystis lacutris*, *Tetraedron minimum*, *Tetradesmus lagerheimii* and *Chrysochromulina parva*.

Diatoms benefited from the reduction of cyanobacteria in spring and early summer of 2014 and 2015, thus their abundance reached maximally almost 6600 and 8500 org mL<sup>-1</sup>, respectively. *Nitschia acicularis* and *Nitschia* sp. were the most abundant as well as centric diatoms. The number of cryptophytes varied year-to year, however, a slight increase was noted. The highest abundances were observed in spring 2011, 2013 and 2015 – over 4600 org mL<sup>-1</sup>, while the most abundant representative was *Rhodomonas lacustris*. Euglenophyceae, Dinophyceae, Xantophyceae and Conjugatophycea consisted of other phytoplankton groups with the lowest abundance in Lake Konin. They were the most abundant only in 2015, exceeding in June and July 400 org mL<sup>-1</sup> (Fig. 6F).

## 3.3. Zooplankton

Great variability in total zooplankton abundance was noted in 2011, with numbers over 40,000 ind  $L^{-1}$  in June, nevertheless, the medians remained similar to those noted in the following years (Fig. 6A). In 2012–2015 maximum zooplankton abundance was <20,000 ind  $L^{-1}$  and the median varied from almost 3000 ind  $L^{-1}$  in 2015 to over 10,000 ind  $L^{-1}$  in 2014. The most abundant (Fig. 6B) rotifers taxa



Fig. 2. Annual changes of water transparency (A), chlorophyll-a concentration (B), the abundance of phytoplankton (C) and suspended solid content (D) in Lake Konin.

were: Anuraeopsis fissa, Keratella cochlearis, K. cochlearis f. tecta, Brachionus angularis, Pompholyx sulcata, Polyarthra dolichoptera.

Cladoceran abundance decreased gradually from 2011 till 2013 by means of median (from 172 to 13 ind  $L^{-1}$ ) as well as min-max values (Fig. 6C). A significant increase was noted in spring 2014 and 2015, up to almost 2100 and 1600 ind  $L^{-1}$ , respectively. The most abundant cladocerans were *Bosmina longirostris*, *B. coregoni*, *Chydorus sphaericus* and *Daphnia cucullata*. Copepod abundance remained low throughout analyzed period with medians varying from 285 ind  $L^{-1}$  in 2013 to 400 ind  $L^{-1}$  in 2011. Maximum values, exceeding 1200 ind  $L^{-1}$  were observed in spring 2011 and 2015 (Fig. 6D). Most abundant were juvenile forms (nauplii and copepodites).

## 3.4. Macrophytes

The total macrophyte area increased gradually from 2011 to 2013 up to over 65,000 m<sup>2</sup>, and then diminished in 2014–2015. Helophytes were mainly observed in Lake Konin. *Typha angustifolia* was the most common, accompanied by *Phragmites communis* and nymphaeids *Nuphar luteum* and *Nymphea alba* (Table 4). The area covered by other helophytes was <950 m<sup>2</sup>, and only three of them were noted each year (*Schoenoplectus lacustris, Carex gracilis, Eleocharis palustre*). Only one representative of submerged macrophytes – *Ceratophyllum demersum* L. – was observed as single individuals in 2011.

The Ecological State Macrophyte Index (ESMI) varied from 0.075 to 0.177, indicating the weak ecological state of the lake. Nevertheless, the lack of submerged macrophytes downgrades the ecological status to bad.

#### 4. Discussion

## 4.1. Strong eutrophication phase (2011–2012)

During this period severe eutrophication was indicated by blooms of Planktothrix agardhii. Large populations of P. agardhii have been noted worldwide (i.a. Rücker et al., 1997, Willame et al., 2005) as well as in Poland (i.a. Stefaniak et al., 2005, Pełechata et al., 2006, Budzyńska et al., 2009, Kozak and Gołdyn, 2014, Kozak et al., 2015). Large populations are formed in temperate climates in late summer and autumn, and they are mostly observed in shallow polymictic lakes like Konin Lake, due to high shade tolerance (Dokulil and Teubner, 2000). However, its dominance over other cyanobacteria, especially Nostocales is reported to be related not only to different light and temperature preferences, but also to the strong competition at high nitrogen concentrations (Pawlik-Skowrońska et al., 2004; Stefaniak et al., 2005). Ammonium N stimulated the growth of oscillatoriaceans (Fig. 7), while the susceptibility of the lake to wind-induced water circulation explains the domination of *P. agardhii* over *Limnothrix* redekei from this group. Oscillatoriacean cyanobacteria are highly abundant in the River Obra, as confirmed for the river section below Lake Konin in 2016 (Budzvńska and Dondajewska, 2017). This explains their high numbers in the studied lake prior to the reconstruction of the dike between lake and river. It is worth mentioning, that P. agardhii is a well-known toxin-producing species, including microcystins (Rohrlack and Utkilen, 2007), thus such high abundances disqualify lake from swimming or angling, and confirm the need for protection and restoration (Grabowska and Mazur-Marzec, 2014).



Fig. 3. The variability of nitrogen concentrations in Lake Konin in 2011–2015: ammonium N (A), the sum of mineral forms (B), organic N (C) and total N (D).

This need was particularly explicit in 2012, when the maximum proliferation of phytoplankton, especially cyanobacteria was observed. This detrimental decrease of water quality was related to both nutrient concentrations and weather conditions. A correlation between cyanobacteria abundance and dissolved nutrients was confirmed statistically in 2011 and 2012 (for mineral N p < 0.01, r = 0.426, n = 36; for orthophosphates p < 0.05, r = 0.365, n = 36). Mineral nitrogen and phosphorus content in water increased, what was attributed to intense internal loading, typical for shallow lakes with a so-called 'active bottom' staying in permanent contact with epilimnetic waters (Søndergaard et al., 2003). Moreover, shallow lakes are susceptible to sediment resuspension, resulting in direct nutrient release to water column (Reddy et al., 1996; Liang et al., 2016) as well as resuspensioninduced unstable redox conditions affecting Na and P cycling. In case



Fig. 4. Annual changes in orthophosphates (A) and total P (B) concentrations.



Fig. 5. The changes in phytoplankton abundance with division into taxonomical groups.

of Lake Konin, wind force was similar (mean in April–October 9.4–10.8 km  $h^{-1}$ ) in all years, however, changes in precipitation were observed, having an impact on phytoplankton growth conditions. 2012 was characterized by lower atmospheric precipitation in comparison to 2011. As cyanobacteria prefer calm and sunny weather, the summer of 2012 was much more conducive for its rapid proliferation.

#### 4.2. Changes induced by external load reduction (2013)

Protective measures by means of dike reconstruction were introduced to Lake Konin in summer 2013, thus the negative impact of the River Obra backwaters was still observed e.g. in high conductivity and elevated mineral N and P concentrations. As a result, cyanobacteria were still dominant in the phytoplankton, although its abundance had decreased two fold. Again these changes might be related to weather conditions as high precipitation was observed in May and June (168 mm totally in comparison to 93 mm in the same period of 2012).

The backwater influenced strongly water conductivity, thus its elimination resulted in its statistically significant decrease (Kruskal-Wallis test, H = 62.79, p < 0.01, n = 102) in the analyzed period, but primarily for the years 2011 and 2013 versus 2012, 2014, 2015 (Table 3).

## 4.3. The lake restoration phase (2014–2015)

Significant alterations in phytoplankton structure began in 2014. Before the application of mudballs haptophytes were most abundant, and this increase was statistically significant (Kruskal-Wallis test, H = 20.63, p < 0.01, n = 99 and Table 3). Dominant *Chrysochromulina parva* occurred throughout the year, although it was usually noted in high densities in winter and spring (Kozak et al., 2013). In Lake Konin its dominance in early spring of 2014 and 2015 was related to the elimination of riverine backwater inflow. As proved in 2016 (Budzyńska and Dondajewska, 2017), cyanobacteria outcompeted other phytoplankton

### Table 2

Changes in the structure of dominant cyanobacteria in Lake Konin expressed as the mean annual abundance of the most numerous taxa [org  $mL^{-1}$ ].

Таха	2011	2012	2013	2014	2015
Aphanizomenon gracile Lemmermann Raphidiopsis raciborskii (Woloszynska) Aguilera, Berrendero Gómez, Kažtoraku Echopique & Salerno	2300 950	5640 520	1490 510	9880 1570	8460 10,310
Limothrix redekei (Goor) Meffert Pseudanabaena limnetica	1580 720	8830 19,860	2240 5790	1060 330	300 360
(Lemmermann) Komarek Planktothrix agardhii (Gomont) Anagnostidis & Komárek	26,660	46,020	18,500	370	170

groups in the River Obra from January to November, thus their inflow into the lake with backwaters resulted in early domination prior to 2014.

Shortly after mudball application green algae dominated the phytoplankton community (its increase was statistically significant, Kruskal-Wallis test, H = 67.54, p < 0.01, n = 99 and Table 3) and cyanobacteria were almost totally eliminated, although they were present in April. Chlorophyta proliferation was stimulated by nitrates coming from organic matter decomposition and nitrification – processes conducted by bacteria implemented with mud-balls. Diatoms and cryptophytes benefited from cyanobacteria diminish as well (Kruskal-Wallis test, H = 34.35, p < 0.01, n = 99, and H = 13.73, p < 0.01, n = 99, respectively, Table 3). Such an increase in phytoplankton species diversity under the influence of external factors fits in with the hypothesis of intermediate disturbance (Padisak et al., 1993).

Nevertheless, Cyanobacteria reappeared in July 2014, and from August again dominated in Lake Konin. At the same time ammonium N concentration increased (Kruskal-Wallis test, H = 18.00, p < 0.01, n = 102) as a result of intensive organic matter decomposition in sediments, leading to oxygen depletion and nitrification inhibition. Windinduced sediment resuspension affecting ammonia N content in lake water was observed in many studies (e.g. Müller et al., 2015; Dadi et al., 2017) under both oxic and anoxic conditions. Almost permanent deposition of fresh, easily degradable organic matter of planktonic origin results in its bacterial mineralization and ammonium N release, especially in muddy sediments (Van Luijn et al., 1999). In Lake Konin EM application increased the microbial consortia in sediments, leading to more intense organic matter decomposition. Released ammonia N was surely undergoing nitrification, nevertheless the uptake of nitrates by highly proliferated phytoplankton was so intense that persistent depletion of N-NO<sub>3</sub> in water column was observed. Increased ammonium N concentrations were usually observed during summer oxygen deficits  $(<1-2 \text{ mgO}_2 \text{ L}^{-1})$  stated already at the depth of 2 m. High ammonia N concentrations in shallow strongly eutrophic lakes were observed in Portugal, China and Poland (Abrantes et al., 2006; Chen et al., 2010; Jóźwiakowski et al., 2009). This mineral N form is willingly utilized by cyanobacteria (Blumquist et al., 1994), including R. raciborskii (Burford et al., 2016), observed as dominant in phytoplankton from August 2014, what was confirmed by CCA analysis (Fig. 7).

The years 2014–2015 were characterized by a complete change in the dominating cyanobacteria, i.e. *R. raciborskii* together with other representative of Nostolaces *A. gracile*, were much more abundant than *P. agardhii* and other Oscillatoriacean like L. *redekei*. This alteration in phytoplankton structure results from the ability of *R. raciborskii* to compete well in low-phosphate conditions (Burford et al., 2016; Ryan et al., 2017), which prevailed in 2014–2015 (median orthophosphate



Fig. 6. Annual changes in zooplankton abundance (A) and the abundance of particular groups: rotifers (B), cladocerans (C) and copepods (D).

concentrations were <0.06 mgP L<sup>-1</sup>, due to statistically significant decrease of concentrations, both for orthophosphates and total P, Kruskal-Wallis test: H = 42.30, p < 0.01, n = 102, and H = 17.88, p < 0.01, n = 102, n = 100, 0.01, n = 102, respectively). Additionally, this species can efficiently store phosphorus, thus surviving periods of low P by using up its intracellular resources and outcompeting other phytoplanktonic organisms (Istvánovics et al., 2000). Research conducted in Rusałka Reservoir in Western Poland indicated that the intense proliferation of R. raciborskii was not related to water temperature or phosphates, nevertheless these factors plays a key role prior to the bloom, stimulating the germination of akinetes (Budzyńska and Gołdyn, 2017). Such a relation was suggested by Wiedner et al. (2007), and similar 'delayed' blooms a month after high water temperature incidents were noted in Lake Balaton (Padisák and Reynolds, 1998). In the case of Lake Konin very high temperatures, reaching over 24 °C were noted in June and July 2015, while intense R. raciborskii proliferation occurred in August.

The bloom of *R. raciborskii* observed in summer 2015 extends the list of water bodies in Europe, where this expansive species, originating from tropical and subtropical regions, has been found. First reported in temperate lakes at the end of the XX<sup>th</sup> century, it has been further observed at many other locations, including Germany (Rücker et al., 2007), Poland (Kokocinski and Soininen, 2012), and even Finland (Komárek, 2013). Similarly to *P. agardhii*, this species is also known to produce toxins (de la Cruz et al., 2013), thus its abundant presence in Lake Konin eliminated this reservoir from recreational purposes. What should be mentioned, in Polish lakes as well as in Germany, *A. gracile* was usually reported as a producer of cylindrospermopsin (CYN, Rücker et al., 2007, Kokociński et al., 2013), not *C. raciborskii. A. gracile* was noted in Lake Konin as well, especially in 2014 and 2015, hence the potential presence of CYN in the studied lake would be rather related to this species.

Seasonal changes in phytoplankton structure in 2014 and 2015 were similar, but in the latter cyanobacteria gained domination over other groups as early as July, and their abundance was higher than in 2014. A very warm summer – with a particularly hot and dry August (mean air temperature 22.8 °C, the low sum of precipitation 7.1 mm) was conducive to rapid cyanobacteria proliferation. Nevertheless, throughout the research period was observed a decreasing abundance pattern of cyanobacteria (Kruskal-Wallis test, H = 48.20, p < 0.01, n = 99 and post-hoc analysis, Table 3), influencing the reduction of total phytoplankton abundance in 2013–2015 (Table 3) and, finally, resulting in a decrease of water pH (Kruskall-Wallis test, H = 22.16, p < 0.01, n = 99). It is also worth mentioning that chlorophyll-a content was much higher than in preliminary studies in 2011–2012, indicating the strong impact of two dominating cyanobacteria species on this parameter.

Neither external load reduction by dike reconstruction, nor EM mudballs application were able to alter the phytoplankton community structure on a long-term basis. The variability of cyanobacteria dominating species seems to indicate that the removal of river backwater was more consequential than restoration by means of biological methods only. EM mudballs managed to reduce the phosphorus concentrations, and partially the cyanobacteria abundance, yet increased nitrogen concentrations were conducive to other cyanobacterial species, typical for low-phosphate environments. Finally, the lake was continuously inaccessible for recreation in 2015, and remained hypertrophic. No changes in phytoplankton structure were noted as a result of the introduction of floating barley straw bales in summer 2014. This might result from the construction of the bales, which



**Fig. 7.** CCA ordination showing the distribution of the dominant species in relation to physico-chemical and biological environmental variables. Abbreviations: temperat – temperature, conducti – conductivity, Cladocer – cladocerans, *Ana. circ – Anabenopsis circulare, Aph.gra – Aphanizomenon gracile, Bacill c – centric diatoms n.det., Chr.par – Chrysochromulina parva, Cry.mars – Cryptomonas marsonii, Cyl. raci – Cylindrospermopsis raciborskii, Des. comm – Desmodesmus communis, Des. spin – D. spinosus, Dic. pulc – Dictyosphaerium pulchellum, Fra. crot – Fragilaria crotonensis, Lim.red – Limnothrix redekei, Mon cont – Monoraphidium contortum, Nit.a.cl – Nitzchia acicularis var. closteroides, Nit. acic – N. acicularis, Nit. sp. – Nitzchia sp., Och.sp – Ochromonas sp., Ooc. lacu – Oocystis lacustris, Pla.aga – Planktothrix agardhii, Pla.lim – Planktolygbya limentica, Pse. acic – Pseudanabaena acicularis, Tet. mini – Tetraedron minimum, Tet. caud – T. caudatum, Uln. acu – Ulnaria acus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)* 

consisted of tightly packed barley straw. As a result the decomposition of lignin, with the release of acting anti-cyanobacterial compounds was present only on the surface of the bales.

The almost constant phytoplankton blooms mainly consisted of cyanobacteria, but other groups were also abundant during its Table 4

Changes in the surface areas of particular macrophytes  $(m^2)$  in Lake Konin, together with ESMI values.

	Area dominated by:	2011	2012	2013	2014	2015
1	Typha angustifolia	26,094.0	44,439.0	54,964.0	40,808.0	33,493.0
2	Phragmites communis	8765.0	3018.5	4607.5	7781.0	7050.0
3	Nuphar luteum and	2221.0	3155.0	4785.0	5714.0	6613.0
	Nymphaea alba					
4	Schoenoplectus lacustris	714.0	745.0	715.0	719.0	944.0
5	Carex gracilis	27.0	98.0	210.5	303.0	344.0
6	Eleocharis palustris	270.0	70.0	215.0	300.0	204.0
7	Sparganium erectum	24.0	30.0	40.0	58.0	-
8	Typha latifolia	-	28.0	-	10.0	25.0
9	Carex acutiformis	-	2.5	15.0	-	-
10	Persicaria amphibia	3.5	-	12.0	10.0	-
11	Iris pseudacorus	2.0	-	2.0	2.0	5.0
12	Carex pseudocyperus and	-	-	135.0	-	-
	Cicuta virosa					
13	Carex riparia	-	-	-	-	10.0
14	Ceratophyllum	+	-	-	-	-
	demersum					
	Total	38,120.5	51,586.0	65,701.0	55,705.0	48,688.0
	ESMI	0.086	0.075	0.100	0.177	0.118

domination (e.g. green algae or haptophytes); they prevented the reappearance of submerged macrophytes in Konin Lake. Their appearance, even in small patches, could enhance positive water quality changes due to the important role of submerged plants i.e. in suppression of phytoplankton growth by nutrient competition or creation of refugees for zooplankton (Meijer, 2000; Kuczyńska-Kippen and Joniak, 2016). Additionally, macrophytes reduce the resuspension of sediments (James et al., 2004), hence decreasing nutrient content in the water column. Nevertheless, the sediments of Konin Lake are of semi-fluid character at its surface, that impedes rooting and further plant growth.

#### 4.4. Environmental factors vs. plankton community

Throughout the analyzed period cyanobacteria were positively correlated with mineral N and P compounds and negatively with water conductivity (Fig. 8). The constant presence of dissolved nutrients in lake water explains the good conditions for cyanobacteria growth,

#### Table 3

Parameters which differed significantly (p < 0.05 in standard font, p < 0.01 in bold) between the pairs of years in Lake Konin. Post-hoc analysis to Kruskal-Wallis test. Abbreviations: Cond – conductivity; Chl-a – concentration of chlorophyll-a; TSS – total suspended solids; NO<sub>2</sub>, NO<sub>3</sub>, SRP and TP – concentrations of nutrients; phyto – total phytoplankton abundance; Cyano – abundance of cyanobacteria; Crypto – cryptophytes; Hapto – haptophytes; Bacill – diatoms; Chloro – green algae; Clad – cladocerans.

	2011		2012		2013		2014		2015	
2011			Cond NO <sub>2</sub> Chl-a	phyto Cyano Hapto	NO <sub>2</sub> NO <sub>3</sub> mineral N Clad		Cond NO <sub>2</sub> mineral N SRP	TP Chloro Bacill Crypto	Cond NO <sub>2</sub> nitrates SRP	TP Chl-a Chloro Bacill
2012	Cond NO <sub>2</sub> Chl-a	phyto Cyano Hapto			pH Cond NO <sub>3</sub> Chl-a	phyto Cyano Hapto	pH organic N SRP TSS	phyto Cyano Chloro Bacill	NH <sub>4</sub> NO <sub>3</sub> SRP phyto	Cyano Chloro Bacill Hapto
2013	NO <sub>2</sub> NO <sub>3</sub> mineral N Clad		pH Cond NO <sub>3</sub> Chl-a	phyto Cyano Hapto			Cond SRP Chloro Bacill Crypto	2	Cond NH <sub>4</sub> SRP	Chloro Bacill Clad
2014	Cond NO <sub>2</sub> mineral N SRP	TP Chloro Bacill Crypto	pH organic N SRP TSS	phyto Cyano Chloro Bacill	Cond SRP Chloro Bacill Crypto				NH4 NO3 Chryso	
2015	Cond NO <sub>2</sub> nitrates SRP	TP Chl-a Chloro Bacill	NH₄ NO₃ SRP phyto	Cyano Chloro Bacill Hapto	Cond NH <sub>4</sub> SRP	Chloro Bacill Clad	NH <sub>4</sub> NO <sub>3</sub> Chryso			



**Fig. 8.** RDA biplot showing relationships between phytoplankton taxonomical groups and selected environmental factors and zooplankton. Abbreviations: temperat – temperature, conducti – conductivity, *Cyanopro* – cyanobacteria, *Dinophyc* – dinoflagellates, *Chloroph* – green algae, *Euglenop* – euglenoids, *Conjugat* – *Conjugatophyceae*, *Xantophy* – *Xantophyceae*, *Cryptoph* – cryptophytes, *Bacillar* – diatoms, *Haptoph* – haptophytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

especially in the case of ammonium N, whose concentrations were periodically higher (e.g. 2014). The correlation of Cyanobacteria with ammonium was also confirmed in other lakes e.g. natural and artificial lakes in Poland (Dondajewska et al., 2017; Rosińska et al., 2017; Pełechata et al., 2016; Kozak et al., 2019). A positive relation was also found for water temperature, that is of paramount importance in the light of climate changes. Higher temperatures will promote phytoplankton proliferation and also enhance internal P loading (Paerl and Paul, 2012), meanwhile temperature and nutrients are considered as two simultaneously acting factors for the development of massive water blooms (Rigosi et al., 2014). Higher water temperature positively influenced the higher occurrence of Dinophyceae, Chlorophyceae or Euglenophyceae (Elliott, 2012). Cyanobacteria can develop intensively both at high and lower water temperatures (Wilk-Woźniak and Mazurkiewicz-Boroń, 2003; Lürling et al., 2013).

Cyanobacteria correlated negatively with conductivity as a result of the uptake of dissolved compounds as well as increased conductivity in spring 2011 and 2013 by riverine backwater inflow, when cyanobacteria abundance was lower than in the following months (Fig. 8). No effect of zooplankton was revealed as it continued to be dominated by Rotifera, feeding mainly on detritus and small bacteria. Cladocerans were correlated with green algae because of their presence in April 2014 and 2015, this being confirmed by a CCA analysis based on particular dominating phytoplankton taxa (Fig. 7).

## 5. Conclusions

The presented results indicated that restoration measures by means of Effective Microorganisms and barley straw were insufficient to reduce both nutrient content and phytoplankton abundance in a shallow lake. The EM application initiated positive changes in the ecosystem by means of excessive organic matter decomposition and increased diversity of phytoplankton, nevertheless a proper reduction of nitrogen and phosphorus content did not occur. Additional restoration methods supporting EM might be considered, as combined treatment used simultaneously e.g. biological, physical and chemical, is reported to be the most effective. For example, sustainable restoration proposed in Western Poland, involves a variety of approaches by combining deep water oxygenation or nitrate treatment, P inactivation and biomanipulation. EM treatment requires supplementary measures aiming at nutrient reduction e.g. magnesium chloride dosage, efficiently binding ammonium N and orthophosphates into insoluble struvite.

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