Decreased nitrogen loading controls summer cyanobacterial blooms without promoting nitrogen-fixing taxa: Long-term response of a shallow lake

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Abstract

The effectiveness of controlling nitrogen (N) to manage lake eutrophication is debated. Long-term, whole-lake case studies are required to determine whether diazotrophic cyanobacteria can fix sufficient N to offset a reduction of N-inputs. We document the recovery of shallow, productive Lake Müggelsee (Germany) over 37 yr (sampling interval 1–2 weeks) during a decrease of N and phosphorus (P) loading of 79% and 69%, respectively. Nitrogen concentrations in the lake responded immediately to loading reduction whereas P concentrations remained elevated for about 20 yr. Total nitrogen (TN) in the lake was always lower than TN in the inflow. Accordingly, estimated denitrification and N-burial rates substantially exceeded N_2 fixation rates in the long term. Phosphorus was growth limiting in spring whereas N was clearly limiting in summer due to high sediment P-release. TN : TP ratios, normalized to phytoplankton biovolume by regression, were 25.5 (weight) in spring and 3.3 in summer. During the study period, dissolved inorganic N (DIN) concentrations in summer decreased and the duration of low DIN concentrations increased by ca. 100 d. The biovolume of cyanobacteria and total phytoplankton decreased by 89% and 76%, respectively. The proportion of N_2-fixing cyanobacteria during summer decreased from 36% to 14% of the total phytoplankton biovolume. The total concentration of heterocysts and estimated total N_2 fixation did not change over time. In the long term, decreasing N-inputs effectively controlled summer cyanobacteria including N_2-fixing taxa, which did not compensate for the N-deficit. A P-only control strategy would not have been as successful.

The role of nitrogen (N) limitation of autotrophs in lakes has been debated for decades. Eutrophication management has focused on controlling phosphorus (P) inputs since the 1970s when its importance was indisputably demonstrated (Schindler 1977). Currently there is a lack of scientific consensus about whether eutrophication is best managed by controlling P alone, or by controlling both N and P (Lewis and Wurtsbaugh 2008; Schindler et al. 2008; Conley et al. 2009; Schindler et al. 2016; Cotner 2017).

Arguments in favor of controlling only P-inputs are based on the premise that P is the ultimate (long-term) limiting nutrient, whereas N-limitation is only temporary and a symptom of P-overfertilization (Wang and Wang 2009; Schindler 2012). N-limitation may favor diazotrophic cyanobacteria, which can satisfy their N-requirements by fixing atmospheric N. This input of atmospheric N is proposed to accumulate in the ecosystem over time and eventually replace any decrease in external N-inputs until P again becomes the primary limiting nutrient (Schindler et al. 2008). According to this perspective, not only is controlling N-inputs expensive, but also futile with the additional side effect of shifting the summer phytoplankton community toward toxic N_2-fixing cyanobacteria (Welch 2009).

Conversely, dual N and P control strategies are based on the argument that N and P co-limit growth (Harpole et al. 2011; Bracken et al. 2015), and N and P enrichment produces strong synergistic effects on eutrophication (Elser et al. 2007; Paerl et al. 2016). Anthropogenic activities have increased N-loading to freshwaters relative to P (Peñuelas et al. 2013). Excess N promotes summer blooms of cyanobacteria (Glibert et al. 2014) and can increase the toxicity of non-diazotrophic forms (Gobler et al. 2016). Accordingly, focusing

only on P may not only be less effective at mitigating eutrophication and reducing harmful cyanobacterial blooms than dual N and P control, but also cause excess N to be exported to downstream N-sensitive ecosystems, like estuaries and coastal areas (Howarth and Marino 2006; Paerl 2009).

Therefore, the effectiveness of managing N-inputs to mitigate eutrophication in lakes hinges on whether diazotrophic cyanobacteria can replace the resulting N-deficit in the long term through N_2 fixation. Some long-term studies demonstrate that N_2 fixation can compensate loading reduction in the long term (Schindler et al. 2008). However, somewhat counter-intuitively, strong N-limitation often does not promote—and may even decrease—diazotrophic cyanobacteria relative to other taxa (Dolman et al. 2012; Gobler et al. 2016). Moreover, evidence suggests that N_2 fixation in lakes only satisfies a small proportion of the annual N-requirements (Ferber et al. 2004; Dolman et al. 2012; Gobler et al. 2016), predominantly due to physical control by temperature, irradiance, and hydrology (Paerl and Otten 2016). Retention time also determines whether fixed N can accumulate in the long term (Schindler 1981; Howarth and Marino 2006; Scott and McCarthy 2010; Scott and Grantz 2013; Grantz et al. 2014).

Although there is much evidence in support of controlling N in freshwater, the rationale behind this has been criticized as being "based solely on short-term, small scale studies, none of them at long-term ecosystem scales" (Schindler et al. 2017). Accordingly, Schindler (2012) stated that "long-term, whole-ecosystem experiments and case histories of lake recovery provide the only reliable evidence for policies to reduce eutrophication." Here, we present a 37 yr ecosystem-scale case history of the recovery of Lake Müggelsee (Germany) after a decrease of N-and P-inputs by 79% and 69%, respectively. Lake Müggelsee is one of Germany's best studied lakes, offering a rare opportunity to observe in detail the long-term effects of nutrient reduction on the phytoplankton community structure and whole-ecosystem response. The aim of our study was to investigate (1) how in-lake N and P concentrations responded to load reduction, (2) which nutrients are growth limiting for phytoplankton, (3) if N-loading reduction favored diazotrophic cyanobacteria, (4) whether N_2 fixation compensated the decrease in external N-load reduction in the long term, and (5) whether dual N and P control can effectively mitigate eutrophication and summer cyanobacterial blooms. Our hypothesis was that decreased N-loading would immediately reduce in-lake N : P ratios and eventually increase N-limitation due to ongoing P-release from sediments of shallow lakes during summer. Moreover, we hypothesized that N_2-fixing cyanobacteria would respond to lower dissolved inorganic N (DIN) concentrations by increasing N_2 fixation per biovolume, but that the absolute N_2 fixation would not exceed denitrification and thus not alleviate summer N-limitation in the long term in this shallow lake.

Methods

Study site and sampling

Lake Müggelsee (52.44°N, 13.65°E) is a shallow (mean depth 4.9 m, maximum depth 8 m), polymictic lake in Berlin, Germany with a surface area of 7.3 km². The lake is fed by the River Spree from a 7000 km² catchment, which is 36% forest and 42% agricultural land and has a population of 720,000. The theoretical retention time of the lake is 6–12 weeks. From 1979 to 1986, water samples were taken from the deepest point of the lake at weekly intervals from spring to autumn and every 2 weeks in winter. Water was sampled with a 5-liter Friedinger sampler at 0.5 m, 4 m, and 7 m depth and volumetrically integrated. From 1987 to 2016, volumetrically weighted integrated samples were taken from 21 subsamples from five different points on the lake. The change in sampling routines had little influence on the concentrations of phytoplankton or nutrients (Driescher et al. 1993). On the same days as the lake sampling, inflow samples were taken in the River Spree just upstream of its mouth into the lake.

Phytoplankton

Phytoplankton samples were fixed with Lugol's solution and abundances of individual species or algal groups were counted with an inverted microscope. The relevant dimensions of at least 50 cells per taxon were measured, and the biovolumes were calculated assuming simple geometrical bodies. The length and number of heterocysts of each filament and the area of each cyanobacterial colony were determined.

Nutrient concentrations, loads, and balance

Dissolved and particulate nutrients were separated by filtration (0.45 μm). Soluble reactive P (SRP) was measured photometrically according to Murphy and Riley (1962). Total P (TP) and dissolved P (DP) were determined as SRP after acid digestion at 160°C for 6 h. Standard methods (DEV 2012) were used to measure the concentrations of ammonium (DIN 38406-5:1983) and nitrate + nitrite by photometry (DIN 38405-9:2011) or ion chromatography (DIN EN ISO 10304-1:2009). Total N (TN) and dissolved N (DN) were measured after catalytic oxidation as chemoluminescence in N analyzers. DIN was calculated as the sum of nitrate (NO_3-N) and ammonium (NH_4-N). Particulate N and P (PN and PP) were calculated as the difference between the total and dissolved concentrations of the respective nutrient. All nutrient ratios given in the text refer to ratios by weight.

Daily discharge data for the inflows (Q) were calculated by the local authorities using permanent records of a gauge 1.2 km upstream of the lake and hydraulic models. The nutrient load was calculated as the product of the daily discharge rates and the inflow concentration (measured weekly, interpolated to daily intervals), aggregated into monthly means. The withdrawal of lake water by direct abstraction (until 1991) and by bank filtration was measured as pumping rates by the

Berlin Water Works supplied as monthly totals. The bank filtration rates were corrected for the mean share of real groundwater based on tracer measurements and models using the monitored groundwater table height. To estimate the nutrient balance, water withdrawal via bank filtration (Q_{filtr}) was assumed to remove only particulate nutrients (c_{part}). Nutrient retention was then calculated as the difference between the nutrient load and nutrient export, which comprises of fluxes via the outflowing river ($[Q - Q_{filtr} - Q_{pipe}] \times c_{total}$), bank filtration ($Q_{filtr} \times c_{part}$), and direct water withdrawal ($Q_{pipe} \times c_{total}$), where Q_{pipe} indicates the volume of surface water taken by the water works (only until 1991) and c_{total} the concentration of total nutrients. We estimated N_2 fixation using published data on fixation rates (either the median of the published rates or calculated by models using median parameter values) per heterocyst (Hadas et al. 2012; Kolzau et al. 2018), or per heterocyst and mean underwater light (Ferber et al. 2004). We then used the median of these three different estimates of fixation rates for the N-balance. Denitrification plus N sedimentation was calculated as N retention plus estimated N_2 fixation.

Physical characteristics

During sampling, vertical profiles of temperature and oxygen were measured every 0.5–1.0 m at the deepest point of the lake. Surface temperature was estimated as the mean temperature in the upper 1 m layer, and bottom temperature as the mean temperature in the 1 m layer centered at the mean lake depth. The mean daily underwater light intensity I_m (photosynthetically available radiation, PAR) in the mixed layer of depth h was calculated according to the Lambert–Beer law as $I_m = I_0(\varepsilon h)^{-1}(1 - e^{-\varepsilon h})$, where ε is the light attenuation coefficient and I_0 is the PAR just below the water surface. I_0 was estimated using global radiation from ERA-Interim climate reanalysis (Dee et al. 2011) and converted to PAR assuming 4.56 μmol photons J^{-1}, a PAR proportion of 50%, and 10% reflection and backscattering at the water surface. The mixed layer depth h was assumed to be 4.9 m, the mean lake depth, when the lake was isothermal. The lake was considered stratified when the difference between surface and bottom temperature exceeded 1°C, in which case h was estimated as the depth of maximum temperature gradient. The attenuation coefficient was measured using two spherical PAR sensors (LI-193SA, LI-COR) at 0.75 and 1.25 m depth at the end of a jetty located on the northern lake shore from 1993 to 2003, and from 2004 on, from a monitoring platform located 300 m from the northern shore. Prior to that and at times when the PAR sensors were unavailable, ε was estimated from the Secchi depth using a relationship based on parallel measurements according to Shatwell et al. (2016) and Nicklisch et al. (2008). Oxygen was measured prior to April 1992 by Winkler titration. From May 1992 to May 2011 vertical oxygen profiles were recorded with H_2O multiprobes (Hydrolab Corporation) and afterwards with optical sensors in multiparameter sondes (Yellow Springs Instruments).
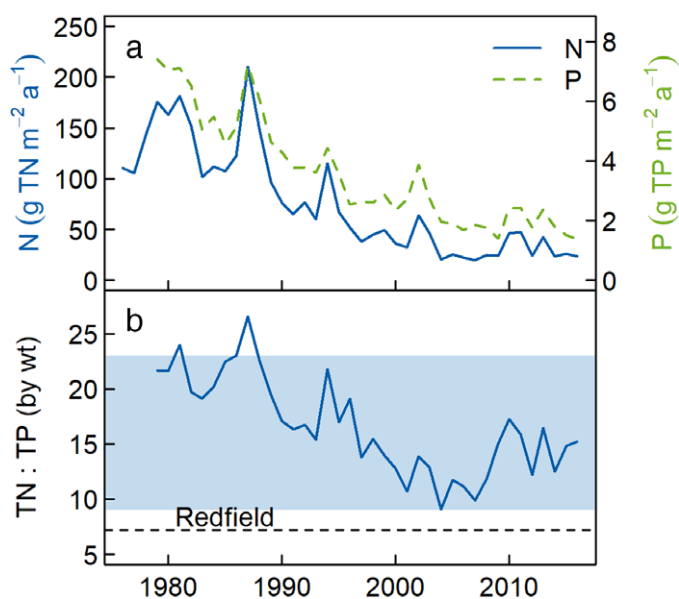

Fig. 1. Annual loads of total nitrogen and total phosphorus (**a**) and their ratio by weight in inflows (**b**). The dashed line in (**b**) indicates the Redfield ratio and the shaded area shows the range of N + P co-limitation ($9 \leq$ TN:TP ≤ 23) suggested by Guildford and Hecky (2000). Strict P- or N-limitation occurs outside this region.

Statistics

Temporal means were calculated by first linearly interpolating the data over time at a daily interval, and then averaging the data over the days in the period of interest. For seasonal means, winter was defined as January to March, spring as April to June, summer as July to September, and autumn as October to December. Beta regressions with a logit link function were used to analyze relationships where the dependent variables were proportions, including the biovolume of N_2-fixing phytoplankton relative to total cyanobacteria biovolume and relative to total phytoplankton biovolume. Beta regressions are useful when the dependent variable is bounded between 0 and 1 and the error distribution cannot be assumed to be binomial, as is the case with relative biovolume. The relationships between phytoplankton biovolume and nutrient concentrations were analyzed using generalized linear models. We used a Gamma error distribution and the identity link function, because biovolume variance increased roughly proportionally to the mean. Standard linear regressions were used in other cases. Nutrient ratios were log-transformed. Analyses were performed with R version 3.4.0 (R Core Team 2017) using the betareg package (Cribari-Neto and Zeileis 2010). Unless otherwise stated, means ± standard deviation are given in the text.

Results

External loading

The external loading of N and P to Lake Müggelsee decreased significantly between the late 1970s and 2016
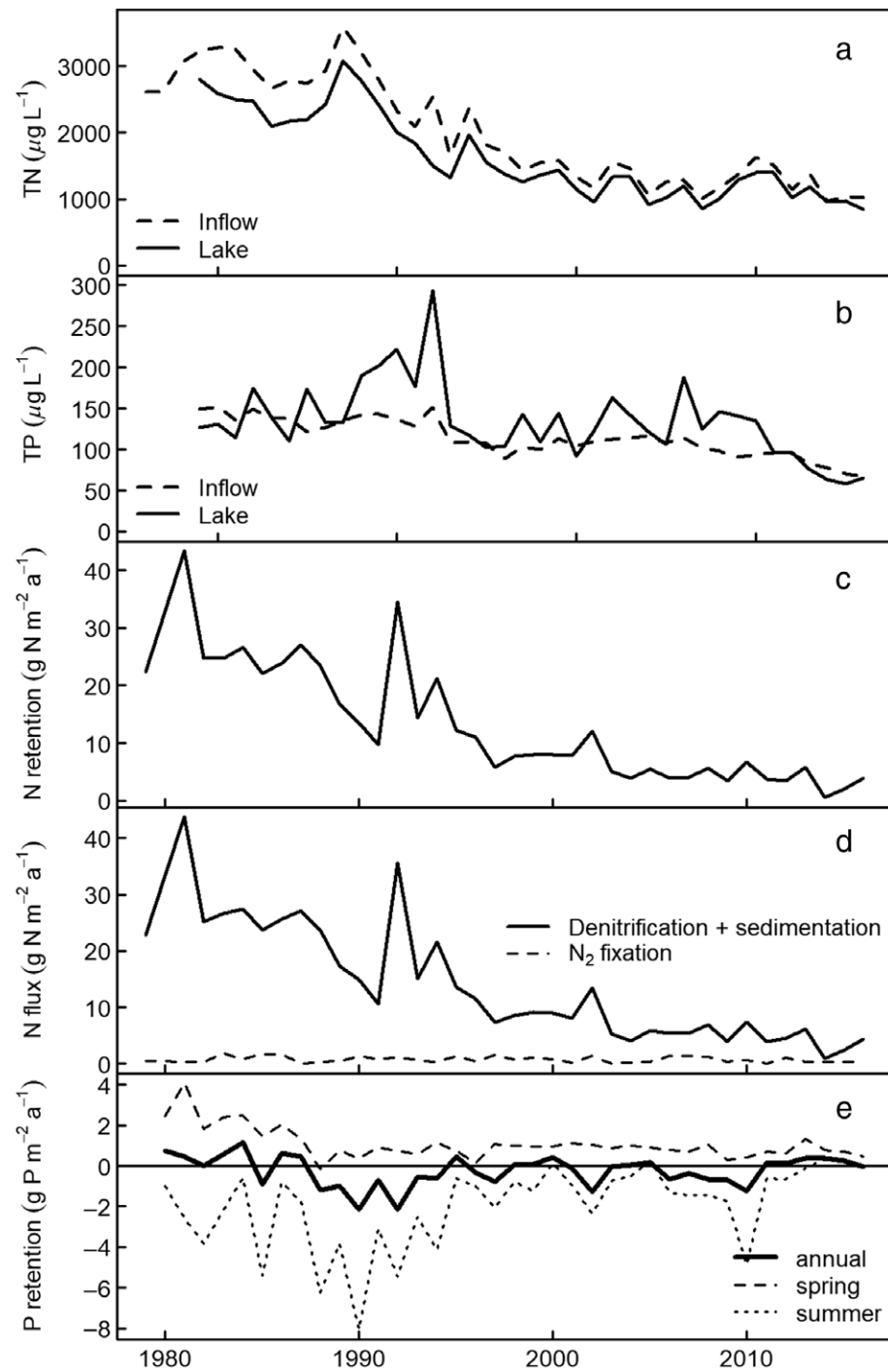
Fig. 2. Annual mean concentrations of total nitrogen (TN; **a**) and total phosphorus (TP; **b**) in the inflow (dashed lines) and in the lake (solid lines), as well as retention of nitrogen (N; **c**) and phosphorus (P; **e**) from the nutrient balance, and the estimated contribution of denitrification plus sedimentation and N$_2$ fixation (**d**).

($p < 0.001$, Fig. 1a). Total N (TN) loads decreased from 140 ± 38 g TN m^{-2} a^{-1} in the 1980s to 30 ± 11 g TN m^{-2} a^{-1} in the decade 2007–2016. Total P (TP) loads decreased from 6 ± 1 to 2 ± 0.4 g TP m^{-2} a^{-1} in the same period. The reasons for the decrease in nutrient loading were a 47% decrease in annual mean discharge from the inflowing River Spree from

9.9 ± 1.6 in the 1980s to 5.2 ± 0.9 m^3 s^{-1} (2007–2016), combined with a decrease in nutrient concentrations in the inflows from 3016 ± 301 to 1224 ± 231 μg TN L^{-1} and 138 ± 9 to 87 ± 12 μg TP L^{-1}. As TN concentrations decreased more rapidly than TP concentrations, TN : TP in the lake inflow decreased accordingly from about 21.9 in the 1980s to

14.1 (by weight) in the decade 2007–2016, but the annual mean ratios did not fall below the Redfield ratio and remained within the range of N + P co-limitation suggested by Guild-ford and Hecky (2000) (Fig. 1b). There were large seasonal variations in the inflow nutrient ratios. In the 1980s, mean inflow TN : TP was 33 in winter compared to 14 in summer. In the decade 2007–2016, TN : TP was 24 in winter compared to 8.3 in summer.

Lake nutrient concentrations and ratios

The TN concentrations in the lake declined immediately and in parallel with the decline in concentrations in the inflow (Fig. 2a). Over the study period, the average TN concentrations in the lake were 15% lower than concentrations in the inflow, with this margin narrowing by about 3% per decade ($p < 0.001$). Thus, lake TN concentrations seemed to approach equilibrium with the inflow concentrations, as particularly evident in recent years. This was reflected in the N-balance of the lake, which always showed net retention of N, but a decreasing amount of retained N over time ($p < 0.001$), which seems to be approaching 0 (Fig. 2c). Accordingly, the estimated retained N was 25.6 ± 7.5 g N m^{-2} a^{-1} in the 1980s, and only 5.6 ± 2.9 g N m^{-2} a^{-1} in the decade 2007–2016. Estimated N$_2$ fixation was on average 0.79 ± 0.52 g N m^{-2} a^{-1} and did not change significantly over time (Fig. 2d). Estimated denitrification plus sedimentation rates were substantially higher than N$_2$ fixation rates and decreased with decreasing N-loading in parallel with N retention (Fig. 2d).

In contrast, following the decrease in external P-loading, in-lake P concentrations declined with a substantial delay (Fig. 2b). In-lake TP concentrations were higher than inflow concentrations in most years, particularly between 1988 and 1992, and only fell steadily below inflow concentrations from 2011 on. The P-balance indicated that the lake released P (i.e., negative retention) on average at annual scales from the mid-1980s until 2010, with variation between years (Fig. 2e). In the 1980s, the P-balance showed strong P-retention in spring and very strong P-release during summer. Spring P-retention then decreased to approximately stable levels by around 1990 and summer P-release roughly stabilized about 5 yr later. During the period 1996 to 2010, spring P-retention was 2.23 mg P m^{-2} d^{-1} whereas summer P-release was 3.65 mg P m^{-2} d^{-1} with an annual average release of 0.36 g P m^{-2} a^{-1}. It appears that the lake required two decades to fully recover from P-loading and only became a net P sink again around 2011.

The lake-internal processes caused a distinct seasonal pattern in the nutrient concentrations (Fig. 3). SRP concentrations were lowest in winter and spring and highest in summer reflecting internal loading from the sediment (Fig. 3a, *see also* Supporting Information Fig. S1). DIN concentrations were highest in winter and lowest in summer (Fig. 3b). There was a decreasing trend in TP and SRP during winter and in TP also during spring
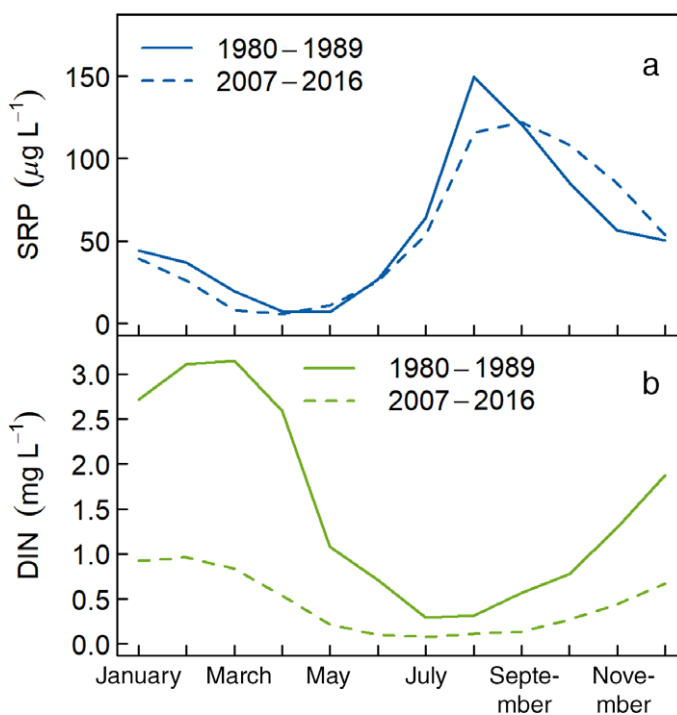

Fig. 3. Monthly means of soluble reactive phosphorus (SRP; **a**) and dissolved inorganic nitrogen (DIN; **b**) for the decades 1980–1989 (solid lines) and 2007–2016 (dashed lines).

and summer, but there was no significant change in the autumn concentrations (Table 1; Fig. 3a). Nitrogen (TN and DIN) concentrations decreased significantly in all seasons (Table 1; Fig. 3b).

Mean seasonal N : P ratios (total and dissolved nutrients) were highest in winter and lowest in summer when they fell below the Redfield ratio (Table 1; Fig. 4). Both TN : TP and DIN : SRP ratios decreased significantly over time. The particulate ratio PN : PP was much less variable than the dissolved nutrient ratio and there was a decreasing trend in spring and summer. Until the early 1990s, PN : PP was generally above Redfield, but afterwards was generally below this value (Fig. 4c). In 2015 and 2016, PN : PP rose above Redfield in winter and spring. Mean seasonal carbon to N ratios (C : N) were between 5.6 and 6.2 by weight, and increased over time by about 2.5% per year in spring and summer and by about 3.5% per year in autumn and winter. During the mid-2000s, the C : N ratio switched from predominantly below to predominantly above the Redfield ratio (Fig. 4d), noting that there were no data before 1999.

The total duration of seasonal low DIN (< 0.2 mg L^{-1}) periods increased significantly ($p < 0.0001$) by around 100 d over the course of the study period (Fig. 5). In the 1980s, DIN was continuously below 0.2 mg L^{-1} on average from mid-July until mid-August each year, whereas in the decade from 2007 to 2016, these periods extended from the end of May until

Table 1. Mean (± SE) nitrogen and phosphorus concentrations and ratios (by weight) in Müggelsee by season for the period 1979–2014 (C : N ratios only since 1999). Trends over time are given in parentheses with the following significance codes: ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. For nutrient concentrations, the table shows arithmetic means and trends in concentration units per year. For nutrient ratios, the table shows geometric means with trends in percent per year.

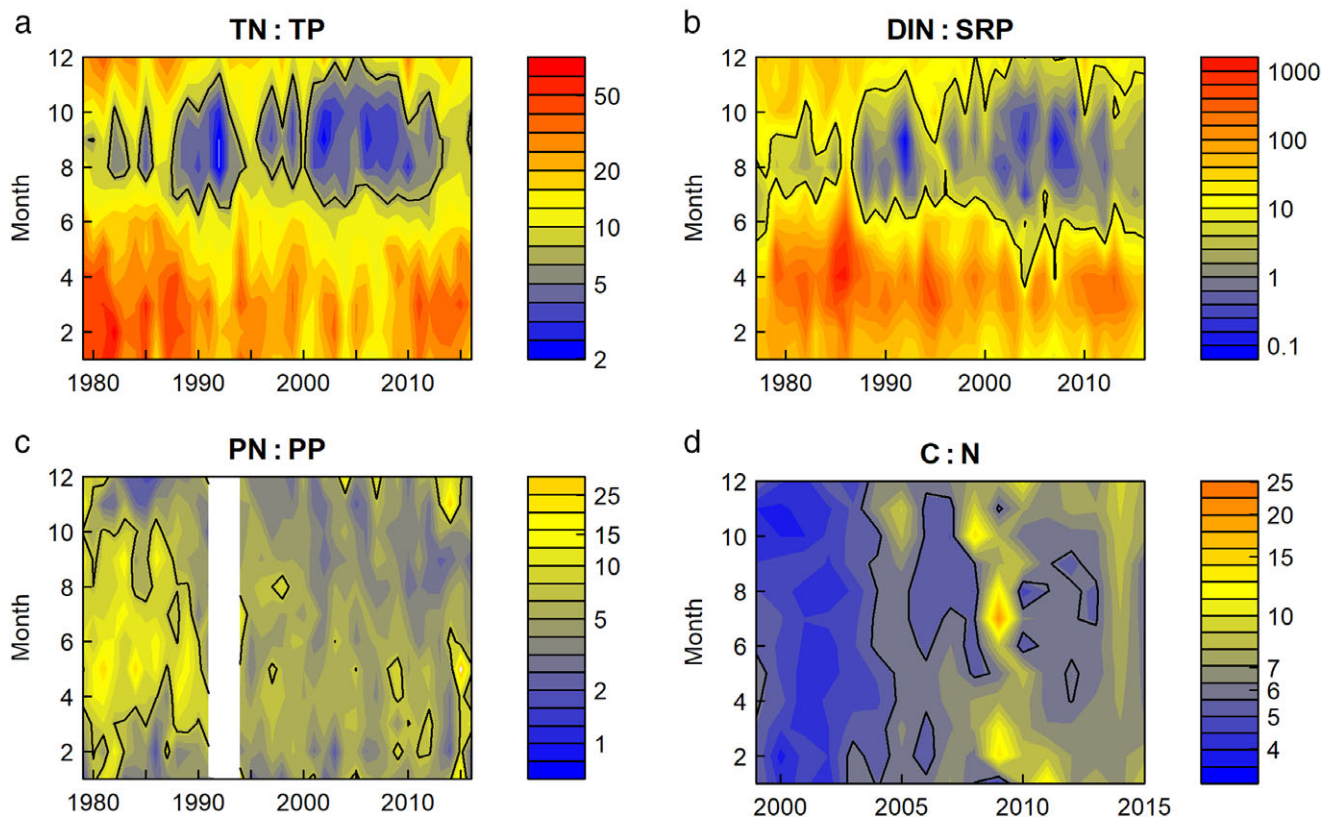|	Winter	Spring	Summer	Autumn	Annual
TP (μg L^{-1})	78±3.5	89±4.9	226±17	144±8.9	134±6.9
	(−1.3 μg L^{-1} a^{-1})***	(−2.0 μg L^{-1} a^{-1})***	(−3.4 μg L^{-1} a^{-1})*	(ns)	(−1.8 μg L^{-1} a^{-1})**
SRP (μg L^{-1})	29±1.3	17±1.2	122±12.5	89±7.9	64.1±5.1
	(−0.33 μg L^{-1} a^{-1})**	(ns)	(ns)	(ns)	(ns)
TN (mg L^{-1})	2.22±0.17	1.61±0.13	1.30±0.07	1.41±0.08	1.64±0.07
	(−0.075 mg L^{-1} a^{-1})***	(−0.061 mg L^{-1} a^{-1})***	(−0.033 mg L^{-1} a^{-1})***	(−0.036 mg L^{-1} a^{-1})***	(−0.050 mg L^{-1} a^{-1})***
DIN (mg L^{-1})	1.68±0.22	0.57±0.06	0.22±0.03	0.77±0.09	0.85±0.08
	(−0.074 mg L^{-1} a^{-1})***	(−0.025 mg L^{-1} a^{-1})***	(−0.010 mg L^{-1} a^{-1})***	(−0.031 mg L^{-1} a^{-1})***	(−0.039 mg L^{-1} a^{-1})***
TN : TP	27.1±6.4%	18.4±5.2%	6.7±5.5%	11.3±7.5%	16.1±5.1%
	(−1.6% a^{-1})**	(−1.1% a^{-1})*	(ns)	(−1.9% a^{-1})**	(−1.4% a^{-1})**
DIN : SRP	75.0±10.5%	67.7±18.0%	2.41±19.5%	10.1±15.9%	41.4±12.9%
	(−2.5% a^{-1})**	(−5.2% a^{-1})***	(−5.3% a^{-1})***	(−5.4% a^{-1})***	(−4.0% a^{-1})***
PN : PP	5.4±5.5%	7.1±7.3%	5.6±6.5%	4.9±6.1%	5.9±4.7%
	(ns)	(−2.0% a^{-1})***	(−2.8% a^{-1})***	(ns)	(−1.3% a^{-1})***
DIN : TP	18.2±9.6%	6.6±12.6%	0.9±13.4%	5.7±11.8%	8.2±9.4%
	(−2.6% a^{-1})***	(−3.0% a^{-1})**	(−3.4% a^{-1})**	(−3.5% a^{-1})***	(−2.8% a^{-1})***
C : N	6.2±5.4%	5.8±4.2%	5.6±4.3%	6.2±6.6%	5.9±4.6%
	(3.3% a^{-1})***	(2.3% a^{-1})***	(2.5% a^{-1})***	(3.9% a^{-1})***	(3.0% a^{-1})***

Fig. 4. Seasonal development of nutrient ratios (by weight). Total nitrogen : phosphorus (**a**), dissolved inorganic nitrogen : soluble reactive phosphorus (**b**), particulate nitrogen : phosphorus (**c**), and particulate carbon : nitrogen (**d**). Black lines delimit areas below the Redfield ratio.
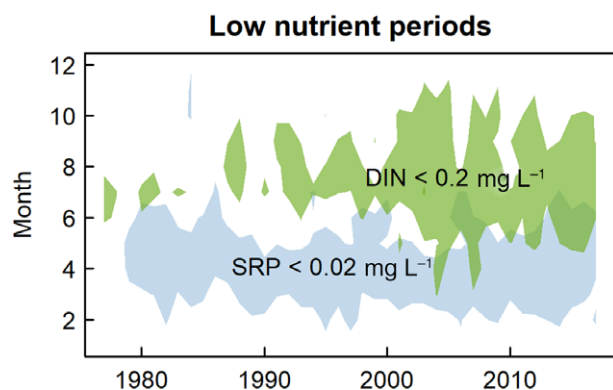
Fig. 5. Occurrence of low dissolved inorganic nutrient concentrations. Shaded areas show periods of soluble reactive phosphorus (SRP) below 20 μg L^{-1} and periods of dissolved inorganic nitrogen (DIN) below 0.2 mg L^{-1}.

Fig. 6. Seasonal course of the biovolume of total phytoplankton (thick lines) and N$_2$-fixing cyanobacteria (Nostocales, thin lines), averaged over the decade 1980–1989 (solid lines) and 2007–2016 (dashed lines).

late September. The total duration of low SRP periods (< 20 μg L^{-1}) did not change significantly over time ($p = 0.23$, Fig. 5). However, there was a trend for continuous periods of low SRP to begin earlier (08 March on average in the 1980s compared to 21 February in 2007–2016, $p = 0.005$).

Phytoplankton

Phytoplankton in Müggelsee generally showed a bimodal seasonal pattern with a spring peak of diatoms around April and a summer peak around late July (Fig. 6). Nitrogen-fixing cyanobacteria (mostly *Aphanizomenon* sp. and *Anabaena* sp. [alias *Dolichospermum* sp.]) showed a small but regular peak in late spring around May, sometimes before or after the clear water phase, and a much stronger development in July and August. This pattern was also reflected in the N$_2$-fixing proportion of the total phytoplankton biovolume (Fig. 6).

The annual mean phytoplankton biovolume decreased significantly by 0.26 ± 0.04 mm^3 L^{-1} a^{-1} ($p \ll 0.001$) from 11.7 ± 3.1 mm^3 L^{-1} in the 1980s to 2.8 ± 1.2 mm^3 L^{-1} in the decade 2007–2016 (Fig. 7a), and the mean summer cyanobacteria biovolume decreased from 10.3 ± 4.8 mm^3 L^{-1} to 1.2 ± 0.6 mm^3 L^{-1} in the same period. The mean summer biovolume of N$_2$-fixing taxa also decreased significantly by 0.18 ± 0.04 mm^3 L^{-1} a^{-1} ($p = 0.0002$) from 6.7 ± 4.2 mm^3 L^{-1} in the 1980s to 0.7 ± 0.3 mm^3 L^{-1} in the decade 2007–2016. Not only did the biovolume of N$_2$-fixing taxa decrease, but also their proportion of the total biovolume ($p = 0.002$) as well as their proportion of the cyanobacteria ($p = 0.03$, Fig. 7b). Whereas in the 1980s, N$_2$-fixing taxa constituted on average 62% of the cyanobacteria and 36% of the total biovolume in summer, by the last decade of the time series they constituted 48% of the cyanobacteria and only 14% of the total summer phytoplankton.

The mean phytoplankton biovolume was significantly related to TP and TN in both spring and summer (Fig. 8; Table 2). There were however considerable differences in these relationships between the seasons. The relationship between
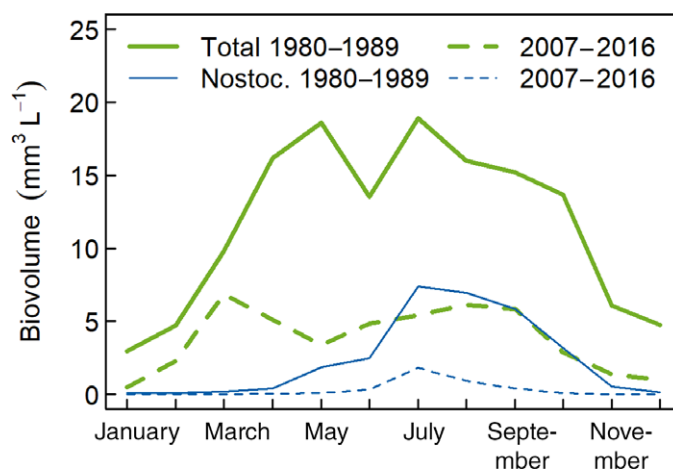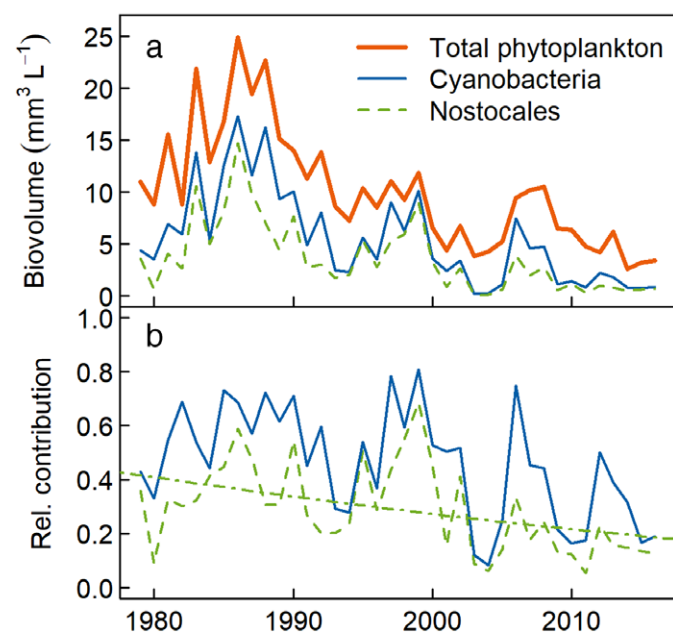

Fig. 7. Annual means of the biovolume of total phytoplankton, cyanobacteria, N$_2$-fixing taxa (Nostocales) (**a**), and the relative contributions of cyanobacteria and Nostocales within the total phytoplankton (**b**). The dot-dashed line in (**b**) is the regression line for Nostocales.

biovolume and TP was stronger in spring than in summer, whereas the relationship between biovolume and TN was stronger in summer than in spring (see pseudo R^2 values in Table 2). Accordingly, spring phytoplankton biovolume decreased proportionally to P, whereas summer biovolume decreased proportionally to N. Moreover, the phytoplankton biovolume yield per unit P (slope of the biovolume vs. TP regression line in Table 2) was four times higher in spring (0.150 mm^3 μg^{-1} TP) than it was in summer (0.037 mm^3 μg^{-1}
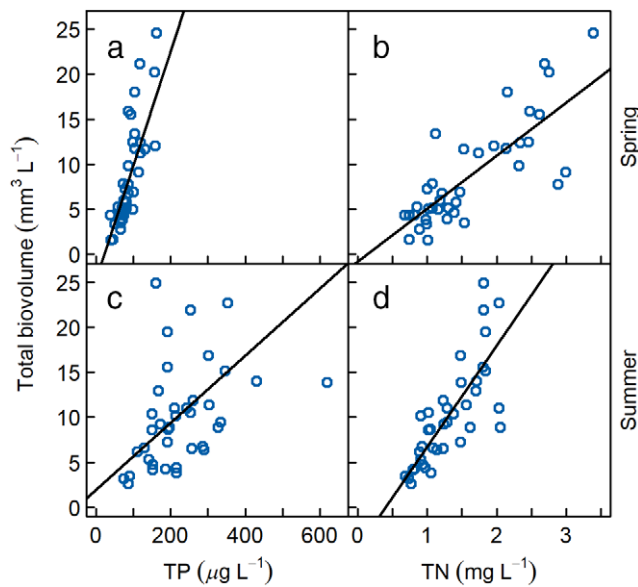
Fig. 8. Relationship between total phytoplankton biovolume and total phosphorus (**a**, **c**) and total nitrogen concentration (**b**, **d**) in spring (**a**, **b**) and summer (**c**, **d**). Circles are seasonal means of observed data, lines are regression fits.

Table 2. Results of regressions of total phytoplankton biovolume (mm^3 L^{-1}) on total phosphorus (TP, μg L^{-1}) or total nitrogen (TN, mg L^{-1}). Values in parentheses are 95% confidence intervals of the coefficients. The pseudo R^2 was calculated as 1 – model deviance/null deviance.

| Regression equation	Pseudo R^2	Degrees of freedom	t	p
Spring biovolume=0.150 (0.12–0.18)×TP–4.74 ([–6.1]–[–3.1])	0.74	35	11.4	≪0.0001
Spring biovolume=5.89 (4.3–7.7)×TN–0.79 ([–2.7]–1.3)	0.62	36	6.7	≪0.0001
Summer biovolume=0.037 (0.011–0.064)×TP +1.92([–2.3]–7.5)	0.24	36	3.6	0.001
Summer biovolume=11.23 (8.87–13.71)×TN–4.48 ([–6.84]–[–1.96])	0.71	36	9.3	≪0.0001
Annual biovolume=0.071 (0.048–0.091)×TP –2.36([–4.21]–0.27)	0.47	36	7.2	≪0.0001
Annual biovolume=4.84 (3.52–6.31)×TN–1.00 ([–2.75]–0.82)	0.60	36	6.7	≪0.0001
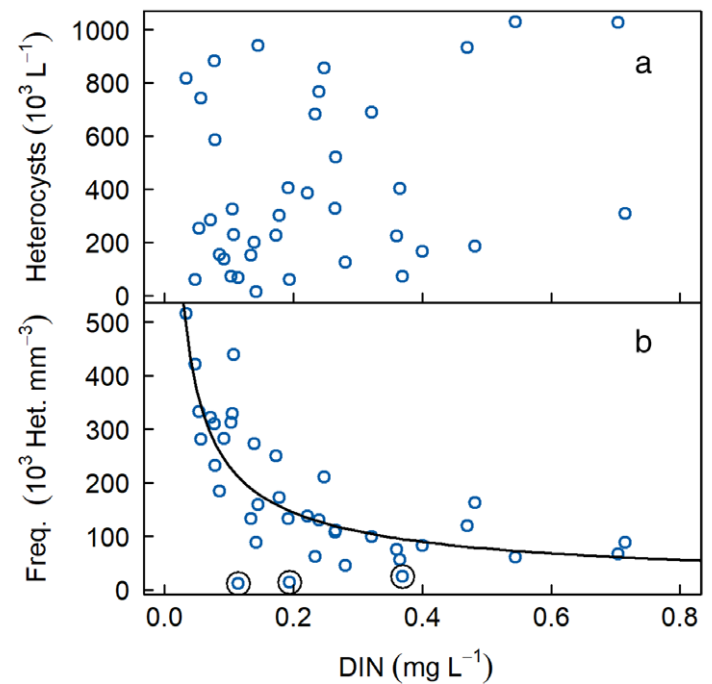
Fig. 9. Mean concentration of heterocysts per liter (**a**), and per unit biovolume of nitrogen-fixing species (**b**) as a function of mean dissolved inorganic nitrogen concentration (DIN) during summer (July–September). Influential years (1987–1989, circled values) were excluded from the regression in (**b**). The black line shows the regression fit.

TP). Conversely, the biovolume yield per unit TN was about twice as high in summer (11.23 mm^3 mg^{-1} TN) as it was in spring (5.89 mm^3 mg^{-1} TN). The ratio of the reciprocals of these slopes gives the nutrient ratio normalized to phytoplankton biovolume. Accordingly, the biovolume-normalized ratio of N to P in spring was 25.5 by weight and thus much higher than the Redfield ratio of 7.2, whereas in summer the normalized ratio was 3.3. At an annual scale, the biovolume-normalized N : P ratio was 14.6.

The mean proportion of N$_2$-fixing cyanobacteria within the total biomass during summer was significantly positively related to the PN : PP ratio ($p = 0.01$), the DIN : SRP ratio ($p = 0.001$), and the DIN concentration ($p = 0.01$). It was negatively related to the C : N ratio ($p = 0.04$, excluding 2009, which deviated from the mean by >3 SDs, or $p = 0.08$ otherwise), and the mean light in the mixed layer ($p = 0.02$). Net specific growth rates of N$_2$-fixing taxa, calculated from the change in biovolume over time as a measure of relative growth performance, were on average highest in April (0.08 d^{-1}) and June (0.09 d^{-1}) preceding the regular May and July maxima. In June, the specific growth rates of N$_2$-fixing taxa were positively related to mean underwater PAR ($p = 0.009$). In April, the difference in specific growth rates of N$_2$-fixing and non-N$_2$-fixing taxa (as a measure of relative advantage of N$_2$-fixers) was positively related to PAR

(p = 0.03). Otherwise the net specific growth rates in April and June were unrelated with nutrients or water temperature.

The mean summer concentration of heterocysts in lake water did not change significantly over time (p = 0.33). Moreover, the concentration of heterocysts in lake water was not related to the DIN concentration (p = 0.10, Fig. 9a). However, the number of heterocysts per unit biovolume of N_2-fixing species increased sharply at low DIN concentrations (Fig. 9b). The frequency of heterocysts was given in terms of DIN concentration (mg L^{-1}) by

Heterocyst frequency (10^3 mm^{-3}) = 48.0($\pm$1.2) $\times$ DIN$^{-0.69\,(\pm0.08)}$ ($p \ll 0.0001$, t = 8.2, df = 33, R^2 = 0.67)

Physical characteristics

The annual mean light extinction decreased by 0.018 m^{-1} a^{-1} ($p \ll 0.001$) from 1.73 $\pm$ 0.30 m^{-1} in the 1980s to 1.16 $\pm$ 0.19 m^{-1} in the years 2006–2014. Accordingly, the mean PAR in the mixed layer (= mean lake depth when unstratified) during summer increased by 0.15 $\pm$ 0.03 mol m^{-2} d^{-1} a^{-1} ($p \ll 0.001$). Mean summer surface temperature increased by 0.051°C a^{-1} (p = 0.002), but there was no significant increase in the difference between surface and bottom temperatures (p = 0.11) suggesting no general increase in stratification. Based on the high-frequency temperature profiles available since 2004, the median and maximum duration of stratification events was 6 h and 20 d, respectively (*see* Supporting Information Fig. S1). We detected no significant increase in the frequency or total duration of summer stratification events longer than 1 d since 2004. Water near the sediment periodically became depleted in oxygen during extended stratification events, but otherwise remained oxic (Supporting Information Fig. S1).

Discussion

While there are many examples of lakes in which reduction of P-loading successfully decreased phytoplankton biomass (Jeppesen et al. 2005), there are very few long-term, whole-ecosystem studies that document whether a combined reduction of N- and P-loading is useful in controlling eutrophication. Moss et al. (2005) found that phytoplankton biomass declined in a shallow lake as N-inputs and concentrations decreased. Köhler et al. (2005) analyzed data in Lake Müggelsee until 2003, and found that phytoplankton biomass decreased in parallel with P-inputs in spring and with N-inputs in summer, but the biomass of the N_2-fixing *Aphanizomenon flos-aquae* did not change. Since the study of Köhler et al. (2005), an additional 13 yr of data have become available for this study, which shows that N and P loads continued to decline after 2003, as did the phytoplankton biomass. The new analysis presented here demonstrated that not only did the biomass of summer cyanobacteria decrease in parallel to TN concentration, but also the proportion of N_2-fixing species within the total phytoplankton decreased. Our results

therefore confirm that a decrease in N- and P-loading, and the concomitant decrease in DIN concentrations in the lake, did not favor N_2-fixing cyanobacteria over other phytoplankton groups. Furthermore, N_2 fixation did not exceed N losses through denitrification and burial and did not compensate for the decrease in N-inputs within the 37-yr study period.

Our results highlighted the importance of seasonality of dissolved nutrient concentrations. At annual scales, total phytoplankton biovolume was significantly correlated with both TN and TP, where a greater percentage of the variance was explained by TN than by TP. Moreover, the TN : TP ratio in inflows was always above the Redfield ratio yet within the range of N + P co-limitation identified by Guildford and Hecky (2000). So which nutrient was responsible for controlling the biomass? We found strong evidence that P was biomass-limiting in spring, whereas N was limiting in summer, which occurs regularly in shallow polymictic lakes (Dolman et al. 2016). The lower TP concentrations in the inflow compared to the lake demonstrated the importance of P-remobilization from the sediments, which delayed a decline in TP for about 20 yr, causing stronger N-limitation in summer.

Our finding of P-limitation in spring and N-limitation in summer was based on regressions of phytoplankton biovolume on total nutrient concentrations. The diagnosis of nutrient limitation on the basis of these kinds of regressions has been criticized for a number of reasons (Lewis and Wurtsbaugh 2008). First, chlorophyll *a* vs. nutrient relationships are often analyzed on log–log plots, which can obscure the enormous variability in the relationship. Second, Chl *a* and N and P are essential components of biomass, so there must necessarily be a relationship between them. This co-linearity means that total N : P ratios, which generally reflect particulate ratios, are not reliable indicators of nutrient limitation (Schindler 2012). We avoided the first issue using linear rather than log-transformed variables, and reduced the second using phytoplankton biomass rather than Chl *a*. Moreover, we can argue that a given quantity of nutrient must necessarily yield a higher biomass as the degree of nutrient limitation increases (internal nutrient quota decreases). Furthermore, the nutrient–biomass relationship should be less variable as the nonstructural internal nutrient pools of phytoplankton are restricted to narrower ranges. Therefore, untransformed biomass–nutrient relationships are useful in diagnosing *relative* degrees of nutrient limitation. Accordingly, our results of a higher yield (slope of the biovolume vs. total N or P regression) and lower residual deviance (higher pseudo R^2) provide strong evidence that P was much more strongly limiting in spring than it was in summer and N was much more strongly limiting in summer than in spring. The ratios of the biomass yields of P and N (regression slopes) are arguably more useful than the N : P ratios themselves, because the yields are normalized to phytoplankton biovolume and thus provide a better indication of the average cellular nutrient status. Based on the nutrient yields, we found that N : P normalized to

biovolume in Lake Müggelsee was well above Redfield in spring and well below Redfield in summer. Schindler (2012) stated that correlations of chlorophyll with one nutrient that are slightly better than with another nutrient are "more likely to reflect relative differences in analytical errors than in nutrient limitations". However, the differences we observed over the nearly four decades are too large and consistent to be attributed to analytical error. The trends we observed in particulate nutrients (falling PN : PP and rising particulate C : N ratios, both crossing the Redfield ratio) also reflected an increasing N-deficit. Combined with the very low observed dissolved N concentrations, we conclude that N was the predominant limiting nutrient in summer, and that the decrease in N-inputs increased N-limitation in the long term without favoring N_2-fixing cyanobacteria.

Internal P-loading can remain high for decades after nutrient input reduction due to legacy loads of P (McCrackin et al. 2017), so that N-limitation during the nonequilibrium phase may only be temporary. In Lake Müggelsee, the absence of decreasing trends in summer SRP concentrations and the fact that the lake was on average a net P-source until around 2010 suggests that the lake may have only very recently reached final equilibrium with P-inputs. This recovery time of about 20 yr seems reasonable compared to other lakes (Jeppesen et al. 2005). The bulk of internal P-loading in summer likely originated from mineralization of freshly sedimented P from the previous winter and spring. Mineralization of fresh organic material under predominantly aerobic conditions can be a substantial internal source of P (Hupfer and Lewandowski 2005; Song and Burgin 2017) or even the driving force of internal P-loading (Sinke et al. 1990). Moreover, undisturbed sediment cores from Müggelsee incubated under aerobic conditions retained P at spring temperatures (15°C) but released P at summer temperatures (20°C) due to a shift in the balance between mineralization and P-iron binding (Hupfer and Lewandowski 2008). This is consistent with the results of our P-balance, showing that P-retention in spring constituted about two-thirds of the P-release in summer, which was too high to induce summer P-limitation. We conclude that the reduction in N-loading and the corresponding strong and persistent N-limitation in summer is what ultimately kept the summer cyanobacterial blooms at bay. Moss et al. (2005) also found that high internal P-release in summer in a shallow lake prevented seasonal P-limitation and caused phytoplankton biomass to decrease in parallel with declining N concentrations and loading. This highlights an important point: although TN : TP ratios in the lake inflows were always above Redfield in the annual mean, the strong seasonal differences and very low summer ratios were ultimately decisive in controlling summer cyanobacteria. Paerl et al. (2016) postulated that lakes like Müggelsee with annual external input N : P ratios between 9 and 23 (weight) and greater denitrification rates than fixation rates should be N + P limited. Our results concur, albeit with seasonal separation of apparently strict N- and P-limitation. N-limitation is prevalent primarily in shallow polymictic lakes

during summer (Dolman et al. 2016). Combined with naturally high seasonal P-release rates, polymictic lakes have potentially high denitrification rates due to a generally good supply of fresh organic material and warmer temperatures above the sediment caused by regular mixing. Therefore reductions in N-inputs, which do not necessarily decrease N : P loading ratios below Redfield, may be effective at controlling summer cyanobacterial blooms in polymictic lakes.

This raises the question of why the N-deficit relative to P was not compensated by N_2 fixation through diazotrophic cyanobacteria. Moss et al. (2013) postulated that N-limitation may persist in lakes simply because N_2-fixing species are unable to develop substantial populations or when fixed N is washed out in winter before it can be incorporated into internal lake cycles. The short water residence times in estuaries affect their sensitivity to N-loading (Howarth and Marino 2006) and can prevent N fixed from the atmosphere or released from the sediment from accumulating enough to reach optimal N : P ratios (Schindler 1981). The same should apply to lakes with short residence times like Müggelsee, particularly given that downstream flushing of N may be more important than P or C on global scales (Maranger et al. 2018). However, flushing was unlikely to have prevented an accumulation of fixed nitrogen in the case of Müggelsee. Dilution by flushing is only possible if inflow concentrations are lower than lake concentrations, but this was never the case. The fact that TN concentrations in the lake were always lower than in the inflow indicates that denitrification plus sedimentation rates permanently exceeded N_2 fixation rates, leading to a net loss of N, even when external N-loading and TN : TP ratios decreased. In fact, our somewhat crude estimates suggest that denitrification plus N-burial rates were many times higher than N_2 fixation rates throughout the case history. It has been argued that N_2 fixation only balances the reactive N pool gradually so that P is the ultimate, long-term limiting nutrient (Schindler 2012; Schindler et al. 2016). Our results do not support this, because N_2 fixation did not exceed N losses in the long term (Scott and McCarthy 2010; Paerl et al. 2016). The theoretical residence times were much longer during summer than on annual mean, and they increased during the study period (from 60 ± 17 d in summers of the 1980s to 109 ± 38 d in 2007–2016). These residence times were long enough to facilitate cyanobacteria growth, and their extension should increase the importance not only of N_2 fixation but also of N losses like denitrification and sedimentation.

Nitrogen losses were not the only reason why decreases in N were not compensated in the long term by N_2 fixation. Increased N-limitation clearly did not favor N_2-fixing species. In fact, all evidence in our results points to the opposite: lower DIN concentrations, lower DIN : SRP, lower PN : PP, and higher C : N all correlated with a lower biovolume and lower proportion of N_2-fixing species, showing that increased N-limitation decreased the relative abundance of N_2-fixing cyanobacteria. This concurs with the results of Dolman

et al. (2012), who found that N_2-fixing taxa reached the highest biovolumes in lakes with high N : P ratios. Paerl and Otten (2016) attributed this phenomenon in many cases to physical control by temperature, irradiance, and hydrology. This may have been the case in our study, because the pre-bloom net specific growth rates of N_2-fixing taxa were correlated with light, suggesting light limitation may have played a role. However, light availability did not play the decisive role because, as the biovolume of N_2-fixing taxa decreased over time, mean underwater light increased due to higher water transparency coinciding with the lake's recovery. Whereas the per heterocyst N_2 fixation estimated according to Ferber et al. (2004) increased over time due to the increase in light availability, total N_2 fixation did not. Moreover there was no correlation between mean underwater light in the summer mixed layer and the proportion of N_2-fixing taxa. It is therefore unlikely that the light supply prevented N_2 fixation from increasing in response to stronger N-limitation. Wagner and Adrian (2009) found that increased stratification duration in Müggelsee promoted N_2-fixing taxa more than non-N_2-fixing cyanobacteria, but only above certain TN and TP thresholds. Conversely, higher temperatures may favor *Microcystis* sp. over N_2-fixing taxa like *Aphanizomenon* sp. (Paerl and Otten 2016). Thus the increasing temperature trend that we observed without a concurrent change in stratification may in part explain the decrease in proportion of N_2-fixing taxa. Higher water transparency decreases summer stratification in Müggelsee (Shatwell et al. 2016), which can explain the absence of trends in stratification despite an increase in water temperature. In general however, we found little evidence that changes in the physical regime constrained N_2 fixation or the abundance of N_2-fixing taxa. Alternative explanations may be limitation by micronutrients (Wurtsbaugh 1988) or the fact that N-limitation may not have been strong enough for N_2 fixation to yield a competitive advantage (Tyrrell 1999).

The sharp increase in the biovolume-specific number of heterocysts we observed at low DIN concentrations is a typical response of N_2-fixing species (Howarth et al. 1988). However, the decrease in biovolume of N_2-fixing species canceled the increase in biovolume-specific heterocyst concentration, so that the total amount of heterocysts did not change over time. Per heterocyst fixation rates tend to be very variable (Hadas et al. 2012), but this finding does not indicate any net increase in N_2 fixation over time in response to decreased DIN concentrations or increased mean light intensity in summer.

Altogether, our results demonstrate that dual N and P control can be an effective strategy to mitigate eutrophication. Particularly in polymictic lakes where summer internal P-loading and denitrification rates are potentially high, the lower N-loading can induce stronger summer N-limitation and in turn effectively control cyanobacterial blooms in the long term. Moreover, increased N-limitation does not necessarily promote N_2-fixing taxa, and in our study, effectively decreased their biomass.

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Conflict of Interest

None declared.