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Biomanipulation of hypereutrophic ponds: when it works and why it fails

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Abstract Phytoplankton, zooplankton, submerged vegetation and main nutrients have been monitored in 48 eutrophic ponds from the Brussels Capital Region (Belgium) between 2005 and 2008. Nine ponds have been biomanipulated in order to improve their ecological quality and prevent the occurrence of noxious cyanobacterial blooms. The 4-year study of a large number of ponds allowed identification of the factors having the strongest influence on phytoplankton growth. Continuous monitoring of the biomanipulated ponds allowed the significance of changes caused by biomanipulation to be tested as well as the main reasons of biomanipulation successes and failures to be elucidated. The main factors controlling phytoplankton in the ponds studied appeared to be grazing by large cladocerans and inhibition of phytoplankton growth by submerged vegetation. Biomanipulation resulted in a significant decrease in phytoplankton biomass in general and biomass of bloom-forming cyanobacteria in particular that were associated with a significant increase in large Cladocera density and size. In six out of nine ponds biomanipulation resulted in the restoration of submerged vegetation. The maintenance of the restored clearwater state in the biomanipulated ponds was strongly dependent on fish recolonisation and nutrient level. In the absence of fish, the clearwater state could be maintained by submerged vegetation or large zooplankton grazing alone. In case of fish recolonisation, restoration of extensive submerged vegetation could buffer, to a considerable degree, the effect of fish except for ponds with high nutrient levels.

Keywords Eutrophic ponds · Biomanipulation · Phytoplankton · Cyanobacteria · Large Cladocera · Submerged vegetation

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Introduction

During the last decades eutrophication has become a serious threat to many lakes and ponds worldwide. Increased productivity has caused considerable degradation of ecological water quality often resulting in the development of potentially toxic cyanobacterial blooms (Paerl 1988; Willame et al. 2005; Hudnell 2008) or profuse growth of filamentous green algae (Irfanullah and Moss 2005).

Many approaches have been used to mitigate the effects of eutrophication. Substantial reduction of nutrient loading can be useful, particularly in cases when nutrients come from point sources (Simons et al. 1994; Moss et al. 1996a; Jeppesen

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et al. 2005, 2007; Søndergaard et al. 2005a), but it might not produce the desired effect or such an effect can be considerably delayed in cases of accumulation of phosphorus in the sediment or diffuse external nutrient sources (Lauridsen et al. 2003; Carpenter 2005; Søndergaard et al. 2007)

A number of studies have demonstrated, however, that sizeable reduction in fish densities shifts lakes and ponds from phytoplankton to submerged vegetation dominance, thus improving the water quality and reducing the risk of cyanobacterial bloom occurrence (Shapiro and Wright 1984; Shapiro 1990, 1995; Gulati and van Donk 2002; Søndergaard et al. 2007; Van Wichelen et al. 2007). Removal of all or most of the fish or addition of piscivores with the aim of reducing phytoplankton biomass is referred to as biomanipulation (Shapiro and Wright 1984; Moss et al. 1996a). It allows phytoplankton control through increase in grazing by large zooplankton released from fish predation. Because of its cost-effectiveness and quick response to the intervention as compared to nutrient reduction measures (Lammens 1999; Lauridsen et al. 2003), biomanipulation has been a primary method of lake and pond restoration (Shapiro and Wright 1984; Jeppesen et al. 1990; Moss et al. 1996a; Gulati and van Donk 2002; Van Wichelen et al. 2007).

Most of the restoration efforts have been focused on lakes. Thus, while a broad understanding of the biomanipulation potential for the restoration of lakes has been gained in the past decades, biomanipulation effects on ponds have been less well elucidated. There is growing evidence, however, that ponds are more than just lakes of small size (Søndergaard et al. 2005b). Their extreme position along the size and depth gradients results in a major shift in the functional coupling of grazers and phytoplankton (Tessier and Woodruff 2002). The small size and low depth greatly increase the chance as well as extent of submerged vegetation restoration (Moss et al. 1996a) and thus enhance top–down control of phytoplankton (Søndergaard and Moss 1998; Søndergaard et al. 2005b). Hence ponds are more likely to shift towards the clearwater state (Moss 2007). Because of their small size and diminished bottom–up control of phytoplankton, ponds respond faster than lakes to changes in their trophic structure. Besides, ponds are usually easy to draw down. This greatly facilitates fish removal, thus insuring that, at least for a while, the plankti-benthivorous fish populations are very small or absent. Because of generally greater isolation, ponds are less likely to be quickly recolonised by fish than lakes (Magnuson et al. 1998). This increases the chances of biomanipulation success.

Given a marked numerical prevalence of ponds over lakes, their important contribution to biodiversity (Williams et al. 2003; Scheffer et al. 2006) and the multitude of amenities they render to local communities, the need of acquiring additional knowledge on biomanipulation potential for restoration of ponds affected by eutrophication is becoming increasingly obvious.

We have studied 48 ponds from the Brussels Capital Region during the period of 2005–2008. Although to a different degree, all of the ponds studied were affected by eutrophication. Many of them were, therefore, prone to mass developments of phytoplankton generally dominated by bloom-forming cyanobacteria. This often led to the formation of extensive surface scums and occasional fish and waterfowl kills. The ponds most affected by eutrophication were biomanipulated in order to restore their ecological quality and prevent bloom development. A comprehensive 4-year study of a large number of ponds with contrasting ecological make-up allowed identification of the factors having the strongest influence on phytoplankton growth. Continuous monitoring of nine biomanipulated ponds allowed the effects of biomanipulation on these factors to be statistically tested as well as the main reasons of biomanipulation successes and failures to be elucidated.

Methods

Study area characteristics

Forty-eight ponds from the Brussels Capital Region (Belgium) have been studied between 2005 and 2008. All the ponds are artificial, created by damming of low order streams in the twentieth century or earlier. They are all shallow (maximum depth <3 m) and flat bottomed and range in surface from 0.1 to 6 ha. A number of ponds

are used for different recreational activities of which fishing and boating are the most common. The ponds are populated by fish communities typical of northern Europe. Many of them have large stocks of plankti-benthivorous fish (mainly common carp: *Cyprinus carpio*, and bream: *Abramis brama*).

Sampling and sample processing

During the warm season (May–September) of the study period, 48 Brussels ponds were sampled on 24 occasions (16 to 33 ponds at a time) for phytoplankton, zooplankton, main nutrients and submerged vegetation. Quantitative samples of phytoplankton, zooplankton, main nutrients (total phosphorus—TP, soluble reactive phosphorus—SRP, nitrate + nitrite—NO_x and ammonium—NH_4), chlorophyll *a* (Chl *a*) as well as conductivity, pH, temperature and Secchi depth (SD) data were collected according to the standard limnological procedures. Mixed water samples based on 10 random sub-samples were taken from each pond with a plastic tube sampler of 4.5 cm diameter and 70 cm length that closes in the lower part. A special extension was fixed to the sampler to reach the deeper parts of the ponds when appropriate. Water samples preserved with Lugol's solution, sodium thiosulfate and buffered formalin (Kemp et al. 1993) were used for phytoplankton identification (genus level) and counting with an inverted microscope (a modified Utermöhl sedimentation technique; Hasle 1978). Biovolumes were calculated using the approximations of cell shapes to simple geometrical forms (Wetzel and Likens 1990).

For zooplankton, 10 L sample consisting of 10 random sub-samples of 1 L was taken from each pond at the same locations and with the same sampler as phytoplankton samples. Zooplankton samples were filtered through a 64-μm mesh net and preserved in 4% formaldehyde (final concentration) before being identified and counted using an inverted microscope. Different levels of identification were used: cladocerans were identified to genus level; copepods were divided into cyclopoids, calanoids and nauplii; rotifers were not discriminated. For the analyses, cladocerans were divided into two groups: "large" (*Daphnia* spp., *Eurycercus* spp., *Sida* spp. and *Simocephalus* spp.) and "small" (*Acroperus* spp., *Bosmina* spp., *Ceriodaphnia* spp., *Chydorus* spp., *Moina* spp. and *Pleuroxus* spp.) (Moss et al. 2003). Predator cladocerans, *Leptodora* spp. and *Polyphemus* spp. that feed mainly on other zooplankters (Reynolds 2006) were not included in the group of large cladocerans. The length of large Cladocera species was measured and taken as an indicator of grazing intensity and size-selective predation (Pourriot 1995; Carpenter et al. 2001).

Surface cover of aquatic vegetation was mapped visually from a boat during each field visit. The presence/absence of the vegetation was verified with a rake when water was not sufficiently transparent. Because submerged macrophytes were often associated with filamentous green algae, which are also known to inhibit phytoplankton growth (Irfanullah and Moss 2005; Peretyatko et al. 2007a), their combined surface cover was used in statistical analyses.

Morphometric variables of the ponds were measured in the field (depth), or using GIS software. Hydraulic retention time was estimated on the basis of the outlet discharge and the corresponding pond volume once in 2006 and monthly in 2007. Monthly measurements showed little variation suggesting that a single measurement can give a reasonably good idea of the retention time in a particular pond outside heavy rain events.

Nine ponds the most affected by eutrophication (persistent phytoplankton blooms often dominated by bloom-forming cyanobacteria, recurrent anoxic/hypoxic conditions and fish and waterfowl kills) were biomanipulated (emptied with fish removal) in order to improve their ecological quality, to reduce phytoplankton biomass and prevent the formation of noxious cyanobacterial blooms. One pond (MlKl) was biomanipulated in early spring 2006 and eight ponds (Beml, Dens, Leyb-a, Leyb-b, PRB2, Sbsk, VKn2 and WPk1) were biomanipulated in early spring 2007. Leyb-a and Leyb-b are two connected ponds separated by a small dam. MlKl was sampled monthly from May to September in 2005–2007 and in May, July and August in 2008. Two ponds (VKn2 and WPk1) were sampled in May, July and August in 2005 and 2008 and monthly from May to September in 2007. The remaining six ponds were sampled in May,

July and August in 2006 and 2008 and monthly from May to September in 2007.

Seven ponds were recolonised by fish after biomanipulation (mainly juvenile planktivorous fish). Due to generally very small size of fish it was difficult to make reliable quantitative estimates of fish density. Fish presence in the biomanipulated ponds was assessed visually and by fishing with a landing net (4 mm mesh size) during each field visit. Ponds where fish reappeared in large numbers were considered as recolonised by fish. Fish presence/absence was used to elucidate changes in zooplankton community composition and size structure.

Statistical analyses

Standard multivariate and univariate tests were used for the analysis of the data. Because the gradient length was below 1.7 standard deviations, suggesting poor unimodality (ter Braak and Smilauer 2002), a linear method—redundancy analysis (RDA), based on average per year environment and phytoplankton data (aggregated to division level) from all the ponds studied, was used to elucidate the relationships between main phytoplankton groups and environmental factors controlling them. Groups of ponds created by the RDA were used to determine in which ponds and years the biomanipulation worked or failed.

Statistical comparisons of phytoplankton, zooplankton, submerged vegetation and main nutrients data averaged per pond per year and corresponding to different biomanipulation outcomes were done with the non-parametrical Kruskal–Wallis test because many of the variables tested were not normally distributed and the variance was not homogeneous. On the basis of the results of the RDA, the data from biomanipulated ponds were subdivided into two main groups: ponds where biomanipulation was successful throughout the study (Beml, Dens, Leyb-a, Sbsk, VKn2 and WPk1) and ponds where biomanipulation failed (Leyb-b, MlKl and PRB2). The first group was further divided into two subgroups: 1b—before biomanipulation and 1a—after biomanipulation. The second group was divided into three subgroups: 2b—before biomanipulation, 2a—after biomanipulation when it worked (MlKl 2006 and 2007; Leyb-b 2007); 2f—after biomanipulation when it failed (PRB2 2007 and 2008; MlKl 2008; Leyb-b 2008). The results of the Kruskal–Wallis tests are shown on the corresponding multipanel boxplots.

Results

All the ponds studied were rich in nutrients (mean TP concentrations above 0.1 mg L^{-1}; Table 1). Phytoplankton biomass varied from 2 to more than 400 μg L^{-1} Chl *a*. Increase in TP concentrations generally corresponded to increase in the total phytoplankton and cyanobacteria biomass. The phytoplankton biomass showed first gradual and then sharp increase along the TP concentration gradient (Fig. 1). High TP concentrations, however, were often associated with low phytoplankton biomass suggesting that other factors can decouple phytoplankton from nutrients.

RDA analysis allowed the factors having the strongest relationships with phytoplankton to be identified (Fig. 2). The first two RDA axes explained 33% and 4% of the variation in the phytoplankton data, respectively (eigenvalues 0.318 and 0.039). As indicated by the Secchi depth arrow, the first axis corresponds to an inverse phytoplankton biomass gradient. Chl *a* and Secchi depth showed a highly significant correlation with phytoplankton biovolumes ($p < 0.01$) suggesting that the latter gives a reasonable estimation of phytoplankton biomass and that turbidity in the ponds studied is mostly phytoplankton induced. To avoid the blurring effect of Chl *a*, Secchi depth and TP (the proxies of phytoplankton biomass) on other environmental variables, they were excluded from the model in forward selection analysis. After their exclusion, six variables showed a significant relationship with the phytoplankton data. These are (in order of percentage variance explained; Table 2): pH, submerged vegetation, large Cladocera length, DIN, SRP and maximum depth. Large Cladocera density was marginally insignificant. The RDA clearly separated the situations before and after biomanipulation (when biomanipulation worked) into two groups along the phytoplankton biomass gradient (Fig. 2). This was consistent with the phytoplankton biomass

Table 1 General characteristics of the biomanipulated ponds before and after biomanipulation

Site	TP (mgP L^{-1})	SRP (mgP L^{-1})	NH4 (mgN L^{-1})	NO_x (mgN L^{-1})	Chl *a* (μg L^{-1})	MD (m)	Cond (μS cm^{-1})	pH	RT (day)
Before biomanipulation									
Beml (1b)	0.673	0.203	0.055	0.082	55.1	1.0	748	7.9	470
	0.402–0.861	0.017–0.479	0.008–0.147	0.018–0.155	14.1–83.8	1.0–1.0	528–875	7.6–8.2	
Dens (1b)	0.351	0.030	0.045	0.048	112.1	0.7	422	8.4	320
	0.084–0.493	0.005–0.072	0.015–0.098	0.005–0.102	77.6–170.0	0.6–0.8	285–517	8.2–8.6	
Leyb-a (1b)	0.506	0.009	0.034	0.202	495	0.6	536	9.0	160
	0.309–0.669	0.000–0.022	0.001–0.053	0.006–0.550	79.1–867.2	0.5–0.7	453–696	8.4–9.4	
Leyb-b (2b)	0.407	0.005	0.028	0.216	398.5	0.8	557	8.8	250
	0.330–0.452	0.000–0.013	0.001–0.053	0.005–0.599	154.2–604.5	0.7–0.8	480–699	8.2–9.3	
MlKl (2b)	0.354	0.019	0.014	0.043	113.3	0.9	473	8.2	11
	0.160–0.495	0.003–0.035	0.000–0.053	0.001–0.280	15.2–225.5	0.8–1.1	336–594	7.9–8.5	
PRB2 (2b)	0.428	0.091	0.204	0.182	56.9	0.8	735	8.0	>1,000
	0.201–0.861	0.007–0.243	0.178–0.238	0.024–0.326	52.8–66.8	0.6–1.2	546–881	7.7–8.1	
Sbsk (1b)	0.426	0.018	0.246	0.149	93.8	0.7	781	8.4	190
	0.301–0.624	0.005–0.037	0.092–0.416	0.039–0.296	36.5–181.4	0.7–0.7	691–848	8.2–8.6	
VKn2 (1b)	0.207	0.004	0.042	0.376	73.7	1.0	562	7.7	4
	0.085–0.390	0.000–0.011	0.004–0.166	0.004–1.013	64.6–82.7	0.9–1.1	509–607	7.3–7.9	
WPk1 (1b)	0.223	0.002	0.014	0.064	45.1	1.1	896	7.8	30
	0.096–0.360	0.000–0.010	0.000–0.038	0.004–0.355	30.3–70.9	0.9–1.2	796–969	7.4–8.0	
After biomanipulation									
Beml (1a)	0.242	0.127	0.535	0.101	18.4	1.0	904	7.6	470
	0.104–0.406	0.017–0.348	0.046–1.291	0.020–0.233	1.1–112.1	0.9–1.1	837–987	7.4–7.9	
Dens (1a)	0.180	0.058	0.852	0.042	13.1	0.6	418	7.8	320
	0.122–0.342	0.012–0.093	0.008–2.552	0.008–0.081	2.7–69.4	0.6–0.7	338–447	7.5–8.7	
Leyb-a (1a)	0.811	0.553	0.223	0.008	50.8	0.5	594	8.4	160
	0.361–1.691	0.284–1.156	0.005–1.375	0.007–0.011	1.9–130.0	0.4–0.6	512–729	7.7–9.3	
Leyb-b (2a)	0.177	0.109	0.099	0.095	56.3	0.9	685	8.0	250
	0.073–0.482	0.015–0.353	0.01–0.378	0.021–0.168	1.1–168.7	0.8–1	580–771	7.9–8.3	
Leyb-b (2f)	0.668	0.235	0.032	0.016	249.2	0.84	567	8.7	250
	0.524–0.922	0.115–0.337	0.014–0.064	0.003–0.026	83.7–550	0.8–0.9	542–590	8.59–8.84	
MlKl (2a)	0.318	0.171	0.185	0.103	13.6	0.9	518	8.2	11
	0.132–0.687	0.026–0.455	0.061–0.424	0.078–0.142	1.5–27.0	0.6–1.2	463–587	7.8–8.5	
MlKl (2f)	0.626	0.331	0.127	0.023	170.8	1.0	448	8.5	15
	0.403–0.865	0.220–0.421	0.024–0.253	0.018–0.029	3.2–464.2	1.0–1.0	379–491	7.7–9.0	
PRB2 (2f)	0.328	0.022	0.128	0.039	141.1	0.8	655	7.9	>1,000
	0.178–0.439	0.004–0.056	0.011–0.521	0.003–0.087	57.8–222.1	0.6–0.9	566–728	7.8–8.3	
Sbsk (1a)	0.220	0.159	0.462	0.070	12.7	0.8	655	7.9	190
	0.059–0.336	0.019–0.313	0.035–1.215	0.028–0.133	2.9–46.2	0.6–0.9	490–872	7.6–8.5	
VKn2 (1a)	0.128	0.049	0.050	0.003	6.9	1.1	487	7.6	4
	0.046–0.266	0.007–0.159	0.010–0.135	0.002–0.004	4.0–9.5	1.0–1.3	398–573	7.3–8.0	
WPk1 (1a)	0.104	0.030	0.291	0.301	10.3	1.1	912	7.7	30
	0.050–0.221	0.002–0.160	0.002–1.515	0.213–0.355	1.6–29.1	0.9–1.2	857–963	7.5–8.0	

Average and range values are given when relevant

MD maximum depth, *Cond* conductivity, *RT* hydraulic retention time; *1b*, *1a* successfully biomanipulated ponds before and after biomanipulation, *2b, 2a, 2f* unsuccessfully biomanipulated ponds before, after when it worked and after when it failed

data. It also showed that in the case of failure the ponds remained (PRB2 2007 and PRB2 2008) or shifted back (Leyb-b 2008 and MlKl 2008) to the pre-biomanipulation group. The samples from these ponds showed high phytoplankton biomass (Secchi depth less than 0.5 m for several consecutive months). This supports the idea that in these ponds biomanipulation failed.

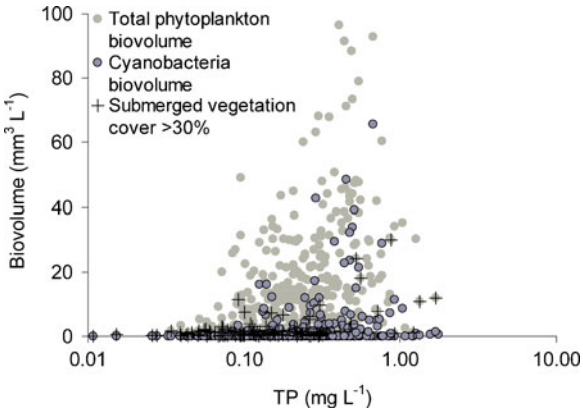


Fig. 1 Relationship between TP and phytoplankton biovolume and submerged vegetation cover (data from 48 ponds studied, TP in log scale). *Crosses* indicate the samples where submerged vegetation cover exceeds 30%

In two ponds, PRB2 and WPk1, numerous fish were observed soon after these ponds were refilled with water, suggesting that not all fish were removed. It should be noted that it was not possible to completely empty these ponds. Fish reappeared in a number of other biomanipulated ponds at different times after biomanipulation (Fig. 3). They came from other ponds or were introduced by humans.

With a single exception of PRB2, all biomanipulated ponds showed a drastic reduction in phytoplankton biomass during the first summer after fish removal. It should be noted that PRB2 was repeatedly polluted by sewage water as a result of sewage overflow into the pond during heavy rains. Such sewage discharge into the pond was

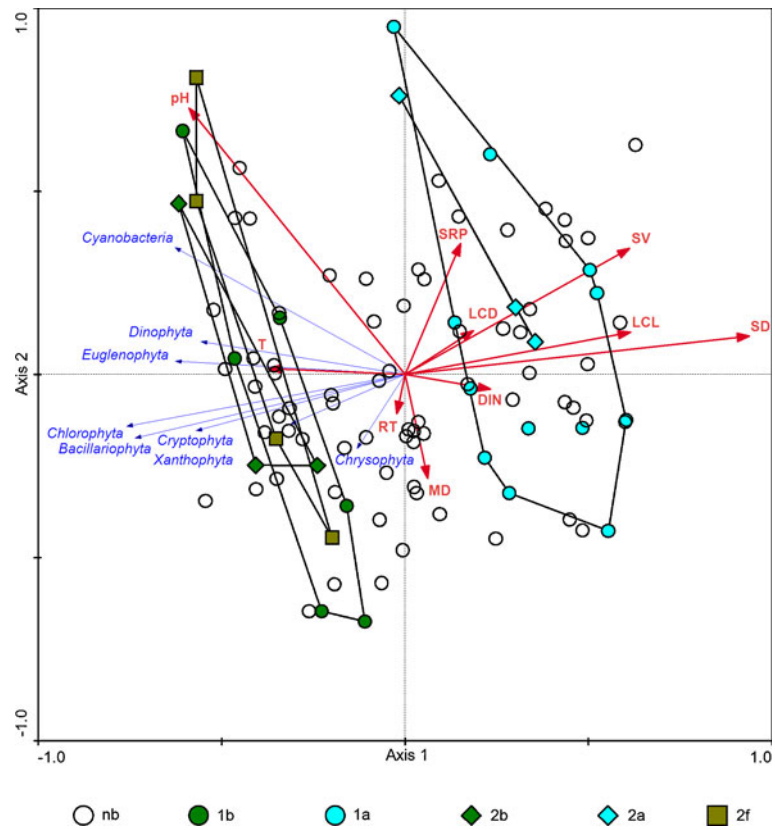


Fig. 2 Redundancy analysis triplot (sites, phytoplankton divisions and environmental variables) based on averaged per year phytoplankton and environment data (2005–2008) from the 48 ponds studied. *Abbreviations*: *DIN* dissolved inorganic nitrogen, *LCL* large Cladocera length, *LCD* large Cladocera density, *MD* maximum depth, *RT* hydraulic retention time, *SD* Secchi depth, *SV* submerged vegetation cover, *SRP* soluble reactive phosphorus, *T* temperature. *Site symbols* indicate biomanipulation status, *nb* non-biomanipulated ponds, *1b*, *1a* successfully biomanipulated ponds before and after biomanipulation, *2b, 2a, 2f* unsuccessfully biomanipulated ponds before, after when it worked and after when it failed

Table 2 RDA forward selection results based on the data from the 48 ponds studied

Marginal effects		Conditional effects		
Variable	Lambda1	Variable	LambdaA	*P*
pH	0.17	pH	0.17	0.002
LCL	0.15	SV	0.12	0.002
SV	0.13	LCL	0.04	0.004
T	0.07	DIN	0.02	0.016
DIN	0.04	SRP	0.02	0.018
LCD	0.03	MD	0.02	0.048
SRP	0.02	LCD	0.02	0.056
MD	0.02	RT	0.01	0.266
RT	0.01	T	0.00	0.364

Marginal effects show the variance explained by each environmental variable alone (Lambda1); conditional effects show the significance of the addition of a given variable (*P*); and the variance explained by the selected variables at the time they were included in the model (LambdaA)

observed soon after biomanipulation. The drop in phytoplankton biomass in the other ponds was accompanied with a marked increase in the density and size of large cladocerans (Fig. 3). Increased zooplankton grazing did not seem to cause any particular pattern in the phytoplankton composition dynamics (Fig. 4).

In five ponds (Beml, Leyb-a, Leyb-b, Sbsk and VKn2), biomanipulation resulted in the restoration of submerged vegetation (mainly *Potamogeton* spp., *Chara* spp., *Nitella* sp., *Ceratophyllum* sp. and green filamentous algae) already during the first year. In WPk1, extensive beds of *Potamogeton* spp. developed only during the second summer after biomanipulation (Fig. 3). No vegetation was restored in Dens, MlKl and PRB2. This can probably be attributed to high phytoplankton turbidity in PRB2 and bird herbivory in Dens and MlKl as these two ponds are very shallow (<1 m deep) and harbour large populations of herbivorous birds (mainly Egyptian geese (*Alopochen aegyptiacus*) and Canada geese (*Branta canadensis*); personal observations).

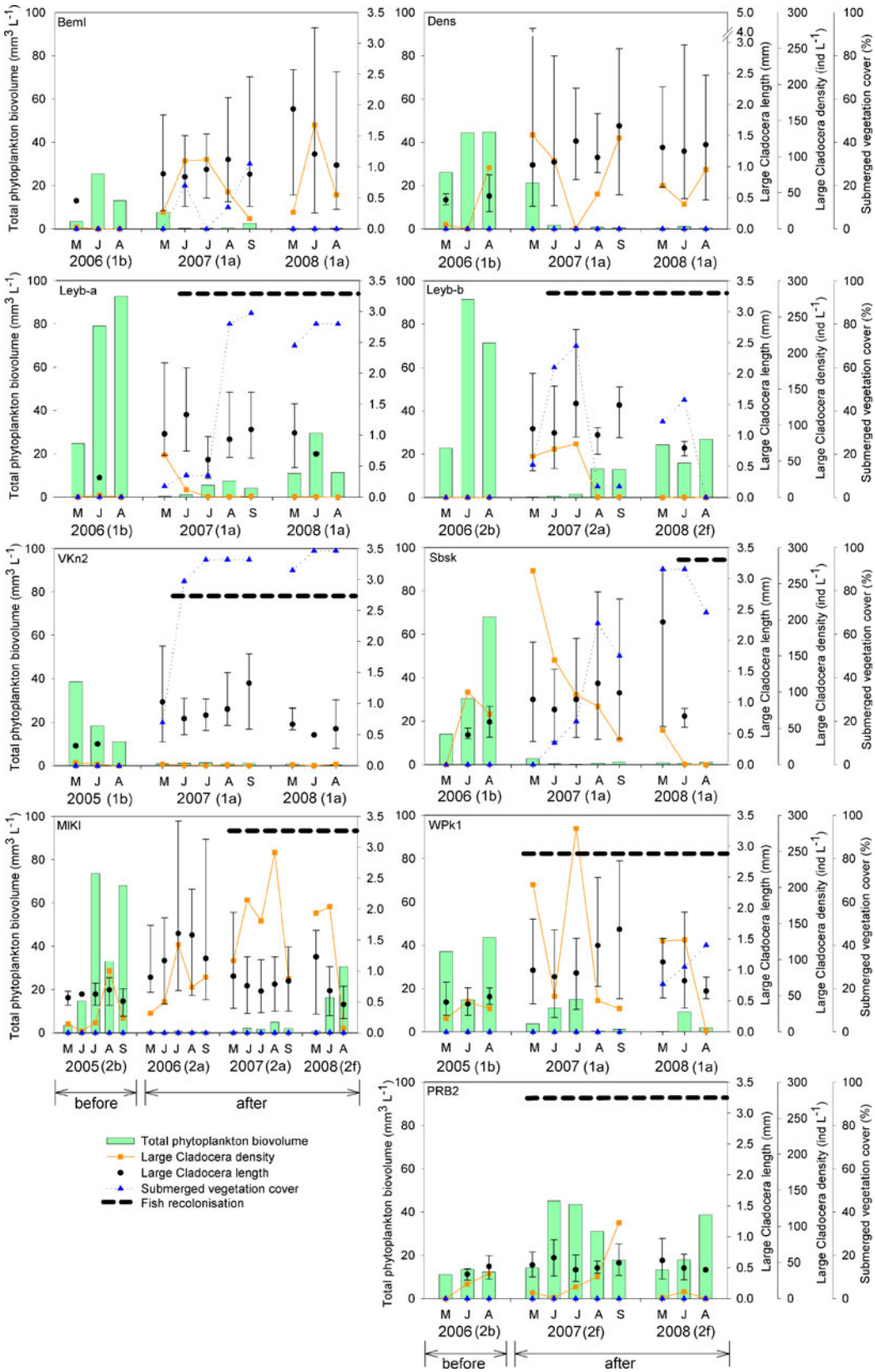
The comparison of the before and after biomanipulation situations with the Kruskal–Wallis test showed that phytoplankton biomass was significantly reduced, whereas the density and size of large cladocerans and submerged vegetation cover significantly increased in the ponds where biomanipulation was successful (1a: Fig. 5) as well as the first year after biomanipulation in Leyb-b and first 2 years in MlKl (2a). Biomanipulation also resulted in a significant decrease in biomass of cyanobacteria.

When biomanipulation failed (2f) phytoplankton biomass significantly increased and density and size of large cladocerans significantly decreased as compared to the years when biomanipulation worked (2a; Fig. 5). The change in the submerged vegetation cover was not significant. It was present, however, only in one pond (Leyb-b), where it collapsed before the shift to the turbid state.

Biomanipulation also affected nutrients. SRP and NH_4 significantly increased when biomanipulation worked (1a and 2a; Fig. 6). TP concentrations decreased significantly only in ponds where biomanipulation was successful (1a). NO_x concentrations did not show significant differences between the before and after biomanipulation situations.

Even though fish removal resulted in a marked decrease in phytoplankton biomass in all but one pond (PRB2) during the first year after biomanipulation, different ponds have shown contrasting phytoplankton dynamics throughout the monitoring period that depended, to a considerable degree, on the recovery of submerged vegetation and fish recolonisation. Ponds that were not recolonised by fish could maintain very low phytoplankton biomass even in the absence of submerged vegetation (Dens and Beml 2008; MlKl 2006). These ponds have shown high densities of large cladocerans. In the absence of fish and submerged vegetation, large cladocerans were the biggest in size (Fig. 3). In the ponds with extensive and dense submerged vegetation, phytoplankton biomass generally remained low despite fish reappearance (VKn2 and Sbsk 2008; WPk1 2008; Fig. 3), whereas when submerged vegetation was lacking, fish reappearance was associated with marked fluctuations in phytoplankton biomass (WPk1 2007; Fig. 3). In MlKl (2008) this resulted in a shift back to the turbid state dominated by dinoflagellates (*Ceratium hirundinella*) and cyanobacteria (*Aphanizomenon* spp.).

In 2008, Leyb-a and Leyb-b showed relatively high phytoplankton biomass despite the presence of extensive *Potamogeton* spp. stands (Fig. 3).


Beml
Dens
Leyb-a
Leyb-b
VKn2
Sbsk
MlKl
WPk1
PRB2
Total phytoplankton biovolume (mm3 L-1)
Large Cladocera length (mm)
Large Cladocera density (ind L-1)
Submerged vegetation cover (%)
M J A
2006 (1b)
2007 (1a)
2008 (1a)
2006 (2b)
2007 (2a)
2008 (2f)
2005 (1b)
M J J A S
2005 (2b)
2006 (2a)
2007 (2a)
2008 (2f)
2007 (1a)
2006 (2b)
2007 (2f)
before
after
Total phytoplankton biovolume
Large Cladocera density
Large Cladocera length
Submerged vegetation cover
Fish recolonisation

◀**Fig. 3** Temporal dynamics of phytoplankton biomass, large Cladocera density and size and submerged vegetation cover in the biomanipulated ponds. *1b*, *1a* successfully biomanipulated ponds before and after biomanipulation, *2b, 2a, 2f* unsuccessfully biomanipulated ponds before, after when it worked and after when it failed

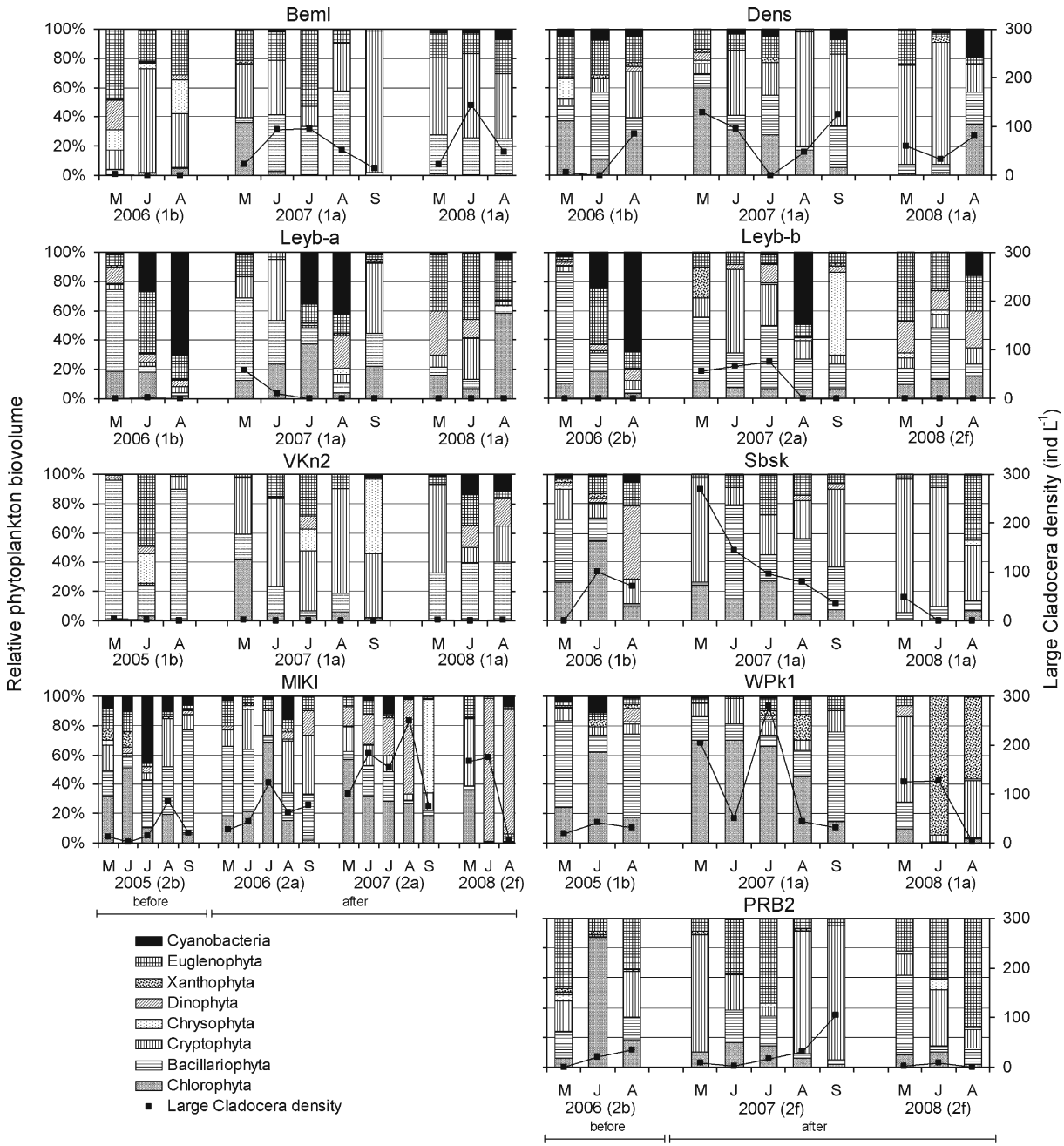


Fig. 4 Temporal dynamics in phytoplankton composition and its relationship to large Cladocera densities in the biomanipulated ponds. *1b*, *1a* successfully biomanipulated ponds before and after biomanipulation, *2b, 2a, 2f* unsuccessfully biomanipulated ponds before, after when it worked and after when it failed

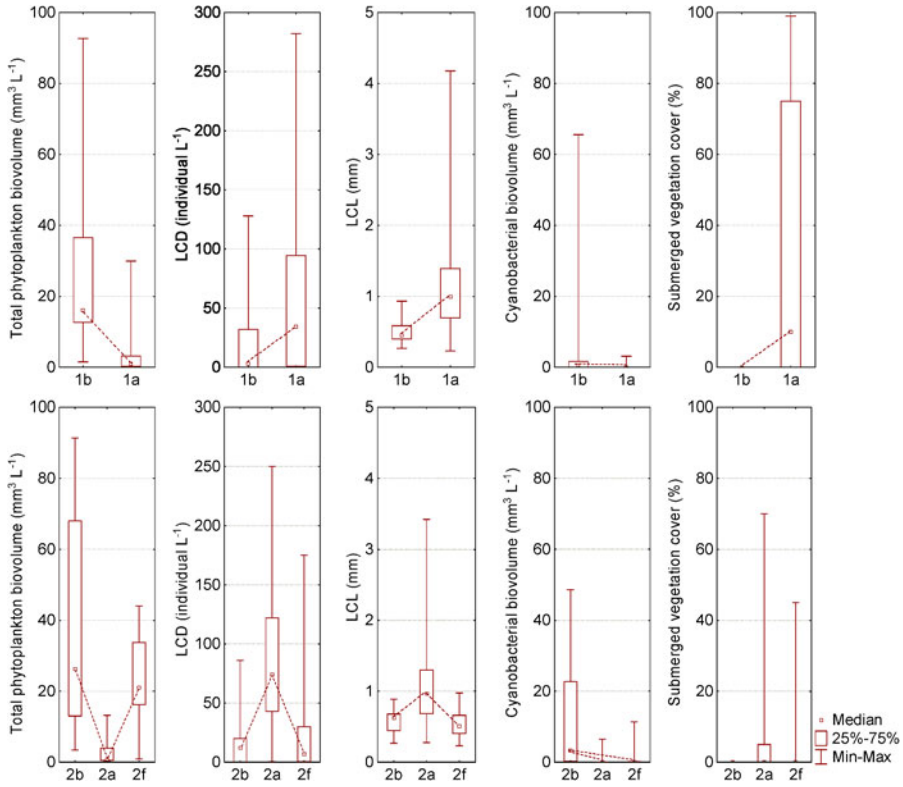


Fig. 5 Comparison of changes in different compartments of the biomanipulated ponds before (*1b*) and after biomanipulation (*1a*) in the ponds where biomanipulation was successful and before (*2b*), after when it worked (*2a*) and after when it failed (*2f*) in the ponds where biomanipulation was not successful. *Lines between boxes* indicate significant changes (Kruskal–Wallis test; $p < 0.05$). For abbreviations see the legend of Fig. 2

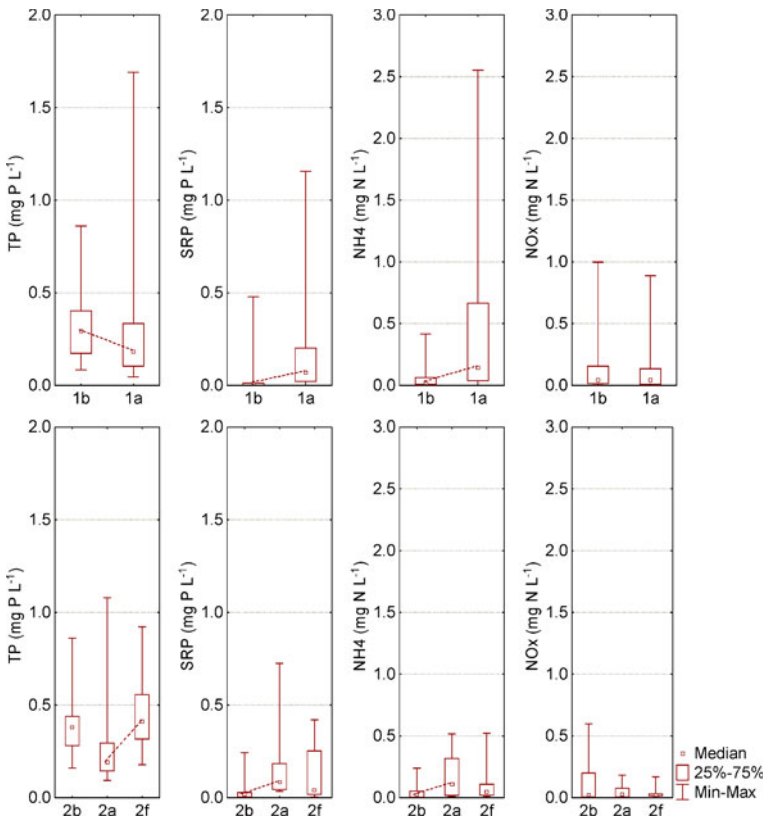


Fig. 6 Comparison of changes in TP, SRP, NH_4 and NO_x in the biomanipulated ponds before (*1b*) and after biomanipulation (*1a*) in the ponds where biomanipulation was successful and before (*2b*), after when it worked (*2a*) and after when it failed (*2f*) in the ponds where biomanipulation was not successful. *Lines between boxes* indicate significant changes (Kruskal–Wallis test; $p < 0.05$)

These two ponds were characterised by high nutrient level (Table 1) due to periodic sewage discharges from the neighbouring households. The phytoplankton assemblages of these ponds were dominated by the flagellated phytoplankters (mainly *Euglena* spp., *Trachelomonas* spp. and, to a lesser extent, *Cryptomonas* spp.). Large Cladocera were observed in very low numbers, except for May–July 2007 in Leyb-b in association with extensive *Potamogeton* spp. stands. In August 2008, large Cladocera were virtually absent in both ponds despite the presence of submerged vegetation, and when *Potamogeton* spp. stands collapsed in Leyb-b, phytoplankton assemblage shifted towards cyanobacterial dominance (*Anabaenopsis* sp.). At this point the managers emptied these ponds in order to prevent further proliferation of cyanobacteria.

Discussion

The ponds studied were nutrient rich and therefore highly productive ecosystems. This productivity potential was realised in some ponds in the development of high phytoplankton biomass that was often dominated by toxic cyanobacteria (all the 2007 and 2008 samples ($n = 46$) from the Brussels ponds analysed for cyanobacterial toxins at Dundee University, Scotland, turned out to be positive for microcystins; Descy et al. 2009). Some ponds were also prone to recurrent anoxic/hypoxic conditions and occasional fish and waterfowl kills. This had a negative effect on the ecological quality (Peretyatko 2007) and, due to the location of these ponds within the densely populated urban area, posed serious public health concerns. Because nutrient reduction was not feasible in the short-term, the local authority managing the ponds resorted to biomanipulation, specifically to its most drastic form—complete fish removal, as it has proven to be able to bring positive results even in nutrient-rich ponds and lakes (Jeppesen et al. 1990; Moss et al. 1996a).

When nutrients are not limiting, phytoplankton is controlled by other factors (Reynolds 2006; Peretyatko et al. 2007b). This idea is consistent with the fact that many Brussels ponds do not develop high phytoplankton biomass despite the apparent nutrient richness. Submerged vegetation and zooplankton grazing appear to be the main factors responsible for phytoplankton control in such ponds (Peretyatko et al. 2007a, b, 2009). This is supported by the RDA results (Table 2, Fig. 2). Submerged vegetation cover and large Cladocera length are the variables having significant negative relationships with phytoplankton; they explained the greater part of the variation in the phytoplankton data.

High densities of plankti-benthivorous fish can prevent the growth of submerged macrophytes (Moss et al. 1996a) and drastically reduce the number and size of large zooplankters (Pourriot 1995), thus shifting a pond or a lake towards a turbid, phytoplankton-dominated state (Scheffer 1998; Scheffer and Jeppesen 1998). Therefore, removal of plankti-benthivorous fish promotes both restoration of submerged vegetation and phytoplankton control by large zooplankton grazing (Moss et al. 1996a; Lammens 1999). This is unequivocally supported by the biomanipulation results. Fish removal led to a significant decrease in phytoplankton biomass associated with a significant increase in large Cladocera densities and size as well as restoration of submerged vegetation in six out of nine biomanipulated ponds (Figs. 3 and 5).

Intense grazing by large cladocerans has not shifted phytoplankton assemblages of the biomanipulated ponds towards grazing-resistant phytoplankters, like bloom-forming cyanobacteria (Fig. 4), as reported by Gliwicz (1990b). Elevated concentrations of cyanobacteria were observed only when large Cladocera populations were decimated by fish. This suggests that when released from predation pressure, large cladocerans can grow big enough (in the fishless ponds the length of large cladocerans exceeded at times 3 mm) to be capable of efficient control of phytoplankton, including bloom-forming cyanobacteria and thus can prevent bloom formation. This is consistent with the report of Gliwicz (1990b) stating that *Daphnia* can control bloom-forming cyanobacteria below critical filament concentration.

Despite the removal of fish, no marked evidence of large Cladocera control by macroinvertebrates, as reported by Benndorf et al. (2000), was observed during the study period. The

fluctuations of large Cladocera densities in the two completely fishless ponds seem to be related to food availability rather than predation. It is possible that macroinvertebrates preyed preferentially on smaller cladocerans and thus biased their size structure towards bigger individuals (Pinel-Alloul 1995) as cladocerans in these two ponds were the biggest in size. This, however, can also be attributed to the absence of predation by fish and low food availability that gives larger filter feeders a competitive advantage (Gliwicz 1990a).

Low phytoplankton biomass in association with low large Cladocera density and size but profuse growth of submerged macrophytes observed in VKn2 after biomanipulation suggests that submerged vegetation alone can strongly inhibit phytoplankton growth when it is sufficiently dense and covers most of the pond surface. Similar reports were made earlier for shallow lakes (Søndergaard and Moss 1998; Blindow et al. 2000). It should be noted, however, that when very abundant, submerged plants can also be considered a nuisance, notably because of inhibition of recreational use and negative effect on the ecological quality owing to the dominance of a single plant species typical for such cases (Moss et al. 2003).

Marked decrease of phytoplankton biomass in the ponds where submerged vegetation was not restored shows that large cladocerans alone can also considerably restrain phytoplankton growth, including that of bloom-forming cyanobacteria. The situation without fish, however, is very unstable as in most cases fish can come from the neighbouring ponds through stream connections, or can be stocked by humans. The results presented here confirm that rapid fish recolonisation can compromise the biomanipulation effort. Fish reappearance in the ponds studied was reflected on the density and size of large cladocerans and subsequently on phytoplankton biomass. This likely caused the shift to the turbid state with high concentration of toxic cyanobacteria in MlKl, a pond where submerged vegetation was not restored 2 years after biomanipulation. The ponds harbouring submerged vegetation, however, could resist to a considerable degree of fish recolonisation. They generally maintained low phytoplankton biomass despite the presence of planktivorous fish. This supports the idea that restoration of submerged macrophytes is crucial for biomanipulation success (Jeppesen et al. 1990; Moss et al. 1996a; van Donk and van de Bund 2002).

It seems, however, that the ability of submerged vegetation to inhibit phytoplankton growth has a strong inverse relationship with the nutrient levels. When nutrient concentrations rise, the vegetation capacity to limit nutrient availability to phytoplankton diminishes and the maintenance of the clearwater state becomes increasingly dependent on grazing by large cladocerans (Moss et al. 1996a). High fish predation can decimate the populations of large cladocerans and thus prevent top–down phytoplankton control and push the system towards the turbid, phytoplankton-dominated state (Pourriot 1995; Moss et al. 1996b).

The significant increase in SRP and NH_4 concentrations despite the restoration of extensive stands of submerged vegetation suggests that nutrient loading levels in the biomanipulated ponds generally exceeded the capacity of submerged plants to sequester the nutrients from the water column to a level that would inhibit phytoplankton growth. This is consistent with the suggestion of Jeppesen et al. (1999) that nutrient limitation of phytoplankton, due to uptake by submerged vegetation, is more likely to occur at lower nutrient loadings. The latter is also supported by the relationship between TP concentrations and phytoplankton biomass and submerged vegetation cover. In the ponds studied, submerged vegetation covering more than 30% of the pond surface was associated with low phytoplankton biomass except for ponds with high TP concentrations (Fig. 1). It should be noted that submerged macrophytes often became senescent rather early in the growing season, mainly due to fouling with epiphytic algae; *Potamogeton* spp. appeared to be the most affected. It therefore seems reasonable to assume that submerged vegetation could have also contributed to the dissolved nutrients pool through mineralisation of dead organic matter.

Thus, the ponds excessively polluted with nutrients often showed elevated phytoplankton biomass despite the presence of extensive submerged vegetation. These ponds were lacking

large cladocerans due to fish recolonisaion, their phytoplankton assemblages being dominated by the flagellated phytoplankters that are less affected by water column stabilisation induced by submerged vegetation (Søndergaard and Moss 1998; Reynolds 2006). This shows that submerged vegetation can buffer the effects of eutrophication only to a certain degree, and implies that increase in nutrient level augments the risk of biomanipulation failure as the likelihood of the development of high phytoplankton biomass in general and cyanobacterial biomass in particular sharply increases up the TP concentration gradient. The fact that fish removal has not resulted in phytoplankton biomass decrease in the pond that was repeatedly polluted by sewage water after biomanipulation supports this idea and suggests that beyond certain nutrient level biomanipulation may have no negative effect on phytoplankton. The latter is consistent with other reports (Jeppesen et al. 1990; Moss et al. 1996a). It seems that in case of excessive nutrient pollution the restoration of ponds to the clearwater state is only possible in combination with a drastic reduction of nutrient loading, as stated earlier for lakes (Jeppesen et al. 1990; Sondergaard et al. 2008).

Conclusions

Biomanipulation has a good potential for short-term restoration of the ecological quality of eutrophic ponds and prevention of the formation of noxious phytoplankton blooms. It can bring a drastic reduction in phytoplankton biomass and restoration of submerged vegetation. The maintenance of the restored clearwater state in the biomanipulated ponds depends to a considerable degree on fish recolonisation. In the absence of fish, the clearwater state can be maintained by large zooplankton grazing alone. In case of fish recolonisation, restoration of extensive submerged vegetation seems to be indispensable for the biomanipulation success. Submerged vegetation, when sufficiently dense and extensive, can considerably restrain phytoplankton growth even in the absence of large zooplankton. The effect of submerged vegetation on phytoplankton in eutrophic ponds seems to be, however, strongly dependent on nutrient level. The more nutrients the less pronounced the effect and beyond certain nutrient level the capacity of submerged vegetation to inhibit phytoplankton growth might be strongly reduced. Therefore, for biomanipulation to be successful in ponds excessively polluted with nutrients, fish removal has to be accompanied with substantial nutrient loading reduction.

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