Beating the Blues by Floc & Lock

J.F.X. van Oosterhout

1. Complex systems are governed by their boundary conditions.
   (this thesis)
2. Phosphorus control suffices to mitigate blooms of cyanobacteria.
   (this thesis)
3. Ecology is a field science.
4. Water column mixing is expressed by the standard deviation estimated from the spatial distribution of the water quality variables
5. Correlations observed in field data are no formal proof of causalities.
6. Economic losses due to failing mitigation efforts should be part of the 'no regret' considerations.
7. A barn of roosters will never yield a golden egg.

Propositions belonging to the thesis, entitled:

Beating the Blues by Floc & Lock

Frank van Oosterhout
Wageningen, June 3rd 2022

Beating the Blues

by

Floc & Lock

J.F.X. van Oosterhout

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Beating the Blues by Floc & Lock

J.F.X. van Oosterhout

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To Charlotte Suzanne St-Martin

April 23, 1959 – December 18, 2011

Art historian, teacher of Dutch literature

Diving Instructor

We were young, playing crocodiles on the beach

I wrote a formula in the sand

You said: we' re having a holiday

Sharks in the ocean. An octopus in the Med

We talked about Leda, Giuditta

We made no vows, but promises

One unfulfilled

This one kept

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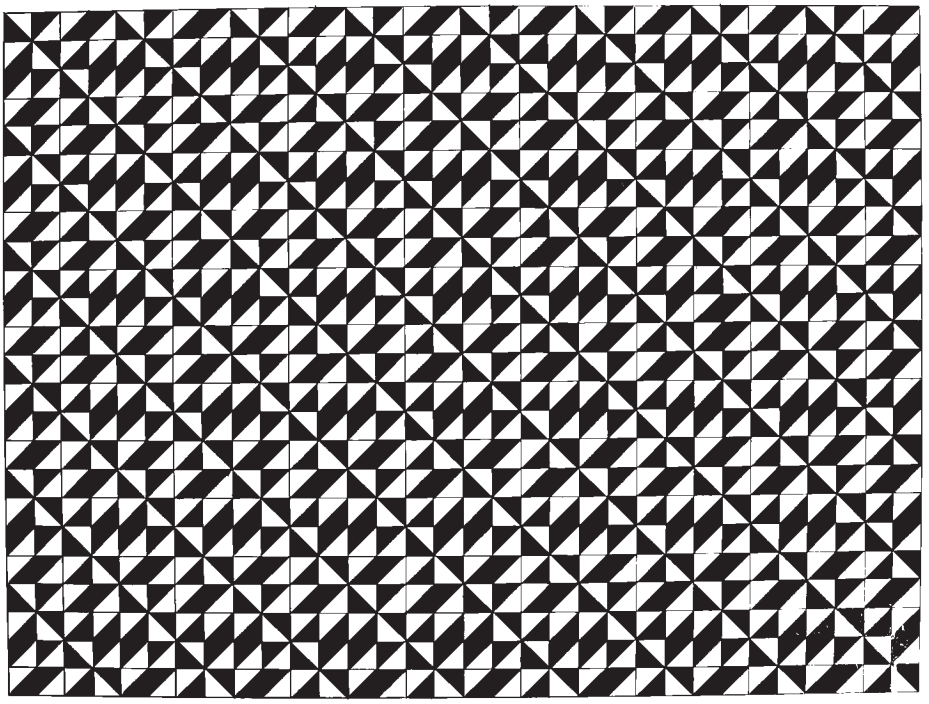

J.F.X. van Oosterhout

Celebrations of a four quarter beat, theme and variations 1.

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Chapter 1

General introduction

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1 General introduction

1.1 Fresh waters, eutrophication, cyanobacteria

Most of the world's natural fresh water is stored in ice caps, glaciers, permanent snow (68.7% of total freshwater) and as groundwater (30.1%), rivers only make up 0.006% of the freshwater, swamps 0.03% and lakes 0.26% (URL1). Despite they only comprise a low share of total freshwater, lakes fulfil important ecosystems services, such as providing water for drinking and industry, irrigation, recreation, aquaculture, amenity. However, many lakes are under anthropogenic stress of which eutrophication is most widespread (Downing 2014). If subjected to eutrophication – i.e. excessive nutrient loading, these waters are often struck by blooms and associated scums of cyanobacteria (Conley et al. 2009; Smith et al. 1999). Several species of cyanobacteria produce potent toxins which pose risks to human (Codd et al. 1999; Dittmann and Wiegand 2006), husbandry and wildlife (Chorus and Bartram 1999). Blooms and scums of cyanobacteria may cause swimming bans (Ibelings et al. 2012), drinking water shortages and result in economic losses from impaired ecosystem services (Dodds et al. 2008; Zhang et al. 2010). In addition, the blooms and scums cause turbid and foul odouring waters and, due to oxygen depletion overnight, can lead to fish kills (Conley et al. 2009; Paerl and Huisman 2008; Smith et al. 1999). Climate change may add to the selective advances cyanobacteria have over other phytoplankton species (Jöhnk et al. 2008; Paerl and Huisman 2008). Still rising nutrient inputs and increasing temperatures (climate change) are expected to interactively intensify eutrophication symptoms – such as blooms of cyanobacteria, in general (Moss et al. 2011). The future predictions are that more standing waters will be struck by more and more intensive blooms of cyanobacteria (Huisman et al. 2018; O'Neil et al. 2012; Paerl and Huisman 2008; Paerl and Paul 2012). Hence eutrophication is considered the most important water quality problem worldwide (Smith and Schindler 2009).

1.2 The need to mitigate

The need to meet up with the good water quality as required by the EU Water Framework Directive - WFD; EU (2000) and the EU Bathing Water Directive. - BWD EU (2006), stresses for safe and efficient mitigation methods. The past century several methods to either remediate nutrient enrichment or to control the densities of nuisance algae have seen the light. Among these methods are the use of algaecides (Jančula and Maršálek 2011), fish stock manipulation (Søndergaard et al. 2008), flushing (Hosper and Meyer 1986; Jagtman et al. 1992; Welch et al. 1972; Welch and Patmont 1980; Welch et al. 1992), artificial circulation (Gächter and Wehrli 1998; Visser et al. 1996), sludge dredging (Björk et al. 2010) and chemical dephosphatization (Cooke et al. 2005). A review of biomanipulation methods applied in the Netherlands lists more failures than successes due to no or insufficient in-lake sediment

phosphorus (P) release (Gulati and Van Donk 2002). A more recent review emphasised the need for in-lake measures in shallow and relatively small Dutch surface waters in which legacies and diffuse nutrient pollution cause recurrent cyanobacterial blooms (Lürling and Mucci 2020).

1.3 Phosphorus control

Based on their common occurrence (e.g. (Reynolds 1984)) it is impossible to permanently eradicate cyanobacteria from a body of water. Hence mitigation of blooms of cyanobacteria aims at imposing a limiting factor on their population growth. Applying Liebig's Law of the minimum (Liebig 1855), such limitation can be achieved by single nutrient reduction. Today's focus is on phosphorus control (Carpenter 2008; Schindler et al. 2008), because phosphorus can be lowered to limiting concentrations more easily than other nutrients (Golterman 1975). I.e. the bioavailability of phosphates can be reduced through the formation of poor to insoluble salts with aluminium, calcium, iron (Cooke et al. 2005) or lanthanum (Peterson et al. 1976), which is not the case with nitrogen (e.g. nitrate, nitrite and ammonium).

As nutrient enrichment is the prime cause of blooms of cyanobacteria in general, an obvious first step to mitigate this nuisance is to reduce the external nutrient inputs, thus, reduction of the external P-loading is considered a prerequisite to achieve good water quality (Hilt et al. 2006). While some lakes recovered after reducing these inputs - e.g. (Mehner et al. 2008), in others, recovery was not observed - e.g. (Björk 1972), or delayed for years to decades, because phosphorus was released (internal loading) from P-rich sediments (Fastner et al. 2016; Gulati and Van Donk 2002; Schindler and Hecky 2009; Søndergaard et al. 2001). After decades of uncontrolled external inflows, the sediments of most lakes are uploaded with legacy-P. Not dealing with the internal P-loading from the sediment is one of the main causes when an attempt to mitigate eutrophication fails (Gulati and Van Donk 2002).

Removing the nutrient-rich sediments by dredging is a straightforward measure to tackle sediment P release (Peterson 1982). However, dredging is relatively expensive (Lürling et al. 2016; Lürling et al. 2020a; Welch and Cooke 2005). Targeting the release of phosphorus (P) from the sediments with a P-fixative is a much cheaper option, which can be achieved by sediment capping (Hart et al. 2003) - either by a physical barrier using clay or sand, or a chemically active barrier. To this effect, aluminium-, calcium- and iron salts have been applied (e.g. (Cooke et al. 1993; 2005)), and recently solid-phase P sorbents (SPS) have gained interest (Spears et al. 2013a). SPS strip ortho-phosphate from the water column and after settling, act as an active barrier that reduces the P release from the sediment (Egemose et al. 2010; Gibbs et al. 2011; Ross and Cloete 2006; Spears et al. 2013a).

1.4 Curative interventions

In most cases where mitigation is needed, blooms (high densities) of cyanobacteria have already established. From the perspective of lake owners, this is a situation in which a curative intervention, a treatment that immediately relieves the nuisance, is wanted. Several curative methods are advocated, reviewed by Lürling and Mucci (2020), however many simply lack effect, have a too short-lived effect or come with toxic side effects, either from the applied compounds or from cell lysis of the toxic cyanobacteria.

During a bloom most of the water column phosphorus is present in phytoplankton, hence not targetable with a SPS. To capture all phosphorus in the water column, this particulate phosphorus fraction must first be forced through the dissolved ortho-phosphate phase. This can be achieved through enforced cell lysis using algaecides (e.g. Jančula and Maršálek (2011)) or by bringing the cells to the sediment where they can be decomposed. The latter can be achieved using a flocculent such as an aluminium salt, which will decrease turbidity within a few hours after being applied to the water column (Cooke et al. 2005), or a flocculent combined with a ballast to overcome the buoyancy of the cyanobacteria (Noyma et al. 2016; 2017; Pan et al. 2006a; Wang et al. 2012; Zou et al. 2006). Besides the removal of all phosphorus from the water column, the precipitation of all phytoplankton is a curative method which immediately relieves the nuisance caused by the bloom present. Subsequent burying the precipitated material under a SPS physically prevents the return of the bloom to the water column and, after senescence of the buried bloom, captures the released phosphates.

1.5 Floc & Lock

The combination of a flocculent (Floc) and a SPS (Lock) differs from classical lake restoration techniques using only iron- or aluminium salts (flocculants), in the sense that in Floc & Lock, the flocculent is used in a low dose to clear the water column of particulate-P in phytoplankton (see for a detailed review (Lürling et al. 2020b). While the SPS also gives the flocs ballast, its main purpose is to intercept the phosphates released from the precipitated material and underlying sediment. This technique was applied in Lake Rauwbraken (Tilburg, The Netherlands) in April 2008 using low dosage poly aluminium chloride (PAC) as a flocculent and the Lanthanum Modified Bentonite (LMB; Phoslock®), as a ballast and P-fixative. Soon after its application, the method was christened Floc & Lock (Lürling and van Oosterhout 2009). The success of 'Floc & Lock' is due to its instantaneous and safe removal of an existing bloom – resulting in water fit for human use and preventing its return for a prolonged period (more than 10 years) by removing the total phosphorus fraction from the water column and strongly reducing the release of phosphates from the sediment.

1.6 Thesis outline

This thesis focusses on the mitigation of blooms of cyanobacteria in Lake Rauwbraken. This mitigation was achieved after a thorough system analysis, which resulted in the Floc & Lock treatment. Despite the long history of studies regarding the mitigation of nuisance blooms of cyanobacteria, there are still several interesting ongoing debates. The overall debate is on single phosphorus control versus nitrogen control or dual reduction (Mucci et al. 2019; Paerl 2008; 2009; Schindler et al. 2008). Among the hottest of the topics are: phosphorus inputs versus internal loading, safe bloom removal, effectiveness and longevity of sediment capping using a SPS, and the fate of lanthanum as we chose the LMB.

Chapter 2 reports on the lake system analysis for Lake Rauwbraken during 2006-2007. Our results show that forty years of relative small external P loads built up a legacy P-pool in the sediment of the lake that directly fuelled the blooms of cyanobacteria, during 2006 - 2007 the lake was in a eutrophic - hypertrophic state.

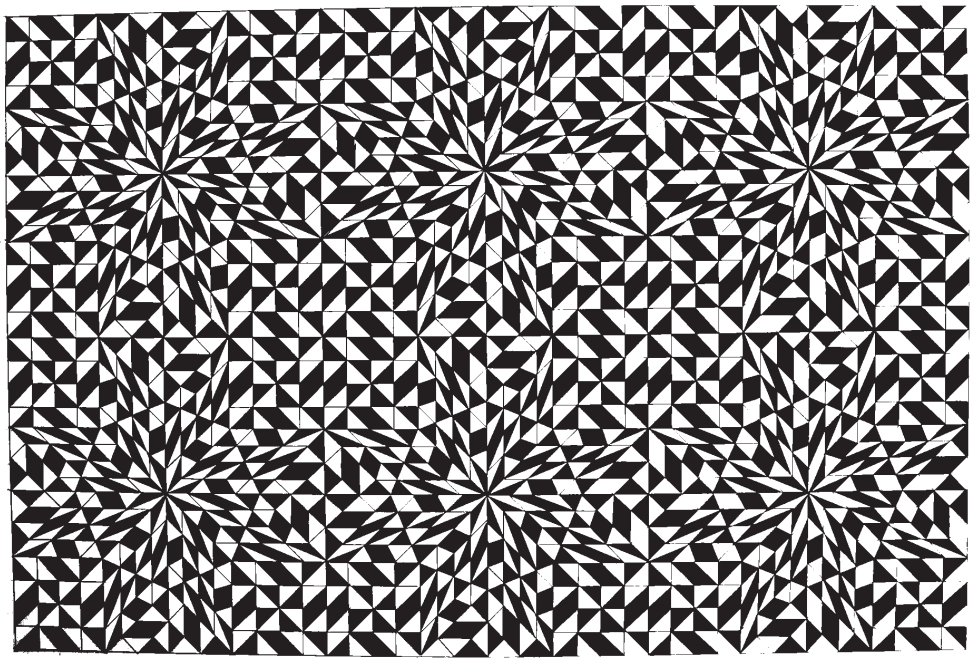
Chapter 3 investigates safe bloom removal using PAC as flocculent and the LMB as sinking weight, the LMB is investigated under laboratory conditions with respect to its effectiveness to bind filterable reactive phosphorus, possible release of nutrients, its effects on pH, electrical conductivity, oxygen saturation and turbidity (NTU). This chapter also investigates the effectiveness of LMB to reduce the growth of phytoplankton species (*Scenedesmus*, *Microcystis* and *Dolichospermum*) and the survival of the rotifer *Brachionus calyciflorus* in the presence of LMB. The results show that a bloom can be safely precipitated using poly aluminium chloride (PAC) with the LMB as sinking weight, the LMB seems promising as SPS.

Chapter 4 reports on the curative effect and direct effects of the Floc & Lock treatment in the field during 2008. The results show that Floc & Lock was a successful curative intervention, the lake was shifted to an oligo - mesotrophic state. A decline in the *Daphnia* population in the lake was observed which was investigated further in laboratory experiments. Here, results show that the effects of Floc & Lock on *Daphnia* were caused by grazing inhibition of flocs and clay, and very low food concentrations acting in synergy. The effects were temporarily, and *Daphnia* recovered from the treatment.

Chapter 5 investigates the effectiveness and the longevity of the treatment through sediment release experiments and 10 years (2008-2018) post-treatment monitoring. The results show an effective reduction in sediment P-release and a durable improvement in the water quality of the lake. Longer-term observation shows that the lake remained in a mesotrophic state, but also indicates that the lake is returning to a eutrophic state due to ongoing and increased external P-loads. A minor low toxic *Microcystis* scum that emerged from the sediment in 2011 is reported but could not lead to a bloom through proliferation due to the imposed P-limitation. The imposed 3 days swimming ban in relation to the Dutch Cyanobacteria Protocol is being discussed.

Chapter 6 investigates the bio-availability of lanthanum to marbled crayfish (*Procambarus fallax* f. *virginalis*) and the fate of lanthanum after the application in Lake Rauwbraken. While the results show that lanthanum is bioavailable to the marbled crayfish, no ill effects were detected. After the LMB application in Lake Rauwbraken, filterable and total lanthanum in the water column were elevated and remained so as compared to the pre-application concentrations. The lanthanum concentrations temporarily exceeded the Dutch maximum permissible concentrations. Lanthanum was taken up by submerged macrophytes. The LMB becomes attached to the submerged macrophytes and was present in chironomid larvae. Three years after the homogeneously application, the LMB was redistributed towards lower densities in the lake's sediment at shallow depths and higher densities at greater depths. Sediment core studies revealed that anaerobic conditions might increase La release into the overlying water column.

Chapter 7 brings the preceding chapters together in a general discussion. This chapter explains how trying to understand the worldwide problem of eutrophication and possible solutions inevitably run into a Gordian knot. Unravelling this knot may start by returning to simple semantics.



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Chapter 2

Lake Rauwbraken: system analysis

Parts of this chapter were published in Inland Waters:

Oosterhout, F. van, Yasseri, S., Noyma, N., Huszar, V., Manzi Marinho, M., Mucci, M. *Waajen G.* and M. Lürling, 2021. Assessing the long-term efficacy of internal loading management to control eutrophication in Lake Rauwbraken. Inland waters. In press.

2 Lake Rauwbraken: system analysis

2.1 Introduction

Lake Rauwbraken is a recreational lake in Berkel-Enschot (municipality of Tilburg, The Netherlands) that started to show clear signs of eutrophication from the beginning of the 21st century. In April 2004, a bloom of the Burgundy blood alga *Planktothrix rubescens* (cyanobacteria) coloured the lake red. The reoccurrence of blooms of *P. rubescens* and summer appearance of *Dolichospermum sp., Microcystis sp.* and *Woronichinia naegeliana* led to prolonged swimming bans in 2005 and 2007, the number of visitors dropped from 20,000 to 1,500 per year. The water quality problem in Lake Rauwbraken comprised of high frequent occurrences of scums and accumulations (George and Edwards 1976) of buoyancy-controlled cyanobacteria (Reynolds et al. 1987; Walsby et al. 1995), leading to extremely high chlorophyll-*a* concentrations in the bathing zone and violation of the EU bathing water requirements (EU 2006). The prospects for the lake as a recreation facility were not good, consequently a mitigation action had to be undertaken.

To find the most feasible method to mitigate the blooms of cyanobacteria in Lake Rauwbraken the lake was studied from January 2006 onwards. While the system analysis of the lake opened from a wide-angle, it soon focused on phosphorus control to limit the proliferation of cyanobacteria in the lake's water column.

This chapter describes the general lake features, the early signs of eutrophication and efforts to rehabilitate the ecology of the lake during 2000-2005; the water and nutrient balances for the lake are reported; water quality variables are reported for the period 2006-2008 before the Floc and Lock treatment. Our system analysis shows that, over 40 years of history, small dispersed sources built up a legacy phosphorus pool in the sediment of Lake Rauwbraken. The resulting internal sediment phosphorus loads directly fuelled the blooms of cyanobacteria. The results presented in this chapter set the baseline for the Floc & Lock treatment of lake Rauwbraken (**Chapter 4**) and our longer-term study (**Chapter 5)**. This chapter describes the status of the lake in text, and full statistics are given where appropriate in chapters 4 and 5.

2.2 Methods

2.2.1 Study site

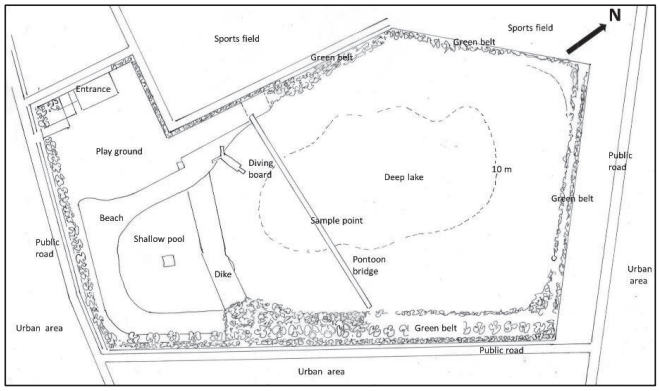


Figure 2.1 Map of Lake Rauwbraken and its premises (see text).

Lake Rauwbraken (Tilburg, The Netherlands) was dug in 1966 as an open mine to excavate sand (Fig. 2.1; Fig. 2.2A). As a result, a small (2.5 ha.) but deep (16 m, dimictic) lake with steep slopes of the banks was made (Fig. 2.2B). Originally, the lake was situated in an agricultural environment. Due to the expansion of the city of Tilburg, Lake Rauwbraken has become an urban lake since 2015. In 2000 the lake was adopted by the Dutch Underwater Parks Foundation (Stichting Nederlandse Onderwaterparken; SNOP).

A) B)

Frans van Boxtel

Figure 2.2 Sand excavation works in 1964 (A) and steep slopes of the banks (B) at Lake Rauwbraken.

The premises of the lake were surrounded by a fence, limiting the access of the public to the shores of the lake. Typical urban activities, like walking the dog, duck feeding, and sports fishing were excluded from the premises. Apart from swimming or diving, no other water-related activities were deployed – i.e. the lake is too small for recreational navigation. The lake had no surface water throughflow, water level fluctuates seasonally, with high water in late winter and low water in autumn (Fig. 2.3). The fluctuating water level in Lake Rauwbraken (see 2.3.3) – although not uncommon in similar isolated sand excavations (quarry lakes) in the province of Noord-Brabant (The Netherlands), was generally considered of extreme magnitude.

A) B)

Figure 2.3 High (A) and low (B) water levels in Lake Rauwbraken

Lake Rauwbraken was surrounded by a greenbelt (Fig. 2.4), which in 2000, had totally overgrown the banks of the lake - leaving the lake's helophyte zone in the shades (Fig. 2.4A). Organic litter from this vegetation fell on the banks of the lake, and through the lake's fluctuating water level, was directly transported into the lake (Fig. 2.4B, C).

A) B) C)

Figure 2.4 Greenbelts at Lake Rauwbraken, shading of helophyte zone (A), organic litter on the dry fallen bank (B), high water picks up organic material (C).

In 2000 the lake was void of both helophytes, and submerged macrophytes. While the helophytes had disappeared due to overgrowing of the shorelines by shrubs and trees (Fig. 2.5A,B), the submerged macrophytes (mainly *Elodea* sp.*)* had been eradicated by the introduction of grass carps *(Ctenopharyngodon idella*, Fig. 2.5C) in the nineteen eighties (Sportbedrijf Tilburg, private archive).

A) B) C)

Figure 2.5 Littoral zone (A) no submerged macrophytes, no helophytes (B), grass carp (C).

The lake was inhabited by perch (*Perca fluviatilis*), common roach (*Rutilus rutilus*), rudd (*Scardinius erythophthalmus*), pike (*Esox lucius*), bream (*Abramis brama*), carp (*Cyprinus carpio*) (2 specimens until October 2007, when one died (110 cm, 37 kg), tench (*Tinca tinca*) and the occasional eel (*Anguilla anguilla*) and spined loach (*Cobitis taenia)*. Since their introduction, most grass carp had died from senescence by 2005, leaving only a few surviving specimens reported occasionally by scuba divers.

The avifauna of Lake Rauwbraken comprised of eurasion coot (*Fulica atra*), mallard (*Anas platyrhynchos*), Great crested grebe (*Podiceps cristatus*) and black-headed gull *(Larus ridibundus)*; one or two not resident grey herons (*Ardea cinerea*) and a great cormorant (*Phalacrocorax carbo*) were daily foragers at the lake. The low number of water birds, especially geese, was explained by the absence of feeding as the premises of the lake (except for the bathing location) are not open to the public. Visiting geese were pro-actively kept away by handclapping until they left.

2.2.2 The early signs of eutrophication

Close inspection of the lake done by SNOP-outside the bathing season, revealed signs of eutrophication, i.e. turbid waters, foul odours and scums in spring and autumn. In the period 2000-2003, spring blooms were observed as low Secchi disk depths and poor visibility by divers. While these blooms did not result in swimming bans at the time, they did hamper the use of the lake as a dive site. As experienced by the divers, the in-water visibility was less than 0.5 m in the epilimnion, below the thermocline - using a torch, it was several meters. During the years 2000-2003, the springtime blooms stratified below the thermocline during April, resulting in increased Secchi disk depths and poor visibility for divers at greater depths (Fig. 2.6).

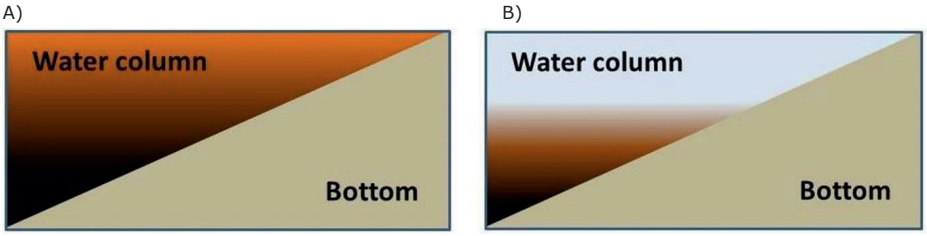


Figure 2.6 Water clarity as experienced by divers; brown = the *Planktothrix* bloom, black = clear water but shaded by the bloom, light blue = clear water; the spring situation with *Planktothrix* stratified at shallow depths (A), the summer situation with *Planktothrix* stratified at greater depths (B).

2.2.3 The first efforts to rehabilitate Lake Rauwbraken

The first efforts to rehabilitate the ecology of Lake Rauwbraken were made in 2001 through an attempt to remove the grass carps using a 300 × 8 m seine deployed from a boat. The fishing yielded a zero catch of grass carps and none of the notoriously known bottom-feeders fish like carp (*Cyprinus carpio*) or bream (*Abramis brama*).

A further effort was made by installing regular maintenance (2001-2003) of the green belts to reduce nutrient inflows through the deposition of organic material and provide sunlight to newly planted helophytes (Fig. 2.7). This maintenance started with an approximate 75% reduction of trees and shrubs from the banks of the lake directly adjacent to the water's edge and the introduction of helophytes - common reed (*Phragmites australis*), common cattail (*Typha sp.*) and tule (*Scirpus lacustris*). Each autumn-winter, the greenbelts and shores of the lake were cleared of leaf litter.

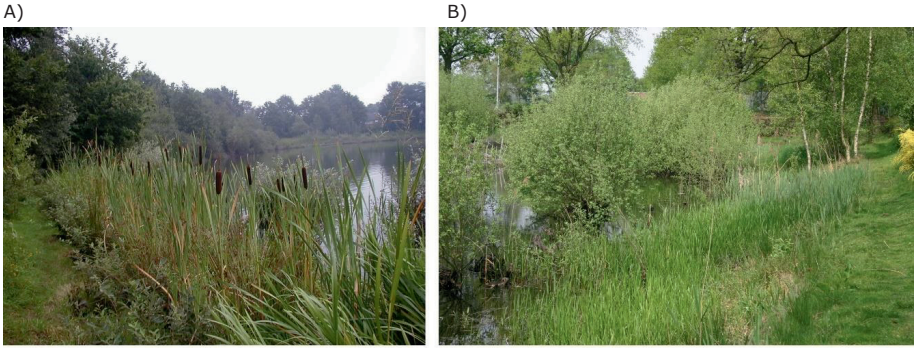


Figure 2.7 Greenbelts and helophyte zone after maintenance.

During 2000-2005, the number of grass carp reduced, and submerged vegetation sporadically returned (mainly *Elodea* sp.). In 2011, maintenance stopped (financial cuts) and in 2013 the coverage of trees and shrubs doubled. After 2011, graylag goose (*Anser anser*), Canada goose (*Branta c. canadensis*) and Egyptian goose (*Alopochen aegyptiacus*) became residents of the lake.

As the lake was void of submerged macrophytes, an attempt was made to reintroduce them. *Elodea* and *Nitella* were harvested from the shallow leisure pool, which due to a dike and dry falling during winter was free of crass carps. The plants were put in nets (Fig. 2.8A) and positioned at several depths (1 to 6 m) in the lake. Although these plants did grow, they disappeared within a month, which was attributed to grazing by the grass carp. Because crass carp were observed to feed on the newly planted helophytes, a bamboo fence (Fig. 2.8B) was built to prevent this grazing.

Figure 2.8 Introduction of plants (A), bamboo fence (B) and breakwater (C).

The lake's fluctuating water level (Fig. 2.3) not only hampered the growth of helophytes (desiccation at low water level), it also resulted in a large erosion zone (Hakanson 1977) along the shoreline of the lake. To prevent erosion of the banks and to protect the newly planted helophytes against wind generated waves (dominant wind comes from the southwest), a breakwater was built from fresh willow branches (Fig. 2.8C) and placed approximately 15 m outside the North-East running shoreline.

After planting common reed (*Phragmites australis*), common cattail (*Typha sp.*) and tule (*Scirpus lacustris*) in 2003, these helophytes expanded but slowly. The tule disappeared within two years after planting. Their slow growth can be attributed to grazing by grass carps and coot (*Fulica atra*), the large summertime drops in water level, local shading and too steep slopes to allow expansion of the root systems. The reeds (*Phragmites australis*) regularly showed signs of stress due to drought during summer, and submerged macrophytes (*Elodea* sp.) in the shallower parts of the littoral zone did not survive the summer season due to desiccation.

At the North-East running bank, the most favourable for helophytes, the reed bed (*Phragmites australis*) expanded less than 1 meter per year. During the years 2001 – 2005 the numbers of grass carps still present, were reduced from an approximate 20 down to 2. The removed specimens were sized 84-92 cm. In 2005 the first patches of submerged macrophytes (*Elodea*) survived the summer season outside the area protected by the bamboo fence – hence the fence was removed in early 2006. In April 2008 *Elodea* had re-established around the lake down to 4 meters depth.

Despite the early efforts to rehabilitate the lake's ecology, the signs of eutrophication prevailed. After the first massive bloom of *P. rubescens* in April 2004 (Fig. 2.9A), subsequent long-lasting water column blooms of *P. rubescens* reoccurred in each year. Especially the frequent occurrences of scums (Fig. 2.9B) and accumulations (Fig. 2.9C) of *P. rubescens* in the bathing zone led to extremely high chlorophyll-*a* concentrations causing a violation of the EU (2006) swimming water quality standards and prolonged closure of the beach bath in 2005 and 2007. Scums of toxic cyanobacteria also occurred in winter. *P. rubescens* scum material collected in Lake Rauwbraken on December 21st 2006 contained 752 µg g^{-1} total microcystins, due to the high cell density in the scum, this made up to a total microcystin concentration of more than 30 mg L^{-1} (Lürling and Faassen 2013), while a swimming ban is advisable at microcystin levels ≥ 20 µg (Schets et al. 2020). Meanwhile, other nuisance cyanobacteria species – belonging to the genera *Dolichospermum, Aphanizomenon*, *Microcystis* and *Woronichinia* –appeared causing irregular blooms.

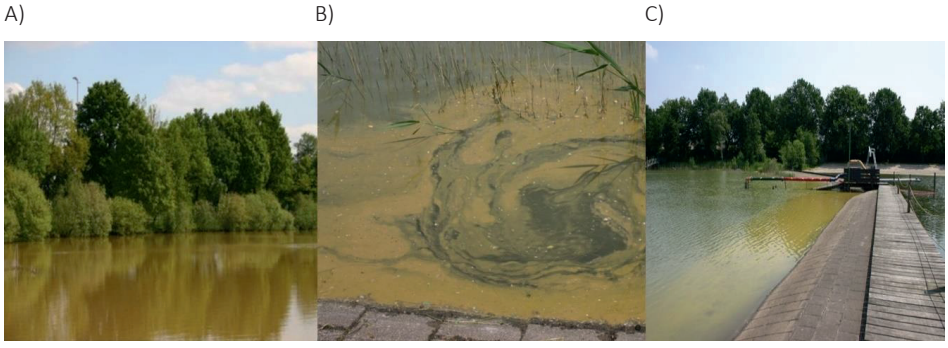


Figure 2.9 *Planktothrix rubescens* in Lake Rauwbraken in 2004: water column bloom (A); Scum (B); Accumulation (C).

The return of submerged macrophytes down to 4 meters depth by April 2008 was not enough to stop the blooms. No doubt, the standing crop of submerged macrophytes removed part of the nutrients from the lake's water column and sediments – making them unavailable to phytoplankton. This newly developed littoral zone only covered a small part of the lake, leaving its pelagic and profundal zones without vegetation to compete with cyanobacteria like *P. rubescens.* I.e. being able to flourish at depths beyond 4 m, *P. rubescens* was directly fuelled by the phosphorus released from the lake's sediment.

Considering the extreme seasonal water level fluctuations in Lake Rauwbraken, a large part of the submerged vegetation stood no chance to survive the growing season due to dry falling of the littoral zone and subsequent desiccation of the plants.

2.3 Lake system analysis

2.3.1 Water column sampling

Water samples were taken by pump and hose (Reich, 12 V Tauch pumpe) in the south-western centre of the lake (Fig. 2.10A, B). Sampling was done biweekly from 0 to 10 m depth at one-meter intervals (9 m at low water levels), from January 1, 2006 to April 20th, 2008.

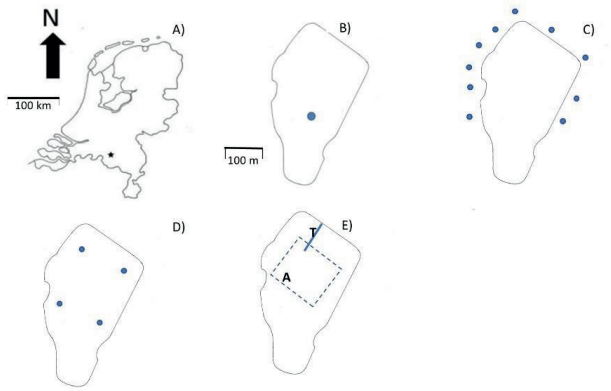


Figure 2.10 Location of Lake Rauwbraken in the Netherlands (A), map of the lake showing the water sampling point (B), sample points organic deposition (C), sediment traps (D), sediment sampling (T = transect, A = core sampling (E).

On-site, temperature, pH, dissolved oxygen concentrations (mg L^{-1}) and saturations (%) were measured using a WTW- multi 350i meter (WTW GmbH & Co. KG, Weilheim, Germany). Water transparency was determined using a 30 cm black and white Secchi-disk. To avoid oversampling during periods of turbid waters, daily Secchi-depths were first computed to weekly medians.

From each depth, five litre water samples were brought to the laboratory. Turbidity was measured using a HACH 2100P turbidity meter (Hach Nederland, Tiel, The Netherlands). Chlorophyll-a (Chl-a) concentrations were measured by hot ethanol extraction spectrophotometric (NNI 2006) with phaeopigment correction as described by Moed and Hallegraeff (1978) and/or with a PHYTO-PAM phytoplankton analyzer (HeinzWalz GmbH, Effeltrich, Germany) calibrated against the Dutch standard

(NEN 6520). The detection limit for the Dutch standard method is 5 µg L^{-1} (NEN, 6520), while for the PHYTO PAM it is below 0.5 µg L^{-1}.

PHYTO-PAM uses four different excitation wavelengths, allowing quantitative measurement of cyanobacteria (BLUE), green algae (GREEN), and diatoms (BROWN) (Heinz Walz GmbH, 2003). Therewith the percentage of cyanobacteria (%Cya) in the total chlorophyll-*a* signal could be estimated as:

$$\%\text{Cya} = 100\text{X} \frac{BLUE}{BLUE + GREEN + BROWN} \ (\%).$$

A detailed description of the capacity of the PHYTO-PAM to distinguish between these phytoplankton groups is given in Lürling et al. (2018).

To identify phyto- and zooplankton species by microscope 1 L water samples were fixated with Lugol, and left to sediment for 7 days, after which the supernatant was gently syphoned off, the remaining 90 mL was put in 100 mL PE bottles and adjusted to 100 mL using a concentrated formalin solution to achieve a final 4% formalin concentration. Phytoplankton species were identified using a Nikon light microscope, zooplankton was identified and counted under a dissecting microscope at 15× magnification.

Suspended solids were determined on GF/F filters through which an appropriate volume (0.5 – 2.0 L) of lake water was filtered. All filters were prepared by washing and subsequent filtration of 0.5 L demi water to remove loose fibres. Hereafter the filters were dried at 105 °C and pre-weighted. The total amount of suspended solids was determined after drying at 105°C, the organic suspended solids were determined as the loss on ignition at 520°C. Each series of determinations included a minimum of 3 control prepared filters.

Total phosphorus (TP; µg L^{-1}) and total nitrogen (TN, mg L^{-1}) were measured in unfiltered water samples, filterable reactive phosphorus (FRP; µg L^{-1}), nitrogen in ammonium (AMM; mg L^{-1}) and nitrite + nitrate (NN) were determined in filtered water samples (0.45 µm membrane filters, Whatman NC45, Whatman International Ltd., Maidstone, UK). The analyses were done by a Skalar SAN++ continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands) following the Dutch standard protocols (NNI 1986; 1990; 1997). TP and FRP were also determined by ICP-MS at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre, The Netherlands). All samples were stored at -16°C until further processing. Nutrient concentrations that fell below their level of detection were replaced by their respective values: TP 10 µg P L^{-1} for SAN++ and 6 µg P L^{-1} for ICP-MS; FRP 4 µg P L^{-1} for SAN++, 1 µg P L^{-1} for ICP-MS; TN 0.2 mg N L^{-1}; AMM 0.02 mg N L^{-1} and NN 0.01 mg N L^{-1}.

For the P balance, the water column P content (kg) is computed as $\mathrm{V\ x\ Mean}_{TP}$, in which V is the volume of the lake (Table 2.1), and $\mathrm{Mean}_{TP}$the mean water column TP concentration taken over all measurements during 2006-2007.

Table 2.1 Lake details.

Lake details	
Location	51°34'57.09"N; 5°07'54.30"E
Surface area (ha)	2.56
Mean depth (m)	8.1 m
Maximum depth (m)	16 m
Volume (m^3)	208,000

2.3.2 Bathymetry

Depth measurements were done with a Llowrange CHIRP and Broadband Sonar™ combined with navigation (Global Positioning System) from a boat. The contours of the lake were measured using the navigation system while walking at the water's edge. The measurements yield a total of 12.501 data points, which were computed into a grid in which each point represents 4 m^2 (using a spline routine proc g3grid in SAS 9.1). For each meter depth the surface area of the bottom (A_d (m^2)) at depth (d (m)) was computed by summation of all 4 m^2 squares at depth = d. The surface area of Lake Rauwbraken is 25,692 m^2.

2.3.3 Water Balance

The water level was measured regularly (mostly weekly) at a fixed point (11.049 m + asl), from 2003 until 2011. Lake Rauwbraken is isolated, fed by ground and rain water, precipitation and evaporation of water counterbalance each other. The water level fluctuates seasonally, following wider scale groundwater levels with high water in late winter and low water in autumn. Water balance calculations for Lake Rauwbraken were based on the equation given by Nõges (2005):

$I + P - E - O \pm \Delta V = 0$, in which I = Inflow (surface runoff, groundwater, surface water), P = Precipitation onto the water surface, E = Evaporation from the water surface, O = Outflow, ΔV = Change in storage during the period in question; all parameters in $m^3\ year^{-1}$.

Over eight years of measuring the mean difference between high and low water levels was 1.66 m (SD = 0.30, n = 8). With a 25,692 m^2 surface area this results in an estimated annual 42,649 m^3 change in water volume (storage).

Daily measurements of the precipitation and evaporation (Makkink 1960) at the meteorological station of Gilze-Rijen Airport (16 km distance from Lake Rauwbraken; 03 April 1987 – 03 April 2008) were used. Evaporation from open water was calculated using the relation between reference evapotranspiration according to Makkink and the open water evaporation according to Penman (Hooghart & Lablans, 1988). Precipitation was 760.6 mm $year^{-1}$, which equals 19,541 m^3 annually. Evaporation was estimated at 572.5 mm $year^{-1}$, which equals 14,709 m^3 annually yielding a precipitation surplus of 4,832 m^3 per year. The rest of the annual water input is assumed groundwater inflow (37817 m^3 per year). Since the water balance is based on data from 8 to 21 years, ΔV is set at 0.
The water balance is then:

Input (groundwater):	37,817 m^3 $year^{-1}$
Precipitation:	19,541 m^3 $year^{-1}$
Evaporation:	14,709 m^3 $year^{-1}$
Outflow (groundwater):	42,649 m^3 $year^{-1}$

2.3.4 Phosphorus (P)-balance

The P balance for Lake Rauwbraken was derived from internal loads (sediment P-release) and external loads through groundwater, organic deposition (leaf litter) from the green belts, precipitation, bathers and water birds. The internal P fluxes were estimated from sediment P release experiments (see 2.3.5 Sediment nutrient release) and sedimentation of P.

2.3.4.1 Phosphorus Outflow

As lake Rauwbraken has no surface water in- or outflow, the single loss of P from the lake is the loss of FRP to the groundwater during periods of declining groundwater level. $P_{out} = \frac{V}{A} \times [FRP]_{mean}$, in which P_{out} is the amount of P lost to the groundwater (scaled to mg P m^{-2} day^{-1}), V is the volume of water lost to the water table (m^3 $year^{-1}$), A the surface area of the lake (m^2) and $[FRP]_{mean}$ the mean FRP concentration during the pre-treatment period (scaled to mg m^{-3}). We assume that groundwater is not a vector for particulate P and that FRP is not bound during its passage through the lake bed.

2.3.4.2 Groundwater

Groundwater was sampled at a tube well, approximately 100 m from the lake. The well was sampled biweekly from December 12, 2005 to October 4, 2007, nutrients were determined after filtration through a Whatman NC45 0.45 µm membrane filter (*see 2.3.1* Water column sampling). Nutrient inflow through groundwater is computed as: $M_{in} = \frac{V}{A} \times C_{groundwater}$, in which M_{in} is the amount of nutrients (scaled

to mg P m^{-2} day^{-1}), V is the volume of groundwater coming in (m^3 $year^{-1}$), A the surface area of the lake (m^2) and $C_{groundwater}$ is the concentration of nutrients (scaled to mg m^{-3}).

2.3.4.3 Organic litter

Organic litter from the lake's greenbelts is directly transported into the lake. Maintenance started with a reduction to one quarter of the original coverage of trees and bushes directly adjacent to the water's edge. Regular maintenance removed 90% of the organic litter, meaning 10% still added to the external loads. The weight of the removed material was obtained from the invoice of the container. In December 2005, 10 random samples (Fig. 2.11C) of organic material were taken from the collected material. The ratio of dry to wet weight material was determined through water loss on drying at 105°C of the complete samples. The dry samples were grinded and stored frozen (-16°C) until further processing. An approximate 20 mg of this material was digested with the combination of Ultrex HNO_3 (65%) and H_2O_2 (30%) (Van Griethuysen et al. 2004). The digestions yielded 2 mL digest of which a 1 mL subsample was diluted 10-fold in which the P concentration was determined by ICP-MS at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre, The Netherlands). The mean P content of the organic litter is calculated in g P kg^{-1} dry material from the 10 samples. The amount of dry material in the refuge container was estimated from the fraction of dry material in the samples and multiplying this by its P content yield the total amount of P removed by maintenance. The maintenance of the greenbelts reduced the annual input of organic litter to one quarter of its historical values. Hence the historical input (i.e. before maintenance was installed in 2000) is computed by multiplying the removed amount of P by 4.

2.3.4.4 Atmospheric deposition

The P concentration in rainwater, which includes inorganic atmospheric deposition, was estimated at 0.031 mg L^{-1} (Waajen et al. 2016a). The annual 760.6 l m^{-2} precipitation of P yielded an annual 0.59 kg P (0.065 mg P m^{-2} day^{-1}) input. Dry deposition for P was neglected.

2.3.4.5 Swimmers

Swimmers or bathers contribute 0.094 g P per day to the water body (Dokulil 2014), referring to (Schulz 1981). With an average of 20,000 swimmer days per year in Lake Rauwbraken this yields an annual input of 1.88 kg P (0.20 mg P m^{-2} day^{-1}). The number of bathers was obtained from registration (daily or season ticket) at entry.

2.3.4.6 Water birds

The model Waterbirds version 1.1 (Hahn et al. 2007; 2008) was used to estimate the P influx via birds. Observations of water birds visiting the lake, estimated numbers, duration of visits, and diet were used as model input (Table 2.2).

Aiming at an overall nutrient balance, we need to distinguish water birds that attribute to nutrient inputs from those that attribute to the output. Until 2011, the main inputs comprised of eurasion coot (*Fulica atra*), mallard (*Anas platyrhynchos*) and black-headed gull (*Larus ridibundus*) (Table 2.2). Grey heron and great cormorant may be a vector of nutrients out of the lake. Assuming the birds transport all nutrients harvested at a lake, the Waterbirds 1.1 model estimates that one grey heron may contribute 0.854 kg P $year^{-1}$ and 3.083 kg N $year^{-1}$ and one Great cormorant may contribute 1.162 kg P and 4.195 kg N to the nutrient balance (either in or output) of a lake. For these birds we assume a neutral contribution to the lake's external nutrient balance (Table 2.2).

Table 2.2 Estimated numbers of water birds visiting, duration of visit, the season of visit and diet yielding annual P and N inputs (kg $year^{-1}$; modelled using Water birds 1.1), # = number.

Bird species	#	when	#days	diet	P (kg $year^{-1}$)	N (kg $year^{-1}$)
Black-headed gull	50	Whole year	365	mix	5.485	27.800
Mallard	10	Whole year	365	grass	0.151	1.848
Mallard	100	Autumn/Winter	183	grass	1.134	13.880
Total					6.770	43.328
Additional After 2011						
Canada goose	40	Autumn/Winter	50	beet	0.2639	1.394
Greylag goose	50	Autumn/Winter	50	beet	0.2552	1.348
Egyptian goose	12	Whole year	365	grass	0.5863	7.179
Total					1.1054	9.921

2.3.4.7 Sedimentation

Sediment traps (Fig. 2.10D) were deployed for three periods of four weeks each. The traps were retrieved on July 7th 2007, August 9th 2007 and March 19th 2008. The traps (PVC pipe) were 40 cm long and had a surface area of 514.5 mm^2. The traps were deployed in a series of 4 mounted on a rig, but as the amounts of collected material were little the contents of four traps per location were pooled. The

entrances of all traps were 1 m above the bottom at 10 m depth. The collected material was centrifuged at 5,000 rpm, decanted and freeze dried. The digestion was done according to Griethuysen et al. (2004). The amounts of phosphorus sedimented (mg m^{-2} per 4 weeks) are computed as: $2x\frac{\frac{M_0}{M}xV\,[P]}{A}$ (mg m^{-2}), in which M_0 (mg) is the total amount of material collected per location, M (mg) the amount of sediment used in the digestion, [P] the phosphorus concentration (mg L^{-1}) in the diluted destruate of volume (V; L) and A (m^2) the total surface area of the 4 traps. The factor 2 originates from the fact that a 1 mL subsample (of a total 2 mL sample) was analysed by ICP-MS (Chemical Biological Soil Laboratory of the Department of Soil Sciences, Wageningen University).

2.3.5 Sediment nutrient release

Sediment nutrient release was determined on intact cores sampled with an Uwitec core sampler (Uwitec, Mondsee, Austria) at depths greater than 10 m (Fig. 2.10E). The release was measured under aerobic conditions in six cores drilled November 29th, 2005 (a) and five cores taken on December 13th, 2005 (b); under anaerobic conditions in five cores drilled April 13th, 2008. Experiments 2005 a/b were done under aerobic conditions only because they were done on site where it was not feasible to work under anaerobic conditions. The 2008 experiments were done at the Aquatic Ecology and Water Quality Management laboratory of Wageningen University. Note: the 2008 cores were part of an effectiveness study which included cores sampled on June 19th, 2008 (post-treatment; Chapter 5).

For experiment 2008, the cores (with their original overlying water) were stored for approximate two years at 4°C in the dark to test both the effectiveness and durability of the treatment. We followed this procedure to test the effectiveness and durability without possible disturbance – e.g. wind or fish induced resuspension, or new sediment being formed on top of the treated sediment. An increase in FRP fluxes in sediment cores taken over time cannot separate LMB efficacy from ongoing external P load (see for instance, figure 10 in Waajen et al. (2016a)). Inasmuch as the ongoing external load, and other influences may impact the net efficacy of the treatment, in 2011 and 2013 cores were taken to reveal the long-term effect of the treatment in the lake while being subject to possible disturbance and new sediment formed.

Depending on the aimed conditions, the experiments started by siphoning off the overlying water and replacing it with either oxygen free or oxygenated water (Table 2.3). The cores were incubated for one day where after the procedure was repeated, which then was done for four consecutive days.

Table 2.3 Sediment release experiments, Experiment = year the cores were sampled, treatment = pre- or post- the Floc & Lock treatment in Lake Rauwbraken, Cores = number of replicates, water Demi = demineralised water, Millipore = Millipore water (Billerica, MA, USA), Volume = volume of water replaced, Incubation time = duration of each incubation, Incubations = number of incubations, condition = oxygen condition maintained during the experiment, T = temperature, controls = number of controls.

Experiment	2005 a/b	2008	2011	2013
Treatment	pre-	pre-/post-	post-	post-
Cores	6/6	5/5	3/3	4/4
Water	Demi	Millipore	Millipore	Millipore
Volume	0.2 l	all overlying water	all overlying water	all overlying water
Incubation time (days)	1	1	1	7
Incubations	4	4	4	5
Condition	aerobic	anaerobic	aerobic/ anaerobic	aerobic/ anaerobic
T (°C)	20	7	7	18
Controls	6	4	12	3

Oxygen free Millipore water was prepared by bubbling with nitrogen gas until the oxygen concentration was below 0.04 mg L^{-1}. Oxygenated Millipore water was prepared by aeration until 100% dissolved oxygen saturation. From the prepared water aliquots were kept as controls to check for background nutrients. Samples from the one day incubations and controls were filtered through a Whatman NC45 0.45 µm membrane filter and analysed on nutrients (see *2.3.1* Water column sampling). Dissolved inorganic nitrogen (DIN) is computed as the sum of AMM and NN. We choose to infer on DIN, rather than on AMM and NN separately, because in this experiment, AMM may be processed into NN and vice versa (Francis et al. 2007).

For each core and incubation period, the total amount of FRP and DIN released (R_{ij}) was computed by: $R_{ij}(mg) = [X_{ij}] \times V_i$, with $[X_{ij}]$ the FRP or DIN concentration (mg L^{-1}) in core i during incubation period j, V_i the volume of water in the core i. With R_i (mg) = SUM(R_{ij}) the total amount of FRP (or DIN) released during the experiment in core i. For each core i, the 24 h sediment release rates were computed

as: $R24_{i.}$ (mg m^{-2} day^{-1}) = $\frac{24x60}{A}\ \frac{R_i}{t}$, with A = surface area of the sediment in the core (0.00181 m^2) and t = total duration (min) of the experiment.

The annual internal nutrient loadings from the sediment are comprised of one part released during thermal stratification in the anaerobic hypolimnion (I_{hyp}), one part that is released in the aerobic epilimnion (I_{epi}) and one part that is released from the whole lake's sediment surface under aerobic condition during the mixed period (I_{mix}). The internal loading is computed as the weighed summation of the I_{hyp}, I_{epi} and I_{mix}. With R_{mean} (mg m^{-2} day^{-1}) is the mean of the $R24_i$. the I_{hyp}, I_{epi} and I_{mix} are computed as: $R_{mean} \times N_{day} \times A$ (kg), N_{day} is 205 days for stratified or 160 days for mixed, A is the sediment surface area (for I_{hyp} A = 15924 m^2, I_{epi} A = 9768 m^2, for I_{mix} A = 25692 m^2). The total annual internal load (kg P) is calculated by summation of I_{hyp}, I_{epi} and I_{mix}; dividing by 365 (days) × 25692 (m^2) will give the weighed summation. For 2008 post-treatment, we measured FRP release under anaerobic conditions only, which reflects a worst-case scenario.

2.3.5.1 Thermal stratification

From January 11, 2003 until December 28, 2010 (number of sample days = 353), SNOP measured the water temperature weekly at sample point 1 (Fig. 2.10B), at every m depth from 0 m to 10 or 9 m depth depending on the water level. Thermal stratification was defined as a difference of 1°C between two consecutive depths. Lake Rauwbraken was stratified for 205 days (summer stratification) and mixed for 160 days each year. The thermocline occurred at 7 m depth.

2.3.6 Sediment phosphorus content

The sediment phosphorus content was determined in the top 2 cm subsamples of 6 sediment cores sampled single fold along a transect (April 13th, 2008) using a Uwitec core sampler (Fig. 2.10E). In two additional cores, the sediment phosphorus content was determined in all 2 cm subsamples down to 16 cm in de sediment. The sediment samples were stored frozen (-16°C) until further processing. From the subsamples, an approximate 1 g wet sediment was subjected to the fractionated phosphorus extraction according to Psenner et al. (1984). From the total scheme the 'Water-soluble phosphorus' (extracted in oxygen-free nanopure water), 'Reductant soluble phosphorus' (extracted in 0.11 M $Na_2S_2O_4$/0.11M $NaHCO_3$) and the humic-bound/organic phosphorus (computed as the difference between the total and filterable phosphorus fraction extracted with 1 M NaOH) were considered as releasable phosphorus – e.g. (de Vicente et al. 2008; Reitzel 2005). The total phosphorus content of the sediment was determined including all fractions from the scheme. We report the sediment phosphorus content as mg P per kg sediment wet weight (WW). To compute this, we use the ratio of dry to wet weight material (f_{dry}), which is determined through water loss on drying at 105°C on separate aliquots from the subsamples.

2.4 Results

2.4.1 Water column

In the period January 2006 – April 2008 (pre-intervention), the mean chlorophyll-a concentration in Lake Rauwbraken was 16.5 µg L^{-1} (SD = 32.4, n = 706). Most of the biomass followed thermal stratification in Lake Rauwbraken, which starts between week 11-15 (end of March, beginning of April) and ends in week 41-47 (mid of October, end of November). This pattern is most obvious in the 2007 chlorophyll-a concentrations (Fig. 2.11). Low Secchi-disk depths were observed by the end of winter and early spring (Fig. 2.12) when shallow spring blooms of *P. rubescens* occurred. The relative higher Secchi-disk depths were observed during late spring and early summer when the *P. rubescens* blooms stratified in the metalimnion. During the years 2000 – 2003, the lowest Secchi-disk depths (dropping below the red 1 m line) occurred shortly before the opening of the bathing season (Fig. 2.12). But water clarity quickly improved due to the stratification of *P. rubescens*. In 2004 the stratification of *P. rubescens* had shifted into the bathing season for two weeks, and in 2005 this happened even later. In the pre-intervention period, towards the end of the bathing season (September), other cyanobacteria, such as *Dolichospermum* sp., *Aphanizomenon* sp., *Microcystis aeruginosa* and *Woronichinia naegeliana*, became more abundant throughout the water column and were observed occasionally each year in late summer scums (as verified by microscope on fresh samples). The contributions of cyanobacteria to the total chlorophyll-a concentration ranged 0 – 100%, with summer means of 82% (SD = 8%; n = 54) in 2006 and 44% (SD = 26%; n = 77) in 2007.

Mean Secchi-depth was 3.5 m (SD = 1.6 m, n = 89), turbidity was 5.4 NTU (SD = 7.5 NTU, n = 557). As with chlorophyll-a (with peaks over 200 µg L^{-1}), high turbidity (up to 51 NTU) occurred in the deep layer. The mean inorganic and organic suspended solids concentrations were 1.1 mg L^{-1} (SD = 1.0 mg L^{-1}, n = 426) and 3.0 mg L^{-1} (SD = 3.8 mg L^{-1}, n = 426).

Mean TP was 134 µg L^{-1} (SD = 132 µg L^{-1} , n = 363), TN was 0.96 mg L^{-1} (SD = 0.99 mg L^{-1}, n = 303), mean FRP concentration was 20 µg L^{-1} (SD = 50 µg L^{-1}, n = 436) , AMM was 0.20 mg L^{-1} (SD = 0.37 mg L^{-1}, n = 452) and NN was 0.08 mg L^{-1} (0.12 mg L^{-1} , n = 450). The mean hypolimnic (months June to September, 7-10 m depth) oxygen concentration was 0.86 mg L^{-1} (SD = 1.72 mg L^{-1}, n = 143), mean saturation was 5% (SD = 15%, n = 119).

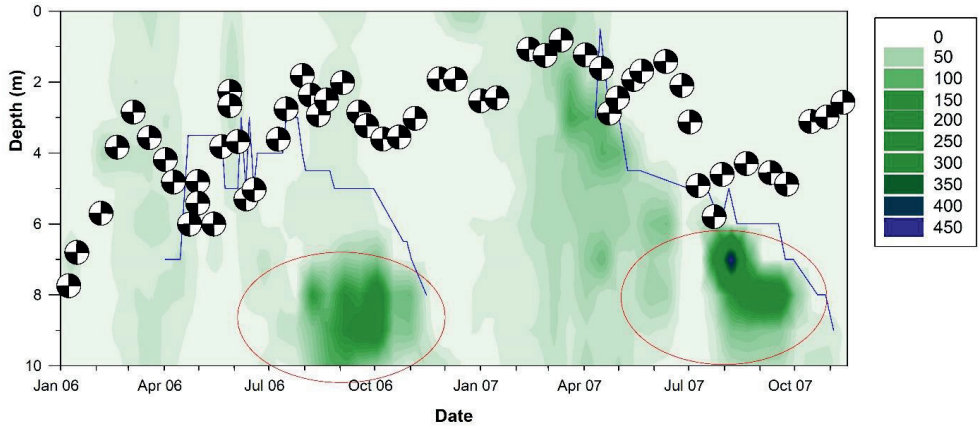


Figure 2.11 Contour plot of water column chlorophyll-a concentrations (µg L^{-1}), Secchi disk depth (black and white quartered circles), thermocline (blue line), and low oxygen - anaerobic depths (red encircled).

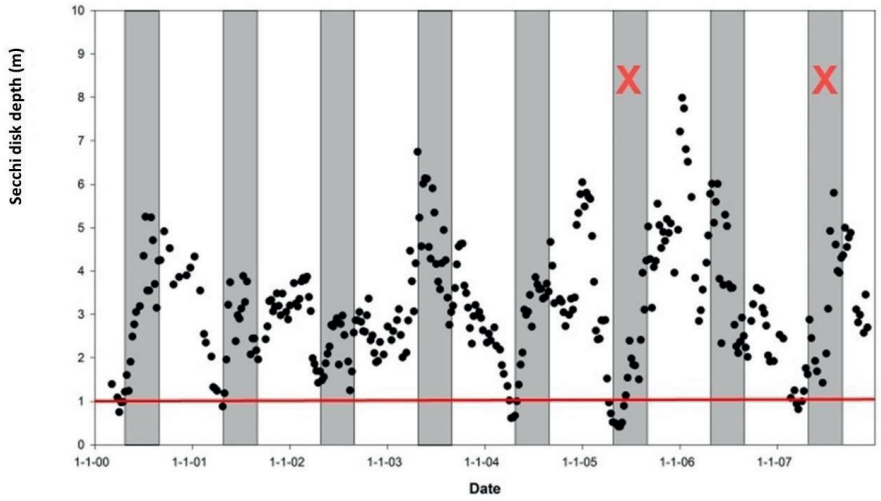


Figure 2.12 The course of Secchi disk depth (m) from 2000 to 2007 (2000-2005 data provided by SNOP), grey bars indicate bathing seasons (May –august), red X indicate years with a swimming ban, the red line indicates 1 m Secchi disk depth.

2.4.2 Sediment nutrient release and internal loading

Experiment 2005a, b yielded a mean FRP release of 2.4 mg m^{-2} day^{-1} (SD = 2.7 mg m^{-2} day^{-1} , n = 6) and 1.3 mg m^{-2} day^{-1} (SD = 2.0 mg m^{-2} day^{-1}, n = 6) , respectively; the mean release of DIN was 31.2 mg m^{-2} day^{-1} (SD = 6.8 mg m^{-2} day^{-1}, n = 6) and 72.9 mg m^{-2} day^{-1} (SD = 11.6 mg m^{-2} day^{-1}, n = 6) of which 82 to 92% was AMM. In experiment 2008 (after 2 years incubation), the FRP release under anaerobic

conditions was 15.1 mg m^{-2} day^{-1} (SD = 5.4 mg m^{-2} day^{-1}, n = 5); the release of DIN was much higher than in experiments 2005, i.e. 126.3 mg m^{-2} day^{-1} (29.1 mg m^{-2} day^{-1}, n = 5). Estimated for the whole lake, the internal P load was 6.82 mg m^{-2} $day^{-1,}$ the internal DIN release was 77.9 mg m^{-2} day^{-1}.

2.4.3 Groundwater FRP and DIN loads

The mean groundwater FRP concentration was 50 µg L^{-1} (n = 19, SD = 46 µg L^{-1}). For DIN it was 19.95 mg L^{-1} (SD = 3.24 mg L^{-1}, n = 12) mg L^{-1}, of which 2% was AMM. Based on the annual 37,817 m^3 inflow of ground water, the FRP inflow approximates 0.2 mg m^{-2} day^{-1}. The DIN inflow approximates 82.2 mg m^{-2} day^{-1}.

The mean FRP concentration in Lake Rauwbraken was 20 µg L^{-1} which may result in a FRP outflow of 0.09 mg m^{-2} day^{-1} based on the fluctuating water level (outflow 42,649 m^3).

2.4.4 Phosphorus from organic deposition

The organic material contained 0.59 g P kg^{-1}. In 2005, 1,244 kg (dry weight) organic material was removed. This was 90% of the total production, which thus was 1382 kg $year^{-1}$, meaning 138 kg was not removed. As no maintenance was done before 2000, the estimated historical dry deposition of organic material from the greenbelts is 5529 kg dry weight per year. The historic load was 0.35 mg P m^{-2} day^{-1}, which was reduced to 0.01 mg P m^{-2} day^{-1} by maintenance.

2.4.5 Sedimentation of phosphorus

Taken over all dates and locations, the mean sedimentation of phosphorus was 3.3 mg m^{-2} day^{-1} (range in individual traps 0.7 – 8.1 mg m^{-2} day^{-1}).

2.4.6 Phosphorus Balance

The total P load was 8.03 mg P m^{-2} day^{-1}. This load comprised of 6.82 mg P m^{-2} day^{-1} (85%) internal load and 1.21 mg P m^{-2} day^{-1} (15%) external load. With an outflow of 0.09 mg P m^{-2} day^{-1} as loss to the ground water, 1.12 mg P m^{-2} day^{-1} remained in the lake.

2.4.7 Macrophytes

Before 2004, no submerged macrophytes were observed. Following the reduction of grass carp, the macrophytes returned. In 2004, single specimens of *Elodea* sp. were observed, but these never survived the growing season. At the shallow sites, the plants were grazed by coots or died because of the falling water level. In 2005, the first patch of *Elodea* (approximately 1 m^2) survived the summer season. In April 2008, *Elodea* had re-established around the lake down to 4 meters depth.

2.4.8 Sediment Phosphorus Content

The phosphorus content in the top 2 cm of the sediment increases from 178 mg P kg^{-1} WW to 1,156 mg P kg^{-1} WW with distance from the lake's shore (Fig. 2.13), which also means with sample depth. On average 24% (SD = 2.0%, n = 6) of this phosphorus is releasable phosphorus.

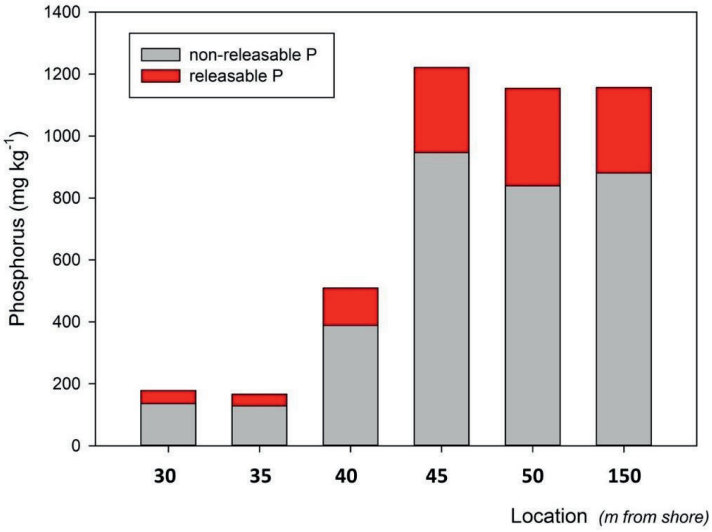


Figure 2.13 Sediment phosphorus (P) content (mg P kg^{-1} wet weight).

2.5 Discussion

2.5.1 The eutrophication of Lake Rauwbraken

Our system analysis revealed that in Lake Rauwbraken, the total P-load (8.03 mg P m^{-2} day^{-1}) to the water column comprised of a small external P-load (1.21 mg P m^{-2} day^{-1}) and a large internal P-load (6.82 mg P m^{-2} day^{-1}). The internal P-load was 85% of the total P-load to the lake. The bigger part (77%) of the internal P-load was released during thermal stratification in the anaerobic hypolimnion. During the mixed period (aerobic conditions), 15% of the annual total internal P load was released. The aerobic 1.2 mg P m^{-2} day^{-1} internal load is small relative to the load during thermal stratification, but it is the same as the total external P-load. Hence, not one to be neglected. In a recent study, Tammeorg et al. (2017) pointed out that a substantial part of the internal P loading can be associated with the aerobic release. From the external P-load, the 0.7 mg P m^{-2} day^{-1} by water birds was the largest. The reduction of the P-load by maintenance (leaf litter, 0.35 mg P m^{-2} day^{-1} to 0.01 mg P m^{-2} day^{-1}) meant only a small achievement on the external P-load, i.e. a reduction from 1.55 mg P m^{-2} day^{-1} to 1.21 mg P m^{-2} day^{-1}. For a recreational lake, the contribution of bathers (urine) is suspected to be a possible important source of nutrients (Dokulil 2014). We calculated a contribution of 0.2 mg P m^{-2} day^{-1} by swimmers, which for Lake Rauwbraken is the same as the contribution by groundwater (0.2 mg P m^{-2} day^{-1}).

While one needs to take into account the size, depth and water residence time for a specific lake, the order of magnitude of external P loads can be much higher than observed in Lake Rauwbraken if sewer effluents are involved. For Rostherne Mere, Carvalho et al. (1995) calculated 12.8 mg P m^{-2} day^{-1} (46.6 kg ha^{-1} $year^{-1}$), and in the case of Lake Søbygaard, Søndergaard et al. (1999) found up to 82.2 mg P m^{-2} day^{-1} (300 kg P ha^{-1} $year^{-1}$). For Lake Mere, Beklioglu et al. (1999) calculated 71.2 mg P m^{-2} day^{-1} (260 kg P ha^{-1} $year^{-1}$). Therefore, we feel safe to say that the external P loads to Lake Rauwbraken were small as compared to lakes with heavy P loads.

Small as the external P loads may be, 1.12 mg P m^{-2} day^{-1} remained in the lake. This is one-third of the measured P sedimentation (3.3 mg m^{-2} day^{-1}), which is affected by the internal load leading to higher TP in the water column than solely expected from external sources. The measured P sedimentation is probably also affected by diver induced sediment resuspension (Hal and Lürling 2004). Evidently, legacy P accumulated in the lake's sediment (up to 1156 mg P kg^{-1} WW) and resulted in the internal load of FRP. The fact that the lake did not respond to a reduction in external P-loading by the maintenance of the greenbelt is explainable from the relatively small reduction and consistent with other lakes which did not respond to a reduction in external P loading (Gulati and Van Donk 2002; Schindler and Hecky 2009; Søndergaard et al. 2001).

The external DIN load from groundwater alone was estimated on 86.2 mg N m^{-2} day^{-1}. This N load mostly comprises of NN, only 2% of the DIN was AMM. For the internal DIN loads we estimated

77.9 mg N m^{-2} day^{-1}. The internal DIN load comprised mostly AMM, ranging from 77% to 97%. Other external N-loads are present, e.g. water birds may contribute 4.7 mg N m^{-2} day^{-1} as estimated by the Waterbirds 1.1 model and swimmers, the atmospheric and organic deposition will add to the N-loads to the lake. However, the identified N sources are already in surplus to the total annual P-load to the lake if one considers which (N or P) may have been limiting the growth of phytoplankton (based on Redfield ratio, (Redfield 1958). I.e. P:N in the Redfield ratio equals 1:16 mol:mol, while in the inflows it equals 1:151, in the internal loading (pre-treatment) P:N equals 1:25. Based on the nutrient loadings to the water column, one may argue the lake is P limited. Nonetheless, the water column average P:N ratio comes to about 1:14 mol:mol implying substantial amounts of N are either bound or lost. The latter is primarily the result of bacterial activity in anoxic environments where denitrification and anaerobic ammonium oxidation (anammox) convert inorganic N into N_2 gas (McCarthy et al. 2016).

2.5.2 Macrophytes

It seemed that the maintenance of the greenbelts and the reduction of the grass carp resulted in some rehabilitation of the ecology of Lake Rauwbraken regarding the return of the helophytes and submerged macrophytes. Although the disappearance of submerged macrophytes is mostly explained by eutrophication and turbid waters due to high benthivorous fish densities (Scheffer 2001), this was not the case in Lake Rauwbraken as the submerged macrophytes were eradicated by the infestation with grass carp. The fact that submerged macrophytes could return to the lake can be explained by the reduction of the grass carps and the seasonal stratification of *P. rubescens,* i.e. the stratification of *P. rubescens* at metalimnic depths provided the macrophytes with clear water during the growth season (as depicted in Fig. 2.6). The return of (submerged) macrophytes did not prevent the blooms of cyanobacteria in spring 2008. In as far as bottom-feeding fish like carp (*Cyprinus carpio*) and bream (*Abramis brama*) may affect water clarity by resuspending sediments into the water column, their densities in Lake Rauwbraken can be considered low (about 30 kg ha^{-1}).

Submerged macrophytes play a key role in the ecology of shallow lakes (Scheffer 2001) and water quality in general (Bloemendaal and Roelofs 1988). In the Dutch deep lakes, quarry lakes, the presence of submerged macrophytes has been overlooked for a long time. Deep lakes can harbour submerged macrophyte communities that are distinctly different from shallow lakes (Seelen et al. 2021). Moreover, macrophytes are observed as deep as 18.9 m depth (Seelen et al. 2021). In Lake Rauwbraken, the submerged macrophytes could only play a minor role, because the surface area that could be covered was limited (dry falling), and turbidity hampered deeper macrophyte colonization. To indicate depths at which macrophytes are light-limited, we use the euphotic zone (z_{eu}). For phytoplankton z_{eu} is the depth beyond which light level falls below 1% of surface irradiation (Reynolds 1984). By approximation $z_{eu} = 1.7 \times$ Secchi-disk depth (Reynolds 1984), with a mean Secchi-disk depth of 3.5 m

during 2006-2007 resulted in z_{eu} = 6.0 m. In a small, but deep lake, like Lake Rauwbraken, the water depth quickly reaches z_{eu} due to the steep slopes of the lake's banks, which is a common feature in quarry lakes (Seelen et al. 2021). This means that the surface area that may be covered by submerged macrophytes is restricted by water clarity in combination with depth (the path length of the penetrating light). Light penetration is limited by suspended solids like organic (living phytoplankton and detritus), inorganic (silt) substances and humic acids (Gilvin) (e.g. (Kirk 1994). In Lake Rauwbraken the concentrations of inorganic material (mean ISS = 1.1 mg L^{-1}) are low as compared to what is generally observed in shallow lakes. For a selection of Dutch shallow lakes, Meijer et al. (1990) reported inorganic suspended solids concentrations of 7.5 - 30 mg L^{-1}, also depending on the amount of benthivorous fish present in the lakes. Hence, our results indicate that in Lake Rauwbraken the phytoplankton densities, as based on organic suspended solids (mean = 3.0 mg L^{-1}), play a bigger role in the light limitation of submerged macrophytes.

The returning submerged vegetation reflected a eutrophic system by its species (*Elodea*) and densities (massive canopy with an abrupt end at 4 m depth).

2.5.3 Sediment release experiments

From our 2005 experiment, it is clear that FRP is released from the sediments under aerobic conditions in the water overlying the sediment, i.e. 2.5-2.9 mg m^{-2} day^{-1}, which supports Tammeorg et al. (2017), that a considerable part of the internal P load may originate from release under aerobic conditions. Note that the redox conditions in the sediment were not measured in our experiments. Hence, we do not know in how far the aerobic conditions in the overlying water may have affected the conditions in the sediment. The 2008 experiment (pre-treatment cores) shows that this FRP release is greatly enhanced by anaerobic conditions, i.e.: 15.12 mg m^{-2} day^{-1}, which is consistent with literature (Boström and Pettersson 1982; Boström 1984; 1988a). The effects of the 2 years incubation are discussed in Chapter 5.

2.5.4 Sediment Phosphorus Content

We found that the top 2 cm of the sediment further away from shore contains more P and more releasable P. Which by itself is an interesting result, because in this observation we have not introduced the amount of sediment present at the sample locations along the transect. It seems that over depth (distance from shore) in Lake Rauwbraken, the sediment has a different composition regarding the amount of P present in the material.

In 2011 (Chapter 6) a similar transect was sampled with more detail in sample depth and replicate samples per depth. From this transect, we know that the thickness of the sediment in Lake Rauwbraken follows a pattern commonly observed in deep lakes, that is according to erosion – transport

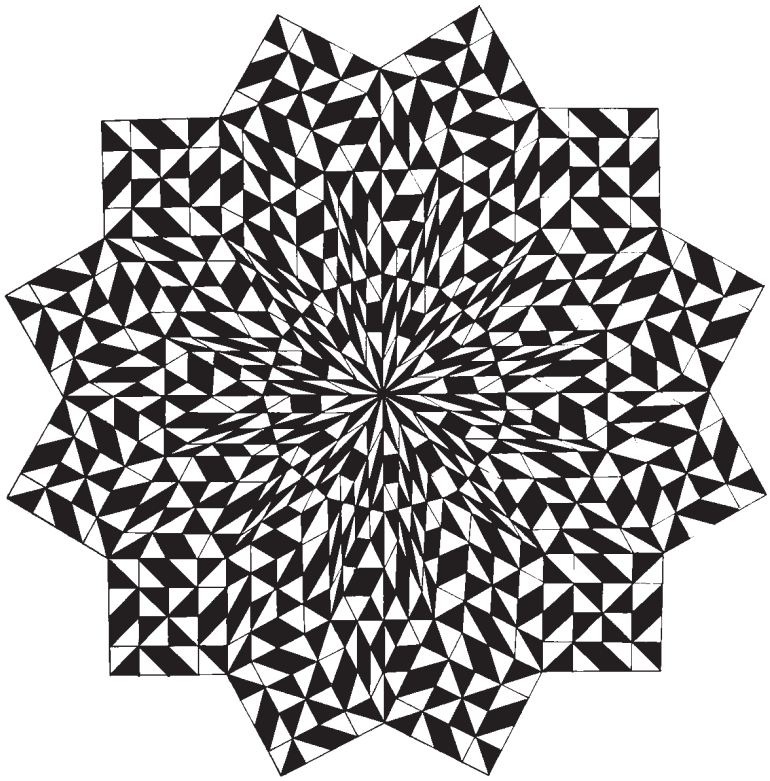
– sedimentation zones (Blais and Kalff 1995). Which means that the amount of sediment in the deeper parts is much larger than in the shallower parts.

2.5.5 Towards mitigation

The water quality problem hampering the use of Lake Rauwbraken as a bathing location, comprised of high frequent, extremely high densities (scums and accumulations) of toxic cyanobacteria. Such problems evolve in two steps: population growth (a biological process) followed by accumulation (a physical process) of buoyancy-controlled cyanobacteria present in the lake (George and Edwards 1976). Instant removal of the standing crop of cyanobacteria could be a curative method to achieve water quality standards (EU 2000; 2006). Eradication by algaecides is not a good idea, because cell lysis of cyanobacteria would release the toxins and hence could lead to the intoxication of humans with cyanobacterial toxins (Azevedo et al. 2002; Hawkins et al. 1985). Alternatively, a bloom of cyanobacteria can be sunk using a low dose of flocculent and sinking weight (Noyma et al. 2016; 2017; Pan et al. 2006a; Wang et al. 2012; Zou et al. 2006), thus avoiding the release of toxins directly into the bathing waters. Applying this method by itself would not prevent new blooms to develop. Imposing nutrient limitation on the population growth of cyanobacteria may suffice to reduce the amount of cyanobacterial biomass that can possibly accumulate. External nutrient load reduction is seen as the state of the art tool to mitigate blooms of cyanobacteria (Hamilton et al. 2016; Paerl 2014; Paerl et al. 2016a), which includes the dual reductions of nitrogen and phosphorus loads. Au contraire this general idea, the most feasible approach to reduce the cyanobacterial nuisances in Lake Rauwbraken, is to reduce the internal P-load (single nutrient reduction, Liebig's law), thus reducing the water column P to limit phytoplankton growth at much lower population densities, because reducing the relatively small external sources is impossible. The case of Lake Rauwbraken underlines that this kind of manmade lake will gradually move to a more nutrient-enriched state as they accumulate nutrients in the sediment.

2.6 Conclusions

- Early signs in Lake Rauwbraken indicated that cyanobacteria were building up high densities, which remained unnoticed during the bathing season as they stratified below the thermocline.
- Lake Rauwbraken built up a large legacy P pool in its sediments through relatively small and dispersed external P inflows over forty years of its history.
- From this sediment P-pool, the internal FRP load made up 85% of the total P load to the lake.
- While the FRP release under anaerobic conditions made up the bigger part of the internal P load, the FRP release under aerobic conditions cannot be ignored, i.e. for Lake Rauwbraken this load was the same order of magnitude as the total external P load.
- The reduction of the external P load by removing leaf litter was not enough to mitigate the blooms of cyanobacteria.
- To mitigate the blooms of cyanobacteria in Lake Rauwbraken, we propose to remove the standing crop of cyanobacteria using a flocculent and to reduce internal P loads by applying a solid phase phosphorus sorbent (Floc & Lock, Chapter 1).



J.F.X. van Oosterhout

Celebrations of a four quarter beat, theme and variations 3.

68 x 49 cm

Acrylic ink on paper

Chapter 3

Lanthanum modified clay: effects on water quality variables and plankton

Parts of this chapter were published in:

Oosterhout, F. and M. Lürling, 2013. Phosphorus binding clay (Phoslock®) in mitigating cyanobacterial nuisance: effects on water quality variables and plankton. Hydrobiologia 710 (1): 265-277.

Lürling, M. and F. van Oosterhout, 2013. Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation. Water Research 47 (17): 6527-6537.

3 Lanthanum modified clay: effects on water quality variables and plankton, the sediment capping mechanism, sinking cyanobacteria

3.1 Introduction

Mitigation of blooms of cyanobacteria currently focusses on phosphorus control (**Chapter 1**). To this effect, in-lake phosphorus control is deemed necessary if internal phosphorus loads hamper lake recovery after external load reductions (**Chapter 1**). In-lake phosphorus control can be achieved by binding bio-available (soluble) orthophosphate in insoluble phosphate-salts. Thus, the search is for the strongest phosphate binder, which is both environmentally safe and unaffected in its phosphate binding capacity by naturally occurring changes in circumstances like pH or redox.

Applications of Aluminium (Al), Calcium (Ca) and (Iron) Fe salts to inactivate phosphate are well known – either in waste water or as in-lake treatment (Cooke et al. 2005). While Al seems the most promising of these (e.g.: (Cooke et al. 1993)), its theoretical stoichiometry to bind phosphate (Al:P =1:1) has never been achieved in the field – the suggested Al:P may range from 4:1 to 10:1 (de Vicente et al. 2008; Reitzel et al. 2005). The affinity of Lanthanum (La) to bind orthophosphates has been known for a long time (e.g.: Peterson et al. (1976)). La and phosphate bind to a stable mineral rhabdophane ($LaPO_4 \bullet nH_2O$), with an extremely low solubility product Ksp = $10^{-24.7}$ to $10^{-25.7}$ $mol^2\ l^{-2}$ at 25°C and infinite dilution (Johannesson and Lyons 1994; Liu and Byrne 1997). The affinity of La to bind phosphate is less affected by deviations from neutral pH as compared to Al and Fe based phosphate binders (Douglas et al. 2004), and the formed mineral rhabdophane is stable over a wide pH range (pH = 3 - 12). Anoxic events, which naturally occur within or above lake sediments, are known to reduce the phosphate binding capacity of Fe (Correl 1998). The phosphate binding capacity of La is not affected by anoxic events (Douglas et al. 2004; Ross et al. 2008).

One difficulty with La as a phosphate binder is to position it on the sediment where it should form an active phosphate-binding layer. However, La can be incorporated in a clay through cation exchange (Douglas et al. 1999). Using this technique, La modified bentonite (LMB; Phoslock®) was developed by the Australian CSIRO (Douglas 2002). LMB is applied as a suspension to the surface of water bodies. LMB removes filterable reactive phosphorus (FRP) from the water, once settled on the sediment, it acts as a 1-3 mm thick active barrier for FRP (URL2; Copetti et al. 2016).

We studied the efficacy of LMB by measuring its capacity to bind Filterable Reactive Phosphorus (FRP) and its release of ammonium, nitrite/nitrate, FRP; its effect on pH, conductivity, oxygen saturation and turbidity (NTU). The pH and turbidity are of special interest, pH because chemical water treatments

(e.g. Al or Fe salts) may cause drops in pH and turbidity because LMB temporarily decreases water clarity - which opposes the aims of mitigation.

We test the sediment capping mechanism in an experiment that compares the release of Filterable Reactive Phosphorus (FRP) in undisturbed sediment cores that received Lanthanum Modified Bentonite (LMB), Aluminium Modified Zeolite (AMZ), unmodified bentonite, unmodified zeolite or no treatment (control). As anaerobic conditions are known to heighten the release of FRP from sediments, e.g.: (Boström et al. 1988b), this experiment was done under both aerobic and anaerobic conditions. The dosages used in this experiment aimed at fully stopping the release of FRP from the sediment. To this effect, we applied an approximate 1.5 times the LMB doses needed to bind all releasable P in the top 10 cm of the sediment. Applying the same amounts of the other compounds, this dosage equals an 10 fold of the recommended dosages for the AMZ.

We investigated the effects of LMB on the growth of *Scenedesmus obliquus* (green alga) and *Microcystis aeruginosa* (cyanobacterium), hypothesizing that LMB concentrations exceeding those that theoretically bind all FRP hinder their growth. Although growth reduction is a good indication of the effectiveness of LMB to bind FRP, several studies show that flocculation with clay can control existing blooms (Anderson 1997; Pan et al. 2006b; 2011a; 2011b; Verspagen et al. 2006). So, we exposed blooming densities of *Anabaena* sp. (cyanobacterium) to increasing concentrations of LMB in the laboratory to test the hypothesis that the more LMB, the stronger the bloom reduction. We studied the population growth of the rotifer *Brachionus calyciflorus* in the presence of LMB.

In addition, we studied the removal efficiency of the flocculent poly aluminium chloride (PAC) as sole PAC and in combination with LMB. To effectively sink PAC flocs that contain positively buoyant cyanobacteria, a sinking weight is needed, for which LBM was used. Hence, LMB acted as a ballast and phosphate fixative. PAC was chosen as a flocculent, because of its strong flocculation and water clearing properties (Delgado et al. 2003) and phosphate absorbing ability (Drabkova 2007; Lopata and Gawrońska 2008). LMB was chosen as a sinking weight and P-fixative because of lanthanum's strong affinity to bind FRP (Johannesson and Lyons 1994; Liu and Byrne 1997). We tested the hypothesis that the combination of the flocculent PAC with LMB would effectively sink a water bloom of cyanobacteria in a laboratory experiment.

3.2 Materials & Methods

3.2.1 Chemicals

The flocculent AquaPAC39 (poly aluminium chloride, $Al_n(OH)_mCl_{3n-m}$, ρ = 1.37 kg L^{-1}, 8.9% Al, 21.0% Cl) and the lanthanum-modified bentonite – (LMB; Phoslock®) 5% La were supplied by Phoslock Europe GmbH (Ottersberg, Germany). The LMB used in the experiments here originated from two batches as industrially produced for field application. The aluminium modified zeolite - Aqual P (AMZ) was obtained from Blue Pacific Minerals, New Zealand. The unmodified bentonite and zeolite were obtained from Aldrich (The Netherlands) respectively from Zeolite products (Varsseveld, The Netherlands).

3.2.2 The Capacity of LMB to bind FRP

Threefold replicates of 200 mL FRP (K_2HPO_4, in nanopure water, pH = 8.12) solution (0.447 mg FRP L^{-1}) received 0.0 (no LMB) 0.1, 0.33, 1.0 and 3.3 g LMB L^{-1} (exact data given in Table 3.1). The suspensions were incubated at 22°C, in darkness and 200 rpm orbital shaking. Initial pH was circum-neutral, oxygen saturation 44 - 75% (Table 3.1). The molybdate-reactive phosphorus concentration (Murphy and Riley 1962) was determined in the filtered (Whatmann GF/F) supernatant of centrifuged (5 min, 3000 rpm) 15 mL samples taken at 0, 1, 2, 3, 4, and 5 hours.

We assumed exponential decline of the FRP concentration: $[FRP_t] = FRP_0e^{-\theta t}$. FRP_0 is the mean FRP concentration at t = 0 for the control (0 mg L^{-1} LMB), t = time (0, 1, 2, 3, 4, 5 hours) and θ the FRP decrease rate (mg L^{-1} hr^{-1}). Because estimates of θ failed Levene's test (equal variances), we analysed log(θ) by one-way ANOVA followed by a Tukey post hoc test in SigmaPlot 11.0. We removed one outlier (0.1 g L^{-1} dose after one hour), as its FRP concentration unexpectedly increased from the initial 0.447 to 0.739 mg L^{-1}. Based on a 4.3% lanthanum content in our batch (Lürling and Tolman 2010), 0.05 g L^{-1} LMB should suffice to remove all FRP (mg).

3.2.3 Nutrient leachates from LMB

From two different LMB batches, triplicate 20 g L^{-1} LMB suspensions (approximately 2 g in 100 mL nanopure water) were tested on their nutrient release. As control three Erlenmeyers contained only 100 mL nanopure water. The Erlenmeyers were closed (parafilm) and placed in darkness at 22 °C and 200 rpm orbital shaking for 24 hours. Afterwards, the suspensions were centrifuged (5 min, 3000 rpm), followed by filtration (0.45 µm membrane filter, Whatman NC45) of the supernatant. In these filtrates ammonium, nitrite/nitrate and FRP concentrations were determined using a Skalar continuous flow analyzer (NNI 1986; 1990; 1997). After correction for the controls, the release per nutrient (mg kg^{-1} LMB) was inferred by T-test based on the means and standard deviations per batch. We applied Welch's test because unequal variances between batches could not be removed by transformation.

3.2.4 Effect of LMB on pH, conductivity and oxygen saturation

We tested algal growth medium (WC medium) and filtered (Whatman GF/C filter) pond water - Kienehoef pond (Sint-Oedenrode, The Netherlands). A stock suspension of 6.4 g LMB in 80 mL of each medium was diluted by each respective medium to 0.625, 1, 1.25, 2, 5, 10, 20 and 40 g L^{-1} (80 mL final volume) in 100 mL plastic containers. An 80 mL control was kept (0 g LMB) for both series. For each dose and medium one experimental unit was obtained. The experiment was done in a laboratory setting at 20^{o}C. pH (WTW-pH320 meter), conductivity (EC) (WTW-Cond 330i meter) and oxygen saturation (Oxyguard oxygen meter) were measured at t = 0, 1 and 2 hours after preparation.

As we found little effect on pH we present median, minimum and maximum values without formal testing. We observed a linear increase in EC with dose, which was independent of time. For this linear EC – dose relation we estimated intercepts and slopes according to y = a+bx (y = mean EC over time, x = dose, a = intercept and b = slope). For the estimated slopes, we tested H_0: b = 0 and compared the slopes in each medium using Graphpad Prism 5.04. As we found no effects on oxygen saturation we did no formal statistical test.

3.2.5 Effect of LMB on turbidity in still water

One gram LMB was added to 1 L of nanopure water in triplicate. Turbidity (NTU) (Hach 2100P Turbidity meter) was measured regularly over 24 hours in still water, hence a maximum undisturbed settling rate was observed. The course of turbidity (NTU) is described by a triple, six parameters exponential decay function (SigmaPlot11.0).

3.2.6 The sediment capping mechanism

In this experiment, we demonstrated and test the effects of the LMB, AMZ, bentonite and zeolite on the release of FRP from the sediment in intact sediment cores. Based on the experiences with the LMB from 3.2.3 we expected nutrient leaching from the compounds used in this experiment. As we had no previous experience with the AMZ or unmodified bentonite and zeolite, we first estimated possible FRP leaching from these compounds, which was done as experiment 3.2.3. In the current experiment the estimated FRP leaching from the LMB and AMZ was exactly 0 mg kg^{-1}, which resulted in non-normality and unequal variances among the FRP leaching among the four compounds. However, for zeolite and bentonite the FRP leaching was non-zero and past on normality and equal variances. Except for the FRP leaching from the LMB and AMZ, we tested the hypothesis that no FRP leaches from the compound using the one sample T-test.

3.2.6.1 Sediment sampling

Forty intact sediment cores (50 cm, 5 cm diameter) were taken – using a Jenkins corer, from the Orion pond – a eutrophic semi-urban pond, in Wageningen. The cores contained a minimum of 10 cm of

sediment. The undisturbed cores were taken to the laboratory where immediately a 5 days sediment release experiment was started. The cores were randomly allocated to either control (no treatment), LMB or AMZ treatment, both were tested under aerobic and anaerobic conditions. Hence for each treatment four replicates were used.

At the start of this experiment, all overlying water was replaced by a fixed volume (300 or 250 ml) nano pure water. For the cores to be incubated under anaerobic conditions, oxygen free nano pure water was prepared through stripping the oxygen by bubbling it with nitrogen gas. For the aerobic incubations the nano pure water was aerated. The treated cores received an approximate 6.87 g of the appropriate compound applied as a slurry at the top of the core (exact data given in the results). As the AMZ is produced as granules the AMZ was grounded using mortar and pestle, both compounds were sieved (International Association for Testing Materials standard, 500 µm). The cores were incubated in the dark in the laboratory at 18.5-23.1°C. All cores were sealed with parafilm, the aerobic incubations were gently aerated during incubation. Each day, before sampling, temperature (°C), pH, conductivity (µS cm^{-1}) and oxygen concentrations (mg L^{-1}) were measured in the cores using a multi-meter (WTW multi 350i). Daily sampling was done by pipetting of 100 ml water from each core and replacing it with either oxygen free or oxygenated nano pure water where appropriate. Samples were kept frozen (-16°C) until analysis. During sampling, the anaerobic incubations were gently bubbled with nitrogen gas to keep them oxygen free. The FRP, AMM and NN concentrations were determined in filtered (Whatman GF/C) 50ml subsamples (Skalar continuous flow analyzer following the Dutch standard protocols (NNI 1986, 1990, 1997).

3.2.6.2 Computations

In case the FRP concentration fell below its level of detection (LOD) its value was replaced by the appropriate LOD. For each core and incubation period (i) the amount of FRP released (R_i ;mg) is computed from that chance in its concentration. I.e. R_i (mg FRP) = ΔCxV , with ΔC= $C_{end} - C_{start}$, in which C_{start} $(mg\ L^{-1})$ and C_{end} $(mg\ L^{-1})$ is the start and end concentrations of FRP for incubation period i, V the volume (L) of water in the core. As only 0.1 L of water was sampled and replaced by 0.1 L nano pure water, the C_{start} is computed for each core and incubation period as: $\mathrm{C}_{start}(mg\ L^{-1}) = \frac{C_{end}\ (V-0.1)}{V}$. As all water was replaced by nano pure water at the start, C_{start} for the first release period (day 1 to day 2) was set equal to 0 mg L^{-1}. For each core the total amount of FRP released (R_{tot}) during the experiment was computed by summation of the R_i. The 24 h release rates were then computed by: R $(\mathrm{mg\ m^{-2}} day^{-1})$ = $\frac{24}{T}\frac{R_{tot}}{A}$ with, T (h) the total duration and A (m^2) the sediment surface area of the core.

The preceding FRP leaching experiment indicated that FRP might leach from the applied bentonite and zeolite. In the sediment release experiment this leaching is measured as a release of FRP. We estimate the release of FRP from leaching as R_{leach} (mg m^{-2} day^{-1}) = (L x G)/A in which L is the amount of FRP leached (mg kg^{-1}) from bentonite or zeolite, G = the amount of the compounds applied (kg) and A the surface area of the cores (m^2). For each compound, G is computed as the mean 8 replicates (4 aerobic, 4 anaerobic) and treated as a constant. As the leachate was determined after 24 h incubation, the R_{leach} may be interpreted as a one day release. We compare the expected FRP releases R_{leach} to the observed FRP release R_1 during the first 24 hours of the experiment.

3.2.6.3 Statistics

The computed FRP releases failed normality (Shapiro-Wilk test). For the log-transformed release of FRP, the transformed data fulfilled both normality and equal variances (Levene's test) requirements for a two-way lay-out ANOVA with condition (aerobic/anaerobic) and compound as main factors. The F-test was done including the condition x compound interaction. The F-test was followed by all pairwise comparison (Holm-Sidak method).

3.2.6.4 Estimation of releasable P

After the sediment release experiment, the top 2 cm of the sediment from the control cores (both aerobic and anaerobic conditions) were sampled, thoroughly mixed which after from each core an approximate 1.5 g (wet weight) was were subjected to the fractionated P extraction (Psenner *et al.* 1988). From the total scheme, the 'Water-soluble P' (extracted in oxygen-free nanopure water) and 'Reductant soluble P' (extracted in 0.11 M Na2S2O4/0.11M NaHCO3) and the humic-bound P (computed TP – FRP, extracted with 1 M NaOH) were considered as releasable P – e.g. (Reitzel 2005; de Vicente *et al.* 2008).

We assume that the release of FRP only involves the top 10 cm (e.g. : (Boström *et al.* 1982) of the sediment and that the specific gravity of the sediments is 1 kg L^{-1} – the latter is also done by (Meis *et al.* 2013). Hence based on a 5 cm diameter and 10 cm of sediment, the cores contained an approximate 0.2 L sediment, equalling 0.2 kg. For the purpose of estimating of the actual amount of releasable P, we base our computation on wet sediment.

For each control core (n=8) used in the sediment release experiment, the total amounts of FRP released was computed as the sum of the releases during the experiment. We assume that this FRP originated from the 0.2 kg sediment and added this amount to the releasable P from the extractions described above. From this estimated P content of the cores we compute the amount of LMB needed based on La:P = 1:1 P binding and 5 % La content in the LMB.

3.2.7 Effect of LMB on the growth of Scenedesmus and Microcystis

Our stock cultures of *Scenedesmus obliquus* and *Microcystis aeruginosa* are mainly comprised of unicells and bicells; *S. obliquus* (green alga) (Turpin) Kützing SAG 276/3a, collection of the University of Göttingen, Germany; *M. aeruginosa* (cyanobacterium) Kützing NIVA-CYA 43, Institute for Water Research Norway. Stock cultures are maintained in the laboratory of the Aquatic Ecology and Water Quality Management Group (Wageningen University).

We prepared three series of WC medium (Fig. 3.1):

A. LMB at 0, 0.005, 0.05, 0.5, 5.0 and 50 g L^{-1}.
B. dilutions (0, 0.001, 0.1, 1, 10 and 100%) of a leachate of a 50 g L^{-1} LMB suspension in WC medium.
C. lanthanum nitrate at 0, 0.025, 0.25. 2.5. 25 and 250 mg La L^{-1}, prepared by dilution from an 1,103 mg L^{-1} $La(NO_3)_3 \cdot 6H_2O$ solution in WC medium.

All dilutions were made with WC medium. The leachate was prepared by 24-hour dark incubation of 50 g L^{-1} LMB at 20°C and 200 rpm orbital shaking, after which the material was centrifuged (5 min at 3000 rpm) and the supernatant filtered (0.45 µm membrane filter, Whatman NC45).

The LMB dosages relate 1 to 1 with the dilutions of the leachate. We interpret the La concentrations in the leachates as resulting from the release of the corresponding dose in the LMB series. The lanthanum nitrate dosages are based on the 5% La content as reported by the manufacturers of LMB. The 0.025 mg La L^{-1} dose has no equivalent in the LMB series, while the 50 g L^{-1} LMB dose has no equivalent in the lanthanum nitrate series. Based on the 5% La content, the total La concentrations in the 0, 0.005, 0.05, 0.5, 5.0 g L^{-1} of the LMB series are approximately the same as the respective 0, 0.25, 2.5, 25 and 250 mg L^{-1} lanthanum nitrate series.

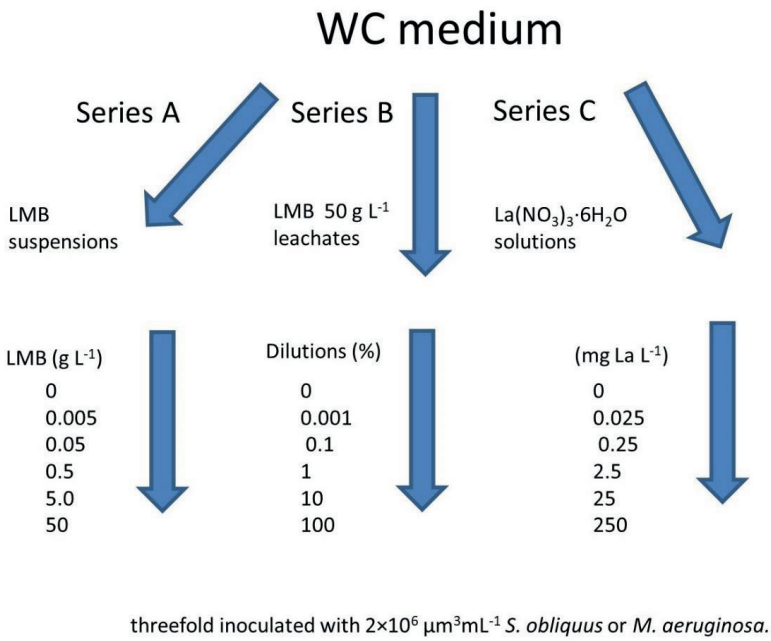


Figure 3.1 Preparation of the three series of medium; series A LMB suspensions, series B LMB leachates, series C lanthanum nitrate solutions.

From each series, 50 mL triplicates in 100 mL Erlenmeyer's were inoculated with 2×10^6 µm^3 mL^{--1} *S. obliquus* or *M. aeruginosa.* As we measured biovolume (µm^3 mL^{-1}), we kept one phytoplankton free Erlenmeyer per concentration as control for the LMB particles. The Erlenmeyer's were closed (cellulose plugs) and incubated for 3 or 7 days for both *S. obliquus* and *M. aeruginosa*, at 21 °C in continuous light (100 µmol quanta m^{-2}s^{-1}) and 25 rpm orbital shaking.

The biovolume (V) (µm^3 mL^{-1}) was determined by Coulter Multisizer II (range 2.5 – 30 µm). The growth rate (µ, day^{-1}) was estimated by the formula $\mu = (\ln(V_t) - \ln(V_0)/ \Delta t$ where V_0 and V_t are the biovolumes at time 0 and t respectively, Δt is the time-lapse (i.e. 3 or 7 days). The *S. obliquus* incubations were also analysed on chlorophyll-*a* concentrations by a PHYTO-PAM phytoplankton analyser (Heinz Walz GmbH, Germany). After three days, settled material was observed in the *S. obliquus* series at a high dosage ≥ 0.5 g L^{-1} LMB, these incubations were analysed again after resuspension of the material.

We chose the non-parametric Kruskal-Wallis method to test the dose effect on µ because ANOVA requirements were not fulfilled (non-normal distribution, unequal variances amongst groups) and could not be met with data transformation. Tukey's test and the Holm-Sidak method were used for pairwise comparisons. We expected µ to reduce with dose, as higher dosages hold less bioavailable FRP

(i.e. more FRP bound by La). We also expected a reduction in μ by shading and clay coagulation in the LMB treatment. Thus, we expected the smallest effect in the leachate, the largest with LMB, and the effect of dissolved lanthanum nitrate to be intermediate.

3.2.8 Effect of LMB on an Dolichospermum (Anabaena) bloom

A late-log culture of *Anabaena cylindrica* (Cyanobacteria, PCC 7122 Pasteur Culture Collection, France) was diluted in WC medium to 212 ± 18 μg chlorophyll-*a* L^{-1}, of which aliquots of 100 mL of were incubated in closed transparent plastic containers at 20 °C, 25 rpm orbital shaking and in a 16:8 h light:dark rhythm of 117 μmol quanta $m^{-2}s^{-1}$ light. Total incubation lasted 18 days, after one day of acclimatization, the containers were dosed with LMB to obtain concentrations of 0, 0.1, 0.25 and 0.5 g L^{-1} in triplicate. Chlorophyll-*a* concentrations (μg L^{-1}) were measured using the PHYTO-PAM on days 0, 1, 3, 6, 9, 12, 15 and 18. The data were subjected to linear regression and slopes were compared in SigmaPlot 11.0.

3.2.9 Effect of LMB on the growth of the rotifer Brachionus calyciflorus

Cysts of *Brachionus calyciflorus* Pallas (Microbiotests Inc. Nazareth, Belgium) were hatched in 100 mL WC medium at 21 °C and continuous light (100 μmol quanta $m^{-2}s^{-1}$). Neonates, 2-3 hours old, were immediately used in the experiments. Two LMB series were prepared using a suspension of *S. obliquus* (2×10^7 μm^3 mL^{-1}, i.e. approximately 10 mg C L^{-1}) in WC medium - *S. obliquus* served as food for *B. calyciflorus*. The experiment was conducted in 24-well culture plates. One received LMB at 0, 0.005, 0.05, 0.1, 0.5 and 5.0 g L^{-1} in fourfold. The other received LMB at 0, 0.005, 0.05, 0.1, 0.2 and 0.4 g L^{-1} in quadruplicate. Concentrations 0, 0.005, 0.05 and 0.1 g L^{-1} were assessed in eight-fold.

Each well was filled with 2.5 mL of the corresponding suspension and inoculated with 2 *B. calyciflorus* individuals. The plates were incubated in the dark at 21 °C, orbital shaking (40 rpm). After 48 hours, the wells were inspected for living animals and population growth was stopped by adding 100 μL of Lugol's solution. Counting was aided by a dissection microscope (15×).

Population growth rate (r) was computed as: $r = (\ln(t_2) - \ln(t_0))/\Delta t)$; t_0,t_2 = density at 0 respectively 48 hours, Δt = 48 hours. Negative r values were avoided by converting r to the finite rate of population increase $\lambda = e^r$ (Gilbert 1996). Treatment effects on r were assessed by one-way ANOVA and Tukey post-hoc comparison test. The homogeneity of variances was tested by Levene's test. The EC_{50} value (50% growth inhibition) was estimated by non-linear regression (4 parameters logistical model) in SigmaPlot 11.0.

3.2.10 Rapid settling of cyanobacteria

The effect of PAC with and without LMB as sinking weight to remove positively buoyant cyanobacteria from a water column was tested in a controlled laboratory experiment (Fig. 3.2). Positively buoyant

cyanobacteria were collected from a surface scum of *Microcystis aeruginosa* on the hypertrophic urban pond De Ploeg (Heesch, The Netherlands, N 51°41'43.70" / E 5°32'10.50""; (Lürling and Faassen 2012). Scum material was diluted and re-suspended in glass-fibre filtered (Whatman GF/C) pond water to approximately 800 µg chlorophyll-*a* L^{-1}. Aliquots of 100 mL of the cyanobacteria suspension were distributed over 18 glass tubes (125 mL). Nine tubes were placed in a "Floc" series and received PAC at 0, 2.2 and 4.4 mg Al L^{-1} (each concentration in triplicate). The other nine tubes were assigned to a "Floc & Lock" series and were treated with the same concentrations of PAC (0, 2.2 and 4.4 mg Al L^{-1}) to which also 39 mg LMB was added. In this series the PAC 0 mg L^{-1} combined with the modified clay is the control for the effect of sole clay treatment. The LMB was added by making a slurry with 5 mL of water from the tube, which was then sprayed on top of the tube. The tubes were placed for two hours in the laboratory at 20 °C.

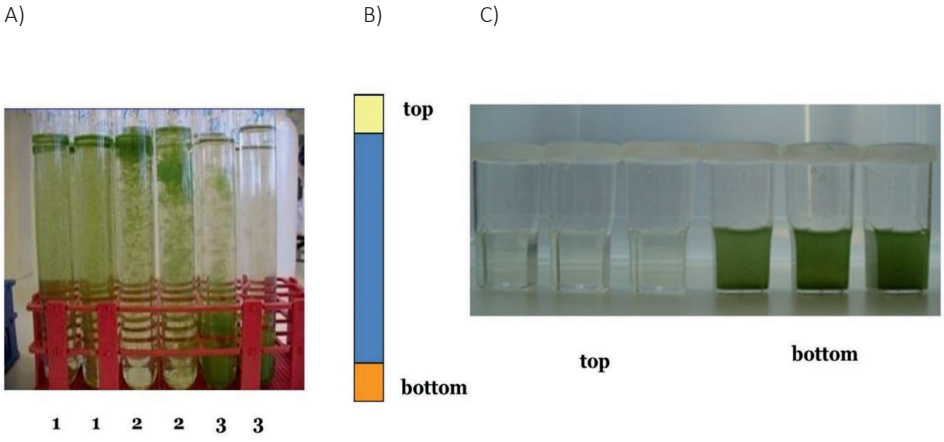


Figure 3.2 The rapid settling experiment, controls (indicated by 1), single PAC (indicated by 2), combined PAC and LMB (indicated by 3) (A); sample locations with the test tubes (B); top and bottom samples of the combined PAC and LMB incubations (C); with permission (Mucci et al. 2019).

Samples (10 mL) were taken at the start and after two hours of incubation from the top and bottom of the tubes. In these samples chlorophyll-*a* concentrations and the Photosystem II efficiencies (PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany) were measured. We measured the Photosystem II efficiency as indicator for cell lysis, in which case a sharp drop in Photosystem II efficiency is expected (Hu et al. 2008; Lürling and Beekman 2010). In the top and bottom samples turbidity (Hach 2100P turbidity meter), conductivity (WTW-Cond 330i) and pH (WTW-pH320) were measured. Data from the top and bottom samples were analysed by two-way ANOVAs using SigmaPlot 12.3 (Systat Software, Inc) with the absence/presence of the LMB and PAC concentration as fixed factors. Data were checked for normality and heteroscedasticity by Normality Test (Shapiro-Wilk)

and Equal Variance test in SigmaPlot prior to the ANOVA. The ANOVAs were followed by pairwise multiple comparison procedures (Holm-Sidak method) to distinguish means that were significantly different ($p < 0.05$).

3.3 Results

3.3.1 Capacity of LMB to bind FRP

The FRP concentrations in the controls remained constant. In the presence of LMB, they decreased exponentially ($[FRP_t] = FRP_0e^{-\theta t}$;Fig. 3.3). The decrease rate (θ) at 22 °C was proportional to the amount of LMB added (Fig. 3.3). Log(θ) passed Levene's test for equal variances ($F_{3,8} = 3.59$, $p > 0.07$). The *F*-test showed a significant dose effect ($F_{3,8} = 70.7$, $p < 0.001$). Tukey's test distinguished 0.1 and 0.33 g L^{-1} from 1 and 3.3 g L^{-1} (Table 3.1). Within one hour the 1 and 3.3 g L^{-1} dosages depleted FRP. The 0.33 g L^{-1} dose depleted FRP within 3-4 hours. While 0.05 g L^{-1} LMB should have been sufficient to bind all FRP, the 0.1 g L^{-1} LMB dose only bound 68% of the FRP in 5 hours (Fig. 3.3; Table 3.1).

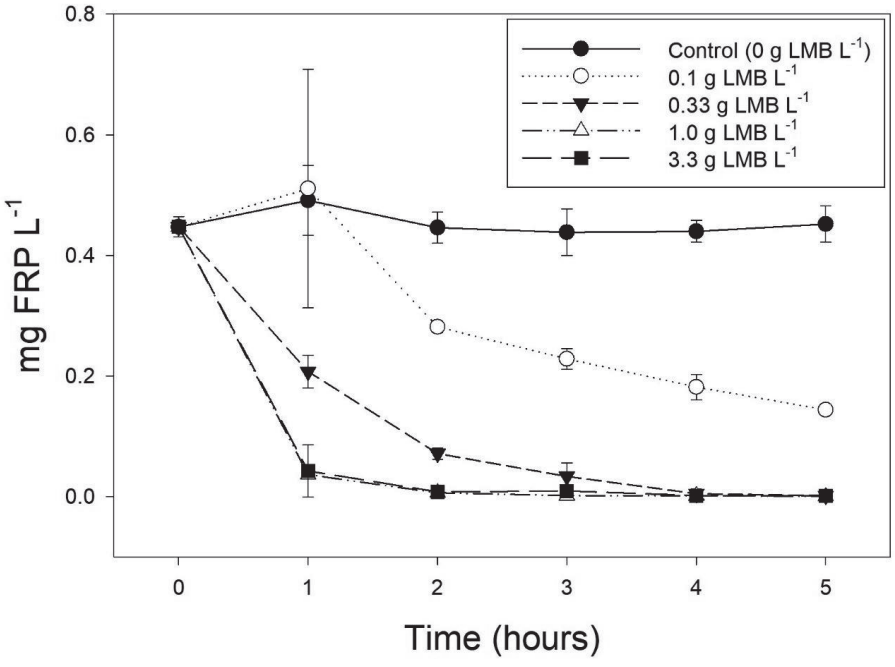


Figure 3.3 Course of the filterable reactive phosphorus (FRP, mg L^{-1}) in incubations exposed to different concentrations of LMB (0 – 3.3 g L^{-1}). Error bars indicate 1 SD (n = 3).

Table 3.1 Mean (SD) (mg) of LMB aliquots, mean (SD) of maximum amount of FRP that theoretically could be bound by this aliquot (max FRP), realized % bound after 5 hrs, estimates of the FRP decrease rate θ (SD) and groups identified by Tukey's test. Entries identified by different characters are statistically significant different according to Tukey's test. Also included are the initial pH and oxygen saturation levels (%).

dose	aliquots	max FRP	bound P	θ	Tukey	Start	Start
g L^{-1}	mg	µg	%	mg L^{-1} h^{-1}	group	pH	O_2 %
0	---	---	---	---	---	6.4	44
0.1	20.3 (0.5)	194 (5)	68 (1)	0.2 (1.1)	A	6.5	48
0.33	65.7 (0.9)	630 (9)	100 (0)	0.8(1.1)	A	6.7	45
1.0	201.2 (0.6)	1929 (5)	100 (0)	2.5(1.1)	B	7.5	50
3.3	660.3 (0.9)	6331 (8)	100 (0)	2.6(1.1)	B	7.9	75

3.3.2 Nutrient leachates from LMB

In nano pure water, LMB seemed to release some FRP, nitrite + nitrate (NN) was not released, and ammonium (AMM) was significantly released. We found significant differences between the two batches in the amounts of FRP and ammonia released (Table 3.2).

Table 3.2 Release of nutrients from LMB (in mg kg^{-1} LMB, mean (SD), n = 6); T = Student's T statistic , df = degrees of freedom, T' = Welch's T approximation, df = degrees of freedom according to Welch-Satterthwaite equation.

	Batch 1			Batch 2			Batch 1 - 2		
	Release	T (df =5)	p	Release	T (df =5)	p	T	df	p
FRP	0.39 (0.20)	4.36	< 0.01	0.05	0.302	0.4	1.98	8	< 0.05
NN	2 (4)	1.12	0.16	4 (4)	2.24	< 0.05	0.87	10	0.80
AMM	224 (3)	167.0	< 0.01	53 (10)	11.85	<0.01	40.1	6	<0.01

3.3.3 Effect of LMB on pH, conductivity and oxygen concentration

In both WC medium and pond water the lowest pH occurred at the highest LMB dose (3.2 g LMB L^{-1}) after two hours. The highest pH occurred in the controls at the start. In WC medium, median pH was 7.1 (range 7.0 - 7.3). In pond water, median pH was 7.8 (range 7.0 - 7.9).

We observed a linear increase conductivity (EC) with dose, which is different for the WC medium and pond water. The regression in WC medium was: EC = 257.4 + 17.77×dose (r^2 = 0.983; $F_{1,6}$

= 351.0, p < 0.01) and in pond water: EC = 249.4 + 9.937×dose (r^2 = 0.983; $F_{1,6}$ = 796.4, p < 0.01). Both slopes deviated significantly from zero, both regressions differed significantly from each other ($F_{1,12}$ = 59.9, p < 0.01).

The mean oxygen saturation in WC medium and pond water was 99% (SD = 1; n = 24), ranges 98 - 101% respectively 97-100%.

3.3.4 The effect of LMB on turbidity in still water

In still water – undisturbed conditions, one gram LMB in 1 L nanopure water raised turbidity from 0.15 to 374 NTU (Fig. 3.4), after 5 minutes turbidity dropped to 48 NTU, after 6 hours below 13 NTU – approximating 1 m Secchi disk depth. After 24 hours, turbidity declined to 6.5 NTU (Fig. 3.4). The decline could best be described by a six-parameter exponential decay function:

Turbidity = 263.89×exp(-6108096×t) + 83.07×exp(-2.9509×t) + 27.03×exp(-0.1419×t) with $F_{5,12}$ = 6648; p < 0.01 and r^2_{adj} = 0.9996 (Fig. 3.4).

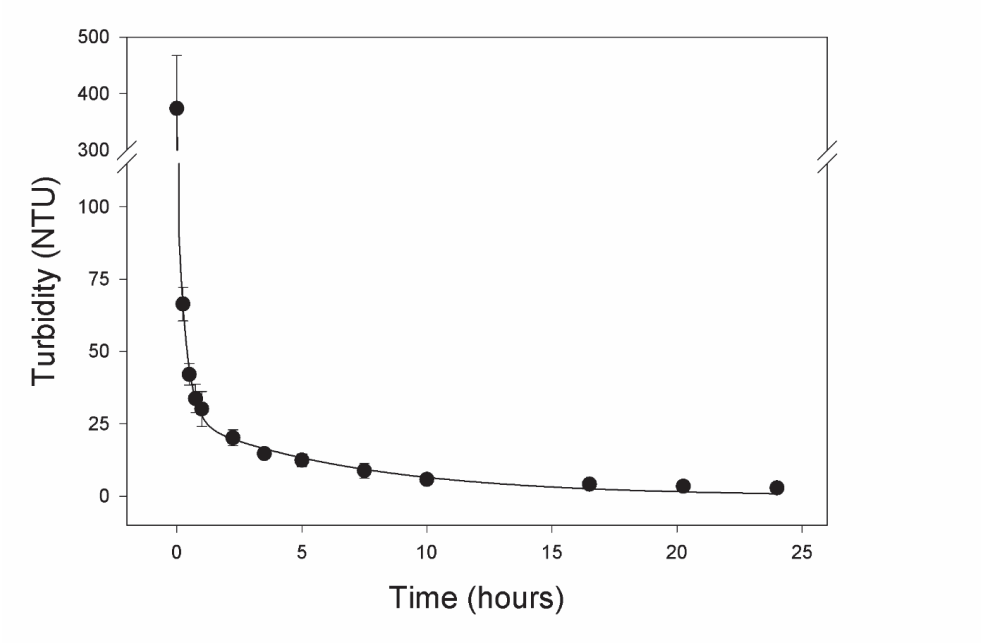


Figure 3.4 Course of the turbidity (NTU) in incubations exposed to 1 g LMB L^{-1}. The solid line represents non-linear regression: Turbidity = 263.89×exp(-6108096×t) + 83.07×exp(-2.9509×t) + 27.03×exp(-0.1419×t); r^2_{adj} = 0.9996. Error bars indicate 1 SD (n = 3).

3.3.5 The sediment capping mechanism

3.3.5.1 Experimental conditions

Taken over all sample days and cores the mean temperature was 20.7°C (SD = 0.9 °C; n = 159; **Appendix**: Table 3.9). The mean oxygen concentration (**Appendix**: Table 3.9) in the aerobic cores was 8.9 mg L^{-1} (SD = 0.3 mg L^{-1}; n = 79), in the anaerobic cores it was 0.8 mg L^{-1} (SD = 0.8 mg L^{-1}; n = 80). Under anaerobic conditions the mean oxygen concentration in the AMZ and LMB treated cores was approximately 1 mg L^{-1} higher than in the controls and zeolite or bentonite treated cores (**Appendix**: Table 3.9). Under aerobic conditions median pH was 7.4 (min-max = 5.4 - 7.9; n = 79), under anaerobic conditions median pH was 7.1 (min-max = 4.7-8.0; n = 80). The lowest pH was observed in the AMZ treated cores under both aerobic (min pH = 5.4) and anaerobic (min pH = 4.7) conditions. EC (**Appendix:** Table 3.9) depended on the treatments. The lowest mean EC was 72.4 µciem cm^{-1} (SD = 16.6 µciem cm^{-1}) in the controls under anaerobic conditions, the highest mean EC was 747.2 µciem cm^{-1} (SD = 218.5 µciem cm^{-1}) in the AMZ treatment (**Appendix**: Table 3.9).

3.3.5.2 FRP leaching

Our leaching experiment indicates that under the conditions in the 24 h leaching experiment significant amounts of FRP are released from both zeolite and bentonite (Table 3.3). As compared to the controls, the zeolite seems to increase the FRP concentration during the first 24 h of the experiment, which is not the case with the other compounds under both aerobic and anaerobic conditions (Table 3.3).

Table 3.3 Mean and standard deviation (SD) of the Filterable Reactive Phosphorus (FRP) leaching from AMZ, LMB, zeolite and bentonite, Expected FRP leaching and first day releases of FRP (See text), n = number of replicates, T = Student T-statistic, P = p-value, β power of the test done with α = 0.05, aerobic = aerobic conditions, anaerobic = anaerobic conditions, SD = standard deviation, NT = not tested.

	FRP leaching from the compounds				Expected FRP leaching	First day FRP release	
	n = 3				n = 4	n = 4	
		$T_{df=2}$	P	β		aerobic	anaerobic
compound	mean (SD) (mg kg^{-1})				mean (SD) (mg m^{-2})	mean (SD) (mg m^{-2})	mean (SD) (mg m^{-2})
control					-	25.7(22.4)	61.2 (5.2)
AMZ	0 (-)	NT	-		0	0.9 (0.2)	0.7 (0.1)
LMB	0 (-)	NT	-		0	0.9 (0.4)	1.3 (0.9)
zeolite	3.76 (0.10)	67.0	< 0.01	1.0	13.2 (0.34)	47.4 (35.9)	34.9 (17.7)
bentonite	3.17 (0.67)	8.37	0.014	0.97	11.3 (2.34)	15.3 (5.1)	32.2 (13.6)

3.3.5.3 FRP release

The FRP release in the controls (untreated cores) was 27.1 mg m^{-2} day^{-1} under aerobic conditions and 74.9 mg m^{-2} day^{-1} under anaerobic conditions (Fig. 3.5; Table 3.4). The FRP release in the cores treated with the AMZ or LMB was 0.6 mg m^{-2} day^{-1} respectively 1.3 mg m^{-2} day^{-1} under aerobic conditions and 0.8 mg m^{-2} day^{-1} respectively 0.7 mg m^{-2} day^{-1} under anaerobic conditions (Fig. 3.5; Table 3.4). In the cores treated with bentonite, the FRP releases were 11.2 mg m^{-2} day^{-1} and 26.5 mg m^{-2} day^{-1} under aerobic respectively anaerobic conditions (Fig. 3.5; Table 3.4). With zeolite the FRP release under aerobic conditions was 34.4 mg m^{-2} day^{-1}, while under anaerobic conditions it was 37.3 mg m^{-2} day^{-1} (Table 3.4).

The ANOVA revealed no significant condition x compound interaction in the transformed data ($F_{4,30}$ = 1.9, p= 0.14), however this test had a low power of 0.23. The F-tests for the effects of aerobic-anaerobic conditions revealed a significant differences between these conditions ($F_{1,30}$ = 4.4, p = 0.04). Except for LMB treated cores, all observed releases were lower under aerobic conditions than under

anaerobic conditions (Fig. 3.5). The F-tests for the effects of the applied compounds revealed a significant effect of the compound ($F_{4,30} = 53.7$, $p < 0.001$). While the FRP releases did not significantly differ between the AMZ and the LMB, the multiple comparisons (Holm-Sidak) revealed that both the AMZ and LMB significantly reduced the release of FRP as compared to the untreated controls, zeolite and bentonite (Fig. 3.5). No significant differences were found between the untreated controls, zeolite or bentonite. With the AMZ, the release under aerobic conditions was slightly lower than under anaerobic conditions, which with the LMB was the other way around (inset; Fig. 3.5).

Table 3.4 Mean FRP release rates (mg m^{-2} day^{-1}).

compound	Aerobic mean (mg m^{-2} day^{-1})	n	SD (mg m^{-2} day^{-1})	Anaerobic mean (mg m^{-2} day^{-1})	n	SD (mg m^{-2} day^{-1})
control	27.1	4	25.9	74.9	4	18.2
AMZ	0.6	4	0.4	0.8	4	0.5
Zeolite	34.4	4	29.3	37.3	4	19.6
LMB	1.3	4	1.4	0.7	4	0.5
Bentonite	11.2	4	4.5	26.5	4	18.7

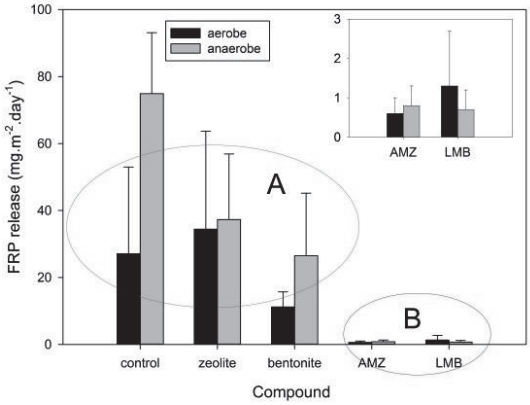


Figure 3.5 Mean FRP releases during the sediment release experiment, groups with similar compound effects (indicated by A,B) are red encircled, the inset shows the mean releases in the AMZ and LMB treatments, error bars indicate 1 SD, n = 4.

3.3.5.4 Releasable P

Taken over all control cores (i.e. both aerobic and anaerobic) and corrected for the FRP released during the experiment, the sediment contained 3.0 (SD = 1.3) g P per kg dry weight. Of this total amount an approximate 72.5 % (SD = 11.3 %) was releasable P. Based on 200 g wet sediment, the cores (top 10 cm) contained 65.38 (SD = 10.33) mg releasable P, which could be bound by 4.5 (SD = 0.7) g LMB per core or 2,316 (SD = 366) g m^{-2}. The applied LMB dosage in this experiment was approximately 1.5 times the amount needed to bind all releasable P present in the top 10 cm of the sediment in the cores.

Table 3.5 Total released = total amount of FRP released during the experiment, Releasable P = total amount of releasable P in 10 cm sediment (mg) from fractionated phosphorus extraction. Released FRP = total amount of FRP released during the experiment (mg), Total = total amount of P in 10 cm sediment (mg).

Releasable phosphorus in the core							
	units	aerobic		anaerobic		all cores	
		n = 4		n = 4		n = 4	
		mean	SD	mean	SD	mean	SD
Total released	mg	0.21	0.20	0.56	0.14	0.38	0.25
Releasable P	mg	66.23	11.81	63.76	10.52	65.00	10.44
total	mg	66.43	11.62	64.33	10.54	65.38	10.33

3.3.5.5 The effects of sediment capping agents on the sediment release of DIN.

The leaching of AMM from the LMB (mean = 248.37 mg.kg^{-1} (Table 3.6) quite stands out from the compounds, of which zeolite (mean = 14.84 mg.kg^{-1}) has the highest AMM leaching). The F-test revealed a significant difference among the compounds tested (Table 3.6). The all pairwise multiple comparisons separated the LMB from the three others as one homogeneous group. In NN leaching, no significant differences among the compounds were observed (Table 3.6).

During the first 24 h of the sediment release experiment, the release of DIN in the controls was (aerobic) 86.1 mg m^{-2} and (anaerobic) 86.2 mg m^{-2} (Table 3.6). In the LMB treated cores the first 24 h DIN release was (aerobic) 530.5 mg m^{-2} (aerobic) and 360.8 mg m^{-2} – hence much higher than in the control cores. However – with the LMB, the DIN release was lower than the 874.0 mg m^{-2} leaching (Table 3.6). While DIN leaching from all other compounds would indicate a higher DIN release as compared to the controls, all first 24 h releases fell below the DIN release in the controls (Table 3.6).

The Kruskal-Wallis test indicated a significant difference in DIN release rates among the controls and compounds. Under aerobic conditions H = 17.33, under anaerobic conditions H = 17.37, both with 4 degrees of freedom and p = 0.002.

Table 3.6 Mean and standard deviation (SD) of the Ammonium (AMM), Nitrite + Nitrate (NN) leaching from AMZ, LMB, zeolite and bentonite, Expected Dissolved Inorganic Nitrogen (DIN) and first day releases of DIN (A, See text), n = number of replicates, T = Student T-statistic, P = p-value, β power of the test done with α = 0.05, aerobic = aerobic conditions, anaerobic = anaerobic conditions, SD = standard deviation, , NT = not tested. Test results (B) Ammonium (AMM) and NN (Nitrite + Nitrate) leaching, Normality ((Shapiro-Wilk test), Equal Variances (Levene's test) , Compound Effect (one-way lay-out ANOVA), p = p-value, β = power of performed test done, α= 0.050.

A)	Leaching from the compounds		Expected DIN leaching	First day DIN release	
	AMM	NN			
	n = 4		n = 4	n = 4	
compound				aerobic	anaerobic
	mean(SD)	mean(SD)	mean(SD)	mean(SD)	mean(SD)
	(mg kg^{-1})	(mg kg^{-1})	(mg m^{-2})	(mg m^{-2})	(mg m^{-2})
control				86.1 (30.3)	86.2 (14.3)
AMZ	5.04 (1.97)	4.47 (5.01)	33.3 (16.6)	27.6 (9.0)	23.8 (6.9)
LMB	248.37 (19.10)	1.15 (0.87)	874.0 (67.0)	530.5 (108.6)	360.8 (29.9)
zeolite	14.84 (11.31)	2.20 (5.01)	59.6 (22.8)	34.3 (6.6)	23.0 (6.9)
bentonite	7.89 (5.39)	4.59 (3.16)	43.7 (27.7)	38.3 (6.6)	49.0 (11.4)
B)	Test results				
				p	β
AMM	Normality			0.6	
	Equal Variances			0.2	
	Compound Effect		$F_{(3,8)}$ = 326.6	< 0.001	1.0
NN	Normality			0.3	
	Equal Variances			0.5	
	Compound Effect		$F_{(3,8)}$ = 0.4	0.7	0.05

3.3.6 Effect of LMB on the growth of Scenedesmus and Microcystis

The growth rates (μ) for *S. obliquus* in the controls used in the different treatment groups were not significantly different (volume-based $F_{2,6}$ = 0.39; p = 0.7; chlorophyll-*a* based $F_{2,6}$ = 2.49; p = 0.2). The overall tests showed a significant dose effect on μ in all series (Table 3.7). In the LMB treatment, dosages ≥ 0.5 g L^{-1}, μ was reduced to negative values (Table 3.7). After resuspending the settled material, μ was significantly elevated (Fig. 3.6). The controls – without *S. obliquus*, revealed this was due to LMB particles (volume-based growth) and LMB fluorescence (chlorophyll-*a* based growth) at the highest dosages (Fig. 3.6).

Tukey's test revealed higher growth rates (μ) for *M. aeruginosa* in the controls of the LMB treatment than in the controls of the leachate and dissolved lanthanum nitrate, which we attribute to small within group variability ($F_{2,6}$ = 8.05, p = 0.02; Table 3.7). The overall tests showed significant dose effects on μ in the LMB and lanthanum treatments but not in the leachates (Table 3.7).

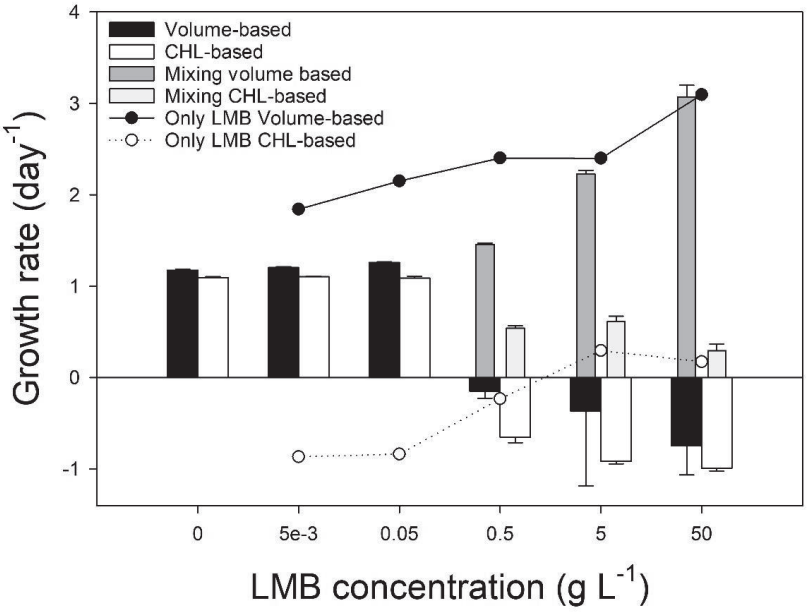


Figure 3.6 Volume-based (black bars) and chlorophyll-a based growth rates (white bars) of the green alga *Scenedesmus obliquus* exposed for three days to different concentrations of LMB. Also included are the growth rates in the highest dosages after thorough resuspension of the sedimented material (grey bars) and the data obtained when 'growth' was estimated from the difference between the initial algal inoculum and the particles in purely LMB incubations (symbols and lines). Error bars indicate 1 SD (n = 3).

Table 3.7 Growth rates (day^{-1}) of the green alga *Scenedesmus obliquus* and the cyanobacterium *Microcystis aeruginosa* exposed for 3 respectively 7 days to different concentrations of A) LMB (dose in g L^{-1}), B) filtrate from a 50 g LMB L^{-1} incubation, and C) lanthanum (dose in mg La L^{-1}). A, B, C, D Pairwise Multiple Comparison Procedures (Holm-Sidak method); a, b, c, d, Pairwise Multiple Comparison Procedures (Tukey Test).

			Scenedesmus		Microcystis
	Treatment	Dose	μ_{VOLUME}	μ_{CHL}	μ_{VOLUME}
			mean (SD)	mean (SD)	mean (SD)
A)	LMB	0	1.18 (0.01)^{ab}	1.09 (0.01)^{A}	0.35 (0.01)^{A}
		0.005	1.20 (0.01)^{ab}	1.11 (0.01)^{A}	0.36(0.03)^{A}
		0.05	1.26 (0.01)^{a}	1.09 (0.02)^{A}	0.40(0.01)^{A}
		0.5	-0.31 (0.12)^{ab}	-0.65 (0.06)^{B}	-0.26(0.04)^{B}
		5	-0.37 (0.82)^{ab}	-0.92 (0.03)^{C}	-0.21(0.04)^{B}
		50	-0.75 (0.32)^{b}	-0.99 (0.03)^{D}	-0.23(0.07)^{B}
	ANOVA		H_5 = 15.3	$F_{5,12}$ = 3578	$F_{5,12}$ = 241.8
	p-value		p < 0.01	*p* < 0.01	p < 0.01
B)	Filtrate	0%	1.19 (0.03)^{A}	1.11 (0.02)^{A}	0.33 (0.01)
		0.01%	1.19(0.01)^{A}	1.10 (0.01)^{AB}	0.34(0.00)
		0.1%	1.19(0.01)^{A}	1.08 (0.01)^{B}	0.31(0.02)
		1%	1.19(0.01)^{A}	1.07 (0.01)^{BC}	0.32(0.02)
		10%	1.15 (0.02)^{AB}	1.05 (0.01)^{C}	0.33(0.01)
		100%	1.12 (0.01)^{B}	1.00 (0.01)^{D}	0.33(0.00)
			$F_{5,12}$ = 11.5	$F_{5,12}$ = 45.4	$F_{5,12}$ = 2.79
	p-value		p < 0.01	p < 0.01	p = 0.07
C)	Lanthanum	0	1.19 (0.00)^{ab}	1.09 (0.01)^{A}	0.32 (0.01)^{A}
		0.025	1.19 (0.00)^{ab}	1.09 (0.02)^{A}	0.33(0.02)^{A}
		0.25	1.19 (0.01)^{a}	1.08 (0.01)^{A}	0.36(0.01)^{A}
		2.5	1.17 (0.02)^{ab}	1.05 (0.01)^{A}	0.34(0.00)^{A}
		25	0.64(0.07)^{ab}	0.16 (0.02) ^{B}	0.27(0.02)^{B}
		250	0.46(0.02)^{b}	0.17 (0.01)^{B}	0.19(0.03)^{C}
			H_5 = 13.7	$F_{5,12}$ = 2739	$F_{5,12}$ = 47.7
	p-value		*P* = 0.02	p < 0.01	p < 0.01

3.3.7 Effect of LMB on an Anabaena bloom

The acclimatization had no effect on the chlorophyll-a concentration of the incubations ($F_{3,8}$ = 1.83; p = 0.2). After treatment, a linear rate of change occurred in the chlorophyll-a concentrations in all incubations (Fig. 3.7). We observed growth in the control and 0.1 g LMB L^{-1}. Bloom termination occurred in the 0.25 and 0.5 g LMB L^{-1} dosages (Fig. 3.7). Adding ≥ 0.25 g LMB L^{-1} to the blooming *Anabaena* reduced the biomass with 11 µg chlorophyll-*a* L^{-1} day^{-1} (Fig. 3.7).

The slopes of the rates of change, were 41.5, 28.4, -9.8 and -12.2 for controls, 0.1, 0.25 and 0.5 g LMB L^{-1}. The test for the equality of the slopes revealed a significant effect of the LMB, $F_{(3,20)}$ = 149, p < 0.01. Three groups of significantly different slopes were identified: 1) control, 2) 0.1 g LMB L^{-1}, 3) 0.25 and 0.5 g LMB L^{-1}. The associated tests were: control versus 0.1, 0.2 and 0.5 g LMB L^{-1}: $F_{1,10}$ = 18.6 (p < 0.01), $F_{1,10}$ = 689.9 (p < 0.001), $F_{1,10}$ = 250.0 (p < 0.01); 0.1 LMB L^{-1} versus 0.25 and 0.5 g LMB L^{-1}: $F_{1,10}$ = 179.4 (p < 0.01), $F_{1,10}$ = 104.0 (p < 0.001); 0.25 versus 0.5 g LMB L^{-1}: $F_{1,10}$ = 0.53; p = 0.5).

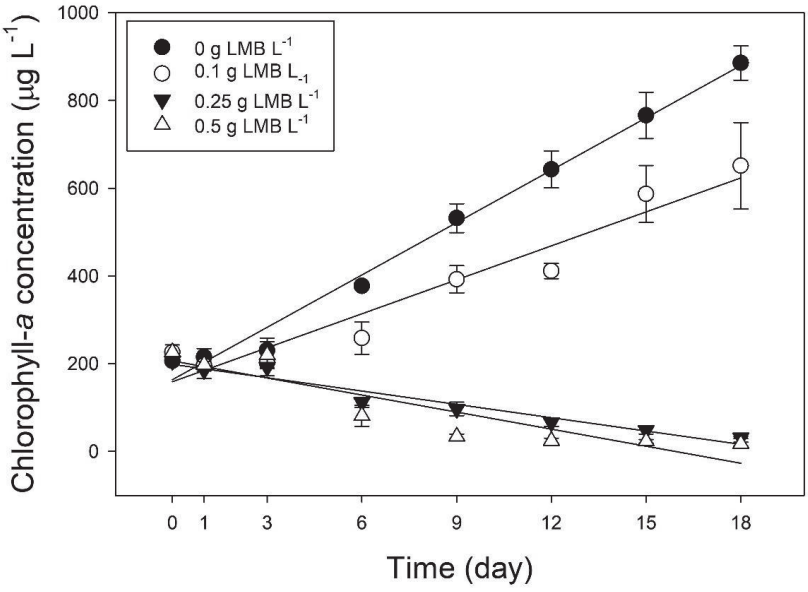


Figure 3.7 Course of the cyanobacteria chlorophyll-a concentration (µg L^{-1}) in *Anabaena cylindrica* populations exposed to different concentrations of LMB (0 – 0.5 g L^{-1}) over a 18 day period. Lines represent linear regressions, while error bars indicate 1 SD (n = 3).

3.3.8 Effect of LMB on growth of the rotifer Brachionus calyciflorus

Population growth of *B. calyciflorus* decreased significantly with increasing LMB concentrations ($F_{6,37}$ = 30.6; p < 0.01; Fig. 3.8). The 0.5 g L^{-1} LMB treatment revealed some mortality, concentrations ≥ 0.2 g L^{-1} significantly inhibited population growth (Fig. 3.8). The 5.0 g LMB L^{-1} dose contained too much clay for reliable observations and was excluded from the analysis. The EC_{50} based on finite rates of population increase was 0.154 (SD = 0.04) g LMB L^{-1} ($F_{3,6}$ = 30.4; p = 0.01; r^2_{adj} = 0.936). The No Observed Effect Concentration was 0.1 g LMB L^{-1} (Fig. 3.8).

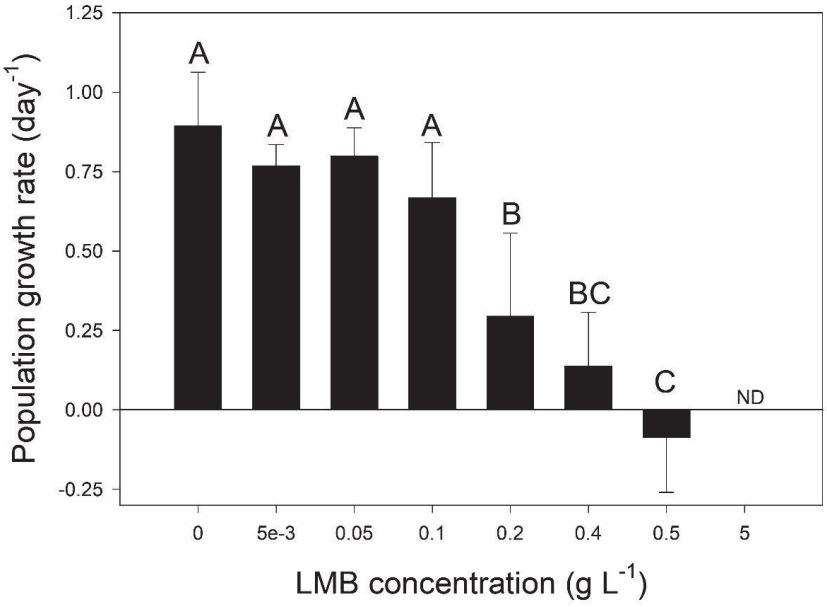


Figure 3.8 Population growth rate (day^{-1}) of the rotifer *Brachionus calycilforus* exposed for 48 hours to different concentrations of LMB (0 – 5 g L^{-1}). Similar symbols indicate homogenous groups (Tukey test), while error bars indicate 1 SD (n = 4 or 8). ND means not determined because of high turbidity.

3.3.9 Rapid settling of cyanobacteria

The chlorophyll-*a* concentrations at the start of the experiment, just before adding the LMB and/or PAC, were on average 845 µg L^{-1} and similar among tubes, as confirmed by a two-way ANOVA (Table 3.8). Two hours after the application of the treatments, most of the positively buoyant cyanobacteria had accumulated in the top layer of the controls and the sole PAC treatments and the sole LMB treatment. In the combined PAC and LMB treatments (Floc & Lock) they had accumulated at the bottom of the tube (Fig. 3.9). The two-way ANOVAs revealed significant LMB, PAC and interaction effects (Table 3.8). Post-hoc tests (Holm-Sidak multiple comparisons) on the chlorophyll-*a* concentrations as well as on turbidity in the top of the tubes revealed three homogeneous groups: 1) chlorophyll-*a* concentration and turbidity in the top of the tubes were highest in the controls, 2.2 mg Al L^{-1} treatment and the sole LMB treatment, 2) significantly lower in the 4.4 mg Al L^{-1} treatment, and 3) the lowest in the combined PAC (2.2 and 4.4 mg Al L^{-1}) and LMB treatments. Chlorophyll-*a* concentrations in the bottom of the tubes were similar in controls and sole PAC treatments, but significantly higher in the LMB treatments with the highest concentrations in the combined treatments of LMB and PAC (Fig. 3.9). The post-hoc tests (Holm-Sidak multiple comparisons) revealed that chlorophyll-*a* concentrations in the bottom of the tubes in the sole LMB treatment were significantly higher than in controls and sole PAC treatments, but significantly lower than in the LMB and PAC treatments. Also, in both LMB and PAC treatments, chlorophyll-*a* concentrations in the bottom of the tubes were significantly different from each other. The turbidity in the bottom of the tubes was not evaluated statistically as values in the combined LMB and PAC treatments exceeded the range of the turbidity meter (>1000 NTU). However, from the comparison of the turbidity in the tops of all tubes, it is evident that most material in the combined LMB and PAC treatments had settled to the bottom of the tubes (Fig. 3.9).

At the start of the experiment, Photosystem II efficiencies of the cyanobacteria were quite similar in the LMB treatments (0.50 ± 0.01) compared to the incubations without LMB (0.52 ± 0.01). Also, in incubations without PAC, 2.2 mg Al L^{-1} and 4.4 mg Al L^{-1} Photosystem II efficiencies were comparable and 0.51 (± 0.01), 0.51 (± 0.02), and 0.52 (± 0.01), respectively. After two hours Photosystem II efficiencies of the cyanobacteria in the samples from the top of the tubes were on average 0.47 (± 0.02) in the controls and sole PAC treatments and 0.51 (± 0.02) in the LMB treatments, while in the bottom samples it was 0.40 (± 0.04) and 0.48 (± 0.03), respectively. The addition of PAC lowered pH from 7.2 in controls to 7.1 in the highest PAC dose, while pH in all LMB treatments was 6.9. Electrical conductivity (EC) was 325 µS cm^{-1} in controls, 327 µS cm^{-1} in the highest PAC dose and 329-333 µS cm^{-1} in the LMB treatments.

Table 3.8 F- and p-values of two-way ANOVAs on start chlorophyll-a concentrations and Photosystem II efficiency in tubes with and without LMB at 0, 2.2 and 4.4 mg Al L^{-1} (PAC39) and on chlorophyll-a concentration, Photosystem II efficiency and the turbidity after 2 hours in the top of the tubes and the bottom of the tubes after two hours incubation. NT = not tested. Also given are results for pH and electric conductivity (EC) in the tubes.

	Chlorophyll-*a* concentration			Turbidity	
Source	Start	Top	Bottom	Top	Bottom
LMB	$F_{1,17}$ = 0.92	$F_{1,17}$ = 28.9	$F_{1,17}$ = 240.3	$F_{1,17}$ = 46.5	NT
	p = 0.4	p < 0.01	p < 0.01	p < 0.01	
PAC39	$F_{2,17}$ = 0.31	$F_{2,17}$ = 21.0	$F_{2,17}$ = 39.7	$F_{2,17}$ = 7.21	NT
	p = 0.7	p < 0.01	p < 0.01	p < 0.01	
LMB × PAC39	$F_{2,17}$ = 0.17	$F_{2,17}$ = 7.26	$F_{2,17}$ = 32.6	$F_{2,17}$ = 12.7	NT
	p = 0.8	p < 0.01	p < 0.01	p < 0.01	
	Photosystem II efficiency			pH	EC
Source	Start	Top	Bottom		
LMB	NT	$F_{1,17}$ = 39.3	$F_{1,17}$ = 28.5	$F_{1,17}$ = 145.7	$F_{1,17}$ = 59.2
		p < 0.01	p < 0.01	p < 0.01	p < 0.01
PAC39	NT	$F_{2,17}$ = 1.55	$F_{2,17}$ = 1.40	$F_{2,17}$ = 2.96	$F_{2,17}$ = 2.51
		p = 0.3	p = 0.3	p = 0.09	p = 0.1
LMB × PAC39	NT	$F_{2,17}$ = 3.47	$F_{2,17}$ = 0.09	$F_{2,17}$ = 4.24	$F_{2,17}$ = 11.3
		p = 0.07	p = 0.9	p = 0.04	p < 0.01

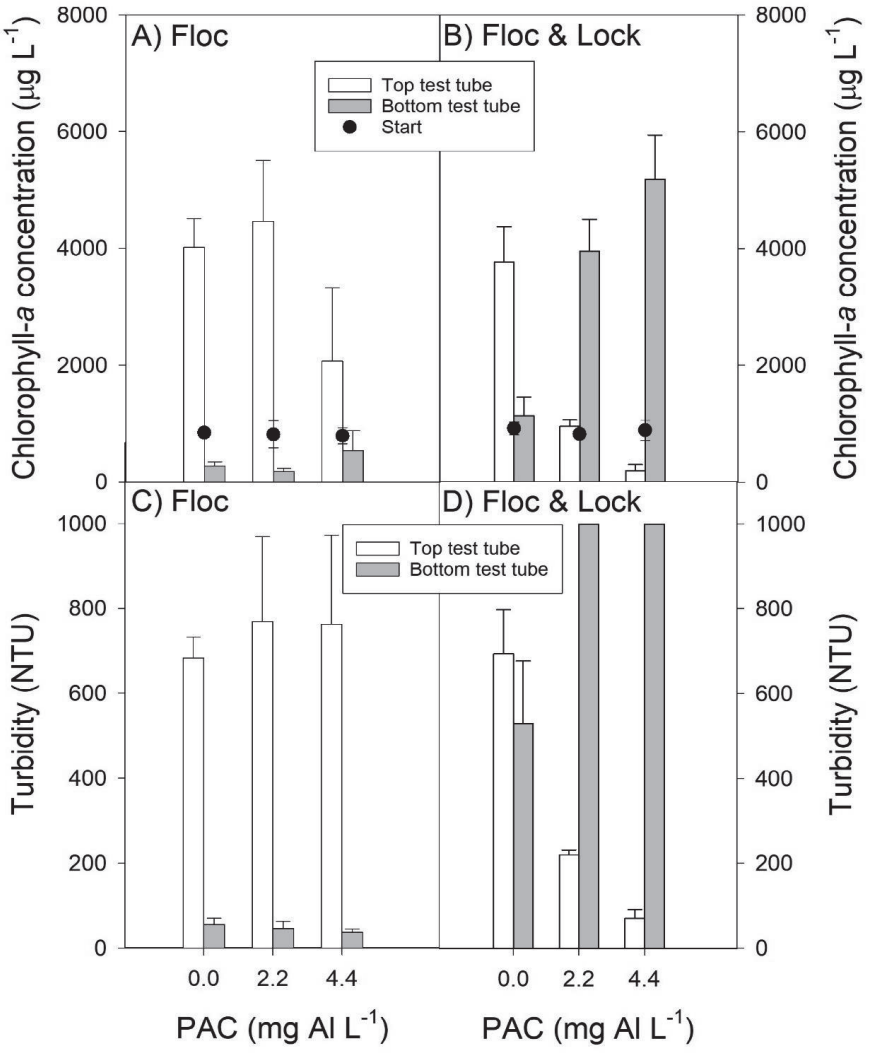


Figure 3.9 Chlorophyll-a concentrations (µg L^{-1}) and turbidity (NTU) after two hours of incubation in the top and bottom of test tubes with positively buoyant Microcystis aeruginosa at different concentrations of the flocculent PAC (A and C, Floc) and in combined treatments of different concentrations of the flocculent PAC and 390 mg L^{-1} of the LMB (B and D, Floc & Lock). Error bars indicate 1 SD (n = 3). The black symbols in the top panels A and B indicate the chlorophyll-a concentrations at the start of the experiment.

3.4 Discussion

3.4.1 Capacity of LMB to bind FRP

Our results confirm the reported FRP binding potential of LMB (Douglas et al. 1999; 2004; Finkler Ferreira and Da Motta Marques 2009; Robb et al. 2003; Ross et al. 2008). LMB was found effective in hampering sediment P release in laboratory settings (e.g. (Ding et al. 2012; Gibbs et al. 2011) as well as *in situ* (Meis et al. 2013).

We observed a dose depending decrease of FRP, which agrees with the expectation that LMB will be more effective in removing FRP from the water the longer it remains in suspension (longer reaction time), or a higher dose than the actual FRP concentration in the water column indicate is applied. In practice, the latter is the case as LMB is dosed according to the total amount of phosphorus present in both water and the top 5 or 10 cm of the sediment (Chapter 4). Our experiment was done with a FRP solution (K_2HPO_4 in nanopure water) lacking compounds which hamper the formation of rhabdophane. The effectiveness of lanthanum to bind FRP is hindered by naturally occurring oxyanions other than phosphate (Johannesson and Lyons 1994) and complex forming humic substances (Lürling et al. 2014; Sonke and Salters 2006; Tang and Johannesson 2003; 2010). Longer-term incubations of LMB and humic acids revealed that the initial hindrance is kinetic in nature and will be overcome in time, meaning that it will take longer for lanthanum in LMB to form rhabdophane with phosphate in the presence of humic acids (Dithmer et al. 2016a). The formation of rhabdophane and to a lesser extent monazite in sediments of lakes that had been treated with LMB underpins that also in situ lanthanum eventually precipitates with phosphate (Dithmer et al. 2016b).

3.4.2 Nutrient leachates from LMB

The amount of lanthanum present in LMB should make our observed release of FRP impossible. The FRP found in the leachates may be an overestimation of the molybdate-reactive phosphorus concentrations due to colloidal bentonite particles that pass through a 0.45 µm membrane filter (Koopmans et al. 2005). This implies both the careful interpretation of FRP concentrations and possible misidentification of other compounds (e.g. La) as being dissolved compounds.

The ammonium release could be attributed to the soluble fraction in the bentonite (Hanway et al. 1956). Gibbs et al. (2011) observed NH_4-N release from LMB treated sediment samples, which was probably caused by an effect on the nitrification process comparable with anaerobic sediments. The implications of the release of ammonium as well as the apparent difference between batches needs further investigation.

3.4.3 Effect of LMB on pH, conductivity and oxygen concentration

The dosing in our pH, EC and oxygen saturation experiments (0.05 to 3.2 g L^{-1} LMB) are high compared to field dosing (0.046 and 0.085 g L^{-1}) applied in The Netherlands (Chapter 4, (Lürling and van Oosterhout 2013; Spears et al. 2013b; Waajen et al. 2016a). Hence, we do not expect a relevant reduction of pH or conductivity during actual applications. The difference between algal growth medium and pond water in dose-effect on EC cannot be explained by our experiment. Although outside the actual field dosing range, it indicates different effects of LMB on EC in waters of different compositions.

3.4.4 The effect of LMB on turbidity in still water

We expect an application of LMB to first cause an increase in turbidity – due to suspended LMB particles, followed by a reduction in turbidity- due to growth limitation of phytoplankton (discussed below). The increase in turbidity is difficult to predict. An application will be in an eutrophicated body of water with elevated NTU, while the settling of LMB particles will depend on turbulent water movements, dictated by local circumstances.

Our turbidity experiment was performed in still water, meaning a maximum undisturbed settling rate of LMB particles in concordance with the results in other studies (Haghseresht 2005; Ross et al. 2008). In Lake Rauwbraken (The Netherlands), adding LMB to the water after a flocculent addition caused a two-day period of elevated turbidity up to 18 NTU (Chapter 4). Having LMB suspended in the water column for a few days may enhance its effectiveness as it depletes the water of FRP further than a rapid sedimentation would.

3.4.5 The sediment capping mechanism

We tested the sediment capping mechanism on intact cores with an approximate 3.0 g P kg^{-1} dry sediment of which 72.5 % was releasable P. Based on 59 lakes reported in literature, the sediment P content may range from 0.47 to 7.5 (24) mg kg^{-1} with a median = 1.7 mg kg^{-1} dry weight (Boström 1984; Kleeberg & Kozerski 1997; Hupfer *et al.* 1998; Burley *et al.* 2001; Berg *et al.* 2004; Schauser *et al.* 2006; Hickey & Gibbs 2009). We assumed that the P content in the upper 2 cm of the sediment is a good proxy for the P content of the deeper lays in the sediment. This may have resulted in an underestimation of the total amount of P in the whole core, e.g. in Lake Søbygaard (Denmark) Søndergaard *et al.* (1999) found higher total P in the deeper sediment layers than in the top 2 cm. Hence the sediment P content of our test cores can be considered quite high compared to those reported in literature.

In this experiment, in the anaerobic incubations, we aimed at oxygen concentration less than 0.01 mg L^{-1}. Our results show that the oxygen concentrations in the anaerobic incubations were above this level (Appendix Table 3.9), which we attribute to the diffusion of oxygen from the surrounding atmosphere into the cores, meaning that our cores were not closed systems. In the cores treated with

AMZ and LMB bacteria were P-limited, hence oxygen consumption was lower than in the control cores and those treated with zeolite and bentonite, where no P-limitation occurred.

The LMB dosage was 1.5 times the amount needed to bind all releasable P from the sediment. For the AMZ, the dosage was a tenfold of the recommended dosage (i.e. recommended dosage 350 g m^{-2} (Gibbs *et al.* 2007). Field applications of the LMB may range 6 to 667 g m^{-2} (median 349 g m^{-2}) (Spears *et al.* 2013), which means that the amount of the LMB that would be needed to bind all releasable P in the sediment used - i.e. 2,316 g m^{-2} (SD = 366 g m^{-2}), is quite above those reported in field applications.

The nutrient leaching experiment indicated that significant amounts of FRP may be released from zeolite and bentonite. If the FRP leaching from zeolite or bentonite is computed to a sediment release equivalent for this experiment, the amount of applied zeolite (6.828 g) could result in a FRP release of 13.2 mg m^{-2} day^{-1} (Table 3.3). Likewise the amount of applied bentonite (6.871) could result in a release of 11.3 mg m^{-2} day^{-1} (Table 3.3). As compared to the control cores, we only observed an increase in the FRP released during the first 24 h in the cores treated with zeolite under aerobic conditions. This may be attributed to FRP leaching from the zeolite.

As computed over the whole sediment release experiment we observed a FRP release of approximately 27 mg m^{-2} day^{-1} in the control cores under aerobic conditions. This release was higher (approximately 74 mg m^{-2} day^{-1}) under anaerobic conditions. (Gibbs *et al.* 2011) used sediments that under aerobic conditions absorbed some P, while under anaerobic conditions it released some 25 mg P m^{-2} day^{-1}. Hence, like the sediment P content, the FRP release in our control cores is high as compared to those reported in literature. The higher FRP release under anaerobic conditions is in agreement with the expectations from literature (i.e.: Boström *et al.* (1988b)). The application of zeolite did not reduce the FRP release under aerobic conditions, which is in agreement with the FRP leaching from zeolite. Otherwise, the application of the unmodified zeolite and bentonite did result in some a reduction of the FRP release. This reduction of FRP release may be due to a physical hampering of the diffusion from FRP, or absorption of FRP by the clays (or both). However, in as far as zeolite (under anaerobic conditions) or bentonite may reduce the release of FRP from the sediment, this effect is too low to make these compounds effective as treatment to mitigate the release of FRP from a lake's sediment.

Our results indicate that – under the test conditions, both the AMZ and LMB have a strong capacity to reduce the release of FRP from a sediment under aerobic and anaerobic conditions. To indicate the order of magnitude of the treatment effects: the sediment FRP release in the controls was approximate 27 mg m^{-2} day^{-1} (aerobic conditions) an 75 mg m^{-2} day^{-1} (anaerobic conditions), with both the AMZ and LMB the FRP releases were reduced down to an approximate 1 mg m^{-2} day^{-1} under both aerobic and anaerobic conditions. Hence under the test conditions of our experiment, the AMZ and LMB can be considered equally effective in reducing the FRP release from the sediment. Noteworthy though,

our results also indicate - that despite the strong reduction, small amounts of FRP may still be released from the sediment. Hence the possible underestimation of the sediments P content may have resulted in a dosages of the AMZ and LMB that were too low to fully stop the FRP release. However, part of the observed FRP release may have been due to humic substances that interfere with the FRP binding capacity by Al of La – which was demonstrated for the LMB by (Lürling *et al.* 2014).

Regarding the experimental testing of candidate compounds to achieve a reduction in FRP release from a lake's sediment it is worth mentioning that the sediment used in the test should actually release meaningful amounts of FRP if untreated. While our experiment was done on cores that released some 75 mg P m^{-2} day^{-1}, Zamparas *et al.* (2013) tested Zenith/Fe as sediment capping agent on cores which – in case not treated, released 0.66 mg P m^{-2} day^{-1}. Hence the 68% reduction of the P release by Zenith/Fe – as reported by Zamparas et al. (2013), can be considered a misleading result.

3.4.6 The effects of sediment capping agents on the sediment release of DIN.

The AMM and NN leaching experiment indicated that all compounds might potentially leach DIN. If one assumes that the leaching of DIN from the compounds would add to the release of DIN from the sediment, the observed release of DIN during the first 24 h would be the sum of the release in the controls plus the leaching. A comparison of the expected DIN leaching to the release of DIN measured during the first 24 h of the experiment revealed that this was not the case. In fact, for the AMZ, zeolite and bentonite the DIN leaching during the first 24 hours fell below the DIN from the control cores, which was not the case for the LMB treated cores. As estimated over the whole experiment sediment release experiment the AMZ, zeolite and bentonite all reduce the release of DIN, while with the LMB an increase is observed. The effects of a sediment capping agent on the sediment release of DIN may run through absorption of DIN (mostly AMM), leaching of DIN or indirectly through P-limitation on the bacterial processes ammonium nitrification, denitrification and anaerobic ammonium oxidation (Anammox).

Regarding the big difference in AMM release between the LMB, AMZ and zeolite a lanthanum modified zeolite, combining the P-binding of lanthanum with the effect of zeolite on AMM release seems an obvious way to go, which was done by (Wang et al. 2017; Yin et al. 2018, see Chapter 7 for a historical perspective of modified clays) .

3.4.7 Effect of LMB on growth of Scenedesmus and Microcystis

Based on the 5% La content given by the manufacturers of LMB the 0, 0.005, 0.05, 0.5, 5.0 g L^{-1} dosages of the LMB series should bind equal amounts of FRP as the respective 0, 0.25, 2.5, 25 and 250 mg L^{-1} dosages of the lanthanum nitrate series. From this, we expected to observe similar reductions in the growth rates of S. *obliquus* respectively *M. aeruginosa* by the LMB suspension and free dissolved lanthanum nitrate series at the related dosages. Based on a 4.3% lanthanum content in our batch

(Lürling and Tolman 2010), the lanthanum nitrate dosages should be more effective than their equivalent LMB dosages. We attribute the larger effect of LMB to the presence of the bentonite particles, which may have caused the coagulation of phytoplankton cells with the bentonite. Several studies have shown that flocculation with natural clay – without modification with lanthanum, can control existing blooms (Pan et al. 2006a; 2011a; 2011b; Verspagen et al. 2006). E.g. a bentonite concentration as low as 15 mg L^{-1} resulted in increased sedimentation of *Microcystis* (Verspagen *et al.* 2006). Otherwise, elevated turbidity due to the presence of LMB will cause strong light inhibition in addition to the coagulation. For this effect we had no control in our experiment. The reduced growth observed with dissolved lanthanum nitrate shows that the La-FRP bond is strong enough to ensure FRP is no longer bioavailable. The leachates of LMB contain between 0.001 and 0.02% of the lanthanum present in LMB (Lürling and Tolman 2010; NICNAS 2001). Thus, the expected growth inhibition based on the binding of FRP to lanthanum is much lower than either the LMB suspension or lanthanum nitrate solution will cause.

3.4.8 Effect of LMB on an Anabaena bloom

Concerning the *Anabaena cylindrica* bloom control experiment similar remarks apply as were described for *S. obliquus* and *M. aeruginosa.* Because we observed rapid FRP binding in the FRP binding experiment, it seems safe to attribute at least part of the observed effects to FRP binding by LMB. Due to the absence of the bentonite control, these experiments cannot be considered as a formal proof of growth inhibition through FRP binding by application of LMB. Still, LMB can be considered effective in reducing phytoplankton growth. The combined effects of both FRP binding and flocculation of phytoplankton cells may prove quite useful in actual field applications.

Our turbidity experiment was done in still water, while our algal growth experiments were done under constant agitation - two extreme opposite ends of the natural conditions. Hence our results on turbidity and reduced algal growth should be interpreted as best-case scenarios, not likely to occur this positive under field conditions.

3.4.9 Effect of LMB on the growth of the rotifer Brachionus calyciflorus

The population growth of the rotifer *B. calyciflorus* was reduced by concentrations of 0.2 g LMB L^{-1} and higher. The observed effect we can only attribute to the presence of LMB, i.e. we cannot distinguish between the effect of particles hampering the feeding or any other possible toxic effect. We consider concentrations of LMB above 0.2 g L^{-1} relatively high as compared to an actual field dosing (e.g. 0.046 and 0.085 g L^{-1} (Chapter 4). However, LMB concentrations during and shortly after the addition through a spray manifold will be much higher than the estimated EC_{50} for growth inhibition (*ca.* 0.15 g L^{-1}). Therefore, effects of a field application on rotifers cannot be excluded.

3.4.10 Rapid settling of cyanobacteria

The combination of LMB and PAC made the cyanobacteria sink to the bottom of the test tubes, whereas they accumulated at the water surface in the controls, the sole PAC treatments and the sole LMB treatment. Hence, in analogy with a SCUBA diver that uses a lead belt to compensate for the buoyancy of his dive suit, the addition of the modified clay provided the PAC-induced flocs with sufficient ballast to counteract the positive buoyancy of the *M. aeruginosa* used in the laboratory experiment. The use of clays as ballast to sink phytoplankton despite the organisms' motility and buoyancy, is well-known in marine environments, where spraying clay as slurry to the water surface is a common measure to mitigate harmful algal blooms (Anderson 1997; Hagström et al. 2010; Sengco and Anderson 2004). However, in freshwater systems, the low ionic strength impairs clay flocculation of cyanobacteria (Han and Kim 2001; Pan et al. 2006a) and removal efficiency furthermore depends strongly on clay type and cyanobacteria stickiness (Verspagen et al. 2006). It is evident from our incubations with solely the modified clay that just LMB addition is ineffective in removing cyanobacteria from the water column.

If the goal is to rapidly remove cyanobacteria from the water column algaecides are frequently used (Jančula and Maršálek 2011). Algaecides induce cyanobacterial cell lysis that subsequently leads to a release of nutrients (Coloma et al. 2017) and intracellular toxins (Jančula and Maršálek 2011; Jones and Orr 1994). Such cell lysis of cyanobacteria has led to the intoxication of humans with cyanobacterial toxins (Azevedo et al. 2002; Hawkins et al. 1985). Hence, preferably, cell lysis is avoided during bloom removal. Alternatives to cell-lysing algaecides are interventions based on flocculation in which cells are glued together and sunk out of the water. To improve the removal efficiency of cyanobacteria such as *M. aeruginosa* addition of the flocculent chitosan to natural soils and clays has been proposed (Pan et al. 2006a; 2006b; 2012; Zou et al. 2006). A study provided evidence for cell lysis of *M. aeruginosa* following a chitosan-clay treatment (Shao et al. 2012) and thus release of cell contents. Another study reported on *M. aeruginosa* cell lysis after alum treatment, but only found effects at a relatively high dosage of 14-15 mg Al L^{-1} where pH effects cannot be excluded and not at 7 mg Al L^{-1} (Han et al. 2012). In our experiment cells were flocculated as intact cells and not lysed as was evident from the Photosystem II efficiency, because in the case of a strong cell lysis a sharp drop in Photosystem II efficiency would have been expected (Hu et al. 2008; Lürling and Beekman 2010). In addition, in this type of experiment, cell lysis can readily be observed as cell lysis would turn the contents of the test tubes homogeneously green or yellow, depending on the species used. We visually observed the accumulation of cells/colonies in either tops or bottoms of our test tubes, homogeneous colouring was absent. This visual observation was confirmed by the Phyto-Pam measurements. Our observation of the absence of cell lysis is fully in line with the results of other studies that showed comparable Al doses (2.4 – 5.8 mg Al L^{-1}) were sufficient to floc *M. aeruginosa* and kept cells viable and intact (Chow et al. 1999) or even higher Al dose that had no effect on cell viability (Drikas et al. 2001).

3.5 Conclusions

- Our results confirm the potential of LMB and AMZ to bind FRP; its effectiveness in natural waters to bind FRP needs further investigation, especially in the presence of other oxyanions and humic substances.
- As the effectiveness depends on both dose and reaction time, LMB seems more promising as a sediment capping agent to reduce the release of FRP from the sediment than as a FRP binder in the water column.
- The small amounts of FRP released from LMB have no consequence on the effectiveness of LMB and most probably is caused by an analytical artefact.
- The implications of the differences between batches of LMB and its release of ammonium need further investigation.
- AMM release from the sediment is an important in-lake nitrogen source, this release can be reduced by sediment capping with a zeolite.
- LMB had no relevant effect on pH and oxygen saturation in the water types we tested. Albeit at concentrations above actual field applications, the effect of LMB on conductivity varies with water types.
- An application of LMB will temporarily increase turbidity. Due to its rapid settlement, prolonged light limitation for submerged macrophytes is not expected from a field application of LMB.
- Under constant agitation, LMB causes a dose-related reduced growth of phytoplankton species *S. obliquus* and *M. aeruginosa* and it was effective in controlling an *Anabaena sp*. bloom during prolonged exposure in our experiment. This effect can be attributed to FRP binding in combination with light limitation and flocculation of the phytoplankton cells with the bentonite, which we might be less under field conditions due to the rapid settlement of the LMB particles.
- The population growth of the rotifer *B. calyciflorus* is reduced by the presence of LMB (EC_{50}= 0.15 g L^{-1}). Overall, the results of our study, i.e. FRP binding and phytoplankton growth reductions in combination with marginal effects on water quality variables, do not give reasons to be reluctant to conduct field trials with LMB.
- The relatively low dose of the flocculent PAC (2.2 and 4.4 mg Al L^{-1}) was insufficient to sediment positively buoyant cyanobacteria. The LMB (390 mg L^{-1}) was insufficient to sediment positively buoyant cyanobacteria. The combination of PAC and LMB effectively sedimented cyanobacteria flocs.

3.6 Appendix

The experimental conditions for the sediment capping mechanism experiment are given in (Table 3.9).

Table 3.9 Amounts of compound added (A, all n =4), Temperature (B), Oxygen concentrations (C), pH (D) and Electrical conductivity (E); B-E all n = 16, [1] n= 15.

A) Amount of compound added				
	aerobic		anaerobic	
compound	mean (g)	SD (g)	mean (g)	SD (g)
AMZ	6.875	0.009	6.873	0.005
zeolite	6.868	0.012	6.873	0.011
LMB	6.874	0.008	6.872	0.008
bentonite	6.871	0.004	6.874	0.005
B) Temperature				
	aerobic		anaerobic	
compound	mean (°C)	SD (°C)	mean (°C)	SD (°C)
control	20.8	0.8	20.5	
AMZ	[1]20.5	1.0	20.8	0.7
zeolite	20.7	1.1	20.8	0.8
LMB	20.5	1.2	20.6	0.8
bentonite	20.6	1.2	20.8	0.8
C) Oxygen concentrations				
	aerobic		anaerobic	
compound	mean (mg L^{-1})	SD (mg L^{-1})	mean (mg L^{-1})	SD (mg L^{-1})
control	8.7	0.3	0.6	0.6
AMZ	[1]8.9	0.2	1.4	1.0
zeolite	8.8	0.3	0.3	0.3
LMB	9.0	0.3	1.3	0.9
bentonite	8.9	0.3	0.6	0.6

Table 3.9 continued

D) pH				
	aerobic		anaerobic	
compound	median	min - max	median	min - max
control	7.5	7.2 - 7.8	7.1	6.8 - 7.4
AMZ[1)]	[1)]7.2	5.4 - 7.6	6.6	4.7 - 7.2
zeolite	7.3	6.8 - 7.8	7.1	5.0 - 7.8
LMB	7.3	7.0 - 7.9	7.2	6.4 - 7.9
bentonite	7.6	7.2 - 7.8	7.3	6.9 - 8.0

E: Conductivity				
	aerobic		anaerobic	
compound	mean (μciem cm^{-1})	SD (μciem cm^{-1})	mean (μciem cm^{-1})	SD (μciem cm^{-1})
control	157.9	95.9	72.4	16.6
AMZ	[1)]747.2	218.5	580.4	101.2
zeolite	203.6	265.9	106.1	158.6
LMB	326.5	95.4	257.6	41.9
bentonite	128.8	61.3	82.5	10.4

J.F.X. van Oosterhout

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Chapter 4

Direct treatment effects of Floc & Lock

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Oosterhout, F. van and M. Lürling, 2011. Effects of the novel 'Floc & Lock' lake restoration technique on *Daphnia* in Lake Rauwbraken (The Netherlands) Journal of Plankton Research 33: 255-263.

Lürling, M. and F. van Oosterhout, 2013. Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation. Water Research 47 (17): 6527-6537.

4 Direct treatment effects of Floc & Lock

4.1 Introduction

External nutrient load reduction is widely seen as the first step to reduce eutrophication, hence to mitigate cyanobacterial nuisances (Hamilton et al. 2016; Paerl et al. 2016b). For Lake Rauwbraken we estimated that the external P load was only 15% of the total P load (8.03 mg P m^{-2} day^{-1}) to the lake's water column (**Chapter 2**). Based on this knowledge, we chose to mitigate the cyanobacterial nuisance by reducing the internal phosphorus loading, without curtailing external nutrient loads. The in-lake Floc & Lock (**Chapter 1**) treatment was applied in April 2008 and comprised of the combination of a flocculent Poly Aluminium Chloride (PAC; Floc) and Lanthanum Modified Bentonite (LMB; Lock) as phosphorus fixative (**Chapter 3**).

Being small businesses, fresh water recreational facilities face serious economic consequences resulting from blooms of cyanobacteria (e.g.: (Dodds et al. 2008)). Thus, for the simple reason of business continuity, there is a call for quick fixes of cyanobacterial nuisances. The current chapter demonstrates the curative effects of the Floc & Lock treatment. We report on the direct treatment effects observed in Lake Rauwbraken during 2008 on chlorophyll-a, cyanobacteria, Secchi-disk depth, turbidity, total phosphorus (TP), total nitrogen (TN), inorganic suspended solids (ISS), dissolved oxygen concentration, and saturation and pH. We treat 2008 as a transitional year between the direct treatment effects and the longer-term study (**Chapter 5**). We choose to do so, because part of 2008 belongs to the pre-treatment period, hence the annual means of water quality variables will be difficult to interpret. Also, the effects of adding the flocculant (PAC) and LMB will disproportionally affect the annual means of the chlorophyll-a concentrations and turbidity.

We proverbially test the hypothesis (see methods) that the Floc & Lock treatment, using PAC and LMB, is an effective curative method to mitigate cyanobacterial nuisance. In the week after the treatment, *Daphnia galeata* (the main cladoceran in Lake Rauwbraken) had disappeared from the water column. We monitored the *Daphnia* population in Lake Rauwbraken before and after the application and did short-term grazing experiment with *D. galeata*, to test the hypothesis that the addition of the flocculent and/or the modified clay reduces the feeding activity of the animals.

4.2 Methods

4.2.1 Chemicals

The flocculent AquaPAC39 (poly aluminium chloride, $Al_n(OH)_mCl_{3n-m}$, ρ = 1.37 kg L^{-1}, 8.9% Al, 21.0% Cl) and the lanthanum-modified bentonite – (LMB; Phoslock®) 5% La were supplied by Phoslock Europe GmbH (Ottersberg, Germany) as was calcium hydroxide that was used for buffering PAC during the whole lake application.

4.2.2 The Floc & Lock treatment in Lake Rauwbraken

The Floc & Lock treatment was applied in three days, from April 21st to 23rd 2008 (Fig. 4.1, Fig. 4.2). On the first day, 2 tons of the LMB were applied, as sinking weight for the flocculation applied the next day. The second day 2 tons of flocculent (poly aluminium chloride, PAC), pH buffered by 75 kg $Ca(OH)_2$, were applied (flocculation, 'Floc'). On the third day, 16 tons of the LMB were applied as sediment capping, to counteract P-efflux from the legacy P in the lake bed ('Lock'). All compounds were applied at the water surface after mixing with lake water from a GPS (Global Positioning System) coordinated barge to cover the whole lake surface. Thus, the LMB was applied as a slurry, and the flocculent was strongly diluted. The application was done by Phoslock Europe GmbH.

The dosage of LMB was based on the amount of P present in the water column and the potentially releasable P fraction from the top 5 cm of the sediment. The sediment P content (1 mg P kg^{-1} wet weight) was determined at the Limnological Institute Dr. Nowak according to ISO 11885-E22:1997-11.

Shortly before the treatment, the mean water column total phosphorus (TP) concentration was 160 µg L^{-1} and with an estimated 214,000 m^3 volume of Lake Rauwbraken (Chapter 2) this yielded approximate 34 kg phosphorus present in the water column. The sediment contained up to an estimated 1 mg P kg^{-1} wet weight, while the 5 cm layer comprised an approximate 150 tons this yield an estimated 150 kg of releasable P. Rounding off to the nearest 5 kg, this yielded a total amount of 185 kg available P to which 18 tons of LMB were applied in a dosing ratio of 100:1 as recommended by the manufacturer.

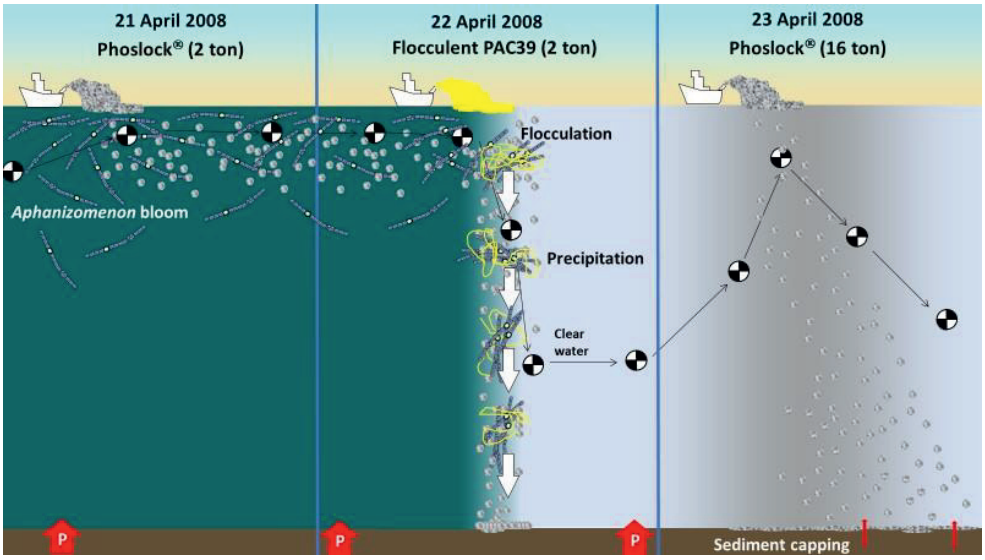


Figure 4.1 The Floc & Lock Treatment of Lake Rauwbraken. See text

Figure 4.2 The Application of LMB at Lake Rauwbraken

4.2.3 Lake Monitoring

Water column sampling, field measurements and analytical methods were described in Chapter 2. Since auto-analyser P determinations are based on molybdate-reactive P, which is sensitive to colloidal bentonite particles (Koopmans et al. 2005), TP and FRP concentrations were also done by ICP-MS at the Chemical-Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre). The La and Al concentrations in non-filtered and filtered samples were determined by ICP-MS at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University). Level of detection (LOD) filterable La 0.02 μg L^{-1}; total La 0.2 μg L^{-1}; filterable Al 0.03 μg L^{-1}; total Al 0.09 mg L^{-1}.

Absorption by humic substances was measured using a Beckman Coulter DU730 photo spectrometer. During and just after application (April 20th -27th) daily samples were taken at depths 1, 3, 5, 7 and 10 m, where after in the remainder of 2008, sampling frequency was reduced to biweekly. Sampling continued in 2009 at depths 0 – 10 m. From these samples, only the *Daphnia* counts are reported in this chapter. From each sampled depth, zooplankton was collected by fixation of 1 L lake water with Lugol's fixative. Fixated samples were kept refrigerated and dark. *Daphnia* was counted under a dissecting microscope at 15× magnification.

We did not statistically test the direct treatment effects of the Floc & Lock treatment on water quality variables. Statistical tests should be based on controlled experiments and random samples of ample size to reach sufficient power. Our study of lake Rauwbraken is a n = 1 study. Hence, from a methodological point of view, our study lacks a control lake. Also, our study is not based on a representable random sample of Dutch lakes, which means that one cannot straightforwardly extrapolate statistical tests done on our results to other lakes.

4.2.4 Grazing experiment

The effects of the flocculent ('Floc') and the modified clay ('Lock') on the grazing activity of *D. galeata* were examined in a short-term grazing experiment. Similarly sized *D. galeata* (1.9 mm ± 0.1 mm; *n* = 40) were placed in filtered (0.45 μm) water from Lake Rauwbraken to which the green alga *Scenedesmus obliquus* was added as food. 'Floc' and 'Lock' were tested separately and in a mixture using the dosages as had been applied in Lake Rauwbraken, i.e. 9 mg L^{-1} 'Floc' and 67 mg L^{-1} 'Lock'; an additional series was included with 10 times the 'Lock' concentration, since the addition as a slurry to the surface of the water column could also have implied a temporarily higher clay concentration.

The animals were collected from a stock culture, maintained in the laboratory and fed three times a week with *S. obliquus*. The green alga *S. obliquus* SAG 276/3a originated from the culture collection of the University of Göttingen (Germany). *S. obliquus* was maintained in 1.0 L chemostat

systems in continuous light of 120 µmol quanta m^{-2} s^{-1} at 20°C on a slightly modified WC medium (Lürling and Beekman 1999) and with a dilution rate of 1.1 day^{-1}. Prior to the experiment, animals were selected, rinsed with filtered Lake Rauwbraken water and placed in Lake Rauwbraken water for three hours. Then individual animals were allocated to one of five treatments in a well in 24 well culture plates. Each well held 2.5 mL food suspension (62 ± 2 µg L^{-1} chlorophyll-a) in lake water with no additive (controls), only flocculent ('Floc'), only P-fixative ('Lock') both flocculent and P-fixative ('Floc & Lock') and ten times the dose of the mean *in situ* treatment (10× 'Lock'). Each treatment was run in eight replicates, while four wells per food treatment without a *D. galeata* served as algal controls. The well plates were incubated for 3-4 h at 22°C in the dark. The clearance rates were calculated from the decrease in chlorophyll-*a* derived from dark-adapted fluorescence using a PHYTO-PAM (Walz, Germany) according to the equation:

$$CR = \frac{[\ln(CHL_{ctrl}) - \ln(CHL_{daphnia})]}{\Delta t} \text{ x } 2.5\text{x}1,$$

in which *CR* = the clearance rate (mL $ind^{-1}h^{-1}$), CHL_{ctrl} = the chlorophyll-*a* concentration (µg L^{-1}) in the control wells after an incubation period *t*, $CHL_{daphnia}$ = the chlorophyll-*a* concentration (µg L^{-1}) in the treatments wells after an incubation period *t* (h), 2.5×1 = the amount of medium per individual *D. galeata* (mL $ind.^{-1}$; ind. = individual). Clearance rates were compared using one-way ANOVA followed by a Tukey's post-hoc comparison test. At the end of the experiment, the body-length of each experimental animal was measured from just above the eye to the base of the tail spine using a dissecting microscope at 40× magnification.

4.2.5 Survival experiment

Since the application was expected to cause a strong reduction in phytoplankton concentration up to 90% (Schumaker et al. 1993), a survival experiment with *D. galeata* was performed. Animals were placed in 1 L jars that contained 800 mL of 0.45 µm filtered Lake Rauwbraken water (No food), lake water with 2 µg L^{-1} CHL-a *S. obliquus* and with 20 µg L^{-1} CHL. Food concentrations were checked after 4 and 8 days using a PHYTO-PAM and readjusted to the desired concentrations. Each treatment was replicated three times and each jar received ten non-egg bearing *D. galeata* from our stock cultures. After 1, 4, 8, 9 to 15 days jars were inspected for survival. In case of reproduction neonates were removed. Death rates were calculated from linear regressions on natural-log transformed survival data. The death rates were compared by one-way ANOVA and significantly different means were distinguished by a Tukey post-hoc comparison.

4.3 Results

4.3.1 Floc & Lock in Lake Rauwbraken

In April 2008, an *Aphanizomenon flos-aquae* bloom was present in the water column throughout the lake. Shortly before the treatment the cyanobacterial nuisance was growing daily. On the first day, before the treatment started, both the Water Framework Directive (WFD; EU 2000) and Bathing Water Directive (BFD; EU 2006) water quality standards were violated by the presence of cyanobacteria in extremely densities. In the bathing zone, where the WFD and BFD target in The Netherlands limited cyanobacterial chlorophyll-*a* to a maximum of 12.5 µg L^{-1} (Ibelings et al. 2012), there were a scum (5 cm thick, 100 m^2 held, approximate 17,000 µg L^{-1} chlorophyll-*a*) and an accumulation (some 500 m^2, exact depth unknown, 600 µg L^{-1} chlorophyll-*a*). As checked by light microscope both scum and accumulation consisted of 100% *A. flos-aquae*. Note: since 2020, the lower boundary for cyanobacterial chlorophyll-*a* of risk level 1 is 12 µg Chl-a L^{-1} (Schets et al. 2020).

The first day of treatment fully suspended both scum and accumulation into the water column. The application of PAC (second day), effectively sunk the former scum and accumulation biomass to the sediment – the latter was checked by visual inspection all-round the lake, confirming their absence elsewhere. Hence, in the bathing zone the treatment effect was a reduction in chlorophyll-*a* ranging from 600 to 17,000 µg L^{-1}. Scuba diving observations shortly after the treatment revealed that a considerable amount of the LMB was accumulated on the sediment in shallower parts of the lake (discussed in Chapter 6).

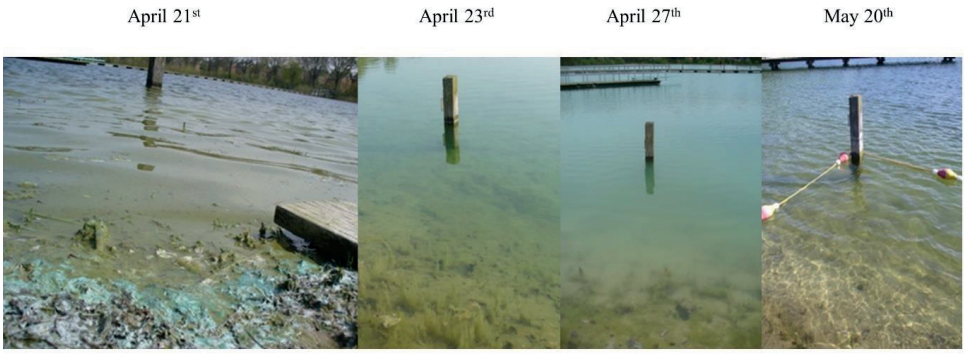


Figure 4.3 Photographs of a surface scum on Lake Rauwbraken (April 21st 2008), the water after the treatment with the flocculent PAC (April 23rd 2008), four days after the surface addition of lanthanum modified clay (April 27th 2008) and after settling of the clay (May 20th 2008), photographs by Miquel Lürling.

4.3.2 Direct treatment effects

Just before the treatment, the mean water column chlorophyll-a concentration (excluding the scum and accumulation) was 6.0 µg L^{-1} (SD = 1.9 µg L^{-1}), directly after the treatment it was 1.2 µg L^{-1} (SD = 0.6 µg L^{-1}) (Fig. 4.4A). The percentage of cyanobacteria increased from a 17% mean (SD = 23%, n = 11) to 27% (SD = 6.4%, n = 11). During the treatment days, the mean concentration of inorganic suspended solids increased from 1.0 mg L^{-1} to 7.2 mg L^{-1} (Fig. 4.4B). During the treatment days, Secchi-disk depth declined from 4.3 m (n = 1) to 0.8 m (n = 1), mean turbidity was elevated from 2.7 NTU (SD = 2.0, n = 11) on the day before the treatment to 7.6 NTU (SD = 4.8, n = 11) on the day after (Fig. 4.4C). Mean TP reduced from 20.4 µg L^{-1} (SD = 19.5 µg L^{-1}, n = 5) to 10.0 µg L^{-1} (SD = 0.0 µg L^{-1}, n =5), mean TN from 0.75 mg L^{-1} (SD = 0.41, n = 5) to 0.26 mg L^{-1} (SD = 0.13 mg L^{-1}, n = 5). On the day before the treatment mean dissolved oxygen saturation was 115% (SD = 18%), concentration 13.43 mg O_2 L^{-1} (SD =1.55 mg L^{-1}, n = 11), on the day after the treatment it was 113% (SD = 18%), concentration 12.70 mg O_2 L^{-1} (SD = 1.86 mg L^{-1}, n =11) (Fig. 4.4D).

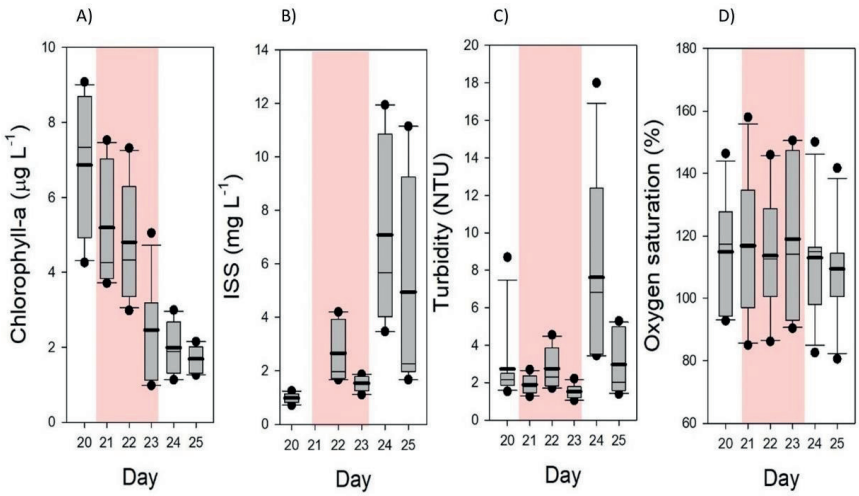


Figure 4.4 The course of chlorophyll-a (µg L^{-1}) (A), turbidity (NTU) (B), inorganic suspended solids (ISS; mg L^{-1}) (C) and oxygen saturation (%) during the treatment (D), Day is day within April, 2008); treatment days indicated in pink. The box indicates the 25th and 75th, whiskers above and below the box indicate the 90th and 10th, thin line = median, and thick line = mean.

Taken over the treatment days, the mean TP (Fig. 4.5A) shows no reduction, while the mean Filterable Reactive Phosphorus (FRP; Fig. 4.5B) shows a minor reduction from 6 µg L^{-1} to 4 µg L^{-1}. In Fig. 4.5A, B,

the increase of the standard deviations of both TP and FRP during the treatment and their reduction shortly afterwards are the most evident. Like with the concentrations of TP and FRP, the TN concentration shows an increase in its standard deviation (Fig. 4.5C), however, the mean TN shows a clear reduction during the treatment. The reduction in TN is not so much present in the filterable fractions – N in ammonium (AMM) and N in nitrite + nitrate (NN; Fig. 4.5C,D,E).

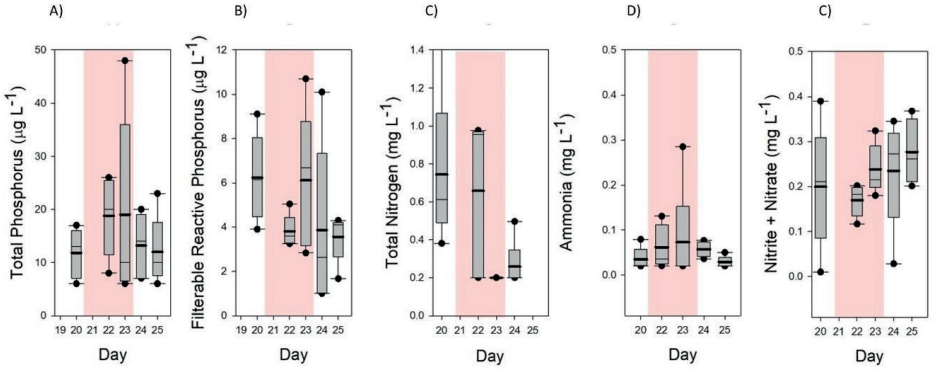


Figure 4.5 The course of total phosphorus (A), filterable reactive phosphorus (B), total nitrogen (C), ammonia (D) and nitrite + nitrate (E) during the treatment (Day is day within April, 2008); treatment days indicted in pink.

4.3.3 Later in 2008

The first phytoplankton to return to the lake were cyanobacteria as indicated by PHYTO-PAM analysis. Subsequent microscopy revealed the absence of filamentous cyanobacteria, but an abundance of tiny cyanobacteria, such as *Cyanobium sp.*, that thrived in the hypolimnion in summer 2008 and later years. While these cyanobacteria caused both high chlorophyll-a and turbidity at greater depth, they had no effect on bathing water quality at shallow depths (Fig. 4.6).

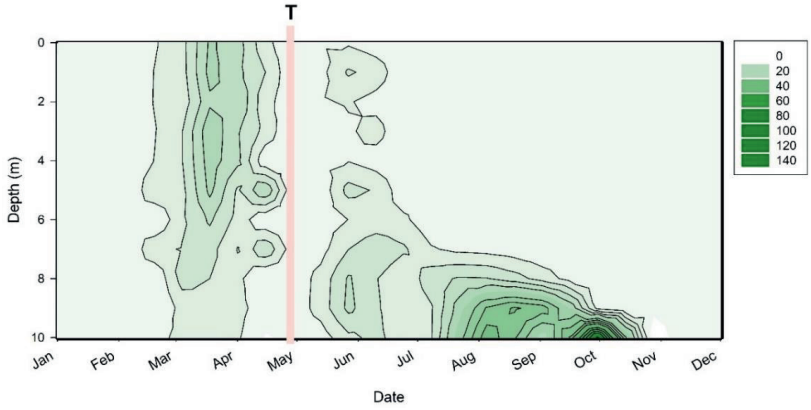


Figure 4.6 Contour plot chlorophyll-a (µg L^{-1}) during 2008, pink bar with T indicates the treatment period.

After the treatment, during spring and summer 2008 the inorganic suspended solid concentration showed a gradual decline (Fig. 4.7). Three weeks after the final treatment day the mean ISS was approximately back to its last pre-treatment value, i.e. on May 14th, the mean ISS was 1.1 mg L^{-1} (SD = 0.2 mg L^{-1}, n = 4). On June 6th the mean ISS was down to 0.4 mg L^{-1} (SD = 0.1 mg L^{-1}, n = 4).

In the days after the application Lake Rauwbraken had a blue-milky colour (Fig. 4.8A), quite like a glacier lake where organic matter content (humic substances) is insignificant (Aas and Bogen 1988). To indicate the amounts of humic substances a scan was made of the light absorption over wavelengths (λ) 250 – 500 nm in filtered water samples from Lake Rauwbraken and Lake the Kuil (The Netherlands). Fig. 4.8B shows the difference between the two lakes regarding light absorption. This blue colour gradually disappeared over the following weeks, where after the lake became more and more clear. The clearest water was observed on November 11, 2008 on which date Secchi disk depth was 10.2 m (bottom at the sample location) (Fig. 4.9B). During these clear waters submerged macrophytes (*Elodea*) grew down to 9 m depth (Chapter 5).

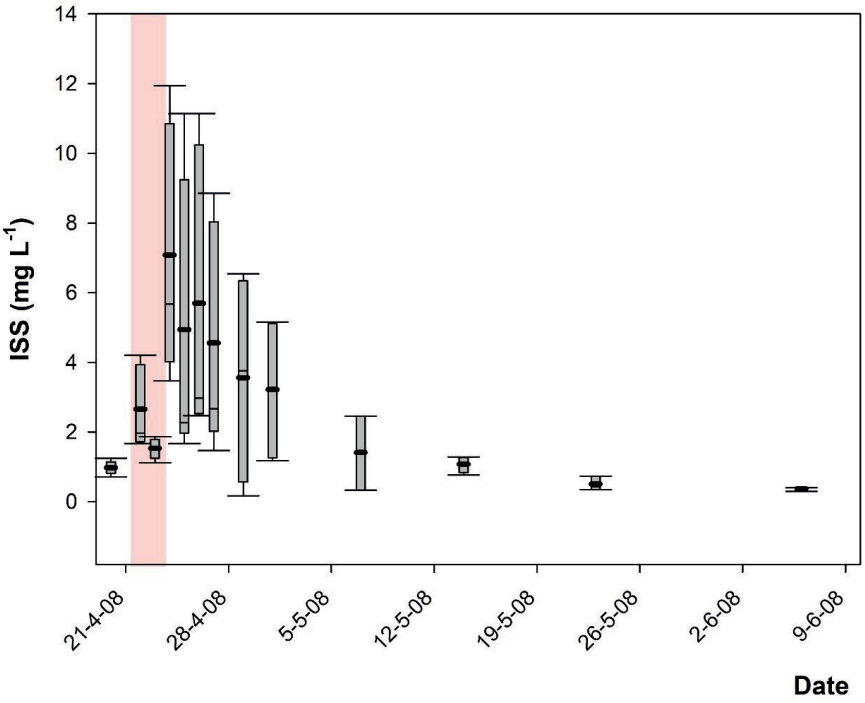


Figure 4.7 The course of inorganic suspended solids during 2008, the pink bar indicates the application days. The box indicates the 25th and 75th, thin line = median, thick line = mean.

A) B)

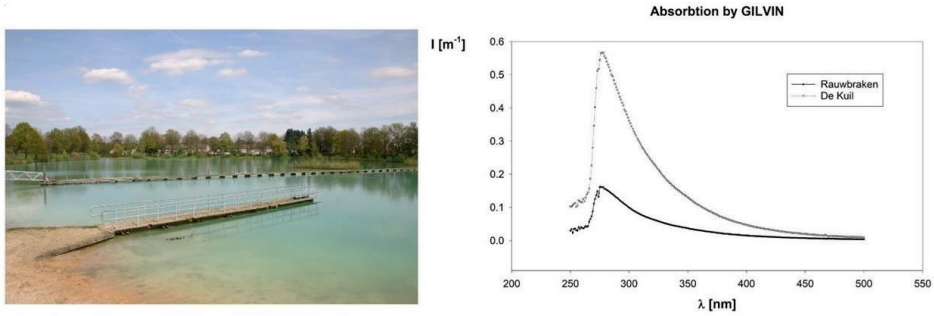


Figure 4.8 The milky blue colour of Lake Rauwbraken after the Floc & Lock treatment in April, 2008 (A) and the light absorption scans of water sampled from Lake Rauwbraken and Lake de Kuil (B).

The general pattern in turbidity (Fig. 4.9A) is the same as observed in the chlorophyll-a concentrations (Fig. 4.6). The high 2008 Secchi-depth (Fig. 4.9) coincided with turbidity below 0.5 NTU.

A) B)

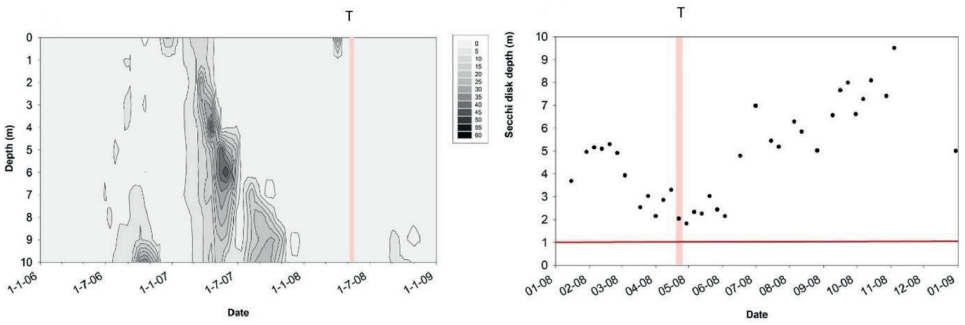


Figure 4.9 Contour plot turbidity (NTU) 2006-2008 (A); The course of Secchi disk depth (m) during 2008 (B), the T and pink bar indicate the treatment.

During the years 2006, 2007 and 2008 the pH followed a distinct pattern with relatively high values in the shallow layer and low values in the deep layer (Figure 4.10 4.10A). However, in 2008 this pattern was shortly interrupted after the treatment. During the pre-treatment years pH could go down to a minimum pH of 6.2 in the deep layer, while in the shallow layer pH could go up to pH = 10.2. Shortly

after the treatment in 2008, pH remained circumneutral, where after the pH developed as in previous years – remaining between pH = 6.3 (in the deep layer) and pH = 8.9 (in the shallow layer). Taken over 2008, post-treatment median pH = 7.2 (n = 230).

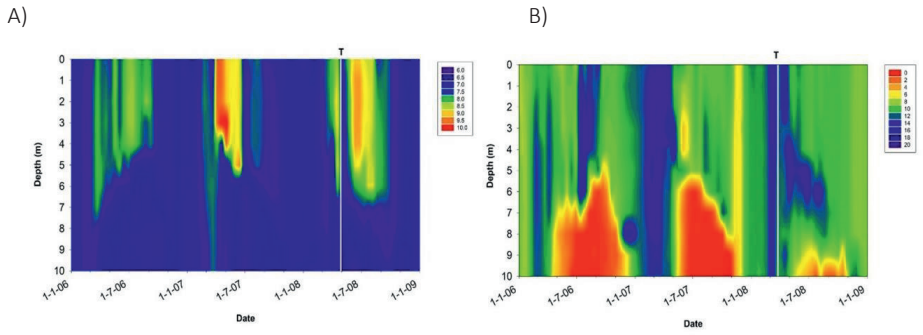


Figure 4.10 Contour plots of pH (A) and oxygen concentration (B) during 2006 – 2008; the T and pink bar indicate the treatment.

Although the oxygen concentration in the deep layer did go down during the summer of 2008 the anaerobic conditions observed in 2006 and 2007 did not occur after the treatment in the summer of 2008. The lower oxygen concentration occurred later in the season and was confined to greater depths (Fig. 4.10B). The lowest oxygen concentration observed was 1.2 mg L^{-1} (deep layer in summer). The course of the oxygen concentration during 2008 is distinctly different from that in 2006 and 2007. In the pre-treatment years the oxygen saturation could go up to 220% in the shallow layer and down to 0% saturation in the deep layer during the summer. During 2008 the oxygen saturation remained between 11% (hypolimnion) and 156% (summer, epilimnion - metalimnion). Although the oxygen concentration in the deep layer did go down during the summer of 2008 the anaerobic conditions observed in 2006 and 2007 did not occur after the treatment in the summer of 2008.

4.3.4 Daphnia in Lake Rauwbraken

D. galeata was present before and during the Floc & Lock treatment (Fig 4.11). Shortly after the treatment it disappeared from the water column which had not been observed in the same periods in 2006, 2007 and 2009 (Fig. 4.12). This disappearance strongly points towards a treatment effect. Daphnids reappeared after several months where some *D. galeata* were observed in the upper water layers, but the most remarkable observation was the explosion of *D. pulex* in late summer, and early autumn in the deep-water layers (Fig. 4.12). In 2009, mostly *D. galeata* were observed (Fig. 4.12).

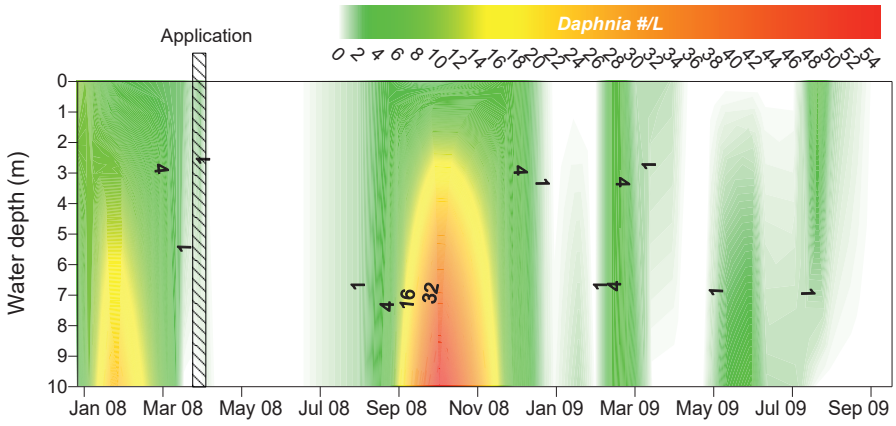


Figure 4.11 Contour plot of Daphnia abundance (individuals L^{-1}) from the surface to 10 m depth in Lake Rauwbraken in the period January 2008 – September 2009. The hatched bar indicates the 'Floc & Lock' treatment, which took place April 21st -23rd 2008.

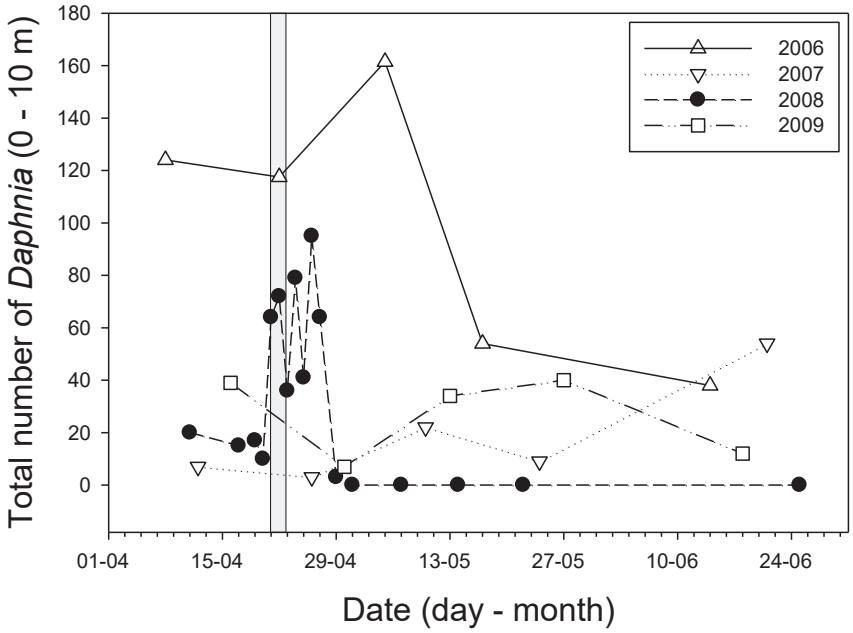


Figure 4.12 Total number of Daphnia in the water column (0 – 10 m) in spring (April – June) 2006 – 2009. The grey bar indicates the 'Floc & Lock' treatment, which took place April 21st -23rd 2008.

4.3.5 Grazing experiment

During the experiment the water quality variables (mean ± 1SD) were similar among treatments: pH 7.5 (± 0.1), conductivity 179 (± 5) µS cm^{-1} and temperature 22.6 (0.5) °C. The body size of the experimental animals was similar among the treatments ($F_{4,35}$ = 0.92; p = 0.461) and on average (± 1SD) 1.9 (± 0.1) mm. The grazing activity, measured as clearance rate (CR) was significantly affected ($F_{4,35}$ = 69.1; p < 0.001) by the treatments (Fig. 4.13). A Tukey test revealed that CR on *S. obliquus* in controls was similar to those in the 'Lock' treatment, but CR were significantly lower in the 'Floc', the 'Floc & Lock' and the '10× Lock' treatments (Fig. 4.13) as compared to both Control and Lock treatments .

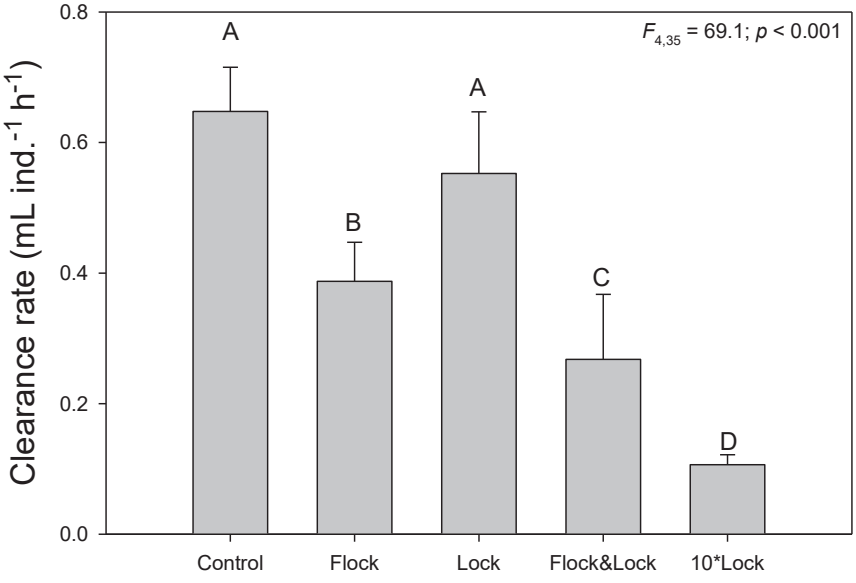


Figure 4.13 Clearance rates (mL $ind.^{-1}$ h^{-1}) of *Daphnia galeata* feeding on *Scenedesmus obliquus* (62 µg L^{-1} CHL) in 0.45 µm filtered Lake Rauwbraken water (Control), in filtered lake water with 9 mg L^{-1} of the flocculent PAC39 (Floc), with 67 mg L^{-1} of modified clay (Lock), in a mixture of 9 mg L^{-1} Floc and 67 mg L^{-1} Lock, and in 10 times the Lock dose (10× Lock, i.e. 670 mg L^{-1}). Error bars indicate 1 SD (n = 8). Different symbols (A,…,D) above bars indicate significant differences at the p = significance of the Tukey post-hoc comparison.

4.3.6 Survival experiment

Animals in the high food treatment (20 μg L^{-1} CHL) had 75% survived until the end of the experiment (Fig. 4.14). In the low food (2 μg L^{-1} CHL) and no food treatments survival was 40% (Fig. 4.14). Death rates were significantly different ($F_{2,6}$ = 11.4; p < 0.01) and two homogenous groups could be distinguished: 1) No food and the 2 μg L^{-1} CHL-a treatments with mean (± 1SD) death rates of 0.40 (± 0.06) day^{-1} and 0.32 (± 0.12) day^{-1}, respectively, and 2) the 2 μg L^{-1} CHL-a and 20 μg L^{-1} CHL-a treatments. Death rates in the later were 0.13 (± 0.02) day^{-1}.

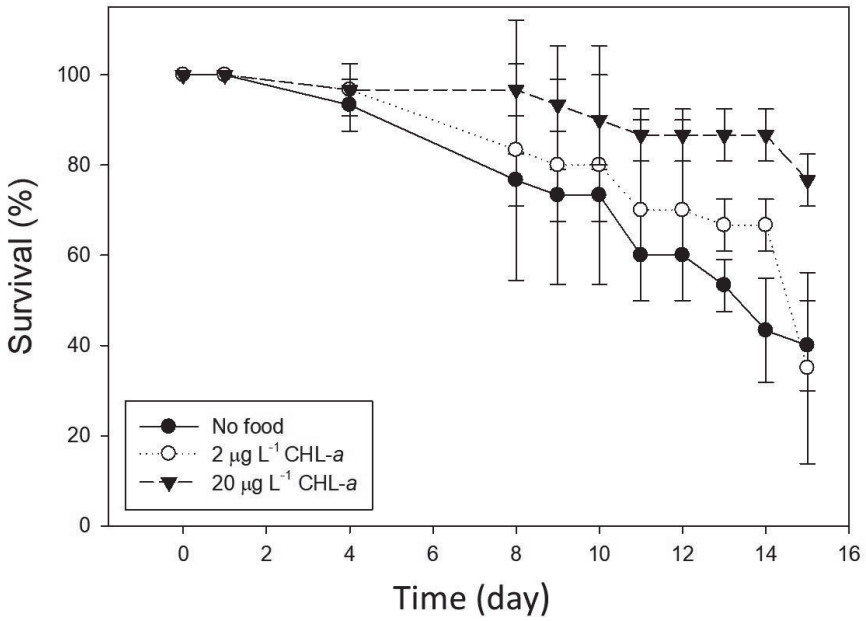


Figure 4.14 Survival (%) of *Daphnia galeata* in 0.45 μm filtered Lake Rauwbraken water (No Food) and in filtered Lake Rauwbraken water with two different *Scenedesmus obliquus* concentrations (2 μg L^{-1} CHL-a and 20 μg L^{-1} CHL-a). Error bars indicate 1 SD (n = 3).

4.4 Discussion

4.4.1 Curative effects

In The Netherlands lakes are important recreational waters. Fulfilling EU bathing water requirements (EU 2000; EU 2006) is a prerequisite for the proprietors of these lakes to run their businesses. While cyanobacteria may build up problematic high densities that go unnoticed during regular bathing water monitoring water (Chapter 2), acceptable water quality may deteriorate from one season to the next, or even within weeks. Whereas it is best to act on early warnings to prevent this problem from occurring in the first place, once it is there, a safe and effective curative method is wanted. The Floc & Lock treatment described in this chapter appeared effective in rapidly removing a bloom and surface accumulation of the cyanobacterium *A. flos-aquae.* Before the application the WFD and BFD were not met due to the *A. flos-aquae* scum (approximate 17,000 µg L^{-1} chlorophyll-*a* and accumulation (600 µg L^{-1} chlorophyll-*a*) in the bathing zone of the lake, a swimming ban would be imposed on the opening day of the bathing season (May 1st). However, no swimming ban needed to be imposed as the cyanobacterial nuisance had vanished within three treatment days. Our experiment in Lake Rauwbraken indicates that Floc & Lock is a useful technique to bring cyanobacterial nuisances to a shortstop (the longevity of the treatment is the subject of Chapter 5).

The PAC and LMB as sinking weight (Floc) were applied before the sediment capping with LMB (Lock) to ensure the bloom was buried beneath the LMB. After the treatment in the course of 2008, Lake Rauwbraken was void of *A. flos-aquae,* indicating that the bloom burying goal was achieved. Later observations with *Planktothrix rubescens* in Lake de Kuil (Lürling et al. 2020c; Mucci et al. 2019; Waajen et al. 2016a) indicated that successful sinking a bloom depends on the species of cyanobacteria present, its buoyancy, the flocculent and the amount of sinking weight applied.

The Floc & Lock application cleared the water column of phytoplankton, including the phosphorus therein. We estimated that the water column contained 34 kg TP (see methods). For Lake Rauwbraken, removing this P is equals the amount of 3 years of external P loading.

4.4.2 Effects on nutrients

We expected the Floc & Lock treatment to clear the water column of total phosphorus. Total phosphorus comprises of a particulate (not filterable) and a filterable fraction (filterable reactive phosphorus; FRP). During the treatment days TP was reduced from a mean of 20 to 10 µg L^{-1}, while FRP was reduced from 6 µg L^{-1} shortly before the treatment to 4 µg L^{-1} on the day after the treatment. The reduction in FRP can be explained by its binding by the LMB – hence moving it to the particulate fraction in the TP pool, which may subsequently have settled on the lake's bottom. Based on the empirical

relation Chl-a ≈ 0.987TP – 6.250 Schindler (1974), the reduction in mean Chl-a (5.19 µg L^{-1}) indicates a 12 µg L^{-1} reduction in mean TP. Hence, in its order of magnitude, the observed reduction in TP (10 µg L^{-1}) is consistent with removed Chl-a. The fact that not all TP was removed can be attributed to not removing zooplankton, a minor amount of remaining phytoplankton, to FRP bound by the LMB still present in the water column, and by the time needed to bind FRP (Chapter 3). While the treatment aimed at TP reduction, it also affected TN as is evident from the TN reduction during the treatment, i.e. from 0.75 mg L^{-1} shortly before to 0.26 mg L^{-1} directly after the treatment. We attribute this reduction in TN to the removal of particulate N (in phytoplankton) as filterable fractions (AMM and NN) increased during the days of treatment.

4.4.3 pH and Oxygen

The pH in the surface layer of Lake Rauwbraken was reduced from 8.3 - 8.7 to 6.4 - 7.1 by the addition of the flocculent and LMB, while it remained between 6.5 and 7.1 near the sediment.

While anaerobic conditions reigned in the lake's hypolimnion during the summers of 2006 and 2007 (Chapter 2), the anaerobic conditions did not occur in summer 2008. The 'Floc' part of the treatment meant a massive sedimentation of organic material, this did however not add to the oxygen depletion in the hypolimnion. After the treatment in 2008, Lake Rauwbraken was considerably low in phytoplankton biomass, meaning that the sedimentation of organic material can be expected to be reduced accordingly. Thus the nutrient supply from the water column, on which the bacterial depletion of oxygen ran, was taken out. Likewise, the addition of the LMB reduced the P supply from the sediment. We thus attribute this change in oxygen regime to the combination of 1) the removal of heterotrophic bacteria from the lake's water column, 2) the strong P-limitation imposed on these and the bacteria in the sediment after the treatment, and 3) the light penetration and photosynthesis by *Elodea* down to 9 m deep and the pico-cyanobacteria near the bottom. The presence of pico-cyanobacteria in a deep chlorophyll maximum (DCM) is fully in line with the prediction by Padisák et al. (2003) of pico-cyanobacteria forming a DCM in lakes where P is limiting and light is still sufficient.

The oxygen concentration (REDOX condition) in the water overlying the sediment in a lake is an important factor steering the release of FRP (internal P load) from the sediment (Stumm and Morgan 1996). The mechanism is mostly seen as one that starts off with the reduction of oxygen concentrations as a result of bacterial breakdown of organic matter (e.g. Wetzel (2001)), i.e. reduced oxygen concentration favours the chemical release of FRP from Fe and Mn (Stumm and Morgan 1996) in the sediment. Conversely, it implies that improving the oxygen regime may reduce FRP release from the sediment as was done in Lake de Ouderkerkerplas (Lürling et al. 2020a). The observed improvement in the hypolimnetic oxygen regime in Lake Rauwbraken directly after the treatment is an important result, because it suggests that reduction of sediment FRP release (thus hypolimnetic FRP concentrations)

improves the oxygen conditions in de overlying water by limiting oxygen consumption by bacteria. While this is in line with and fully expected from current knowledge (Wetzel (2001, chapter 9)), seeing the oxygen regime actually improve after reduction of FRP in a whole lake experiment remains an eye opener.

4.4.4 Water Clarity

4.4.4.1 Turbidity and Secchi disk depth

Water clarity is an important water quality variable from both ecological, i.e. the depth at which submerged macrophytes can grow, and recreational points of view, i.e. clear waters are more attractive than turbid ones. While the application of PAC resulted in clear waters overnight, the application of LMB made the lake, albeit temporarily, turbid. During the three weeks after the treatment, water clarity improved as the LMB (inorganic suspended solids concentration) settled on the sediment of the lake. Considering the 9 m depth at which submerged macrophytes were observed later in 2008 (Chapter 5), these three weeks to reach clear water seems acceptable.

4.4.4.2 Humic substances

The use of PAC had the additional advantage that besides being a potent flocculent (Delgado et al. 2003) and precipitating the cyanobacteria, it also reduced the concentration of humic substances (Kuo and Amy 1988; Liu et al. 2009; Yan et al. 2008). Although humic substances were not measured around the application, the blue-milky colour of the Lake after the application (*see* Fig. *4.8*A) made it resemble a glacier lake where organic matter content is insignificant (Aas and Bogen 1988). As a proxy for humic substances absorption at 380 nm (A_{380}) can be used which varies in Dutch inland waters from about 1 to 63 m^{-1} (Buiteveld 1995). In Lake Rauwbraken A_{380} was on average 2.0 m^{-1} in 2006 (range 1.0 -7.3 m^{-1}), but 0.6 m^{-1} in 2008 (range 0.5 – 0.7 m^{-1}) after the Floc & Lock application. Hence, humic substance concentration in Lake Rauwbraken was already low and further reduced by the Floc & Lock treatment.

4.4.5 N=1

We are aware that our study comprises of one lake, which hampers the generalization of our results. While not tested formally, the results presented in this chapter indicate that the Floc & Lock treatment might be effective in other lakes. From this idea, a formal hypothesis can be formulated that 'Floc & Lock is an effective in-lake treatment as a curative method to mitigate cyanobacterial nuisance'. Meanwhile, several other studies have shown the potential of the Floc & Lock approach in laboratory experiments (Miranda et al. 2017; Noyma et al. 2016) and field applications (Su et al. 2021; Waajen et al. 2016a). While the longer-term effects and effects of the treatment on sediment phosphorus release

are presented in Chapter 5, the results presented in the current chapter only allow us to hypothesise on the curative (direct and short term) effects.

4.4.6 Daphnia

The Floc & Lock method as applied to Lake Rauwbraken, caused a temporal disappearance of *Daphnia* in this lake. No *Daphnia* were found in water samples for at least three months after the application, whereafter daphnids recovered. In laboratory experiments, Lürling and Tolman (2010) found no detrimental effects La on *Daphnia magna*. However, *D. magna* kept for 5 days at a realistic dosage of LMB were 7-13% lighter than their conspecifics in controls experiments (Lürling and Tolman 2010), which indicates an effect of the clay carrier. Since filter feeding zooplankton such as *Daphnia* are generalist feeders and thus do not discriminate between food and clay particles, ingestion of clay can have detrimental effects on the organisms. Several studies have revealed that suspended clays cause decreased feeding, lower survival and reduced growth in *Daphnia* (Cuker and Hudson 1992; Kirk and Gilbert 1990; Kirk 1991; 1992; McCabe and O'Brien 1983; Robinson et al. 2010).

Several factors may have caused the disappearance of *D. galeata* following the Floc & Lock treatment. First, animals might have been trapped in the aluminium hydroxide flocs and precipitated to the sediment (Minzoni 1984; Schumaker et al. 1993). This possibility is, however, not supported by the field observations as animals, and especially juveniles, increased during and the few days after the application. The vanishing of *D. galeata* about one week after application also rules out the acute toxicity of the Floc & Lock treatment. Acute toxicity of the active ingredient in the flocculent, aluminium, was also not expected at the applied dose of 1 mg Al L^{-1}, because the same and a double dose had no acute effects on *D. magna* (Tomasik et al. 1995). At 5 mg L^{-1} some immobilization was detected by Tomasik *et al*. (1995), while in hard water and neutral pH the EC_{50} for immobilization was about 60 mg Al L^{-1} (Khangarot and Ray 1989). In another study, at neutral pH the LC_{50} of aluminium to *D. magna* was 3.9 mg L^{-1} and 1.4 mg L^{-1} for 48 h and 21 d exposures, respectively (Biesinger and Christensen 1972). These authors found a 16% inhibition of reproduction at 0.32 mg L^{-1} (Biesinger and Christensen 1972). In soft water with a pH of 6.5 a similar concentration of 0.32 mg L^{-1} appeared acutely toxic to *D. magna*, but not when calcium was increased (Havas 1985). In general, a low pH as a consequence of aluminium addition is considered the main factor of *Daphnia* mortality (Havas 1985; Minzoni 1984; Tomasik et al. 1995). In our treatment, pH never became less than 6.5, which means that over 99% of the added aluminium was present as insoluble $Al(OH)_3$, while the maximum concentration of filterable aluminium was only 63.4 μg L^{-1}. Nonetheless, the flocculent, PAC, might be toxic to *Daphnia* as our grazing experiment clearly revealed significantly reduced clearance rates. Undoubtedly the $Al(OH)_3$ solids were ingested by *Daphnia* and the observed effect on reduced clearance rates could be caused by an insufficient time for the flocs to age into insoluble flocs (Cooke et al. 1993). Effects caused by impurities

in the PAC39 give another possibility. Finally, also the flocs themselves might interfere with the feeding process directly and impair movements of *Daphnia* (Peterson et al. 1974), or indirectly through efficient enveloping algae which were no longer available to the *Daphnia's*.

The addition of a low amount of the buffering agent $Ca(OH)_2$ (approximately 0.25 mg L^{-1}) probably had no effect on *Daphnia*, as lime applications with $Ca(OH)_2$ up to 78 mg L^{-1} had no effect on *Daphnia* (Prepas et al. 2001; Zhang et al. 2001).

A study with *Daphnia* by Lürling and Tolman (2010) yielded a no-observed effect concentration (NOEC), based on reproduction, of 100 µg La L^{-1}, which was similar to the result found in a 21 d *Daphnia* reproduction test (Sneller et al. 2000). The maximum concentration of filterable lanthanum in Lake Rauwbraken (90.8 µg L^{-1}) was close to the NOEC. Taking among species variability of sensitivity to lanthanum in consideration, effects on reproduction of *D. galeata* cannot be excluded. The increase in juveniles during and straight after the application was most likely from broods that had been deposited in the animals brood pouch prior to the treatment of the Lake.

When exposed to the modified clay, somatic growth of juvenile *Daphnia* was affected at concentrations exceeding 100 mg clay L^{-1}, while at 100 mg clay L^{-1} animals were already slightly lighter (7 – 13%) than their conspecifics in controls and treatments up to 50 mg L^{-1} (Lürling and Tolman 2010). These values are close to the whole-lake averaged dosage of 67 mg L^{-1}. However, the application takes place through a spray manifold where the slurry is brought into the upper water layer ((Robb et al. 2003); Fig. 4.2). Hence, in the upper water layers, LMB concentrations during and shortly after the addition will be much higher than the ones calculated over the whole water body. This was also reflected in turbidity-depth profiles during and straight after the application. Turbidity declined rapidly as a consequence of sedimentation of the clay particles (Haghseresht 2005; Ross and Cloete 2006). Nevertheless, the short period (1 – 2 days) of high turbidity (with maxima of around 18 NTU) might have affected the feeding rate of the animals, as shown by our grazing experiment, in which a clay dosage of ten times the whole-lake averaged one, caused a strong feeding inhibition. This is corroborated by studies that found strongly depressed feeding rates in *Daphnia* by suspended clay (Kirk 1991) and at turbidity above 10 NTU (McCabe and O'Brien 1983). However, whether short exposures to relatively high clay concentrations, and consequently low food intake, have strong impact on the *Daphnia* population is not clear (Robinson et al. 2010).

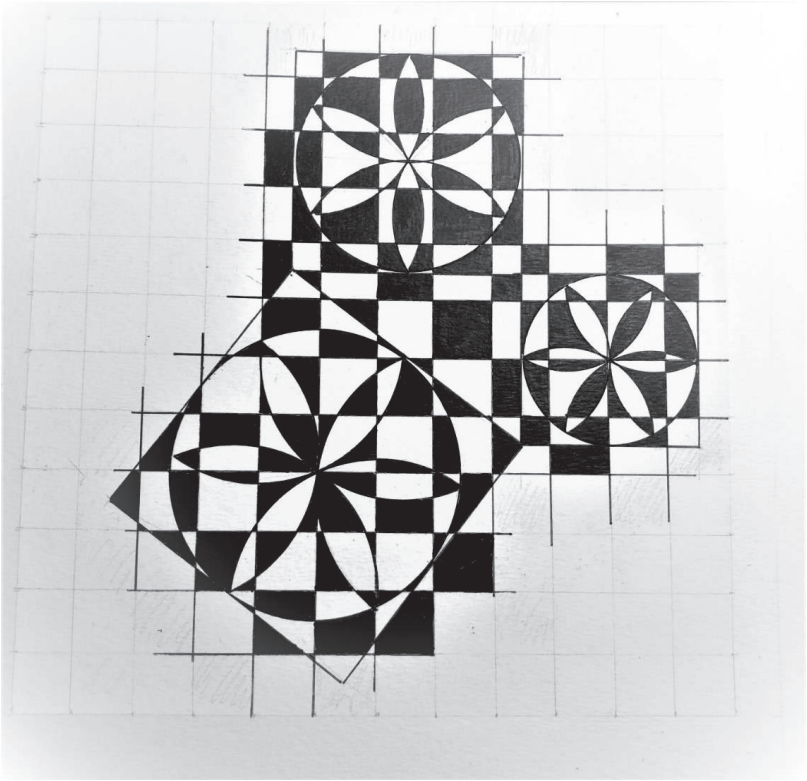
Then it should also be noted that the turbidity before the application was mainly a result of phytoplankton, whereas after the application it was mostly fine clay. Reductions in juvenile *Daphnia* survival and growth by these clay concentrations are plausible (Kirk and Gilbert 1990), but also the generally low food concentrations themselves seem to be a major factor in the disappearance of *D. galeata*. This is corroborated by the significantly reduced survival of *D. galeata* in the low food

treatments (2 μg L^{-1} CHL) that resemble the post treatment concentrations *in situ* compared to the pre-treatment concentrations (~20 μg L^{-1} CHL).

Interestingly, similar observations on *Daphnia* abundances have been made in Newman Lake (Washington, USA) following an aluminium treatment, where two weeks after the treatment especially cladocera, such as *Daphnia* disappeared, but recovered in the months afterwards (Schumaker et al. 1993). In Lake Rauwbraken, recovery took longer which might be explained by high predation pressure of visually hunting fish in the crystal-clear water during the spring and summer months. Because no anoxia up to 10 m depth was observed, and water transparency was over 7 m during summer, a deep-water refuge from visually hunting predators was most probably absent. In October large-bodied *D. pulex* appeared in the lake, and also *D. galeata* reappeared, probably since the biomass of the main visually hunters 0^{+}-fish had been decreased substantially by then (Van Gool and Ringelberg 2002).

4.5 Conclusions

- In Lake Rauwbraken, the Floc & Lock combination of PAC and LMB had a strong curative effect, water quality instantaneously improved, resulting in good water quality according to the BWD.
- The treatment reduced FRP, TP, TN and chlorophyll-a but not AMM and NN.
- Post-treatment the hypolimnion remained aerobic as a result of P-limitation of heterotrophic bacteria.
- The application of the LMB (Lock) temporarily (three weeks) reduced water clarity.
- The effects of Floc & Lock on *Daphnia* in Lake Rauwbraken seemed to be caused by several factors acting in synergy. Physical effects of flocs, grazing inhibition of flocs and clay, very low food concentrations, and absence of predation refuge all might have played a role. However, the effects were temporarily, and *Daphnia* recovered from the treatment.
- The Floc & Lock treatment is a promising curative method to mitigate cyanobacterial nuisance in lakes. A formal proof of this curative effect needs underpinning by extending the experiment to a larger, randomly selected set of lakes, including different water types and other species of cyanobacteria.



J.F.X. van Oosterhout

Pythagoras in transitions.

Incomplete oeuvre of unfinished works

Acrylic ink on paper

50 x 29.5 cm

Chapter 5

The long-term efficacy Floc & Lock

Parts of this chapter were published in:

Oosterhout, F. van, Yasseri, S., Noyma, N., Huszar, V., Manzi, M., Mucci, M., Waajen, G. and M. Lürling, 2021. Assessing the long-term efficacy of internal loading management to control eutrophication in Lake Rauwbraken. Inland Waters. In press.

5 The long-term efficacy

5.1 Introduction

In April 2008, Lake Rauwbraken (Chapter 2) was Floc & Lock treated (Chapter 1, 4) to reduce nuisances caused by blooms of cyanobacteria. With the goal to impose phosphorus limitation on the population growth of cyanobacteria, the treatment cleared the water column of P and cyanobacteria (Floc) and subsequently reduced the internal P loads from the sediment (Lock). The study of Lake Rauwbraken provides us with an opportunity to assess the efficacy and longevity of controlling internal loading, without reducing external nutrient loads (Chapter 2,4). This chapter reports on the long term water quality, and ecosystem response crucial to determine cost-effectiveness and longevity of the treatment (Spears et al. 2013b; 2016).

We use ten years of monitoring data (post-treatment) to address the following hypotheses: (i) starting in 2009 there are no trends in the annual means of chlorophyll-a, the percentage of cyanobacterial chlorophyll-a, Secchi disk depth, turbidity, total phosphorus, filterable reactive phosphorus, total nitrogen, nitrogen in ammonium, nitrogen in nitrite and nitrate, hypolimnic oxygen concentration and saturation (ii) that sediment nutrient fluxes (dissolved nitrogen and phosphorus) were not effectively controlled following the Floc & Lock treatment.

Submerged macrophytes play a key role in the ecology of shallow lakes (Scheffer 2001) and water quality (Bloemendaal and Roelofs 1988) in general. We report on submerged macrophytes and explain their coverage from the lake's fluctuating water level and water clarity; we explain from macrophyte P content how they could only play a minor role in the water quality of Lake Rauwbraken. We discuss the implications of the long-term responses reported in terms of long-term management of internal loading in Lake Rauwbraken, and potentially others.

While water quality remained good for a decade after the treatment in 2008, a minor *Microcystis* scum was observed in August 2011. We added a sediment incubation experiment to test the hypothesis that the *Microcystis* in this scum may have originated from the lake's sediment. In the appendix, we also added an algal growth experiment done with lake Rauwbraken water to show that phytoplankton was phosphorus limited.

5.2 Methods

5.2.1 Study site, increased external P-loads

The maintenance of the greenbelts (Chapter 2) stopped in 2011, three years after the treatment. Over the following years, the greenbelts grew back to their original coverage and so did the P-inflows from it. Geese became resident at the lake adding 0.12 mg P m^{-2} day^{-1} to the P inflows. Due to an urbanization project near the lake, P-polluted rainwater is now fed into the ground water table some 100 m distance from the lake, resulting in a 10% higher ground water inflow to the lake. Rainwater generally is low on P, however discharges of rainwater from a hard surface in urbanized areas can still be an important P source (Waajen et al. 2016b; 2019). Hence, the external P-loads to Lake Rauwbraken are returning to their historical values and are currently probably higher due to the inlet of this urban run-off water through the groundwater table, and the growing population of geese.

5.2.2 Lake monitoring

Water column sampling and analyses were done as described in Chapter 2. From 2009 onwards, sampling was done at 0 to 10 m depth. During 2010 the sampling aimed at once every three weeks, and in 2011 every month. From 2012 to 2018 the sample frequency was (aiming at monthly): 2012 April-September, n = 5, 2013 March -December, n = 8, 2014 February-September, n = 10, 2015 March-September, n = 8, 2016 February – October, n = 10, 2017 February-October, n = 10 and 2018 January-September, n = 9.

5.2.3 Sediment release

Sediment core sampling and release experiments were described in Chapter 2. Release was measured under anaerobic conditions in five cores drilled on April 13th, 2008 (pre-treatment, Chapter 2), June 19th, 2008 (post-treatment); and under both aerobic and anaerobic conditions in six cores drilled in 2011 and eight cores drilled in 2013.

5.2.4 Submerged Macrophytes

From 2000 to 2011 macrophytes were monitored during scuba dives throughout the dive season April-October. From 2008 to 2017, each year in the third week of June, coverage and species composition were estimated along 6 to 12 transects (Fig. 5.1, red lines) perpendicular to the shore using a rake (< 1 m depth) and bathyscope from a boat (< 2 m depth) and/or scuba diving (> 3 m depth). Each year the same transects were sampled.

On October 10th, 2008 and April 23rd, 2009, macrophytes were sampled along a 0 to 9 m depth transect (Fig. 5.1, blue line). All macrophytes within a hoop (Ø 0.5 m) were collected (scuba diving), rinsed with lake water and dried at 50 °C. The P content was determined as described for organic

deposition (see 2.3.4.3). We computed the P contained in submerged macrophytes to kg P m^{-2} and extrapolated to the total amount of P (kg) contained in submerged macrophytes in the lake based on the bathymetry of the lake.

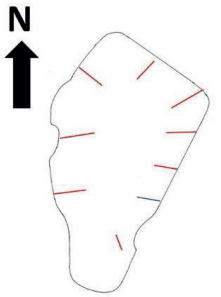


Figure 5.1 Macrophyte sample locations. Redline = rake and bathyscope, blue line = sampling 2008, 2009.

To indicate depths at which macrophytes are light-limited we use the euphotic zone (z_{eu}). For phytoplankton, z_{eu} is the depth beyond which light level falls below 1% of surface irradiation (Reynolds 1984). By approximation z_{eu} = 1.7 × Secchi disk depth (Reynolds 1984), we estimate z_{eu} using the mean pre-treatment Secchi-depth.

5.2.5 Mystery Scum

5.2.5.1 Microcystins analysis.

On August 3rd 2011, scum and waters samples were collected and extracted and analysed for eight microcystins (MC) variants (dm-7-MC-RR, MC-RR, MC-YR, dm-7-MC-LR, MC-LR, MC-LY, MC-LW and MC-LF) and nodularin (NOD) by Liquid Chromatography (Agilent 1200 LC) with tandem Mass Spectrometry detection (LC-MS/MS on an Agilent 6410A QQQ, Waldbronn, Germany) as described in Lürling and Faassen (2013). MC variants were quantified against calibration curves made with certified calibration standards (DHI LAB Products, Hørsholm, Denmark) and subsequently corrected for recovery.

5.2.5.2 Sediment incubations

In August 2011, two sediment cores (diameter 6 cm) were drilled using a Uwitech Cores sampler (Fig. 29E, area indicated by A, Chapter 2). Core I was taken from 12.1 m depth and core II from 11.8 m depth. In the laboratory, the over standing water was removed, and the cores were sliced using a core cutter. The following slices were collected: Core I, the top layer (~0-2 mm), ~5-7 mm, ~10-12 mm and ~20-22

mm; core II, top layer (~0-2 mm), ~2-5 mm, ~5-7 mm and ~20-22 mm. Aliquots of 25 µL sediment were pipetted using a wide mouth pipet-tip into individual 3 mL wells on 24-welled plates that contained 2 mL algal growth medium (Lürling and Beekman 2006). For each sediment layer as well as for samples of the above standing water three replicates were used. As positive control served three wells to which 25 µL of a *Scenedesmus obliquus* culture were added. The plates were incubated in a Sanyo Gallenkamp incubator (SANYO Electric Co., Ltd., Osaka, Japan) for 7 days at 20°C in 120 µmol m^{-2} s^{-1} light provided from above in a 16:8 hours light: dark cycle and continuously shaken at 100 rpm. After 7 days cyanobacterial and eukaryote algae chlorophyll-a concentrations were measured using the PHYTO-PAM phytoplankton analyzer (PHYTO-ED, system II version, Heinz Walz GmbH, Effeltrich, Germany).

5.2.5.3 Statistical testing

For TN, TP, Chl-*a*, the percentage of cyanobacteria, turbidity and Secchi-depth we tested the hypothesis: no post-treatment trend using Pearson rank order correlation (not assuming a form of response) of whole water column yearly means against year. For the trends in hypolimnetic oxygen concentration during summer stratification, we selected the oxygen concentrations measured from June to September at depths 7 to 10 m.

We did not control for differences in core incubation between experiments, statistical tests were done within experiments, hence tests were not affected by differences between the experiments. We tested the hypothesis that there was no difference between the before and the after treatment sediment nutrient releases (2008) by T-test. In the 2011 and 2013 experiments, we tested the hypothesis: no difference between aerobic and anaerobic conditions on FRP and DIN release rates using a T-test. In case data failed homogeneity of variance (Levene's test) they were log transformed (FRP 2008 and 2011). All tests were done in SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

5.3 Results

5.3.1 Chlorophyll-a, cyanobacteria

The treatment strongly reduced chlorophyll-*a* concentrations (Fig. 5.2A) to a mean concentration of 5.5 µg L^{-1} over the entire post-treatment period (Table 5.1). During the first post-treatment years (2009-2012) elevated chlorophyll-a concentrations were observed during summer near the bottom (Fig. 5.2A). PHYTO-PAM analysis indicated dominance of cyanobacteria, subsequent microscopy revealed absence of *P. rubescens*, but abundance of tiny cyanobacteria, such as *Cyanobium sp.* From July 2013 and onwards the pronounced elevated chlorophyll-a concentrations in the deep layer during summer seem to disappear, while higher chlorophyll-a concentrations occur over the entire water column during mixing in winter/spring (Fig. 5.2A) as a result of proliferation of *Ceratium* and *Dinobryon*. In Spring 2016 we observed a low biomass of *Planktothrix rubescens.* No trend was found in the annual means of chlorophyll-a (Table 5.1; Fig. 5.3A). The percentage of cyanobacterial chlorophyll-a showed a steep increase from 2013 onwards (Fig. 5.3B) with a significant trend up (Table 5.1; Fig. 5.3B).

Table 5.1 Yearly mean values and standard deviation (SD) of Chlorophyll-a , percentage of cyanobacteria (Cyanobacteria) , Secchi-depth, turbidity, total phosphorus (TP) , Total nitrogen (TN), Filterable reactive phosphorus (FRP), Ammonia (AMM), Nitrite + Nitrate (NN), Hypolimnic oxygen concentration (Hypo oxygen), hypolimnic oxygen saturation (Hypo oxygen sat) and trends (trends) in post-treatment yearly means (corr. = correlation coefficient, n = number of samples), except for Hypolimnic oxygen concentration and hypolimnic oxygen saturation which are mean values from June to September at depths 7 to 10 m. n.s. = not significant at α-level 0.05. The pre-treatment = period from Jan. 1, 2006 to April 20, 2008, post treatment = April 24, 2008 to Dec. 31, 2018, for nutrients to Dec. 31, 2017.

Variables	pre-treatment			post-treatment			trends		
	mean	(SD)	n	mean	(SD)	n	corr.	p	n
Chlorophyll-a (µg L^{-1})	16.5	(32.4)	706	5.5	(8.6)	1485	0.52	n.s.	11
Cyanobacteria (%)	64	(32)	411	17	21	1339	0.61	< 0.05	11
Secchi disk depth (m)	3.5	(1.6)	89	4.0	1.9	147	-0.92	< 0.05	11
Turbidity (NTU)	5.4	(7.5)	557	2.2	1.8	1246	0.72	< 0.05	11
TP (µg L^{-1})	134	(132)	363	14	14	1189	0.89	< 0.05	11
TN (mg L^{-1})	0.96	(0.99)	303	0.50	0.42	1024	-0.15	n.s.	11
FRP (µg L^{-1})	20	(50)	436	6	(10)	1188	-0.09	n.s.	11
AMM (mg L^{-1})	0.20	(0.37)	452	0.14	(0.14)	1139	-0.02	n.s.	11
NN (mg L^{-1})	0.08	(0.12)	450	0.08	(0.08)	1139	-0.36	n.s.	11
Hypo oxygen (mg L^{-1})	0.86	(1.72)	143	4.55	(4.29)	228	-0.78	< 0.05	11
Hypo oxygen sat (%)	5	(15)	119	41	(39)	220	-0.81	< 0.05	11

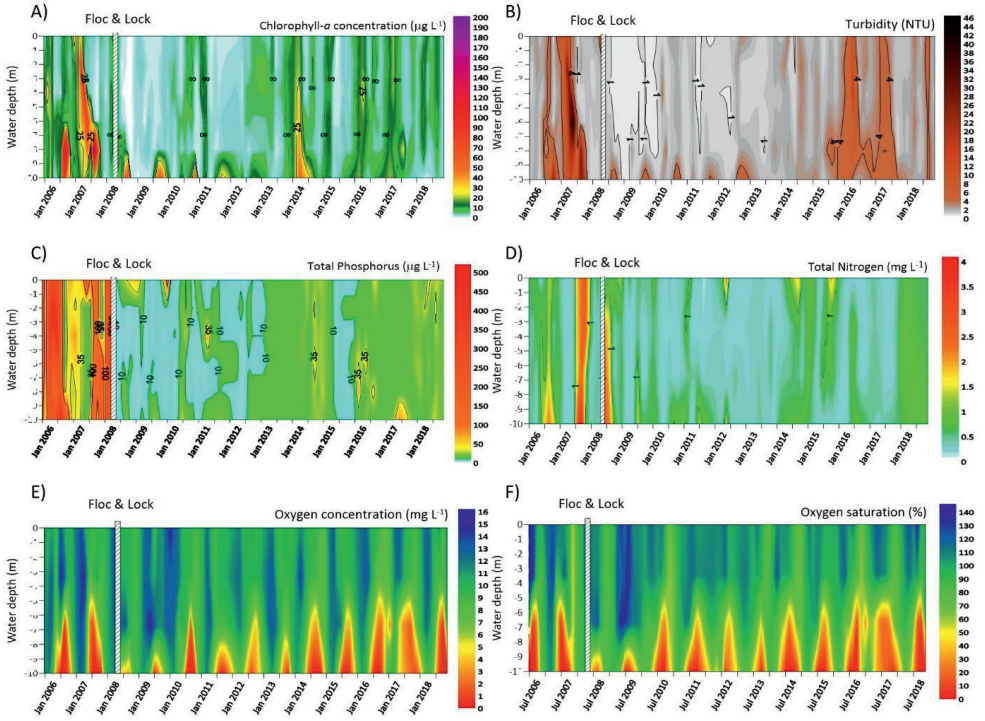


Figure 5.2 Contour plots of chlorophyll-a concentration (A), turbidity (B), total phosphorus concentration (C), total nitrogen concentration (D), dissolved oxygen concentration (E), and dissolved oxygen saturation (F) from 2006 to 2018 in Lake Rauwbraken. Vertical bar indicates the Floc & Lock treatment.

5.3.2 Water clarity

Post-treatment 2008, Secchi disk depth gradually increased to reach a maximum of 10.2 m (bottom) in November. The mean post-treatment (2009 – 2018) Secchi-depth was 4.0 m; there was a significant trend downwards (Table 5.1; Fig. 5.3C). Post-treatment mean turbidity was 2.2 NTU. The post-treatment turbidity (Fig. 5.3B) follows a similar pattern as chlorophyll-a (Fig. 5.3A), with a significant upward trend (Table 5.1).

5.3.3 Nutrients

The pre-treatment mean TP concentration was 134 µg L^{-1}, which was reduced to 14 µg L^{-1} over the entire post-treatment period (Fig. 5.3E; Table 5.1). Mean TN was reduced from 0.96 mg L^{-1} to 0.50 mg L^{-1} (Fig.5.3F; Table 5.1). In the first post-treatment years (up to and including 2012), TP remained below LOD with some higher values at the surface and in the mid-water column (Fig. 5.2C). There was a significant trend upward in the annual TP means (Table 5.1; Fig. 5.3E). This increase seems to start in 2013, five years after the treatment (Fig. 5.2C). Shortly after the treatment (summer 2008) there was a

short period with relatively high TN throughout the water column, while in subsequent years higher TN values were sparse (Fig. 5.2E). There was no trend in the annual mean TN (Table 5.1,Fig. 5.3 F).

The pre-treatment mean FRP concentration was 20 µg L^{-1}, which was reduced to 6 µg L^{-1} over the entire post-treatment period (Table 5.1; Fig. 5.3I), AMM was reduced from 0.20 mg L^{-1} to 0.14 mg L^{-1}; NN was unchanged at 0.08 mg L^{-1} (Table 5.1; Fig. 5.3J, K). There were no significant trends in AMM and NN (Table 5.1).

5.3.4 Oxygen

Post treatment, the hypolimnion oxygen regime remained improved (aerobic) until 2013. There were significant trends downwards in both oxygen concentration and saturation (Table 5.1). Judging from both Fig. 5.2 (E, F) and Fig. 5.3 (G, H) it appears that a trend towards anaerobic conditions in the hypolimnion started from 2014 onwards.

5.3.5 Sediment release of filterable reactive phosphorus and dissolved inorganic nitrogen

In experiment 2008, the T-test on the log transformed data revealed a significant difference; anaerobic sediment FRP release was 85% lower in cores collected after treatment than those from before the treatment (Table 5.2A). In experiment 2011, the mean FRP released under anaerobic conditions was 3.0 mg m^{-2} day^{-1}, and under aerobic conditions it was 1.2 mg m^{-2} day^{-1} (Fig. 5.4A, Table 5.2A). The T-test on the log transformed data revealed a significant difference between the anaerobic and aerobic conditions (Table 5.2A). In experiment 2013, the mean FRP released under anaerobic conditions was 4.6 mg m^{-2} day^{-1}, while under aerobic conditions it was 1.6 mg m^{-2} day^{-1} (Fig. 5.4A, Table 5.2). The T-test on untransformed data failed to reach statistical significance, however this test had a low power (Table 5.2A).

In experiment 2008 (after 2-years incubation), the release of DIN was much higher than in experiments 2005, 2011 and 2013 (all fresh cores) (Fig. 5.4B, Table 5.2B). Post treatment in 2011, the sediment release of DIN was 43.92 mg m^{-2} day^{-1} (86% AMM, aerobic condition) and 44.09 mg m^{-2} day^{-1} (94% was AMM, anaerobic conditions, Fig. 5.4B). In 2013, the releases of DIN were 27.33 mg m^{-2} day^{-1} (77% AMM, aerobic conditions) and 24.12 mg m^{-2} day^{-1} (87% was AMM, anaerobic conditions). In both years, the T-test indicated no significant difference between the DIN release rates measured under aerobic and anaerobic conditions (Table 5.2B). However, in both cases the test had a low power.

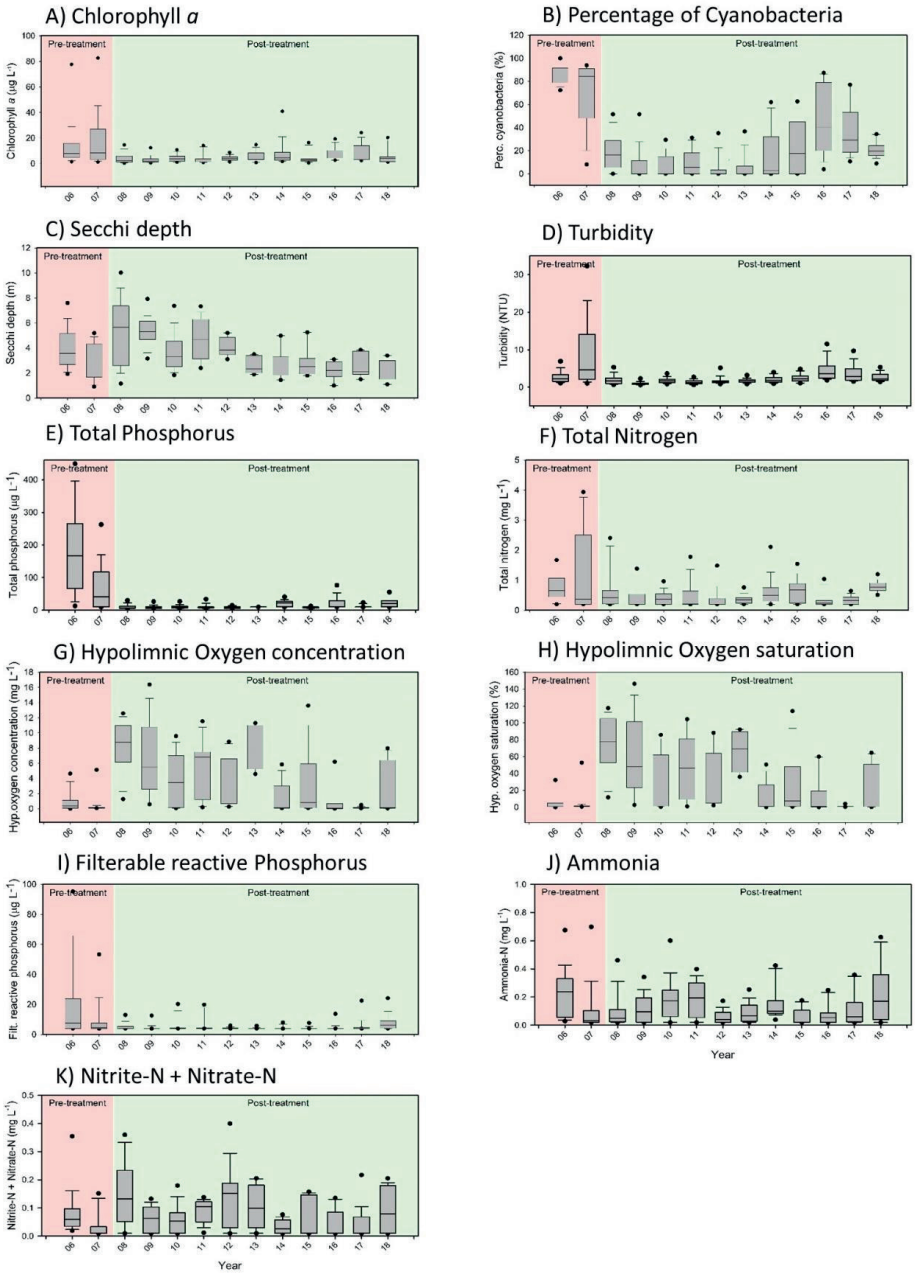


Figure 5.3 Box plots per year of the chlorophyll-a concentration (A) , percentage of cyanobacteria (B), Secchi disk depth (C), turbidity (D), total phosphorus(E), total nitrogen (F) during the whole year, and Hypolimnic oxygen concentration(G) and hypolimnetic oxygen saturation (H), during June to September, at depths 7 to 10 m, during pre and post treatment. The line inside the box is the median, the limits of the box encompass the 25th and 75th percentiles ages, the whiskers are 10th and 90th percentiles, and the dots indicate the 95% confidence interval. Each box encompasses the temporal variability in each year.

Table 5.2 A: Release of Filterable Reactive Phosphorus (FRP). B: Test results release of Dissolved Inorganic Nitrogen (DIN); n = number of replicates, SD = standard deviation, T = test statistic, df= degrees of freedom, release p = two-tailed p-value, β = power of the test with alpha = 0.050.

A)FRP release							
Exp.	Mean (SD)	Mean (SD)	n	T	df	p	β
	a	b					
2005	2.4 (2.7)	1.3 (2.0)	6	Not tested			
	before	after					
2008	15.1 (5.4)	2.3 (0.6)	5	[1)] 8.4	8	< 0.01	
	anaerobic	aerobic					
2011	3.0 (0.7)	1.2 (0.1)	3	[1)] 6.4	4	< 0.01	
2013	4.6 (2.7)	1.6 (1.0)	4	2.1	6	0.08	0.43
B) DIN release							
Exp.	Mean (SD)	Mean (SD)	n	T	df	p	β
	a	b					
2005	31.2 (6.8)	72.9 (11.6)	6	Not tested			
	before	after					
2008	126.3 (29.1)	145.3 (76.7)	5	-0.52	8	0.6	0.07
	anaerobic	aerobic					
2011	44.1 (3.2)	43.9 (5.8)	3	0.05	4	1.0	0.05
2013	24.1 (5.8)	27.3 (4.6)	4	-0.86	6	0.4	0.11

[1)] T test was done on log transformed data

A)

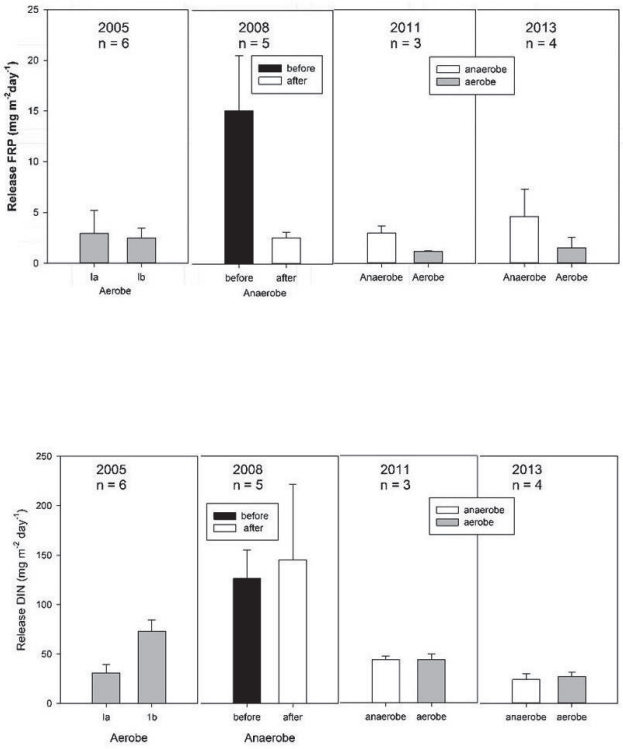


Figure 5.4 The sediment release of Filterable Reactive Phosphorus (FRP) (A); Dissolved Inorganic Nitrogen (DIN) (B); The year 2005 - 2013 refer to the experiments in the text. 2005 a = cores sampled in November, b = cores sampled in December. 2005 a, b and 2008 black bar = before treatment. 2008 white bar and 2011, 2013 = post-treatment.

5.3.6 Internal loading of filterable reactive phosphorus and dissolved inorganic nitrogen

Estimated for the whole lake, in 2008 before the treatment, the internal P load was 6.82 mg m^{-2} day^{-1} (Chapter 2). After the treatment this was reduced to 2.3 mg m^{-2} day^{-1}, based on the anaerobic worst-case scenario. In 2011 it was 1.8 mg m^{-2} day^{-1} and in 2013 it was 2.6 mg m^{-2} day^{-1} (Table 5.3). In the 2011 and 2013 experiments, the FRP release under aerobic conditions was about half that under anaerobic conditions (Table 5.3). During 2008 the hypolimnion remained aerobic, meaning that the worst-case scenario of 2.3 mg P m^{-2} day^{-1} sediment release under anaerobic conditions can be assumed to be its probable half because of extended *in-situ* aerobic conditions (1.15 mg P m^{-2} day^{-1}). The 2008 post-treatment internal load thus becomes 1.55 mg P m^{-2} day^{-1}. Before the treatment, the internal DIN release was 77.9 mg m^{-2} day^{-1}. In 2011 this was 50.0 mg m^{-2} day^{-1} and in 2013 it was 26.2 mg m^{-2} day^{-1}.

Table 5.3 Internal Phosphorus Loads to Lake Rauwbraken. Year = year for which the internal loading is computed, period = period of thermal stratification, conditions refers to anaerobic hypolimnion or aerobic area, A = sediment surface area, release = sediment FRP release (mg m-2 day^{-1}), N_{day} = number of days, Load = internal load per period (kg); total = total internal P load (kg year^{-1}).

	Conditions	A (m^2)	Release (mg m^{-2} day^{-1})	N_{day} day	Load (kg)		
Before 2008	I_{hyp}	15,924	15.1	205	49.4		
	I_{epi}	9,768	2.4	205	4.8		
	I_{mix}	25,692	2.4	160	9.9	+	
	total				64.0	=	6.82 (mg m^{-2} day^{-1})
After 2008	I_{hyp}	15,924	2.3	205	7.5		
	I_{epi}	9,768	2.3	205	4.6		
	I_{mix}	25,692	2.3	160	9.5	+	
	total				21.6	=	2.3 (mg m^{-2} day^{-1})
2011	I_{hyp}	15,924	3.0	205	9.8		
	I_{epi}	9,768	1.2	205	2.4		
	I_{mix}	25,692	1.2	160	4.9	+	
	total				17.1	=	1.8 (mg m^{-2} day^{-1})
2013	I_{hyp}	15,924	4.6	205	15.0		
	I_{epi}	9,768	1.6	205	3.2		
	I_{mix}	25,692	1.6	160	6.6	+	
	total				24.8	=	2.6 (mg m^{-2} day^{-1})

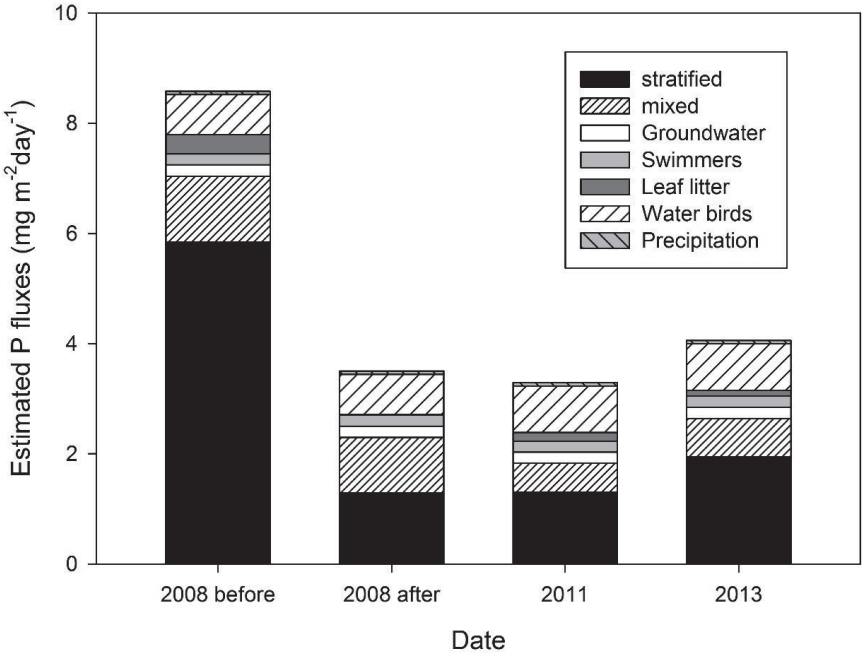


Figure 5.5 Phosphorus fluxes (mg m^{-2} day^{-1}) in Lake Rauwbraken; stratified = internal P loading during thermal stratification, mixed = internal P loading during mixed period.

5.3.7 Macrophytes

From 2006 to 2008, coverage by *Elodea* increased. One week prior to the Floc & Lock treatment, *Elodea* was present as a massive canopy around the lake down to 4 m depth, further some specimens of *Nitella* sp. and *Potamogeton* sp. were present. During the clear waters observed in 2008, *Elodea* grew down to 9 m depth (albeit at low densities at this depth). Post-treatment the *Elodea* density gradually became less and reached less deep (Fig. 5.6), while *Nitella* sp. became more abundant (Fig. 5.6B, C). In 2015, the invasive species *Crassula helmsii* was observed, and in 2016 its coverage increased (Fig. 5.6D). Taken over the whole post treatment period, coverage by submerged macrophytes first increased, while during later years coverage reduced.

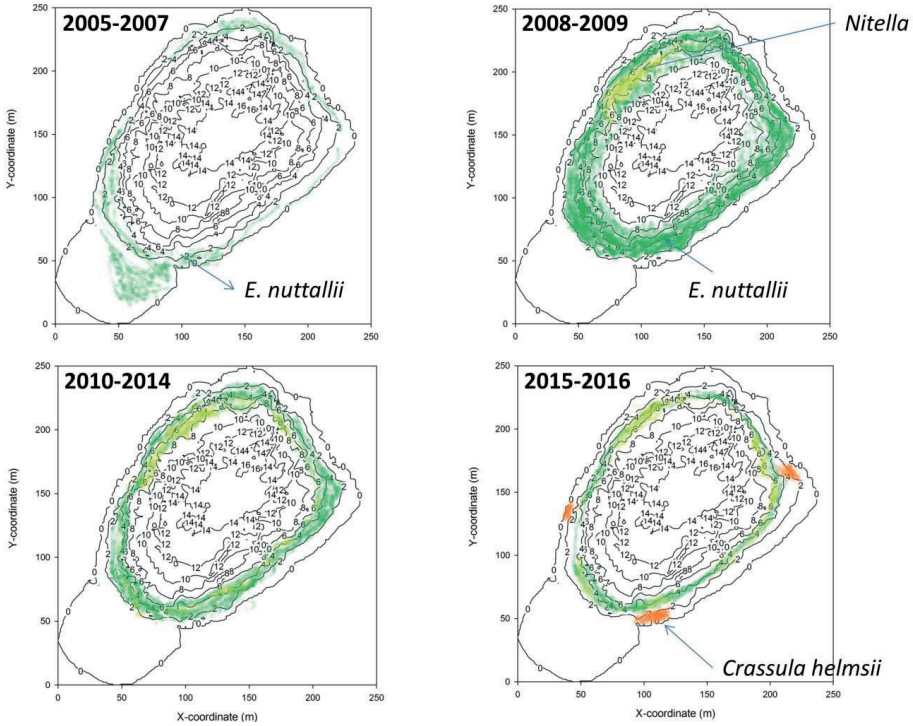


Figure 5.6 Macrophytes coverage in Lake Rauwbraken pre (2005-2007) and post treatment (other panels), solid lines = depth, dark green = *Elodea nutallii*, light green = *Nitella* sp., orange = *Crassula helmsii*.

During summer, water level drops to 1.66 m (on average) below the high winter level. As a result, the submerged macrophytes in this shallow zone fall dry and die due to desiccation (Fig. 5.7). By approximation (z_{eu}, Chapter 2), post-treatment, the submerged macrophytes were light-limited below 6.4 m.

Assuming the macrophytes may fill the water column down to 4 m depth, less than 8% of the lake volume may benefit from their positive effects on the water quality. Based on the transect in October 2008, this zone may contain approximately 4.2 kg P present in submerged macrophytes. Compared to the 34 kg total in the water column, submerged macrophytes played a minor role as P-compartment as compared to the water column TP content. This thus explains how the uptake of P by the newly established submerged macrophytes was not enough to prevent the blooms of cyanobacteria.

In 2011, maintenance (Chapter 3) stopped and the 1,382 kg of organic material remained on the lake's shores. By 2013, this load had doubled to 2,488 kg year^{-1}. The external P loads from organic material are given in Table 5.4.

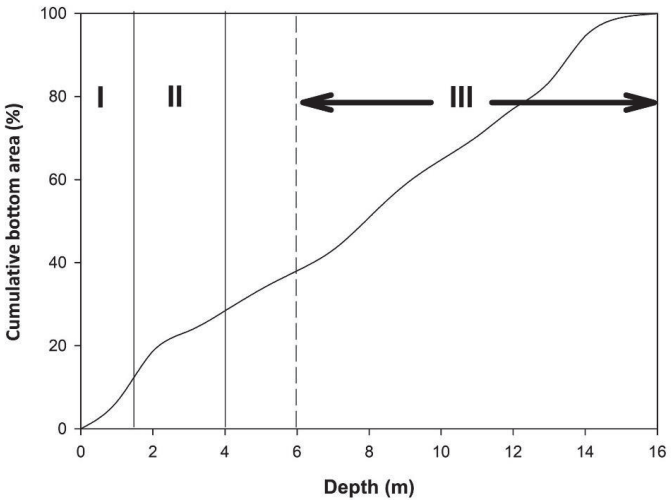


Figure 5.7 Physical factors limiting coverage of submerged macrophytes in Lake Rauwbraken, the solid line indicates the percentage of the lake's bottom surface area at the depth relative to the highest water level observed, the area indicated with I is the dry-falling zone, the area indicated with II is the area covered by submerged macrophytes in April 2008, the dashed line indicates zeu. Zeu is based on the pre-treatment mean Secchi disk depth (1.66 m). Area III (depths deeper than the dashed line) indicates light limited area.

Table 5.4 Phosphorus loads from organic material.

Year	Organic litter	Phosphorus
	(kg year^{-1})	(mg m^{-2} day^{-1})
2000	5,528	0.35
2005	138	0.01
2011	1,244	0.08
2013	2,488	0.16

5.3.8 Mystery scum

5.3.8.1 Microcystins

On August 3rd 2011 the regional water authority observed about 3.5 million cyanobacterial cells mL^{-1} (3.4 million being *Microcystis*), cyanobacterial biovolume 287 mm^3 L^{-1} (of which 266 mm^3 L^{-1} *Microcystis*) in a water sample from Lake Rauwbraken and a swimming ban was imposed. According to our analysis the yellow ochre accumulated material (indeed consisting mainly of *Microcystis*) had a cyanobacterial chlorophyll-a concentration of 1,208 µg L^{-1} with mean total MC concentration of 1.35 (± 0.35) µg L^{-1} (n = 3), or 0.72 (± 0.19) µg g^{-1} based on dry weight (Fig. 5.8). Three MC variants were detected: dmMC-LR, MC-LR and MC-YR. Meanwhile, the cyanobacterial chlorophyll-a concentrations over the first 8 m water column were less than 0.5 µg L^{-1} and total chlorophyll-a was less than 2 µg L^{-1}, close to the sediment at 9 m chlorophyll was about 10 µg L^{-1} (Fig. 5.9) with a Secchi-depth of 4.5 meters.

The mystery scum came about without preceding water column proliferation of cyanobacteria (Fig. 5.10A, B). The 2011 water column TP (Fig. 5.10C) was too low to support such proliferation both before and after the occurrence of the scum.

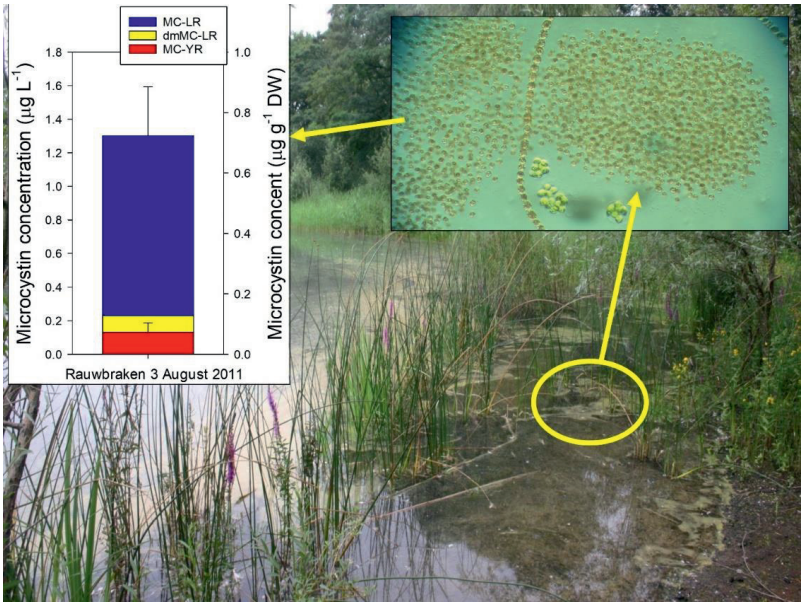


Figure 5.8 The near shore surface accumulation in Lake Rauwbraken on August 3rd 2011 consisted mostly of *Microcystis* that produced primarily microcystin-LR (MC-LR) with a total MC concentration of 1.3 µg L^{-1}.

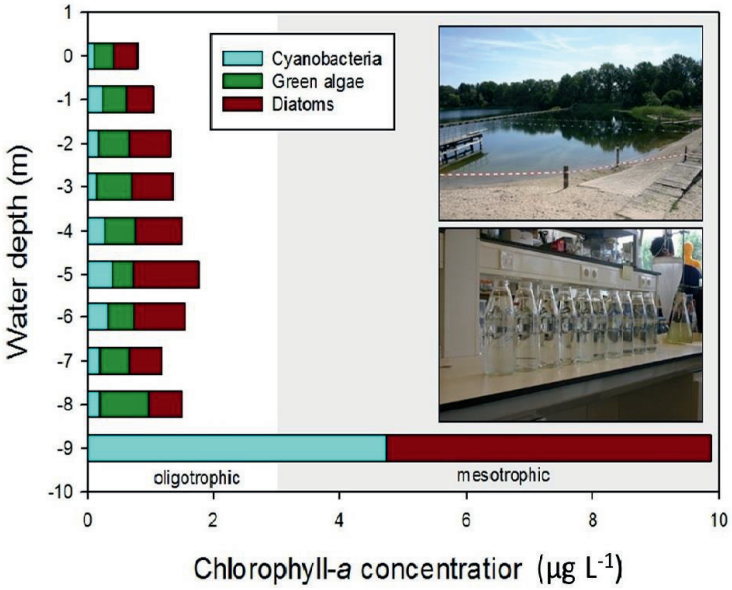


Figure 5.9 Chlorophyll-a concentrations over the water column in Lake Rauwbraken on August 3rd, 2011. The inserted photos show the clear water.

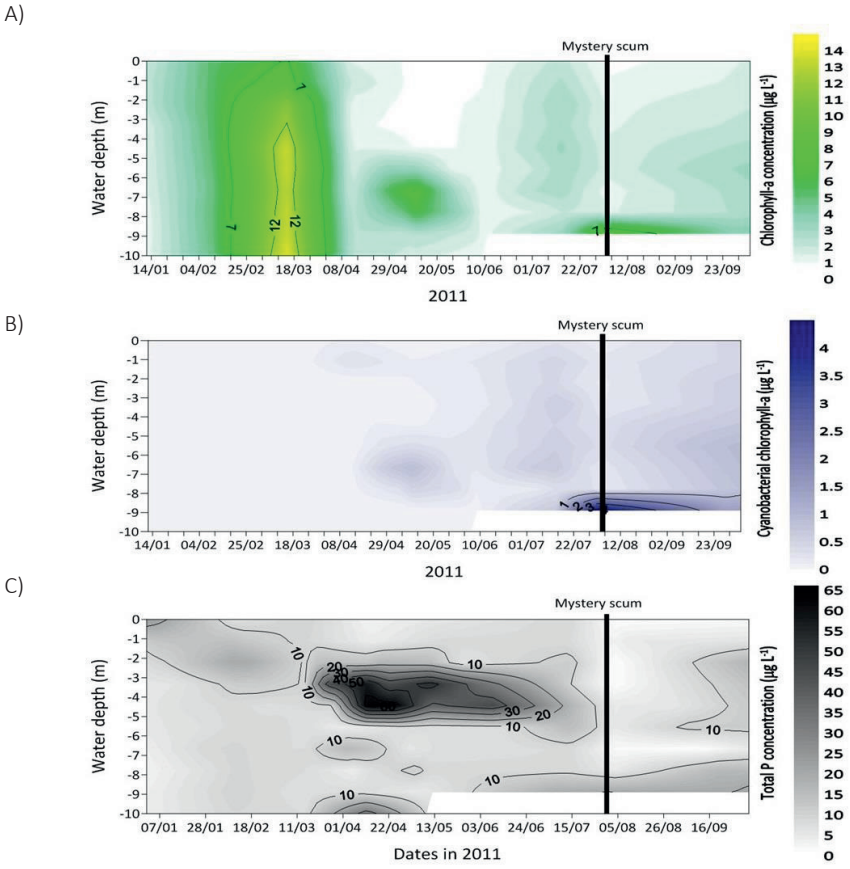


Figure 5.10 The chlorophyll-a concentration (µg L^{-1}), cyanobacterial chlorophyll-a (µg L^{-1}) and total phosphorus (µg L^{-1}), during 2011 in Lake Rauwbraken over the water depth and in time; chlorophyll-a concentration (A), Cyanobacterial Chlorophyll-a concentration (B),Total phosphorus concentration (C). The vertical bar indicates the occurrence of the mystery Microcystis scum.

5.3.8.2 Sediment incubation experiment

The initial chlorophyll-a concentrations in the 25 µL sediment suspensions taken at 20-22 mm sediment depth from core I were 8.4 (± 0.3) µg L^{-1} for cyanobacteria, 3.0 (± 0.4) µg L^{-1} for green algae and 8.8 (± 0.5) µg L^{-1} for diatoms; core II 15.3 (± 3.0) µg L^{-1} for cyanobacteria, 9.2 (± 1.1) µg L^{-1} for green algae and 10.5 (± 2.2) µg L^{-1} for diatoms. The overall Photosystem II efficiency was 0.26 (± 0.05). The algal growth medium yielded no Photosystem II efficiency and had an average background fluorescence corresponding to a total chlorophyll-a concentration of 0.39 (± 0.04) µg L^{-1}. After 7 days of incubation in

all sediment suspensions a phytoplankton community had developed consisting of cyanobacteria, green algae and diatoms with corresponding chlorophyll-a concentrations exceeding the start values (Fig. 5.11).

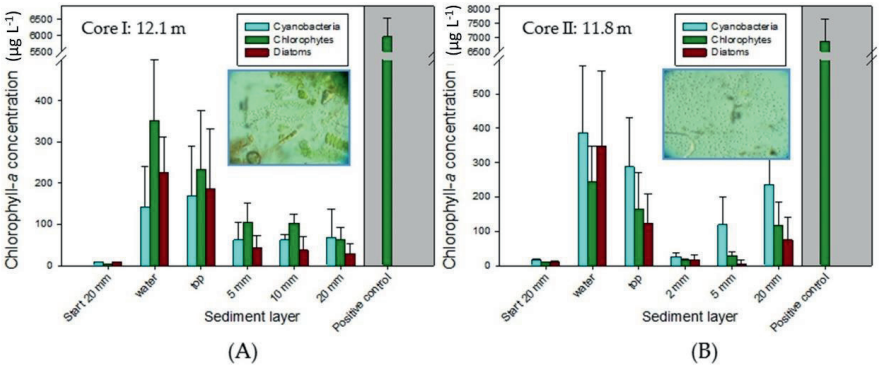


Figure 5.11 Chlorophyll-a concentrations (µg L^{-1}) for cyanobacteria, green algae and diatoms in sediment suspensions of algal growth medium with sediment taken at different depths from cores from Lake Rauwbraken (top layer, 2 or 5 mm deep, 10 mm and 20 mm) as well as the over standing water. The positive control is a culture of Scenedesmus obliquus inoculated in the algal growth medium. Error bars indicate one standard deviation (n = 3);material from a core taken at 12.1 m deep (A), material from a core taken at 11.8 m deep (B).

5.4 Discussion

5.4.1 Change in trophic status

The 2008 Floc & Lock application in Lake Rauwbraken (Chapter 4) resulted in ten years of successful mitigation of cyanobacterial nuisances, meaning that as a bathing site, the lake fulfilled its main ecosystem service. Our study shows a long term effective reduction in FRP and TP concentrations, according to OECD criteria (OECD 1982), Lake Rauwbraken has shifted from a eutrophic to a predominantly mesotrophic status (Fig. 5.12). We attribute these longer-term effects to the P binding by the LMB. The PAC applied as flocculent (Chapter 4) undoubtedly will have bound phosphates (Lopata and Gawrońska 2008). Based on the 11:1 Al:P weight ratio (Rydin et al. 2000) or a molar Al:P ratio of minimally 10:1 (de Vicente et al. 2008), the two tons of PAC (178 kg Al) could immobilise 16-17 kg P.

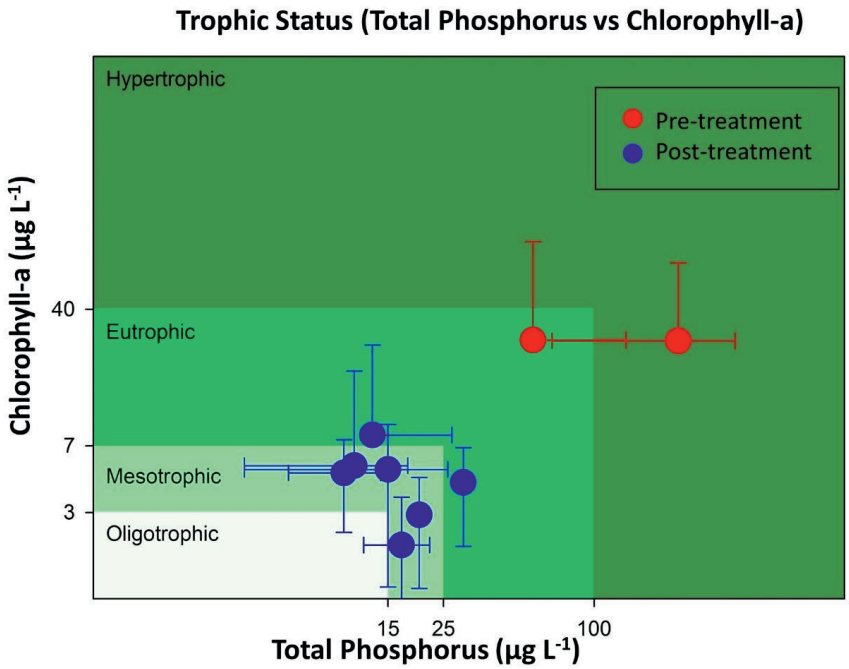


Figure 5.12 Pre- and post-treatment trophic status of Lake Rauwbraken.

5.4.2 Longer-term treatment effects

5.4.2.1 Chlorophyll-a, cyanobacteria

We did not find a significant trend in the annual mean chlorophyll-a concentrations, however the percentage of cyanobacterial chlorophyll-a showed a significant trend up. It thus seems that at low total

phosphorus concentrations (mean = 14 µg L^{-1}, SD = 14 µg L^{-1}) cyanobacteria may outcompete other phytoplankton groups (green algae or diatoms).

It is, clear that the cyanobacteria are building up biomass (as the increase of the percentage of cyanobacteria indicates) to a density that may result in nuisance and new swimming bans. As no effort was done to reduce the external P loads, this return is fully expected from recent (post-treatment) external P loading. As our sediment release experiments 2011 and 2013 (see below) show, the lake is building up a new mobile P- pool. The first signs are evident and the 2016 spring appearance of low biomass of *Planktothrix rubescens* further underpins this.

5.4.2.2 Single in-lake P reduction

We observed a significant trend upwards in TP, but not in FRP, TN, AMM or NN. It seems that the increase in the contribution of cyanobacteria is linked to the increase in TP. Considering the fact that this observation is made on a single lake, our data do not allow to test for a causal relation. The observation that both the contribution of cyanobacteria and TP both increase, while TN, AMM and NN remained without significant trend, is in line with our idea that single P control suffices to control cyanobacteria. While noting that the particulate N fraction was reduced by the Floc & Lock application, bio-available N (DIN) was not reduced (**Chapter 4**). The algal growth experiments done in 2011 confirm this P limitation (*see* **Appendix 5.6.2**: algal growth experiment).

5.4.2.3 Water Clarity

Our results indicate that summer Secchi disk depth improved shortly after the Floc & Lock treatment and remained improved for four years after treatment. Later observations show a significant trend downwards and we expect that Secchi disk depth is returning to its pre-treatment value. In bathing water a minimum of 1 m Secchi disk depth is widely accepted as safety requirement by Dutch Water Authorities, but not demanded by legislation (EU 2006). During five years in the period 2000-2008 (pre-treatment) Secchi disk depth fell below 1 m just before the bathing season started. While we can set aside 2008 as a transitional year, Secchi-depths below 1 m were not observed during 2009 -2018. In 2008 (post-treatment), the lowest Secchi disk depth (0.7 m) was found during the application of the LMB. The lowest Secchi disk depth post treatment was 1.5 m (before the bathing season, February 2014). Comparing Fig. 5.2 A to C, it is clear that turbidity quite closely follows the chlorophyll-a concentrations, indicating that the underwater light climate is mostly governed by the phytoplankton density.

5.4.2.4 Oxygen

Towards the end of the post-treatment period, anaerobic periods in the hypolimnion seem to intensify, which might be a result of the gradual build-up of newly deposited organic matter combined with lower transparency of the water column and thus less photosynthesis in the hypolimnion during stratification implying that oxygen consumption processes are prevailing. The effect of the treatment on the hypolimnetic oxygen regime thus lasted five to six years.

5.4.2.5 Internal Loading

The treatment significantly reduced the internal P loading as is evident from the before – after comparison on the 2008 cores. We reject hypothesis ii, meaning that the Floc & Lock treatment reduced the internal FRP load, which as above, we attribute to the LMB. However the FRP release increased over the post-treatment years (2011 - 2013). A similar effect was found in the Lake de Kuil (Netherlands; Floc & Lock treated in 2009) where the sediment P release decreased from 5.2 mg P m^{-2} day^{-1} (before) to 0.4 P m^{-2} day^{-1} mg (after) and increased in later years (Waajen et al. 2016a). As could be seen from the sediment P release experiment, P release was not completely zero and hence some growth is to be expected. The LMB dose aimed to immobilize all potential releasable P in the top 5 cm of the sediment (**Chapter 4**), yet a remaining, but far less, P efflux implies the sediment P release was not completely blocked.

The most difficult part in treating a lake with a P adsorbent to counteract sediment P-release is the estimation of the communicating sediment depth. In Lake Rauwbraken the communicating depth was estimated to be 5 cm (**Chapter 4**), which turned out a good proxy. I.e. in June 2014, the majority of the lanthanum applied to the lake, was found back in the top 5 cm of the sediment (Dithmer et al. 2016b). Hence, the increased FRP release as found in the sediment P release experiment over time is probably not caused by a deeper mixing of the LMB into the sediment. As no effort was done to reduce the external P loads, the increase in internal FRP loading post treatment is fully expected from recent (post-treatment) external P loading.

The sediment DIN release, both pre- and post-treatment, as well as under both anaerobic and aerobic conditions occurred mostly as AMM. The DIN releases in experiment 2008 (both pre- and post-treatment) are higher than in all other experiments. The DIN concentrations in the overlying water after two years of core incubation (experiment 2008) as well as the measured release of DIN during experiment 2008 revealed a significant difference between the cores sampled before and after the treatment. The 2008 DIN release rates were higher compared to the ones sampled in 2011 (3 years after the treatment) and 2013 (5 years after the treatment). The fact that DIN may have accumulated in the 2008 cores during their incubation indicates that the release of DIN in this experiment cannot be

interpreted as equivalent to DIN release in the field. Hence, we did not include them in the calculations of the DIN flux calculations above. It seems DIN release was not affected by oxygen concentration, since no difference was observed in aerobic and anaerobic conditions.

Although our "anaerobic conditions" contained enough oxygen (> 0.1 mg O_2 L^{-1}) for ammonium nitrification (Carlucci and McNally 1960; Goreau et al. 1980), we cannot rule out anaerobic conditions in the treatment, thus denitrification and anaerobic ammonium oxidation (Anammox) that may lead to some loss of nitrogen as N_2 (e.g.: Francis et al. (2007)). Thus, with respect to nitrogen, our experimental units are not closed systems, meaning that the estimated amount of released DIN are best interpreted as minimum amounts of DIN released.

5.4.3 Phosphorus Balance

In 2008 before the treatment, the total P load was 8.03 mg P m^{-2} day^{-1} (Fig. 5.5). This load comprised of 6.82 mg P m^{-2} day^{-1} (85%) internal load (Table 5.3) and 1.21 mg P m^{-2} day^{-1} (15%) external load (**Chapter 2**). With an outflow of 0.09 mg P m^{-2} day^{-1} as loss to the ground water, 1.12 mg P m^{-2} day^{-1} remained in the lake. After the intervention and based on the worst-case scenario, the internal P load was reduced to 2.3 mg P m^{-2} day^{-1} (Table 5.3). However, the bigger part of the reduction in internal P load was achieved in the lake's hypolimnion. In 2008, no aerobic incubations were done, but using the 2011 and 2013 data yield on average 36% of the flux under anaerobic conditions. Since the hypolimnion was not anoxic in 2008, a proxy of $2.3 \times 0.36 = 0.8$ mg P m^{-2} day^{-1} can be obtained.

Accordingly, the total P load was reduced to 3.51 (mg P m^{-2} day^{-1}) (Fig. 5.5). While the contributions from ground water, swimmers and rain remained unchanged during the study, the external P loads from water birds and leave litter increased in 2011 and 2013 (Table 5.4; Table 2.2). After 2011, maintenance of the greenbelts stopped and geese became resident to the lake (**Chapter 2**), changing the external nutrient loads to the lake. Also, the internal P loads increased in 2011 and 2013 (Table 5.3). Despite the increase, the internal load remained below its pre-treatment values (Table 5.3; Fig. 5.5).

5.4.4 Macrophytes

Post-treatment during 2008, this eutrophic character of the vegetation remained at shallow depths, while during the period of clear waters allowed *Elodea* to grow down to 9 m depth. The growth of submerged vegetation at these greater depths reflects an oligotrophic system. While water clarity during 2010-2014 allowed growth of submerged macrophytes, the coverage reduced and the species changed from *Elodea* to *Nitella*, which can be seen as a change from an eutrophic vegetation to an oligotrophic vegetation (Lambert-Servien et al. 2006). Whereas the Floc & Lock treatment instantaneously shifted the water quality from eutrophic to oligo-mesotrophic, it seems that this shift was followed with at least one year delay by the submerged vegetation. The gradual decline in

vegetation cover matches the steady reverse to more eutrophic conditions. The sudden appearance of the invasive macrophyte *Crassula helmsii* is not entirely unexpected. It is first appearance dates back to 1995 also in the province of Noord-Brabant (Brouwer and den Hartog 1996) and it has been spreading ever since.

5.4.5 Mystery scum

On August 3rd 2011, after three years without swimming bans and good water quality according to the BWD, a scum was observed in the bathing area. The regional water authority issued a swimming ban based on cyanobacterial biomass in this scum, the total MC concentration of 1.35 (± 0.35) µg L^{-1} indicated no health risks. This scum was considered a mystery because it occurred without a preceding water column proliferation.

The accumulated material (approximately 50 m^2 and maximally 0.5 cm thick; (Fig. 5.8)) could be identified as a category II scum according to the Dutch Cyanobacteria Protocol (Ibelings 2005). The relatively low MC concentration (1.35 µg L^{-1}), is regarded as posing little risk with low probability of adverse health effects below 10 µg L^{-1} (Chorus et al. 2000). Evidently, not all cyanobacterial scums or other high concentrations of cyanobacteria coincide with high concentrations of toxins (Faassen and Lürling 2010; Sivonen and Jones 1999). Hence, the observed scum qualified as a false positive observation. This mystery scum vanished rapidly and the swimming ban was lifted one week after it had been imposed. Fig. 5.10 (A,B) shows there was no water column proliferation in the months before and after the occurrence of the mystery scum. The fact that the cyanobacteria were not able to proliferate can be explained by the low water column TP (Fig. 5.10C), which we attribute to the intervention, i.e. the treatment had reduced mean water column TP from 134 µg P L^{-1} pre-intervention to 14 µg P L^{-1} post (April 24th 2008 – December 31st 2016) (Table 5.1).

While the scum formation can be explained by the accretion of cyanobacteria at the lake surface due to buoyancy control and accumulation at the lee side of the lake, the origin of the cyanobacteria themselves needs to be explained. One possibility is that they were growing slowly in the water column while an alternative is recruitment from the sediment. Cyanobacteria were present in the water column, albeit at very low biomass with on average 0.35 µg L^{-1} chlorophyll-a in July and August 2011 (Fig. 5.10B). Nonetheless, accumulation of all this in 0.5 cm at the water surface would yield around 600 µg L^{-1} chlorophyll-a and thus the 1200 µg L^{-1} observed in the nearshore accumulation could have originated from such process combined with further accumulation by wind action. Thousand-fold to million-fold concentrations of cyanobacterial cell populations may occur by such accumulation and wind effects (Falconer et al. 1999). By rough estimation the scum had a 0.25 m^3 (0.005 m thick x 50 m^2 = 0.25 m^3) volume, with 1208 µg L^{-1} cyanobacterial chlorophyll-a the total content of the scum was 302 mg (250 L x 1208 µg L^{-1}). If the scum originated from accumulation from the whole lake (V = 207912 m^3), this would

imply a hardly noticeable change of (302÷207912) 0.0015 µg L^{-1} water column chlorophyll-a (Phyto Pam LOD = 0.01 µg L^{-1}). Hence, a mystery scum like the one described here may easily develop in the best of all waters, e.g. oligotrophic waters may still hold up to 3 µg L^{-1} chlorophyll-a (classification according to (Forsberg and Rydin 1980)), which easily allows for the formation of a mystery scum. Such processes are most probably causing the nearshore cyanobacteria accumulations in oligotrophic North-Patagonian lakes (Nimptsch et al. 2016). Where those accumulations occur regularly (Nimptsch et al. 2016), the sudden appearance in Lake Rauwbraken also opens a possibility for an alternative explanation via sediment recruitment. The survival of cyanobacteria cells or akinetes in the sediment together with their ability to emerge from the sediment, e.g. through gas vesicle based buoyancy control makes the benthic connection an important part of the ecology of cyanobacteria (Reynolds et al. 1981). Viable *Microcystis* colonies may reside in the sediment of lakes up to several cm deep in the sediment (Brunberg and Boström 1992; Latour and Giraudet 2004; Reynolds et al. 1981). Also, in Lake Rauwbraken viable cyanobacteria (and eukaryote algae) were present in the sediment (Fig. 5.11). Hence, the sudden cyanobacterial scum in Lake Rauwbraken may have been a release of *Microcystis* from the sediment. While circumstances were in favour to emerge from the sediment, this inoculum found no grounds to proliferate, which under more nutrient-rich conditions it could do (Reynolds et al. 1981).

On the observation of a scum of cyanobacteria the obvious thing to consider is its size. The 2011 scum was not that massive (see Fig. 5.8). A small amount of accumulated cyanobacteria can look seriously bad when sampled (Fig. 5.13). If at all there is in this case a problem, it is of the same order of magnitude as some geese droppings on a beach – which may involve *Campylobacter* (Gorham and Lee 2016), hence a risk for human health, a problem easily dealt with. In this case in 2010, the few cm^2 accumulation (Fig. 5.13) was scooped off with a bucket and deposited in the green belt, which solved the problem.

A) B) C)

Figure 5.13 A very small scum of cyanobacteria in Lake Rauwbraken in 2010 (A). This accumulation may look more severe when zoomed in (B) and particularly very dense when sampled (C). The few dozen cm^2 accumulation was scooped off with a bucket and deposited in the green belt. Photos by Frits Smit.

5.5 Conclusions

- The Flock & Lock treatment changed Lake Rauwbraken from hypereutrophic (pre-treatment) to oligo-mesotrophic (post-treatment).
- The 2008 Floc & Lock application in Lake Rauwbraken significantly reduced the internal P-load. We reject the hypothesis that there was no difference between the before and after treatments sediment FRP releases. We consider the longer-term reduction in chlorophyll-a a result of phosphorus limitation. As the treatment did not affect the bio-availability of nitrogen in ammonia or nitrite + nitrate and their fluxes to the lake were not changed. We consider the reduction in TN a concomitant effect of the imposed phosphorus limitation.
- Post-treatment we observed significant trends in the percentage of cyanobacterial chlorophyll-a, TP, Secchi disk depth, turbidity and hypolimnic oxygen concentration and saturations, hence for these variables we reject our hypothesis of no trend.
- No trends were found in Chlorophyll-a, FRP, TN, AMM or NN.
- The overall effects of the Floc & Lock treatment lasted ten years, which makes the technique an economically feasible method.
- Our results also indicate that the lake is returning to a eutrophic system which is due to the phosphorus inflows, a maintenance treatment of Lake Rauwbraken is needed.
- In 2011 a sudden, relatively small accumulation of cyanobacteria was discovered near the shore. A swimming ban was issued, which we found no support in either the Dutch Cyanobacteria Protocol nor in the microcystin content of the accumulated material. A true positive hazard can only follow from toxin analysis, hence rather than crying wolf on the observation of a local high density of cyanobacteria, it makes sense to measure their toxicity. Measuring toxins will also exclude the false positive. If an accumulation or scum is present the toxin analysis should be done on the high-density material.
- Our whole lake experiment adds to the body of evidence that phosphorus control can mitigate eutrophication and consequently strongly reduce harmful cyanobacteria biomass.

5.6 Appendix

5.6.1 Sediment release experiments.

The experimental conditions of the sediment release experiments are given in Table 5.5.

Table 5.5 Sediment release experiments, Experiment = year the cores were sampled, treatment = pre- or post- the Floc & Lock treatment in Lake Rauwbraken, Cores = number of replicates, water Demi = demineralized water, Millipore = Millipore water (Billerica, MA, USA), Volume = volume of water replaced, Incubation time = duration of each incubation, Incubations = number of incubations, condition = oxygen condition maintained during the experiment.

Experiment	2005 a/b	2008	2011	2013
Treatment	pre-	pre-/post-	post-	post-
Cores	6/6	5/5	3/3	4/4
Water	Demi	Millipore	Millipore	Millipore
Volume	0.2 l	all overlying water	all overlying water	all overlying water
Incubation time (days)	1	1	1	7
Incubations	4	4	4	5
Condition	aerobic	anaerobic	aerobic/ anaerobic	aerobic/ anaerobic
T (°C)	20	7	7	18
Controls (n)	6	4	12	3

5.6.2 Algal growth experiment

5.6.2.1 Introduction

As we set out to impose phosphorus limitation on the phytoplankton in Lake Rauwbraken, it bears relevance to show that this goal was achieved. To show that, post-treatment in Lake Rauwbraken, phytoplankton was phosphorus limited an algal growth experiment was done with water samples taken in 2011 from lake Rauwbraken. We test the hypothesis: adding N or P does not affect the growth rates.

5.6.2.2 Methods

In 2011 (January 14, February 11, March 15, April 14, May 15, June 10 and July 15), aliquots were taken from Lake Rauwbraken samples obtained during regular sampling from two and nine m depths. Without processing, 12 the aliquots for each depth were divided in 50 ml subsamples in 100 ml Erlenmeyer's. For each depth, three Erlenmeyer's were kept as control (no additions), three were spiked with 50 µL KNO_3 , three with K_3PO_4 and three with both (as positive control). The nutrient solutions were obtained from stock solutions (N = 14 g N L^{-1}, P = 1.2 g P L^{-1}). The Erlenmeyer's were closed (cellulose plugs) and incubated 7 days at 20ºC, 130 µmol quanta m^2s^{-1},16:8 h light : dark, 60 rpm. Before spiking all and after seven days incubation chlorophyll-a was measured by Phyto-Pam phytoplankton analyser (Heinz Walz GmbH, Germany). The growth rate (λ, day^{-1}) was estimated by the formula: $\lambda = \frac{\ln(Chla_t) - \ln(Chla_0)}{\Delta t}$, in which, t = time (days; t = 0, 7), $\mathrm{Chla_t}$ the chlorophyll-a concentration (µg L^{-1}) measured at day = t, Δt = time lapse (7 days).

In the statistical analyses, the incubations from 2 m and 9 m depths are treated as different experiments. We treat the samples taken on the different dates as replicates. As the estimated growth rates (λ) failed normality, and transformation solved this problem only for 2 m depth, we chose to do a Kruskal-Wallis one way Analysis of variance on ranks.

5.6.2.3 Results and discussion

During most of the months growth was observed in the controls (no nutrients added). However, some negative growth rates were observed, e.g. in January, March at 2 m depth (Fig. 5.14A). Whether phytoplankton was so poorly nourished that it died or was grazed by zooplankton we cannot say. Adding both N and P (positive control) resulted in higher (positive) growth rates than in the controls (Fig. 5.14 A,B), meaning that our samples contained healthy phytoplankton that could grow if well nourished. The fact that N+P incubations resulted in the highest growth rates confines nutrient limitation in the controls (no nutrients added) to either N or P. Comparing them to the controls (no nutrient added), adding only N makes P the limiting nutrient in the incubations, likewise adding only P makes N the limiting nutrient.

A) B)

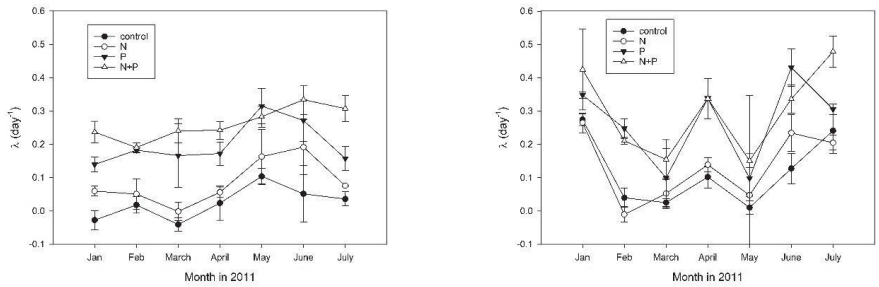


Figure 5.14 Algal growth experiments during 2011, 2 m depth (A), 9 m depth (B). Control = no nutrients added, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added.

The analysis of variance on ranks revealed significant effects of the added nutrients (2 m: $H_{df=3} = 57.3$, $p < 0.01$; 9 m: $H_{df=3} = 36.0$, $p < 0.01$). We reject the no-effects of added nutrients hypothesis. For both depths, the all pairwise multiple comparison (Tukey Test) revealed two significantly differing groups: group 1 = N+P, P added, group 2 = controls, N added.

We conclude that in the water samples taken from Lake Rauwbraken phytoplankton growth rates were P-limited.

J.F.X. van Oosterhout

Aquarium Ruin

Incomplete oeuvre of unfinished works

Pencil on paper

50 x 29.5 cm

Chapter 6

The Fate of Lanthanum

Parts of this chapter were published in:

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6 The Fate of Lanthanum

6.1 Introduction

Although the lanthanum modified bentonite (LMB) is reported as a promising solid phase phosphorus binder (Akhurst et al. 2004; Copetti et al. 2016; Douglas et al. 2004; Haghseresht et al. 2009; Robb et al. 2003; Ross et al. 2008; van Oosterhout and Lürling 2013), there are concerns regarding the environmental safety of La modified clays. Depending on the chemical water composition and concentration of its dissolved or free ion forms, La can be toxic to aquatic organisms (Akhurst et al. 2004; Barry and Meehan 2000; Douglas et al. 2004; NICNAS 2001). Much of the environmental safety of LMB is derived from the fact that La is stored in the bentonite matrix and the extreme low solubility (Johannesson and Lyons 1994; Liu and Byrne 1997) of the lanthanum-phosphate complex (Rhabdophane), from which it is deducted that La will not be bioavailable after an application of LMB. Lürling and Tolman (2010) reported La leaching from the LMB in a laboratory setting and, a review of 16 case study lakes confirms the release of filterable La to the water column following the LMB (Spears et al. 2013b).

The safety of LMB is described in NICNAS (2001), however, the bioavailability of La from the LMB is not tested (NICNAS 2001). There is evidence that La could be taken up or stored in blue mussels (*Mytilus edulis)* (Lobel et al. 1991) and in duckweeds (*Spirodela polyrhiza*), Cladocerans (*Daphnia magna*), goldfish (*Carassius auratus L.*) and shellfish (*Bellamya aeruginosa*) (Yang et al. 1999). As the LMB is to settle on the lake sediment, it is expected that bottom-dwelling organisms will be exposed to relatively high concentrations of the LMB and potentially to La (Traunspurger and Drews 1996). Crayfish - benthic crustaceans, have been proposed and used as a bioindicator for contamination by heavy metals (Alikhan et al. 1990; Bagatto and Alikhan 1987a; 1987b; Faria et al. 2010).

To test if exposure to the LMB might result in uptake of La and possibly negative effects on the biota, we conducted a parallel groups experiment in which the La contents of unexposed (controls) and LMB exposed marbled crayfish (*Procambarus fallax* f. *virginalis*; (Martin et al. 2010)) are compared after 0, 14, and 28 days exposure. To exclude potential sorption of lanthanum to the outside of the animals and to address possible tissue specific distribution of La samples of gills, hepatopancreas, carapace, ovaries and abdominal muscle were analysed. For each tissue we test the hypothesis that there is no difference in La concentration (µg g^{-1}) between the controls and exposed animals after 0, 14, and 28 days.

In The Netherlands, La is subjected to a maximum permissible concentrations (MPC) legislation (Sneller et al. 2000), which applies to both the water column and sediments. In freshwater systems, the Dutch MPCs are 10.1 µg L^{-1} for nominally filterable (<0.45 µm) La, and 150.1 µg L^{-1} for total La in the water column, while for sediment the MPC is 500.1 mg La kg^{-1} DW (URL3, (Sneller et al. 2000). With

these MPCs effectively regulating the application of LMB, there is a requirement for a detailed understanding of the distribution and concentrations of La in water, sediment and biota following LMB application. The rationale behind La determination and the MPCs is that ecotoxicology of La is assumed to occur in the dissolved form (e.g. $La^{3+}_{(aq)}$) in aqueous solution, where the trivalent La-ion possesses the greatest risk for adverse biological effects (Das et al. 1988). However, Reitzel et al. (2017) demonstrated that "any La found in solution after LMB treatment in hard water lakes is associated with colloids." In addition, it was demonstrated (Mucci et al. 2020) that in waters containing carbonate that La may be present as lanthanite ($La_2(CO_3)_3.8H_2O$) associated with the LMB, quite apart from that bound to phosphate as rhabdophane, or that bound by humic substances. On this basis, for many natural freshwaters, little, if any La is present in the uncomplexed, trivalent state. Nonetheless, further investigation of the nature (speciation) of La present in waters following LMB application, particularly over ecologically –relevant timescale (years to decades) is required to further elucidate the potential for the biogeochemical transfer of La applied as LMB through water, sediment and biota.

While a large number of whole lake LMB applications have been reported in the literature, few studies have focussed on the role of La, and in particular in longitudinal studies relevant to the annual inputs and cycling of P in aquatic systems. Insight in the behaviour and fate of La is important to determine treatment efficacy, longevity and the occurrence, if any, potentially undesirable side effects. After a decade of monitoring, this chapter reports on the fate of La via the analysis of water column and sediment samples in addition to that within macrophytes and chironomid larvae following the LMB application in Lake Rauwbraken. We investigate the spatial and depth heterogeneity of the LMB, and the extent of the La release. We have also used a filtration experiment to test the hypothesis that part of filterable La (FLa) is particulate La.

6.2 Methods

6.2.1 Marbled crayfish

6.2.1.1 Ethical statement

Under Dutch law, experiments with freshwater crayfish do not require approval of the Animal Experiment Committee (in Dutch: Dier Experiment Commissie, DEC). Animal welfare was monitored daily and at termination of the test period animals were culled quick and humanely to avoid any unnecessary suffering.

6.2.1.2 Experimental design

Sixty adult marbled crayfishes (*Procambarus fallax* f. *virginalis* (Martin et al. 2010)) obtained from stock culture (Alterra, Wageningen UR) were randomly assigned to either control or LMB treatment group (Fig. 6.1). The LMB used was Phoslock®, obtained from Phoslock Europe GmbH (Ottersberg, Germany). Randomisation was stratified according to body length (from tip of rostrum to tip of telson). The experiment was conducted according to a parallel group design, i.e. one control and one treatment with a 7-day run-in phase, followed by either a 14 or 28-day exposure phase (Fig. 6.1). After run-in, the LMB treated group received approximately 1000 mg L^{-1} LMB per experimental unit, while the controls were kept without LMB. The LMB dosage was based on practical feasibility – e.g. added LMB in combination with aeration yields turbid experimental units which impairs visual inspection of the crayfish. From each treatment group 10, animals were sampled at the end of run-in (day 0), day 14, and day 28, respectively. All sampled animals were individually kept 4 days in clean containers with copper-free water devoid of LMB to empty their gut (depuration) before further processing. Dutch tap water contains copper which can result in toxic effects on especially crustaceans - e.g. (Hubschman 1967b; 1967a), hence we used copper-free water. Anticipating possible deaths, we chose to enter the maximum number (60 crayfish) practically feasible into the experiment. Of these, the tissues of 36 crayfish were analysed for La- hence from each treatment group, the tissues of 6 animals were analysed as sampled at the end of run-in (day 0), day 14 and day 28, respectively.

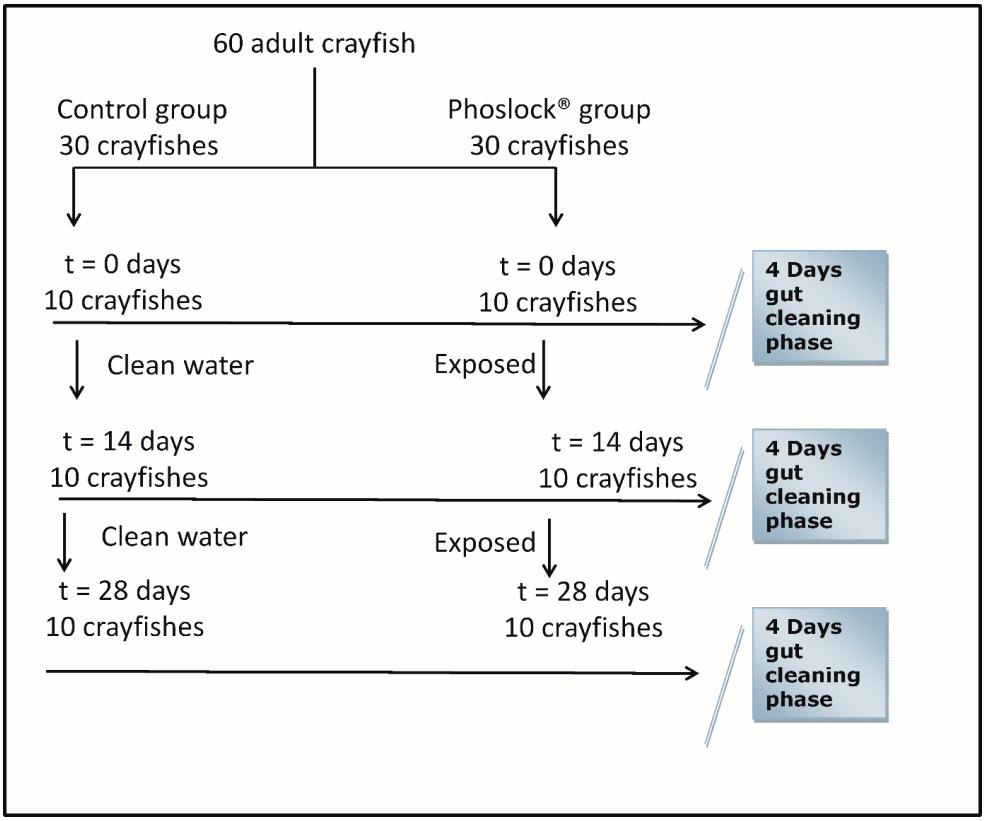


Figure 6.1 Experimental design, see text.

6.2.1.3 Experimental set-up

Individual crayfish were placed in 13.5 × 11.0 × 9.0 cm (1.3 L transparent plastic containers and kept under climatized conditions (20 °C, semi dark, i.e. 12 hours indirect diffuse light, 12 hours dark, constant aeration) in 1 litre copper-free tap water (pH = 7.5 and EC = 194.5 µs cm^{-1}) for 7 days (run-in) prior to the experiment. The containers were closed with a transparent lit. To facilitate both adequate mixing and oxygen saturation each container was aerated through a small hose fitted through the lit. To avoid toxicity due to accumulation of waste products like ammonia, each container was refreshed weekly: copper free tap water for controls or a new 1000 mg L^{-1} LMB suspension in copper free tap water. The animals were fed two times per week (approximately 12 mg per crayfish) commercial fish food pellet (a product of Trouw Nutrition Nederland B.V.). The general health status of the crayfish with respect to death, moulting or egg bearing was observed daily.

After depuration the animals were euthanized by burying them in ice for 30 minutes. To give the tissues a better texture for dissecting, the crayfish were immersed in hot tap water for 2 minutes

after euthanisation. To remove possibly attached LMB from the outside of the carapace, the crayfishes were rinsed two times with copper free water. Prior to dissection, the total body weight and body length of all animals were measured. The specimens were dissected to obtain tissues samples from carapace, gills, ovaries, hepatopancreas and abdominal muscle. Tissue samples were individually put in 30 mL plastic containers, closed with a lid, weighed and stored at (-20 °C). Subsequently, the tissues were freeze-dried (-60 °C) for 24 hrs, crushed (using pestle and mortar), where after approximately 20 mg per tissue sample was destructed with the combination of Ultrex HNO_3 (65%) and H_2O_2 (30%) (Van Griethuysen et al. 2004). The La concentration in the destruates was determined by ICP-MS at the Chemical-Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre). As a control for the origin of La in the tissues we tested 4 copper free water samples and 3 fish food samples, all processed as described above.

6.2.1.4 Water quality variables

Temperature (°C), dissolved oxygen (mg L^{-1}), pH, electric conductivity (EC, µS cm^{-1}) and turbidity (NTU) were measured using Oxy Guard Handy Gamma oxygen meter, WTW 320 pH meter, Cond 315i WTW conductivity meter, and HACH 2100P turbidity meter, respectively. Except for week 4 - in which they were assessed once, these measurements were taken twice per week.

As ammonia is known to be toxic to crayfish at concentrations above 50 mg L^{-1} (Meade and Watts 1995), we monitored the ammonia concentration in our experiment. The ammonia concentration (mg L^{-1}) was determined in a random subset of 10 experimental units per treatment per week. The filterable (0.45 µm, Whatman NC45) lanthanum concentration was determined in a random subset of 4 experimental units per treatment per week. The samples were taken shortly before refreshing. The filtered (0.45 µm, Whatman NC45) samples were analysed for ammonia concentration using a Skalar continuous flow analyzer following the Dutch standard protocols (NNI 1990), filterable La concentration was determined by ICP-MS at the Chemical-Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre). The ICP-MS level of detection for La in the matrix (HNO_3) is 0.2 µg L^{-1}, in water it is 0.02 µg L^{-1}. Tissue concentrations are expressed as (µg g^{-1}) dry weight (DW).

Cross contamination between, as well as within the groups was prevented by following strict operational procedures. Controls were always handled or measured before LMB treated experimental units, after replenishing or measurements the container was directly closed with its lit, and the probes were rinsed with copper-free and demi water between measurements. The depuration procedure was followed to avoid possible contaminations of tissue samples through gut content during subsequent dissection. During the euthanisation and hot water procedure, cross contamination was prevented by first putting individual animals in a small sealed plastic bag. Throughout dissecting, crushing and destruction control samples were processed before LMB treated samples, using clean dissection

equipment for each animal, and cleaning the pestle and mortar with demi water and 80% ethanol using tissue paper between samples.

6.2.1.5 Data analysis

For the water quality variables temperature, dissolved oxygen concentration, pH, EC, NTU, ammonia concentration and filterable lanthanum we present descriptive statistics only. As the number of deaths and egg bearing animals in both control and LMB treated groups numerically came out quite the same we did not formally test these. The number of moults was compered by chi-square test. As body length (mm), body weight (g) and their growths per week as well as the tissue lanthanum concentrations were non-normally distributed we compared the treatment groups by Mann-Whitney U test, we report median and min, max values in conjunction with the Mann-Whitney U test. The lanthanum concentration in the selected tissues of the crayfish are reported as median (min-max) (µg g^{-1}) dry weight (DW). together with the test results. For convenience the results are also presented as means and standard deviations in a graph. Statistics were done in SigmaPlot 11.0. All statistical tests were conducted with a level of significance $\alpha = 0.05$. Unless stated otherwise the number of observations (*n*) for water quality variables is 119 and 120 in the control and LMB group, respectively.

6.2.2 Lake Monitoring

6.2.2.1 Water column sampling

Water column sampling was done as described in **Chapters 2, 4 and 5.** La was measured until 2017. Samples were filtered through filters (0.45 µm membrane filters, Whatman NC45, Whatman International Ltd., Maidstone, UK) and analysed for their FLa concentration. Unfiltered samples were used to measure TLa. La concentrations were measured via ICP-MS in the Chemical-Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University, The Netherlands).

We present monthly-based boxplots for the whole study period. Post application trends were tested by Spearman Rank Order Correlation in SigmaPlot v12.5. During the post-application period, three extreme values in FLa concentrations and two TLa concentrations were observed which were included in our analysis. We also investigated the effect of the extreme TLa and Fla concentrations on the Spearman Rank Order Correlation.

6.2.2.2 LMB Sedimentation

In April 2008, shortly before the PAC and LMB application in Lake Rauwbraken, 16 PVC 40 cm long pipe sediment traps with a surface area of 5.1 cm^2 were suspended 1 m above the lake bed of Lake Rauwbraken. One set of four single sediment traps (Fig. 6.2A, series A) was equally spaced along a

transect running across the lake - North-East to South-West, and was deployed from April 20 to May 14, 2008. Four sets of sediment traps (Fig. 6.2, series B) were equally spaced over the lake bottom from March 19 to May 21, 2008. The series B were four traps put on a small rig with the traps in close proximity. The entrances of traps A1 and A2 were at 12 m depth, A3 at 11 m and A4 at 7 m depth, for series B all sediment traps were placed at 9 m depth. Material collected in the sediment traps comprised flocculated suspended particulate material and sedimented LMB. Sediment trap samples were centrifuged (5,000 rpm) and freeze dried at -60°C. From the dry material an approximate 20 mg was digested in a combination of Ultrex HNO_3 (65%) and H_2O_2 (35%) (Van Griethuysen et al. 2004). The La concentration in the sediment trap digests was determined by ICP-MS at the Chemical- Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre).

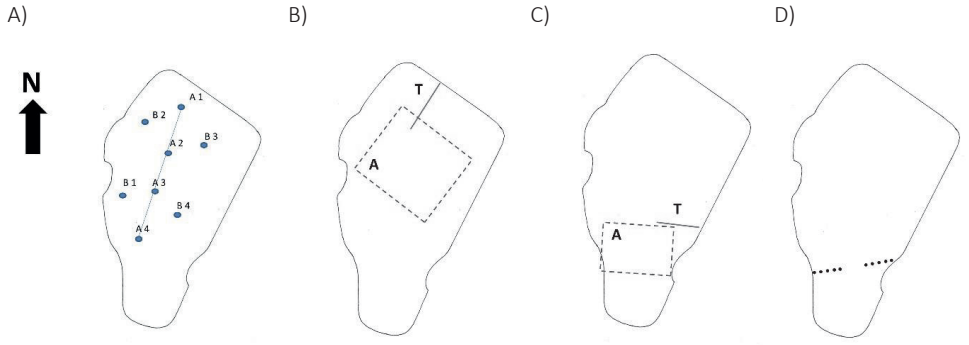


Figure 6.2: Sample locations: Sediment traps, series A and B (A); Sediment cores, A = area, T = transect (B); Macrophytes , A = area, T = transect (C), Chironomidae (D).

La concentrations measured in the digested sediment trap material were used as a tracer to determine the spatial distribution of the LMB after the application. Based on the total of 18 tons of applied LMB to the 25,692 m^2 of lake surface, we expect ~0.7 kg LMB m^{-2} to settle homogeneously on the lake bottom. Assuming a 5% La (weight/weight) concentration in the LMB, this gives an estimated concentration of 35 g La m^{-2}. The distribution of LMB using La as a tracer was evaluated using a Kruskal-Wallis one-way ANOVA on ranks in SigmaPlot v12.5, with the location of the sediment traps as factor.

6.2.2.3 Spatial distribution of sedimentary lanthanum

Two intact sediment cores were sampled prior to the LMB and PAC application (April 13, 2008). Three years after the application (2011), 46 intact cores were sampled along a transect (Fig. 6.2B) using an Uwitec core sampler. In 2011, replicate cores were sampled at one-meter water depth intervals and fixed distances from shore from just above the water's edge down to 14 meters water depth (90 m

offshore). While depths 5 and 10 m were not obtained, the sampling yielded two cores for 9 m depth; 3 cores for 0, 1, 3, 4, 11, 12 and 13 m depth; 4 cores for 7, 8 m depth and 6 cores for 2 m and 14 m depth. In all cores the thickness of the sediment layer was recorded. All sediment cores were divided into 2 cm subsamples. If less than 2 cm of sediment was present, the available amount was sampled. Sediment was defined as any material on top of the underlying sand. From the subsamples of the 2008 cores, approximately 1 g of wet sediment was subjected to a P fractionation analysis according to Psenner (Psenner et al. 1984), and the La concentrations were determined in the extracts. The 2011 subsamples were subjected to the extraction method according to Houba et al. (1997). Whereas the Psenner extraction uses a series of increasingly aggressive steps, the Houba method consists of one step of 15 ml of 0.43 M nitric acid added to approximately 1.5 g of dry sediment (dried at 30 °C), agitated for 2 hours, centrifuged for 10 minutes at 3000 rpm, and filtered through a Whatman Aqua 30/0.45 CA filter Unit.

The La concentrations in the extracts were determined by ICP-MS at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University). For the Psenner (1984) La extractions the ICP-MS detection limits (LOD) ranged from 0.002 to 0.3 µg L^{-1} depending on the step. The La and P concentrations in the Houba et al. (1997) extraction were determined by ICP-AES with LODs La = 0.01 mg L^{-1}, P = 0.1 mg L^{-1}.

Wet (M_w (g)) and dry sediment weights (M_{dw} (g)) were determined after 24 h drying at 105°C, from which the fraction of dry sediment ($f_{dry} = \frac{M_{dw}}{M_w}$, dimensionless) in the subsamples was calculated. For each subsample, the La concentration is presented in mg kg^{-1} DW. For the 2008 cores, this involved the summation of the five fractions (H_2O, bicarbonate/dithionite, NaOH, HCl and final digestion with H_2SO_4). For each subsample, the amount of La present (La_{sub}, (g)) was calculated based on the volume of the sediment subsamples, the surface area of the cores and a specific gravity (SG) of the sediment. The SG was estimated using SG = 1+1.2*f_{dry} (kg m^{-3}), in which f_{dry} (dimensionless) is the fraction of dry material in the sediment subsample by regressing f_{dry} on the SG. The concentration of La (g m^{-2}) in the sediment both pre- and post-LMB application, was estimated by summation of the (La_{sub}, (g)), per core and scaled to (g m^{-2}). We investigate the spatial distribution of La (g La m^{-2} per depth) over the lake's bottom and within the sediment as mg La kg^{-1} sediment per cm depth in the sediment.

6.2.2.4 Sediment lanthanum release

Undisturbed sediment cores were sampled as described in **Chapter 2**. The release of La was measured in the 2008 and 2011 cores.

The FLa release rates passed on normality (Shapiro-Wilk test) and equal variances (Levene's test). T-tests were done in SigmaPlot 12.5 to investigate any differences in FLa release before and after the LMB and PAC application (cores 2008) and between aerobic and anaerobic conditions (cores 2011).

6.2.3 Macrophytes

Leaves and roots of *Nymphaea* (floated leaf), *Phragmites, Scirpus* and *Typha* (emergent) were collected on April 20, 2008 (pre-PAC-LMB application), and August 5, 2008 (post application). The plant material was thoroughly rinsed with demineralized water. From the roots only, the inner tissue was selected to avoid possible attached LMB. All material was frozen immediately following collection and subsequently freeze dried. On October 12, 2008 and March 23, 2009 submerged macrophytes were sampled by scuba diving along a transect (Fig. 6.2C) from 1 to 9 m depth, (9 depths, (12.10.2008) and 6 depths (23.03.2009). All macrophytes present (*Elodea nuttallii*) were collected within a 0.5 m diameter area. The macrophytes were thoroughly rinsed with lake water at the time of sampling. After weighing, the material was dried in an oven at 50°C. The macrophyte materials were digested and analysed for their La concentration as described for the sediment traps.

6.2.4 Chironomid larvae

Sediment samples were regularly taken at 10 locations using an Ekman-Birge grabber (0.04 m^2) along a transect across Lake Rauwbraken (Fig. 6.2D). From April 18, 2008 (shortly before the PAC-LMB application, one sample date) until May 15, 2011 (3 years after the application) with a total of 26 samplings. Upon return to the laboratory, the sediment samples were washed through a 0.5 mm sieve using tap water. The collected Chironomid larvae were then thoroughly rinsed with Millipore water and visible particles on the outside of the larvae were removed. No attempt was made to clean the guts of the larvae. For each sample location the larvae were counted, pooled and freeze-dried at -60°C. The digestion and analysis of the La in the chironomid larvae were done as described for the sediment traps.

6.2.5 Filtration experiment

The environmental safety of the LMB is based on the incorporation of derived La is in the bentonite clay and the extreme low solubility (K_{sp} = $10^{-24.7}$to $10^{-25.7}$ mol^2 L^{-2}) (Johannesson and Lyons 1994; Liu and Byrne 1997) of the La-phosphate (Rhabdophane). Hence it is not expected that free La^{3+} ions will be bioavailable after an application of LMB. However, from a review of 16 case study lakes it is clear that filterable La (FLa) increases in the water column after an application of the LMB (Spears et al. 2013b). The pore size used in the filtration of water samples may range from 0.2 µm to 1.2 µm (Reitzel et al. 2017). While it is assumed that filterable La is equivalent to dissolved La, the use of different sized filters caused a lack of consistency across studies (Reitzel et al. 2017). Moreover Reitzel et al. (2017) used 1.2 µm, 0.45 µm and 0.2 µm, and finally ultracentrifugation to separate colloidal La from dissolved La.

Lake Kleine Melanen (The Netherlands) is an eutrophic urban pond that suffered blooms of cyanobacteria. In 2010 an enclosure experiment was done to test sediment techniques with the LMB (Waajen et al. 2019). An experiment to test the effects of different techniques to reduce sediment phosphorus release was done in 11 Perspex (acrylic) cylinders (diameter 1.05 m, height 1.30 m) that were put in the lake's sediment and were open to the sediment and air. The tested techniques were: sand capping + LMB (n = 3) and sand capping + LMB + PAC (n = 3), in which the sand capping was first applied where after the LMB was added and finally (where applicable) PAC was used as flocculent. In this experiment 292 g LMB m^{-2}. From each enclosure a water sample was filtered through 0.45, 0.30, 0.15, 0.10, 0.05, and 0.025 µm pore size. The 0.45 µm filters served as control. The La concentrations in the filtered and unfiltered samples were determined by ICP-MS at the Chemical- Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University Research Centre). The ICP-MS level of detection (LOD) for filterable La was 0.02 µg L^{-1} and for total La it was 0.20 µg L^{-1}. La concentrations below LOD were replaced by their LOD.

After log transformation the La concentration in the filtrates fulfilled equal variances and normality requirements for analysis of variance. The ANOVA (proc glm, SAS 9.2) was run with treatment and pore size as factors and included replicate nested within treatment to deal with variability observed within the two treatments. The ANOVA was followed up by all pairwise comparison (Tukey's test) to reveal differences between the La concentrations as obtained after filtration with different pore sized filters.

6.3 Results

6.3.1 Marbled crayfish

No relevant differences were observed between the control and LMB treated groups regarding water temperature, dissolved oxygen concentration, pH, EC, and ammonia, (Table 6.1). Turbidity in the LMB treated group was much higher than in the control group (Table 6.1).

Survival in the LMB treatment groups was 100%, in the control group two crayfish died during run-in (which were replaced) and one during week 1 (which was not replaced). The number of moulting ($X^2_{df=1}$ = 1.4, p = 0.2) and egg bearing crayfish were similar amongst the two treatment groups. In the control group (n = 19) 14 moulted and 4 bore eggs during 28 days experiment. In the LMB treated group (n = 20) 10 animals moulted and 5 bore eggs.

Table 6.1 Descriptive statistics for temperature (T, °C), dissolved oxygen (DO2, mg L^{-1}), pH, conductivity (EC, µs cm^{-1}), Turbidity (NTU), ammonium (NH4-N, mg L^{-1}) and filterable lanthanum (FLa, µg L^{-1}) for both control (Control) and lanthanum modified bentonite (LMB) groups throughout the experiment; except for pH, mean and standard deviations (SD) are given for all variables, for pH median, minimum (min) - maximum (max) values are given.

	Control		LMB	
	n = 119		n = 120	
	mean	SD	mean	SD
T	20.0	(0.2)	20.0	(0.2)
DO_2	8.8	(0.3)	8.8	(0.2)
pH median (min-max)	8.1	7.4-8.2	8.0	7.4-8.2
EC	181.0	(32.0)	183.0	(24.7)
NTU	0.8	(0.4)	100.2	(114.3)
	n = 40		n = 40	
NH_4-N	0.1	(0.4)	0.9	(1.2)
	n = 16		n = 16	
FLa	0.04	(0.03)	16.80	(32.95)

No statistically significant differences were observed between the groups with respect to body length, weight and their growth. Median (25%-75% percentiles) of body length at the start were 54.8 mm (48.3-58.0 mm) and 52.9 mm (51.1-57.2 mm) in the control respectively LMB group (U = 181, p = 0.6), median (25%-75% percentiles) of body weight at the start were 3.9 g (2.6-4.8 g) respectively 3.6 g (3.1-4.3 g) (U = 193, p = 0.9). Median (25%-75% percentiles) growth in body length were 0.5 mm $week^{-1}$ (0.2-1.1 mm $week^{-1}$) and 0.6 mm $week^{-1}$ (0.2-1.3 mm $week^{-1}$ in the control respectively LMB treated

groups (U = 174, p = 0.5). In both groups, median (25%-75% percentiles) growth in body weight were 0.1 g week^{-1} (0.0 – 0.2 g week^{-1}) (U = 190, p = 0.8).

The copper-free tap water used in both treatments contained 0.04 (SD = 0.03, *n* = 4) µg L^{-1} La. The fish food used in both treatment groups contained 58.3 (SD = 56.0, *n* = 3) µg g^{-1} dry weight lanthanum. The LMB treated group received 1.0038 g L^{-1} (SD = 0.0030, *n* = 60) LMB per experimental unit per week. FLa concentration in the water of the control group was 0.04 (SD = 0.03, *n* = 16), while in the LMB group it was 16.80 µg L^{-1} (SD = 32.95, *n* = 16) (Table 6.1).

In all tissues sampled at day 0 – before treatment, we found a background La concentration in both treatment groups (Table 6.2), which was slightly higher in the control group than in the LMB treated group. The difference in the median La concentrations between the two treatment groups (LMB - control group) at day 0 were: carapace -0.4 µg g^{-1}; gills -0.3 µg g^{-1}; ovaries – 1.0 µg g^{-1}; hepatopancreas -0.1 µg g^{-1} and abdominal muscle -0.1 µg g^{-1}. These differences reached statistical significance between the treatment groups for carapace, gills and abdominal muscle (Table 6.2).

After 14 and 28 days of the experiment, no relevant increase was observed in the control group (Table 6.2). In the LMB treated group a significant increase in the lanthanum concentration per dry weight (DW) was found in all tissues after both 14 and 28 days of exposure (Fig. 6.3; Table 6.2). The differences between the two treatment groups were statistically significant between treatment groups for all five tissues (Table 6.2). Expressing the treatment effect as the difference of the median concentration between the two treatment groups (LMB minus control group) yields the following effects, the plus sign (+) indicating an increase in the La concentration (µg g^{-1} DW):

Day 14: carapace +10.5 µg g^{-1} DW; gills + 112 µg g^{-1} DW; ovaries + 2.6 µg g^{-1} DW; hepatopancreas + 32.9 µg g^{-1} DW and abdominal muscle + 3.2 µg g^{-1} DW.

Day 28: carapace +17.9 µg g^{-1} DW; gills + 182 µg g^{-1} DW; ovaries + 2.2 µg g^{-1} DW; hepatopancreas + 41.9 µg g^{-1} DW and abdominal muscle + 7.6 µg g^{-1} DW.

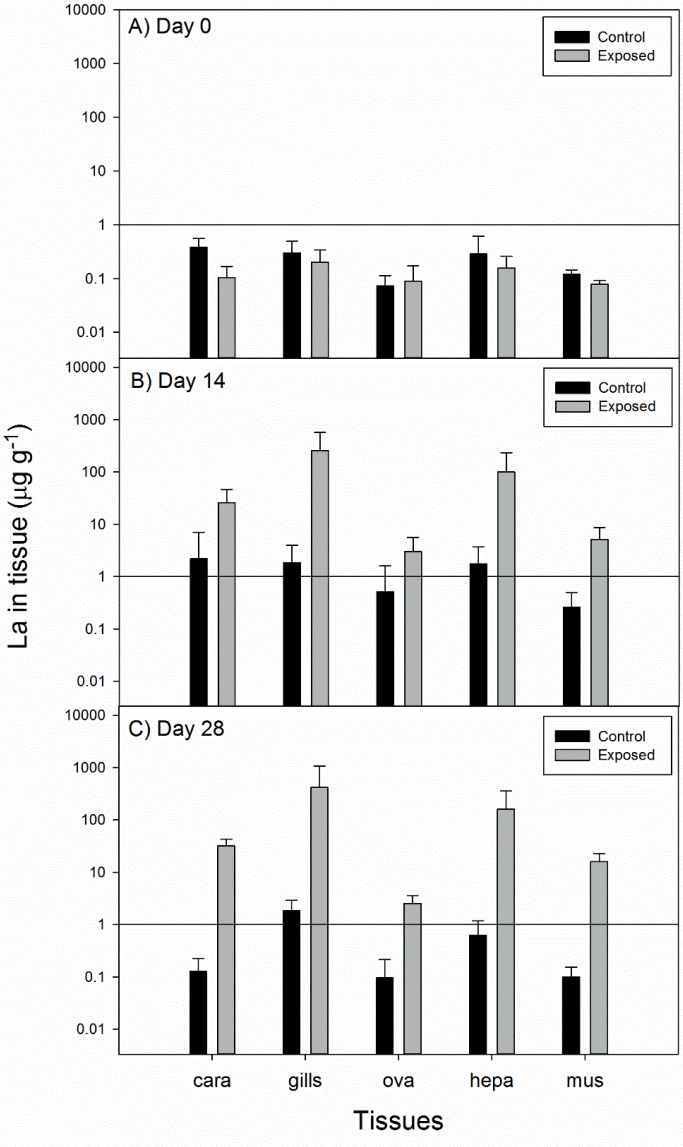


Figure 6.3 Lanthanum concentration (µg g^{-1} DW) in carapace (cara), gills (gills), ovaries (ova), hepatopancreas (hepa) and muscle (mus) tissue of crayfish at day 0 (A), day 14 (B), and day 28 (C); bars indicate mean, whiskers indicate standard error, all n = 6.

Table 6.2 Median, minimum (min), and maximum (max) of lanthanum concentrations (µg g^{-1} DW) in carapace, gills, ovaries, hepatopancreas and muscle tissue of crayfish at day 0, 14, and 24; U = Mann-Whitney U statistic, the associated n is given in the header; p = p-value of the test.

		Control n = 6		LMB n = 6			
	Day	median	(min - max)	median	(min - max)	U	p
Carapace	0	0.4	(0.2 - 0.6)	0.0	(0.0 - 0.1)	0	< 0.01
	14	0.1	(0.0 - 6.6)	10.6	(2.1 - 35.1)	1	< 0.01
	28	0.1	(0.0-0.2)	18.0	(8.0 - 23.8)	0	< 0.01
Gills	0	0.5	(0.5 - 1.2)	0.2	(0.1 - 0.6)	4	0.03
	14	2.1	(0.4 - 7.6)	114.0	(51.6 - 600.2)	0	< 0.01
	28	1.5	(0.6 - 3.5)	183.5	(125.2 - 962.0)	0	< 0.01
Ovaries	0	1.1	(0.3 - 1.4)	0.1	(0.0 - 1.5)	8	0.13
	14	0.1	(0.0 - 1.5)	2.7	(1.5 - 4.3)	0	< 0.01
	28	0.1	(0.0 - 0.8)	2.3	(0.8 - 7.7)	0	< 0.01
Hepatopancreas	0	0.2	(0.1 - 0.9)	0.1	(0.0 - 0.2)	8	0.13
	14	0.8	(0.1 - 2.7)	33.3	(4.7 - 200.0)	0	< 0.01
	28	0.2	(0.1 - 0.9)	44.1	(19.2 - 306.3)	0	< 0.01
Abdomial muscle	0	0.1	(0.0 - 0.1)	0.0	(0.0 - 0.1)	1	< 0.01
	14	0.1	(0.0 - 0.4)	2.3	(1.1 - 6.2)	0	< 0.01
	28	0.0	(0.0 - 0.1)	7.6	(4.4 - 13.6)	0	< 0.01

6.3.2 Lake water column La

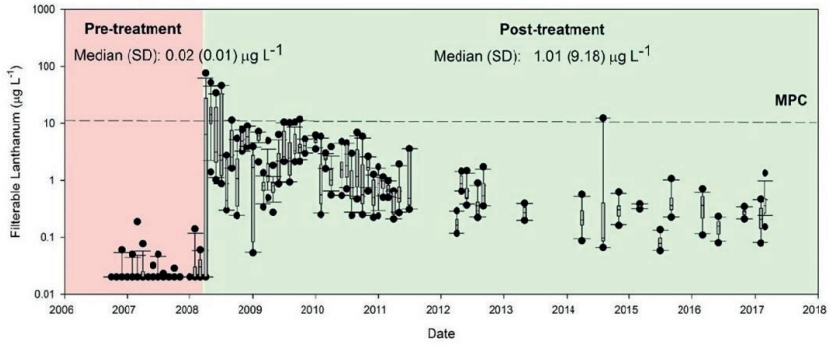
In 2006/7, prior to the LMB application, the mean water column FLa concentration was 0.02 µg L^{-1} (SD = 0.01 µg L^{-1}, n = 323), while the TLa was 0.22 µg L^{-1} (SD = 0.32 µg L^{-1}, n = 323). On the day after the application, the mean water column FLa was 44 µg L^{-1} (SD = 35 µg L^{-1}; n = 5), while the TLa was 528 µg L^{-1} (SD = 508 µg L^{-1}; n = 5), 2200 times and 2400 times the preceding FLa and TLA concentrations respectively. Using a three-parameter (FLa_{final}, $FLa_{0,}$ b) exponential decay model the decay rates were 0.04 day^{-1} and 0.13 day^{-1} for FLa and TLa, respectively (Appendix A).

During the post-application period (2009 and later), we observed three extreme FLa concentrations (out of 622) and two in TLa concentrations (out of 603). The observed extreme values were on: April 2, 2012 FLa at 3 m depth was 182 µg L^{-1} while TLa at 9 m depth was 200 µg L^{-1}, May 25, 2012 FLa at 3 m depth was 22 µg L^{-1} and TLa at 10 m was 46 µg L^{-1}. On August 26, 2014, the FLa concentration at 2 m depth was 12.6 µg L^{-1}. Including the three extreme values the post-application FLa concentrations ranged from 0.05 µg L^{-1} to 182 µg L^{-1} with mean of 2.0 µg L^{-1}, (SD = 7.6 µg L^{-1}, n = 622).

Excluding the extremes FLa, the concentrations ranged from 0.05 µg L^{-1} to 11.8 µg L^{-1} with mean of 1.7 µg L^{-1}, (SD = 2.02 µg L^{-1}, n = 619). Including the two extreme concentrations, the post-application TLa ranged from 0.20 µg L^{-1} to 200 µg L^{-1} with mean 5.5 µg L^{-1}, (SD = 9.8 µg L^{-1}, n = 603), excluding the extremes, FLa concentrations ranged from 0.20 µg L^{-1} to 39.1 µg L^{-1} with mean 5.1 µg L^{-1}, (SD = 5.5 µg L^{-1}, n = 601).

While the overall post-application pattern is of a gradual decline in concentration, there are fluctuations in the monthly means of both FLa and TLa during the post-application period (Fig. 6.4). Initially the FLa and TLa concentrations increase as a result of the LMB application. Taken over the whole post-application period (2008 and later), both TLa and FLa show a significant downward trend. For FLa and TLa the Spearman Rank Order Correlation with year were -0.77 ($p < 0.01$, n = 10) and -0.73 ($p < 0.01$, n = 10), respectively. Excluding the extreme values in FLa and TLa concentrations, the Spearman Rank Order Correlation were -0.78 for FLa and -0.77 for TLa, respectively (both $p < 0.01$).

A)



B)

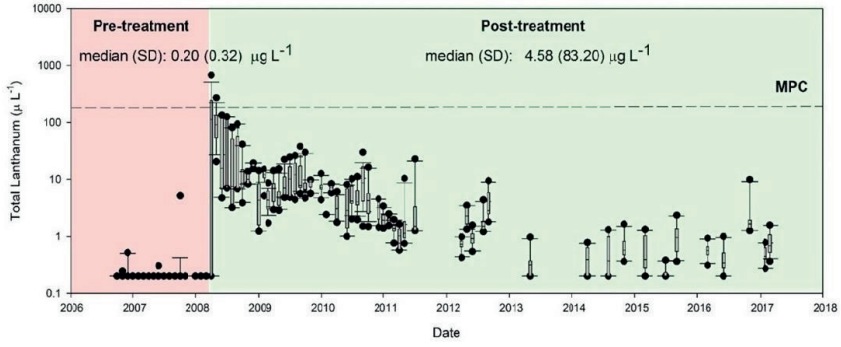


Figure 6.4 Monthly box and whisker plots of Filterable (FLa; A) and Total Lanthanum (TLa; B) during 2006-2017).

6.3.3 LMB Sedimentation

On May 14, 2008, 10.5 g La m^{-2} (SD = 2.5 g m^{-2}, n = 4) and on May 21, 2008 16.2 g La m^{-2} (SD = 2.8 g m^{-2}, n = 12) had settled (Fig. 6.5). The Kruskal-Wallis one-way ANOVAs on ranks testing (H_n) the differences between locations failed to reach a significance, i.e. on May 14 H_3 = 3.0, p = 1.0 and on May 21 H_3 = 7.7, p = 0.05. While the difference between the two retrieval dates suggests not all LMB had settled on the bottom by May 14, the theoretical coverage of 35 g La m^{-2} was not attained.

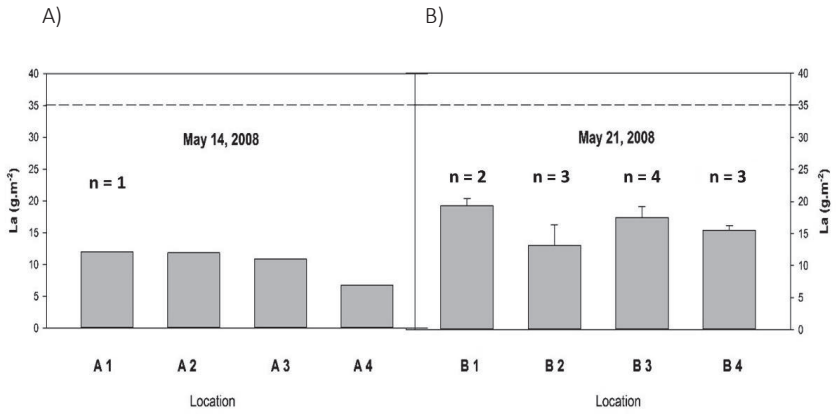


Figure 6.5 Sedimentation of Lanthanum; traps retrieved on May 14 (A),traps retrieved on May 21 (B), n=number of replicates per location, dashed horizontal line expected amount of La (g m^{-2}).

6.3.4 Spatial LMB distribution in lake bottom sediment

Sampled along the transect (Fig. 6.2B), between 0 and 5 m depth less than 1 cm sediment (defined as any material on top of the underlying sand) was found. From 5 to 10 m the sediment layer was 2 – 5 cm thick and below 12 m the sediment was more than 15 cm thick (Fig. 6.6A).

Before application, the sediment La concentration between 0 and 5 m depth ranged from 0.03 g m^{-2} to 0.07 g m^{-2}, between 5 to 10 m from 0.14 g m^{-2} to 0.20 g m^{-2} and below 10 m it ranged between 0.20 g m^{-2} and 1.86 g m^{-2} Fig. 6.6B). Three years after the application (2011), the sediment La concentration between 0 and 5 m depth ranged from 0.2 g m^{-2} to 5.4 g m^{-2}, between 5 to 10 m from 24.7 g m^{-2} to 30.6 g m^{-2} and below 12 m from 30.7 g m^{-2} to 40.0 g m^{-2} (Fig. 6.6B). At 11 m depth the sediment La concentration was lower relative to surrounding depths. These changes corresponded to mean increases in La concentrations in the 0 to 5 m, 5 to 10 m and below 10 m depth intervals of 56, 90 and 33 times, respectively.

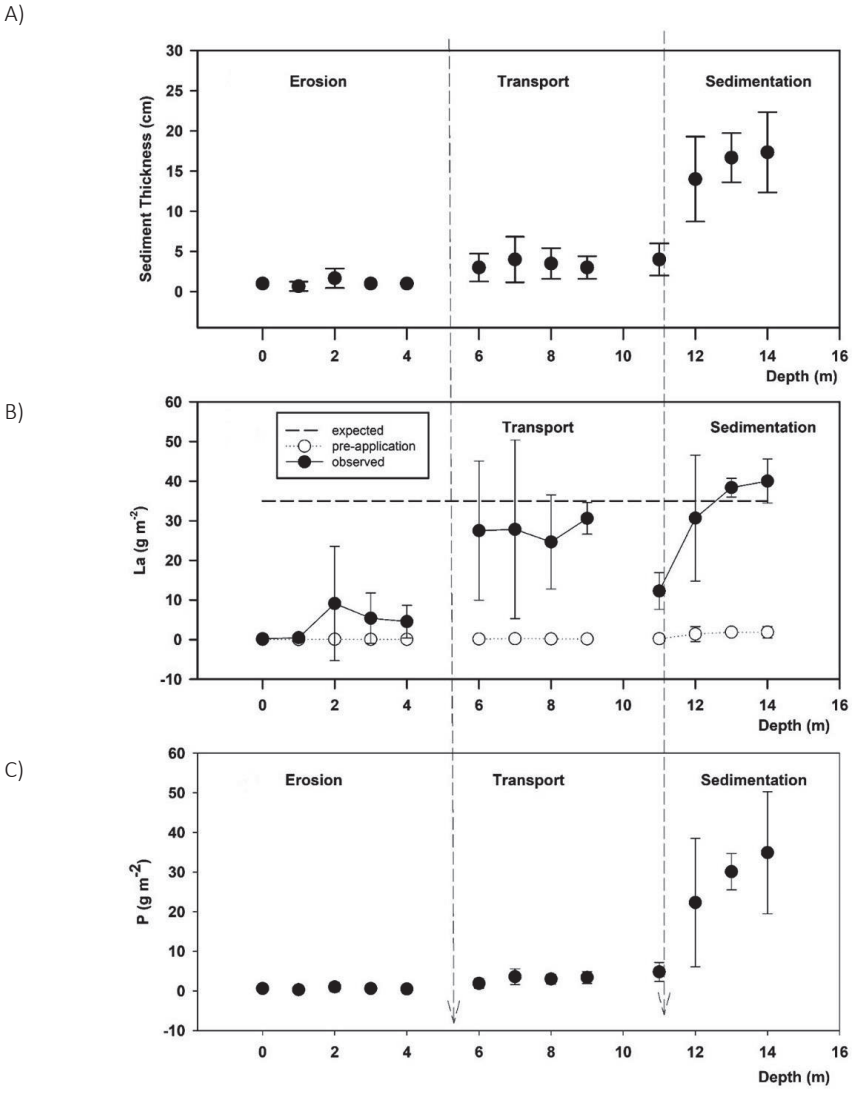


Figure 6.6 Depth versus sediment thickness (A),sediment lanthanum concentration (B) and sediment P (C), whiskers indicate 1 SD; the expected 35 La g m^{-2} (broken line) are defined for post-application. The thickness of the sediment shows a pattern commonly observed in deep lakes. This pattern is usually divided into erosion – transport – sedimentation zones (Blais and Kalff 1995).

The amount of sediment (Fig. 6.6A) and sediment-P (Fig. 6.6B) found along the transect are unevenly distributed over depth. From 0-4 m depth there was approximately 1 cm sediment in the cores sampled, from 6-11 m there was 2 cm and below 12 m depth there was more than 15 cm sediment. Based on this transect the sediment of Lake Rauwbraken contains an approximate 225 kg P.

6.3.5 Vertical distribution of La in lake bottom sediment.

The pre-application La concentration slightly increased from 0.5 mg kg^{-1} between 0 and 2 cm to 7 mg kg^{-1} between 14 and 16 cm depth in the sediment (Fig. 6.7). The post-application La sediment concentrations decreased monotonically from 6702 mg kg^{-1} between 0 and 2 cm to 7 mg kg^{-1} between 12 and 14 cm. At 16 cm depth in the sediment it was similar to background concentrations (Fig. 6.7). Based on an assumed La concentration of 5% La in the LMB this equates to the 0 to 2 cm layer post-application containing approximately 13% LMB. This also corresponds to 13,400 times increase in sediment La over pre-application concentrations.

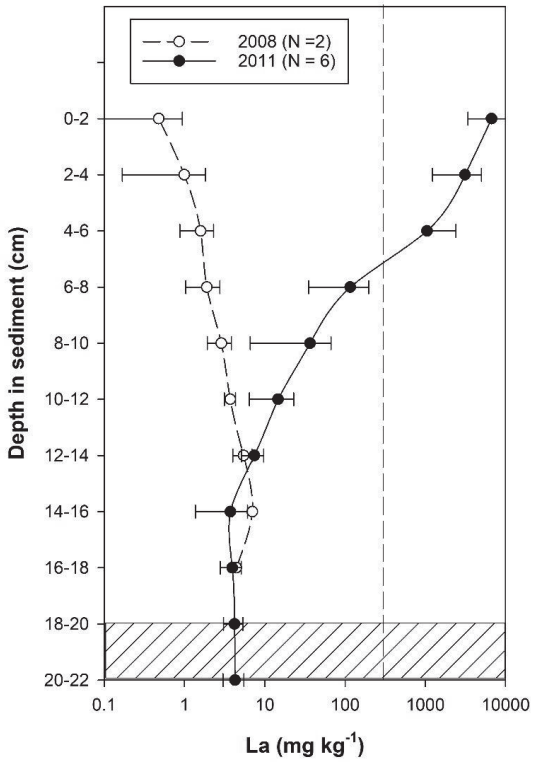


Figure 6.7 Sediment La profiles (log scale). whiskers indicate 1 SD, dashed vertical line indicates the 500.1 mg kg^{-1} DW MPC, crosshatched area indicates the sand bed below the sediment; 2008 = pre-application, 2011 = post-application.

6.3.6 Sediment release of Lanthanum

The average FLa release from the cores sampled before the application of LMB was 0.006 mg La m^{-2} day^{-1} (Table 6.3, experiment 2008), which exceeds the LOD 0.003 mg m^{-2} day^{-1} based on the LOD for FLa. The post-application 2008 anaerobic release of FLa was 1.02 mg m^{-2} day^{-1}, which was 17 times higher than the pre-application FLa release, $T_{df = 8}$ = -3.39, $p < 0.01$ (Table 6.3, experiment 2008). In the core samples from 2011, the anaerobic release of FLa of 0.063 mg m^{-2} day^{-1} was lower than that measured in the 2008 post-application cores (Table 6.3, experiment 2008). The FLa release under anaerobic conditions (0.063 mg m^{-2} day^{-1}) was significantly higher than under aerobic conditions (0.006 mg m^{-2} day^{-1}), $T_{df = 4}$ = 2.85, $p < 0.05$ (Table 6.3, experiment 2011).

Table 6.3 Sediment release of Lanthanum; n = number of cores, Norm = p value Shapiro-Wilk normality test, Eq. var. = p value Levene's test for equal variance.

Year	Application	La- Release Mean (SD) (mg m^{-2} day^{-1})	n	Norm.	Eq. var.
2008	anaerobic before	0.006 (0.001)	5	0.08	0.10
	anaerobic after	1.02 (0.67)	5		
2011	anaerobic	0.063 (0.035)	3	0.43	0.13
	aerobic	0.006 (0.003)	3		

6.3.7 Macrophytes

In four species of emergent macrophytes (*Nymphaea alba, Phragmites australis, Scirpus lacustris, Typha latifolia*) collected before the application of the LMB in 2008 the La concentrations were between 0.82 and 5.05 mg La kg^{-1} DW (Table 6.4A). Post-application the La concentration was substantially elevated. For floated leave and emergent macrophytes the increase was 6 to 130 times higher, ranging between 22.6 and 136 mg La kg^{-1} DW, respectively. Before the LMB application the La concentration in the submerged macrophyte, *Elodea nuttallii* was 7.5 mg La kg^{-1} DW. After the application DW concentrations increased up to 1,764 and 2,925 mg La kg^{-1}, corresponding to 235 to 389 times higher La concentration, respectively (Table 6.4B).

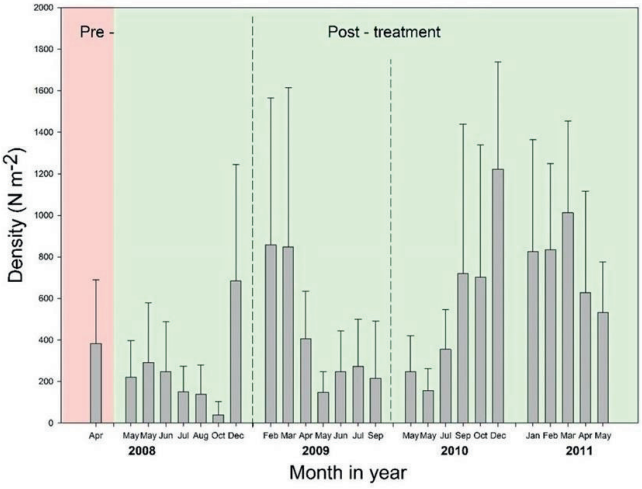
Table 6.4 A: Lanthanum concentration in leaf and roots of macrophytes (n=1) (n.d. means not determined). B: Lanthanum concentration in Elodea (± standard deviation) sampled in different moments. Specimen means a single specimen sampled (n=1) and Transect means all Elodea sampled in a plot (n=9).

A) Lanthanum concentration in floated leaves and emergent macrophytes			
Species	Part	La concentration (mg kg^{-1})	
		Pre-application	Post-application
Nymphea	Root	2.3	136
	Leaf	n.d.	22.6
Phragmites	Root	0.8	107
	Leaf	2.7	71.4
Scirpus	Root	2.3	29.8
	Leaf	5.1	32.3
Typha	Root	n.d.	122.4
	Leaf	n.d.	24.8
B) Lanthanum concentration in *Elodea (submerged macrophyte)*			
Data	Part	La concentration (mg kg^{-1})	
		Pre-application	Post-application
20-04-2008 Specimen	Root and Leaf	7.51	
05-08-2008 Specimen	Root and Leaf		2356 (±1228)
12-10-2008 Transect	Root and Leaf		1764 (±963)
23-03-2009 Transect	Root and Leaf		2925 (±1785)

6.3.8 Chironomid larvae

Following the application of the LMB in 2008, the density of chironomid larvae fluctuated seasonally with higher numbers in winter, and lower numbers in summer (Fig. 6.8A). The La concentration in the chironomid larvae increased from 1.7 (SD = 0.6) µg g^{-1} DW pre-application to 1421 (SD = 956) µg g^{-1} DW one month after the application or 836 times the pre-application concentration (Fig. 6.8B). In the last sample (May 2011), La concentrations had decreased to 206 (SD = 81) µg g^{-1} DW (Fig. 6.8B).

A)



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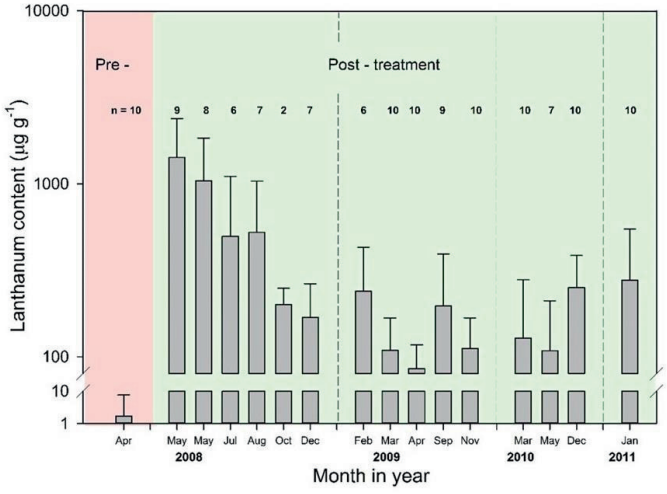


Figure 6.8 Density of Chironomid larvae (N m^{-2} = mean number of larvae per square meter;(A), Lanthanum concentration in Chironomid larvae (B), Whiskers indicate 1 SD.

6.3.9 Lanthanum in the lake

In April 2008, 18 tons LMB (4.5% La) was applied to Lake Rauwbraken, equivalent to 810 kg La. Assuming that during the application the LMB spread equally over the lake and shallow pool (810 kg on 30,092 m^2). This means about 132 kg La may have settled in the shallow pool, which leaves 678 kg for the lake. This comes quite close to the 619 kg estimated in the sediment (Table 6.5). The submerged macrophytes contained 2.4 kg La (2008) and 2.1 kg La (2009) and water column TLa was 1.9 kg in 2009 with a loss to the groundwater of 0.2 kg.

Table 6.5 Sediment Lanthanum.

Sediment	La before ($g\ m^{-2}$)	La after ($g\ m^{-2}$)	La added ($g\ m^{-2}$)	m^2	kg La
0-5m	0.05	2.8	2.75	9229	25.37975
5-10m	0.17	27.65	27.48	9608	264.0278
>10m	1.03	35.35	34.32	9616	330.0211
Total					619

6.3.10 Filtration experiment

In the unfiltered water samples the mean La concentrations were 1,327.5 µg L^{-1} (SD = 276.8 µg L^{-1}, n = 3) for sand capping + LMB and 295.5 µg L^{-1} (SD = 61.0 µg L^{-1}, n = 3) for sand capping + LMB + PAC. The F-tests revealed significant application ($F_{1, 25} = 646.6$, $p < 0.01$), replicate within application ($F_{4, 25} = 184.1$, $p < 0.01$) and pore size effects ($F_{5, 25} = 21.9$, $p < 0.01$). In the filtered samples, the La concentration declined with pore size of the filter used (Fig. 6.9). The all pair wise comparisons indicated that the FLa in the filtrates of both 0.45 µm and 0.30 µm filters were different from all others, 0.15 µm was different from all others except 0.10 µm and no difference was observed between 0.10 µm, 0.05 µm or 0.025 µm.

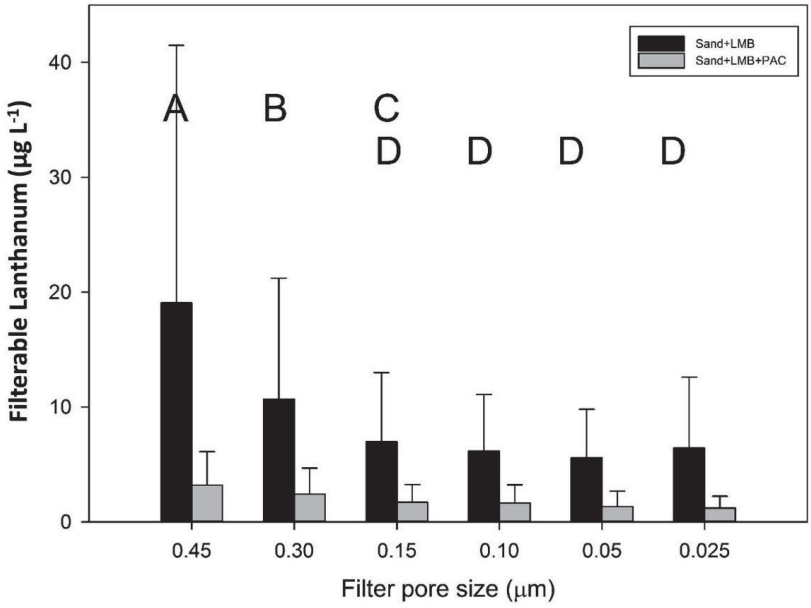


Figure 6.9 Filterable lanthanum after filtration with different pore sized filters. Characters indicate homogeneous (Tukey) groups for the effect of filter pore size.

6.4 Discussion

6.4.1 Marbled crayfish

After exposure to LMB, the lanthanum concentration in the crayfish tissues of the LMB treated group had gone up several orders of magnitude, e.g. as compared to the control group the lowest increase was 23-fold in abdominal muscle after 14 days exposure, while a 122-fold increase was observed in the gills after 28 days. Hence, the observed marginal difference between the two groups at the end of the run-in – before treatment, is of little meaning. The observed elevated La concentrations in the gills and carapace may – at least partly, be due to LMB particles attached to the outside. This is not the case for ovaries, hepatopancreas and abdominal muscles. Hence the increase in La concentration in the ovaries, hepatopancreas and abdominal muscle of the crayfish after exposure to LMB straightforwardly shows that La from the LMB is bioavailable and taken up by the crayfish. Therefore, we reject the hypothesis that exposure of the crayfish to this LMB does not change the La content of the tissues we selected.

Although one specimen died in the controls, none of the exposed crayfish died during the experiment, hence the observed increased lanthanum concentrations in the five tissues do not result in acute lethal toxic effects. This, however, does not exclude possible chronic effects, e.g. as measurable by reduced offspring or less fertile offspring, as these endpoints were not included in our experiment. Nonetheless, based on the number of moulting, egg bearing crayfish and observed growth with no significant difference between the treatment groups, we consider all animals to have developed normally and equally among the control and LMB treated groups during our experiment.

The temperature, dissolved oxygen, pH and EC in both treatments treatment groups encompass the ranges where the crayfish can be best cultured (Powell and Watts 2006; Seitz et al. 2005; Trouilhé et al. 2007). The higher EC, NTU, ammonium and filterable FLa concentrations in the water of the LMB group are due to the presence of LMB (Oosterhout and Lürling 2013); Chapter 3). The ammonia concentration was well below the range at which toxic effects are to be expected (Meade and Watts 1995). The NTU is elevated because LMB – which is a fine-grained bentonite, is resuspended due to the combination of the aeration of the test systems and the activity of the crayfish - hence in the controls the NTU is not elevated. During an actual field application, the LMB is applied as a suspension to the water surface – i.e. from a barge through a spray manifold. While its settling rate depends on local circumstances, the application of the LMB elevates the NTU for some time. The LMB settles on the sediment to form a 1-3 mm thick chemical barrier for FRP (Solutions 2014), where wind induced water movements or bioturbation are expected to be able to resuspend the LMB. Although one might expect some effects of the elevated NTU on survival, moulting, egg bearing or growth, this was not observed in our experiment.

The background concentration of rare earth elements such as La is generally assumed to be fall well below detection limits (which is 0.02 µg L^{-1} for FLa). Our results show there is detectable lanthanum in both the copper-free water and fish food we used. One may argue that the 0.04 µg L^{-1} La in the copper free water is fairly low; this is not the case for the 58.3 µg g^{-1} La in the fish food. Rare earth metals are applied as micro fertilizers to promote seed germination, stimulate the growth of roots, increase the content of chlorophyll and enhance the resistance of crops (Hu et al. 2002; Tyler 2004), which may account for at least part of the La found in the fish food. Thus, the La found in the tissues of the control group and part of the La we found in the LMB treated group originates from both copper free tap water and fish food. The fact that there is a detectable La concentration in the tissues of the crayfish - before treatment, is already an indication of the bioavailability of La to the crayfish. Similarly, in carps (*Cyprinus carpio*) background concentrations of La were 0.01 µg g^{-1} in muscle tissue, 0.02 µg g^{-1} in bone and 1.04 µg g^{-1} in liver tissue (Hao et al. 1996). Also Qiang et al. (1994) found background La concentrations of 0.04 µg g^{-1} in muscle tissue of carps, 0.14 µg g^{-1} in bone and 0.12 µg g^{-1} in organ tissues. Livers of rainbow trout (*Oncorhynchus mykiss*) had background La concentrations between 0.1 and 0.55 µg g^{-1} (Landman and Ling 2007).

While externally attachment of LMB particles can be excluded as possible route for La into the ovaries, hepatopancreas and abdominal muscle, the increased La concentration is due to the ingestion of the LMB – which may run through attachment of the LMB to food particles. As ingested food goes from the stomach directly to hepatopancreas (not just producing enzymes delivered to the gut in crayfish), the La concentration measured in this tissue can also be partially influenced. Hence clear physiological isolation is true just for ovaries and muscles. Using as input for chemical equilibrium modelling (Cheaqs Pro; (Verweij 2013)) the hardness (3 mmol $CaCO_3$) and pH (8.0) of the used water, and a 5% La content in the LMB - assuming all La will be dispersed in the water, yields that maximally 0.01% of the lanthanum would be dissolved as $LaCO_3^+$, and 99.99% would precipitate as $La_2(CO_3)_{3(s)}$. From this we infer that the uptake of La in the dissolved phase after leaching from the LMB without ingestion is unlikely in our experiment. However, this may not be the case after field applications. Spears et al. (2013) describe that the FLa concentrations in water overlying the sediments of 16 lakes are elevated for 3 – 12 months after the LMB application (Spears et al. 2013b). Bearing in mind that our filtration experiment (discussed below) and (Reitzel et al. 2017), indicate that FLa is not equal to dissolved La, the actual occurrence of dissolved La after a field application of the LMB remains an open question depending on local circumstances. Hence, whether or not truly dissolved La is a possible route applying to gills and carapace in addition to possible attachment of LMB particles in the field equally remains an open question.

Aquatic invertebrates may take up dissolved trace metals into their body through their permeable body surface (Rainbow 2007), for which the gills are one of the pathways (Bryan 1971;

Marsden and Rainbow 2004) and from the diet (Wang et al. 1996). These studies also show there are distinct differences in the accumulation of heavy metals between tissues – e.g. copper, nickel and cadmium in hepatopancreas, gills, carapace, abdominal muscle et cetera. (Alikhan et al. 1990; Bagatto and Alikhan 1987a). This, however, does not hold for all metals – e.g. while zinc was found to accumulate in the hepatopancreas, iron was found to be evenly distributed over the hepatopancreas, exoskeleton, abdominal muscle, digestive gut and viscera (Bagatto and Alikhan 1987b). Meanwhile, magnesium and manganese do not always accumulate according to the pattern observed for other metals - e.g. Mg concentration was highest in the exoskeleton, while that of Mn was highest in the digestive gut (Bagatto and Alikhan 1987b). While the crustacean hepatopancreas has a function in food absorption and secretion of digestive enzymes (Vonk 1960), it is also identified as the principal site for the accumulation of metal ions (Brown 1982). Part of the La concentration found in the gills and carapace may be due to direct sorption of La from the surrounding water. The relatively high concentration of La found in the gill tissue of the LMB treated group, suggests that direct uptake of La through the gills may be a possible path. Still, as our experiment does not discriminate between precipitated La of any form on the outside of the gill tissue and dissolved La having passed the gill membrane. The path through the gills however, is supported by literature (Bryan 1971; Rainbow 2007). When carps were exposed for 45 days to 0.5 mg L^{-1} lanthanum nitrate, especially in gills and internal organs lanthanum concentrations were elevated, but also in muscle tissue and the skeleton (Qiang et al. 1994), this indicates that La can be taken up and incorporated in fish tissue. Support for the bioavailability of La from the LMB is obtained from two LMB applications in Lake Okareka (New Zealand). After the application of LMB in 2006, elevated La concentrations in livers of trout were observed (Landman and Ling 2007).

Incorporation in clay minerals, not only provides ballast required for La to reach the prime target site, which is the bottom sediment, but also has been suggested to overcome the toxicity of La to some aquatic organisms (Haghseresht et al. 2009) and to dramatically reduce the availability of its free form (Groves 2010). However, LMB leachate in synthetic soft water had an effect on the tiger prawn *Macrobrachium* sp. with a 14 day LC50 of 800 mg L^{-1} and a 21 day LC_{50} of 700 mg L^{-1} (Groves 2010). This might point to toxicity exerted by the La^{3+} ion (Das et al. 1988) of which the concentration will be higher in soft water than in hard water (Reitzel et al. 2017). Evidently, the chemical characteristics of the water and conditions at the water sediment interface determine the liberation of lanthanum from the clay matrix and its speciation. Nonetheless, in laboratory tests using non-soft waters no adverse effects of LMB on most aquatic biota have been found so far (e.g., (Clearwater 2004; Groves 2010; Lürling and Tolman 2010; Watson-Lung 2009)), but the population growth of the rotifer *Brachionus calyciflorus* was reduced at LMB concentrations of 0.2 g L^{-1} and higher ((van Oosterhout and Lürling 2013) (**Chapter 3**).

So far, the LMB has been applied to at least 200 lakes (Copetti et al. 2016). Actual dosage applied are between 60 g m^{-2} (Lake Okareka, New Zealand) and 667 g m^{-2} (Lake Kleiner See, Germany) (Spears

et al. 2013b)). As recommended by its manufacturers, the LMB is applied to bind all phosphorus present – based on the total phosphorus content of the water column and in the top cm of the sediment, which indicates that actual field dosing can even be higher than the 667 g m^{-2} as applied in Lake Kleiner See. Hence, the LMB dosage used in our experiment - approximately 1 g L^{-1} or 67.3 g m^{-2} is quite low compared to dosing at field applications.

Here, we have shown that lanthanum concentrations were elevated strongly in crayfish tissues of specimens exposed to LMB. Inasmuch as lanthanum tissue concentrations seemed to increase over time, longer term exposure experiments could be advised. In general accumulation of metals in crayfish tissues appears dose- and time-dependent and could be influenced by depuration (Kouba et al. 2010), making long-term studies crucial in evaluating potential negative effects on the population and community level.

6.4.2 Lake monitoring

Our results show that La was present in Lake Rauwbraken before the application of the LMB in 2008, in all water and sediment samples, macrophytes and chironomid larvae. This observation is consistent with (Waajen et al. 2017a) and important in view of the belief in the Netherlands that LMB application implies "the introduction of foreign substances to the water" (Deelen 2013; Heerdt et al. 2012) that are "naturally absent in sediment" (Gogh 2014). La belongs to the rare earth elements, which by no means implies it is extremely rare, e.g. it is more abundant than lead and occurs in Dutch surface waters and sediments in median concentrations of 21 ng L^{-1} (range 16-204 ng L^{-1}) and 27 mg kg^{-1} (range 6-130 mg kg^{-1}), respectively (Moermond et al. 2001).

Directly after the application, the Dutch MPC's for water column FLa and TLa were temporarily exceeded. Based on the mean water column concentration FLa declined below the MPC for dissolved La of 10.1 µg L^{-1} after 75 days and TLa declined below its MPC of 150.1 µg L^{-1} after 15 days (**Appendix** 6.6.1). During the 10 post-application years, both FLa and TLa remained elevated compared to the pre-application values. We attribute these longer terms elevated concentrations to sedimentation and resuspension. In the period immediately following LMB application, unconsolidated PAC-LMB flocs were probably able to be resuspended with sediment redistribution in Lake Rauwbraken. Labile La associated with LMB or potentially (partially) displaced from the LMB interlayers via ion exchange may have been complexed with humic substances to form colloidal species (Reitzel et al. 2017) that may have a long residence time within the water column. The colloidal nature of FLa was checked and confirmed in an experiment with water from LMB treated enclosures (**Appendix** B) in Lake Kleine Melanen (Waajen et al. 2019). Over time FLa declined, but at a slower rate than TLa (**Appendix** 6.6.1). Stronger sedimentation of the larger particles is reflected in the FLa : TLa ratio that increased over time from ca. 0.2 to 0.6 (Fig. 6.10).

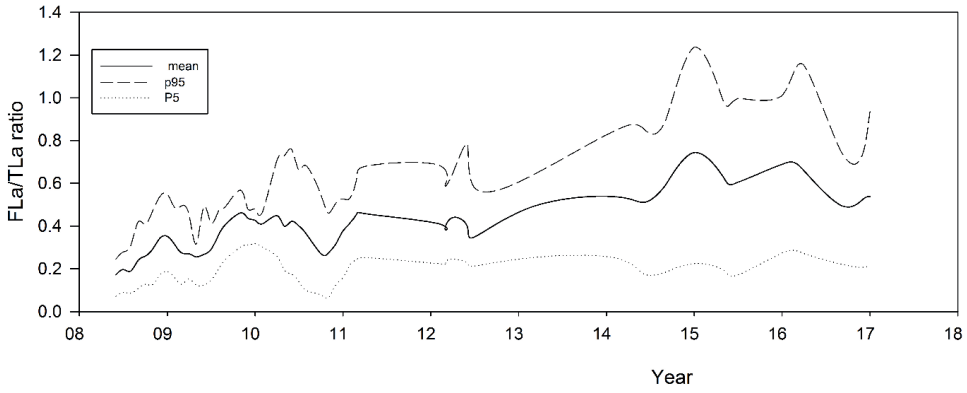


Figure 6.10 Smoothed Fla/TLa ratio, smoothing done by moving average, two months back-two months forward). P5 = 5th percentile, p95 = 95th percentile.

The phosphate binding capacity of La is not affected by anaerobic conditions (Douglas et al. 2004; Ross et al. 2008). Significantly, however, anaerobic incubations revealed 10 times more La release from LMB amended sediment than in aerobic incubated sediment cores. While the cause is not certain, it may be that Fe^{2+} ions formed under anaerobic conditions displace La from the interlayer. Recently, a relatively strong adsorption of Fe^{2+} by LMB has been found with maximum adsorption capacity of 8.5 mg Fe^{2+} g^{-1} LMB (Ding et al. 2018), similar to the La contained within LMB. Anaerobic conditions also stimulate methanogens and methane ebullition (Bergen et al. 2019), which could transport colloidal La from the sediment into the over-standing water. Evidently, more research is needed to decipher what is causing the elevated La release from amended sediments under anoxia.

The decline of FLa and TLa below their MPCs is important, because The Netherlands is the only country that has La standards for water and sediment (Sneller et al. 2000). The La MPCs were introduced because of industrial emission of rare earth elements in de Nieuwe Waterweg, the Netherlands (Sneller et al. 2000). The FLa standard is based on the NOEC determined in a 21 days *Daphnia* reproduction test (NOTOX 1995) divided by 10 (Sneller et al. 2000). The NOEC of 100 µg La L^{-1} was calculated as the geometric mean $(A \times B)^{1/2}$, with A and B as the limits of the exposure range (NOTOX 1995), whereas using all six measurements presented in the NOTOX report yielded a mean of 232 µg La L^{-1} and a median of 195 µg La L^{-1}. Thus, twice the NOEC was used. More concerning is that in the experiment, the animals were supposed to be fed daily with 1 x 10^6 cells mL^{-1} *Chlorella*, but from day 5 to 13 this was reduced to 0.5 x 10^6 cells mL^{-1}, whereas after the feeding with 1 x 10^6 cells mL^{-1} was restored (NOTOX 1995). The animals were cultured in Elendt M7 medium, which contains 0.143 mg L^{-1} KH_2PO_4 and 64.8 mg L^{-1} $NaHCO_3$ (Samel et al. 1999). Consequently, precipitation of algal food in the higher La treatments cannot

be excluded (Lürling and Tolman 2010). This seems to be corroborated by a Parallel Lines Analysis showing that the slopes of the cumulative reproduction in each NOTOX treatment were similar ($F_{4,35}$ = 0.47; P = 0.8; Fig. 6.11). Evidently, the difference is caused in the first reproductive days until day 13 (Fig. 6.11). Hence, the Dutch La standards are based on a single experiment that suffers from a severe methodological flaw.

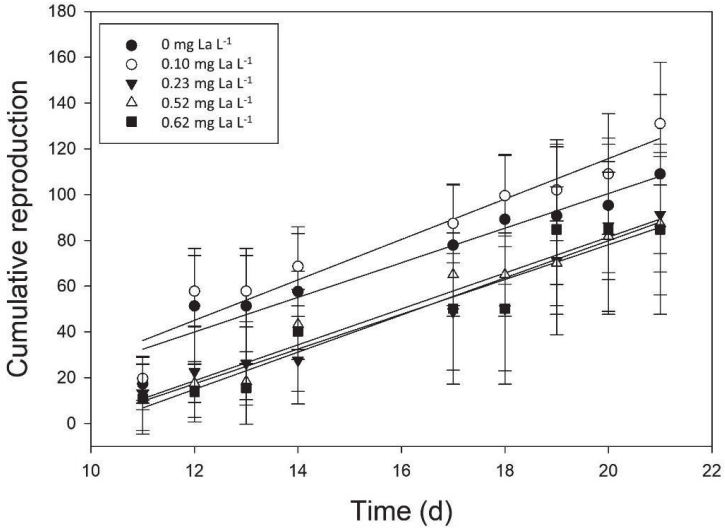


Figure 6.11 Cumulative reproduction of Daphnia at different Lanthanum concentrations. Reproduced from NOTOX (1995).

6.4.3 Sediment traps

The amounts of La retrieved in the sediment traps that were deployed during the treatment fell quite below the expected 35 g La m^{-2} – which is equivalent to 700 g LMB m^{-2}. Based on the amounts of TLa in the water column we expected that by May 14 and 21 a minimum of 34 g La m^{-2} had settled. In-water inspection (scuba diving) revealed that part of the LMB was accumulated on the sediment in shallower parts of the lake (Chapter 2). Based on this observation, the Floc & Lock application in Lake de Kuil (Waajen et al. 2016a) was done through hypolimnetic injection as opposed to through a spray manifold at the surface of the lake.

We used the same sediment traps in 2007 and 2008 before the LMB application which yielded an average sedimentation rate of suspended solids of 25 g m^{-2} (SD = 15 g m^{-2}, n = 12) per 4 weeks. To sample these low rates of sedimentation, sediment traps with a small opening and a long tube are considered the best option (Bloesch and Burns 1980). The entry of material into a sediment trap is hampered by friction, i.e. for each particle that settles in the trap an equal volume of water must leave

the trap. The latter is slowed down by friction of the water with the inner wall of the trap (Bloesch and Burns 1980). Under low sedimentation rates this friction is not considered a major problem. However, after the LMB application in Lake Rauwbraken the majority of the approximately 700 g m^{-2} of the LMB settled within a few days. During such a massive sedimentation, the friction at the entrance of the traps is likely to cause parts of the material to be directed away from the trap (Bloesch and Burns 1980). Thus, the traps used may also have resulted in an underestimation of the amounts of LMB (La) that settled on the sediment.

6.4.4 Sediment-La

According to its manufacturers, the LMB should be homogeneously distributed over a lake's sediment, moreover its placement on the sediment is described as a thin layer on top of the sediment – which then should result in its maximum effective sediment capping function. Yasseri and Epe (2016) reported that wind drift and internal currents might affect the spatial distribution of the LMB. While our sediment traps indicated a homogeneous distribution of La over the lake's sediment directly after the application, three years later La was redistributed towards low La at shallow water depths (i.e. La < 5.4 g m^{-2}) and higher La in the deeper parts of the lake (i.e. La > 30 g m^{-2}). The spatial La distribution in the sediment revealed that, after the application, the first 6 cm of the sediment exceeded the MPC of 500.1 mg kg^{-1} (Fig. 6.7). It seems the LMB is relocated towards the deeper parts of the lake. We attribute this relocation to the ongoing processes of re-suspension and sedimentation, naturally occurring in deep lakes resulting in the distinct erosion-transport-sedimentation zones as described by (Hakanson 1977). The deep sediment (e.g. below 10 m water depth) may also be resuspended by wind driven water movements (Wetzel 2001), whilst bioturbation (e.g. fish and chironomid larvae) may affect transport processes within a sediment. Collectively, these processes are likely to affect the distribution of the LMB within the sediment – with the result that not all LMB remains on the surface of the sediment after 3 years, as observed here (Fig. 6.6). A similar result was found by (Dithmer et al. 2016b) Arguably, the sediment sample method (UWITEC core sample) may have relocated minor amounts of the LMB deeper within the sediment, i.e. as the tube is forced into the sediment, friction between the tube and the sediment may cause small amounts of material forced downwards with the tube. Also, the effectiveness of the LMB may benefit from its redistribution towards the deeper sediments as most of the sedimentary P resides here (Fig. 6.6). Given the above, to ensure longevity of effective dosing of the LMB should be based on an estimated communicating sediment depth, which is a difficult parameter to determine. In Lake Rauwbraken a communicating depth of 5 cm was assumed (Chapter 4), which was a fair estimated given the La as tracer measure in this study (Fig. 6.6) and in Dithmer et al. (2016b). We estimated this depth based on the bathymetry of the lake and the paucity of bottom-dwelling fish.

However, if fish such as bream (*Abramis brama)* or carp (*Cyprinus carpio*) are present, their foraging behaviour may substantially increase communicating sediment depths (Huser et al. 2016).

6.4.5 Sediment FLa release

The pre-application sediment release of FLa was 0.006 mg La m^{-2} day^{-1}. Post-application in 2008, the release of FLa was 1.016 mg m^{-2} day^{-1}. We reject the hypothesis that FLa is not released from the sediment, which is consistent with Gibbs et al. (2011). In the cores sampled in 2011 (3 years after application) the release of FLa was much lower. However, under anaerobic conditions, this release was an order of magnitude higher than under aerobic conditions: 0.063 versus 0.006 mg m^{-2} day^{-1}, respectively. We reject the hypothesis that the release of Fla is not affected by anaerobic-aerobic conditions. Based on the high binding capacity of La for FRP (Johannesson and Lyons 1994; Liu and Byrne 1997) and the fact that anaerobic conditions do not affect this binding, the differences in the release of FLa between aerobic and anaerobic conditions could be caused by ferrous iron ions displacing unconsolidated La from the clay interlayers and by methane ebullition under anaerobic conditions. The difference between the releases of FLa under aerobic and anaerobic conditions may result in seasonality in the lake FLa concentrations, i.e. as a result of thermal stratification. The highest mean Fla concentration was measured in late year (mean FLa = 2.17 µg L^{-1}), and the lowest in the early year (mean FLa = 1.36 µg L^{-1}; Table 6.6). If split by depth the highest FLa occurred in the deep layer during summer and late year (mean Fla = 3.18 µg L^{-1} and 3.04 µg L^{-1}; **Appendix** 6.6.2).

6.4.6 Macrophytes

We observed an increase in La in macrophytes. For the submerged macrophytes and *Nymphaea* leaves we cannot exclude that this is caused by LMB particles on the outside of the plants. For the emergent parts (stems) and inner tissues of the roots it is unlikely that this increase is caused by attached LMB. Thus, it is likely that La is taken up by macrophytes, which was also found by Yang et al. (1999) and Weltje et al. (2002). The La concentration in the inner tissues of the roots is higher than in the leaves.

The background La content in the submerged macrophyte *Elodea nuttallii* was 7.5 mg La kg^{-1} DW, which is the same as has been found by (Waajen et al. 2017a). Likewise, after the application La concentrations increased up to similar orders of magnitude (Waajen et al. 2017a) underpinning that La, either through bio-accumulation or attached LMB, becomes associated with macrophytes. This means that macrophytes can be a vector for La transfer to herbivorous fish and water birds. Earlier observations in Lake Het Groene Eiland (The Netherlands), treated with the LMB in 2008, revealed that after one year droppings of herbivorous birds, Canada Geese (*Branta canadensis*), contained between 0.03 and 69.5 µg La per g dry weight (median 0.4 µg La g^{-1}) (Lürling and van Oosterhout 2013). Thus, herbivorous birds may be a vector for transport of La away from the treated location.

6.4.7 Chironomid larvae

With only one pre-application sample of chironomid larvae, it cannot be determined if the density of these larvae was changed after the LMB application. Notably, however, the La concentrations in the chironomid larvae post-application were several orders of magnitude higher than pre-application. Similarly, Waajen et al. (2017a) reported La concentrations of chironomid larvae over twice as high after LMB application than pre-application. The chironomid larvae were analysed without gut cleaning and thus the elevated La concentrations may be due to direct ingestion. Our results indicate that chironomid larvae may be a vector for either the LMB or La to other animals in the food web – e.g. fish predating these larvae.

6.4.8 *Filtration experiment*

Our filtration experiment shows that the FLa concentrations in filtered samples depend on the pore size of the filters - smaller pores result in lower FLa concentrations, which confirms a similar result by (Reitzel et al. 2017). We attribute these differences to small $LaPO_4$ particles that may pass through 0.45 µm filters but not through the smaller pore sized filters. Our results indicate these particles are between 0.15 µm and 0.45 µm. While the exact chemical-physical nature of these particles is yet to be resolved, this does shed new light on how to interpret filterable fractions of compounds in general. Mostly 0.45 µm filtered samples are interpreted as representative of the dissolved and bioavailable forms of the compound(s) at hand, i.e. $La^{3+}_{(aq)}$ and $PO^{3-}_{4\,(aq)}$, not just filtered samples that may contain La in any form. It seems likely that the 0.45 µm filtered La fraction overestimates the potential bioavailable La.

The difference between the two applications (sand + LMB and sand + LMB +PAC) is noteworthy. We explain the lower FLa concentrations in the application with PAC as a result of flocculation – hence larger particles that do not pass the used filters.

6.4.9 General

Depending on water composition, concentration and hence, speciation, La can potentially be toxic to aquatic organisms (Akhurst et al. 2004; Barry and Meehan 2000; Douglas et al. 2004; NICNAS 2001). In hard water lakes and in eutrophic waters with elevated pH, however, virtually all FLa will be colloidal (Reitzel et al. 2017), because at higher alkalinity La will precipitate with (bi)carbonate and at elevated pH insoluble La-hydroxide will form. In soft water lakes, Reitzel et al. (2017) showed that complexation of La with humic substances might occur. How much truly dissolved La^{3+} will be present depends on water chemistry, yet even if some trivalent ions are present, no detrimental effects of a LMB application on aquatic organisms are to be expected (D'Haese et al. 2019).

The environmental safety of LMB is related to the low solubility (K_{sp} = $10^{-24.7}$ to $10^{-25.7}$ mol^2 L^{-2}) (Johannesson and Lyons 1994; Liu and Byrne 1997) of the La-PO_4 mineral (rhabdophane). The presence of La within the bentonite interlayers reduces the bioavailability and formation of FLa with bioavailability

further reduced after binding with P. A review of LMB is given in (NICNAS 2001) however, the bioavailability of La was not evaluated. There is evidence in literature that La could be accumulated by the amphipod *Corophium volutator* (Moermond et al. 2001); blue mussels (*Mytilus edulis) (Lobel et al. 1991)*; in duckweeds (*Spirodela polyrhiza*), Cladocerans (*Daphnia magna*), goldfish (*Carassius auratus L.*), shellfish (*Sinotaya (Bellamya) aeruginosa*) *(Yang et al. 1999)* and Carp (*Cyprinus carpio)* (Qiang et al. 1994). The uptake of La from the LMB has also been demonstrated in rainbow trout (*Oncorhynchus mykiss*) and koura (*Paranephrops planifrons*) (Landman and Ling 2007), and the Marbled crayfish (*Procambarus fallax* f. *virginalis*) (This Chapter). However, in none of those experiments the La speciation has been determined or any potential toxic effects determined. Whilst the free La^{3+} cation carries the greatest risk of biological effects (Das et al. 1988), this ion will be virtually absent in eutrophic waters. There is also little evidence of La toxicity in humans as it is strongly protein bound in the plasma (Damment and Pennick 2007; 2008). Indeed, lanthanum generally is considered to be of low toxicity, and depending on its chemical form, the acute oral dose of lanthanum as assessed in rats varies from 3400 mg kg^{-1} to > 10000 mg kg^{-1} body weight (Redling 2006). The absence of any ecotoxicity in whole lake applications (Waajen et al. 2016a; 2017a) is therefore not surprising. Still, in analogy to the debate on ocean iron fertilization (Cullen and Boyd 2008), we advocate that the use of lanthanum modified clays in lakes to restore them should be accompanied by a thorough study on potential side effects.

La based eutrophication management tools are powerful, since La precipitates with phosphate forming an extremely stable mineral in sediments (Dithmer et al. 2016b), whilst it remains active until virtually all La has precipitated as rhabdophane regardless presence of confounding factors that compete with binding (Dithmer et al. 2016a). Consequently, the amount of P that will be immobilized can be calculated far more accurately than for other commonly used mitigation compounds, such as alum, which has Al : P binding ratios varying between 2:1 to 13:1 (Huser et al. 2011) and where flock ageing impairs the adsorption capacity (de Vicente et al. 2008), or phosphate bound to iron that can easily be released under anoxia or elevated pH (Søndergaard et al. 2003). The vast majority of the introduced La could be retrieved from the sediment, only a fraction is lost to groundwater. The calculated amount of La in the sediment of the deep lake (619 kg) corresponds with an immobilization of 138 kg P. The strongly improved water quality underpins that the introduced La has strongly reduced the internal P load in Lake Rauwbraken.

6.5 Conclusions

- La was present at low concentrations in stock cultured marbled crayfishes (*Procambarus fallax* f. *virginalis* (Martin et al. 2010)), copper-free water and fish food and in Lake Rauwbraken sediment, water column, macrophytes and chironomid larvae before the LMB application.
- Our laboratory study shows that La from the LMB is bio-available to and taken up by the marbled crayfishes (*Procambarus fallax*) f. *virginalis*.
- After the application of the LMB, both filterable and total lanthanum in the water column were elevated and remained so as compared to the pre-application concentrations. While the water column FLa and TLa concentrations exceeded maximum permissible concentrations as defined for the Netherlands, they did return to below these maximum permissible concentrations.
- The LMB that reached the deep sediment was distributed homogeneously over the lake's bottom during its application. Three years after the application, the LMB was redistributed towards lower densities in the lake's sediment at shallow depths and higher densities at greater depths.
- La from the LMB was taken up by submerged macrophytes. Otherwise, the LMB becomes attached to the submerged macrophytes and is present in the gut concentration of chironomid larvae. Meaning that submerged macrophytes and chironomid larvae may be a vector for La in the food chain.
- Sediment core studies revealed that anaerobic conditions might increase La release into the overlying water column. The precise mechanism is yet undetermined and requires further investigation.
- La concentrations in filtered samples depend on the pore size of the filter suggesting that various colloidal forms of La are present. The implication is that the true concentration of FLa may be lower than previously estimated. The seasonality in FLa and TLa needs further investigation.
- The dimensions of the sediment traps (as deployed in our study) to sample massive sedimentation events such as an LMB application need to be reconsidered.
- We advocate that the application of in-lake chemical water treatments to mitigate eutrophication should be accompanied by a thorough study of potential side effects.

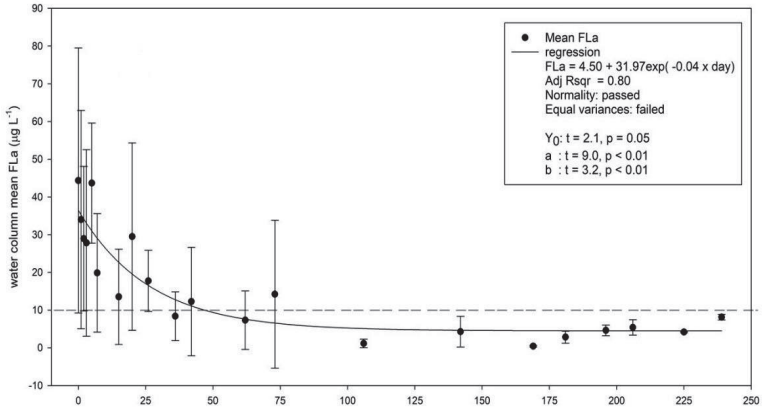
6.6 Appendix

6.6.1 *Post application decline of water column FLa and TLa*

To investigate the decline of FLa and TLa concentrations (which exceeded the Dutch MPCs directly after the application) we fitted a three parameter exponential decay to the pooled 1 and 3 m depth data during 2008 in SigmaPlot v12.5 using the equation: $FLa\ (t) = FLa_{final} + FLa_0 e^{-bt}$ (Equation 1), in which FLa_{final} is the asymptotic value assumed for the period of decay, (FLa_{final} + FLa_0) the FLa concentration at t = 0 and b the rate of decay.

The mean water column FLa concentration decayed in an exponential-like pattern during the weeks after the application (Fig. 6.12). After 75 days FLa concentrations declined below the MPC criterion of 10.1 µg L^{-1}. Using a three parameter (FLa_{final}, FLa_0, b) exponential decay model, the final 2008 mean FLa concentration was estimated to be 4.5 µg L^{-1} (Fig. 6.12). Similarly, the final 2008 TLa concentration was estimated using the three-parameter model to be 33.7 µg L^{-1} (Fig. 6.12). After 15 days TLa declined below the maximum permissible concentration (MPC) criterion, but remained elevated as compared to the 2006/7 pre-application concentration.

A)



B)

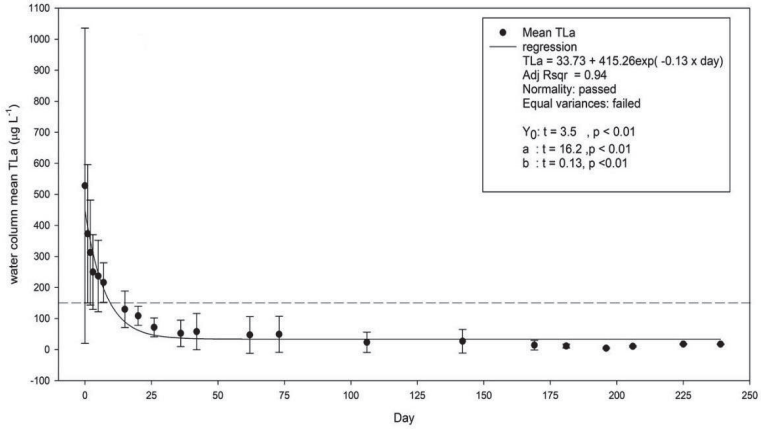


Figure 6.12 Filterable Lanthanum (FLa) during 2008 (~250 days post PAC-LMB application). A: FLa, dashed horizontal line indicates MPC 10.1 µg L^{-1}) and B: Total Lanthanum (TLa), dashed horizontal line indicates MPC 150.1 µg L^{-1}. The regression line is given by: $FLa\,(t) = FLa_{final} + FLa_0 e^{-bt}$, t = student t-statistic, p = p value for H_0: estimate = 0; Day = days after the application; Adj Rsqr = adjusted R square.

6.6.2 *Post application seasonality in Filterable (FLa) and Total Lanthanum (TLa)*

We divided our study in three periods: pre-application (January 1th, 2006 – April 20th, 2008), transitional period (April 24, 2008 – December 31, 2008) and post-application (January 1th, 2009 – 31-December 2017). To infer on possible seasonality in FLa and TLa for both the pre- and post-application periods we present means and standard deviations of our data pooled according to the period in the year: early year (early; months January to May), summer (months June to September) and late year (late; months October to December); and two depth layers: shallow (depths 0 – 6 m) and deep (7-10 m). During summer the shallow and deep layers reflect thermal stratification. The extreme values described in 6.3.2 are excluded from Table 6.6 but described in the text.

Based on the whole water column, the highest mean Fla concentration was measured in late year (mean FLa = 2.17 µg L^{-1}), the lowest in early year (mean FLa = 1.36 µg L^{-1}; Table 6.6). If split by depth the highest FLa occurred in the deep layer during summer and late year (mean Fla = 3.18 µg L^{-1} and 2.66 µg L^{-1}; Table 6.6). Based on the whole water column post-application, the highest mean TLa occurred in during summer (mean TLa = 6.95 µg L^{-1}), the lowest in early year (mean TLa = 3.92 µg L^{-1}; Table 6.6). When split by depth the highest TLa occurred in the deep layer during summer and late year (mean TLa = 10.66 µg L^{-1} and 8.03 µg L^{-1}; Table 6.6). For FLa we observed three extreme values out of 622 measurements, for TLa we observed two out of 603 measurements. If the extreme values are included the results are: FLa early year shallow mean 2.10 (SD = 12.63, n = 208), deep mean = 1.77 (SD = 2.61, n =117) and summer shallow mean = 1.37 (SD = 1.61, n = 140); TLa early year shallow mean 3.56 (SD = 4.53, n = 197), deep mean = 6.81 (SD = 19.38, n =107). In or excluding these extreme values does not change the general picture that FLa and TLa tend to be higher in the deep layer during thermal stratification.

Table 6.6 Post application seasonality in Filterable and Total Lanthanum, SD = standard deviation, n = number of observations.

	Season											
	Early			Summer			Late			Whole Period		
layer	mean ($\mu g\ L^{-1}$)	n	SD ($\mu g\ L^{-1}$)	mean ($\mu g\ L^{-1}$)	n	SD ($\mu g\ L^{-1}$)	mean ($\mu g\ L^{-1}$)	n	SD ($\mu g\ L^{-1}$)	mean ($\mu g\ L^{-1}$)	n	SD ($\mu g\ L^{-1}$)
Filterable Lanthanum												
shallow	1.23	207	1.55	1.29	139	1.31	1.92	55	2.01	1.34	401	1.56
deep	1.59	116	1.85	3.18	74	3.06	2.66	28	3.04	2.27	218	2.58
column	1.36	323	1.67	2.67	214	2.38	2.17	83	2.41	1.67	619	2.02
Total Lanthanum												
shallow	3.34	196	3.37	4.66	140	4.06	4.59	56	4.36	3.88	392	3.81
deep	4.99	106	4.52	10.66	75	9.07	8.03	28	7.02	7.43	209	7.27
column	3.92	302	3.89	6.95	215	6.94	5.74	84	5.59	5.12	601	5.53

J.F.X. van Oosterhout

Grape study

Acrylic paint on newspaper

15 x 15 cm

Chapter 7

General discussion

7 General discussion

7.1 Introduction

In April 2004, Lake Rauwbraken (The Netherlands) suddenly colored red by a bloom of the Burgundy blood alga (*Planktothrix rubescens,* Cyanobacteria) (Chapter 2). The reoccurrence of similar blooms led to prolonged swimming bans in 2005 and 2007. While the 2008 bathing season started with a nuisance bloom of *Aphanizomenon* (cyanobacteria), the future of the lake as a recreational facility looked rather grim.

A lake system analysis (Chapter 2) revealed that, over forty years of history, a small external inflow of phosphorus had loaded the lake sediment, which then became the main source of phosphorus (internal loading) in the lake. Based on this system analysis, the Floc & Lock treatment was developed especially for Lake Rauwbraken. The water column was cleared of P (Floc) and the efflux of P stored in the sediment (internal P-load) was impeded (Lock). Sediment P-release under anoxia showed an 80-85% reduction over the years after application compared to before. The treatment was successful as an instantaneous cure (Chapter 4). While the Water Framework Directive ((EU 2000); WFD) and Bathing Water Directive (EU 2006) were violated when the treatment started in April 2008, Floc & Lock effectively precipitated the *Aphanizomenon* bloom where after WFD and BWD were met. The effects of the intervention were durable (Chapter 5) and, according to the criterion by Cooke et al. (2005), can be considered a long term success as it resulted in strongly reduced P concentrations at least for more than ten years.

Other than a 3-day swimming ban in 2011 due to a mystery scum (Chapter 5), no swimming bans were issued due to cyanobacterial nuisances. Post-treatment, from 2008 to 2018, annual as well as mean summer chlorophyll-*a* concentrations and TP concentrations were strongly reduced as compared to the pre-treatment period (2006, 2007). Lake Rauwbraken shifted from a eutrophic to a predominantly mesotrophic status and water quality met the WFD and BWD demands (Chapter 5). This study demonstrates the strong point of a lake system analysis (Cooke et al. 2005; Lürling et al. 2016; Mucci et al. 2019), which pinpointed the mitigation effort to reducing the internal P-load, rather than the paradigmatic reduction of external nutrient loads only.

To remove P from the water column of a lake, a solid phase P binder like LMB (Chapter 1), is best applied when particulate P is low and FRP high. This situation typically occurs outside the blooming season (winter) and thus limits the time window during which a solid phase phosphorus binder has its highest effectiveness in the water column. The combination with a flocculent, precipitating total P, widens the applicability to all year round, particularly the Floc & Lock technique can be applied as an instantaneous cure during a cyanobacterial bloom.

While other lakes in the Netherlands suffered from blooms of cyanobacteria for decades and mitigation attempts resulted in more failures than successes (Gulati and Van Donk 2002), the problem in Lake Rauwbraken was mitigated after a single intervention, within four years after the first nuisance bloom. The Floc & Lock technique seems promising in mitigating blooms of cyanobacteria in relatively small, thermally stratifying lakes, where legislation (e.g., the EU Water Framework Directive -WFD; (EU 2000)- and the EU Bathing Water Directive. -BWD; (EU 2006) demands good water quality for recreational use. Although Floc & Lock stands a good chance to be applicable in other lakes, it probably will be one part of a total mitigating package, i.e. Floc & Lock does not reduce external inputs of phosphorus to a lake – which in most cases is the first to achieve.

The costs for treating Lake Rauwbraken in 2008 were €50,000, which was only 10% of the estimated costs for dredging or sediment capping using sand that varied between €500,000 and €600,000.

7.2 Floc & Lock revisited

Over time 'Floc & Lock' has become a synonym for the combined application of poly aluminum chloride (PAC) and lanthanum modified bentonite (LMB). At the time (2008), PAC and LMB seemed the best available compounds as flocculent and solid phase phosphorus sorbent (**Chapter 3**). Clearly, the effectiveness of the Floc & Lock treatment in Lake Rauwbraken can be attributed to the excellent performance, in Lake Rauwbraken, of the compounds chosen. These compounds however, do not define Floc & Lock as a mitigation technique, as the compounds used may be replaced by others - which may be better for other situations. Cheaper alternatives for lanthanum, such as aluminum modified clays, might expand the applicability cost-wise to larger systems. However, such solid phase P-sorbents need thorough testing first (Gibbs et al. 2011; Spears et al. 2013a).

7.2.1 Solid phase phosphorus binders

The LMB pretty much coined the novel technique to apply a solid phase phosphorus sorbent to remove phosphate from the water column combined with a strong reduction of its release from a lake's sediment. After the introduction of the LMB (Douglas et al. 1999; 2002), other modified clays swiftly followed, i.e. kaolinite enriched with lanthanum (Yuan et al. 2009), zeolite with aluminium (Gibbs et al. 2011) and bentonite with iron (Zamparas et al. 2012), and recently zeolite with lanthanum (Wang et al. 2017; Yin et al. 2018), zirconium modified zeolite (Yang et al. 2014; 2015), but also modified natural soils (Pan et al. 2012) and unmodified natural products, such as red soils (Noyma et al. 2016), or industrial by products (Douglas et al. 2016; Spears et al. 2013a).

In the LMB, lanthanum precipitates with phosphate forming the mineral rhabdophane, which is resistant to weathering over geological time (Douglas et al. 2004). The phosphate binding capacity of lanthanum is not affected by anoxic events and is effective over a much wider pH range than Fe or Al

salts (Douglas et al. 2004; Ross et al. 2008). Anoxic events, which naturally occur within or above sediments, are known to greatly reduce the phosphate binding capacity of iron (Correl 1998). Hence, iron-enriched clays (Zamparas et al. 2012) might be considered far less suitable, and because of their redox sensitivity, no long-lasting control of sediment P release is to be expected from Fe (Cooke et al. 2005).

A major drawback in using natural clays/soils is that they will not, or only marginally, influence P in the water column and will not hamper P-release from the sediment unless the physical barrier is sufficiently thick, e.g., at least 5 cm to resist ebullition and shear stress (Quandt 2001). An enclosure experiment with chitosan-modified local soils in Lake Taihu showed higher P concentrations after bloom removal, which was considered a remaining problem of the technique (Pan et al. 2006b). Therefore, modified clays enriched with aluminum (Gibbs et al. 2011) or lanthanum (Douglas et al. 2004; Yuan et al. 1999) that target the release of P from the sediments due to their strong P-binding capacity might be far more promising than using unmodified soils and clays.

7.2.2 *Lanthanum modified bentonite and confounding factors*

While the recommended LMB dose was effective in controlling phosphorus in Lake Rauwbraken, in an enclosure experiment (Lürling and Faassen 2012) and in the first LMB application in the Netherlands (Het Groene Eiland), LMB did not meet the expectations (Lürling and van Oosterhout 2013). In Het Groene Eiland, which was dammed off from a larger lake, cyanobacterial blooms did not occur after the intervention, but P concentrations were not reduced due to ongoing inputs. The enclosure experiment was started in mid-summer during a strong cyanobacteria bloom (Lürling and Faassen 2012) and thus, as explained before, no effect is to be expected since all P is already inside the cells. In some experiments, the advised 100:1 dose appeared insufficient to reduce phosphate concentrations to low levels (Reitzel et al. 2013a, b). Several factors might influence the (short-term) P sorption capacity of LMB of which pH, alkalinity and humic substances seem the most important ones (Geurts et al. 2011; Lürling et al. 2014; Reitzel et al. 2013b; Ross et al. 2008; Sonke and Salters 2006; Tang and Johannesson 2003; 2010). It should be noted that competition with hydroxyl ions (high pH), carbonates (high alkalinity) and complexation by humic substances will only delay the La-P binding, it will not prevent it (Dithmer et al. 2015). Indeed, none of these factors played a major role in Lake Rauwbraken (Chapters 2,4,5). In fact, analysis of sediment collected from 10 LMB treated lakes across Europe, including Lake Rauwbraken, revealed that most La was bound to P as rhabdophane (Dithmer et al. 2016b).

7.2.3 The fate of Lanthanum

Despite the demonstrable, and sustained improvement in water quality in Lake Rauwbraken following the application of LMB, the presence of and fate of both TLa and FLa is worthy of a detailed investigation to satisfy concerns in terms of both its environmental fate and public acceptance of LMB as a viable in-

lake eutrophication management option. This is also pertinent in light of the potential for trophic transfer of La (Chapter 6) and the potential, albeit slight, for La ingestion by recreational lake users (D'Haese et al. 2019).

7.2.4 Flocculants

The addition of flocculants to clays is recognized as a powerful tool to sink harmful marine algae out of the water column (e.g., Hagström and Granéli 2005; Pierce et al. 2004; Sengco and Anderson 2004) and are used in freshwaters to enhance removal of cyanobacteria (Pan et al. 2006a; b; 2012; Zou et al. 2006). Regarding the application of a flocculant to remove a bloom of cyanobacteria there are three points to mention: safety, effectiveness and durability.

The safe removal of the bloom means the cyanobacterial cells must remain intact during the process, i.e. in two lakes in Australia cell lysis of toxic cyanobacteria by the algaecide copper sulphate, led to liver diseases in humans (Falconer et al., 1983; Hawkins et al., 1985). For the purpose and safety, avoiding cell lysis, a we chose a relative low dose of PAC (Chapter 4). As an alternative for inorganic flocculants such as PAC or iron(III)chloride, the organic flocculant chitosan is promoted as 'non-toxic and eco-friendly' (Li and Pan, 2013). However, at a realistic low dose, chitosan causes cell lysis in *Cylindrospermopsis raciborskii* and *Planktothrix agardhii* (Mucci et al., 2017).

During 2004-2007 *Planktothrix rubescens* was the main culprit in Lake Rauwbraken, at the time of the Floc & Lock treatment in 2008, the lake was struck by a bloom of *Aphanizomenon*. The Floc & Lock treatment, with PAC as flocculant, effectively sunk this bloom, and the water column remained void of filamentous cyanobacteria for several years. The Floc & Lock treatment of Lake de Kuil in 2009 was done during a bloom of *Aphanizomenon* (Waajen et al. 2016a), with iron(III)chloride as flocculant (Waajen et al., 2016a) and as ongoing diffuse P inputs led to new nuisance blooms the treatment was repeated in 2017 (Mucci et al. 2019), now during a bloom of Planktothrix with PAC as flocculant. While in both cases, the treatment resulted in good water quality (Mucci et al. 2019; Waajen et al. 2016a) and the first observations indicated that *Planktothrix w*as sunk to the bottom, it reappeared in the water column after two weeks, albeit in a much lower concentration. It seems as if some of the entrapped filaments had escaped the flocs (Lürling et al. 2020b). Thus, what may suffice as dosage of flocculant and sinking weight for one species of cyanobacteria may not for another species.

The application of the flocculant (PAC) with sinking weight (LMB) was done to force all particulate P to the sediment of the lake and to safely remove the existing bloom of cyanobacteria (Chapter 4). A low dose of PAC often results in an only temporarily relieve, because the dosage is simply too low to bind all P, but also because the amorph aluminium flocs transform into crystalline forms (ageing), in which case the particulate P returns to the water column as FRP after the decay of the precipitated bloom

(Berkowitz et al. 2006; de Vicente et al. 2008). The combination of a flocculant with a solid phase phosphorus binder (Floc & Lock) thus prolongs the curative effect of a flocculent (Chapter 4) to a durable effect (Chapter 5). Later work by Huser et al. (2016), revealed that a relatively higher dosage of Al in thermally stratifying lakes with a high residence time results in 17 to 44 years of longevity.

7.2.5 *Baby steps*

Promising as the experiences with Floc & Lock in Lake Rauwbraken and Lake the Kuil (Waajen et al. 2016a) are, as whole lake experiments they also show that minor differences between lakes can have a relevant effect on the result of a treatment. E.g. by the examples of the humic substances and different species of cyanobacteria above, it makes, by all means, sense to take one or two small steps before copy-paste a successful treatment from one lake to the next. In the case of a flocculent and a solid phase P-sorbent, these steps may comprise of testing the compounds on water and sediment from the lake at hand shortly before the treatment is to be applied. If at all the lake has a surprise in store, the results of these experiments will allow for changes in the compounds to use, their dosing or indeed refrain the application if necessary.

7.2.6 Maintenance

While in the Netherlands it is accepted that even nature reservations need some sort of maintenance, natural waters are mostly seen as a free resource which can be used without maintenance. As the story of Lake Rauwbraken tells (this thesis), things can go fine for decades, but then a problem not anticipated in the business plan for the recreational facility arrives (blooms of cyanobacteria). Based on current knowledge, lake owners are best advised that at some point in time (which can to some extent be predicted) a budget is needed to diagnose and mitigate blooms of cyanobacteria.

Maintenance of lakes, e.g. counterbalancing the external phosphorus loads by immobilisation, is necessary to keep water quality acceptable, simply because none of the waters is fully isolated from its environment and, as in seepage lakes like Lake Rauwbraken, diffuse nutrient loads are key to the water quality problem. The fact that lakes developed serious eutrophication symptoms, clearly demonstrates that maintenance is necessary to keep water quality such that the specific lake can fulfil its ecosystem services. E.g. Epe et al. (2017) obtained good results for Lake Bärensee (Germany) in which the trophic level was reduced by an initial dose of LMB and the subsequent smaller reapplications (Epe et al. 2017).

If one considers the costs of the treatment (€50,000) of Lake Rauwbraken, the 2008 investment boils down to some €5,000 per year. Arguably, the 2008 investment was the price paid for >30 years of good water quality. It may seem odd to compute the annual costs of a treatment in such a reverse way. However, if one draws up a business plan for a recreational facility, one does include maintenance like lawn mowing and pruning the hedges of which the integral costs are quite beyond the €50,000. The

established external load reduction by the maintenance of the greenbelts cost more than €10,000 per year in man hours alone, whereas the 4 months closure due to the swimming ban in 2007 implied €150,000 loss of revenue.

7.3 The Gordian knot

Dealing with nuisance cyanobacteria, limnologists tend to start by defining 'eutrophication' as the problem to tackle. The proliferation of cyanobacteria is nutrient driven (O'Neil et al. 2012; Smith et al. 1999; Watson et al. 1997), hence the link to 'eutrophication' (Chorus and Bartram 1999; Conley et al. 2009; Smith et al. 1999). 'Eutrophication' is considered a complex problem. This complexity starts with the fact that there is no consensus regarding the definition of eutrophication (see below). Next, there is no consensus regarding what the problem is. It is of interest to note that Vollenweider (1968) made a point on this subject back in 1968: 'Is it eutrophication or one of its symptoms, among which blooms of cyanobacteria?' And, when we're dealing with cyanobacteria, is it their presence as such, or their alleged toxicity? To which I may add that there is no consensus on what a bloom is either (Smayda 1997). Moreover, while we are in the mist on the problem site of the equation, we're also stuck in a paradigmatic swamp when it comes to solutions. E.g. source or effect-oriented mitigation, should we target nitrogen, phosphorus or both? We're caught in a Gordian knot.

7.3.1 Eutrophication

Most definitions of '*eutrophication*' include cause and effect – nutrients and biomass, in one (Box 1). In the early years, analytic methods to determine nutrient concentrations were considered not sufficient, hence Naumann (1919) used phytoplankton production as a proxy for the nutrient (trophic) status (Hutchinson 1969). As analytical methods have improved since the times of Naumann, nutrient concentrations are now commonplace to identify the trophic status of a lake. Still, phytoplankton densities, measured as chlorophyll-a ($\mu g\ L^{-1}$) are forever associated with the trophic status of surface waters.

Box 1 Some definitions of eutrophication.

During the 20th century it was observed that of these waters changed from relatively clear waters to green soups. This process of change was called eutrophication, for which several definitions have seen the light. Most definitions of 'eutrophication' include cause and effect – nutrients and biomass, in one. E.g. (Rohlich 1969) states: '*Eutrophication refers to the natural or artificial addition of nutrients to bodies of water and to the effects of added nutrients*. In 1971, at the ASLO symposium 'The Limiting Nutrient Controversy' A.F. Bartsch provided a working definition of eutrophication: '*Eutrophication is the nutrient enrichment of waters, which frequently results in an array of symptomatic changes, among which increased production of algae and other aquatic plants, deterioration of fisheries, deterioration of water quality, and other responses, are found objectionable and impair water use' (Bartsch, 1970).* In (Wetzel 2001): *Eutrophication is simply the alteration of the production of a lake along a continuum from low to high values, that is, from oligotrophy to eutrophy.* Many other definitions go around, which in the end resulted in lack of consensus of what eutrophication is. Moss (1980) actually goes as far as not using the terminology, i.e. he simply states that some lakes are more fertile than others (Moss 1980).

7.3.2 Symptoms

In fresh water lakes, '*Eutrophication*' may lead to several alleged symptomatic nuisances. E.g. massive stands of macrophytes, or absence of macrophytes, turbid waters, high densities of cyanobacteria or floating plants (Bloemendaal and Roelofs 1988). These symptoms may have multiple causes, of which only one is nutrient enrichment. Turbidity comprises of the combined effects of inorganic suspended solids, detritus (humic compounds) and phytoplankton. Meaning that a high turbidity may be caused by bottom-feeding fish as well as by phytoplankton growth or brownification (e.g. (Graneli 2012)). Submerged macrophytes may disappear as a result of turbid waters – which may or may not be the result of nutrient enrichment. In Lake Rauwbraken, they disappeared as a result of grazing by Grass carp (Chapter 2). In most nuisance cyanobacteria, buoyancy control is base to water column accumulations (George and Edwards 1976) and surface scums (Reynolds et al. 1987; Walsby et al. 1995). The density of cyanobacteria in the water column of a lake is thus governed by two different processes: proliferation (a biological process) and accumulation (a physical process). Thus, even a low biomass of cyanobacteria in the water column may cause a nuisance through scums or accumulations and locally impair ecosystem services in a lake (Chapter 5). Hence, the step from blooms of cyanobacteria to '*eutrophication*' should be made with care, distinguishing proliferation from accumulation.

7.3.3 Unravelling the Gordian knot

To unravel the Gordian knot, one may choose to not use one word, 'eutrophication', to indicate both cause and effects, thus separating the process of nutrient transport from the trophic status of a lake. Whether or not such nutrient transport into the system will lead to a higher trophic status still depends on the amounts flowing out of the lake. In case the inflow of a nutrient is larger than its outflow, one may speak of eutrophication, in case the outflow is larger it would be oligotrophication. Eutrophication then becomes a straightforward nutrient enrichment of a (aquatic) system or external nutrient load. In this case, 'excessive' nutrient loads mean nutrient loads that are in excess of the outflows, not excessive because the lake show symptoms of a (hyper) eutrophic status. There is the concept of a permissible P load (Vollenweider and Dillon 1974) or critical P load (Vollenweider 1968; Vollenweider 1975; Vollenweider 1976), which is an external P load that delineates between oligotrophic and mesotrophic lakes, respectively between mesotrophic and eutrophic lakes. However, the trophic status linked to permissible and critical loads is based on symptoms (e.g. Secchi disk depth, chlorophyll-a concentration, hypolimnic oxygen concentration, frequency of algal blooms), not on nutrient concentrations (Vollenweider and Dillon 1974) . Moreover, a small external P load, seemingly permissible, not accompanied by symptoms of a (hyper) eutrophic system, is likely to fire back as it integrates to (builds up a) legacy sediment P-pool if not counterbalanced by a similar outflow (Chapter 2).

7.3.4 Mitigation of eutrophication

Mitigation of eutrophication thus becomes the reduction of the external nutrient loads to a lake or indeed enlargement of the nutrient outflow. In its most common occurrence, eutrophication of a freshwater lake is the enrichment with all three macro nutrients: P, N and C, which in its base, is a result of human activity (Jančula and Maršálek 2011; Smith et al. 1999), that is: nutrient emissions from households, agriculture and industry. The ultimate source-oriented mitigation of eutrophication would be to stop the emissions. The past century has resulted in a legacy of nutrient 'freely' cycling in our atmosphere, ground and surface waters. Meaning that even if nutrient emission are stopped, the eutrophication of our surface waters will go on for decades to centuries (Goyette et al. 2018; Jarvie et al. 2013).

Reduction of external nutrients loads is advocated as the silver bullet in mitigating cyanobacterial nuisance (Hamilton et al. 2016; Paerl 2014; Paerl et al. 2016a), which may work in lakes that have an ongoing external load or a short residence time and no major loading from the sediment (Edmondson 1970). Reduction of external nutrient input is only feasible when sources are identifiable, such as point sources. When the external sources are diffuse, full external load control may not be feasible and in-lake measures will turn the management option of first choice (Huser et al. 2016; Lürling and Mucci 2020).

7.3.5 What makes-up the trophic status

The trophic statuses of lakes (oligotrophic, mesotrophic, eutrophic; (Naumann 1919; Weber 1907) generally focus on water column nutrient concentrations (and chlorophyll-a). Which ignores the fact that nutrients may reside in other compartments, such as the lake sediment or submerged macrophytes. Internal nutrient fluxes may just as well drive the proliferation of cyanobacteria (**Chapter 2**). In case external inputs have loaded the sediment with nutrients, recovery may take decades after applying just external load reduction due to massive P recycling (Cooke et al. 2005; Gulati and Van Donk 2002; Søndergaard et al. 1999; 2001).

Expanding the trophic status of a lake to include all bioavailable nutrients within the system may provide better predictive insights for mitigation efforts. To mitigate the eutrophic status is to reduce the bioavailable nutrients present in the system. As the proliferation of cyanobacteria is nutrient driven, this would be cause (not source) oriented mitigation.

7.3.6 Dealing with symptoms

While all nutrients must be available in ample amounts to allow cyanobacteria to proliferate to high densities, Liebig's law (Liebig 1855) implies this proliferation can be stopped by making a single nutrient growth limiting. Hence, mitigation of cyanobacterial nuisances by limiting the proliferation of cyanobacteria is not the same as mitigating eutrophication because proliferation can be stopped by reducing a single nutrient to limiting concentrations within a lake. Applying an in-lake treatment without curtailing the external loads to a lake may seem like mopping up the floor without also turning off the faucet. However, if the external loads are small relative to the internal loads, practical and economic feasibility are likely to rule over the paradigmatic external load reductions (**Chapters 2,4,5**).

The approach of dealing with a symptom (like blooms of cyanobacteria) is met with critics because it is not source-oriented. Meaning that source-oriented mitigation appears to be the preferred way to go. However, source-oriented mitigation actually means one wants to solve all the world's problems all at once (see: Mitigation of eutrophication, above). Which, obviously, runs into a non-feasible solution from a practical point of view. In the context of practical mitigation, the time until the effort shows its effects (as good water quality) is of importance, because not meeting up with water quality standards implies further economic losses for the proprietors of a lake or – indeed, more people suffering diseases like gastroenteritis. Dealing with the symptom (i.e. blooms of cyanobacteria) may thus be a way to buy time to set up the more elaborate effort of source oriented mitigation.

7.3.7 Lakes and views: matters of socio-limnology

Given the worldwide number of lakes (~117 million lakes; 5 million km^2 area; (Verpoorter et al. 2014)), their drainage basins, local forms of use, appreciations of qualities et cet. the global '*eutrophication*'

problem falls apart in an astronomical number of unique local problems. The human use of these waters (e.g.: (Palermo and Maynord 1998) roughly splits into forms that need good water quality (irrigation, drinking water production, recreation, ornamental, natural habitats), and forms of use that affect water quality negatively as recipients of waste (industrial, drainage of agricultural fields and household effluents). Stakeholders directly linked to a lake may therefore have conflicting interests. Mitigation of blooms of cyanobacteria by reduction of external nutrient loads involves stakeholders that may not directly be linked to the lake, their numbers depending on the size of the catchment area. Moreover, stakeholders may have strong ideas regarding the general approach of mitigation, meaning that in their view some forms of mitigation are off-limits - and that geoengineering depends on a toolbox rather than one single product. It thus means that a stakeholders analysis is a prerequisite to mitigation efforts. If we (limnologists) stick to our science, a stakeholders analysis is not part of our methodology. Considering poor water quality as a problem that must be solved, then anything that lies between the problem and its solution must be known and understood. Which thus opens the field of socio-limnology: the science of inland waters and human views on their use.

7.4 Lake System Analysis

7.4.1 Measure toxins

The issue with cyanobacteria lies in their potent toxins, which pose health risks to humans, husbandry and wildlife (Dittmann and Wiegand 2006; Sivonen and Jones 1999). However, not all species of cyanobacteria produce toxins and species that have toxin producing strains may also have strains that do not produce toxins (Janse et al. 2004) or may produce variants differing in toxicity (Chorus et al. 2000). A common situation with cyanobacteria is their sudden occurrence as a nuisance in surface scums or accumulations, like the first bloom in Lake Rauwbraken in 2004 (Chapter 3), or the mystery scum (Chapter 5). Based on the extremely high densities of cyanobacteria in scums and accumulations, and if toxic, these are also the most hazardous to human health.

The very first step in diagnosing a nuisance bloom should be to measure its toxicity and assess its societal impact. In a third world country where people suffer gastroenteritis (vomiting and diarrhea) (Chorus et al. 2000), because their single water resource is teeming with toxic cyanobacteria is quite a different problem as compared to an ornamental urban pond in a first world country where the mal odouring scums are not wanted for esthetical reasons. The route to prevent human exposure to cyanobacteria may thus vary from providing clean drinking water to putting up a warning sign. Having established that the water quality problem to mitigate is a high density of toxic cyanobacteria, a lake system analysis should be done before deciding on mitigating efforts (Cooke et al. 2005; Lürling et al. 2016; Mucci et al. 2019).

7.4.2 Nutrient balance

A system analysis should include a nutrient balance – paramount to anything else, revealing both external and internal nutrient sources to a lake's water column. A prerequisite for the nutrient balance is the water balance that reveals inflows and outflows, yielding insight into the residence time. The nutrient balance will identify if the external load is an issue, if internal load is an issue, if both are an issue or if both are not the main drivers of cyanobacterial nuisance. The biological characteristics of a water body identify the changes from a desired condition, for instance the fish stock, abundance and composition of submerged macrophytes, the condition of the banks and the presence of waterfowl.

7.4.3 Societal cost-benefit

The societal cost-benefit analysis includes the use of the water body – drinking water source, irrigation, fisheries and aquaculture, industrial process water, recreational – and success and fail factors to high benefits and low costs. Altogether the system analysis will yield insight into which sources need to be tackled to achieve the highest chance for success and will guide to a most promising set of measures to achieve success, yet also leaving a well-founded 'do-nothing' as an option. Hence, a system analysis is valuable for underpinning the prioritization of lake management strategies.

7.4.4 You can't touch this

Most stakeholders are people or groups of people that operate in the world of legislated use of a lake and as such behave as legislation prescribes. To a great extent our view on the world is based on processes that are within legislation. Indeed we cannot deal with everything, however cutting our diagnosis of a system short at this point is an oversimplification. The roles of benthivorous fish carp (*Cyprinus carpio*) and bream (*Abramis brama*) in water quality in general is well known (e.g.: (Meijer et al. 1989; Meijer et al. 1990)). And biomanipulation by fish stock reduction has become a useful tool in water quality management (Gulati and Van Donk 2002; Søndergaard et al. 2008). While sport fishing organizations are quite cooperative stakeholders, in many lakes attempts to reduce fish stocks are simply boycotted by some individuals (referring back to socio-limnology above) as has become quite clear from the work of Waajen (Waajen 2016b).

Water birds as modes of nutrient transport are well known (Hahn et al. 2007; Hahn et al. 2008), meanwhile, birds are widely protected (2009/147/EG 2009). If a system analysis indicates that water birds play a relevant role in the nutrient fluxes into a lake, a mitigation effort may run into a deadlock – either from legislation or from a non-cooperative attitude of the stakeholders involved.

7.5 Looking for Trouble

Whole lake experiments, such as those presented in this thesis, are different from off the shelf mitigation attempts. A system analysis for the purpose of an off the shelf mitigation of a nuisance caused

by cyanobacteria, typically, can only run from known problems to known solutions. Such practical diagnosis is not the same thing as scientific research in the field of limnology. In a limnological study, one would like to go wide, sample about everything and at a high frequency. In fact, one is looking for trouble. That such efforts pay-off is illustrated by the *Daphnia* study (Chapter 4) and our knowledge of humic substances as measured in Lake Rauwbraken, which at least gave a hint to their effects in other lakes (Lürling et al. 2014). Conversely, and in hind sight, the mitigation effort in Lake Rauwbraken could have been underpinned without knowledge of nitrogen. Obviously, if one is to show that single phosphorus control suffices to mitigate blooms of cyanobacteria, one needs to show that nothing happened with the nitrogen concentrations in the lake.

System analysis heavily draws on diagnostic tools. Of these, the most powerful is field observation. Having said that immediately raises the questions: what should we look for? And how often should we look? The latter may be extended to the spatial distribution and number of replicates that should be included in an observation. Fractionated phosphorus extraction and sediment phosphorus release experiments likewise are powerful tools, yet they seem too elaborate to be included in standard profiling lake conditions. One thing that might come from a detailed study like this thesis is a list of minimum requirements to understand the lake system. If one considers the societal problems and economic losses involved there is a lot to gain from acting on early warnings – e.g. such as the illusive *Planktothrix rubescens* described in Chapter 2. By the examples of three lakes, Spears et al. (2021) demonstrate that preventing ecological degradation is preferable over attempting to restore lakes after degradation. Although there are protocols to profile lakes in more depth – such as the Dutch manifests bathing water profile and the cyanobacteria protocol (Anonymus 2006; Anonymus 2008), current water quality monitoring is not enough to prevent problems to occur. Working according to these protocols cannot lead to preventions as their base entry is to see if a known problem, such as a bloom of cyanobacteria (Anonymus 2008) is present or not. Meaning that actions can only be taken after the occurrence of a water quality problem. One solution is to provide monitoring protocols that are written from the perspective thinking beyond today. By the example of Lake Rauwbraken (Chapter 2): one yearly snapshot of sampling this lake at its deepest point at regular depth intervals would have been enough to pick up the developing cyanobacterial problem. If one bears in mind that acting on the early signs could have helped to prevent swimming bans (and financial losses) there is much to be gained.

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Summary

Blooms and scums of cyanobacteria led to prolonged swimming bans in Lake Rauwbraken (The Netherlands). We mitigated this nuisance by imposing phosphorus limitation on the lake. Our lake system analysis revealed that, over 40 years history, small dispersed sources built up a legacy phosphorus pool in the sediment and that the internal sediment release of phosphorus was the main phosphorus-source to the cyanobacteria (**Chapter 2**). The practically and economically most feasible approach was to reduce the internal phosphorus load by applying a solid phase phosphorus sorbent (SPS). We combined the flocculent poly aluminium chloride (PAC; Floc; **Chapter 3**) with the SPS lanthanum modified bentonite (Lock; Phoslock®; **Chapter 3**) as sinking weight to sink a present bloom, additional lanthanum modified bentonite was applied to the reduce internal phosphorus load. The treatment first stripped the water column of phosphorus using a flocculant (Floc) (**Chapters 3, 4**), shifting the lake from a (hyper) eutrophic state to a mesotrophic state. It also provided an instantaneous cure to the nuisance and lifted the imposed swimming ban (**Chapter 4**). Longer term, water quality remained good for more than a decade, but due to ongoing and increased external phosphorus loads, the lake is returning to a eutrophic state (**Chapter 5**). Directly after its application, the treatment caused a temporarily decrease in the *Daphnia* population in the lake (**Chapter 4**). Four years after the treatment *Microcystis* cells emerged from the lake sediment and produced a minor low-toxic scum. The event did not lead to a proliferation into a nuisance bloom (**Chapter 5**). Other than this mystery scum, the lake remained free of cyanobacterial nuisances. Directly after the application the Dutch maximum permissible filterable lanthanum concentration was exceeded for after 75 days (**Chapter 6**). Three years after the application, part of the LMB is relocated to the deeper part in the lake (**Chapter 6**). The lanthanum from the LMB is taken-up by Crayfish (*Procambarus fallax* f. *virginalis)* and macrophytes (**Chapter 6**), no ill effects were observed in the Crayfish nor in the lake itself. Successful mitigation of high densities of toxic cyanobacteria depends on strict definition of the problem, lake system analysis and knowledge of the human factor: socio-limnology (**Chapter 7**).

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Jean François Xavier (Frank) van Oosterhout (born in Delft, 1960), graduated at Rijks Universiteit Leiden (Biology) in 1988. After working as a database administrator – statistician in pharmaceutical industry, Frank became a diving instructor, founded and chaired Stichting Nederlandse Onderwater Parken and was caretaker of Lake Rauwbraken from 2000 to 2012. From 2005 onwards Frank is guest working (part time) at the Aquatic Ecology and Water Quality Management Group of Wageningen University and Research Centre.

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