Hydrobiologia (2017) 795:35–48
DOI 10.1007/s10750-017-3110-x

PRIMARY RESEARCH PAPER

Nitrogen or phosphorus limitation in lakes and its impact on phytoplankton biomass and submerged macrophyte cover

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Received: 9 November 2016 / Revised: 10 January 2017 / Accepted: 27 January 2017 / Published online: 6 February 2017

Abstract We used data on nutrients, chlorophyll *a* (Chla) and submerged macrophyte cover from up to 817 Danish lakes to elucidate seasonal variations in nitrogen (N) and phosphorus (P) concentrations and to study the impact of N or its role in combination with P. In both deep and shallow lakes, we found marked seasonality in the ratio between total N and total P (TN:TP) and in the inorganic concentrations of nitrogen (DIN), indicating that N more easily becomes a limiting nutrient as summer proceeds. TN:TP reached its lowest values of <7 (by mass) in August in 25% of the shallow lakes. Chla generally related more strongly to TP than to TN, but at high TP concentrations TN explained more of the variability in Chla than TP. Macrophyte cover tended to decrease at increasing TN when TP was between 0.1 and 0.4 mg/l. At macrophyte cover above 20%, Chla was considerably lower compared with lakes with low macrophyte cover. We conclude that P is of key importance for the ecological quality of Danish lakes but that increased N concentrations, particularly in shallow lakes with moderate to high TP, may have significantly adverse effects on lake water quality and ecological status in summer.

Keywords Danish lakes · Nutrients · Seasonality · Chlorophyll a · TN:TP ratio

Introduction

The key role played by phosphorus (P) in the structure and ecological quality of lakes has long been recognised (Schindler, 1977; OECD, 1982; Vitousek & Howarth, 1991). Increased loading of P has led to loss of water clarity, increased blooms of harmful algae and decreased biodiversity in many parts of the world (Paerl, 1988; Carpenter et al., 1998; Jeppesen et al., 2000). Strong empirical relationships can usually be established between phytoplankton biomass or Chla and P loading and concentrations (Vollenweider, 1976; Phillips et al., 2008; Søndergaard et al., 2011). In lake management, a reduction of P availability with the aim to decrease phytoplankton biomass is therefore often the first measure introduced to combat and reverse cultural eutrophication.

Recently, however, besides the role of P, also the importance of nitrogen (N) limitation for lake productivity has been more intensively studied and

Handling editor: Karl E. Havens

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discussed (Sterner, 2008; Conley et al., 2009; Moss et al., 2013; Olsen et al., 2015a). Worldwide, huge amounts are invested in reducing nutrient loading to aquatic ecosystems, and targeting eutrophication efficiently and cost effectively is of utmost importance (Schindler & Hecky, 2009). While some studies conclude that lakes are overwhelmingly P limited, for example the Great Lakes (Dove & Chapra, 2015), others suggest that N and P produce a dual effect (James et al., 2003; Lewis & Wurtsbaugh, 2008). Large-scale meta-analyses of bioassay enrichment experiments often show that both N and P can increase autotroph production and that a simultaneous increase in both nutrients leads to higher production in nearly all situations (Elser et al., 2007). Pearl et al. (2011) and Müller & Mitrovic (2015) concluded that ecosystem conservation efforts should take a balanced approach to N and P abatement and that the best strategy for lake managers to adopt would be to pursue a double focus on nitrogen and phosphorus. The paradigm that lake phytoplankton productivity is naturally P limited has thus been challenged. In this respect, it has been argued that the reason why P is often found to be the most limiting nutrient for phytoplankton productivity is that most lakes over the years have received increased loading of human-derived N from the atmosphere or other sources such as fertilisation in agriculture (Camarero & Catalan, 2012; Moss et al., 2013). P limitation would then largely concern lakes where the balance between N and P has been changed because of increased anthropogenic input of N, and P limitation in unproductive lakes is a derived character induced by atmospheric N deposition (Bergström & Jannson, 2006).

Concerning ecosystem functioning, increased N concentrations have been suggested to affect ecological quality in several ways. For instance, cyanobacteria biomass and dominance may increase (Xu et al., 2010; Jeppesen et al., 2011; Davis et al., 2015) and, in shallow lakes, the growth and species diversity of submerged macrophytes may be negatively impacted due to enhanced shading by phytoplankton and/or periphyton or effects of toxic stress on plant metabolism (Barker et al., 2008; Yu et al., 2015; Olsen et al., 2015b). Total nitrogen (TN) concentrations above 1.2–2 mg/l have been suggested to be critical for the abundance of submerged macrophytes in temperate lakes when total phosphorus (TP) is higher than 0.1–0.2 mg/l (Gonzalez Sagrario et al., 2005). In shallow lakes, reduced growth of submerged macrophytes may have a number of secondary effects on the structure and function of the entire ecosystem, with subsequent negative effects on ecological state and lake water quality (Carpenter & Lodge, 1986; Jeppesen et al., 1998; Søndergaard et al., 2010).

When lake monitoring data are used to elucidate the role of P versus N limitation, mean summer or yearly concentrations are often used as well as the theoretical nutrient demand based on the Redfield ratio (Redfield, 1958). Moss et al. (2013) reported that a distribution range of 3.6–13.5 (TN:TP, by mass) is commonly used to separate definite P limitation from definite N limitation, Abell et al. (2010) identified potential N limitation at TN:TP < 7 (by mass), and based on a broad range of lakes and ocean sites Guildford & Hecky (2000) concluded that N-deficient growth was noticeable at TN:TP < 9 (by mass), whereas P-deficient growth consistently occurred when TN:TP was >22.6 (by mass). It has been stressed that TN:TP ratios should be used with caution in the prediction of nutrient limitation in individual systems as they do not always identify the limiting nutrient as nutrient bioassays do (Nikolai & Dzialowski, 2014). A drawback of using only yearly or summer means of N or P concentrations and their ratios is that they hide pronounced seasonal variations in both nutrient loading and the internal cycling of nutrients. Also it masks the changes in phytoplankton assemblages and other biological components, which may have diverse nitrogen and phosphorus demands and respond differently to increased nutrient loading according to lake type (Smith & Bennet, 1999; Donald et al., 2013). For example, in four German shallow lakes Kolzau et al. (2014) observed a shift from P limitation in spring to N or light limitation later in the year, whereas deep lakes remained predominately P limited. In large eutrophic Lake Taihu (China), Xu et al. (2010) found a significant positive effect on phytoplankton growth by N addition during summer and in autumn bloom periods.

In this study, we used nutrient, chlorophyll *a* and macrophyte data from more than 800 Danish lakes encompassing more than 3000 lake years and covering a large gradient of N and P. Cyanobacteria can constitute a significant part of total phytoplankton biomass in eutrophic Danish lakes (Søndergaard et al., 2011), but their role as nitrogen fixers for the total N budget is regarded as minor and heterocystous cyanobacteria do not dominate at low TN:TP ratio or low inorganic nitrogen concentrations (Jensen et al., 1994). Although empirical analyses cannot reveal causal mechanisms between nitrogen and phosphorus availability and primary producers, the use of a large dataset including lakes covering a large nutrient gradient and a wide range of ecological conditions allows us to establish a more general picture of when and where and to what extent N plays a role in lakes.

Our aim was to describe the general seasonal pattern in nitrogen and phosphorus concentrations and to establish empirical evidence of how different nitrogen concentrations at contrasting phosphorus concentrations influence phytoplankton biomass and submerged macrophyte cover and, with it, the overall lake water quality. First, we investigated when nitrogen limitation is likely to occur using a stoichiometric approach and, secondly, we looked for effects of changing nitrogen availability on phytoplankton biomass and macrophyte cover.

Materials and methods

The data used in this study are derived from the Danish monitoring programme on the aquatic environment. This ongoing programme came into operation in 1989 and is based on well-defined and comparable sampling and analytical procedures (Svendsen et al., 2005). We used only data from freshwater (conductivity <100 mS/m), non-coloured (colour <100 mg Pt/l) lakes > 1 ha. Deep and shallow lakes were separated according to mean depth being deeper or shallower than 3 m as in the EU lake typology (Poikane et al., 2014). In Denmark, almost all lakes with a mean depth below 3 m are polymictic and most lakes with a mean depth above 3 m are dimictic. Only samples from the epilimnion were included in the analyses.

Chemical data included chlorophyll *a* (Chla), TP, dissolved inorganic phosphorus (DIP), TN and dissolved inorganic nitrogen (DIN) calculated as the sum of the concentrations of ammonium, nitrate and nitrite. All chemical analyses were performed using standard analytical procedures; see Søndergaard et al. (2005) for further details. The ratio between Chla and TP (Chla:TP, µg/l/µg/l) was calculated to express the phytoplankton yield per unit of phosphorus. Data on submerged macrophytes included mean cover of rooted macrophytes and filamentous green algae (cover_fil) as the percentage of the whole lake area. Except for seasonal data, all chemical data presented are mean summer concentrations calculated as weighted averages from 1 May to 30 September or as mean July–August concentrations ($TN_{Jul–Aug}$, $TP_{Jul–Aug}$, $DIN_{Jul–Aug}$).

The dataset included chemical data and morphological data from 3211 lake years with data on minimum TP, TN and Chla corresponding to 817 lakes. Each lake was monitored for 1–27 years during the period from 1989 to 2015. In 38% of lakes, data were available for 1 year, in 18% for 2 years, in 11% for 3 years and in 9% for more than 10 years. Eighty-six percent of the lakes were shallow with a mean depth below 3 m and mostly small (median area = 10 ha). A majority of the lakes were eutrophic with a median TP of 0.115 mg/l and a median Chla of 41 µg/l, TP (10–90% fractiles) ranging by a factor of 15 and the 10–90% fractile of Chla by a factor of 17 (Table 1). Monthly data on TN and TP were based on biweekly summer samplings and monthly winter samplings in 18 lakes (12 shallow and 6 deep) for 18–24 years, representing 4313 lake months. Data on submerged macrophytes from shallow lakes with a mean depth ≤3 m were available from 327 lakes (676 lake years). Macrophytes were monitored once every year (July–August) by measuring cover and cover_fil at 150–375 locations (number increasing with lake size) in each lake. On each location, cover and cover_fil were determined using a 7-point scale ranging from absent to complete cover. Cover_fil was 0 in 32% and only >10% in 13% of the lakes. To some extent, cover and cover_fil may depend on the previous year's macrophyte abundance, but this was not considered in our analyses as data were seldom available from several years in a row.

In the analyses of relationships between N and Chla and cover, TP and TN were divided into two categories, each defined by the median values of TP and TN in the whole dataset (lake years). This yielded four nutrient groups for which both TP and TN data were available: TP_low and TN_low ($TP < 0.107$ mg/l, $TN < 1.37$ mg/l, $n = 1178$), TP_low and TN_high ($TP < 0.107$ mg/l, $TN > 1.37$ mg/l, $n = 419$), TP_high and TN_low ($TP > 0.107$ mg/l, $TN < 1.37$ mg/l, $n = 435$) and TP_high and TN_high ($TP > 0.107$ mg/l, $TN > 1.37$ mg/l, $n = 1179$).

Table 1 Descriptive data of lakes included in the analyses. Summer mean values (1 May—30 September, presented as 10, 50 and 90% percentiles). Shallow lakes with macrophytes are lakes with a mean depth <3 m where submerged macrophytes were recorded. n = number of lakes

Variable	Lakes with nutrient and chlorophyll *a* data				Shallow lakes with macrophytes			
	N	10%	50%	90%	*N*	10%	50%	90%
Area (ha)	689	2.3	10.1	153.0	285	3.0	12.4	156.0
Mean depth (m)	645	0.5	1.3	4.6	260	0.5	1.2	2.4
TP (mg/l)	811	0.030	0.115	0.434	284	0.022	0.090	0.266
DIP (mg/l)	610	0.004	0.017	0.232	158	0.003	0.011	0.094
TN (mg/l)	809	0.69	1.38	2.80	284	0.60	1.16	1.99
DIN (mg/l)	458	0.030	0.149	1.065	129	0.030	0.104	0.678
Chla (μg/l)	810	7.6	40.7	126.7	282	5.8	33.2	103.5
Cover (%)	–	–	–	–	531	0.3	17.8	63.0
Cover_fil (%)	–	–	–	–	522	0.0	0.4	12.1

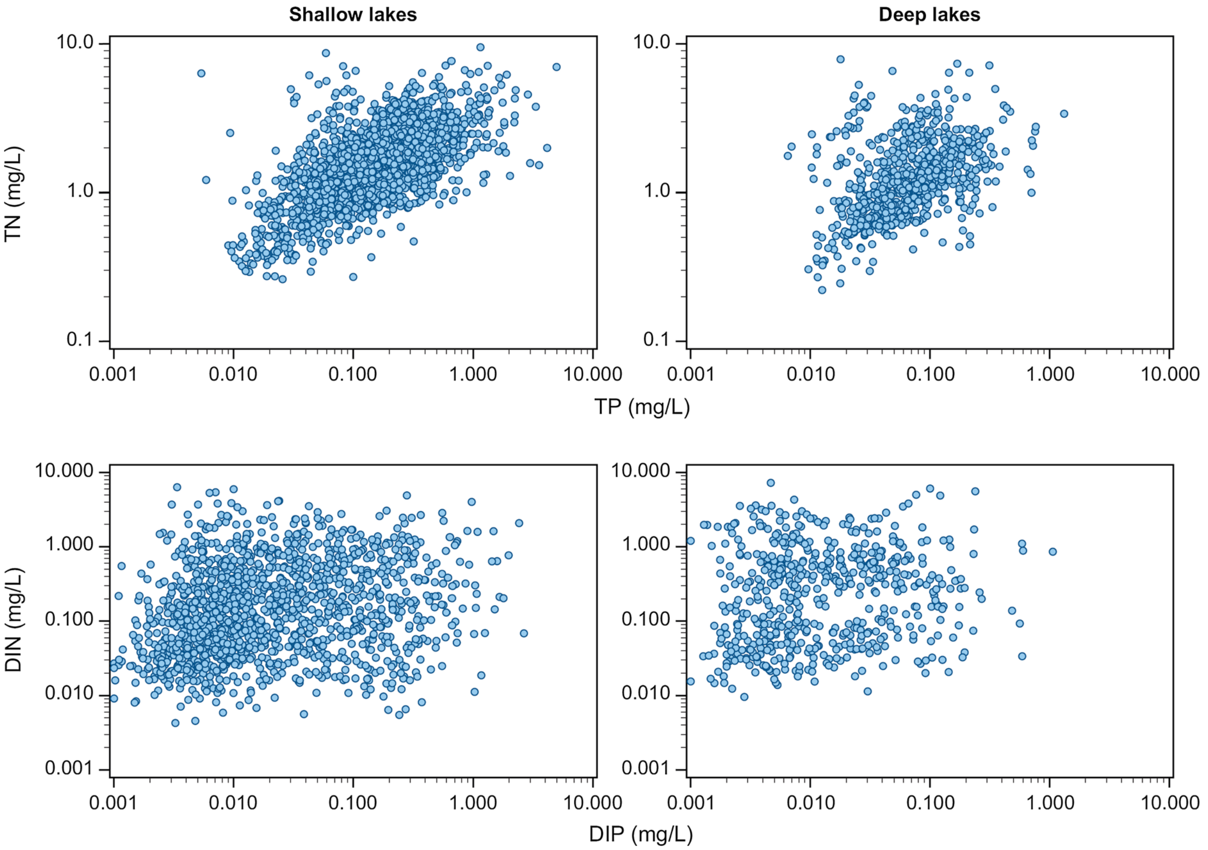


Fig. 1 Nitrogen versus phosphorus concentrations in shallow ($n = 679$, lake years = 2198) and deep ($n = 120$, lake years = 743) lakes. *Upper* TN and TP (shallow lakes: $\log TN = 0.50 + 0.37 * \log TP$, $r^2 = 0.40$, $P < 0.001$; deep lakes: $\log TN = 0.42 + 0.29 * \log TP$, $r^2 = 0.17$, $P < 0.001$). *Lower* DIN and DIP (shallow lakes: $\log DIN = -0.51 + 0.20 * \log DIP$, $r^2 = 0.05$, $P < 0.001$; deep lakes: $\log DIN = -0.35 + 0.16 * \log DIP$, $r^2 = 0.02$, $P < 0.001$)

Regression analyses between Chla and N and P were performed using SAS Proc Reg. N and P concentrations were log10 transformed before the regression. In the multivariate regression, we used a stepwise forward selection including TP, TN, mean depth and lake area as explanatory variables.

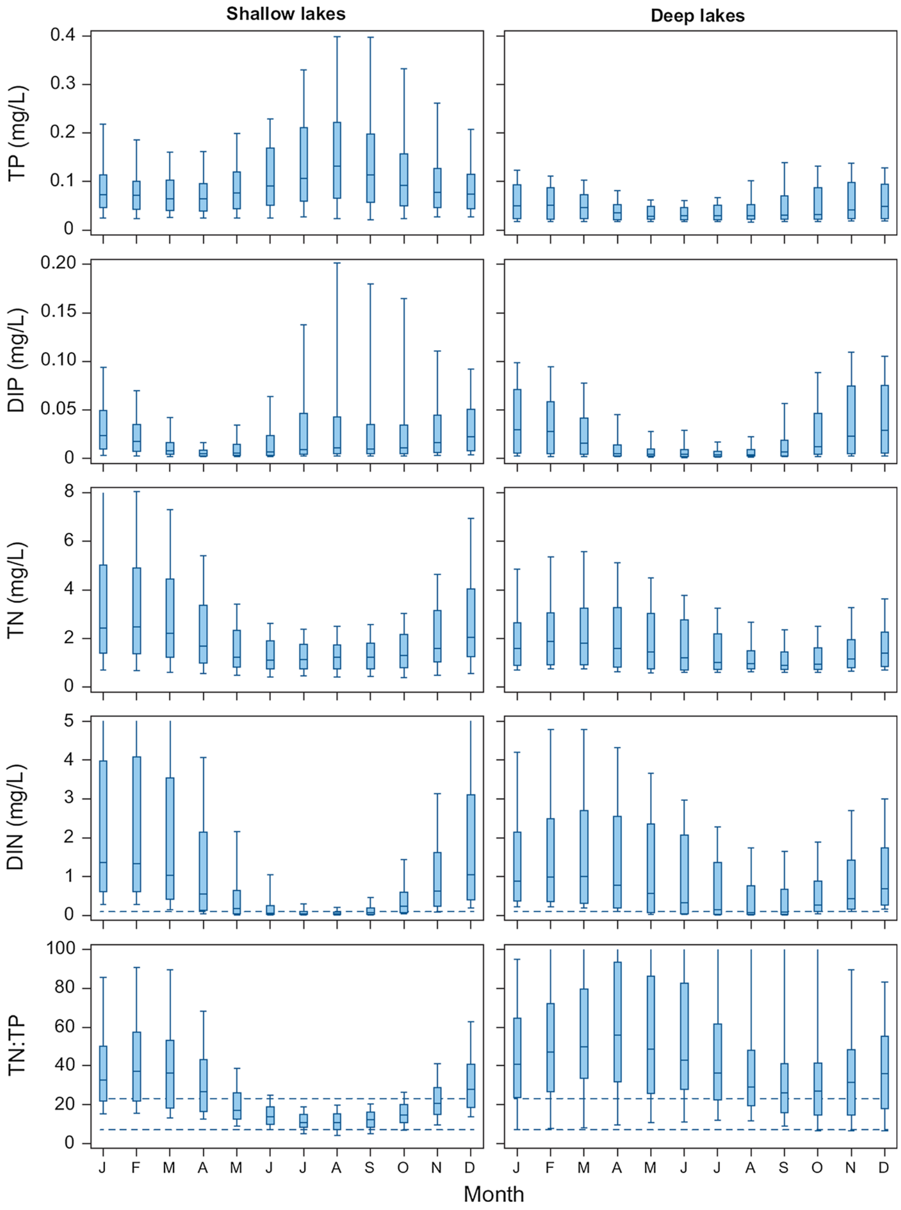


Fig. 2 Seasonal TN:TP ratios and DIN concentrations in shallow ($n = 12$, lake months = 2590) and deep ($n = 6$, lake months = 1723) lakes. The *box plots* show 10, 25, 75 and 90% fractiles. The line DIN = 0.1 mg/l indicates a boundary of potential DIN limitation (Camarero & Catalan, 2012). The two lines in the TN:TP figures display the TN:TP ratio (by mass), which can be used to identify potentially N-limited (TN:TP < 7) (Abell et al., 2010) or P-limited (TN:TP > 22.6) lakes (Guildford & Hecky, 2000)

Results

N and P concentrations

In the full dataset, TN and TP concentrations were significantly and positively related in both shallow and deep lakes (Fig. 1). The relationship was strongest in the shallow lakes, which had a larger nutrient gradient, however. Median TN:TP (by mass) was 11.8 (10–90% quantiles: 4.2–25.6) in the shallow lakes and 11.4 (10–90% quantiles: 7.4–45.0) in the deep lakes. The inorganic fractions of phosphorus and nitrogen were also significantly, but less strongly, related (Fig. 1).

Seasonal N and P concentrations

N and P concentrations were highly variable over the season in both the shallow and the deep lakes (Fig. 2). In the shallow lakes, TP and DIP increased during summer, whereas in the deep lakes the lowest TP and DIP concentrations were recorded during summer. TN and DIN concentrations decreased during spring and

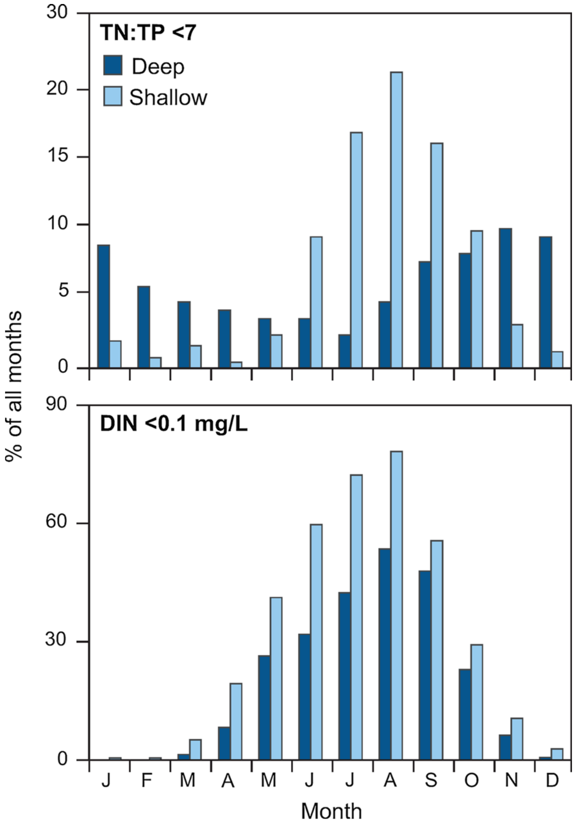


Fig. 3 Frequency distribution of lake months (% of all) with TN:TP < 7 (*upper*) and months (% of all) with DIN < 0.1 mg/l (*lower*). Number of lake years per month = 216 (shallow lakes) and 144 (deep lake) >1 ha

summer in both the shallow and the deep lakes, but particularly in the shallow lakes DIN reached low levels in July, August and September.

The TN:TP mass ratio varied extensively over the season in both the shallow and the deep lakes (Fig. 2), but the ratio was generally lowest and most variable in the shallow lakes. The ratio was highest during December–March and reached its minimum in July, August and September, where the TN:TP ratio in the shallow lakes was below 23 in all observed lake years. The number of lake months with TN:TP <7 changed during the season in both the shallow and the deep lakes (Fig. 3). During December–April, the percentage of lake months with TN:TP <7 in shallow lakes was less than 2%, but during summer the percentages increased up to 25% (August). In the deep lakes, the highest percentages with TN:TP <7 occurred in November (12%) and the lowest percentage in July (3%). The number of months with DIN <0.1 mg/l was less than 3% during winter in both the deep and the shallow lakes but increased during summer to a maximum in August in 78% of the shallow lakes and in 53% of the deep lakes (Fig. 3).

Chlorophyll a in relation to N and P

Calculated from all TP and TN concentrations, Chla was highly significantly and positively related to both TP and TN (Table 2), with a stronger relationship to TP ($r^2 = 0.49$) than to TN ($r^2 = 0.35$). In the four nutrient categories, TP was a strong predictor of Chla, particularly so at low TP and TN, but Chla was also positively and significantly related to TN. At low TP and TN, r^2 between Chla and TN was 0.25 and at high TP and TN it was 0.12 (Fig. 4; Table 2). In the TP_low, TN_high category, r^2 was much higher when related to TP than to TN, but in the TP_high, TN_low category Chla did not relate significantly to TP but to TN ($r^2 = 0.10$). A Chla regression with $\mathrm{TN_{Jul-Aug}}$ and $\mathrm{DIN_{Jul-Aug}}$ instead of TN and DIN did not increase r^2. The abundance of filamentous algae (cover_fil) related weakly to TP ($P = 0.04$, $r^2 = 0.01$, $n = 522$) and not to TN.

The 3-d plot relating Chla to both N and P demonstrated a clear impact by both nutrients on the Chla concentrations, particularly by P at TN above 0.5 mg/l and by N at TP above 0.1 mg/l (Fig. 5). Including only lakes with TP < 0.1 mg/l and a finer TP scale (Fig. 5, middle), Chla was affected by increased TN when TP was above 0.04–0.06 mg/l. Here, mean Chla was about 25 µg/l at TN below 0.8 mg/l but increased to more than 40 µg/l at higher TN. When using July–August concentrations, the impact of both nutrients was even more marked, and N also had a positive impact on Chla at $\mathrm{TP_{Jul-Aug}}$ above 0.05 mg/l (Fig. 5, lower).

In the multiple regression using both TP and TN, TN only increased the overall r^2 from 0.49 to 0.55 (Table 3; Fig. 4). At high TP (low and high TN), the correlation between Chla and TP was very low ($r^2 = 0.00–0.01$), the correlation with TN being higher, though still low ($r^2 = 0.10–0.11$). Including mean depth and lake area in the multiple regression increased the overall model r^2 only slightly or not at all.

Table 2 Single-factor regression analyses of Chla versus TP and TN performed for all lakes and the four groups of low/high TP and low/high TN lakes. In the regression with $\mathrm{TN}_{\mathrm{Jul-Aug}}$ and $\mathrm{DIN}_{\mathrm{Jull-Aug}}$, we used mean July–August concentrations of TN, DIN and Chla. Chla, P and N concentrations were log 10 transformed before regression analyses

Nutrient level	Chla vs.	Log Chla=	*N*	r^2	*P*
All	TP	2.30 + 0.77 * log TP	3179	0.49	<0.001
	TN	1.41 + 1.12 * log TN	3175	0.35	<0.001
	$\mathrm{TN}_{\mathrm{Jul-Aug}}$	1.49 + 1.17 * log $\mathrm{TN}_{\mathrm{Jul-Aug}}$	2811	0.32	<0.001
	DIP	2.02 + 0.25 * log DIP	2288	0.11	<0.001
	DIN	Not significant	–	–	–
	$\mathrm{DIN}_{\mathrm{Jul-Aug}}$	Not significant	–	–	–
TP: low	TP	2.71 + 1.11 * log TP	1186	0.50	<0.001
TN: low	TN	1.34 + 1.25 * log TN	1190	0.25	<0.001
	$\mathrm{TN}_{\mathrm{Jul-Aug}}$	1.40 + 1.21 * log $\mathrm{TN}_{\mathrm{Jul-Aug}}$	1044	0.18	<0.001
	DIP	1.72 + 0.23 * log DIP	495	0.05	<0.001
	DIN	1.45 + 0.22 * log DIN	499	0.08	<0.001
	$\mathrm{DIN}_{\mathrm{Jul-Aug}}$	Not significant	–	–	–
TP: low	TP	2.70 + 1.03 * log TP	434	0.37	<0.001
TN: high	TN	1.56 − 0.35 * log TN	434	0.02	=0.003
	$\mathrm{TN}_{\mathrm{Jul-Aug}}$	Not significant	–	–	–
	DIP	1.78 + 0.14 * log DIP	308	0.02	=0.015
	DIN	1.43 − 0.15 * log DIN	234	0.06	<0.01
	$\mathrm{DIN}_{\mathrm{Jul-Aug}}$	1.49 − 0.10 * log $\mathrm{DIN}_{\mathrm{Jul-Aug}}$	260	0.02	0.011
TP: high	TP	Not significant	–	–	–
TN: low	TN	1.59 + 1.26 * log TN	436	0.10	<0.001
	$\mathrm{TN}_{\mathrm{Jul-Aug}}$	1.68 + 0.83 * log $\mathrm{TN}_{\mathrm{Jul-Aug}}$	390	0.05	<0.001
	DIP	1.30 − 0.23 * log DIP	278	0.12	<0.001
	DIN	Not significant	–	–	–
	$\mathrm{DIN}_{\mathrm{Jul-Aug}}$	Not significant	–	–	–
TP: high	TP	2.09 + 0.26 * log TP	1173	0.05	<0.001
TN: high	TN	1.68 + 0.73 * log TN	1173	0.12	<0.001
	$\mathrm{TN}_{\mathrm{Jul-Aug}}$	1.78 + 0.68 * log $\mathrm{TN}_{\mathrm{Jul-Aug}}$	1019	0.11	<0.001
	DIP	1.85 − 0.08 * log DIP	906	0.02	<0.001
	DIN	1.91 − 0.07 * log DIN	723	0.02	<0.001
	$\mathrm{DIN}_{\mathrm{Jul-Aug}}$	1.93 − 0.07 * log $\mathrm{DIN}_{\mathrm{Jul-Aug}}$	777	0.02	<0.001

Submerged macrophyte and filamentous algae cover relative to TN and TP in shallow lakes

The 3-d plot relating cover to both N and P showed a clear trend towards higher cover at decreasing nutrient concentrations, particularly so for P. At TP between 0.1 and 0.4 mg/l, cover tended to increase at both decreasing TN and $\mathrm{TN}_{\mathrm{Jul-Aug}}$ (Fig. 6).

The 3-d plot showing mean Chla in relation to TP or TN showed increasing Chla at decreasing cover when TP was above 0.025 mg/l (Fig. 7). The increase in Chla at increasing TP was highest when cover was below 10–20%. Also in relation to TN, the effect of cover on Chla was most pronounced when cover was below 20%.

Chla:TP in relation to TN and cover

In the regression analyses between Chla:TP and TN, cover and both TN and cover, the relationships were generally weak and not or only very weakly correlated with TN (Table 4). The strongest (and negative) correlation between Chla:TP was with cover ($r^2 = 0.16$–0.17 at low TP and $r^2 = 0.11$–0.12 at high TP).

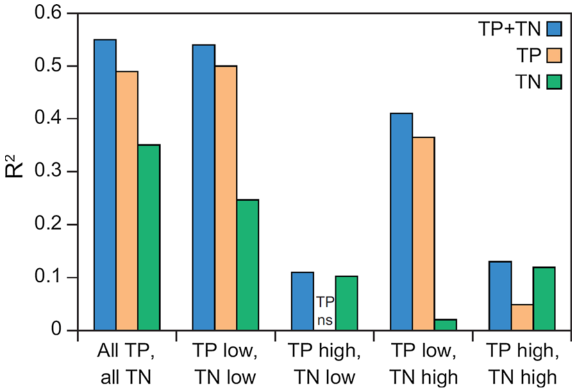
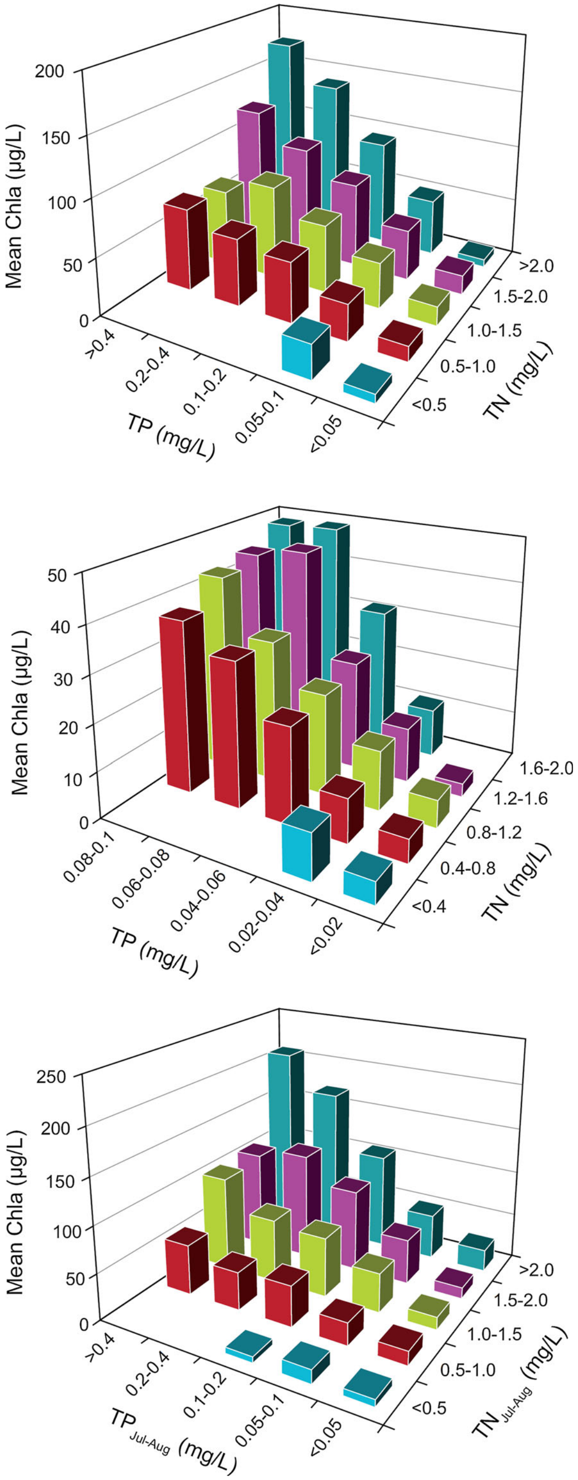


Fig. 4 *R*-squares in the single- and multi-factor regression analyses between Chla and TP and TN. The correlations are shown for all TP and TN concentrations and for the four groups with high and low TP and TN. *ns* not significant



Discussion

The seasonal changes in the concentrations of N and P were particularly pronounced in shallow lakes where TN:TP and DIN fell to low levels during most of the summer. Correspondingly, Dolman et al. (2016) concluded that N limitation in a large set of German lakes was much more common in polymictic than in stratified lakes. A less strong TN:TP seasonality in deep lakes probably reflects the reduced impact on the internal P loading of epilimnetic water in stratifying lakes compared with shallow eutrophic lakes that often have 2–3 times higher TP concentrations during summer than in winter due to P release from the sediment (Jeppesen et al., 1997; Søndergaard et al., 1999, 2013). In both types of lakes, seasonal change is also influenced by seasonal variation in loading and denitrification, the latter increasing at higher temperatures and with increasing sedimentation of easily degradable organic matter, leading to lower N concentrations during summer (Windolf et al., 1996; Saunders & Kalff, 2001). The effect of denitrification will be strongest in shallow lakes where the sediment surface area to water volume ratio is higher than in deep lakes. Overall, this implies that phosphorus most likely will be a limiting nutrient during spring and early summer, whereas nitrogen expectedly will become limiting later in the summer and in early autumn, particularly in shallow lakes. Similar results have been found in four German lakes where nitrogen tended to become increasingly limiting during summer (Kolzau et al., 2014) and in Maumee Bay of Lake Erie where Chaffin et al. (2014) found symptoms of N-limited growth during August and September. Also, in a number of ponds from the plains of Eastern

◀**Fig. 5** Mean Chla in shallow lakes with various TP and TN categories. *Upper* Chla relative to summer mean of TN and TP (total number of lake years = 1743, no lakes with TN < 0.5 mg/l at TP above 0.1 mg/l. *Middle* As the upper, but with TP < 0.1 mg/l and TN < 2 mg/l (total number of lake years = 649). *Lower* July–August mean of TN and TP (total number of lake years = 1797, no lakes with $TN_{Jul–Aug}$ < 0.5 mg/l at $TP_{Jul–Aug}$ above 0.2 mg/l)

Colorado, Mischler et al. (2014) suggested the occurrence of a transition from N and P co-limitation to N limitation across the growth season. Furthermore, our study revealed a marked reduction in DIN during spring and early summer, not least in the shallow lakes, and, according to the DIN threshold around 0.1 mg/l between N-limited and P-limited lakes proposed by Camarero & Catalan (2012), this means that a majority of the shallow and many of the deep Danish lakes are N limited for a large part of the summer. Generally, low N availability in shallow lakes, in particular during the main growing season of submerged macrophytes, suggests that changed N loading at this time of the year might have a significant impact on the overall ecological quality.

In our study lakes, TP was a strong predictor of Chla and generally also stronger than TN, which is in agreement with the findings in other multi-lake studies (Guildford & Hecky, 2000; Håkanson et al., 2007; Phillips et al., 2008). Some studies, such as that of Downing & McCauley (1992), concluded that P versus N limitation depends on nutrient levels and that N limitation was significantly more frequent in lakes with TP >0.03 mg/l. We also found that TN added significantly to the variation in Chla when TP was high. The impact of N was even more pronounced when we only used July–August concentrations,

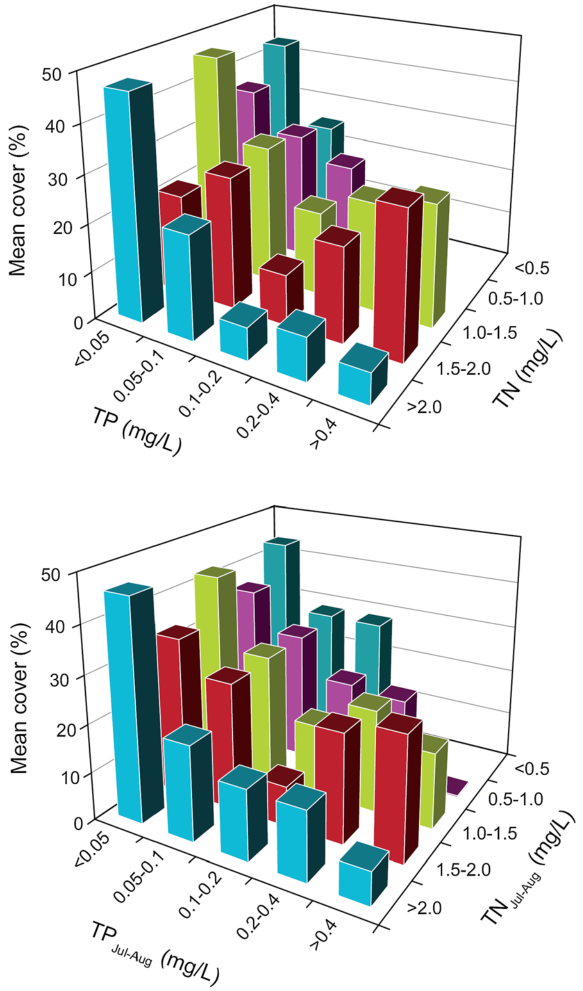


Fig. 6 Mean cover in shallow lakes with various TP and TN categories. *Upper* Cover relative to summer mean of TN and TP (total number of lake years = 532, no lakes with TN <0.5 mg/l at TP above 0.1 mg/l and no lakes with TN between 0.05 and 0.1 mg/l at TP above 0.4 mg/l). *Lower* July–August mean of TN and TP (total number of lake years = 569, no lakes with $TN_{Jul–Aug}$ <0.5 mg/l at $TP_{Jul–Aug}$ above 0.2 mg/l)

Table 3 Multiple regression analyses between log 10 Chla and TP, TN, mean depth (Z, m) and lake area (ha) with parameter estimate, partial *R*-square for the included variables and the total model *R*-square. All variables were log 10 transformed before regression analyses. *ns* not significant

Nutrient level	Log Chla=	Partial r^2				Model r^2
		TP	TN	*Z*	Area	
All	2.08 + 0.61 * TP + 0.55 * TN + 0.07 * z	0.49	0.05	0.00	ns	0.55
TP: low, TN: low	2.51 + 0.98 * TP + 0.46 * TN + 0.16 * z − 0.02 * area	0.50	0.02	0.02	0.00	0.54
TP: low, TN: high	2.82 + 1.21 * TP + 0.22 * z	0.37	ns	0.04	ns	0.41
TP: high, TN: low	1.70 + 1.22 * TN + 0.07 * area	ns	0.10	ns	0.01	0.11
TP: high, TN: high	1.82 + 0.15 * TP + 0.61 * TN + 0.07 * z	0.01	0.11	0.00	0.00	0.13

thereby emphasising the seasonal factor and the fact that varying N availability during the growing season is important in controlling phytoplankton biomass, particularly so at high nutrient levels. It is also clear, however, that factors other than N and P are important for the phytoplankton biomass, and inclusion of both TP and TP in the empirical analyses still did not increase r^2 to values above 0.55. One such factor is the presence or absence of submerged macrophytes as demonstrated in many studies (Jeppesen et al., 1998) and in this analysis as well where lower Chla per unit of phosphorus was recorded at all nutrient levels when cover increased.

There may be various mechanisms behind the positive effect of N on phytoplankton biomass, particularly at medium–high TP levels, one of them being that high N concentrations can negatively impact the growth of submerged macrophytes as suggested by, for example, Smith & Lee (2006), Özkan et al. (2010) and Olsen et al. (2015b). This may trigger a shift from a macrophyte- and clear water-dominated state with low Chla to a turbid and phytoplankton-dominated state with high Chla. In a recent study of Danish lakes, Søndergaard et al. (2016) found that when the cover of submerged macrophytes was below approximately 20%, the amount of Chla produced per P unit was twice as high as in lakes with high cover (>20%). Another explanatory mechanism could be that N at high P concentrations has the potential to increase the phytoplankton biomass even further because phytoplankton is often co-limited by N and P (Sterner, 2008; Müller & Mitrovic, 2015). Elser et al. (2007) showed that enrichment of both N and P had a higher response of autotrophs than one of the nutrients alone.

The impact of N and P on submerged macrophyte abundance was less clear than the impact on phytoplankton Chla, probably reflecting that macrophyte abundance not only depends on turbidity related to nutrient concentrations but also on several other factors such as lake depth, lake area, sediment type and grazing by waterfowl (Jeppesen et al., 1998). No TN effects on cover were detected at low TP, but at higher TP levels, and at levels between 0.1 and 0.2 mg P/l, there were indications of a significant negative impact of TN on macrophyte cover. The impact seems to occur gradually over the TN range from 0.5–1.0 to >2 mg/l and it was difficult to identify thresholds as those recorded by Gonzalez Sagrario et al. (2005).

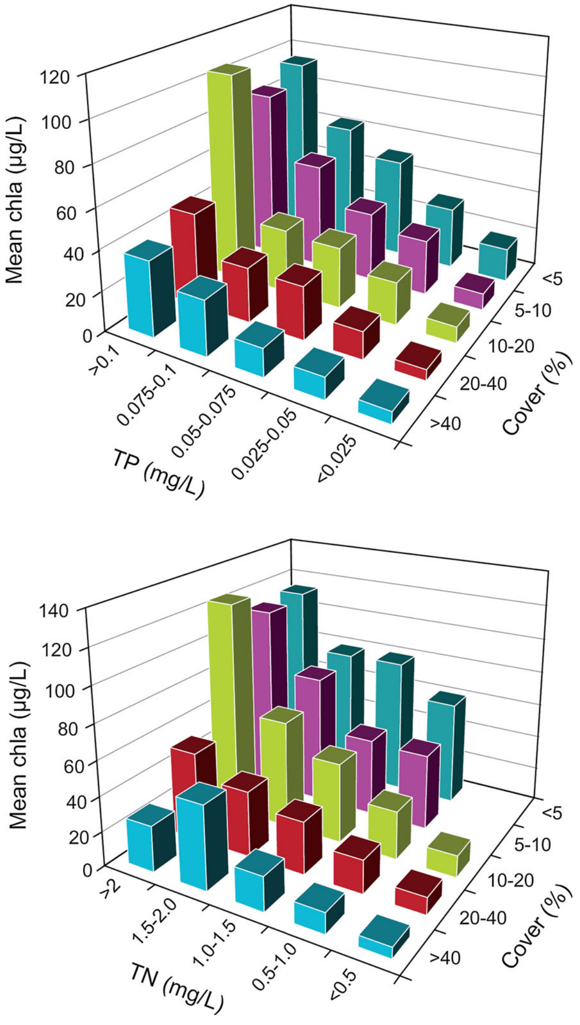


Fig. 7 Mean Chla relative to cover and TP (*upper*, number of lake years = 528) or TN (*lower*, number of lake years = 528)

Nutrient dynamics inside submerged macrophyte beds are, however, complex and depend on a number of interacting mechanisms as well as on the density and type of macrophytes (Barko & James, 1998; Kufel & Kufel, 2002; Holmroos et al., 2015), and empirical analyses may not properly reveal these kinds of interactions. A further challenge is the tight coupling between biological structure and nutrient cycling, as previously demonstrated in Danish biomanipulated lakes, where the establishment of clear water conditions with higher cover of macrophytes also greatly impacts the concentrations of both nitrogen and phosphorus (Søndergaard et al., 2002). This illustrates a "chicken-or-egg" problem, implying that it is difficult to clearly define the dependent variable as

Table 4 Regression analyses between Chla:TP (µg/l/µg/l) and log TN, log $\mathrm{TN}_{\mathrm{Jul\text{-}Aug}}$ and cover in shallow lakes using the two categories of TP and TN. In the regression with $\mathrm{TN}_{\mathrm{Jul\text{-}Aug}}$, we used mean July–August concentrations of TP and Chla. In the multiple regressions, *P* value and partial r^2 are given in parentheses for each variable

Nutrient level	Chla:TP vs.	Chla:TP=	*N*	r^2	*P*
All	TN	0.42 + 0.12 * logTN	3171	0.01	<0.001
	$\mathrm{TN}_{\mathrm{Jul–Aug}}$	0.49 + 0.07 * log $\mathrm{TN}_{\mathrm{Jul–Aug}}$	2976	0.00	=0.03
	Cover	0.53 − 0.0045 * cover	577	0.14	<0.001
	TN, cover	TN not significant	–	–	–
TP: low	TN	0.48 + 0.42 * logTN	1167	0.05	<0.001
TN: low	$\mathrm{TN}_{\mathrm{Jul\text{-}Aug}}$	0.58 + 0.45 * log $\mathrm{TN}_{\mathrm{Jul–Aug}}$	1083	0.03	<0.001
	Cover	0.58 − 0.0051 * cover	297	0.16	<0.001
	TN, c over	TN not significant	–	–	
TP: low	TN	Not significant	–	–	–
TN: high	$\mathrm{TN}_{\mathrm{Jul\text{-}Aug}}$	Not significant	–	–	–
	Cover	0.60 − 0.0054 * cover	66	0.17	<0.001
	TN, cover	TN not significant	–	–	–
TP: high	TN	0.31 + 0.38 * logTN	430	0.02	=0.005
TN: low	$\mathrm{TN}_{\mathrm{Jul\text{-}Aug}}$	Not significant	–	–	–
	Cover	0.43 − 0.0044 * cover	89	0.12	=0.001
	TN, cover	TN not significant	–	–	–
TP: high	TN	Not significant	–	–	–
TN: high	$\mathrm{TN}_{\mathrm{Jul–Aug}}$	0.43 + 0.11 * log $\mathrm{TN}_{\mathrm{Jul–Aug}}$	1091	0.00	=0.04
	Cover	0.51–0.0045 * cover	126	0.11	<0.001
	TN, cover	TN not significant	–	–	–

both N and Chla depend on a number of mutually interacting mechanisms.

The combined effects of N and P on submerged macrophytes in shallow lakes suggest that high cover can be obtained at low TP irrespective of N concentrations, but that high cover at intermediate–high TP concentrations may more easily be achieved at low TN concentrations. The relationship between submerged macrophytes and phytoplankton suggests that high cover is more important than low N concentrations at a given TP for the phytoplankton biomass. Filamentous green algae have been associated with increased N loads (Valiela et al., 1997), but there was no clear evidence for that in our study. However, filamentous algae often demonstrate rapid changes in abundance over the season, so our analyses may not be detailed enough to capture the possible effects on different levels of N availability.

Conclusions

There is still considerable uncertainty regarding when N and P alone or in combination affect the ecological state of lakes, and multiple investigations have been carried out to elucidate the role of N. The sources and concentrations of nitrogen and phosphorus are usually closely related, which makes it difficult to fully separate their specific ecological impacts using empirical analyses. The data analysed here, representing mainly eutrophic, shallow lakes in an overall intensively agricultural landscape, indicate that both N and P are important nutrients.

In our lakes we found (1) a marked seasonality in the availability of N and P, which means that N more easily becomes an important and limiting nutrient as summer proceeds; (2) that phytoplankton biomass (chlorophyll *a*) was overall most closely related to phosphorus, but at high TP concentrations enhanced N levels further augmented Chla, although other factors apart from N and P must somehow contribute to explain the variability in Chla; (3) that in shallow lakes, the abundance of submerged macrophytes varied substantially in a wide range of N and P concentrations, indicating that other factors are likely also important; generally, however, macrophyte cover tended to respond more clearly to P than to N. High macrophyte cover (>20%) in shallow lakes reduces Chla to considerably lower levels compared with lakes with low macrophyte cover.

Part of the controversy regarding N or P limitation in lakes may originate from the fact that the various investigations represent several lake types and that lakes respond differently to N and P loading depending on factors such as morphology, nutrient loading and its seasonality, trophic structure and season. This should be acknowledged further in the discussions of the importance of N and P in the management of lakes.

Acknowledgements The project was supported by the EU project MARS (Managing Aquatic ecosystems and water Resources under multiple Stress) funded under the 7th EU Framework Programme and by CLEAR (a Villum Kann Rasmussen Centre of Excellence project). The Danish regional environmental authorities are acknowledged for their careful lake monitoring and data collection. Anne Mette Poulsen and Tinna Christensen are acknowledged for editorial and layout assistance.

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