

RESEARCH ARTICLE

Succession of submerged vegetation in a hydrologically reclaimed opencast mine during first 10 years

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Succession of submerged vegetation was monitored from the early stages for a period of 10 years by Self-Contained Underwater Breathing Apparatus (SCUBA) divers in Milada Lake. Milada Lake is the result of a flooded surface coal mine, the first large-scale hydrological recultivation in the Czech Republic. The main focus was on apparent changes in the percentage cover of primary producers (macrophytes and macroalgae) during early successional stages. In total, data on 33 environmental factors (chemical, physical, and biological) were collected and the importance of their impact was statistically analyzed. Besides macrophytes, we also monitored macroalgae that mainly occupied deep areas (down to 12 m) and have been neglected in literature in contrast to macrophytes. Uniquely, the trophic state of the study site decreased in contrast to most water bodies worldwide. Changes in the plant species composition during succession, including replacement of the dominant group of Characeae with *Vaucheria* sp. (both macroalgae), correlated with a decrease in nitrate concentration, N-NO₃. Furthermore, the herbivorous fish biomass and availability of uncovered lake bottom played important roles in the succession of macrophytes and macroalgae. Although some changes in submerged vegetation during succession of a newly created lake are to be expected, the main shifts observed apparently correlated with the studied factors. The conclusions may be useful for the prediction of succession in similarly created lakes in the future.

Key words: Characeae, environmental factors, herbivorous fish, macroalgae, macrophytes, *Vaucheria*

Implications for Practice

- In post-mining lakes characterized by small catchment area and high retention time, the decrease in trophic level, that is, oligotrophication, has to be expected. Macrophytes and macroalgae must be considered as important structural and functional component of the littoral habitat.
- On the other hand, the composition of submerged vegetation is not easy to predict as many factors contribute and their effect is highly species-specific. Among the most important belong inter-specific competition realized via competition for light, nutrients, and involvement of allelopathic effects, and herbivory by fish.
- The presence of rudd (*Scardinius erythrophthalmus*, native herbivore in temperate zone of Europe) in the post-mining lake fish community can affect the submerged vegetation composition towards herbivory-resistant species.

Introduction

Many large water bodies are created by hydrological recultivation after completion of coal mining (Blodau 2006). The number of such ecosystems is increasing and will increase worldwide during the following decades (Linke & Schiffer 2002). Moreover, the cost of EU emission allowances has increased the pressure to discontinue mining and the rate of formation of post-mining water bodies is exceeding initial assumptions (Garcia et al. 2020). Successfully completed recultivation

provides important knowledge for the initiation of new projects and may predict some consequences (Vejříková et al. 2018). Disturbances and particularly the formation of a new ecosystem causes partial or total removal of primary producers and initiates succession (Glenn-Lewin & van der Maarel 1992; Johnson & Miyanishi 2007). The entire succession dynamic is based on well-known trade-offs and constraints (Connell & Slatyer 1977; Tilman 1990), the most important of which is the competition-colonization trade-off (Levins & Culver 1971; Wilson 2011).

The succession of both natural and man-made lakes or reservoirs can be characterized by four basic stages (Hilt et al. 2013): (i) The initial stage is characterized by the pioneer species with high fecundity and ability to spread readily (Roxburgh et al. 2004). The community of freshwater ecosystems commonly includes Characeae in the temperate climate zone (Portielje & Roijackers 1995) and is shortly accompanied by submerged macrophytes. The originally bare bottom in the shallow zone (0–5 m) becomes overgrown by continuous

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macrophyte cover and the size of the area increases quickly (Schutten et al. 1994). Typical pioneer macrophytes are e.g. *Potamogeton* spp., *Callitriche* spp., or *Utricularia* spp. (Schutten et al. 1994; Périllon et al. 2018). They effectively colonize the bottom by both rhizomatic growth and rooting of vegetative fragments. For example, *Potamogeton perfoliatus* has a more mixed strategy combining re-rooting fragments and rhizomatic growth (31 and 41%, respectively). *Potamogeton pectinatus* (*Stuckenia pectinata*) prefers colonization by rhizomes (84%). These vegetative reproductive strategies mostly prevail over sexual strategies in the initial stages of succession (Hilt et al. 2016; Vári & Tóth 2017). (ii) A stage with a high number of submerged macrophytes and macroalgae species, usually around 25 species, follows the initial stage of succession. Rapidly increasing biomass of the macrophytes and macroalgae, and low lake trophy is typical for this stage (Vestergaard & Sand-Jensen 2000; Sayer et al. 2010a, 2010b). The most common dispersal vectors of seeds, oospores, and vegetative propagules of submerged macrophytes are by water, followed by wind and animals (Krahulec et al. 1987; Portielje & Roijackers 1995; Boedeltje et al. 2003; Soons et al. 2008; Xie et al. 2010). As in terrestrial ecosystems, the early succession stages in aquatic ecosystems are the most diverse in terms of number of species (Willby et al. 2001; Murphy 2002). (iii) A stable stage follows and is characterized by a long period of macrophyte dominance during part of the year (May–September), keeping phytoplankton at a constant level. The seasonal changes in phytoplankton abundance are mostly dampened with low chlorophyll-*a* concentrations ($<10\text{--}15\ \mu\text{g L}^{-1}$) during the entire summer (Sayer et al. 2010a). At the same time, macrophyte species richness is reduced likely due to a decline in the number of plant reproductive strategies (Sayer et al. 2010a). (iv) The last stage, before phytoplankton dominance, is the commonly named “crashing lakes” stage. Common dominant species are e.g. *P. pectinatus* and *P. pusillus*. The macrophyte-cover period is shortened (2 months) and is consistently followed by a mid-summer crash in the plant population. Seasonality of nutrient and chlorophyll-*a* concentrations in the crashing lakes are similar to turbid lakes without submerged macrophytes. Subsequently, most lakes become dominated by phytoplankton. However, a stage of floating or emergent macrophytes (Krahulec et al. 1987; Portielje & Roijackers 1995) or periphytic and filamentous algae (Phillips et al. 1978; Jeppesen et al. 1992; Ibelings et al. 2007) can occur in shallow lakes. The described succession stages can last from 10 to 100 years in shallow lakes (Sayer et al. 2010a).

Many environmental factors (chemical, physical, and biological) play important role in the succession and the changes in the factors affect the course of succession. In terms of chemical factors, both nitrogen and phosphorus are limiting nutrients for primary producers in freshwater lakes. However, phosphorus is not the main limiting nutrient for macrophytes rooting in the sediment with additional access to the bioavailable form of phosphorus stored there. Nitrogen concentration (in the form of nitrate N-NO_3) is a major predictor of vegetation cover, mostly more important than the concentration of phosphorus in the water (James et al. 2005; Lambert & Davy 2011). With higher nitrate

availability, higher competition pressure occurs and leads to the mentioned prevalence of floating macrophyte species or phytoplankton algae that results in light limitation. A decrease in macrophyte species richness occurs commonly under the threshold concentration of $1\text{--}2\ \text{mg N-NO}_3\ \text{L}^{-1}$ in winter (James et al. 2005) or $0.6\ \text{mg N-NO}_3\ \text{L}^{-1}$ under experimental conditions (Barker et al. 2008). Nitrate can have a toxic effect on Characeae (Lambert & Davy 2011). The observed decrease in Characeae species richness was above the mean annual concentration threshold of approximately $2\ \text{mg N-NO}_3\ \text{L}^{-1}$ (Lambert & Davy 2011). In terms of biological factors, grazing of herbivorous fish and waterfowl can directly influence biomass and species composition of vegetation cover depending on the locality (Moore et al. 2010; Vejříková et al. 2018). Furthermore, allelopathy, documented in all groups of primary producers (cyanobacteria, micro- and macroalgae, and angiosperms), affects species richness and mostly has an inhibitive effect (Gross 2003; Hilt & Gross 2008). Furthermore, submerged vegetation is also dependent on many hydrological factors such as lake variables, including lake area, altitude, shoreline complexity, connectivity, water quality (water transparency, CO_2 concentration, alkalinity, and water conductivity) and sediment composition (Bakker et al. 2013 and related studies).

The present study is focused on vegetation succession in a newly created lake with four abundant species of macrophytes, two species of macrophytes with rare occurrence, and two species of macroalgae that dominated during monitoring. The number of studies focusing on submerged vegetation is increasing (Bakker et al. 2013, 2016); however, the presence of macroalgae (except Characeae) in aquatic ecosystems is under-examined even though they are common and visible macroscopically (Messyasz et al. 2018). Monitoring of a wide spectrum of environmental factors provides insight on their importance during succession and reveals the triggers of species transition within succession. Based on the literature, we supposed the largest impact to be that of nutrient concentration (e.g. Portielje & Roijackers 1995; Lambert & Davy 2011). Most studies conducted under natural conditions describe situations with trophic state increase, whereas nutrient concentration decrease was recorded in our study site, a typical phenomenon for post-mining lakes. Furthermore, we expected a significant impact from grazing by herbivores, which was observed in a manipulative experiment (Vejříková et al. 2018), and also of competition for light due to the possible fatal consequences of the shading effect (Bakker et al. 2013).

Methods

Study Site

The study was conducted in Milada Lake ($50^\circ39'\text{N}$, $13^\circ58'\text{E}$), Czech Republic, that was newly created by the flooding of an abandoned open cast brown-coal mine (Fig. 1). It was the first large hydrological recultivation in the Czech Republic and one of the first in central Europe (Čech et al. 2009). It is an oligotrophic lake with mean summer total phosphorus in the surface

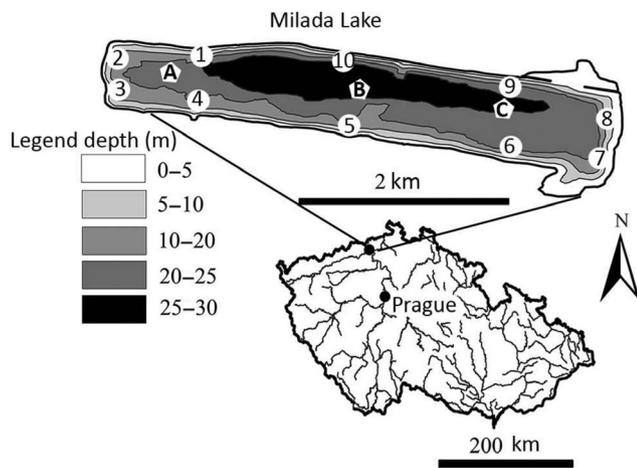


Figure 1. Location of Milada Lake in the Czech Republic and bathymetric map of the lake with 10 transects (numbers 1–10) of macrophyte monitoring, and three profiles (A–C) where water was sampled for subsequent chemical analyses.

layer of $<10 \mu\text{g L}^{-1}$. It has an area of 250 ha, a volume of 0.036 km^3 , and a maximum depth of 25 m. Aquatic restoration of the coal mine started in 2001 and was finished in 2010 with the final water level of 146 m a.s.l. The annual volume of water inflow varied during restoration (Fig. 2). Forty-one percent of the water volume between the years 2007 and 2009 was supplied by controlled inflow from Kateřina Reservoir. In 2010, only 10% came from Kateřina Reservoir and the remainder from the catchment basin. The highest water inflow from the catchment basin was in 2010, 60% more than in other years between 2007 and 2016 (Fig. 2; Rous 2016).

From 2007 to 2016, macrophytes were present up to 8 m depth (only sporadic rooted fragments incapable of permanent growth were found deeper, to 12 m). Macroalgae were present at 11–12 m depth and covered 5–30% of the bottom. At 12–13 m depth, macroalgae were rare. Macrophytes consisted of *Myriophyllum spicatum*, *Potamogeton pectinatus*, *P. crispus*,

Elodea canadensis, and two rare species *M. verticillatum* and *P. pusillus*. The macroalgae present are Characeae (dominated by *Chara globularis* >90%) and *Vaucheria* sp. (Vejříková et al. 2018). The fish community consisted of rudd (considered to be the main herbivores), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), pike (*Esox lucius*), European catfish (*Silurus glanis*), tench (*Tinca tinca*), and pike-perch (*Sander lucioperca*).

Monitoring of Submerged Vegetation

Monitoring of submerged vegetation was conducted annually in September 2007–2016. Monitoring started at a depth of 2 m because a steep stone wall, to a depth of 2 m, was created encircling the entire lake to prevent damage to the shoreline by waves. The lake bottom is covered with sediment and has a gradual slope below 2 m. To obtain a qualitative assessment of submerged vegetation, two SCUBA divers visually assessed the occurrence of the species present. Monitoring was conducted on 10 transects evenly distributed throughout the lake (Fig. 1); however, between 2007 and 2011, fewer transects were monitored in exceptional circumstances (not less than five evenly distributed throughout the lake). The transects were perpendicular to the shore and were marked using measuring tapes at the bottom to a depth of 12 m. Submerged vegetation commonly did not reach 12 m depth, only in a few cases, macroalgae cover continued to a depth of 13.5 m. Both divers swam independently and visually assessed the percentage cover of each macrophyte species and the uncovered bottom area, up to 1 m on both the left and right sides of the measuring tape. The assessments were recorded at 1-m depth intervals and the sum of the values added together was 100%. The final value of percentage cover was the mean of four values for each meter of depth (values from two divers, from the left and right sides). The deviation of the percentage cover between divers was 2–15%, on average 4%. For better interpretation, we present depth intervals 2–4, 4–6, 6–8, and 8–10 m, thus, we averaged two 1-m depth intervals for the final values. The percentage cover of species corresponds to the mean values of all depth intervals, not the amount of area of monitored bottom.

Monitoring of Environmental Factors

Between the years 2002 and 2015, chemical parameters were analyzed monthly from water sampled at 2-m intervals from three profiles marked by anchored buoys. From 2016, chemical parameters were analyzed only from the central profile and three depths (water surface, middle, bottom; Fig. 1). All controlled inflows were concurrently monitored. Monitoring of 27 chemical parameters was conducted by the company Labe River Authority, state enterprise, OVHL Hradec Králové according to the Czech technical standards (BOD 5, pH, conductivity 25, ANC-4.5, BNC-8.3, N-NO₂, N-NH₄, N-NO₃, TN, N org. drain, TP, Cl, SO₄, DOM, POM, CHOD Cr, HCO₃, CO₃, TOC, CO₂ dis., Na, K, Fe, Mn, Ca, and Mg). The most important chemical parameters were determined as follows: nitrate (N-NO₃) by continuous flow analysis and spectrophotometric

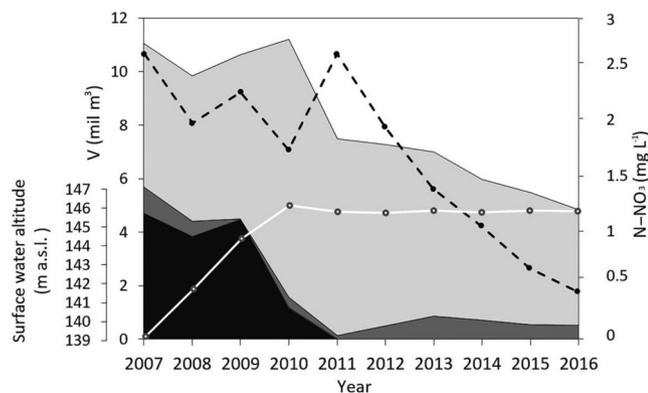


Figure 2. Volume of water inflow from Kateřina Reservoir (black), deep wells (dark gray), and from the rest of the catchment basin (light gray) in 2007–2016. The white line shows surface water altitude, and the black dashed line shows the concentration of N-NO₃ in the lake (mg L^{-1}).

method, bicarbonate (HCO_3^-) using acid neutralizing capacity and pH, Ca^{2+} by complexometric titration and total phosphorus by continuous flow spectrophotometry (total phosphorus was under the detection limit [$<10 \mu\text{g L}^{-1}$] during most measurements and no trend of decrease/increase could be recorded, thus, it could not be included in the statistical analysis due to inapplicable data).

The monitored biological parameters were chlorophyll-*a* concentration and fish community composition. The fish community (including herbivorous rudd used as a biological environmental factor) was sampled annually in September by standardized multi-mesh gillnets. For details, see Vejřková et al. (2016).

Furthermore, volumes of water inflows, volume percentage of new water, water level, and percentage of uncovered bottom were used as physical environmental factors (see sections Study site and Monitoring of submerged vegetation for description).

To simplify graphical interpretation, we present only representative years 2007, 2010, 2013, and 2016 (Fig. 5, Table S1).

Statistical Analysis

Redundancy analysis (RDA) was used to test the effects of environmental factors on the percentage cover of each species within the monitored years. The automatic log transformation, plus centering and standardization was provided in the program CANOCO 5 (Šmilauer & Lepš 2014). Statistical significance was analyzed using Monte Carlo permutation tests (number of permutations: 4,999). The age of the lake was used as a time series factor of the conditioning variable and water level as a covariate. The following data were used as environmental variables: concentration of N-NO_3 , Na, K, Fe, Mn, Ca, Mg, pH, SO_4 , HCO_3^- , chlorophyll-*a*, herbivorous fish biomass, volumes of water inflows, volume percentage of new water, and percentage of uncovered bottom. A method of forward selection was performed to select only five environmental variables with the highest explained variation.

A chi-square test was used to compare the percentage cover of macrophytes versus macroalgae in three depth intervals (2–4, 4–6, and 6–8 m) in four representative years (2007, 2010, 2013, and 2016). A paired *t*-test was used to compare percentage cover of Characeae and *Vaucheria* sp. in the east versus west

side of lake. The chi-square test and *t*-test were conducted using Statistica 13 (TIBCO Software Inc. 2017).

In Figures 3 and 6, the percentage of uncovered bottom was included among the environmental variables and the plant cover was recalculated so that the sum of all species was 100%. In Figure 5, uncovered bottom was intentionally kept emphasizing the decrease in uncovered bottom and increase in plant cover.

Results

Changes in Key Environmental Factors

Based on RDA forward selection, five variables with the highest explained variation were selected, specifically concentration of N-NO_3 , HCO_3^- , Ca^{2+} , herbivorous fish biomass, and uncovered bottom (Table 1). Except Ca^{2+} , a significant decrease in values of the selected variables was observed with time. N-NO_3 , explained 48% of total variability, it had a relatively stable concentration for the first 6 years (during filling of the lake). The concentration subsequently decreased by 87% over the next 4 years (Table 1; Fig. 2). Herbivorous fish biomass, explaining 15.2% of total variability, showed two peaks in the first and sixth years of monitoring. The herbivorous fish biomass was 38 kg/1,000 m^2 nets in the first year, and it decreased by 82% during the second year. The biomass subsequently increased again and reached 68% of the original size during the sixth year. This was followed by a significant decrease to only 2.8% of the initial herbivorous fish biomass. Ca^{2+} , explaining 8.7% of total variability, showed an opposite trend. The highest concentration was in the last monitored year. It increased by 49% compared to the average of a steady slight decline during the previous 9 years. HCO_3^- , explaining 7.2% of total variability, decreased by 8.7% during the first 3 years, and it subsequently showed a relatively stable trend with small fluctuations. Percentage of uncovered bottom, the last selected variable explaining 4.6% of total variability, decreased markedly with time. During the first 3 years, the percentage of uncovered bottom decreased from 70 to 20%. During the following years, the decline continued at a slower rate to only 5% in the last monitored year.

Table 1. Five environmental factors (concentration of N-NO_3 , herbivorous fish biomass, uncovered bottom, concentrations of HCO_3^- , and Ca^{2+}) with major impact on succession of submerged vegetation in Milada Lake according to RDA (forward selection).

Year	N-NO_3 mg L^{-1}	Herbivorous fish kg 1,000 m^{-2} of nets	Uncovered bottom %	HCO_3^- mg L^{-1}	Ca^{2+} mg L^{-1}
2007	2.58	38.01	70.30	324.21	58.44
2008	1.93	6.91	46.47	309.05	56.85
2009	2.23	6.55	18.17	295.97	56.23
2010	1.68	8.85	22.08	303.71	55.26
2011	2.58	17.36	13.66	304.07	59.13
2012	1.90	25.86	5.23	296.50	50.92
2013	1.31	9.05	15.09	294.86	50.16
2014	0.97	8.26	8.89	300.98	51.96
2015	0.57	2.15	22.30	297.62	46.33
2016	0.35	1.07	5.11	297.62	72.57

Changes in Species Composition of Submerged Vegetation during Succession

Three apparent changes in species composition of submerged vegetation were observed between the years 2007 and 2016 in Milada Lake. The first apparent change was observed in 2008 when a marked decrease in cover of *Potamogeton crispus* and *Myriophyllum spicatum* was recorded. In 2007, 61.6% of total cover was comprised of *P. crispus* and *M. spicatum* (Fig. 3). According to RDA (forward selection), the percentage cover of these species was positively correlated with three environmental factors (Fig. 4). Out of 83.7% of total exploratory variables, 15.2, 7.2, and 4.6% were explained by herbivorous fish biomass, concentration of HCO_3^- , and percentage of uncovered bottom, respectively. However, the impacts were statistically insignificant ($p \geq 0.09$) due to the high statistical significance of the concentration of N-NO_3 that plays an important role in the following change (see below). Trends of these environmental factors are illustrated in Table 1. From 2008, macroalgae dominated in the lake.

The second significant change was observed in 2011 when *Vaucheria* sp. appeared and its cover rapidly increased in the following years. *Vaucheria* sp. comprised 9.1 and 60.8% of total vegetation cover in 2011 and 2016, respectively. *Vaucheria* sp. dominated in deep parts (6–8, 8–10 m) where no macrophytes prospered, similarly to Characeae. Percentage cover of Characeae gradually decreased from 72% in 2011 to 10.5% in 2016 with increasing cover of *Vaucheria* sp. The transition was highly correlated with the concentration of N-NO_3 in the water. The concentration of N-NO_3 started to decrease from 2010 when controlled flooding was stopped (Fig. 2). As revealed by RDA (forward selection), the decrease in N-NO_3 concentration was the key and highly significant factor affecting the succession of submerged vegetation in the lake ($F = 6.5$; $p = 0.0004$). The concentration of N-NO_3 explained 48% of

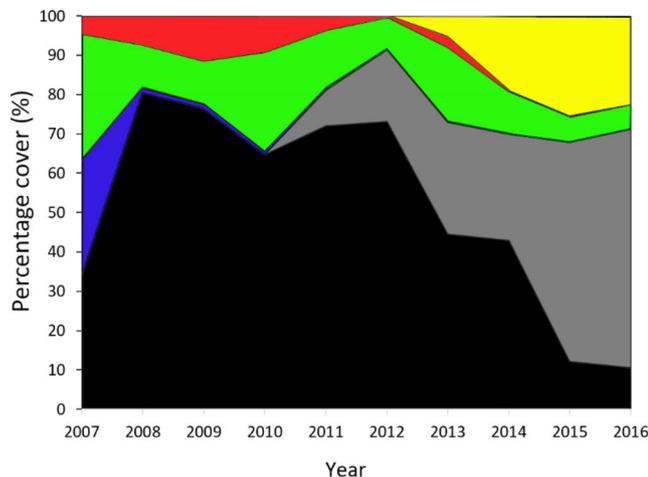


Figure 3. Species composition of submerged vegetation in Milada Lake throughout the 10-year monitoring period counted as mean cover values in depths 2–10 m: Characeae (black), *Vaucheria* sp. (gray), *Potamogeton crispus* (blue), *Myriophyllum spicatum* (green), *Elodea canadensis* (red); *P. pectinatus* (yellow). Rare species *P. perfoliatus* and *M. verticillatum* (cover <1% in all years) were not included.

total variability. A similar trend was recorded for the decrease in Ca^{2+} concentration in water; however, only 8.7% of total variability was explained by this environmental factor ($p = 0.3$; Fig. 4).

The third apparent change occurred in 2013 when *P. pectinatus* was first recorded with cover 4.9%, the cover increased to 25.6% in 2015 (Fig. 3). In contrast to the increase in *P. pectinatus*, a decrease in *Elodea canadensis* (11.2% in 2009, 0% in 2014) and *M. spicatum* (19.1% in 2013, 6.1% in 2016) was recorded. All mentioned changes led to the dominance of *Vaucheria* sp. (60.8%) in 2016, followed by *P. pectinatus* (22.6%).

Depth and Spatial Distribution of Species of Submerged Vegetation in the Lake

The percentage of uncovered bottom decreased, and submerged vegetation spread to deeper parts with time (Fig. 5). Maximum depth of occurrence of *Vaucheria* sp. and Characeae in 2016 was 13.5 and 12.7 m, respectively. Occurrence of macrophytes versus macroalgae varied with the depth gradient. Macroalgae dominated mainly at depth 6–8 m except for 2007 when macroalgae dominated at 4–6 m depth. In contrast, macrophytes dominated mainly at 2–4 m. All mentioned dominances were statistically significant (Table S1). No macrophyte species occurred deeper than 8 m.

Significant differences in the spatial distribution of both types of macroalgae were found. *Vaucheria* sp. was first recorded in 2011 in the west of the lake, and its percentage cover was significantly higher in the west than in the east for all monitored years ($t = 3.55$, $df = 5$, $p < 0.05$). In contrast, the percentage cover of Characeae was lower in the west than in the east ($t = 5.47$, $df = 5$, $p < 0.005$; Fig. 6). Moreover, a decrease in Characeae with time was more apparent in the western part of the lake. This is probably not caused by the concentration of N-NO_3 , as the difference between east and west ranged only between 2.5 and 8%, without any clear trend. In contrast, it may be caused by

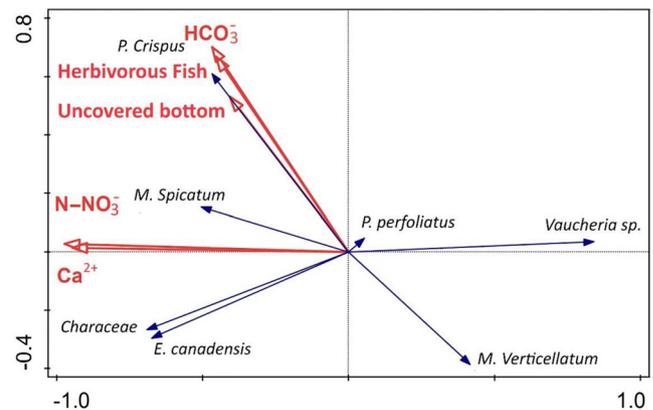


Figure 4. Redundancy analysis (RDA) of the percentage cover of six macrophytes and two macroalgae in Milada Lake in relation to the concentration of N-NO_3 , the concentration of Ca^{2+} , and concentration of HCO_3^- , herbivorous fish biomass, and percentage of uncovered bottom throughout years 2007–2016.

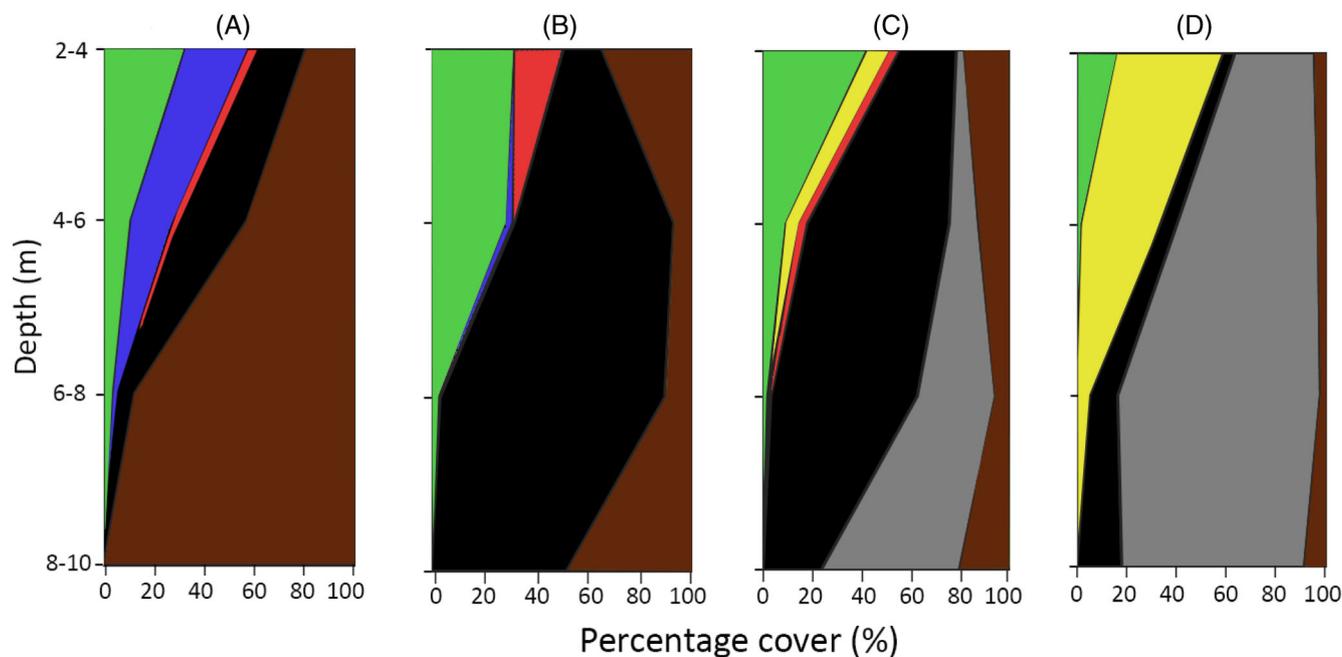


Figure 5. Spreading of submerged vegetation (in percentage) to deeper parts (depths 2–4, 4–6, 6–8, and 8–10 m) and by contrast decrease of uncovered bottom (brown) with time in (A) 2007, (B) 2010, (C) 2013, and (D) 2016 is illustrated. Percentage cover of *Myriophyllum spicatum* (green), *Potamogeton crispus* (blue), *Elodea canadensis* (red), Characeae (black), *Vaucheria* sp. (gray), *P. pectinatus* (yellow) is shown.

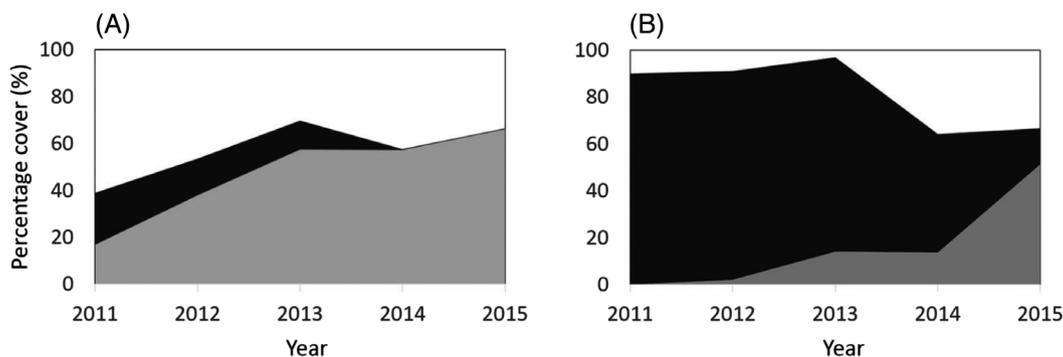


Figure 6. Percentage cover of dominant macroalgae *Vaucheria* sp. (gray) and Characeae (black), and macrophytes (white) in 2011–2015 from depths 2–10 m on (A) west and (B) east side of the lake.

Table 2. Mean temperature (°C) at depth 6–10 m during a year in the west and east sides of the lake, averaged from years 2011–2016.

	January	February	March	April	May	June	July	August	September	October	November	December
East	3.1	1.45	1.6	6.9	8	9.75	12.1	18.45	13.35	13.1	8.4	5.4
West	3.2	1.8	1.6	6.85	8.75	9.65	11.6	12.3	13.8	13.5	8.5	5.5

differences in the mean temperature at 6–8 and 8–10 m depths during the year (Table 2).

Discussion

Our study was conducted in a newly created lake and thus changes in the macrophyte community are to be expected.

However, the observed correlations, especially the correlation with N–NO₃ concentration decrease, apparently influenced the process. Succession of primary producers in a freshwater ecosystem is often connected with an increase in the trophic state (Portielje & Roijackers 1995) that leads to the very common problem of eutrophication (van Straten 1986). In contrast, the trophic level decreased in Milada Lake. In the initial succession

stage, the high influx of nutrients was caused by inflow from Kateřina Reservoir and by apparent degradation of terrestrial plants that were suddenly submerged (personal observation). Subsequently, nutrient concentration decreased due to accumulation in sediment and in aquatic organisms. Thus, the main changes in submerged vegetation were related to a decrease in nutrient concentration, in contrast to most recently formed freshwater bodies.

A high percentage cover of *Potamogeton crispus* and *Myriophyllum spicatum* was already observed before monitoring in 2006 (Čech et al. 2009). During the first monitored year 2007, *P. crispus* and *M. spicatum* comprised 62% of total cover. However, a rapid decrease was recorded in 2008 for both *P. crispus* (from 30 to 1%) and *M. spicatum* (from 31 to 11%) and is described in Čech et al. (2010). In contrast, the percentage cover of Characeae increased from 34 to 80%. This first apparent change was related to a decrease in herbivorous fish biomass probably caused by stocking of predatory fish (Vejřík et al. 2017). Consequently, the biomass of palatable *Elodea canadensis* (van Donk & Otte 1996) and Characeae (Lake et al. 2002) increased due to decreased pressure from herbivorous fish. *E. canadensis* and Characeae covered a substantial part of the uncovered bottom (Čech et al. 2011), and thus competition for light, carbon, and available space increased (Den Hartog & Segal 1964; Carignan & Kalff 1980). This is in accordance with the results of our underwater manipulation experiment (Vejříková et al. 2018), where the increase of cover of *M. spicatum* and *P. crispus* was positively correlated with a decrease in competition by the removal of dominant macroalgae. High abundances of *M. spicatum* and *P. crispus* in the presence of high herbivorous fish biomass in the initial stage of succession were apparently caused by the chemical and physical defenses of both species. *Myriophyllum spicatum* contains high concentrations of phenolic compounds (Dorenbosch & Bakker 2011; Kapuscinski et al. 2014) and *P. crispus* tries to defend itself with tough and partially spine-like leaves (Vejříková et al. 2018). Other species in the lake lack similar defense mechanisms. The second reason for the decrease and subsequent vanishing of *P. crispus* is the decrease in nutrient concentration given that this species prefers a high trophic state (Cao et al. 2018). The second and most apparent change in appearance of the submerged vegetation was dominance of *Vaucheria* sp., and by contrast decrease in Characeae which corresponded with a change in hydrological level. Controlled inflow from Kateřina Reservoir was stopped in 2010. The water from Kateřina Reservoir was rich in nutrients and the nutrient concentration started to decrease rapidly since 2012. During the rapid decrease in nutrient concentration, the percentage cover of Characeae decreased from 65 to 11% in 2016. The same trend continued and no Characeae were found in the lake in 2018 (Vejřík, L. & Peterka, J. unpublished data). In contrast, cover of *Vaucheria* sp. increased from 11% in 2011 to 61% in 2016. The third apparent change during succession was the occurrence of *P. pectinatus* in 2013 and its gradual increase in percentage cover. Due to the decrease in nutrient concentration in the lake, *P. pectinatus* had an advantage over *E. canadensis*, which has

higher nutrient requirements (Portielje & Roijackers 1995). Moreover, *P. pectinatus* profited from the decrease in herbivorous fish biomass caused by predatory fish (Vejřík et al. 2017). *Potamogeton pectinatus* is a competitively successful species (Vejříková et al. 2018), however, it is vulnerable to grazing (Hilt 2006; Vejříková et al. 2016) due to lack of chemical defense (Dorenbosch & Bakker 2011). For instance, the biomass of *P. pectinatus* decreased by over 90% due to grazing, recorded by Hilt (2006). Furthermore, preference of *P. pectinatus* by fish was observed in Matsalu Bay in Estonia (Hidding et al. 2010a), and in Lauwersmeer in the Netherlands (Hidding et al. 2010b). Low herbivorous pressure positions *P. pectinatus* as a more competitively successful species than *M. spicatum* that must invest energy in chemical defense (Vejříková et al. 2018).

Although Characeae commonly dominate in low trophic lakes (Portielje & Roijackers 1995; Van den Berg et al. 1999) and have strong allelopathic activity (Vermaat et al. 2000; Hilt & Gross 2008), this group was replaced by *Vaucheria* sp. The transition happened probably due to a large decrease in the concentration of N-NO₃ to 0.35 mg L⁻¹ in 2016, when N-NO₃ decreased under the critical limit due to accumulation of present N-NO₃ in other primary producers and low input of new nutrients from tributaries. Characeae are very sensitive to high concentrations of N-NO₃, however, they are not able to grow in water with too low concentrations. The optimal concentration for Characeae seems to be 0.5–2 mg L⁻¹ (Lambert & Davy 2011). *Vaucheria* sp., like Characeae (van den Berg et al. 1999), has more intensive growth in winter than in summer (Ozimek 2006). Therefore, N-NO₃ from dead macrophytes during the winter season is readily accumulated by *Vaucheria* sp. (Vejříková in prep.). *Vaucheria* can accumulate redundant nutrients in a nutrient sink (Simons et al. 1994), and thus, it can grow in waters with very low nutrient concentration (Schagerl & Kerschbaumer 2009). The unresolved question is whether Characeae were not suppressed by allelopathy from *Vaucheria* sp. Unfortunately, allelopathic compounds have not been tested in our study and information about this species is still limited (Messyasz et al. 2018). The observed uneven spatial distribution of macroalgae, that is, *Vaucheria* sp. prevailing in the west and Characeae in the east of the lake, is remarkable, however, it is not fully clear why this is the case. It is obviously not affected by the N-NO₃ because no trend was observed in the east–west direction. Neither does water transparency have an effect as it almost did not differ between the sides. Limnological factors that come into the question are (i) the inflow/outflow orientation, as all tributaries are located in the west and, conversely, the outflow from the lake is on the eastern side, (ii) the orientation of the prevailing winds, as western winds are constantly prevalent in this area causing relatively intense waves in the east of the lake and affecting the mean temperature and thermocline depth (Čech et al. 2011; Čech et al. 2012). As a result, the temperature at 6–8 and 8–10 m depths (macroalgae dominance) is slightly higher in the west with *Vaucheria* sp. dominance. However, this trend is rapidly changed by the wind in July and August, the warmest months, when the mean temperature at

these depths rises to 20°C in the east, which is on average 6°C higher than in the west. Therefore, this period probably represents an annual threshold preventing more intensive spreading of *Vaucheria* sp., which prefers cold water (Schagerl & Kerschbaumer 2009).

The area of uncovered bottom decreased during succession. Therefore, competition among species increased with the increasing shading effect of competitively successful species (Bakker et al. 2013). This is probably the reason why macroalgae smaller in size occurred in deeper parts where macrophytes do not meet the requirements for photosynthetically active radiation (Portielje & Roijackers 1995). Macroalgae such as Characeae (van den Berg et al. 1999; van Nes et al. 2002; Bakker et al. 2010) and *Vaucheria* sp. (Schagerl & Kerschbaumer 2009) have low requirements for temperature and photosynthetically active radiation. Thus, the fluctuation of biomasses is low during the year, they can survive in winter and also in deeper parts than macrophytes. Specifically, *Vaucheria* sp. has the typical ability to absorb blue light (Kataoka et al. 2001). This depth segregation of macrophytes and macroalgae has a positive impact on the structuring of the ecosystem that leads to an increase in biodiversity in the lake (Jeppesen et al. 1992).

The important impact of nutrients, herbivory, and competition on succession in the Milada macrophyte community was according to our expectations. However, only nutrient concentration was statistically significant, specifically, the concentration of N-NO₃ that decreased during succession. The importance of nutrient concentration is in accordance with Barker et al. (2008) and Lambert and Davy (2011), which we confirmed for natural succession in post-mining lake with nutrient concentration decrease. The other environmental factors, specifically herbivory and competition (presence of uncovered bottom), were statistically insignificant most probably due to the high significance of N-NO₃. Nevertheless, the impact of these factors is apparent and is in accordance with other studies, namely with Marklund et al. (2002) and Vejříková et al. (2018) in terms of herbivory and Stiers et al. (2011), and Bakker et al. (2013) in terms of competition.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Statistical significance of dominance of macrophytes and macroalgae.

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