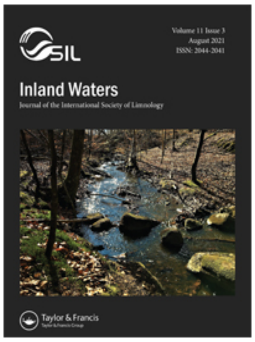

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Assessing the long-term efficacy of internal loading management to control eutrophication in Lake Rauwbraken

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ABSTRACT
Lake Rauwbraken was impacted by eutrophication caused by diffuse external phosphorus (P) loads (total 1.21 mg m^{-2} d^{-1}, estimated in 2008). Over 40 years, this load built up a legacy pool in the sediments, resulting in 6.82 mg m^{-2} d^{-1} PO_4-P internal load (estimated in 2008), causing cyanobacterial blooms and swimming bans. To address the internal load in this lake, a low dose treatment of flocculant (polyaluminium chloride) combined with a solid phase phosphate fixative (lanthanum-modified bentonite) was applied in 2008. We examined the chemical and ecological responses to this treatment to demonstrate the efficacy of controlling internal loading without reducing external loading. Based on 2 years pre- and 10 years post-treatment monitoring, the mean Secchi disk depth (3.5–4.0 m) and the hypolimnetic oxygen concentration (0.86–4.55 mg L^{-1}) increased while decreases occurred in turbidity (5.4 to 2.2 NTU), chlorophyll *a* (16.5 to 5.5 µg L^{-1}), contribution of cyanobacteria (64% to 17% of chlorophyll *a*), total phosphorus (134 to 14 µg L^{-1}), and total nitrogen (0.96 to 0.50 mg L^{-1}). The treatment reduced the PO_4-P release from sediment under anoxic conditions from 15.1 to 1.7 mg m^{-2} d^{-1} post-treatment in 2008, 2.3 mg m^{-2} d^{-1} in 2011, and 4.7 mg m^{-2} d^{-1} in 2013. Post-treatment, submerged macrophytes reached high coverage in 2008 and 2009. Longer term, post-treatment macrophyte cover was reduced. The lake is returning to a eutrophic state as a result of ongoing external P loads 10 years following the control of internal loading.

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cyanobacteria; eutrophication control; in-lake; lake restoration; sediment P release

Introduction

Eutrophication of fresh waters often leads to blooms of potentially toxic cyanobacteria, which are a threat to human and animal health (Smith et al. 1999, Dittmann and Wiegand 2006). Blooms of cyanobacteria may cause swimming bans (Ibelings et al. 2012), drinking water shortages, and economic losses from impaired ecosystem services (Dodds et al. 2009, Zhang et al. 2010). The water quality demands of the European Water Framework Directive (WFD) and Bathing Waters Directive (BWD) require that safe and efficient management measures are implemented in impacted waterbodies (European Commission 2000, 2006).

Before implementing measures, a diagnosis of the mechanisms causing the nuisance should be made (Cooke et al. 2005, Lürling et al. 2016). Such diagnosis (i.e., an analysis of nutrient fluxes to a lake system) can be used to identify important sources and fluxes of nutrients and to develop effective management interventions to control them.

Reducing nutrient inflows may mitigate eutrophication in lakes where internal loading of nutrients from bed sediments is low (Edmondson 1970). If the sediment phosphorus (P) content and internal loading are high, recovery may take decades after external load reduction due to P recycling between bed sediments and the water column (Rolighed et al. 2016). The reduction of external nutrient load is feasible when sources are identifiable, such as point sources. Where point sources (e.g., from sewage treatment systems) have been controlled or are low, and diffuse external P sources (e.g., run-off from urban or agricultural land) are high and difficult to control, in-lake measures may be the only option with which to address blooms of

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cyanobacteria, especially where internal loading is a significant source of P (Huser et al. 2016, Lürling et al. 2020a). Commonly, eutrophication management in fresh waters have focused on P loading reduction (Carpenter 2008, Schindler and Hecky 2009), and various measures have been developed to address internal P loading in lakes, such as sludge dredging, hypolimnetic withdrawal, oxygenation and chemical precipitation, or P fixation (Lürling et al. 2020b).

Lake Rauwbraken (Fig. 1a) is one of 500 deep sand excavations (Osté et al. 2010) in the Netherlands; most have a recreational function and are subject to the BWD. In Lake Rauwbraken, blooms of *Planktothrix rubescens* have resulted in elevated human health risk leading to management responses, including swimming bans and the development of mitigation measures.

Prior assessments of nutrient loading to Lake Rauwbraken confirmed that internal P loading was a major driver of poor water quality leading to the implementation of measures, termed here “Floc & Lock” (Lürling and van Oosterhout 2013). Our current work deals with the detailed system analysis and long-term water quality and ecosystem responses crucial to determine cost effectiveness and longevity of the treatment (Spears et al. 2013, 2016). This treatment provides an opportunity to assess the efficacy of controlling internal loading only, with respect to longevity of effect and effect size over a 10-year period, making this one of the most comprehensive assessments of this approach.

We utilized long-term monitoring data spanning a 12-year period (i.e., 2 years pre-application and 10 years post-application) to examine the chemical and ecological responses following the treatment conducted in 2008. We addressed the following specific hypotheses: (1) that sediment nutrient flux (dissolved nitrogen [N] and P) was effectively controlled following the Floc & Lock treatment, and (2) that this effect would extend beyond the 10-year monitoring period. We also report on submerged macrophytes and discuss their role in the water quality of Lake Rauwbraken. We discuss the implications of the long-term responses reported in terms of longer-term management of internal loading in this site and potentially others.

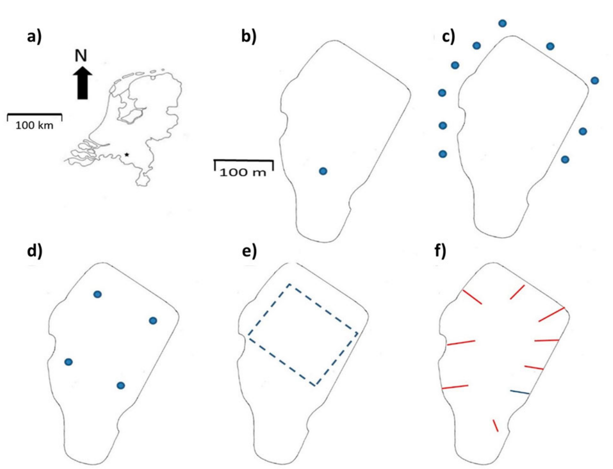

Figure 1. (a) Location of Lake Rauwbraken in the Netherlands, (b) water sampling point, (c) sample points for organic deposition, (d) location of sediment traps, (e) location of core sampling, and (f) macrophyte transect (red line = rake and bathyscope, blue line = sampling).

Material and methods

Greenbelt clearing

Lake Rauwbraken (Fig. 1a, Table 1) is an isolated, small (2.5 ha) thermally stratifying lake constructed in 1966. The lake is surrounded by a greenbelt, which in 2000 had overgrown the banks of the lake, shading the helophyte zone. The greenbelt runs from the water’s edge to a fence around the premises, varying 5–25 m in width. Organic litter from this vegetation falls on the banks of the lake and through the lake’s fluctuating water level is directly transported into the lake. In 2000, 75% of the terrestrial vegetation (all trees with a trunk <30 cm diameter at 1.5 m height) was cleared to reduce nutrient inflow from the riparian zone and to provide sunlight to the helophyte zone. In the same year, all shrubs were cut and thereafter pruned annually until 2011. The green belt was maintained at a low water level (see water balance) from late autumn to late winter. During maintenance, small organic material (leaves and twigs) was collected using a wind blower and subsequently taken to a refuse container. The amount collected was weighed at the city recycling centre, and the wet weight of material removed was reported. We estimated by visual inspection that ∼90% of all organic material was removed from the riparian zone during maintenance periods. In 2011, all work stopped due to

Table 1. Lake Rauwbraken details and management interventions.

Lake details	
Location	51°34′57.09″N; 5°07′54.30″E
Surface area (ha)	2.56
Mean depth (m)	8.1
Maximum depth (m)	16
Volume (m^3)	208 000
Management	
2000–2005	Reduction greenbelts, planting helophytes
2000–2011	Removal 75% leaf litter annually
21–23 April 2008	Floc & Lock intervention: 2 t PAC (coagulant, Floc), 75 kg $Ca(OH)_2$ (buffer), 16 t Phoslock (LMB, Lock)

financial cuts. In 2013 the coverage of trees and shrubs had approximately doubled, as observed during monthly surveys.

Grass carp

Submerged macrophytes had been eradicated by the introduction of grass carp (*Ctenopharyngodon idella*), total 300 kg, each ~0.5 kg (Sportbedrijf Tilburg 1988, the Netherlands), to remove nuisance macrophytes (*Elodea* sp.) in the 1980s. During 2000–2005, the grass carp population had fallen to only a few specimens as a result of natural mortality, in line with their life expectancy in temperate waters (Caves et al. 2021), and the water weed *Elodea nuttallii* sporadically returned in 2005.

Floc & Lock intervention

The Floc & Lock treatment (April 2008) was based on a lake system analysis during 2005–2008. Two tonnes lanthanum-modified bentonite (LMB; Phoslock; Douglas 2002) was applied as ballast, followed by 2 tonnes coagulant polyaluminium chloride (PAC) to clear the water column; 16 tonnes LMB were then added (Table 1), creating a P barrier on the sediment (Lürling and van Oosterhout 2013). Dosing was based on the water column and releasable sediment P determined by Limnological Institute Dr. Nowak (Germany; ISO 11885-E22:1997-11). The application was done by Phoslock Europe GmbH from 21–23 April 2008.

Constructing the P mass balance

We compiled available empirical data on potential P sources in the catchment, including from groundwater, waterfowl, atmospheric wet and dry deposition, and organic matter fall from riparian vegetation and bathers (Table 2). We supplemented these data with measurements of sediment P flux (release and sedimentation) and water column and macrophyte P content to produce mean estimates of fluxes and stocks of P into and out of the lake and between the sediment and the water column. Modeling of internal loading was considered but discounted in favour of our empirical approach because the base assumption for lakes as a "completely mixed reactor" (e.g., Vollenweider 1975) were not met. Lake Rauwbraken is an isolated lake (no surface water inflow or outflow, no point sources), mainly fed by rain and groundwater, that thermally stratifies each summer (hence not a mixed system), and a relevant part of the nutrient inflow is organic material and bird droppings, meaning these nutrients are not directly available throughout the lake (i.e., time lag is due to mineralization of this nutrient source). In addition, such models draw heavily on empirical data from similar lakes (Vollenweider 1968, 1976, Vollenweider and Dillon 1974), which are not available for artificial waterbodies like those created from Dutch sand excavations (Seelen et al. 2021).

However, our studies (Lürling and van Oosterhout 2013, Waajen et al. 2016a) have demonstrated that our empirical approach for P fluxes sufficiently reveal that fluxes from the lake sediment are sensitive to the Floc & Lock treatment in such quarry lakes.

Table 2. The external and internal mass balance terms; FRP = filterable reactive phosphorus.

Source	Term	Method
External	precipitation	water balance, literature
	groundwater (in)	water balance, tube well sampling
	organic deposition	field samples
	water birds	counts, model
	bathers	counts, literature
Outflow	groundwater (out)	water balance, water column FRP
Internal	sediment release	laboratory experiments
	sedimentation	field, sediment traps

Water balance

The lake has no connection with other waterbodies, and inflow is primarily via ground water. The lake's water level fluctuates seasonally with high water in late winter and low water in autumn. Over 8 years of measurement, the mean difference between high and low water level was 1.66 m (standard deviation = 0.30, n = 8), based on weekly measurements during 2003–2011. The 25 692 m^2 surface area and detailed bathymetry (discussed later), results in an estimated annual 42 649 m^3 difference; not all of the difference is a result of groundwater because precipitation and evaporation are also involved. Daily measurement of the precipitation and evaporation (Makkink 1960) at the meteorological station of Gilze-Rijen Airport (16 km distance from Lake Rauwbraken; 3 Apr 1987 to 3 Apr 2008) were used. Precipitation was 760.6 mm yr^{-1}, equalling 19 541 m^3 annually. Evaporation was estimated at 572.5 mm yr^{-1}, equalling 14 709 m^3 annually, yielding a precipitation surplus of 4832 m^3 yr^{-1}. The remaining annual water input is assumed groundwater inflow (37 817 m^3 yr^{-1}). Because the water balance is based on data from 8 to 21 years, the change in volume (ΔV) was set at 0. The lake receives 37 817 m^3 yr^{-1} groundwater and 19 541 m^3 yr^{-1} precipitation, and loses 4709 m^3 yr^{-1} to evaporation and 42 649 m^3 yr^{-1} to groundwater. The groundwater (tube well) was sampled every 2 weeks (12 Dec 2005 to 4 Oct 2007), and nutrients were determined after filtration through a Whatman NC45 0.45 μm membrane filter. Nutrient inflow was based on the volume of groundwater coming in and its nutrient concentration.

Precipitation

The P load due to precipitation on open water was calculated using the annual precipitation data from the nearby meteorological station Gilze-Rijen, the average phosphate concentration in rain water (Stolk 2001), and assuming the total P (TP) concentration in rainwater is 2 times the concentration of phosphate (Buijsman 1989).

Organic deposition

In December 2005, 10 random samples (Fig. 1b) of organic material were taken from the collected leaf litter. The ratio of dry to wet weight material was determined through water loss on drying at 105 °C of the complete samples. After drying, the samples were ground and stored frozen (−16 °C) until further processing. Approximately 20 mg of the grinded 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, and P concentration was determined by ICP-MS at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University, the Netherlands). The mean P content of the organic litter was calculated in g kg^{-1} dry material from the 10 samples. The amount of dry material in the container was estimated from the fraction of dry material in the samples, and multiplying this fraction by its P content yielded the total amount of P removed by maintenance.

As in 2005, the estimated P input by organic litter was one-fourth of its historical value (before 2000); the estimated historical dry deposition of organic material was computed by multiplying the removed amount of P by 4.

Water birds

During 2000–2011, water birds were observed throughout the year during daily inspections of the lake and its premises. While most counting was done by direct observation, flocks of gulls were counted from photographs. P influx via birds was estimated with the program Waterbirds 1.1 (Hahn et al. 2007, 2008). Until 2011, waterfowl inputs were composed of Eurasian coot (*Fulica atra*), mallard (*Anas platyrhynchos*), and black-headed gull (*Larus ridibundus*; Supplemental Material Table S1). For resident grey heron (*Ardea cinerea*, $n = 2$) and great cormorant (*Phalacrocorax carbo*, $n = 1$), we assumed a neutral contribution (Supplemental Material Table S1). After 2011, greylag goose (*Anser anser*), Canada goose (*Branta* c. *canadensis*), and Egyptian goose (*Alopochen aegyptiacus*) became resident, adding to external nutrient loads.

Bathers

The P contribution from bathers was estimated from an input of 0.094 g d^{-1} per bather (Dokulil 2014, referring to Schulz 1981) and multiplied with the total number of bathers days per year. The number of bathers were obtained from registration (daily or season ticket) at entry.

Sediment nutrient release

Sediment nutrient release was determined for cores sampled with a Uwitec core sampler (Uwitec, Mondsee, Austria) at depths >10 m (Fig. 1c). The release was measured under aerobic conditions in 6 cores sampled 29 November 2005 (Exp. A; Table 3) and 5 cores taken on 13 December 2005 (Exp. B; Table 3); under anoxic conditions in the laboratory in 5 cores sampled 13 April 2008 (pre-treatment, Exp. A) and 19 June 2008 (post-treatment, Exp. B); and under both aerobic (Exp. B) and anoxic conditions (Exp. A) in 6 cores sampled in 2011 and 8 cores sampled in 2013 (Supplemental Material Tables S2 and S3). Overlying water was siphoned and replaced with oxygen-free or oxygenated water (Supplemental Material Table S2) as appropriate. The cores were incubated for 4 days, during which the overlying water was replaced each day.

Oxygen-free Millipore water was prepared by bubbling with N gas until the oxygen concentration was <0.04 mg L^{-1}. Oxygenated Millipore water was prepared by aeration until 100% oxygen saturation. From the prepared Millipore water, aliquots were kept as controls to check for background nutrients. Samples from the 1-day incubations and controls were filtered (Whatman NC45 0.45 μm membrane filter) and analysed for nutrients. Dissolved inorganic N (DIN) was computed as the sum of N in ammonium (AMM) and nitrite/nitrate (NN; analytic methods are described later). We choose to use DIN because in this experiment AMM may be transformed into NN and vice versa (Francis et al. 2007). In these experiments, denitrification and anoxic ammonium oxidation (anammox) may lead to some N loss as N_2 (Francis et al. 2007). Hence, for DIN our experimental units are not closed systems, and DIN release may be underestimated.

Table 3. Release of filterable reactive phosphorus (FRP) and dissolved inorganic nitrogen (DIN) in sediment cores taken from <10 m depth in Lake Rauwbraken in different years.

	FRP release						
Year	Mean (SD) (mg m^{-2} d^{-1})	Mean (SD) (mg m^{-2} d^{-1})	*n*	*t*	df	*p*	β
FRP release	Exp. A	Exp. 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	8.4[a]	8	<0.01	
	anoxic	aerobic					
2011	3.0 (0.7)	1.2 (0.1)	3	6.4[a]	4	<0.01	
2013	4.6 (2.7)	1.6 (1.0)	4	2.1	6	n.s.	0.43
DIN release	Exp. A	Exp. 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	n.s.	0.07
	anoxic	aerobic					
2011	44.1 (3.2)	43.9 (5.8)	3	0.05	4	n.s.	0.05
2013	24.1 (5.8)	27.3 (4.6)	4	−0.86	6	n.s.	0.11

Note: Exp. A and B (2005) are 2 repeated experiments (aerobic incubation) at diferent times; before and after (2008) indicate that cores were taken just before and 2 months after the intervention (both anoxic incubations); 2011 and 2013 cores were incubated both under aerobic and anoxic conditions; *n* = number of replicates, SD = standard deviation, *t* = *t*-test statistic, df = degrees of freedom, *p* = 2-tailed *p* value (n.s. = not significant at α = 0.05), β = power of the test with α = 0.050.
[a]*t*-test conducted on log transformed data

The filterable reactive P (FRP) and DIN release rates were computed separately for each experiment and condition. For each core, incubation, and period, the total FRP and DIN released (R_{ij}) was computed by:

$$R_{ij.}(\mathrm{mg}) = [X_{ij}] \times V_i, \tag{1}$$

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; and R_i (mg) = SUM($R_{ij.}$) is 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:

$$R24_{i.}(\mathrm{mg\ m^{-2} d^{-1}}) = \frac{24 \times 60}{A}\frac{R_i}{T}, \tag{2}$$

with A = surface area of the sediment in the core (0.00181 m^2) and T = total duration (min) of the experiment.

Results of aerobic and anoxic incubations were used to determine internal loading. To this end, internal nutrient loadings comprised release during thermal stratification in the anoxic hypolimnion ($I_{hyp,}$ 160 days, depths >7 m), release in the aerobic epilimnion ($I_{epi,}$ 160 days, depths ≤7 m), and release from the whole-lake sediment surface under aerobic condition during the mixed period (I_{mix}, 205 days; see thermal stratification). The internal loading was computed as the summation of the I_{hyp}, I_{epi}, and I_{mix}; R_{mean} (mg m^{-2} d^{-1}) is the mean of the $R24_i$. The I_{hyp}, I_{epi}, and I_{mix} are computed as:

$$R_{mean} \times n_{day} \times A(\mathrm{kg}), \tag{3}$$

with n_{day} representing 205 days for mixed or 160 days for stratified, and A the sediment surface area (for I_{hyp} A = 15 924 m^2, I_{epi} A = 9768 m^2, and I_{mix} A = 25 692 m^2). The total annual internal load (kg P) was calculated by summation of I_{hyp}, I_{epi}, and I_{mix}; dividing by 365 (d) × 25 692 (m^2) yielded the average internal P load per day. For 2008 post-treatment, we measured FRP release under anoxic conditions only, which reflects a worst-case scenario. These cores were kept in the incubator (7 °C, dark) for ~2 years. Treated cores were expected to have retained low FRP concentrations (i.e., maintained FRP trapped in the sediment). 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.

Bathymetry

Measurements were taken from a boat using a Llowrange CHIRP and Broadband Sonar combined with navigation (Global Positioning System). The contours of the lake were measured using the navigation system while walking at the water's edge. The measurements yielded 12 501 data points, which were computed into a grid with each point representing 4 m^2 (using a spline routine PROC G3GRID in SAS 9.1).

The surface area of the epilimnion and hypolimnion were computed by summation of all 4 m^2 squares between 0 and 7 m depth and summation of all 4 m^2 squares between 7 and 16 m depth, respectively. The total surface area (25 692 m^2) was obtained by summation of all 4 m^2 squares.

Thermal stratification

From 11 January 2003 to 28 December 2010 (number of sample days = 353), the water temperature was measured weekly at the single water monitoring point (Fig. 1d) at 1 m intervals from 0 m to 10 or 9 m depth, depending on the water level. After 2011, water temperature measurements were less frequent (i.e., at water quality sampling dates). Thermal stratification was defined as a difference of 1 °C between 2 consecutive depths. The thermocline occurred at 7 m depth.

Sediment traps

Sediment traps (Fig. 1e) were deployed during 2 periods of 4 weeks in 2007 and 1 period of 4 weeks in 2008. The traps were 40 cm long PVC pipes with a surface area of 5.1 cm^2. The entrances of all traps were 1 m above the bottom at 10 m depth. The collected material was centrifuged at 5000 rpm, decanted, and freeze dried. The freeze-dried material was analysed according to Van Griethuysen et al. (2004), as described for organic deposition. The amount of P sedimented (mg m^{-2} per 4 weeks) was computed as:

$$\frac{\frac{M_0}{M} \times V\,[\mathrm{P}]}{A} \tag{4}$$

where M_0 (mg) is the amount of material collected, M (mg) the amount digested, [P] the phosphorus concentration (mg L^{-1}) in the decanted volume (V; L), and A (514.5 mm^2) the total surface area of the traps.

Assessing responses in water column to Floc & Lock treatment

From 2003 onward, temperature profiles (starting Jan 2003, 0–10 m depth), Secchi depth (starting Mar 2003), and water level (starting Jan 2003) were measured weekly.

Water sampling (Fig. 1d) was conducted every 2 weeks from 0 to 10 m depth at 1 m intervals (9 m at low water levels) from 17 November 2005 to 20 April 2008. During and just after application (20–27 Apr 2008) daily samples were taken at depths 1, 3, 5, 7, and 10 m; during May–December 2008, sampling was conducted every 2 weeks; from 2009 onward, sampling was conducted from 0 to 10 m depth at 1 m intervals. During 2010 the sampling was conducted once every 3 weeks and in 2011 every month. From 2012 to 2018 the sample frequency was April–September 2012 ($n = 5$), March–December 2013 ($n = 8$), February–September 2014 ($n = 10$), March–September 2015 ($n = 8$), February–October 2016 ($n = 10$), February–October 2017 ($n = 10$), and January–September 2018 ($n = 9$).

On site, dissolved oxygen concentrations (mg L^{-1}) and saturation (%) were measured using a WTW-multi 350i meter (WTW GmbH & Co. KG, Weilheim, Germany). Water transparency was determined using a Secchi disk. Water samples of 2 L were brought to the laboratory, where 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 spectrophotometrically (NEN 6520) as described by Moed and Hallegraeff (1978) and/or with a PHYTO-PAM phytoplankton analyser (HeinzWalz GmbH, Effeltrich, Germany) calibrated against the Dutch Chl-*a* standard (NEN 2006). The detection limit is 5 µg L^{-1} for the Dutch standard method (NEN 2006) and 0.5 µg L^{-1} for PHYTO-PAM. A detailed description of the PHYTO-PAM capacity to distinguish between phytoplankton groups is given in Lürling et al. (2018). The percentage of cyanobacteria was computed as:

$$100 \times \frac{\mathrm{Cya}}{\mathrm{Tot\ Chl}\text{-}a}, \tag{5}$$

with Cya = concentration of cyanobacterial Chl-*a* and Tot Chl-*a* the total Chl-*a* as measured by PHYTO-PAM.

To identify phytoplankton species by microscope, monthly 1 L water samples (0–10 m depth) were taken and fixated with Lugol and left to sediment for 7 days, after which the supernatant was gently siphoned 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. Species were identified using a Nikon light microscope.

TP (µg L^{-1}) and total N (TN; mg L^{-1}) were measured in unfiltered water samples; filterable reactive P (FRP; µg L^{-1}), ammonium-N (AMM; mg L^{-1}), and nitrite + nitrate-N (NN; mg L^{-1}) were determined in filtered water samples (0.45 µm membrane filters, Whatman NC45, Whatman International Ltd., Maidstone, UK) using a Skalar SAN++ continuous flow analyser (Skalar Analytical B.V., Breda, the Netherlands) following Dutch standard protocols (NNI 1986, 1990, 1997).

Because auto-analyser (molybdate) P determinations are sensitive to colloidal bentonite (Koopmans et al. 2005), TP and FRP concentrations during and post-treatment were done by ICP-MS at the Chemical-Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University), which has a quality system based on the ISO-17025 standard. Samples were stored at −16 °C until analysis, which always took place within the same calendar year. Nutrient concentrations below level of detection were replaced by their respective values of detection levels, which were 10 $\mu g\ L^{-1}$ TP for SAN++ and ICP-MS; 4 $\mu g\ L^{-1}$ FRP for SAN++ and ICP-MS; 0.2 $mg\ L^{-1}$ TN; 0.02 $mg\ L^{-1}$ AMM; and 0.01 $mg\ L^{-1}$ NN. Note that the level of detection for TP and FRP on ICP-MS is 6 and 1 $\mu g\ L^{-1}$, respectively; to avoid skewing our data we applied the higher SAN++ values to correct for level of detection (LOD).

Submerged macrophytes

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

To indicate depths at which macrophytes are light limited, we used the euphotic zone (z_{eu}), which for phytoplankton 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), we estimated z_{eu} using the mean pre-treatment Secchi disk depth.

On 10 October 2008 and 23 April 2009, macrophytes were sampled along a 0–9 m depth transect (Fig. 1f, blue line). All macrophytes within a hoop (0.5 m diameter) were collected (scuba diving), rinsed with lake water, and dried at 50 °C. The P content was determined according to Van Griethuysen et al. (2004) as described for organic deposition. We computed the P contained in submerged macrophytes to $kg\ m^{-2}$. Assuming macrophyte coverage found along the transect in 2008 was representative for the whole lake, we extrapolated the amount of P m^{-2} to the total amount of P (kg) contained in submerged macrophytes in the lake based on the bathymetry of the lake.

Statistical testing

For TN, TP, Chl-*a*, percentage of cyanobacteria, turbidity, and Secchi disk depth, we tested the hypothesis that there were no post-treatment trends 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 calculated the mean oxygen concentrations measured from June to September at depths 7–10 m.

Because we did not control for differences in the experimental design of the individual incubations, we did not test for differences between them. In the 2008 experiment, we tested the hypothesis that there was no difference between the before- and the after-treatment sediment nutrient releases using a *t*-test. In the 2011 and 2013 experiments, we tested the hypothesis that there was no difference between aerobic and anoxic conditions on FRP and DIN release rates using a *t*-test. When data failed homogeneity in variance (Levene's test), rates were log transformed (FRP 2008 and 2011), after which they fulfilled normality and homogeneity in variance. All tests were conducted in SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

Results

Constructing the phosphorus balance

The mean groundwater FRP (measured as P) concentration was 50 (standard deviation 46) $\mu g\ L^{-1}$ ($n = 19$) and for DIN (measured as N) was 19.95 (3.24) $mg\ L^{-1}$ ($n = 12$), of which 2% was AMM. Based on the annual 37 817 m^3 inflow of ground water, the FRP inflow was ~0.2 $mg\ m^{-2}\ d^{-1}$ and DIN inflow ~82.2 $mg\ m^{-2}\ d^{-1}$. The mean FRP concentration in Lake Rauwbraken was 20 (45) $\mu g\ L^{-1}$ ($n = 439$) resulting in an FRP outflow of 0.07 $mg\ m^{-2}\ d^{-1}$ based on the fluctuating water level (outflow 42 649 m^3). Pre-treatment, the internal DIN release was 77.9 $mg\ m^{-2}\ d^{-1}$ and post-treatment was 50.0 $mg\ m^{-2}\ d^{-1}$ (2011) and 26.2 $mg\ m^{-2}\ d^{-1}$ (2013). Rain water contained on average 0.031 $mg\ L^{-1}$ P, which yielded 0.065 $mg\ m^{-2}\ d^{-1}$ on open water from precipitation.

The mean P content of the organic material was 0.59 (0.06) $g\ kg^{-1}$ ($n = 10$). After the 75% reduction of the greenbelts, the maintenance removed ~1244 kg (dry weight) organic material per year, ~0.72 $kg\ yr^{-1}$. Because no maintenance was done before 2000, the estimated historical dry deposition of organic material from the greenbelts was 4976 kg dry weight per year. Hence the historical P loading through leaf litter was ~2.88 $kg\ yr^{-1}$ (Supplemental Material Table S4). Based on an estimated P contribution of 0.094 $g\ d^{-1}$ per bather (Dokulil 2014; referring to Schulz 1981), 20 000 bather days per year yields a P load of 0.20 $mg\ m^{-2}\ d^{-1}$.

Sediment release of filterable reactive phosphorus and dissolved inorganic nitrogen

Experiment A and B in 2005 yielded an FRP release of 2.4 and 1.3 mg m^{-2} d^{-1} of P, respectively (Table 3). In the 2008 experiment, pre-treatment and post-treatment anoxic FRP release was 15.1 and 2.3 mg m^{-2} d^{-1}, respectively (Table 3). The *t*-test revealed a significant difference between the before and after treatment sediment P releases (Table 3).

In the 2011 experiment, the anoxic and aerobic mean FRP release was 3.0 and 1.2 mg m^{-2} d^{-1}, respectively (Table 3). The *t*-test (log transformed data) revealed a significant difference between the anoxic and aerobic conditions (Table 3). In the 2013 experiment, the mean anoxic and aerobic FRP release was 4.6 and 1.6 mg m^{-2} d^{-1}, respectively (Table 3). The *t*-test failed to reach significance; however, this test had a low power (Table 3).

From the 2005 (Exp. A and B) experiment, the pre-treatment DIN release was 31.18 and 72.92 mg m^{-2} d^{-1}, respectively, of N, of which 82 and 92% was AMM (Table 3). In the 2008 experiment (after 2-year incubation), the DIN release was much higher than in the 2005, 2011, and 2013 experiments (all fresh cores; Table 3). Post-treatment in 2011, the DIN release was 43.92 mg m^{-2} d^{-1} (86% AMM, aerobic) and 44.09 mg m^{-2} d^{-1} (94% AMM, anoxic). In 2013, the DIN releases were 27.33 mg m^{-2} d^{-1} (77% AMM, aerobic) and 24.12 mg m^{-2} d^{-1} (87% AMM, anoxic). In both years, the *t*-test indicated no difference between aerobic and anoxic conditions (Table 3); however, it had low power.

Sedimentation of phosphorus

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

Phosphorus balance

The pre-treatment (2008) TP load was 8.03 mg m^{-2} d^{-1}, which included 6.82 mg m^{-2} d^{-1} (85%) internal load (Table 4) and 1.21 mg m^{-2} d^{-1} (15%) external load (Table 5). With 0.07 mg m^{-2} d^{-1} as a loss to the ground water, 1.14 mg m^{-2} d^{-1} was retained in the lake. Post-treatment based on the release under anoxic conditions, the internal P load was 2.3 mg m^{-2} d^{-1} (Table 4). Based on the 2011 and 2013 data, the aerobic flux was 36% of the anoxic flux. Because the hypolimnion was not anoxic in 2008 post-treatment, a proxy of $2.3 \times 0.36 =$ 0.83 mg m^{-2} d^{-1} was obtained. Accordingly, the TP load was reduced to 2.03 mg m^{-2} d^{-1}. While the contributions from ground water, bathers, and rain remained unchanged, the external P loads from water birds and leaf litter increased in 2011 and 2013 (Table 5); the internal P loads also increased in 2011 and 2013 (Table 4). Despite the increase, the internal loads remained below pre-treatment values (Table 4).

Table 4. Internal phosphorus loads to Lake Rauwbraken before and after the Floc & Lock intervention in 2008; I_{hyp} = release from the sediment in the anoxic hypolimnion during stratification, I_{epi} = release from the shallow aerobic areas of the lake during stratification, and I_{mix} = release during the period of complete mixing of the lake. A = sediment surface area, release = sediment FRP release (mg m^{-2} d^{-1}), n_{day} = number of days, Load = internal load per period (kg yr^{-1}); total = total internal P load (kg yr^{-1}).

	Conditions	A	Release	n_{day}	Load	Release
Before	I_{hyp}	15 924	15.1	205	49.4	
2008	I_{epi}	9768	2.4	205	4.8	
	I_{mix}	25 692	2.4	160	9.9	
	total				64.0	6.82
After						
2008	I_{hyp}	15 924	2.3	205	7.5	
	I_{epi}	9768	2.3	205	4.6	
	I_{mix}	25 692	2.3	160	9.5	
	total				21.6	2.3
2011	I_{hyp}	15 924	3.0	205	9.8	
	I_{epi}	9768	1.2	205	2.4	
	I_{mix}	25 692	1.2	160	4.9	
	total				17.1	1.8
2013	I_{hyp}	15 924	4.6	205	15.0	
	I_{epi}	9768	1.6	205	3.2	
	I_{mix}	25 692	1.6	160	6.6	
	total				24.8	2.6

Water quality variables

TP before the intervention was high, with a mean pre-treatment TP of 134 µg L^{-1}, but it was strongly reduced by the intervention, yielding a post-treatment TP mean of 14 µg L^{-1} (Table 6). In the first post-treatment years (up to and including 2012), TP remained <10 µg L^{-1} (LOD) with some higher values at the surface and in the mid-water column (Fig. 2a). From 2013 onward, TP increased with a significant trend in the annual means (Table 6). TN was reduced from an average of 0.96 mg L^{-1} before to 0.50 mg L^{-1} after the intervention, but there was no trend in the annual mean TN (Table 6).

Table 5. External P loads from different sources to Lake Rauwbraken before and after the Floc & Lock intervention.

	External P load (mg m^{-2} d^{-1})		
Source	Before (2008)	After (2011)	After (2013)
Birds	0.7	0.8	0.8
Groundwater	0.2	0.2	0.2
Leaf litter	0.01	0.1	0.2
Bathers	0.2	0.2	0.2
Rainfall on open water	0.1	0.1	0.1
Total	1.21	1.4	1.5

Table 6. Yearly mean values and standard deviation (SD) of water quality variables (variables): chlorophyll *a* concentration, percentage of chlorophyll *a* as cyanobacteria (Cyanobacteria), Secchi disk depth, turbidity, total phosphorus (TP), total nitrogen (TN), filterable reactive phosphorus (FRP), ammonium (AMM), nitrite + nitrate (NN), hypolimnetic oxygen concentration (Hypo oxygen), hypolimnetic oxygen saturation (Hypo oxygen sat), and their trends (trends) in post-treatment yearly means (corr. = correlation coefficient, p = p-value, n.s. = not significant at $\alpha = 0.05$, n = number of samples), except for hypolimnetic oxygen concentration and hypolimnetic oxygen saturation, which are mean values from June to September at depths 7–10 m. The pre-treatment period is from 1 January 2006 to 20 April 2008; the post-treatment period is from 24 April 2008 to 31 December 2018, and for nutrients to 31 December 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 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	0.82	<0.01	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} N)	0.20	(0.37)	452	0.14	(0.14)	1139	−0.02	n.s.	11
NN (mg L^{-1} N)	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

The intervention also reduced Chl-*a* concentrations (Fig. 2c) and the percentage of cyanobacteria (Table 6). Before the intervention, the highest Chl-*a* concentrations occurred in the deep layer during summer due to *P. rubescens*. At the water surface, accumulations of *Dolichospermum flos-aquae*, *Microcystis aeruginosa*, and *Woronichinia naegeliana* were detected almost daily. During 2009–2012, picocyanobacteria such as *Cyanobium* sp. were observed near the bottom. In spring 2016, *P. rubescens* reoccurred in samples but declined

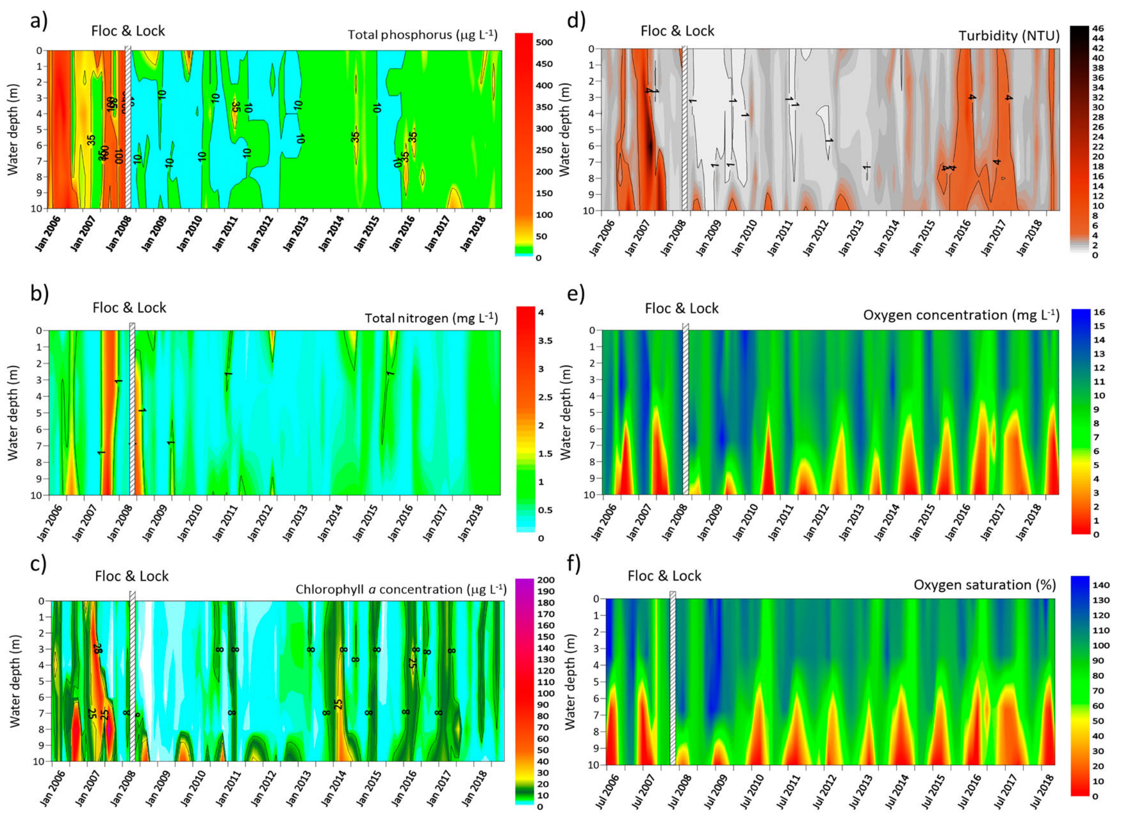

Figure 2. (a) Contour plots of total phosphorus concentration, (b) total nitrogen concentration, (c) chlorophyll *a* concentration, (d) turbidity, (e) dissolved oxygen concentration, and (f) dissolved oxygen saturation from 2006 to 2018 in Lake Rauwbraken. Vertical bar indicates the Floc & Lock treatment.

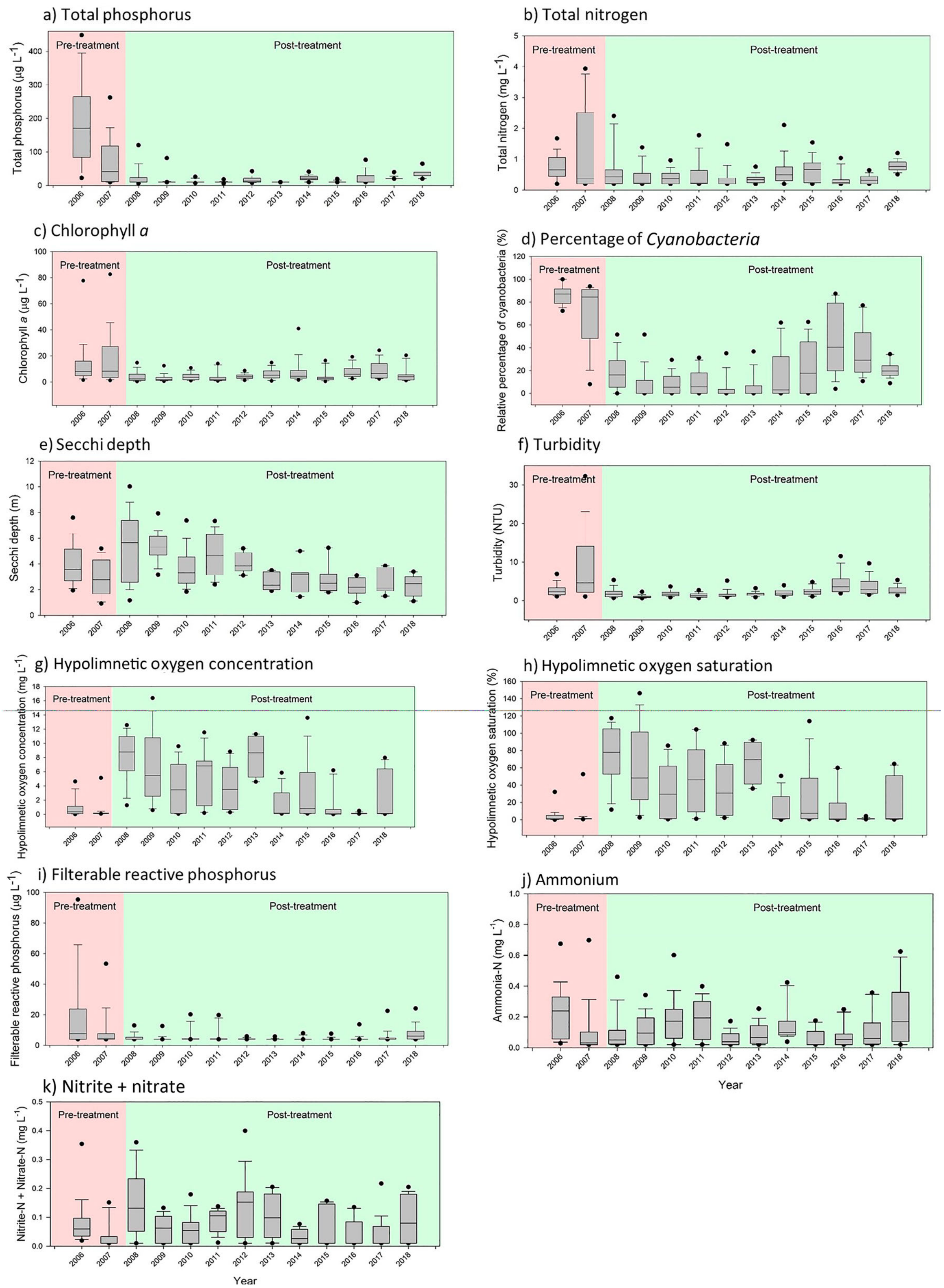

Figure 3. Box plots per year of (a) whole water column total phosphorus concentration, (b) total nitrogen concentration, (c) chlorophyll *a* concentration, (d) percentage of cyanobacterial Chl-*a*, (e) Secchi disk depth, (f) turbidity during the whole year, (g) hypolimnetic dissolved oxygen concentration (h) and hypolimnetic dissolved oxygen saturation during June to September at water depths 7–10 m, and (i) filterable reactive phosphorus concentration, (j) ammonium-N concentration, and (k) nitrite-N and nitrate-N concentration over the whole year and whole water column. The pink plane indicates the pre-treatment period, the green plane the post-treatment period. The line inside the box is the median, the limits of the box encompass the 25th and 75th percentiles, the whiskers are the 10th and 90th percentiles, the dots indicate the 5th and 95th percentiles.

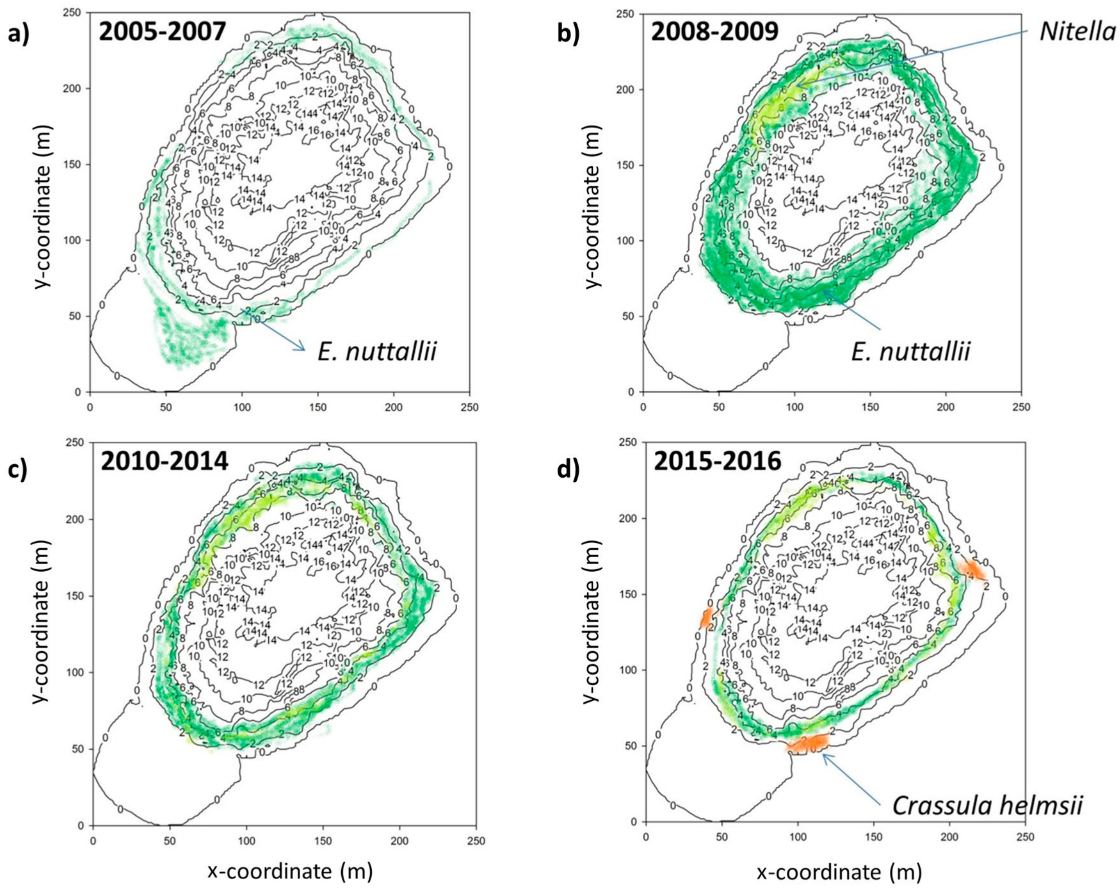

Figure 4. Macrophyte coverage in Lake Rauwbraken. Pre-treatment period (a) 2005–2007, (b) 2008–2009, (c) 2010–2014; and post-treatment period (d) 2015–2016; solid lines = depth, dark green = *Elodea nutallii*, light green = *Nitella* sp., orange = *Crassula helmsii*.

during 2017–2018. In 2014, a spring bloom of *Ceratium* and *Dinobryon* occurred that vanished before summer. No trend was found in Chl-*a* concentrations (Table 6). The percentage of cyanobacteria in the total Chl-*a* showed a steep increase from 2013 onward with a significant upward trend (Fig. 3b, Table 6).

Secchi disk depth improved by the intervention and increased to a maximum of 10.2 m in November 2008 and thereafter had a significant downward trend (Fig. 3c, Table 6). As with Chl-*a*, high pre-treatment turbidity (up to 51 NTU) occurred in the deep layer (Fig. 3d). The post-treatment turbidity followed a similar pattern as Chl-*a*, with a significant upward trend (Fig. 3a, Table 6).

Mean hypolimnetic dissolved oxygen concentration and saturation were higher after the intervention than before (Fig. 3g–h), with a significant downward trend after the intervention (Table 6). Post-treatment, the hypolimnion oxygen regime remained aerobic until 2013, and thereafter anoxic conditions were more common (Fig. 3g–h).

The mean post-treatment FRP was 70% lower than pre-treatment FRP, but no significant trend was found (Fig. 3i, Table 6). AMM and NN seemed less affected, and no significant trends in AMM and NN were found (Fig. 3j–k, Table 6).

Macrophytes

In 2005, the first patch of *Elodea* (1 m^2) occurred (Fig. 4a). In April 2008 prior to the treatment, a dense and water column-filling *Elodea* canopy occurred around the lake to 4 m depth, with some *Nitella* sp. and *Potamogeton* sp. During the clear water phase in 2008, *Elodea* grew down to 9 m depth (albeit at low densities at this depth; Fig. 4b). Post-treatment the *Elodea* density decreased and was not as deep, while *Nitella* sp. became more abundant (Fig. 4b–c). In 2015, the invasive species *Crassula helmsii* was observed and increased in coverage in 2016 (Fig. 4d). Over the whole post-treatment period, coverage by submerged macrophytes first increased but decreased during later years. The summer water level was 1.66 m below the winter level. Submerged macrophytes in this zone fall dry and die (Fig. 5). Based on the Reynolds (1984) z_{eu} approximation, the submerged macrophytes were light limited below 6 m. In 2008,

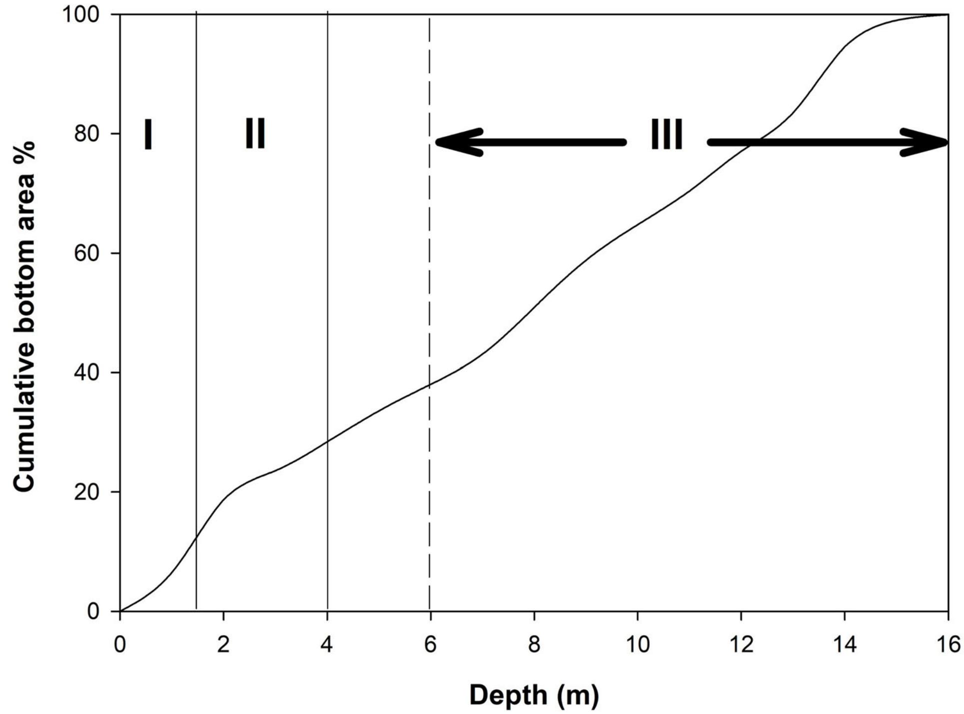

Figure 5. Physical factors limiting coverage of submerged macrophytes in Lake Rauwbraken. The solid line indicates the percentage of the lake's bottom surface area at the depth relative to the highest water level observed; area I is the dry-fall zone; area II is the area covered by submerged macrophytes in April 2008; the dashed line indicates z_{eu}, which is based on the pre-treatment mean Secchi disk depth (1.66 m); and area III (depths deeper than the dashed line) indicates the light-limited zone.

when coverage and densities were highest, ~4.2 kg P was present in submerged macrophytes.

Discussion

The eutrophication of Lake Rauwbraken

The pre-treatment TP load (8.03 mg m^{-2} d^{-1}) to Lake Rauwbraken included a relatively small external P load (1.21 mg m^{-2} d^{-1}) and a large internal P load (6.82 mg m^{-2} d^{-1}; 85% of the TP load); 48% of the internal load was released under aerobic conditions. This 2.4 mg m^{-2} d^{-1} internal load was twice as large as the external load, and therefore not one to be neglected, and in line with Tammeorg et al. (2017), who noted that a substantial part of the internal P loading may stem from aerobic release. From the external P load, the 0.7 mg m^{-2} d^{-1} by water birds was the largest. The reduction of the P load by maintenance (leaf litter from 0.35 to 0.01 mg m^{-2} d^{-1}) represented only a small improvement on the external P load, which explains why the lake did not respond to this action. In Lake Rauwbraken, the contribution of bathers (0.2 mg m^{-2} d^{-1}) was the same as the contribution by groundwater. From the external P loads, 1.14 mg m^{-2} d^{-1} remained in the lake, building up legacy P in the lake sediment and resulting in the internal FRP load. Lake Rauwbraken is an example of a lake where a relatively small external P load over time accumulated in a relatively large internal P load. As such it has similarities to lakes that did not show signs of recovery after the external P load had reduced due to a large internal P load (Søndergaard et al. 2001, Schindler and Hecky 2009, Rolighed et al. 2016). Given the nature of the external P load, P reduction is not feasible.

The external P loads to Lake Rauwbraken are currently increasing. After maintenance stopped (2011), the greenbelts grew back to their original coverage, resulting in higher P loads from organic deposition. Geese became resident (since 2013), adding 0.12 mg m^{-2} d^{-1} to the external load. An urbanization project near the lake now feeds P-polluted rainwater into the ground some 100 m distance from the lake (since 2014), resulting in a 10% higher ground water inflow to the lake. Rainwater has low P; however, discharges of rainwater from a hard surface in urbanized areas can be an important P source (Waajen et al. 2016b, 2019). Consequently, internal P storage is building up.

The Floc & Lock intervention

The Floc & Lock intervention effectively removed an *Aphanizomenon* bloom (Lürling and van Oosterhout

2013), and the lake remained void of filamentous cyanobacteria until spring 2016. During 10 years of post-treatment monitoring, water quality remained improved as evidenced by the reductions in TP, FRP, Chl-*a*, and the percentage of cyanobacteria (Table 6). This improvement was achieved because LMB strongly reduced water column P and anoxic sediment FRP release by 85% (before–after comparison of 2008 cores). These results emphasize the reported strong efficiency of LMB in reducing the FRP concentrations in the water column and the P flux from sediments (Copetti et al. 2016).

Sediment nutrient release and internal loading

The anoxic FRP release (2008 pre-treatment) was much larger than the aerobic release (2005 experiment), consistent with the literature (Boström et al. 1982). Nonetheless, aerobic pre-treatment P release rates (1.3–2.4 mg m^{-2} d^{-1}) support Tammeorg et al. (2017), who noted that a considerable part of the internal P load can be released under aerobic conditions. In our experiments, however, sediment sampled from the deeper parts (>10 m depth) was incubated under aerobic and anoxic conditions, whereas in situ, not only will the redox state differ at different depths of the lake, but also the amount of releasable P. We determined the sediment P release on sediment sampled deeper than 10 m depth, which may result in an overestimation of the internal loading for shallower depths (Kowalczewska-Madura et al. 2019). Nonetheless, the internal P load evidently exceeded the external P load, emphasising the necessity of reducing the internal P load.

FRP release increased during 2011–2013, a result also found in Lake de Kuil (the Netherlands) where a Floc & Lock intervention decreased sediment P release from 5.2 to 0.4 mg m^{-2} d^{-1} but increased in later years (Waajen et al. 2016a). Considering the ongoing post-treatment diffuse external P loading, we hypothesise that Lake Rauwbraken is building up a new mobile P pool. The increased FRP release is therefore probably not caused by underdosing of LMB (Van Oosterhout et al. 2020). The difficulty in treating a lake to counteract sediment P release is estimating the relevant sediment depth, which is the depth below the sediment surface from which P may diffuse to the overlying water column. In Lake Rauwbraken this depth was estimated as 5 cm (Lürling and van Oosterhout 2013), a good proxy because in June 2014 lanthanum profiles the majority of the lanthanum was in the top 5 cm (Dithmer et al. 2016, van Oosterhout et al. 2020).

Our sediment release experiments indicate that the treatment did not affect DIN release. Under anoxic conditions in our experiments, release of DIN was mostly AMM, consistent with Austin and Lee (1973). Although our "aerobic conditions" contained enough oxygen in the over-standing water (>0.1 mg L^{-1}) for ammonium nitrification (Carlucci and McNally 1960, Goreau et al. 1980), we cannot rule out anoxic conditions in the sediment. Moreover, denitrification and anoxic ammonium oxidation (anammox) may have led to some loss of N as N_2 (Francis et al. 2007). Because our experimental units are not closed systems for DIN, the estimates of released DIN are best interpreted as minimum amounts.

The much higher DIN release in cores incubated for 2 years compared to fresh cores taken in 2005, 2011, and 2013 reflects gradual organic matter decomposition. This process occurred in both treated and non-treated cores, whereas FRP remained low in the treated cores and thus demonstrated the efficacy of LMB in immobilizing FRP in the sediment even under prolonged periods of gradual organic matter decomposition. It also strongly suggests that the gradual increase in sediment FRP release observed in the 2011 and 2013 cores was a result of internal storage buildup due to ongoing external P load.

Water quality and trends

Water clarity

Secchi disk depth improved after the Floc & Lock treatment and remained improved for 5 years. Later observations show a significant downward trend, indicating a return to its pre-treatment value. In bathing water, a minimum of 1 m Secchi disk depth is a safety requirement by Dutch Water Authorities (not demanded in BFD). From 2000 to 2008, Secchi disk depth fell below 1 m in 5 years. In 2008, after the treatment, Secchi disk depth soon fell below 1 m due to the application of the LMB. Secchi disk depths <1 m were not observed during 2009–2018. Turbidity closely followed Chl-*a* concentrations, indicating the underwater light climate is mostly governed by the phytoplankton density.

Chlorophyll a and nutrients

We observed a significant upward trend in the annual means of TP; no trends were found in Chl-*a* or TN post-treatment (2008–2018). Chl-*a* concentrations remain low, but an increase in the percentage of cyanobacteria indicates the lake is gradually moving back to more eutrophic conditions. The reoccurrence of *P. rubescens* in 2016 can be viewed as an early warning signal of a return to eutrophic conditions.

Oxygen conditions

The lake's hypolimnion was anoxic during summer 2006 and 2007 but was aerobic in 2008. After the treatment in 2008, phytoplankton primary production was low; hence, sedimentation of organic material can be expected to be reduced accordingly, indicating that the fuel for the bacterial depletion of oxygen was removed by P limitation on the whole water column. While the "Floc" part of the treatment resulted in a massive sedimentation of organic material, it did not add to the oxygen depletion in the hypolimnion. We 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* until 9 m deep and the picocyanobacteria near the bottom. The presence of picocyanobacteria forming a deep chlorophyll maximum (DCM) is in line with the prediction of Padisák et al. (2003) that picocyanobacteria form a DCM in lakes where P is limiting and light is still sufficient. As seen from the sediment P release experiment, P release was not zero, and hence some growth is expected. The LMB dose aimed to immobilize all potential releasable P in the top 5 cm of the sediment (Lürling and van Oosterhout 2013), yet a remaining, but far lower, P efflux implies the sediment P release was not completely blocked. 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. Toward the end of the post-treatment period, anoxic periods in the hypolimnion seemed to intensify, which we attributed to reduced hypolimnetic oxygen production from photosynthesis as a result of lower water column transparency in combination with increased oxygen consumption through decomposition of newly deposited organic matter.

Submerged macrophytes

In Lake Rauwbraken, the submerged macrophytes were eradicated through introduction of grass carp, and their return after reduction of grass carp did not prevent cyanobacterial blooms in 2007 and 2008. Bottom-feeding fish like carp (*Cyprinus carpio*) or bream (*Abramis brama*) may affect water clarity by resuspending sediments, but their densities in Lake Rauwbraken are low (about 30 kg ha^{-1}). Submerged macrophytes play a key role in the ecology of shallow lakes (Scheffer 2001). In Lake Rauwbraken they played a minor role in the pre-treatment period because their coverage was limited by a lowered water level during the growing season, and submerged macrophytes that grew in shallow parts of the littoral zone in the early season died from desiccation when this zone became a terrestrial habitat during summer. Meanwhile, light limitation hampered macrophyte growth at greater depth during the early season, mostly caused by *P. rubescens* that stratifies in the meta-hypolimnion (Van Hal and Lürling 2004; also see year 2007 in Fig. 2c). Because of the steep slopes in Lake Rauwbraken, water depth quickly reaches z_{eu}. If macrophytes fill the water column down to 4 m depth, <8% of the lake volume may benefit from their positive effects. This zone may contain ~4.2 kg of P present in submerged macrophytes, which is low compared to the 32 kg in the water column, and explains why P storage in macrophytes could not prevent cyanobacterial blooms.

The pre-treatment vegetation in 2008 reflected a eutrophic system by its species (*Elodea*) and density (massive canopy with an abrupt end at 4 m depth). In 2008 (post-treatment), clear waters allowed *Elodea* to grow to 9 m depth. During 2010–2014 the species changed from *Elodea* to *Nitella*, reflecting a eutrophic to oligotrophic change (Lambert-Servien et al. 2006). Whereas Floc & Lock shifted water quality from eutrophic to oligo-mesotrophic (based on Chl-*a* and nutrients) in 3 days, this shift was followed with some years delay by the submerged vegetation. The gradual decline in vegetation matches the steady reverse to eutrophic conditions. The appearance of the invasive macrophyte *C. helmsii* was not unexpected. It first appeared in the province of Noord-Brabant in 1995 (Brouwer and den Hartog 1996) and has been spreading since.

A general view on the need for preventative management

To mitigate eutrophication, the first step is to draw up a nutrient balance for the system, which includes external (both point and dispersed sources) and internal loads. The diffuse external loads to Lake Rauwbraken are difficult to control. External sources usually fall outside the area of operation where the water manager of the lake has full authority and may involve many stakeholders, their numbers depending on the size of the catchment area, meaning that the time until the effort can be achieved is longer because negotiations take longer. Not all stakeholders may cooperate, meaning that the catchment area approach cannot be realized to its full extent. For instance, stopping P applications in agricultural fields will only be achieved if it does not impair farm profitability (Dodd et al. 2012). However, the legacy of nutrients emitted during the past century means that even if emission were stopped, the eutrophication of our surface waters will go on for decades to centuries (Jarvie et al. 2013, Goyette et al. 2018). Hence, regular maintenance of lakes is necessary to keep water quality acceptable because no waterbody is fully isolated from its environment. Such a strategy was implemented successfully in Bärensee (Germany), in which the trophic level was reduced by an initial dose of LMB and the subsequent smaller reapplications (Epe et al. 2017).

The costs for internal loading control in Lake Rauwbraken was €50 000, equating to an annual treatment cost of about €5000 per year for the decade during which no closures were reported. The buildup to the internal loading causing the water quality problems took ~30 years. The 2008 investment could be seen as the price paid for >30 years of good water quality. Computing the annual costs of a treatment in such a reverse way may seem counterintuitive, but a business plan for a recreational facility, for example, must include maintenance like lawn mowing and pruning the hedges, an integral cost beyond the €50 000. In contrast to other costs associated with running a recreational waterbody, this cost is relatively low. For example, the maintenance of the riparian areas cost >€10 000 per year in staff time, whereas the 4-month closure due to the swimming ban in 2007 incurred €150 000 loss of revenue. However, even though our results indicate that the lake remained in a good bathing water conditions for at least 10 years post treatment, they also indicate that the lake is returning to its eutrophic state, and thus future measures will be necessary. Inasmuch as the diffuse external load cannot be controlled, and based on the monitoring data following the 2008 intervention, a maintenance dosage of LMB to bind the P that gradually accumulates over time is recommended.

Conclusions

Over a 40-year history, small dispersed P sources built up a legacy sediment P pool that delivered a high internal P load to Lake Rauwbraken, resulting in a eutrophic status and blooms of cyanobacteria. A combined in-lake P precipitation and fixation resulted in 10 years of good water quality according to the European Bathing Waters Directive. The treatment significantly reduced the internal P load. Post-treatment, no significant trends in the percentage of Chl-*a*, turbidity, TP, TN, AMM, or NN were found. However, FRP showed a significant upward trend, and Secchi depth and oxygen concentration showed significant downward trends. Because the lake is returning to its eutrophic state due to ongoing diffuse P inflows, a second intervention in Lake Rauwbraken is needed.

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