Brussels Instituut voor Milieubeheer
Institut bruxellois pour la gestion
de l' environnement

Vrije Universiteit Brussel
Vakgroep Biologie

Algemene Plantkunde
en Natuurbeheer

Ecologische beoordeling van het effect van biomanipulatie op langere termijn in enkele vijvers in het Brussels Hoofdstedelijk Gewest

Sylvia DE BACKER, Anatoly PERETYATKO, Samuel TEISSIER and Ludwig TRIEST

Eindverslag

01.04.2009– 31.03.2010

Inhoudstafel

Samenvatting

Gedurende de voorbije decennia zijn vele ondiepe meren en vijvers in bepaalde mate onderhevig geweest aan eutrofiëring ten gevolge van menselijke activiteiten. Dit kan een overgang veroorzaken naar een troebele, fytoplanktongedomineerde watertoestand, wat vaak resulteert in een ernstige bloei van potentieel toxische cyanobacteriën. De Brusselse vijvers vertonen over het algemeen een grote variatie aan fytoplanktonbiomassa, ondanks de over het algemeen hoge nutriëntenconcentraties (eutroof tot hypereutroof volgens totale fosfaatconcentraties). Dit impliceert dat de Brusselse vijvers een goed potentieel hebben voor restauratie door biomanipulatie.

In deze studie werd de ecologische status van 13 Brusselse vijvers, gebiomanipuleerd tussen 2005 en 2009 (i.e. drooglegging van de vijvers en verwijdering van vis), bestudeerd aan de hand van een onderzoek van het fytoplankton, het zoöplankton, de macrofytenvegetatie alsook de nutriëntenconcentraties. Om de context van het onderzoek te verbreden, werden 17 extra vijvers gedurende dezelfde periode bestudeerd ter vergelijking.

Initieel bracht de biomanipulatie een positief resultaat in 11 van de 12 vijvers. De meeste vijvers vertoonden een merkbare verbetering in verschillende aspecten van hun ecologische kwaliteit. De biomanipulatieresultaten bevestigen de impact van (plankti- en benthivore) vissen op de ecologische kwaliteit van vijvers en tonen aan dat wanneer vijvers beïnvloed worden door eutrofiëring, een belangrijk gedeelte van de ecologische kwaliteit kan hersteld worden door het manipuleren van de visgemeenschappen. Vissen spelen een centrale rol in het structureren van zoöplankton- en submerse vegetatiegemeenschappen die, op hun beurt, een cruciale rol spelen in de controle van fytoplankton in eutrofe vijvers.

Een belangrijke factor die het succes van biomanipulatie op langere termijn kan ondermijnen is de herkolonisatie door vissen. De herkolonisatie van kleine planktivore vissen had echter enkel een significant effect op de fytoplanktonbiomassa in vijvers met een submerse vegetatie met een bedekkingspercentage van minder dan 30%. Dit was niet het geval in vijvers met een vegetatiebedekking van meer dan 30%, waar, ondanks het negatieve effect van vissen op de densiteiten en de lengte van de grote cladoceren, de fytoplanktonbiomassa niet verhoogde na herkolonisatie. Dit benadrukt het belang van het herstel van submerse vegetatie na biomanipulatie, die, ook al wordt niet voldoende bescherming geboden voor de grote cladoceren, toch in staat is het fytoplankton efficiënt te controleren indien het bedekkingspercentage groter is dan 30%. Het belang van submerse vegetatie voor de voortplanting van snoeken en de stabilisatie van het systeem na biomanipulatie in acht genomen, zijn maatregelen om een dense submerse vegetatie te herstellen van belang voor de stabilisatie van de helder watertoestand na biomanipulatie.

Hoewel submerse macrofyten hebben aangetoond dat ze in staat zijn om een stijging van de fytoplanktonbiomassa te voorkomen, is een beperking van de nutriëntenconcentraties in zekere mate nodig. Boven een zekere grenswaarde van TP in de waterkolom (een ruw gemiddelde van 350 µg L^{-1} voor Brusselse vijvers) zijn macrofyten niet meer in staat om het fytoplankton efficiënt te controleren gedurende de hele zomer, wat uiteindelijk resulteert in het verdwijnen van de vegetatie. Deze grenswaarde werd ook gebruikt voor het opstellen van een schema voor het bepalen van de geschikte restoratiemethode voor vijvers in Brussel, waarbij geadviseerd wordt om voor biomanipulatie, indien mogelijk, de nutriëntentoevoer te reduceren als TP > 350 µg L^{-1}. In dit schema worden ook maatregelen voorgesteld om de stabiliteit van de helder watertoestand te verbeteren door het herstel van vegetatie te stimuleren of piscivore vissen toe te voegen. Regelmatige monitoring van succesvol gerestaureerde vijvers is nodig indien men verslechtering van de situatie vroeg wil opsporen en zo tijdig maatregelen te kunnen nemen opdat de situatie niet verder verslechtert.

Abstract

During the past decades, many shallow lakes and ponds have been subject to a considerable degree of eutrophication, as a result of human activities, which can cause a transition to a turbid, phytoplankton-dominated state, often resulting in severe blooms of potentially toxic cyanobacteria. Brussels ponds in general show a considerable variation in phytoplankton biomass, despite the overall high nutrient concentrations (eutrophic to hypereutrophic when considering total phosphorus). Therefore, these ponds have a potential for restoration by means of biomanipulation.

The aim of this study was to investigate the ecological state of 13 Brussels ponds biomanipulated in 2005 – 2009 (i.e. pond drawdown and fish removal) by means of assessing changes in phytoplankton, zooplankton, macrophytic vegetation communities and main nutrient concentrations. In order to investigate the biomanipulated ponds within a broader context, 17 additional ponds were studied during the same period.

Initially, the biomanipulation brought positive results in all but one pond. Most of the biomanipulated ponds have shown a marked improvement in several aspects of their ecological quality. The biomanipulation results confirm the importance of fish in determining the ecological quality of eutrophic ponds and indicate that when pond ecosystems are impaired by eutrophication, a considerable degree of their ecological quality can be restored through manipulation of the fish community. Fish play a central role in structuring zooplankton and submerged macrophyte communities that, on their turn, play a crucial role in controlling phytoplankton in eutrophic ponds.

An important factor altering the positive result of biomanipulation on the longer term is the recolonization of fish. However, the reappearance of small zooplanktivorous fish did only have a significant effect on phytoplankton biomass in ponds with a submerged macrophyte cover of < 30%. This was not the case in ponds where submerged vegetation cover was > 30%, where, despite the considerable impact of fish on large Cladocera densities and length, phytoplankton biomass did not increase significantly upon fish recolonization. This highlights the importance of submerged vegetation recovery after biomanipulation, that, although it did not seem to provide sufficient shelter for large cladocerans, was able to control phytoplankton biomass if cover was > 30%. Taking into account the importance of submerged vegetation recovery, efforts should be made to enhance their restoration after biomanipulation.

Although submerged macrophytes have shown to be able to prevent a phytoplankton biomass increase after fish recolonization, nutrients should be limited to a certain extent. Above a certain threshold of nutrient concentration in the water column (a rough average TP concentration of 350 µg P L^{-1}), macrophytes are no longer able to efficiently control phytoplankton biomass during the whole summer, resulting eventually in their disappearance. Based on this threshold, a decision tree was developed as a guideline for choosing appropriate restoration measures for ponds, advising nutrient reduction before biomanipulation when the average TP concentration is more than 350 µg P L^{-1}. Once biomanipulation is performed, several steps are incorporated into the decision tree considering stabilization of the clear-water state, such as measures to stimulate macrophyte recovery or the addition of piscivorous fish. Regular monitoring of the successfully restored ponds is necessary in order to detect any deterioration of the situation and to enable additional adequate measures to be taken in order to avoid further deterioration of the system.

1. Introduction

1.1 The importance of small lakes and ponds

During the past decades, many small lakes and ponds have been subject to a considerable degree of eutrophication as a result of human agricultural and industrial activities. Eutrophication, a process of increase in nutrient concentrations (mainly phosphates and nitrates), can cause a transition to a turbid, phytoplankton-dominated state in ponds and lakes, often resulting in severe blooms of potentially toxic cyanobacteria (Scheffer *et al.*, 1993; Willame *et al.*, 2005).

Despite the fact that small lakes and ponds represent a great majority of all discrete standing waterbodies in Europe (Oertli *et al.*, 2005), they have often been overlooked by scientists and less ecological and conservation related research has been conducted on ponds. Only recently, ponds are considered as a particular type of waterbody, which can be defined as 'waterbodies between $1m^2$ and 2 ha in area which may be permanent or seasonal, including both man-made and natural waterbodies' (Biggs *et al.*, 2005). Despite their small size, ponds are valuable for biological diversity conservation, harboring often considerably more unique and more uncommon species, and overall more species in general than other waterbody types (Williams *et al.*, 2004; Biggs *et al.*, 2005: Linton and Goulder, 2000). Next to their value for biodiversity conservation, ponds are also valuable for recreational purposes such as boating and fishing. Considering the importance of small lakes and ponds in many aspects, more attention should be paid to their restoration when their ecological quality is impaired as a result of eutrophication.

1.2 Alternative stable states

Many ecosystems in general are strongly influenced by external conditions, such as climate changes, exploitation by humans, loss or increase in biodiversity, habitat fragmentation and an input of toxic substances or nutrients (Scheffer *et al.*, 2001). Depending on the condition of these external factors, an ecosystem will adapt. In some cases such adaptations take place gradually, in other cases they can be very sudden. In some ecosystems, two different stable stadia can exist at similar environmental conditions. In each of these stadia, different processes exist that stabilize the present state. Such processes provide a certain resilience, that allows changes in the environment without visually effecting the ecosystem state. Still, such changes in the environment can reduce the resilience of the ecosystem, thereby making the system more vulnerable to changes and shifting it to the other state with only the slightest disturbance. Once the system shifts, it will be very difficult to return to its former state, since the new state will also stabilize itself by a number of mechanisms.

This principle can be illustrated by the 'marble-in-a-cup' model (Figure 1). The ecosystem is represented by a landscape consisting of two valleys and a marble in one of both. Each valley represents an alternative state of the ecosystem. The deeper the valley, the more stable the state. When changing external conditions, the valley in which the marble resides will rise up i.e. the ecosystem will lose some resilience. A slight disturbance is now sufficient to let the marble roll over into the other valley and thus shift the ecosystem to the other state.

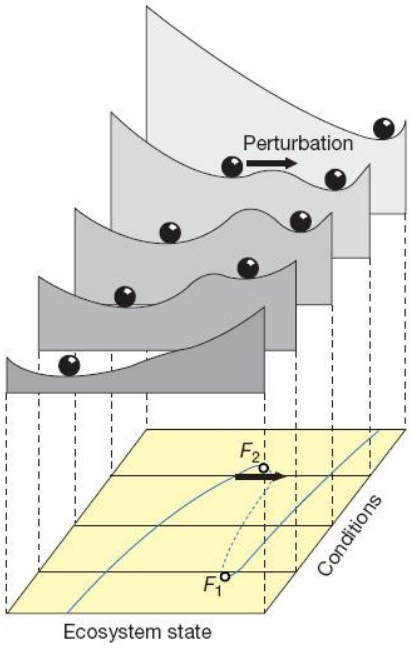

Figure 1 Illustration of the 'marble-in-a-cup' model (from Scheffer *et al.*, 2001)

Shallow lakes and ponds are an example of such an ecosystem with multiple stable states. A pond can reside in a clear-water, macrophyte dominated state with only poor nutrient concentrations, or in a turbid, highly eutrophic phytoplankton dominated state having poor or no submerged vegetation. The relationship between eutrophication, turbidity and submerged vegetation is visualised in (Figure 2) and is based on three basic principles:

eutrophication increases turbidity

(1) submerged vegetation reduces turbidity

(2) submerged vegetation disappears once a certain turbidity threshold is crossed

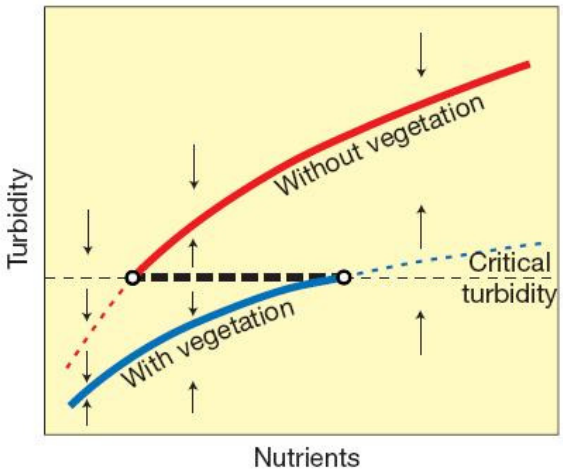

Figure 2 Graphical model of the two alternative stable states in shallow lakes (from Scheffer *et al.*, 2001)

At low nutrient concentrations, transparency will be high because of low phytoplankton growth, allowing submerged vegetation to grow. High nutrient concentrations on the other hand, will increase phytoplankton growth and turbidity, and thereby promote the disappearance of submerged vegetation that suffer from reduced light conditions. At intermediate nutrient concentrations, ponds can reside in both alternative states. (Scheffer *et al.*, 2001)

In the diagram (Figure 2), both states (with and without vegetation) are described as a function of turbidity and nutrients. A clear-water pond with submerged vegetation has a certain resilience, allowing a small increase in nutrients and turbidity without loss of macrophytes taking place (the lower equilibrium line). Once a certain threshold of critical turbidity is passed, the vegetation will disappear and the water will become turbid (the upper line). Once in the turbid state, the pond will not return easily to the clear-water state, as this state also has a certain resilience, and will only become clear again when nutrients are reduced very strongly (Scheffer *et al.*, 2001).

1.3 The role of submerged vegetation in maintaining a clear-water state

Submerged macrophytes play a crucial role in the stabilization of the clear-water state in shallow lakes and ponds. Van Donk and van de Bund (2002) have summarized the most important mechanisms that are responsible for the impact that submerged macrophytes can have on the ecosystem. The presence of submerged macrophytes generally has a negative impact on phytoplankton growth, caused by several mechanisms. First of all, macrophytes are a part of the ecosystem, competing with phytoplankton and periphyton for nutrients and light. Indirectly, macrophytes can promote competition with phytoplankton by serving as a surface for growth of periphyton, also competing with phytoplankton for nutrients. Secondly, macrophytes provide a shelter for plant associated invertebrates and zooplankton that feed on periphyton and phytoplankton, preventing their predation by planktivorous fish. The increased grazing of zooplankton and plant associated invertebrates can be the main factor controlling phytoplankton biomass. Macrophytes also play a role in increasing sedimentation and lowering resuspension of nutrients from the sediment. Another reason for the lack of phytoplankton inside vegetation beds is because of shading by macrophytes, providing the phytoplankton with insufficient light to survive. This is especially the case for floating macrophytes, floating leaved vegetation or very dense vegetation. Field data also suggests that macrophytes use allelopathic substances to influence phytoplankton biomass, although it is difficult to distinguish allelopathic effects from other competitive interactions (van Donk and van de Bund, 2002).

1.4 Food web concept: the basis of biomanipulation

The food chain theory is based on the idea that organisms in a system can be categorized into trophic levels and that organisms at a specific trophic level feed on the trophic level below, and in turn are fed upon by the organisms in the trophic level above. The actual patterns that we see are based on this but are much more complex and rather can be seen as a complex food web instead of a chain. Too simplify such food webs, we can assign each organism to a specific trophic level i.e.

primary producers, herbivores, predators etc. (Brönmark and Hansson, 1998). Following the developing knowledge about food web interactions in the 1980s, the trophic cascade concept was introduced (Carpenter *et al.*, 1985). A trophic cascade is an indirect interaction characteristic of linear food chains where a predator species A has an indirect positive effect on a plant species C by reducing the abundance of the herbivore species B. This theory is called the *trophic cascade hypothesis* and partly explains the variation in primary productivity that remains unexplained by the effects of nutrient input as a result of trophic interactions in the food web. More specifically, changes in fish assemblage structure at the top of the food web, can eventually cascade down to the level of primary producers such as phytoplankton (Figure 3).

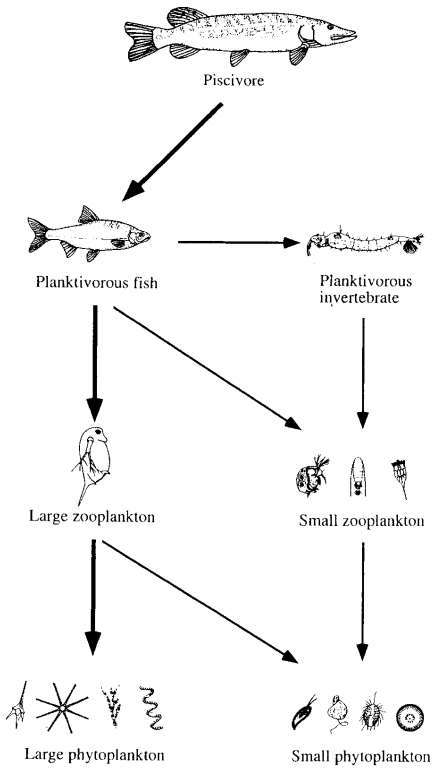

Figure 3 An example of a simplified pelagic foodweb. The thickness of the arrows indicates the importance of the relationship (from Brönmark & Hansson, 1998)

Biomanipulation of ponds is based on the idea of the food web concept. Fish removal will have an effect on lower trophic levels that will eventually cascade down to the level of phytoplankton, shifting the ecosystem back to the clear-water state. Removal of benthivorous fish, that dwell up the sediment and thereby release nutrients into the water column will increase sedimentation, as the removal of planktivorous fish will release the predation pressure on large zooplankton that allows them to control phytoplankton biomass, resulting in a shift to the clear-water state (De Backer *et al.*, 2008).

1.5 State-of-the-art on biomanipulation of shallow lakes

In recent years, many restoration efforts have been made to restore shallow lakes to a clear-water state (Meijer *et al.*, 1999; Skov *et al.*, 2003). Biomanipulation is often used as a restoration tool additional to nutrient reduction and control (Hosper & Jagtman, 1990). Fish play a central role in biomanipulation of shallow lakes and ponds because they are much easier to manipulate than nutrients, phytoplankton or zooplankton (Lammens, 1999). Different methods of fish manipulation can be used: complete or partial removal of fish stock, removal of benthivorous and planktivorous fish or addition of piscivorous fish (often pike) are the common techniques.

On a short term, the response of lakes to biomanipulation can be very drastic. Total or partial removal of fish stock appears to have marked effects on lakes and ponds (Lammens, 1999), resulting in a switch from turbid to clear water, caused by increased zooplankton grazing (mainly *Daphnia* spp.). This is often followed by the recovery of submerged vegetation and a slight decrease in nutrients (Van Donk *et al.*, 1990; Ozimek *et al.*, 1990).

A second phase after obtaining a clear-water state is the reintroduction of piscivorous fish. Addition of piscivores is commonly used to stabilize the system because they are able to control small planktivorous fish that feed on zooplankton (Lammens, 1999). Pike (*Esox lucius* L.) are often used for this purpose because they are able to eat larger prey than most other piscivores of similar size and they are cannibalistic which implies that they will not suffer from reduced growth if they are overpopulated (Hunt & Carbine, 1951). Pike stocking has proven to control or decrease the planktivorous fish population significantly and can be considered an effective tool for biomanipulation of shallow lakes (Berg *et al.*, 1997).

Although biomanipulation by planktivorous fish removal generally succeeds in causing a shift to the clear-water state on a short term, this is not always the case on a longer term. A return to turbid condition often occurs a few years after biomanipulation (Søndergaard *et al.*, 2007). One of the main factors assuring the stable clear-water state is the recovery of extensive submerged vegetation (Jeppesen *et al.*, 1990; Hosper & Jagtman, 1990). If this is not the case, the return to the turbid state is very likely.

Beside the absence of stable submerged macrophyte communities, insufficient external loading reduction and internal phosphorus loading are the most probable causes for a return to pre-biomanipulation conditions (Søndergaard *et al.*, 2007). Insufficient removal or reintroduction of planktivorous fish also promotes the turbid state (Meijer *et al.*, 1994). Lack of predation by the added piscivorous fish can also be a reason for biomanipulation failure (Skov *et al.*, 2003) In some cases, the addition of pike only affects the lake status during the season they were added (Søndergaard *et al.*, 1997). The return to a turbid state is often a gradual process that is first manifested by increase in periphyton overgrowth on macrophytes, decrease in surface cover of submerged macrophytes, decrease of mean length of *Daphnia* spp. and increase in total fish stock (Meijer *et al.*, 1994). As the outcome of biomanipulation on the longer term is often uncertain, more research is needed on the causes of such a return to a turbid state in order to prevent biomanipulation failure in the future.

2. Project aim and objectives

2.1 Aim

The aim of this study was to investigate the ecological state of 13 Brussels ponds one to five years after biomanipulation (i.e. pond drawdown and fish removal) by means of assessing changes in phytoplankton, zooplankton, macrophytic vegetation communities and main nutrient concentrations.

In order to investigate the biomanipulated ponds within a broader context and to take into account inter annual variation, 17 additional ponds were studied during the same period. PchR and TrSG, two ponds where no fish were removed (or large fish immediately returned), were studied because of recent pike additions in order to improve their ecological quality. They were however not considered as 'biomanipulated ponds' in this report.

2.2 Specific objectives

1. Acquisition of phytoplankton, zooplankton, macrophyte and environmental (main nutrients, pH, conductivity, temperature, maximum depth, Secchi depth and hydraulic retention time) data from 30 ponds according to the methodology used in De Backer *et al.* (2008) on three occasions (May, July and August).

2. Detailed statistical analysis of the collected data (cluster analysis, RDA, Kruskal-Wallis ANOVA test) and description of all biomanipulated ponds including their arrangement within the range of all ponds studied in 2009.

3. Determination of the ecological status of all the ponds studied according to the Ecoframe scheme (Moss *et al.*, 2003) based on selected variables and comparison of the ecological status of the biomanipulated ponds to previous years.

4. Comparison of different after biomanipulation situations using phytoplankton, zooplankton and nutrient data.

5. Overall conclusion and recommendations on biomanipulated ponds based on the results of this study.

2.3 Deliverables

1. Report including a detailed description of the used methodology (terrain, laboratory, statistics), the data acquired from all samples (values of the variables examined), determination of the ecological status and the arrangement of these ponds within the gradient of ponds from previous projects i.e. "City Ponds" and "Prospective Research Ponds" (Peretyatko & Triest, 2005; 2006).

2. Database comprising the data obtained in the frame of this project in 2009 on the biomanipulated ponds in a digital version.

3. Materials and Methods

3.1 Study area characteristics

All ponds that were selected for this study, are located in the immediate surroundings of or inside the Brussels urban area, some of them located in the Sonian forest, others in parks (Table 1). The ponds range in their surface area from several acres to more than 5 hectares and in depth from 0.5 to 3 m (Table 2).

Table 1 Names and general characteristics of the studied ponds.

Pond name	Pond name short	Location	Estimated surface area (m^2)	Year of bio-manipulation*	Total n° of pike added in 2008
Bemelvijver	Beml	Park	4277	2007	10
Denisvijver	Dens	Park	3317	2007	10
Leybeekvijver a	Leyb-a	Park	2838	2007/2009	8
Leybeekvijver b	Leyb-b	Park	2881	2007/2009	5
Kleine Mellaertsvijver	MlKl**	Park	9572	2006	26
Koning Boudewijnparkvijver 1	PRB1	Park	2539	2009	-
Koning Boudewijnparkvijver 2	PRB2	Park	6280	2007	15
Grote Neerpedevijver 1	NrPd1	Park	52233	2009	-
Sobieskivijver	Sbsk	Park	2481	2007	10
Verdronken Kinderen 1	VKn1	Forest	4449	2005	10
Verdronken Kinderen 2	VKn2	Forest	1106	2007	3
Woluwepark 1	WPk1	Park	23045	2007	50
Watermael-Bosvoordevijver	WtMl	Forest	29700	2005	-
Grote vijver Ter Kameren Bos	BCmb	Park	57805	-	-
Ixellesvijver 1	IxP1	Park	11539	-	-
Ixellesvijver 2	IxP2	Park	18041	-	-
Neerpedevijver 2	NrPd2	Park	13538	-	-
Neerpedevijver 4	NrPd4	Park	23729	-	-
Pêcheries Royales	PchR	Park	14770	-	176
Poelbosvijver	Plbs	Forest	1767	-	-
Rood Klooster 2	RKl2	Forest	22979	-	-
Rood Klooster 3	RKl3	Forest	17384	-	-
Rood Klooster 4	RKl4	Forest	7120	-	-
Rood Klooster 5	RKl5	Forest	37760	-	-
Ter Coignes	TrCg	Park	3282	-	-
Ter Lindenvijver	TrIn	Park	980	-	-
Tournay-Solvay Grand	TrSG	Forest	3250	-	10
Ten Reuken	Tenr	Park	29700	-	-
Ter Bronnenvijver	TrBr	Park	8244	-	-
Woluwepark 2	WPk2	Park	19629	-	-

* biomanipulation took place in early spring of the year indicated
** MlKl was emptied but not refilled in 2009 and therefore will not be mentioned when discussing 2009 results

3.2 Sampling and sample processing

Quantitative phytoplankton, main nutrient (total phosphorus – TP, soluble reactive phosphorus – SRP, SiO_2, NO_x (*i.e.* NO_2 and NO_3) and NH_4^+), chlorophyll *a* (Chl *a*) and zooplankton samples were collected on 3 occasions in each pond during late spring and summer 2009 (in May, July and August). Conductivity, pH, temperature, Secchi depth, maximum depth and hydraulic retention time were measured *in situ*. Aquatic vegetation was estimated semi-quantitatively during each field visit.

Mixed water samples based on ten random subsamples 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. An extension was fixed to the sampler to reach the deeper parts of the ponds when appropriate. After stirring the collected water, 500 mL were taken for phytoplankton identification and enumeration, 1L for chemical analyses and 1L for Chl *a* analysis. Samples for Chl *a* analysis were filtered onto Whatman GF/C filters and stored at -18°C for several days before analysis. Pigments were extracted in 90% acetone in the dark for 8 hours. Pigment concentrations were measured spectrophotometrically. Nutrient and Chl *a* concentrations were measured according to standard methods (APHA-AWWA-WEF, 1995). Phytoplankton samples were fixed in the field with alkaline lugol, sodium thiosulfate and buffered formalin (Kemp *et al.*, 1993) and stored in the dark before identification and enumeration to genus level using inverted microscopy. Biovolumes were calculated using the approximations of cell shapes to simple geometrical forms (Wetzel and Likens, 1990). Secchi depth was measured using a 30 cm diameter disk. When Secchi depth reached the bottom, 0.5 or 0.1 m were added to the measured depth, depending on whether the disk was well or partially visible, respectively. Conductivity, pH and temperature were measured *in situ* using a portable Multi 340i meter (WTW). Hydraulic retention time was estimated on the basis of the discharge measured at the outlet of each pond and the corresponding pond volume.

For zooplankton, 10 subsamples of 1L were collected with the same sampler used for phytoplankton and nutrients. The samples from a given pond were mixed and filtered through a 64 µm-mesh net and preserved in 5% formaldehyde (final concentration) at 4°C before being identified and enumerated using inverted microscopy. Different levels of identification were used: cladocerans and rotifers were identified to genus level, copepods were divided into cyclopoids, calanoids and nauplii. For the analysis, cladocerans were divided into large (*Daphnia* spp., *Diaphanosoma* spp., *Eurycercus* spp., *Polyphemus* spp., *Sida* spp., and *Simocephalus* spp.) and small (*Acroperus* spp., *Alona/Biapertura* spp., *Alonella* spp., *Bosmina* spp., *Camptocercus* spp., *Ceriodaphnia* spp., *Chydorus* spp., *Disparalona* spp., *Graptoleberis* spp., *Pleuroxus* spp., and *Scapholeberis* spp.) Cladocera.

Macrophytes were identified to genus level. Percentage coverage was estimated per vegetation type: submerged macrophytes (mainly *Potamogeton pectinatus*, *Chara* spp. and *Ceratophyllum demersum*), floating leaved macrophytes (mainly *Nuphar lutea* and *Nymphaea alba*), floating macrophytes (mainly *Lemna* spp.) and green filamentous algae (mainly *Spirogyra* spp.).

Similar data were obtained for all the studied ponds during previous years (2004 – 2009) and were used in the analyses (Peretyatko & Triest, 2005;2006 and De Backer et al., 2008; 2009).

3.3 Data treatment and statistical analyses

Phytoplankton biovolume and Chl *a* concentrations were highly significantly positively correlated (Spearman rank $r_s = 0.92$; $p < 0.001$) and showed similar spatial-temporal distribution patterns. Phytoplankton biovolume was identified to genus level and thus has a greater discriminative power than Chl *a*. Therefore it was used as a proxy for phytoplankton biomass instead of Chl *a*.

For multivariate tests, averaged values per year were used to avoid a blurring effect due to occasional shifts in phytoplankton biomass. Rare phytoplankton genera were not included in the analyses. Hierarchical cluster analysis (farthest-neighbour method) based on Sorensen distance measure (PC-ORD 4.0; McCune and Mefford, 1999) and redundancy analysis (RDA, CANOCO 4.5; ter Braak and Smilauer, 1998) were used to explore the phytoplankton data and to estimate their relationship to the measured environmental variables. Phytoplankton biovolumes were aggregated to division level and log transformed for RDA analysis. The automatic forward selection procedure was used to select the environmental variables that contributed most to the explanation of phytoplankton data.

Statistical comparisons of phytoplankton and zooplankton data were done to estimate the significance of the changes in the ponds after biomanipulation. When data were not normally distributed, non-parametric tests were used (mainly Kruskal-Wallis ANOVA)

The ecological status of each pond studied was estimated according to the Ecoframe scheme (version 8; Moss *et al.*, 2003) on the basis of selected variables (pH, total phosphorus, Secchi depth, chlorophyll *a*, phytoplankton diversity, plant community, plant diversity, plant abundance and large to total Cladocera ratio). Most of the ponds studied fit into the 17-th ecotype. A number of ponds showed conductivity above 800 µS cm^{-1} (Table 7) that normally reflects some saline influence. All these ponds are located close to the highways and therefore their elevated conductivity can be related to the deicing of the highways in winter. Because the natural conductivity of the water in these ponds is most likely below 800 µS cm^{-1} they were put into the 17^{th} ecotype. Lake shore structure was not included in the estimate because its assessment is based on the % cover of natural vegetation and is difficult to apply to the artificial water bodies like Brussels ponds.

4. Results and discussion

4.1 General results of all the ponds studied in 2009

All the ponds studied were characterized by high nutrient concentrations. Average TP concentrations were well above 0.1 mg L^{-1} (Figure 4; Table 2), corresponding to hypereutrophic conditions for ponds. Here, we use the threshold of 0.1 mg L^{-1} that is generally used for ponds (Brönmark & Hansson, 1998) rather than the threshold of 0.15 mg L^{-1} that has been determined for lakes (UNEP, 2003). Overall, TP concentrations tended to increase up the phytoplankton biomass gradient. Conversely, SRP concentrations tended to decrease as phytoplankton biomass increased. Dissolved inorganic nitrogen (DIN) concentrations varied from pond to pond (Figure 5) and, although lower than reported from some other European lakes and ponds, mostly exceeded the level at which phytoplankton growth could be slowed down due to nitrogen limitation (Reynolds, 1994).

The biomanipulated ponds generally showed higher levels of dissolved nutrients (SRP, DIN) in comparison to non-biomanipulated ponds. This indicates that in biomanipulated ponds, phytoplankton uptake does not compensate nutrient input (internal and external loadings), suggesting that factors other than nutrients control phytoplankton biomass in these ponds.

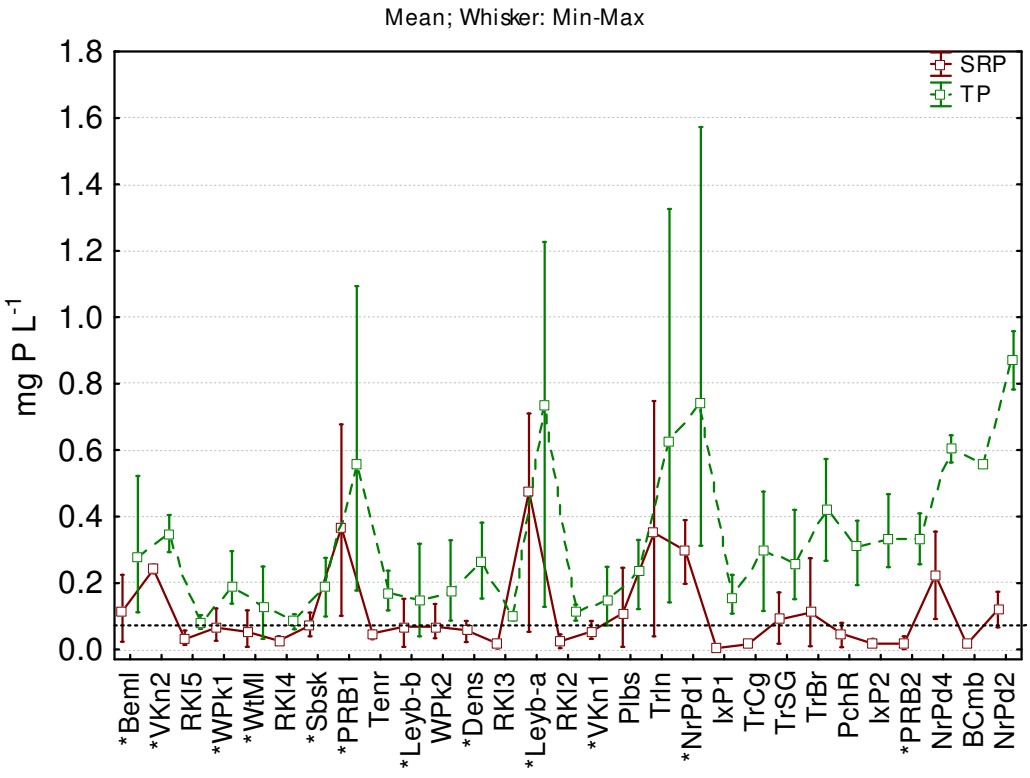

Figure 4 Inter- and intra-pond variation in TP and SRP concentrations in 2009. Ponds are arranged according to phytoplankton biovolume increase. A dotted line indicates the boundary between eutrophic and hypereutrophic conditions. * = biomanipulated ponds

Table 2 Environmental variables of the studied ponds. Average and ranged values are given. Abbreviations used: TP – total phosphorus, SRP – soluble reactive phosphorus, Chl *a* – chlorophyll *a*, Cond – conductivity, SD – Secchi depth, MD – maximum depth, RT – hydraulic retention time, T – temperature

Site	**TP** (mg P L^{-1})	**SRP** (mg P L^{-1})	**NH4** (mg N L^{-1})	**NOx** (mg N L^{-1})	**Chl a** (µg L^{-1})	**Cond** µS.cm^{-1}	**pH**	**SD** (m)	**MD** (m)	**RT** (day)	**T** (°C)	**O_2** (mg L^{-1})
DATA OF 2009												
BCmb	0.555	0.016	0.018	0.007	262.76	451	8.4	0.2	0.8	200	20.8	8.70
	0.555 - 0.555	0.016 - 0.016	0.018 - 0.018	0.007 - 0.007	262.76 - 262.76	451 - 451	8.4 - 8.4	0.2 - 0.2			20.8 - 20.8	8.70 - 8.70
*Beml	0.277	0.116	0.901	0.036	2.14	819	7.5	2.6	1.0	350	21.6	4.29
	0.112 - 0.522	0.023 - 0.224	0.322 - 1.351	0.009 - 0.052	1.87 - 2.67	764 - 859	7.4 - 7.6	1.8 - 3.0			17.7 - 24.7	3.68 - 5.15
*Dens	0.262	0.058	0.366	0.027	19.29	399	7.9	2.0	0.5	220	23.7	7.94
	0.153 - 0.382	0.022 - 0.086	0.053 - 0.809	0.009 - 0.054	1.34 - 51.21	318 - 449	7.5 - 8.2	0.9 - 3.0			20.1 - 28.0	4.91 - 9.91
IxP1	0.153	0.006	0.024	0.043	62.22	802	8.3	0.5	1.8	200	19.3	10.22
	0.109 - 0.225	0.002 - 0.011	0.013 - 0.035	0.005 - 0.084	28.61 - 99.52	757 - 878	8.2 - 8.5	0.4 - 0.7			15.7 - 23.4	9.44 - 10.75
IxP2	0.306	0.013	0.020	0.005	170.93	667	8.4	0.4	1.2	250	19.3	8.90
	0.231 - 0.467	0.001 - 0.032	0.011 - 0.033	0.001 - 0.009	54.64 - 478.28	648 - 694	8.3 - 8.5	0.3 - 0.6			15.6 - 24.0	7.96 - 9.70
*Leyb-a	0.738	0.476	0.046	0.015	23.92	551	8.6	2.3	0.4	15	22.5	10.35
	0.128 - 1.227	0.053 - 0.710	0.022 - 0.079	0.004 - 0.031	3.74 - 63.21	504 - 624	8.0 - 9.0	1.5 - 3.0			19.5 - 26.8	9.30 - 11.35
*Leyb-b	0.149	0.069	0.033	0.082	14.23	582	8.2	2.4	0.8	20	21.8	12.96
	0.039 - 0.318	0.008 - 0.153	0.024 - 0.045	0.001 - 0.137	2.14 - 34.41	509 - 663	7.8 - 8.6	1.7 - 3.0			18.2 - 25.9	11.92 - 14.08
*NrPd1	0.745	0.298	1.245	1.047	78.67	607	8.6	1.5	0.8	0	24.9	11.91
	0.313 - 1.572	0.198 - 0.389	0.022 - 3.472	0.005 - 2.909	3.93 - 225.68	467 - 684	7.8 - 9.7	0.4 - 3.1			22.7 - 28.7	5.52 - 16.47
NrPd2	0.870	0.120	0.021	0.012	129.05	527	9.0	0.2	0.5	0	26.3	16.21
	0.783 - 0.958	0.066 - 0.173	0.018 - 0.024	0.002 - 0.021	61.19 - 196.91	513 - 541	8.9 - 9.1	0.2 - 0.2			23.6 - 29.0	15.40 - 17.01
NrPd4	0.604	0.224	0.017	0.004	150.79	551	8.8	0.4	0.7	100	25.2	13.70
	0.563 - 0.645	0.092 - 0.355	0.014 - 0.020	0.004 - 0.004	73.33 - 228.24	541 - 560	8.7 - 8.9	0.2 - 0.5			21.6 - 28.7	13.29 - 14.10
PchR	0.309	0.047	0.094	0.007	70.48	649	8.1	0.5	1.0	30	22.0	8.93
	0.194 - 0.387	0.007 - 0.080	0.017 - 0.246	0.000 - 0.018	60.79 - 77.96	629 - 659	8.0 - 8.1	0.5 - 0.6			19.1 - 24.7	7.35 - 10.41
Plbs	0.239	0.109	0.178	0.152	37.36	940	7.8	1.7	1.5	20	18.8	5.36
	0.122 - 0.330	0.008 - 0.246	0.062 - 0.346	0.023 - 0.221	1.34 - 59.98	933 - 944	7.7 - 7.8	0.8 - 3.0			17.0 - 20.9	3.91 - 6.73
*PRB1	0.558	0.367	0.017	0.003	11.63	927	8.5	2.0	0.8	30	22.8	11.47
	0.178 - 1.094	0.102 - 0.677	0.009 - 0.033	0.002 - 0.004	5.09 - 21.46	913 - 945	8.0 - 9.1	1.9 - 2.2			21.2 - 25.6	6.70 - 14.63
*PRB2	0.332	0.018	0.202	0.023	89.18	735	8.0	0.4	0.8	100	21.9	7.07
	0.257 - 0.409	0.001 - 0.039	0.019 - 0.567	0.002 - 0.065	63.67 - 117.11	687 - 762	7.9 - 8.2	0.4 - 0.5			20.0 - 25.4	5.36 - 8.17
RKl2	0.115	0.024	0.072	0.296	23.34	558	8.0	1.0	2.0	13	18.8	9.77
	0.087 - 0.135	0.004 - 0.046	0.011 - 0.189	0.002 - 0.882	18.55 - 28.18	404 - 641	7.5 - 8.2	0.8 - 1.2			15.1 - 21.4	7.85 - 10.75

(continuation of table 2)

Site	TP (mg P L^{-1})	SRP (mg P L^{-1})	NH4 (mg N L^{-1})	NOx (mg N L^{-1})	Chl a (µg L^{-1})	Cond µS.cm^{-1}	pH	SD (m)	MD (m)	RT (day)	T (℃)	O_2 (mg L^{-1})
DATA OF 2009												
RKl3	0.099 0.087 - 0.110	0.018 0.002 - 0.030	0.070 0.010 - 0.164	0.222 0.000 - 0.661	24.33 12.83 - 38.14	546 460 - 600	8.0 7.7 - 8.1	1.1 1.0 - 1.2	3.0	14	20.3 16.0 - 23.0	8.69 7.56 - 10.35
RKl4	0.085 0.061 - 0.106	0.026 0.016 - 0.039	0.147 0.036 - 0.365	0.233 0.044 - 0.584	6.91 3.20 - 13.25	580 541 - 606	7.8 7.7 - 8.0	2.4 2.0 - 3.0	1.0	0	19.6 16.5 - 22.3	8.68 7.52 - 9.93
RKl5	0.081 0.062 - 0.103	0.030 0.013 - 0.057	0.027 0.010 - 0.054	0.079 0.002 - 0.204	2.67 1.60 - 3.47	508 474 - 526	7.8 7.5 - 8.3	3.4 3.0 - 4.1	1.1	11	20.6 17.3 - 24.4	11.87 8.80 - 13.90
*Sbsk	0.186 0.099 - 0.275	0.072 0.040 - 0.110	0.179 0.019 - 0.489	0.003 0.002 - 0.005	8.18 4.54 - 11.47	557 480 - 636	7.9 7.3 - 8.7	2.0 1.4 - 2.6	0.8	50	22.6 19.4 - 26.3	8.64 3.11 - 14.12
Tenr	0.165 0.118 - 0.237	0.049 0.031 - 0.058	0.144 0.010 - 0.388	0.052 0.014 - 0.113	13.42 1.07 - 26.70	488 444 - 537	7.9 7.7 - 8.2	1.8 0.8 - 3.2	1.4	12	22.9 19.2 - 27.5	10.33 6.90 - 13.16
TrBr	0.423 0.267 - 0.573	0.111 0.009 - 0.274	0.280 0.016 - 0.802	0.108 0.004 - 0.285	237.84 35.39 - 525.69	676 647 - 714	8.0 7.6 - 8.4	0.7 0.3 - 1.1	1.0	30	20.6 16.8 - 23.4	10.90 5.43 - 14.65
TrCg	0.295 0.116 - 0.475	0.016 0.008 - 0.024	0.166 0.020 - 0.311	0.025 0.006 - 0.044	121.93 52.48 - 191.38	503 415 - 591	8.0 7.8 - 8.3	0.5 0.4 - 0.7	1.0	30	23.4 22.4 - 24.4	9.79 9.06 - 10.51
TrIn	0.629 0.141 - 1.326	0.350 0.040 - 0.747	0.157 0.023 - 0.422	0.063 0.010 - 0.164	49.65 22.59 - 80.59	698 684 - 719	7.8 7.7 - 8.0	1.0 0.7 - 1.6	0.8	5	21.0 19.2 - 24.3	6.55 4.46 - 9.57
TrSG	0.257 0.151 - 0.420	0.090 0.017 - 0.172	0.011 0.009 - 0.013	0.004 0.003 - 0.004	76.57 49.59 - 129.45	553 544 - 569	8.1 8.0 - 8.1	1.1 0.9 - 1.5	1.4	12	19.7 16.3 - 23.1	11.43 10.62 - 11.87
*VKn1	0.148 0.072 - 0.248	0.054 0.032 - 0.086	0.114 0.036 - 0.225	0.053 0.017 - 0.122	98.40 3.47 - 174.25	442 415 - 468	7.5 7.2 - 7.8	2.7 0.8 - 4.3	1.2	34	17.8 16.0 - 20.1	6.82 3.37 - 10.34
*VKn2	0.343 0.293 - 0.405	0.240 0.229 - 0.257	0.237 0.194 - 0.313	0.039 0.010 - 0.093	29.98 7.72 - 72.47	487 455 - 540	7.4 7.3 - 7.6	2.4 2.0 - 3.0	1.0	9	17.9 14.9 - 20.6	6.87 6.18 - 7.72
*WPk1	0.191 0.138 - 0.296	0.065 0.026 - 0.123	0.398 0.042 - 0.837	0.082 0.075 - 0.092	7.13 3.74 - 9.64	952 840 - 1,023	7.7 7.6 - 8.0	2.6 1.8 - 4.0	1.0	30	21.9 18.5 - 25.3	9.23 4.52 - 12.90
WPk2	0.175 0.087 - 0.329	0.069 0.033 - 0.136	0.555 0.274 - 0.911	0.120 0.071 - 0.215	10.38 1.87 - 25.01	784 744 - 851	7.8 7.6 - 8.1	2.7 1.2 - 4.0	1.1	20	23.2 19.0 - 27.1	7.48 4.80 - 10.15
*WtMl	0.127 0.032 - 0.250	0.053 0.008 - 0.117	0.152 0.011 - 0.429	0.063 0.002 - 0.175	14.06 4.01 - 22.96	467 333 - 537	8.1 7.7 - 8.8	2.6 1.5 - 4.0	1.3	7	21.0 17.9 - 24.4	10.53 7.17 - 15.87

Table 3 Environmental variables of the ponds in the year before biomanipulation. Average and ranged values are given. Abbreviations used: TP – total phosphorus, SRP – soluble reactive phosphorus, Chl a – chlorophyll a, Cond – conductivity, SD – Secchi depth, MD – maximum depth, RT – hydraulic retention time, T – temperature

Site	**TP** (mg P L^{-1})	**SRP** (mg P L^{-1})	**NH4** (mg N L^{-1})	**NOx** (mg N L^{-1})	**Chl a** (µg L^{-1})	**Cond** µS.cm^{-1}	**pH**	**SD** (m)	**MD** (m)	**RT** (day)	**T** (°C)	**O_2** (mg L^{-1})
DATA BEFORE BIOMANIPULATION												
Beml	0.673	0.203	0.055	0.082	52.1	748	7.9	0.7	1.0	470	20.9	3.47
	0.402 - 0.861	0.017 - 0.479	0.008 - 0.147	0.018 - 0.155	5.5 - 83.8	528 - 875	7.6 - 8.2	0.6 - 0.7			18.4 - 25.9	2.14 - 5.60
Dens	0.351	0.030	0.045	0.048	87.8	422	8.4	0.4	0.7	320	22.2	4.01
	0.084 - 0.493	0.005 - 0.072	0.015 - 0.098	0.005 - 0.102	4.6 - 170.0	285 - 517	8.2 - 8.6	0.3 - 0.5			19.4 - 27.7	2.90 - 4.84
Leyb-a	0.506	0.009	0.034	0.202	469.7	536	9.0	0.3	0.6	150	21.5	6.60
	0.309 - 0.669	0.000 - 0.022	0.001 - 0.053	0.006 - 0.550	3.0 - 867.2	453 - 696	8.4 - 9.4	0.2 - 0.6			18.7 - 26.1	6.08 - 6.95
Leyb-b	0.407	0.005	0.028	0.216	348.6	557	8.8	0.3	0.8	250	21.2	6.47
	0.330 - 0.452	0.000 - 0.013	0.001 - 0.053	0.005 - 0.599	4.2 - 604.5	480 - 699	8.2 - 9.3	0.2 - 0.6			18.0 - 25.9	6.20 - 6.60
NrPd1	2.516	0.430	4.198	0.344	1403.50	636	8.4	0.2	0.4	25	20.7	13.43
	1.554 - 4.235	0.217 - 0.637	2.584 - 5.348	0.165 - 0.592	602.73 - 2680.6	533 - 751	8.0 - 8.7	0.1 - 0.2			20.4 - 21.3	9.78 - 17.70
PRB1	0.946	0.320	0.028	0.005	310.23	801	8.7	0.3	0.8	30	20.6	10.71
	0.343 - 1.760	0.054 - 0.677	0.018 - 0.041	0.002 - 0.007	119.62 - 491.28	746 - 849	8.5 - 8.9	0.2 - 0.5			19.4 - 22.3	8.58 - 12.05
PRB2	0.428	0.091	0.204	0.182	40.2	735	8.0	0.6	0.8	5000	21.2	2.97
	0.201 - 0.861	0.007 - 0.243	0.178 - 0.238	0.024 - 0.326	2.4 - 66.8	546 - 881	7.7 - 8.1	0.4 - 0.9			18.4 - 26.6	1.96 - 4.10
Sbsk	0.426	0.018	0.246	0.149	82.8	781	8.4	0.6	0.7	50	21.4	4.25
	0.301 - 0.624	0.005 - 0.037	0.092 - 0.416	0.039 - 0.296	3.6 - 181.4	691 - 848	8.2 - 8.6	0.4 - 0.7			18.9 - 26.2	3.60 - 5.55
VKn1	0.213	0.004	0.173	0.185	20.1	546	7.8	0.7	1.1	34	18.4	7.24
	0.092 - 0.326	0.000 - 0.007	0.025 - 0.463	0.043 - 0.442	5.8 - 32.5	529 - 561	7.6 - 8.1	0.7 - 0.7			15.9 - 21.4	4.80 - 10.87
VKn2	0.207	0.004	0.042	0.376	54.3	562	7.7	0.8	1.0	4	17.3	7.59
	0.085 - 0.390	0.000 - 0.011	0.004 - 0.166	0.004 - 1.013	8.2 - 100.4	509 - 607	7.3 - 7.9	0.6 - 1.0			12.2 - 22.1	4.85 - 9.64
WPk1	0.223	0.002	0.014	0.064	41.4	896	7.8	0.6	1.1	31	20.5	10.64
	0.096 - 0.360	0.000 - 0.010	0.000 - 0.038	0.004 - 0.355	22.7 - 73.6	796 - 969	7.4 - 8.0	0.6 - 0.8			15.1 - 25.4	9.80 - 12.50
WtMl	0.161	0.003	0.054	0.184	13.9	532	7.9	1.2	0.7	7	19.4	8.81
	0.043 - 0.261	0.000 - 0.005	0.000 - 0.133	0.110 - 0.264	4.1 - 28.7	488 - 596	7.7 - 8.4	0.5 - 1.5			15.8 - 23.8	5.50 - 13.60

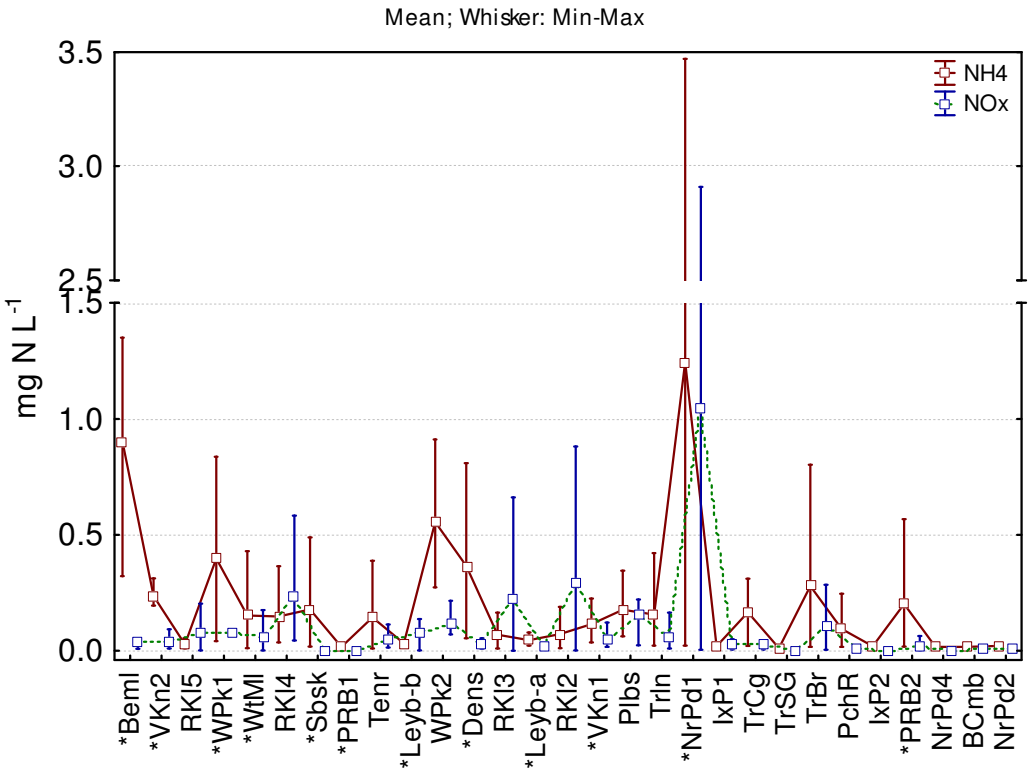

Figure 5 Inter- and intra- pond variation in NH_4^+, and NOx (NO_2^- and NO_3^-) concentrations in 2009. Ponds are arranged according to phytoplankton biovolume increase. * = biomanipulated ponds

In agreement with previous research on the ponds (Peretyatko et al., 2007b), phytoplankton biovolumes showed a gradient ranging from several to more than 220 mm^3 L^{-1}, despite the apparent nutrient richness (Figure 6). A similar pattern was shown by Chl a concentrations (Figure 7) covering a range from oligotrophic to hypereutrophic conditions (UNEP, 2003).

Cluster analysis of mean phytoplankton biovolumes did not construct clear groups that would correspond to the alternative stable states as hypothesized for shallow lakes by Scheffer *et al.*, (1993) (Figure 8). Therefore phytoplankton biovolume thresholds as defined by Peretyatko *et al.* (2007b) were used to split the ponds into three groups: clear (< 2 mm^3 L^{-1}), intermediate (between 2 mm^3 L^{-1} and 20 mm^3 L^{-1}) and turbid (> 20 mm^3 L^{-1}) (Figure 6). Consequently, the group of clear ponds includes Beml, VKn2, RKl5, WPk1, WtMl, RKl4 and Sbsk. TrBr, PchR, IxP2, PRB2, NrPd4, BCmb and NrPd2 were considered as turbid ponds. All 15 remaining ponds were considered as intermediate ponds. These groups are shown on the cluster analysis dendrogram in three different colors (Figure 8). The cluster analysis constructed two rather than three groups of ponds, one group consisting of all clear ponds and some of the more clear intermediate ponds, and one group consisting of all turbid ponds and the remaining intermediate ponds.

All of the biomanipulated ponds can be considered as clear to intermediate ponds, except for PRB2 where biomanipulation failed and the average phytoplankton biovolume is high (38 mm^3 L^{-1} in 2009). Therefore, PRB2 can be considered as a turbid pond (Figure 6).

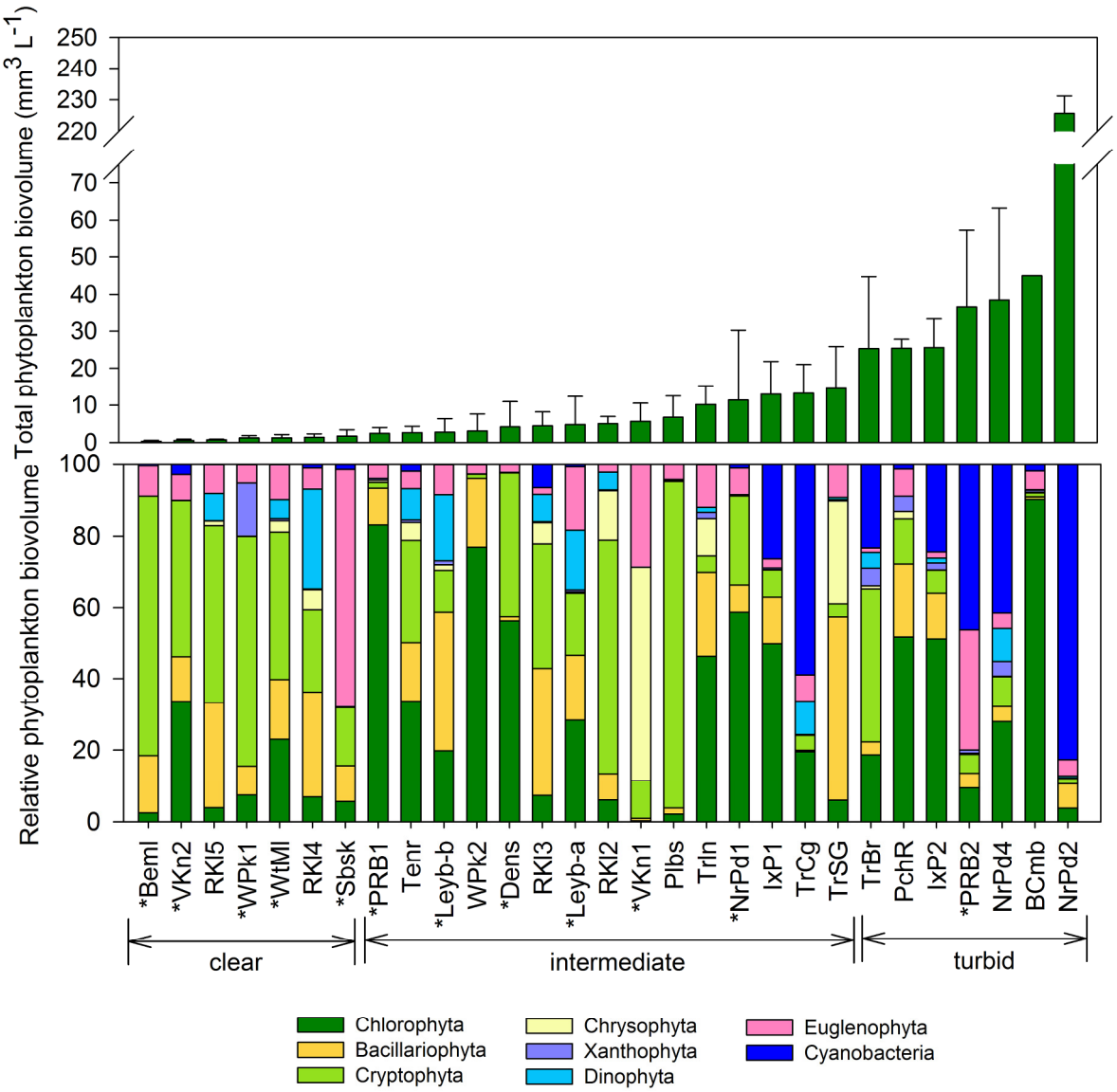

Figure 6 Mean total and relative phytoplankton biovolume among the studied ponds in 2009. Ponds are arranged according to phytoplankton biovolume increase. * = biomanipulated ponds

Large Cladocera density shows a considerable inter-pond variability and is not significantly correlated to phytoplankton biomass (R_s = 0.064, n = 29, p = 0.74). Conversely, large Cladocera length tends to decrease when phytoplankton biomass increases (R_s = -0.53, n = 29, p = 0.003). The ratio of large Cladocera to total zooplankton density decreases with a phytoplankton biomass increase (R_s = -0.51, n = 29, p = 0.004), probably as a result of fish predation on the larger forms in these ponds, shifting the community to smaller zooplankton such as copepods, rotifers and small Cladocera (Figure 9). This is supported by the fish removal data. All the biomanipulated (and formerly turbid) ponds were previously overstocked with plankti-benthivorous fish (> 600 kg per hectare).

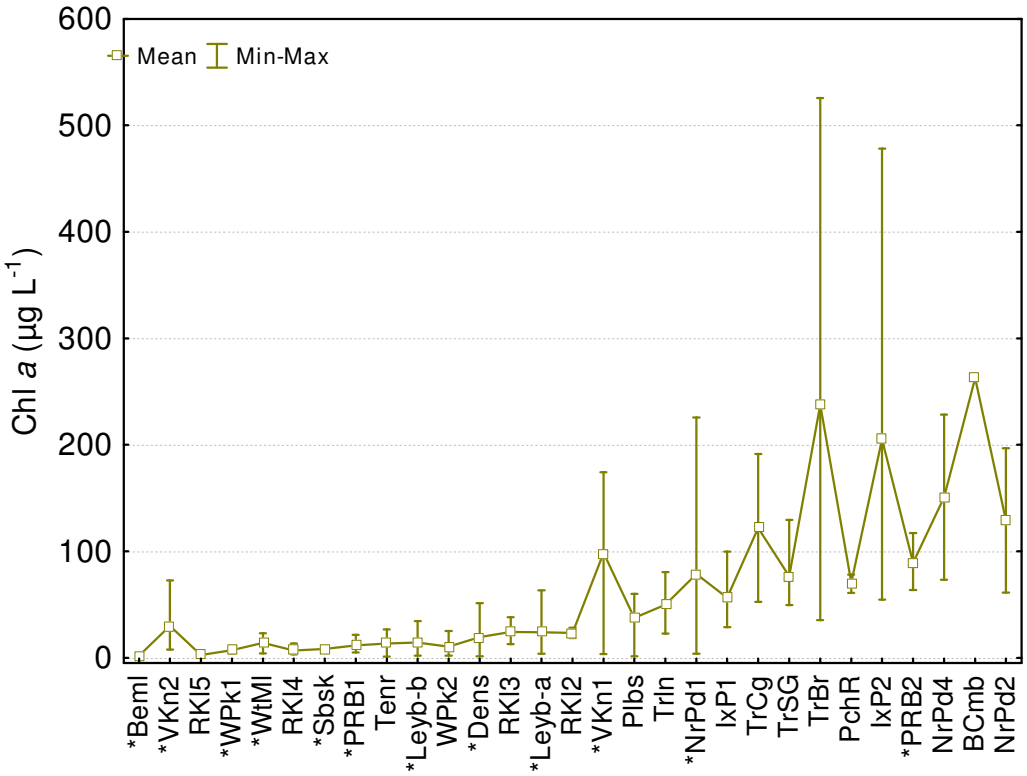

Figure 7 Inter- and intra- pond variation in chlorophyll *a* concentrations in 2009. Ponds are arranged according to phytoplankton biovolume increase. * = biomanipulated ponds

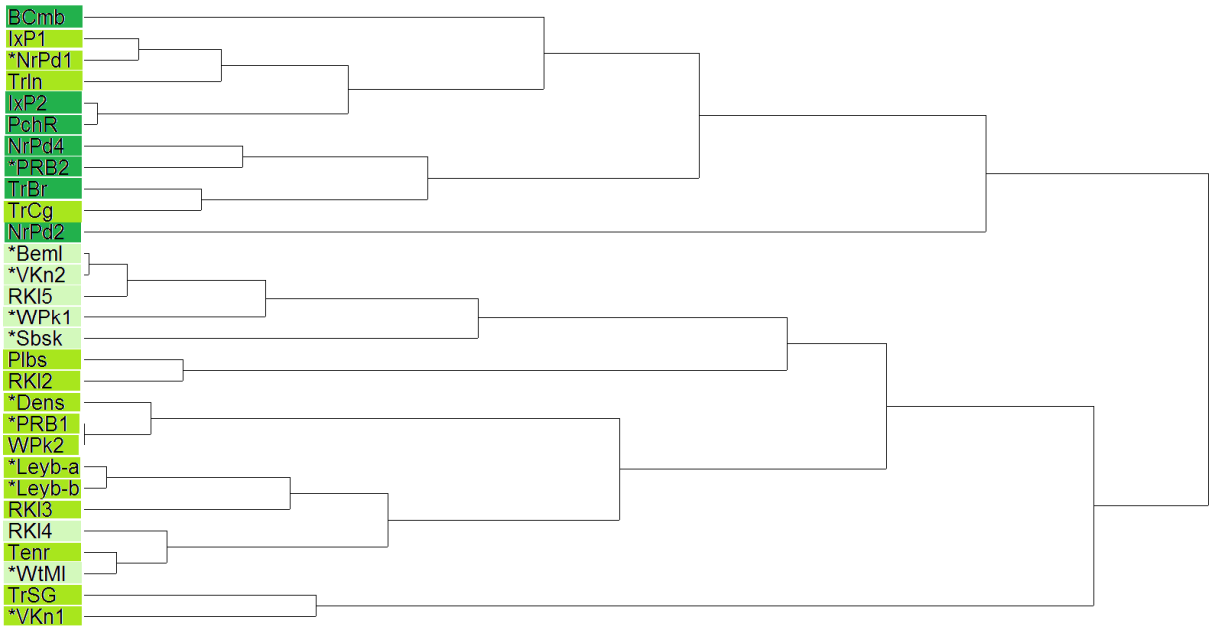

Figure 8 Cluster analysis based on mean phytoplankton biovolume data of 2009 with Farthest Neighbour as a group linkage method and Sørensen distance as dissimilarity measure; light green - clear, green - intermediate, dark green – turbid. * = biomanipulated ponds

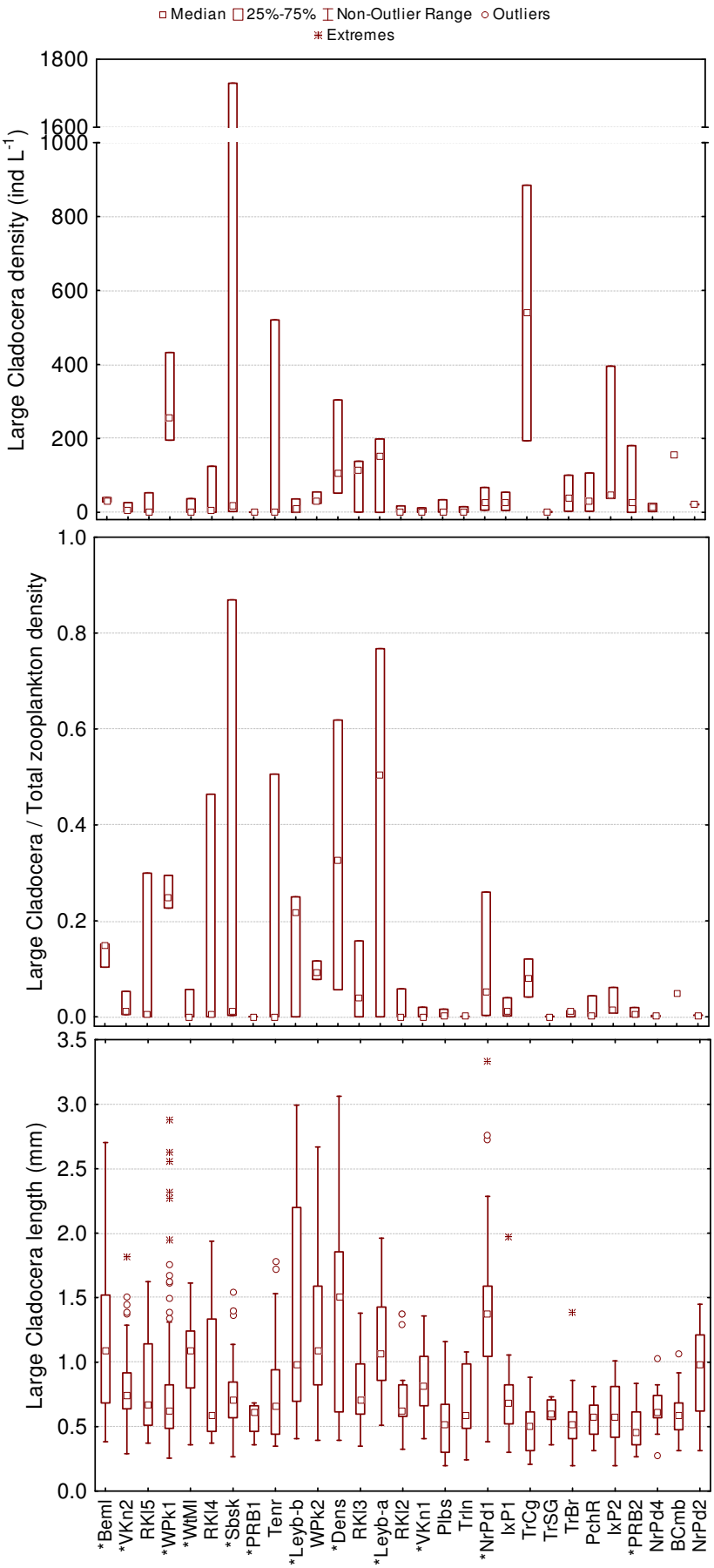

Figure 9 Inter- and intra- pond variation in large Cladocera density, length and ratio of large Cladocera to total number of zooplankton in 2009. Ponds are arranged according to phytoplankton biovolume increase. * = biomanipulated ponds

A redundancy analysis (RDA) based on averaged per year (2005 – 2009) phytoplankton and environmental data gave further insight into the effects of biomanipulation and the situation one to five years after biomanipulation. Data from ponds studied in previous reports were added to the analysis, including data from the additional ponds studied in 2009 to enlarge the dataset. The first two axes of the RDA explained 38% of the variation in the phytoplankton data, of which 35% by the first and 3% by the second axis (Table 4).

Table 4 Summary of the RDA analysis results

Axes	1	2	3	4	Total variance
Eigenvalues :	0.348	0.028	0.017	0.015	1.000
Species-environment correlations :	0.808	0.468	0.483	0.454	
Cumulative percentage variance					
of species data :	34.8	37.5	39.2	40.7	
of species-environment relation:	83.4	90	94	97.7	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.417

As indicated by the Secchi depth arrow, the first axis corresponds to a phytoplankton biomass gradient. The second axis roughly corresponds to a pH gradient. Chl *a* and Secchi depth showed highly significant relationships with phytoplankton biovolumes ($p<0.01$; data not shown), suggesting that the latter gives a reasonable estimation of phytoplankton biomass and that the turbidity in the ponds studied is mostly phytoplankton induced. Variables that can be considered as a proxy for phytoplankton biomass (such as Secchi depth, Chl *a*) could blur the effect of other environmental variables and therefore were not included into the forward selection procedure, but are shown on the RDA biplot. TP was also left out of the forward selection procedure as it did not show considerable variation among the studied ponds, that were all eutrophic to hypereutrophic.

Seven other variables showed significant relationships with the phytoplankton data after exclusion of Chl *a*, Secchi depth and TP (Table 5). These variables are pH, submerged vegetation cover (SV), large Cladocera length (LCL), maximum depth (MD), SRP, temperature (T) and large Cladocera density (LCD). They explained 20%, 12%, 3%, 2%, 1%, 1% and 1% respectively (LambdaA;Table 5).

These variables seem to play an important role in the control of phytoplankton biomass and composition. Large Cladocera length (LCL) alone explained significantly more than large Cladocera density (LCD), showing the importance of size of large cladocerans for control of phytoplankton. Except for SRP that was significantly negatively correlated with phytoplankton biovolumes, nutrients showed a poor relationship with phytoplankton, suggesting again that factors other than nutrients control phytoplankton in these ponds (Table 5).

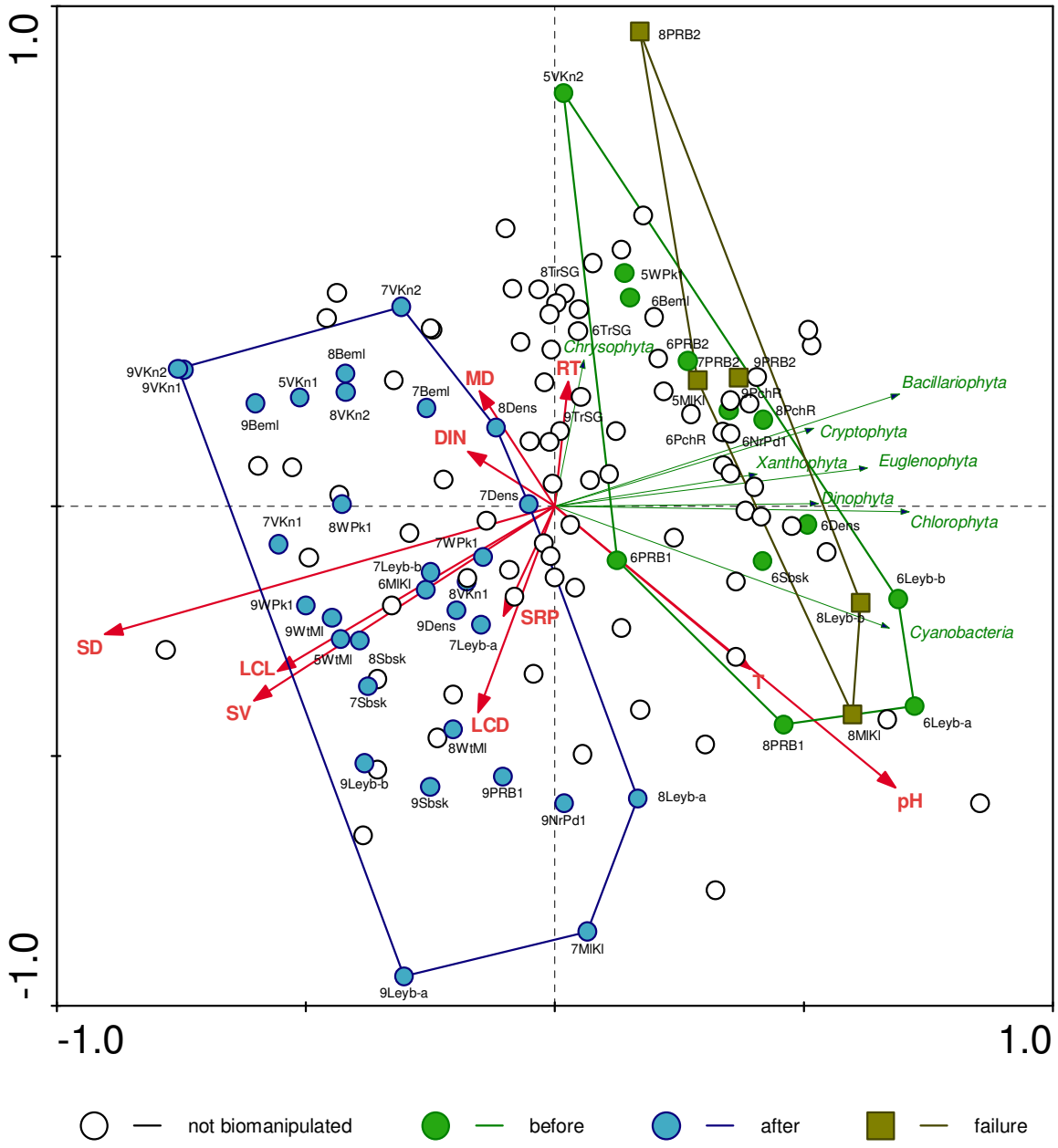

Figure 10 Redundancy analysis biplot (sites and environmental variables) based on averaged phytoplankton and environmental data per year (indicated before pond name) from ponds before and after biomanipulation. City ponds and Prospective Research ponds are added. Ponds before biomanipulation are indicated with green circles, ponds after biomanipulation with blue circles and failures by green squares. Lines connecting circles indicate group boundaries. Abbreviations used: SRP – soluble reactive phosphorus, DIN – dissolved inorganic nitrogen, SD – Secchi depth, MD – maximum depth, RT – hydraulic retention time, T – temperature, LCD – large Cladocera density, LCL – large Cladocera length, SV – submerged vegetation

Table 5 RDA forward selection results. 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 additional variance explained at a time the variable was included into the model (LambdaA)

Marginal Effects		**Conditional Effects**		
Variable	Lambda1	Variable	LambdaA	p
pH	0.20	pH	0.20	**0.002**
SV	0.15	SV	0.12	**0.002**
LCL	0.13	LCL	0.03	**0.002**
T	0.07	MD	0.02	**0.010**
MD	0.02	SRP	0.01	**0.012**
LCD	0.02	T	0.01	**0.090**
DIN	0.01	LCD	0.01	**0.080**
SRP	0.01	RT	0.01	0.272
RT	0.01	DIN	0.01	0.450

With a single exception of PRB2, all ponds biomanipulated in 2007 have shown a drastic reduction in phytoplankton biomass the first summer after fish removal (De Backer *et al.*, 2008). The drop in phytoplankton biomass was accompanied with a marked increase in large Cladocera density and size. Similar patterns were observed for two additional ponds, NrPd1 and PRB1, that were biomanipulated in 2009. It should be noted that PRB2 was polluted by sewage water soon after biomanipulation as a result of sewage overflow into the pond during a particularly heavy rain period.

In 2008 and 2009, most of the ponds biomanipulated in 2007 maintained a lowered phytoplankton biomass in comparison with the situation before biomanipulation. In the RDA results, they are situated on the left side of the diagram, in the direction of decrease of phytoplankton biomass, as indicated by the Secchi depth arrow. Leyb-b and MIKl however, shifted back to the right side of the diagram in 2008. Therefore, they can be considered as failures, since they regained a high phytoplankton biomass in 2008. Rebiomanipulation of Leyb-b in end of summer 2008 resulted in a lowered phytoplankton biomass in 2009 and again a shift to the left side of the diagram.

Biomanipulation in PRB2 was never successful, and therefore this pond is situated on the left side during the whole study period. The group of ‘failures’ in the RDA diagram overlaps almost completely with the ‘before’ situations, suggesting these ponds completely returned to their turbid state comparable to how it was before biomanipulation.

The RDA results confirm the idea that length of large Cladocera is more important than their numbers for phytoplankton control. Large Cladocera length alone explained 13%, while large cladoceran densities explained only 2% of the variation in the phytoplankton data (Table 5). High densities of large Cladocera were observed in turbid as well as in intermediate or clear-water ponds. Large cladocerans however were only found in clear-water ponds, suggesting that it is not abundance but average length of large cladocerans that is important for phytoplankton control (Figure 9).

Submerged macrophytes are known to be able to control phytoplankton through different mechanisms (Søndergaard and Moss, 1998; van Donk and van de Bund, 2002; Peretyatko *et al.*, 2007a). Our results confirm the importance of vegetation for phytoplankton control and as a consequence for biomanipulation success. Submerged vegetation cover (SV) alone explained 15% of the variation in the phytoplankton biomass, highlighting the importance of vegetation to maintain a clear-water state. Phytoplankton biovolume was negatively correlated to submerged vegetation (R_s = -0.60, n = 29, $p < 0.001$). This is shown by Table 6, where ponds are arranged according to phytoplankton biovolume increase, showing a general decrease of total submerged vegetation cover with increasing turbidity.

Submerged macrophytes are known to compete with phytoplankton for nutrients (Scheffer *et al.*, 1993) and as such limit phytoplankton biomass. This was observed in VKn2, a clear-water pond with a very dense *Ceratophyllum demersum* vegetation (Table 6) lacking any large cladocerans to graze on phytoplankton. The low phytoplankton biomass in this pond shows that submerged vegetation alone is able to control phytoplankton biomass in some cases. Phytoplankton can also be controlled by zooplankton alone, as we observed in some other ponds (MlKl in 2007; Beml and Dens from 2007 – 2009). If however, planktivorous fish recolonize such ponds, zooplankters, having no refugia, might quickly be preyed down, which can lead to a rapid increase in phytoplankton biomass. This was the case in MlKl, a pond where soon after planktivorous fish recolonization (in spring 2008) a shift to the turbid state took place. Although a dense submerged vegetation seemed to protect large Cladocera from predation in Leyb-a in 2008 (see De Backer et al., 2009), in general large Cladocera densities and length are reduced by predation even in ponds with a submerged vegetation cover of more than 60%. However, if submerged vegetation cover was > 30%, phytoplankton biomass did not significantly increase after fish recolonization, suggesting that submerged macrophytes were able to control phytoplankton by other means than by providing refuge to zooplankton (see 4.2.2 The importance of submerged vegetation recovery).

Other environmental variables, such as pH, temperature and hydraulic retention time, also affect phytoplankton (Peretyatko et al., 2007b). The first two have shown a significant positive relationship to phytoplankton biovolume in the RDA. The effect of retention time could be blurred by other factors acting in the opposite direction (such as presence of submerged vegetation in ponds with a long retention time) or could have a greater effect on phytoplankton composition rather than on biomass (i.e. selection against slow growing phytoplankters).

Table 6 Aquatic plant diversity and mean % cover of different vegetation types in 2009 in the ponds studied. Ponds are arranged according to phytoplankton biovolume increase. Used abbreviations: FA – filamentous algae, SM – submerged macrophytes, FLM – floating leaved macrophytes, FM – free floating macrophytes. * = biomanipulated pond

	*Beml	*VKn2	RKl5	*WPk1	*WtMl	RKl4	*Sbsk	*PRB1	Tenr	*Leyb-b	WPk2	*Dens	RKl3	*Leyb-a	RKl2	*VKn1	Plbs	Trln	*NrPd1	lxP1	TrCg	TrSG	TrBr	PchR	lxP2	*PRB2	NrPd4	BCmb	NrPd2
Chara spp.			+		+	+	+	+	+							+													
Nitella spp.																													
Callitriche obtusangula						+																							
Ceratophyllum demersum		+			+	+			+																				
Elodea nuttallii					+			+																					
Lemna spp.					+		+									+													
Nuphar lutea	+			+	+								+				+						+						
Nymphaea alba	+			+																									
Potamogeton pectinatus			+	+	+	+	+		+	+				+		+													
Potamogeton pusillus							+	+	+										+										
Spirodela spp.					+																								
Spirogyra spp.			+	+		+	+			+																			
Zannichellia palustris																			+										
unknown emergent species								+																					
FA			5	17		12	7			5																			
SM		89	92	10	46	63	58	37	55	70				82		47			33										
FLM	53			20	5								0.1				18					5	30						
FM					12		46									99													
Total cover % (no FM)	53	89	97	47	51	75	65	37	55	75	0	0	0.1	82	0	47	18	0	33	0	0	5	30	0	0	0	0	0	0

4.2 Biomanipulated ponds

4.2.1 General overview of all ponds biomanipulated during 2005 – 2009

During the period 2005 – 2009, a total of 13 ponds were biomanipulated (i.e. fish removal and pond drawdown) and refilled in early sping: VKn1 and WtMl were biomanipulated in 2005; MlKl in 2006; Beml, Dens, Leyb-a, Leyb-b, PRB2, Sbsk, VKn2 and WPk1 in 2007 and NrPd1 and PRB1 in 2009 (end of summer 2008). Statistical comparison of the variation in phyto- and zooplankton variables from year to year for each pond was not useful, as in most of the years only three values were obtained for each variable, which is insufficient to obtain any statistical significance. In general, similar changes were observed after biomanipulation in all ponds except PRB2 (Figure 14). Phytoplankton biovolume decreased after biomanipulation, corresponding to an increase in large Cladocera density and length. This was not the case for PRB2, where biomanipulation failed in 2007, possibly due to insufficient fish removal or nutrient rich inflowing sewage water (see De Backer et al., 2008). VKn1, WtMl, Beml, Dens, Sbsk, VKn2 and WPk1 retained a phytoplankton biomass below 5 mm^3 L^{-1} during the whole study period after biomanipulation as a result of increased zooplankton grazing and/or submerged vegetation recovery. All other ponds (MlKl, Leyb-a, Leyb-b, NrPd1, PRB2 and PRB1) have shown elevated phytoplankton biovolumes (15 mm^3 L^{-1} or more) at certain times during the period after biomanipulation.

Previous research has shown that phytoplankton biomass can be efficiently controlled by submerged vegetation alone in the absence of large Cladocera, as is the case for VKn2, a pond with almost no large zooplankters and a low phytoplankton biomass that has developed a dense *Ceratophyllum demersum* vegetation and is able to maintain a clear-water state. This suggests that vegetation alone is capable of controlling phytoplankton and maintain a clear-water state. However, in such case, it should be taken into account that the vegetation can become too dense, resulting in de-oxygenation of the water (Figure 11). This was the case for VKn2 in 2009 (showing an O_2 concentration of sometimes only 2 mg L^{-1} during daytime; Teissier et al., 2010).

Figure 11 Dense *Ceratophyllum demersum* vegetation in VKn2, causing low O_2 concentrations

On the other hand, our results have shown that large zooplankton alone can also control phytoplankton biomass. Some ponds where no submerged vegetation has recovered (Dens, MlKl 2007), have developed very high densities of large zooplankton (Figure 12). Grazing by large Cladocera alone resulted in a very low phytoplankton biomass, despite the absence of vegetation. This is however a very unstable situation. If such ponds are recolonized again by fish, large cladocerans will quickly be preyed down and no longer be able to control phytoplankton, shifting the pond back to the turbid state as was the case for MlKl in 2008.

Figure 12 High densities of large Cladocera as a result of fish removal

In Leyb-b, phytoplankton biomass increased again after fish recolonization in 2008, coinciding with a decrease in large Cladocera length and density (Figure 15). Although a considerable cover of submerged vegetation was present in this pond in May (~ 40% cover), Leyb-b returned to a turbid state. Leyb-a, a pond with a higher submerged vegetation cover in general (~ 70%), also developed a high phytoplankton biomass in 2008, although to a lesser extent than Leyb-b (Figure 15). The elevated levels of phosphorus (TP and SRP) in both ponds in 2008 (Figure 16) suggest that when nutrient levels were too high, submerged vegetation is no longer able to efficiently control phytoplankton and will eventually disappear due to shading by epiphytic overgrowth, as was the case for Leyb-b in August 2008 (see also section 4.2.3 When is nutrient reduction particularly desirable?). Although the extensive cover of submerged vegetation in Leyb-a did not prevent an increase in phytoplankton biomass, the increase in turbidity was less severe as in Leyb-b.

Figure 13 Epiphytic overgrowth on submerged macrophytes in Leyb-b in 2009

Rebiomanipulation of Leyb-a and Leyb-b in 2009 restored a clear-water situation, but small fish immediately recolonized both ponds. The outcome for the coming years is uncertain, as in Leyb-a TP concentrations were still above 0.5 mg L^{-1} in 2009 (Figure 16). TP concentrations were considerably lower in Leyb-b, suggesting that the macrophytes present in Leyb-a remove a significant amount of nutrients from the water before flowing into Leyb-b.

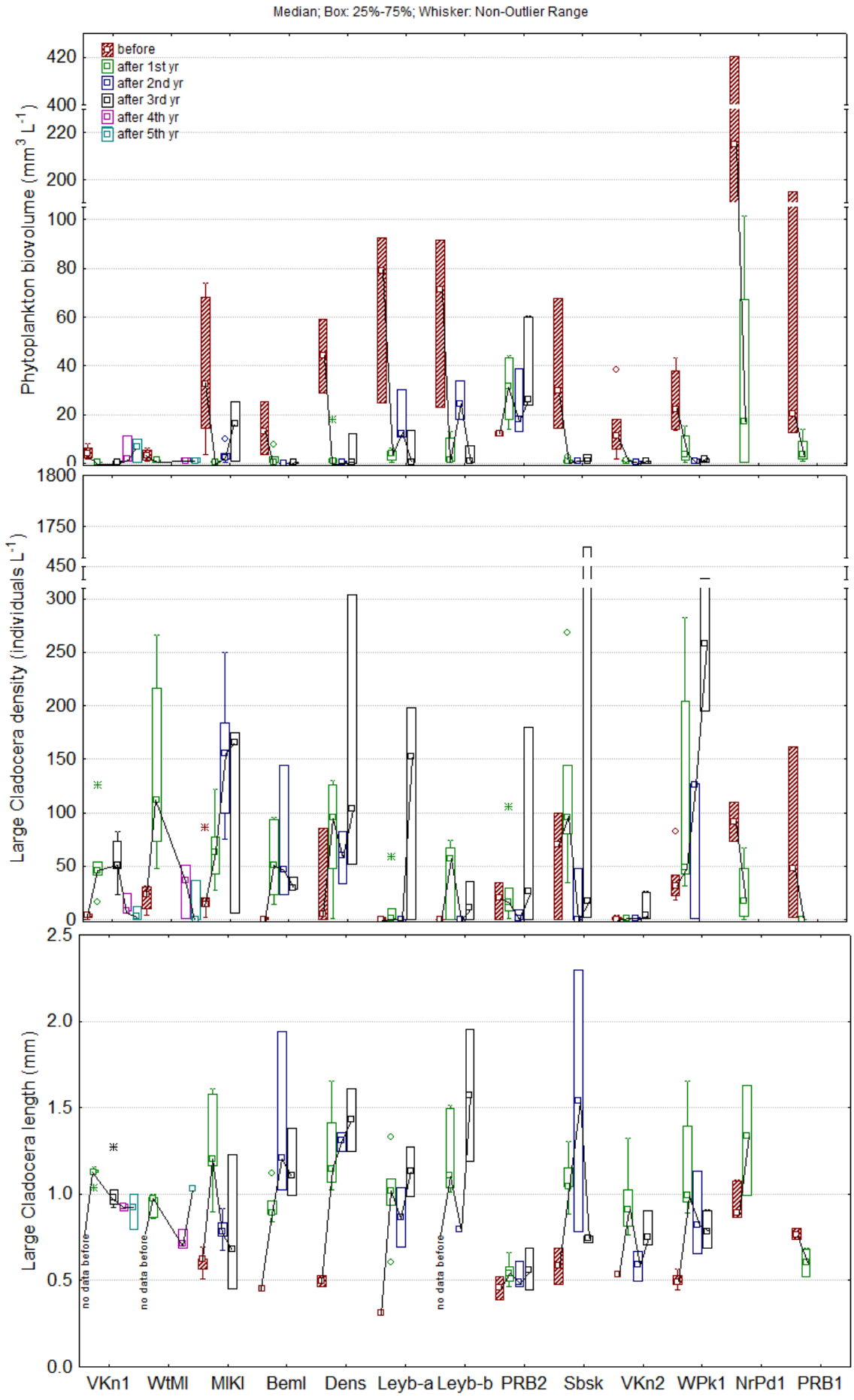

Figure 14 Boxplots showing phytoplankton biovolumes, large Cladocera density and large Cladocera length before and after biomanipulation for all ponds biomanipulated during 2004-2009

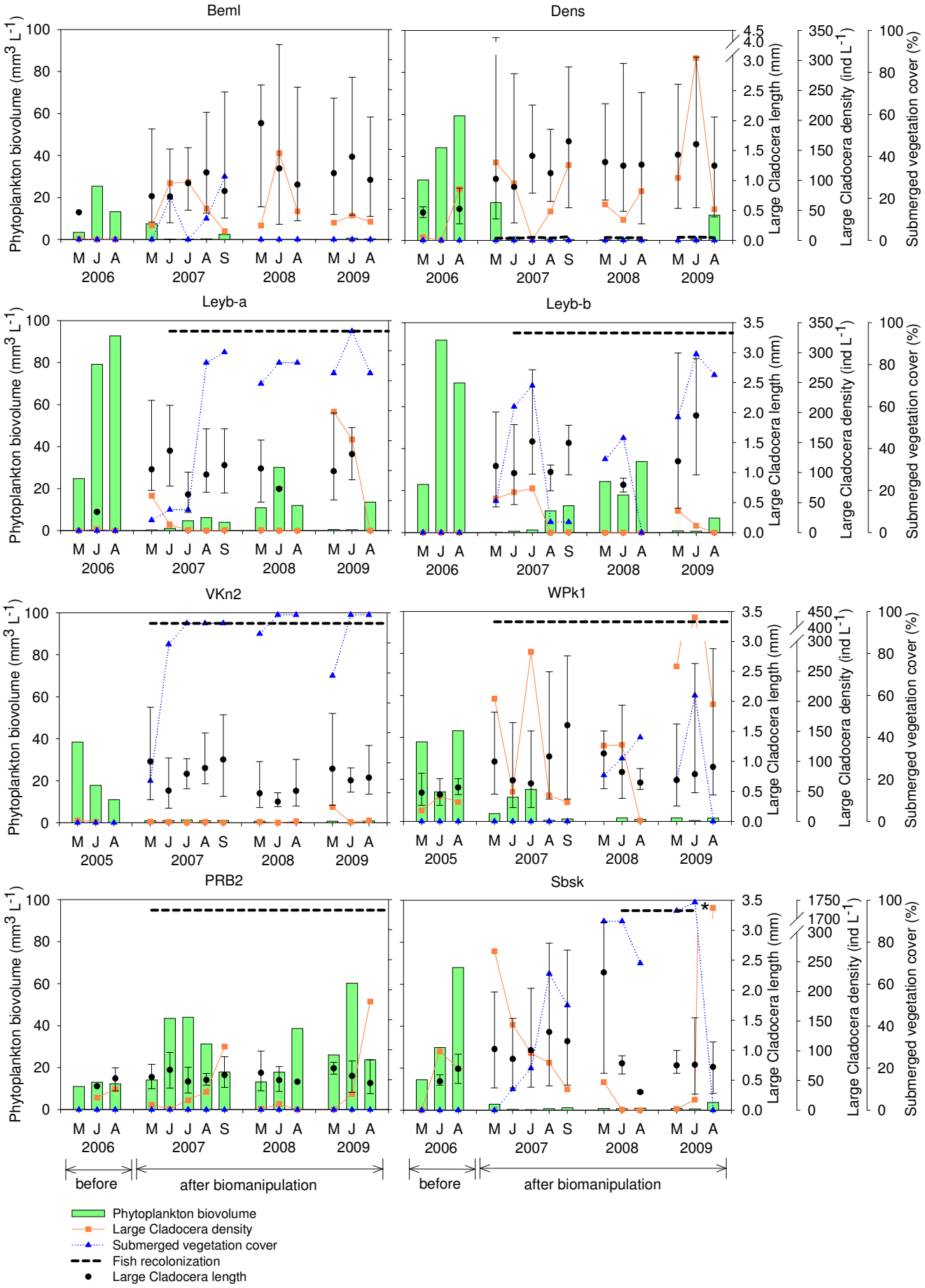

Figure 15 Temporal variation in phytoplankton biovolume, large cladocera density and size, submerged vegetation cover and fish recolonization in all ponds biomanipulated in 2007 (2006 – 2009). * = fish kill in Sbsk due to low oxygen concentrations caused by the thick layer of *Lemna* spp. on the water surface

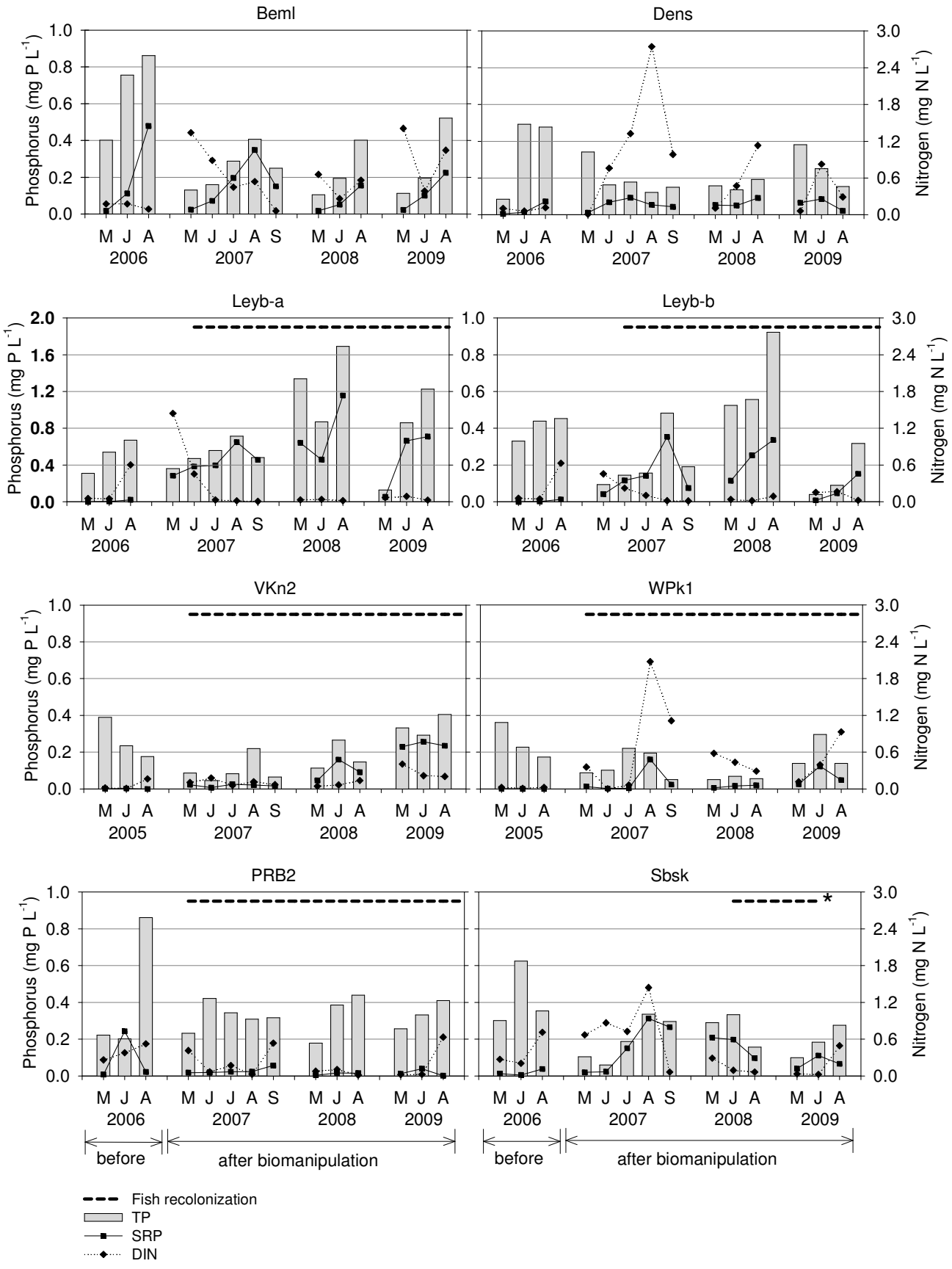

Figure 16 Temporal variation in TP, SRP, DIN and fish recolonization in all ponds biomanipulated in 2007 (2006 – 2009) * = fish kill in Sbsk due to low oxygen concentrations caused by the thick layer of *Lemna* spp. on the water surface

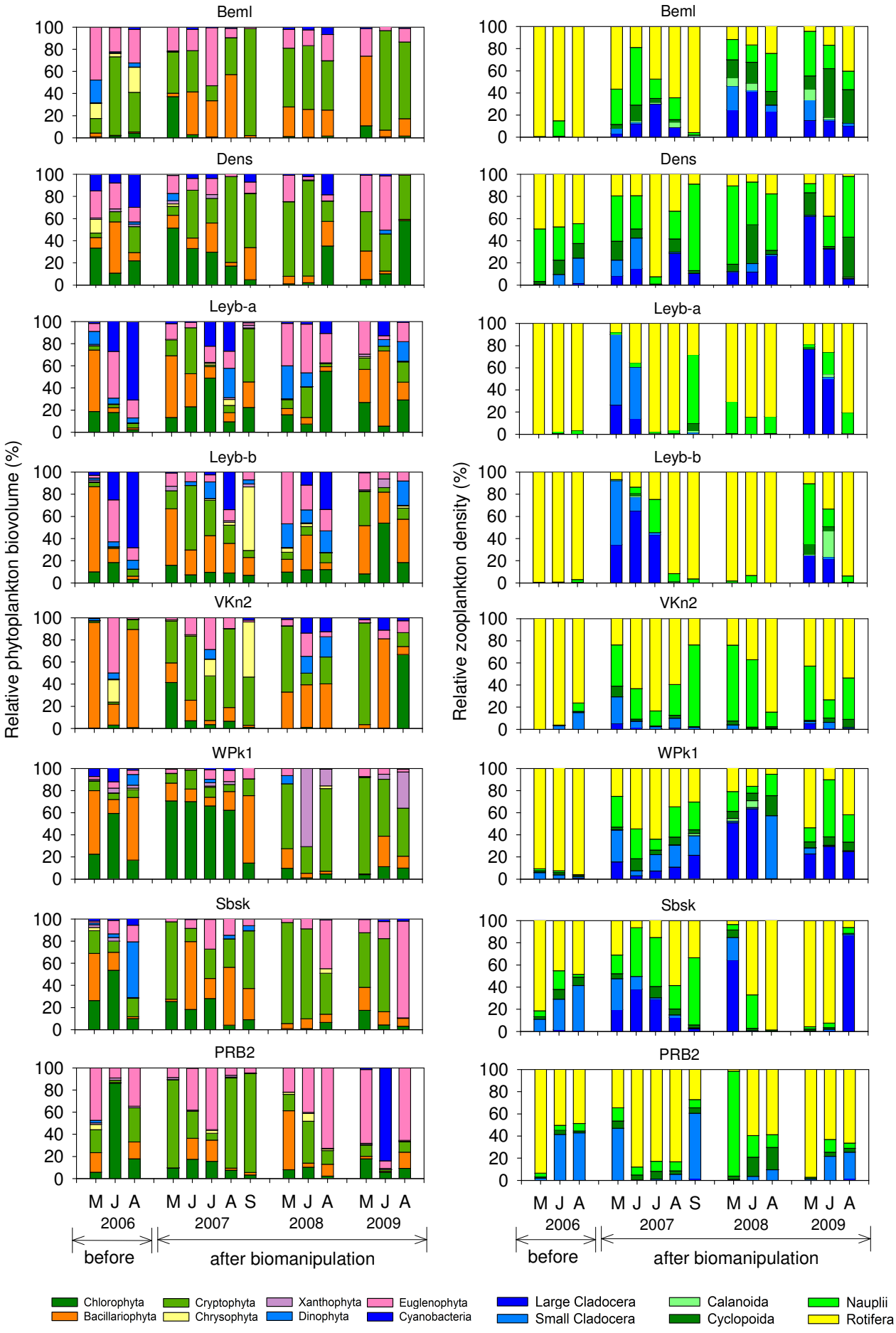

Figure 17 Temporal variation in relative phytoplankton biovolume and zooplankton density in all ponds biomanipulated in 2007 (2006 – 2009)

VKn1 and WtMl, two ponds that were biomanipulated in 2005, have been studied for a longer period after biomanipulation (Figure 20; Figure 21; Figure 22). It should be noted that in these ponds, before biomanipulation, phytoplankton biomass was already below 10 mm^3 L^{-1} (Figure 20) and no cyanobacterial blooms were observed (Figure 22). In 2008, four years after biomanipulation, both ponds had an extensive submerged vegetation cover, harboring several species (Table 6). In combination with low nutrient concentrations (Figure 21), phytoplankton biomass in these ponds was low, despite the recolonization by fish in WtMl. In 2009, WtMl did not change considerably. VKn1 however, developed an extensive cover of different *Lemna* and *Spirodela* species, forming a thick mat on the water surface (Figure 18). As a result, all submerged macrophytes disappeared in August due to light limitation, reduced oxygen concentrations or a combination of both. A similar situation was found in Sbsk, where macrophytes died off in august (Figure 19). Fish died in summer in Sbsk because of low oxygen concentrations, resulting in a revival of large Cladocera densities in July (Figure 15).

Figure 18 Thick mat of several *Lemna* and *Spirodela* species in VKn1 in 2009

PRB1 and NrPd1, two ponds that were biomanipulated only recently (spring 2009), both initially showed a shift towards a lower phytoplankton biovolume (Figure 20). In NrPd1, this coincided with a strong increase in large Cladocera length but not in their numbers, as their density was already rather high before biomanipulation. Small juvenile fish recolonized this pond almost immediately after biomanipulation, resulting in a gradual decrease of large Cladocera density and size. Although submerged vegetation recovered to more than 50%, it was unable to control phytoplankton biovolume that increased again to more than 100 mm^3 L^{-1}, coinciding with a collapse of the submerged vegetation in September. A similar situation was found in PRB1 (Figure 20). In this case, biomanipulation showed no visible effect on large cladocerans because of the reoccurrence of small planktivorous fish in this pond, feeding on the larger zooplankters. Although initially phytoplankton biomass had decreased after biomanipulation, it increased again in late summer. The fact that submerged vegetation in both ponds seemed incapable of controlling phytoplankton biomass, is most likely the result of the highly elevated levels of nutrients (mainly phosphorus, but also nitrogen in NrPd1; Figure 21) to more than 1 or 2 mg L^{-1} of TP found in PRB1 and NrPd1 respectively. This suggests again that submerged

macrophytes can control phytoplankton, but only when nutrients are not above a certain threshold (see also section 4.2.3 When is nutrient reduction particularly desirable?). When nutrients are high, submerged macrophytes will become overgrown with epiphyton and suffer from reduced light availability due to shading, resulting eventually in their disappearance as was also the case for Leyb-b.

Figure 19 100% cover of *Lemna* during summer 2009 in Sbsk

Two ponds that were not biomanipulated through fish removal, but where pikes were added, were investigated to study the impact of pike addition alone. In PchR , no fish were removed, however, a large amount of pike (176 1^+ individuals) was added in 2008. In TrSG, a pond that where the water was drawdown in 2007 but where fish were only partially removed, 10 1^+ pike were added in 2008. Pike addition did not seem to have any effect on the ecological quality of the two ponds when considering phytoplankton biovolume, large Cladocera length or densities (Figure 20) or nutrients (Figure 21), nor in phytoplankton or zooplankton composition (Figure 22). Based on calculations made in a previous report on pike addition (De Backer et al., 2009), it is indeed unlikely that the amounts of pike that were added in PchR and TrSG were sufficient to reduce juvenile planktivorous fish. As pike need vegetation for their reproduction, mainly for attachment of the eggs (Craig, 1996), and to provide shelter for the young pike to protect them from older pike (Casselman and Lewis, 1996), it is unlikely that reproduction will take place in these ponds.

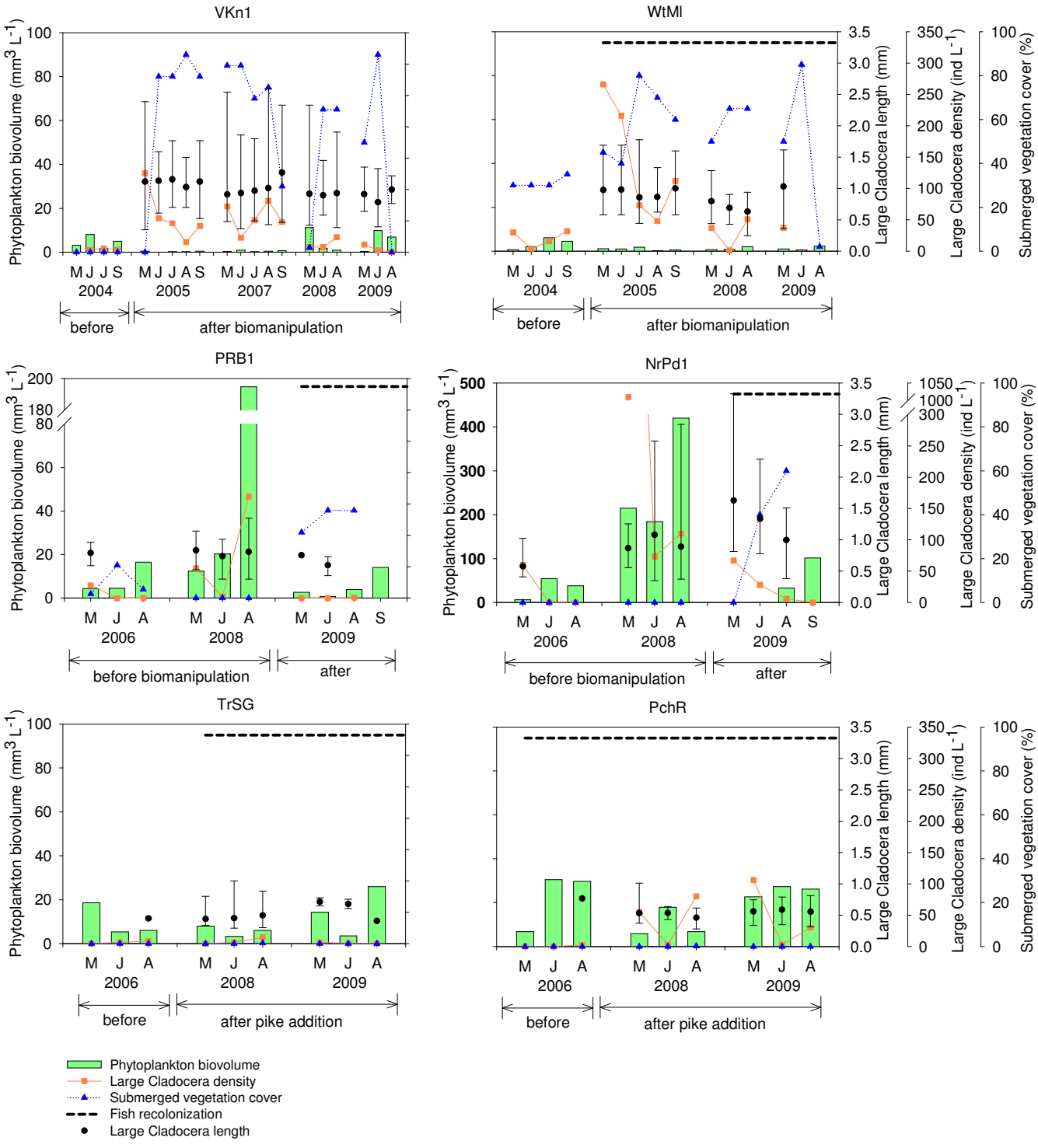

Figure 20 Temporal variation in phytoplankton biovolume, large cladocera density and size, submerged vegetation cover and fish recolonisation in two ponds biomanipulated in 2005 (VKn1 and WtMl), two ponds biomanipulated in 2009 (PRB1 and NrPd1) and two ponds where only pike were added (TrSG and PchR). Phytoplankton data was also available for September 2009 for PRB1 and NrPd1

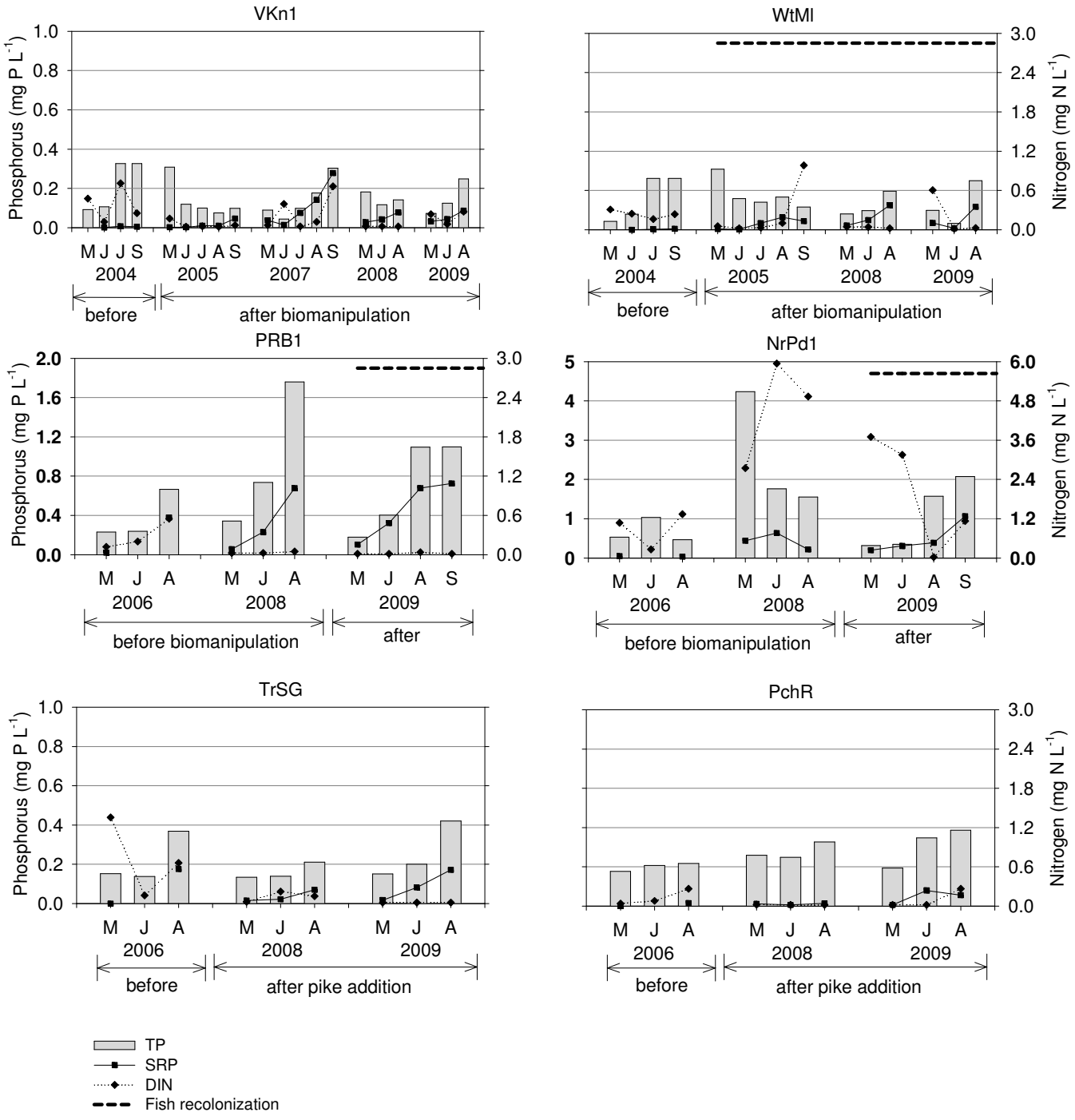

Figure 21 Temporal variation in TP, SRP and DIN in two ponds biomanipulated in 2005 (VKn1 and WtMl), two ponds biomanipulated in 2009 (PRB1 and NrPd1) and two ponds where only pike were added (TrSG and PchR) For PRB1 and NrPd1, data was also available for September 2009

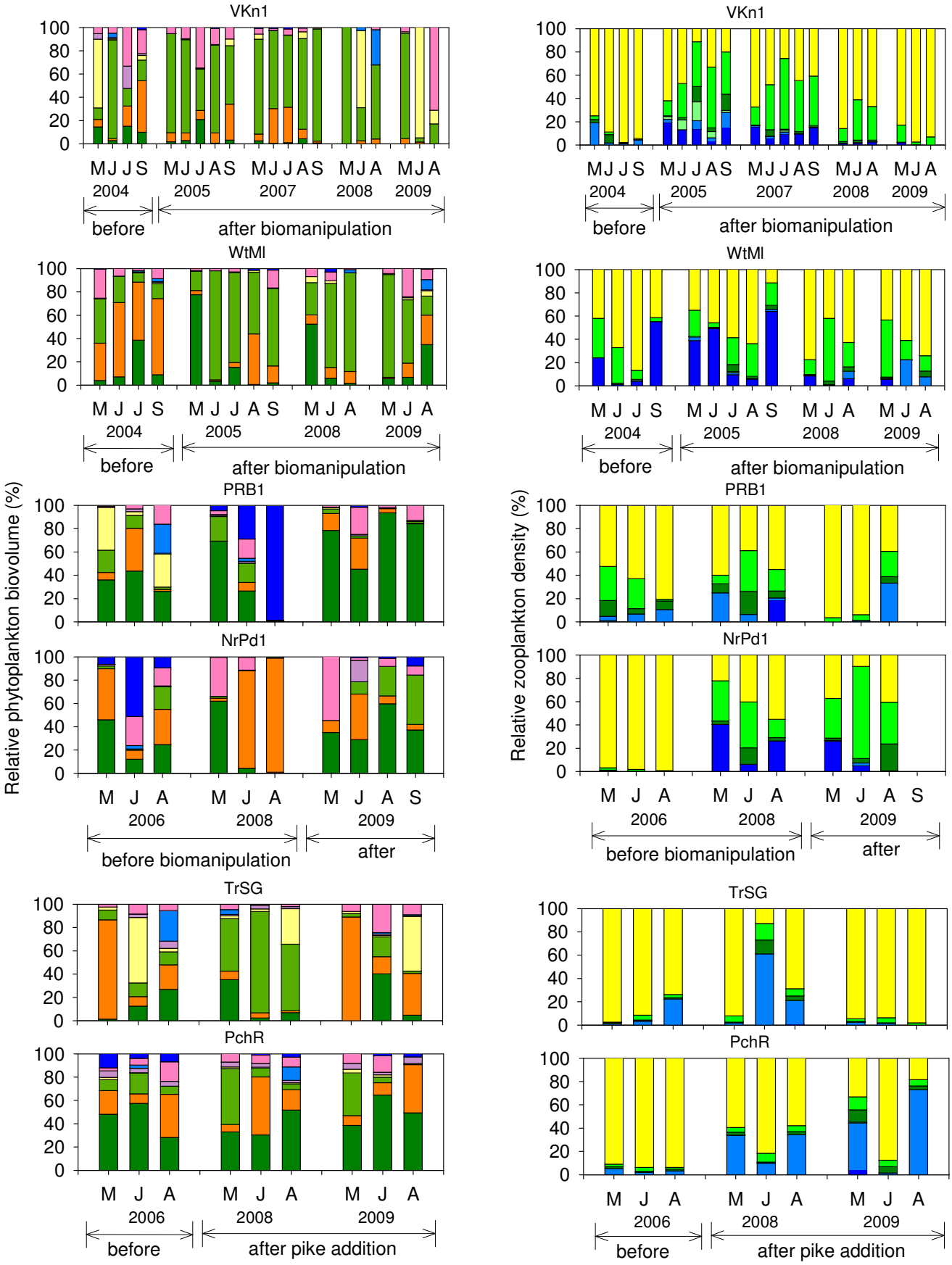

Figure 22 Temporal variation in relative zooplankton density and phytoplankton biovolume in two ponds biomanipulated in 2005 (VKn1 and WtMl), two ponds biomanipulated in 2009 (PRB1 and NrPd1) and two ponds where only pike were added (TrSG and PchR). Phytoplankton data was also available for September 2009 for PRB1 and NrPd1

4.2.2 The importance of submerged vegetation recovery

Initially, biomanipulation through fish removal and water drawdown was successful in 12 out of 13 ponds (except for PRB2; De Backer et al., 2008). A marked decrease in phytoplankton biomass and a strong increase in density and size was observed immediately after biomanipulation. 10 out of 13 ponds have developed submerged vegetation to a certain extent. Although the initial result of biomanipulation was promising, in the period after biomanipulation the ponds have shown very different dynamics, mainly defined by planktivorous fish recolonization and presence of submerged vegetation (De Backer et al., 2009).

Phytoplankton biomass can be controlled in different ways. Previous research on the studied ponds has shown that dense vegetation alone, in absence of high densities of large zooplankters, is capable of maintaining a low phytoplankton biomass. Submerged macrophytes are known to be able to control phytoplankton biomass through several mechanisms such as competition for nutrients, allelopathy, increased sedimentation and providing a refuge from predation for zooplankton (van Donk and van de Bund, 2002). In ponds where no submerged vegetation has recovered (Dens, Beml, MIKI 2007), very high densities of large zooplankton had developed. In general, grazing by large Cladocera alone resulted in a very low phytoplankton biomass, despite the absence of vegetation (De Backer et al., 2009). This is however a very unstable situation. If such ponds are recolonized again by fish, large cladocerans will quickly be preyed down and no longer be able to control phytoplankton, shifting the pond back to the turbid state (as was the case for MIKI in 2008).

The results presented here show that the recolonization of small juvenile planktivorous fish (20-50 mm) in some of the ponds, feeding on large zooplankters, had a significant effect on large cladoceran density and length and consequently phytoplankton biomass. The impact of recolonizing fish however depended on the extent of the submerged vegetation cover (Figure 23). In non-vegetated ponds, total phytoplankton biovolume increased significantly in ponds where juvenile fish returned (Figure 23). In general, turbidity increased to such an extent that all ponds without submerged vegetation shifted back to the turbid state.

This is well exemplified for the case of MIKI, a non-vegetated pond where juvenile fish recolonization resulted in a shift to the turbid state (Figure 24a). This pond had significantly improved after biomanipulation: phytoplankton biomass decreased considerably, as large Cladocera density and length increased. As a result of fish recolonization however, phytoplankton biomass increased again. Length of large Cladocera decreased significantly. Their density, on the contrary, increased due to the presence of fish (Figure 24a), shifting the population towards smaller but more numerous individuals due to the selective feeding of fish on larger individuals. This suggests that size of large Cladocera seems to be more important for phytoplankton control than their density, as mentioned previously.

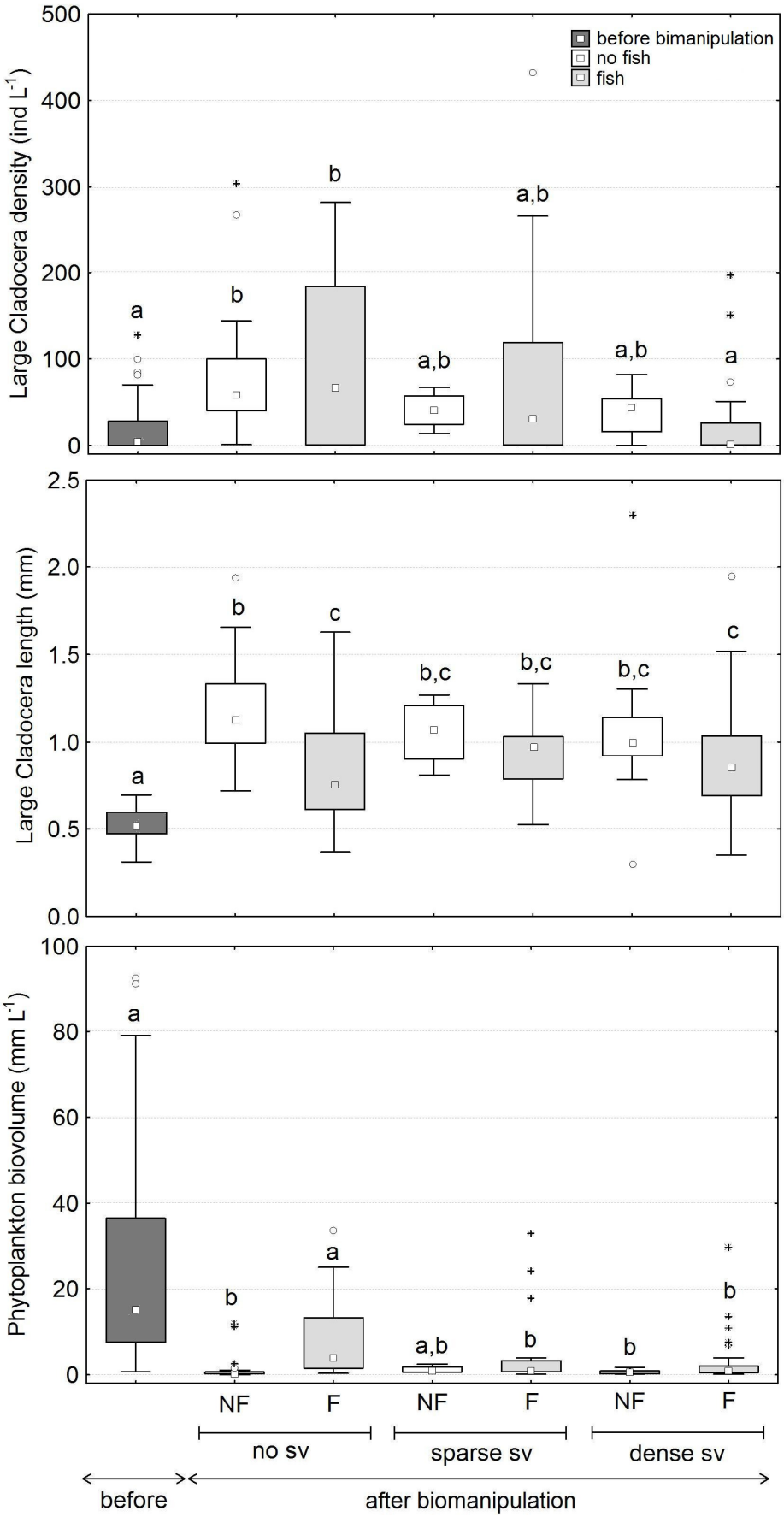

Figure 23 Phytoplankton biovolume and large Cladocera density and length in presence (F) and absence (NF) of fish and different cover of submerged vegetation. The situation before biomanipulation is also shown. Different characters indicate significant differences using Kruskal-Wallis ANOVA

The effect of fish recolonization was different in sparse- or densely vegetated ponds, where the pond remained clear with an average phytoplankton biovolume of only 1 mm^3 L^{-1} after fish recolonization (Figure 23). A considerable decrease of large Cladocera length after fish recolonization was still noted in sparse- and densely vegetated ponds, although less steep than in non-vegetated ponds (Figure 23). No distinct change of large Cladocera densities was seen in non- or sparsely vegetated ponds after fish recolonization. In densely vegetated ponds, a significant decrease was shown to almost an absence of large cladocerans after fish recolonization. Both length and density results suggest that large Cladocera are not protected by submerged vegetation, as they almost completely disappeared in ponds with a very high vegetation cover.

This is well exemplified by the case of Sbsk, a pond with a dense submerged vegetation cover of > 70% on average, where fish recolonization after biomanipulation did not lead to a shift to the turbid state, although large Cladocera decreased considerably in average length and density (Figure 24b).

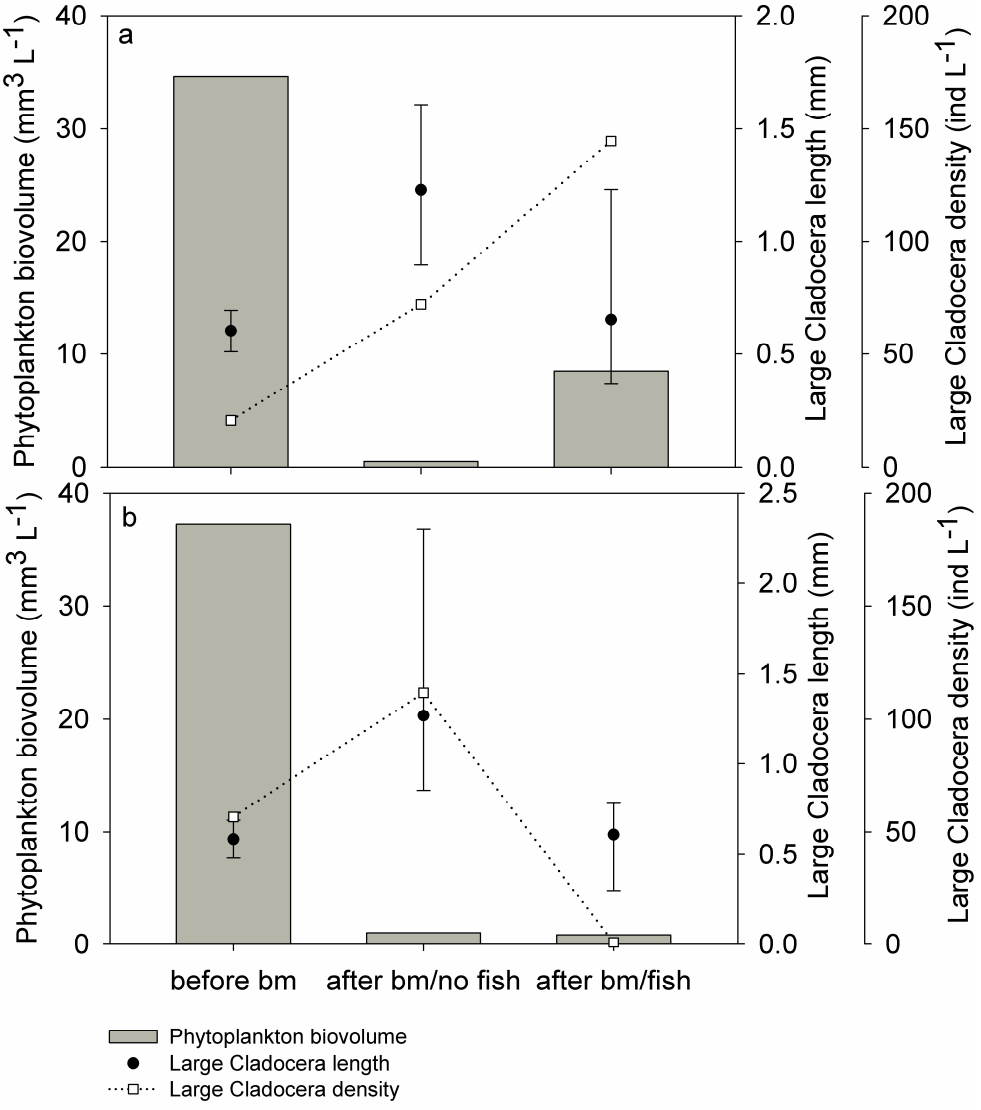

Figure 24 Impact of fish recolonization on large Cladocera length and density and phytoplankton biovolume in (a) a non-vegetated (MlKl) and (b) a densely vegetated (Sbsk; > 70 % submerged vegetation cover) pond

Thus, the reappearance of small juvenile planktivorous fish in some of the ponds can alter the success of biomanipulation. Phytoplankton biomass increased upon fish recolonization to such an extent that some of the ponds returned to a turbid state. The extent of the increase however depended on submerged vegetation cover, since densely vegetated ponds did not return to the turbid state as was the case for ponds lacking submerged vegetation. However, the strong decline in large Cladocera densities and length after fish recolonization in ponds with a dense vegetation to even a total disappearance in most cases, suggests that submerged vegetation did not provide sufficient shelter for zooplankton against fish predation. Despite the lack of any refuge for large Cladocera and their decline in size and density, independent of the extent of submerged vegetation cover, submerged macrophytes can control phytoplankton biomass and prevent a significant increase in turbidity after fish recolonization.

Taking into account the importance of submerged macrophytes in stabilizing the after biomanipulation situation, measures should be taken to restore a (preferably dense) submerged vegetation in those ponds where macrophytes did not recover. Moss et al. (1996) defined three main reasons for the absence of submerged vegetation after biomanipulation:

(1) Natural inocula (propagules and seeds) are absent and must be introduced,

(2) Natural inocula are present but are prevented from being established by destruction by birds,

(3) Natural inocula are present but sediment conditions are preventing establishment.

Different measures can be taken to establish vegetation, such as the introduction of seeds and propagules when they are absent. To provide protection from bird herbivory, exclosures can be used to prevent bird damage during the early phases of macrophyte establishment (Moss *et al.*, 1996). It is also important to have numerous ponds that are vegetated within the same area. Birds will then not be concentrated on only one pond but will be spread over several. In most cases, sediment quality should be sufficient to provide plant growth unless there has been a contamination with pollutants such as toxic heavy metals (Moss *et al.*, 1996).

4.2.3 When is nutrient reduction particularly desirable?

There are several causes for biomanipulation failure, most of which are related to the presence of fish. First of all, if the amount of fish that was removed was insufficient, or when fish recolonized after biomanipulation and no submerged vegetation is present, large zooplankton grazers will quickly be preyed down, resulting in a phytoplankton biomass increase. As discussed in the previous section (4.2.2 The importance of submerged vegetation recovery), a significant cover of submerged vegetation (> 30%) can efficiently control phytoplankton biomass to a certain extent, even when fish recolonize such ponds. However, it appears that when nutrient concentrations are too high, submerged vegetation is no longer able to control phytoplankton efficiently, resulting in epiphytic overgrowth and consequently a reduced light availability, eventually leading to a total disappearance of macrophytes. This was observed in several ponds, such as Leyb-b in 2008 or NrPd1 in 2009.

To estimate the threshold of nutrients above which macrophytes can no longer control phytoplankton biomass efficiently, the relationship between phytoplankton biovolume and TP was studied, based on averaged per year data for each of the ponds studied during 2004 – 2009 (Figure 25). For each case, a cross is shown when maximum submerged vegetation cover during that year was ≥ 30% i.e. which have developed an extensive cover of submerged vegetation at some point during the summer.

The threshold of TP concentrations above which submerged vegetation can no longer control phytoplankton biomass sufficiently is situated around 350 µg P L^{-1} for Brussels ponds. The cases with submerged vegetation above this threshold are still having an impact on phytoplankton biomass, as their total phytoplankton biovolume is considerably lower than that of many cases with similar TP concentrations without vegetation, but this does not prevent phytoplankton biomass from exceeding 10 mm^3 L^{-1}. Consequently, submerged macrophytes in this situation will be shaded by phytoplankton and epiphyton and eventually collapse during summer.

The efficiency of submerged vegetation to control phytoplankton thus seems to be related to the level of nutrient loading. This explains the high phytoplankton biomass in presence of vegetation in Leyb-a and Leyb-b in 2008, that both showed elevated TP concentrations during that year (Figure 16). This was also the case in NrPd1 after biomanipulation in 2009, where macrophytes disappeared and the water became turbid again in September with a Secchi depth of < 0.3 m and a phytoplankton biovolume of > 100 mm^3 L^{-1} (Figure 20).

Therefore, when attempts are made to restore eutrophic ponds through biomanipulation, nutrient reduction should always be considered, particularly if the average TP concentration is above 350 µg L^{-1}. More details on how the TP threshold concentration of 350 µg L^{-1} was determined statistically can be found in Teissier et al. (2010).

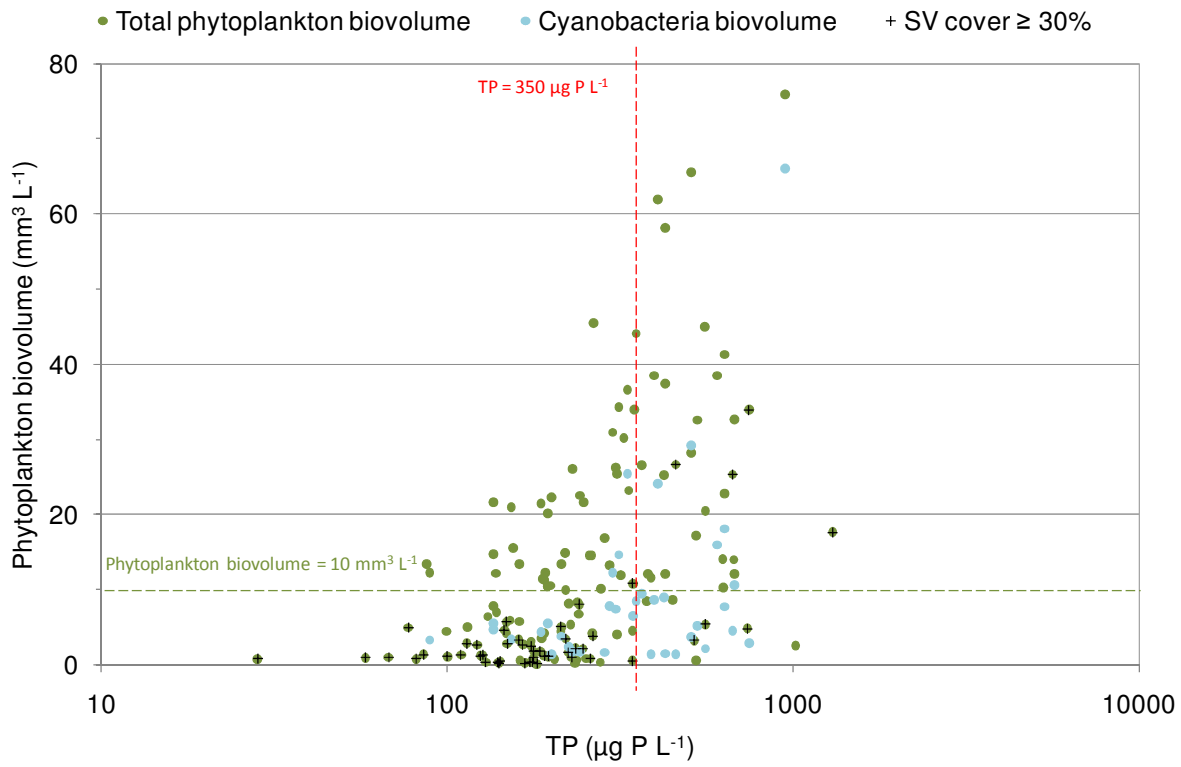

Figure 25 Relationship between phytoplankton biovolume and TP based on averaged per year data of all ponds studied from 2004 to 2009 (n = 143). Total phytoplankton biovolume as well as cyanobacterial biovolume are shown on the graph. A cross is shown for each total phytoplankton biovolume where maximum cover of submerged vegetation was > 30%. TP is given on a logarithmic scale.

4.3 Determination of the ecological status using ECOFRAME

New European legislation, Water Framework Directive (2000/60/EG), sets as a goal that all European water bodies should meet the criteria for good ecological status by the end of 2015. For implementation of the Water Framework Directive, a panel of leading European fresh water ecologists and limnologists developed and tested a system (ECOFRAME) to determine ecological status of shallow lakes and ponds (Moss *et al.*, 2003). The system is based on biotic and abiotic variables that determine the ecological make-up of a lake or a pond. Although ponds and small lakes are not explicitly covered by the Directive, their ecological quality should be assessed and, in case of need, restored because they represent the majority of standing freshwater bodies in many European countries.

Table 7 Ecological status of the ponds studied in 2009. Ponds arranged in order of phytoplankton biomass increase; dark blue - high, blue - good, green - moderate, yellow - poor, brown - bad ecological status. * = water level was lowered

	Biomanipulated	Site	Overall ecological status 2009	Ecotype no.	Conductivity (µS cm^{-1})	pH (log units)	TP (µg L^{-1})	Secchi depth (m)	Chlorophyll a (µg L^{-1})	Phytoplankton diversity	Plant community	Plant diversity (species no.)	Plant abundance	Cladocera (no. Large: no. total)
ECOLOGICAL STATUS 2009	X	BemI	poor	17	819	7.5	277	2.6	2.1	A	CanNym	2	1	0.7
	X	VKn2	poor	17	487	7.4	343	2.4	30.0	A	CanNym	1	3	0.6
		RKI5*	moderate	17	508	7.8	81	3.4	2.7	A	Char	2	3	0.3
	X	WPk1	moderate	17	952	7.7	191	2.6	7.1	A	ElPo	3	2	0.9
	X	WtMl	poor	17	467	8.1	127	2.6	14.1	A	ElPo	5	2	0.1
		RKI4*	moderate	17	580	7.8	85	2.4	6.9	A	ElPo	4	3	0.3
	X	Sbsk	moderate	17	557	7.9	186	2.0	8.2	A	ElPo	3	3	1.0
	X	PRB1	bad	17	927	8.5	558	2.0	11.6	A	ElPo	3	2	0.0
		Tenr	moderate	17	488	7.9	165	1.8	13.4	A	CanNym	4	2	0.5
	X	Leyb-b	poor	17	582	8.2	149	2.4	14.2	B	ElPo	1	3	1.0
		WPk2	bad	17	784	7.8	175	2.7	10.4	A	Absent	0	0	1.0
	X	Dens	bad	17	399	7.9	262	2.0	19.3	A	Absent	0	0	1.0
		RKI3	bad	17	546	8.0	99	1.1	24.3	B	Absent	0	0	0.1
	X	Leyb-a	poor	17	551	8.6	738	2.3	23.9	B	ElPo	1	3	1.0
		RKI2	bad	17	558	8.0	115	1.0	23.3	A	Absent	0	0	0.0
	X	VKn1	poor	17	442	7.5	148	2.7	98.4	A	Char	2	2	0.7
		Plbs	bad	17	940	7.8	239	1.7	37.4	A	CanNym	1	1	0.0
		Trln	bad	17	698	7.8	629	1.0	49.6	A	Absent	0	0	0.0
	X	NrPd1	bad	17	607	8.6	745	1.5	78.7	A	ElPo	2	2	0.8
		IxP1	bad	17	835	8.3	137	0.7	54.5	B	Absent	0	0	0.1
		TrCg	bad	17	503	8.0	295	0.5	121.9	C	Absent	0	0	0.2
		TrSG	bad	17	553	8.1	257	1.1	76.6	B	CanNym	1	1	0.0
		TrBr	bad	17	676	8.0	423	0.7	237.8	B	CanNym	1	1	0.0
		PchR	bad	17	649	8.1	309	0.5	70.5	B	Absent	0	0	0.0
		IxP2	bad	17	682	8.4	296	0.4	177.8	B	Absent	0	0	0.1
	X	PRB2	bad	17	735	8.0	332	0.4	89.2	B	Absent	0	0	0.1
		NrPd4	bad	17	551	8.8	604	0.4	150.8	B	Absent	0	0	0.0
		BCmb	bad	17	451	8.4	555	0.2	262.8	B	Absent	0	0	0.1
		NrPd2	bad	17	527	9.0	870	0.2	129.1	C	Absent	0	0	0.0

high	good	moderate	poor	bad

A number of selected variables acquired within this study (pH, TP, Secchi depth, chlorophyll *a*, phytoplankton diversity, plant community structure, plant abundance and large to total Cladocera ratio) were processed according to the ECOFRAME system (version 8; Moss *et al.*, 2003) to estimate the ecological status of the ponds studied. Averaged per study period values (warm months only) were used in the system. Table 7 presents the results of the ecological status assessment for the 29 ponds studied in 2009. No pond showed an overall good ecological quality. Only 5 out of 29 ponds (RKl5, WPk1, RKl4, Sbsk and Tenr) can be attributed a moderate ecological status; 6 ponds (Beml, VKn2, WtMl, Leyb-b, Leyb-a and VKn1) correspond to a poor and the remaining 18 ponds to a bad ecological status (Table 7).

Of the 12 biomanipulated ponds studied in 2009, 2 were attributed a moderate status (Sbsk and WPk1), 6 correspond to a poor (Beml, Leyb-a, Leyb-b, VKn1, VKn2 and WtMl) and 4 to a bad ecological status (Dens, NrPd1, PRB1 and PRB2)(Table 8). Beml, VKn2, VKn1 and WtMl are only marginally poor, meaning that an improvement in one or two variables can improve the ecological quality to moderate. NrPd1 and PRB1 are only marginally bad and could improve to the poor status with only a slight improvement.

In 2009, the ecological status of Leyb-a and Leyb-b improved again from bad to poor after biomanipulation performed end of August 2008 (Table 8), as a result of increased transparency and a higher ratio of Cladocera to total zooplankton density. Sbsk improved from poor to moderate due to an increase in plant diversity. VKn1 on the contrary, deteriorated to a poor status mainly due to an increased Chl *a* concentration after disappearance of submerged macrophytes due to light limitation caused by a thick mat of free-floating macrophytes (a mixture of *Lemna* and *Spirodela* spp.). All other ponds maintained the same ecological status as in 2008. Recovery of submerged vegetation, providing structural diversity and thus favoring biological diversity and strengthening the resilience of a pond ecosystem (van Donk and van de Bund, 2002), is crucial for the restoration of ecological quality. This is difficult to achieve in shallow ponds with large populations of herbivorous birds such as Dens or Beml. The overall ecological quality of this pond remains bad despite considerable improvement in several variables after biomanipulation.

The ecological quality of NrPd1 and PRB1, two ponds that were biomanipulated in late summer 2008, improved considerably on several aspects (Table 8). Despite these improvements, the overall ecological status remained bad in both ponds. This is the result of the TP concentrations that remained high after biomanipulation in both ponds, coinciding with a low large to total Cladocera ratio in PRB1 and a high Chl *a* concentration in NrPd1.

Table 8 Ecological status before and after biomanipulation. Dark blue - high, blue - good, green - moderate, yellow - poor, brown - bad ecological status

	Site	Overall ecological status	Ecotype no.	Conductivity (µS cm^{-1})	pH (log units)	TP (µgL^{-1})	Secchi depth (m)	Chlorophyl a (µgL^{-1})	Phytoplankton diversity	Plant community	Plant diversity (species no.)	Plant abundance	Cladocera (no. large:no. total)
BEFORE BIOMANIPULATION	Beml	bad	17	748	7.9	673	0.7	52.1	A	CanNym	2	3	0.1
	Dens	bad	17	422	8.4	351	0.4	87.8	B	Absent	0	0	0.1
	Leyb-a	bad	17	536	9.0	506	0.3	469.7	C	Absent	0	0	0.0
	Leyb-b	bad	17	557	8.8	407	0.3	348.6	C	Absent	0	0	0.0
	MlKl	bad	17	473	8.2	354	0.4	113.3	B	Absent	0	0	0.0
	NrPd1	bad	17	636	8.4	2516	0.2	1403.5	B	Absent	0	0	1.0
	PRB1	bad	17	801	8.7	946	0.3	310.2	C	Absent	0	0	0.1
	PRB2	bad	17	735	8.0	428	0.6	40.2	B	Absent	0	0	0.0
	Sbsk	bad	17	781	8.4	426	0.6	82.8	B	Absent	0	0	0.0
	VKn1	bad	17	546	7.8	213	0.7	20.1	A	ElPo	2	1	0.0
	VKn2	bad	17	570	7.7	174	0.8	54.3	A	Absent	0	0	0.0
	WPk1	bad	17	895	7.8	204	0.6	41.4	B	CanNym	2	1	0.1
	WtMl	bad	17	532	7.9	161	1.0	13.9	A	Absent	0	0	0.9
AFTER BIOMANIPULATION 2007	Beml	moderate	17	935	7.7	247	1.4	28.7	A	CanNym	4	3	0.8
	Dens	bad	17	433	7.9	191	0.9	18.0	A	Absent	0	0	0.7
	Leyb-a	bad	17	634	8.3	517	1.2	19.8	B	ElPo	2	2	0.2
	Leyb-b	poor	17	661	8.0	213	1.5	25.7	B	ElPo	1	2	0.5
	MlKl	bad	17	448	8.5	626	1.4	170.8	B	ElPo	2	1	0.7
	PRB2	bad	17	624	8.0	324	0.4	151.1	A	Absent	0	0	0.1
	Sbsk	poor	17	711	7.8	196	1.8	7.0	A	ElPo	2	2	0.8
	VKn1	moderate	17	480	7.8	142	2.1	6.0	A	Char	5	3	0.9
	VKn2	poor	17	525	7.6	100	1.3	7.3	A	CanNym	1	3	0.2
	WPk1	poor	17	924	7.8	131	1.2	14.5	A	CanNym	2	1	0.4
AFTER BIOMANIPULATION 2008	Beml	poor	17	851	7.5	233	1.8	1.2	A	CanNym	2	3	0.9
	Dens	bad	17	394	7.6	162	1.3	4.9	A	Absent	0	0	0.9
	Leyb-a	bad	17	528	8.7	1300	0.8	102.5	B	ElPo	2	2	0.1
	Leyb-b	bad	17	567	8.7	668	0.6	249.2	B	ElPo	1	2	0.1
	MlKl	bad	17	448	8.5	626	1.4	170.8	B	ElPo	2	1	0.7
	PRB2	bad	17	708	7.8	334	0.4	124.4	A	Absent	0	0	0.0
	Sbsk	poor	17	560	8.0	260	1.8	22.0	A	ElPo	2	2	0.7
	VKn1	moderate	17	417	8.1	146	1.8	23.6	A	Char	5	3	1.0
	VKn2	poor	17	423	7.6	176	1.9	6.1	A	CanNym	1	3	0.3
	WPk1	moderate	17	891	7.7	58	2.2	3.4	A	Can Nym	3	1	0.5
AFTER BIOMANIPULATION 2009	Beml	poor	17	819	7.5	277	2.6	2.1	A	CanNym	2	1	0.7
	Dens	bad	17	399	7.9	262	2.0	19.3	A	Absent	0	0	1.0
	Leyb-a	poor	17	551	8.6	738	2.3	23.9	B	ElPo	1	3	1.0
	Leyb-b	poor	17	582	8.2	149	2.4	14.2	B	ElPo	1	3	1.0
	NrPd1	bad	17	607	8.6	745	1.5	78.7	A	ElPo	2	2	0.8
	PRB1	bad	17	927	8.5	558	2.0	11.6	A	ElPo	3	2	0.0
	PRB2	bad	17	735	8.0	332	0.4	89.2	B	Absent	0	0	0.1
	Sbsk	moderate	17	557	7.9	186	2.0	8.2	A	ElPo	3	3	1.0
	VKn1	poor	17	442	7.5	148	2.7	98.4	A	Char	2	2	0.7
	VKn2	poor	17	487	7.4	343	2.4	30.0	A	CanNym	1	3	0.6
	WPk1	moderate	17	952	7.7	191	2.6	7.1	A	ElPo	3	2	0.9
	WtMl	poor	17	467	8.1	127	2.6	14.1	A	ElPo	5	2	0.1

high	good	moderate	poor	bad

5. Conclusions and recommendations

Initially, the biomanipulation of 12 Brussels ponds carried out between 2004 and 2009 in order to restore their ecological quality brought positive results in all ponds except PRB2. Most of the biomanipulated ponds have shown a marked improvement in several aspects of their ecological quality. The biomanipulation results confirm the importance of a balanced fish community in determining ecological quality of ponds and indicate that when pond ecosystems are impaired by eutrophication, considerable degree of their ecological quality can be restored through the manipulation of fish community. Fish play a central role in structuring zooplankton and submerged macrophyte communities that, in turn, play a crucial role in controlling phytoplankton in eutrophic ponds.

An important factor altering the success of biomanipulation on the longer term is the recolonization of planktivorous fish. Despite the lack of any refuge effect of submerged vegetation on large zooplankters, phytoplankton biomass increase was only significant in ponds with a submerged vegetation cover < 30% after fish recolonization. The fact that submerged macrophytes have shown the ability to prevent a significant increase in phytoplankton biomass, emphasizes the importance of their recovery after biomanipulation. Different measures can be taken to establish vegetation if it remains absent: (i) the introduction of seeds and propagules, (ii) the pond can be deepened if depth is < 1m (iii) exclosures can be placed to exclude birds and prevent bird damage in early phases of macrophyte establishment.

Although submerged macrophytes can prevent a phytoplankton biomass increase after fish recolonization, nutrients should be limited to a certain extent. Above a certain threshold of nutrient concentration in the water column (a rough average TP concentration of 350 µg L^{-1} as obtained from the data on Brussels ponds), macrophytes are less able to efficiently control phytoplankton biomass during the whole summer.

A decision tree was developed as a general guideline for selecting appropriate restoration measures for Brussels ponds, advising nutrient reduction before biomanipulation, particularly when the average TP concentration is more than 350 µg L^{-1} through reduction of external nutrient loading and/or sediment removal. (Figure 26). Once biomanipulation is performed, several steps are incorporated into the decision tree considering stabilization of the clear-water state, such as measures to stimulate macrophyte recovery or the addition of piscivorous fish. If the restoration measures (biomanipulation and/or nutrient reduction) were successful, the system should be maintained (i.e. no plankti-benthivorous fish stocking etc.). It should be noted that this is a general guideline and that the final decision for appropriate restoration measures is case-dependent. Because Brussels ponds are highly dynamic and large fluctuations can occur over short periods, regular monitoring of the successfully restored ponds is necessary in order to detect any deterioration of the situation. The decision tree enables adequate measures to be taken in order to avoid further deterioration of the system.

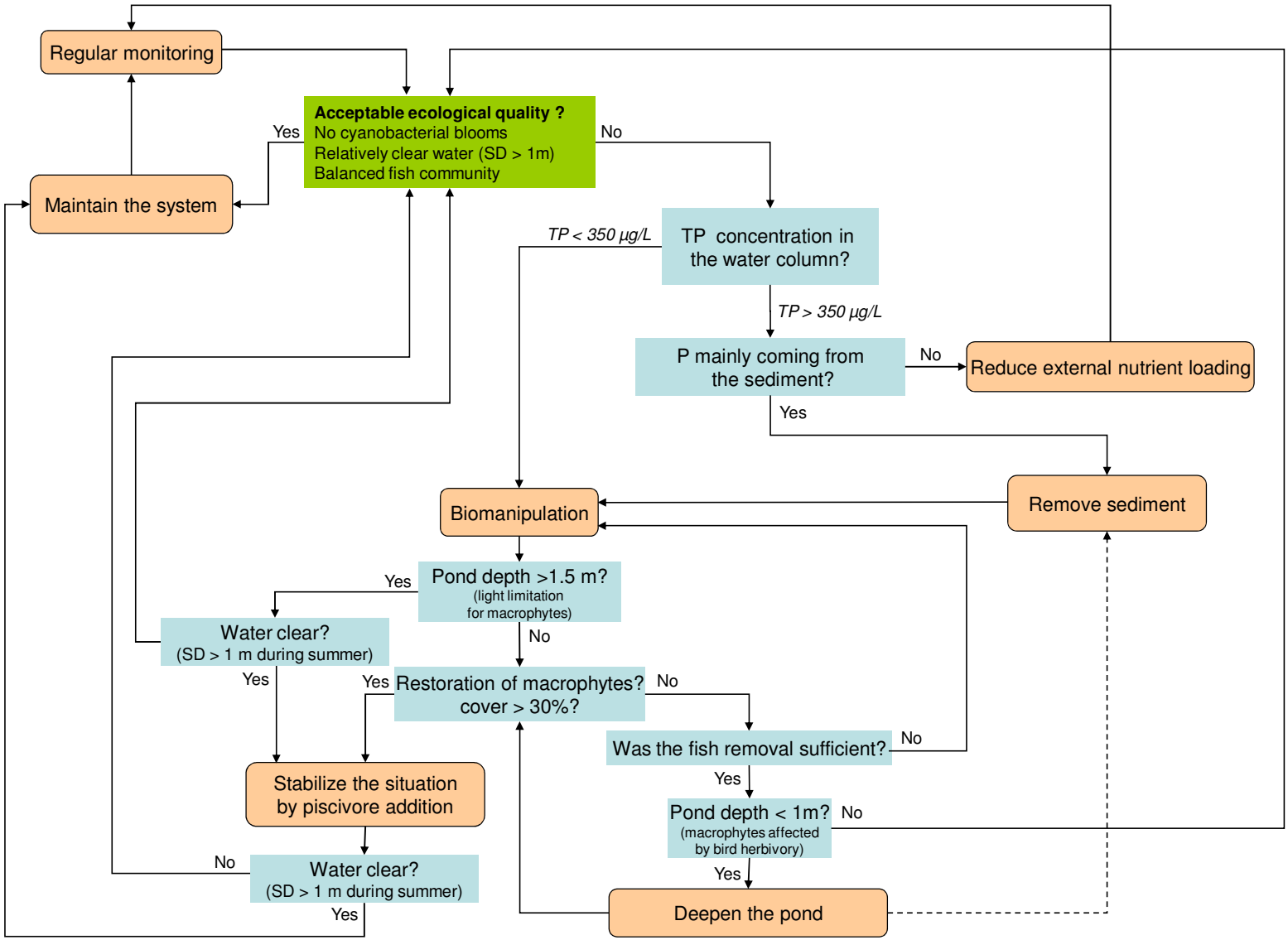

Figure 26 General decision tree for selecting restoration measures in Brussels ponds

6. References

APHA-AWWA-WEF. 1995. *Standard methods for the examination of water and wastewater.* American Public Health Association: Washington D.C.

Berg S, Jeppesen E, Søndergaard M. 1997. Pike (*Esox lucius* L.) stocking as a biomanipulation tool 1. Effects on the fish population in Lake Lyng, Denmark. *Hydrobiologia.* **342**/**343**: 311–318.

Biggs J, Williams P, Whitfield M, Nicolet P, Weatherby A. 2005. 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. *Aquatic Conservation-Marine and Freshwater Ecosystems.* **15**: 693-714.

Brönmark C, Hansson L-A. 1998. *The biology of lakes and ponds.* Oxford University Press: Oxford.

Carpenter SR, Kitchell JF, Hodgson JR. 1985. Cascading trophic interactions and lake productivity: fish predation and herbivory can regulate lake ecosystems. *Bioscience.* **35**: 634-639.

Casselman JM, Lewis CA. 1996. Habitat requirements of northern pike (Esox lucius). *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 161-174.

Craig JF. 1996. *Pike*: *Biology and exploitation (Fish & Fisheries Series).* Chapman & Hall: London.

De Backer S, Peretyatko A, Teissier S and Triest L. 2008. *Ecologische opvolging van het actief biologisch beheer van sommige vijvers beheerd door het B.I.M*. Brussels, APNA, VUB, 57p.

De Backer S, Peretyatko A, Teissier S and Triest L. 2008. *Ecologische evaluatie van het bepoten van snoek in de biologisch gemanipuleerde vijvers van het Brussels Hoofdstedelijk Gewest.* Brussels, APNA, VUB, 51p.

Hosper SH, Jagtman E. 1990. Biomanipulation additional to nutrient control for restoration of shallow lakes in The Netherlands. *Hydrobiologia.* **200**/**201**: 523-534.

Hunt BP, Carbine W F, 1951. Food of young pike, *Esox lucius* L., and associated fishes in Peterson's ditches, Houghton Lake, Michigan. *Trans. am. Fish. Soc.* **80**: 67–83.

Jeppesen E, Jensen JP, Kristensen P, Søndergaard M, Mortensen E, Sortkjaer O, Olrik K. 1990. Fish manipulation as a lake restoration tool in shallow, eutrophic, temperate lakes .2. Threshold levels, long-term stability and conclusions. *Hydrobiologia*. **200**: 219-227.

Kemp PF, Sherr BF, Sherr EB, Cole JJ. 1993. *Handbook of Methods in Aquatic Microbial Ecology.* Lewis Publishers: Boca Raton, FL.

Lammens EHHR. 1999. The central role of fish in lake restoration and managment. *Hydrobiologia*. **395**/**396**: 191-198.

Linton S, Goulder R. 2000. Botanical conservation value related to origin and management of ponds. *Aquatic Conservation-Marine and Freshwater Ecosystems. **10**: 77-91.*

McCune B, Mefford MJ. 1999. *Multivariate analysis of ecological data (PC-ORD for Windows, version 4.0).* MjM Softwater: Gleneden Beach, OR.

Meijer M-L, de Boois I, Scheffer M, Portielje R, Hosper H. 1999. Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies. *Hydrobiologia* 408/409: 13-30.

Moss B, Madgwick J, Phillips G. 1996. *A guide to the restoration of nutrient-enriched shallow lakes.* The Broads Authority: Norwich.

Moss B, Stephen D, Alvarez C, Becares E, Van de Bund W, Collings SE, Van Donk E, De Eyto E, Feldmann T, Fernandez-Alaez C, Fernandez-Alaez M, Franken RJM, Garcia-Criado F, Gross EM, Gyllstrom M, Hansson LA, Irvine K, Jarvalt A, Jensen JP, Jeppesen E, Kairesalo T, Kornijow R, Krause T, Kunnap H, Laas A, Lille E, Lorens B, Luup H, Miracle MR, Noges P, Noges T, Nykanen M, Ott I, Peczula W, Peeters E, Phillips G, Romo S, Russell V, Salujoe J, Scheffer M, Siewertsen K, Smal H, Tesch C, Timm H, Tuvikene L, Tonno I, Virro T, Vicente E, Wilson D. 2003. The determination of ecological status in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive. *Aquatic Conservation-Marine and Freshwater Ecosystems*. **13**: 507-549.

Meijer M-L, Jeppesen E, van Donk E, Moss, B, Scheffer M, Lammens E, van Nes E, van Berkum JA, de Jong GJ, Faafeng BA, Jensen JP. 1994. Long-term responses to fish-stock reduction in small shallow lakes: interpretation of five-year results of four biomanipulation cases in The Netherlands and Denmark. *Hydrobiologia.* **275**/**276**: 457-466.

Oertli B, Biggs J, Cereghino R, Grillas P, Joly P, Lachavanne JB. 2005. Conservation and monitoring of pond biodiversity: Introduction. *Aquatic Conservation-Marine and Freshwater Ecosystems.* **15**: 535-540.

Ozimek T, Gulati RD, van Donk E. 1990. Can macrophytes be useful in biomanipulation of lakes? The Lake Zwemlust example. *Hydrobiologia* **200**/**201:** 399-407, 1990.

Peretyatko A, Triest L. 2005. *Biomonitoring in the pond-river complex of the Woluwe catchment: a tool for management and restoration within the framework of the blue and green networks.* Brussels, APNA, VUB: 69p.

Peretyatko A, Triest L. 2006. *Bepaling van de ecologische status van sommige vijvers beheerd door de A.G.R. van het B.I.M.*. Brussels, APNA, VUB: 49p.

Peretyatko A, Symoens J-J, Triest L. 2007a. Impact of macrophytes on phytoplankton in eutrophic peri-urban ponds, implications for pond managment and restoration. *Belgian Journal of Botany*. **140**: 83-99.

Peretyatko A, Teissier S, Symoens JJ, Triest L. 2007b. Phytoplankton biomass and environmental factors over a gradient of clear to turbid peri-urban ponds. *Aquatic Conserv: Mar. Freshw. Ecosyst.* **17**: 584–601.

Reynolds CS. 1994. The ecological basis for the successful biomanipulation of aquatic communities. *Archiv für Hydrobiologie.* **130**: 1-33.

Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E. 1993. Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*. **8**: 275-279.

Scheffer M. 1998. *Ecology of shallow lakes.* Cluwer Academic Publishers: Dordrecht.

Scheffer M, Carpenter S, Foley JA, Folke C. and Walker B. 2001. Catastrophic shifts in ecosystems. *Nature* **413**: 591-596.

Skov C, Lousdal O, Johansen PH, Berg S. 2003. Piscivory of 0+ pike (*Esox lucius* L.) in a small eutrophic lake and its implication for biomanipulation. *Hydrobiologia.* **506–509**: 481–487.

Søndergaard M, Jeppesen E, Berg S. 1997. Pike (*Esox lucius* L.) stocking as a biomanipulation tool 2. Effects on lower trophic levels in Lake Lyng, Denmark. *Hydrobiologia* **342**/**343**: 319–325.

Søndergaard M, Moss B. 1998. Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes. In T*he structuring role of submerged macrophytes in lakes,* Jeppessen E, Søndergaard M, Christoffersen K (eds). Springer: New York; 115-133.

Søndergaard M, Jeppesen E, Lauridsen TL, Skov C, Van Nes EH, Roijackers R, Lammens E, Portielje E. 2007. Lake restoration: successes, failures and long-term effects. *Journal of Applied Ecology.* **44**: 1095-1105.

Teissier, S., A. Peretyatko, S. De Backer & L. Triest, 2010. Relargage des nutriments par les vases et classement de 25 étangs pour la priorité de curage., Brussels, APNA - Vrije Universiteit Brussel (VUB). 54p.

ter Braak CJF, Smilauer P. 1998. *Canoco reference manual and user's guide to Canoco for Windows: software for canonical community ordination (version 4).* Microcomputer Power: Ithaca.

UNEP, 2003. *Planning and management of lakes and reservoirs: an integrated approach to eutrophication*, in UNEP Newsletter.

Van Donk E, Grimm MP, Gulati RD, Klein Breteler JPG. 1990. Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia* **200**/**201:** 275-289.

van Donk E, van de Bund WJ. 2002. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany*. **72**: 261-274.

Wetzel GR, Likens EG. 1990. *Limnological analyses.* Sringer-Verlag: New York.

Willame R, Jurczak T, Iffly JF, Kull T, Meriluoto J, Hoffmann L. 2005. Distribution of hepatotoxic cyanobacterial blooms in Belgium and Luxembourg. *Hydrobiologia.* **551**: 99-117.

Williams P, Whitfield M, Biggs J, Bray S, Fox G, Nicolet P, Sear D. 2004. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in southern England. *Biological Conservation*. **115**: 329-341.