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Lake-type-specific seasonal patterns of nutrient limitation in German lakes, with target nitrogen and phosphorus concentrations for good ecological status

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SUMMARY

1. Eutrophication is a global environmental problem that leaves many lakes with impaired ecological status. Human activity has increased the total concentrations of both nitrogen and phosphorus in aquatic systems, but their relative influence on phytoplankton biomass is uncertain. Their action as alternative limiting resources complicates assessment of their relative influence and disagreement may be in part due to seasonal shifts and lake-type-specific differences in the prevalence of limitation by nitrogen versus phosphorus. Debate continues as to whether measures to reduce nitrogen would be beneficial in addition to controls placed on phosphorus.
2. We used a piecewise model to test whether total nitrogen (TN) concentrations, in addition to total phosphorus (TP), influence phytoplankton biomass in 369 lowland German lakes. The piecewise model predicts biomass from TN for low N : P ratio lakes, and from TP for high N : P ratio lakes. We tested three N : P mass ratios to divide lakes: dissolved inorganic nitrogen to TP (DIN : TP), DIN to dissolved reactive phosphorus (DIN : DIP) and TN : TP. TN was a better predictor of biomass than TP when either the DIN : TP ratio was below 1.6, DIN : DIP was below 8.4, or TN : TP below 29; predictions were most accurate when using the DIN : TP ratio.
3. To investigate seasonal and lake-type-specific patterns of N and P limitation, we used the DIN : TP ratio, together with absolute concentrations of DIN and DIP, to predict the limiting nutrient at each lake in each month of the vegetation period. N limitation was much more common in polymictic than stratified lakes. While a high proportion of both stratified and polymictic lakes were P limited in early spring (60–70%), for polymictic lakes, we found a strong shift from P limitation to N limitation in summer: more than 50% of polymictic lakes were N limited between June and September and only 15–30% were P limited.
4. To obtain lake-type-specific nutrient targets we estimated the average TN and TP concentrations at which lakes of different types achieved good ecological status according to EU water framework directive criteria. Stratified lakes achieved good ecological status at concentrations of 400–500 μg L^{-1} TN or 20–35 μg L^{-1} TP, while for polymictic lakes values of 500–1000 μg L^{-1} TN, or 35–75 μg L^{-1} TP were required.
5. We estimate that nitrogen has an important influence on phytoplankton biovolume, and thus ecological status, for many polymictic lakes in Germany. While there is some uncertainty in the nutrient targets required to achieve good ecological status, this uncertainty is small compared with the range of concentrations currently observed, and lakes with moderate or worse status have concentrations of both TN and TP that are far above these current target estimates.

Keywords: limitation, nitrogen, nutrient targets, phosphorus, phytoplankton

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Introduction

Anthropogenic eutrophication is a global environmental problem in which an increase in the supply of nutrients to aquatic and marine systems relaxes the normal restrictions on primary productivity, thereby allowing phytoplankton to attain much higher biomass than would otherwise be possible (Smith, 2003). Eutrophication is a major reason why the current ecological states of a large proportion of European lakes are significantly degraded relative to their background or pre-industrial state (European Environment Agency 2012). After eutrophication gained recognition in the 1960s as a widespread problem (Lund, 1967), a consensus view developed that phosphorus was the nutrient most commonly controlling phytoplankton biomass in freshwater systems (reviewed by Lewis & Wurtsbaugh, 2008) and it was argued that, due to the ability of some cyanobacteria to fix atmospheric nitrogen (N_2 fixation), eutrophication could not be controlled by restricting nitrogen import to fresh waters (Schindler *et al.*, 2008). A focus on phosphorus control led to substantial improvements in many lakes and rivers (e.g. Edmondson & Lehman, 1981; Jeppesen *et al.*, 2005) but recently there has been renewed interest in the role that nitrogen reductions could play in limiting phytoplankton biomass. Overall, nitrogen and phosphorus limitation are found with similar frequencies in fresh waters (Elser *et al.*, 2007) but the prevalence of N versus P limitation varies seasonally (Kolzau *et al.*, 2014), regionally (Abell *et al.*, 2012), between lakes of differing morphology (e.g. Chaffin, Bridgeman & Bade, 2013; Kolzau *et al.*, 2014), and according to catchment land use, which in turn influences N : P ratios along trophic gradients (Downing & McCauley, 1992; Hayes *et al.*, 2015). In particular, the large seasonal exchanges of nutrients between sediment and water column seen in shallow waterbodies (Grüneberg *et al.*, 2015) may mean that for certain lake types, control by nitrogen is more effective during specific periods of the year. A better understanding of the frequency of nitrogen limitation, and any lake-type-specific seasonal patterns of limitation, would be useful when deciding whether to regulate nitrogen.

The European Water Framework Directive (WFD) commits all member states to ensuring that their waterbodies have a good ecological status by 2015 (EC 2000). Targets for in-lake nutrient concentrations can focus efforts around specific aims and serve as 'yardsticks' to measure progress towards good ecological status. An extensive process is underway in the EU to determine metrics of ecological status in fresh waters (Birk *et al.*, 2012; Carvalho *et al.*, 2012), establish common standards for 'good ecological status' (Poikane *et al.*, 2014), and to estimate target phosphorus and nitrogen concentrations to achieve this good status (Carvalho *et al.*, 2013). There are several alternative approaches to determining nutrient targets (see US EPA, 2010 for a good overview). The pressure-response approach involves estimating statistical relationships between nutrient concentrations and some numeric measure of ecological status, and using these relationships to calculate the nutrient concentrations at which good status is achieved for some desired proportion of waterbodies. This method has the advantage that nutrient targets are linked explicitly to some predefined ecological outcome, such as a certain phytoplankton biovolume, chlorophyll *a* concentration, or value of a more complex multimetric index of ecological status. For lakes, all proposed WFD metrics have some measure of the abundance of phytoplankton as an important component, such as mean biovolume or chlorophyll *a* concentration (Birk *et al.*, 2012), but also may include metrics for the taxonomic composition of the phytoplankton such as trophic indicator lists or proportion of cyanobacteria.

The statistical pressure-response relationship between phosphorus and phytoplankton biovolume (or chlorophyll *a*) is often stronger than that for nitrogen, and this has also been used as an argument for focussing on phosphorus control (OECD 1982; Magumba *et al.*, 2014). However, in regions where lakes with low N : P predominate, nitrogen is the better predictor of phytoplankton biomass (White, 1983; Abell, Özkundakci & Hamilton, 2010). Using a model that estimates the N : P ratio at which phytoplankton biomass shifts between being better predicted by total nitrogen (TN) than total phosphorus (TP), Dolman & Wiedner (2015) show that the joint TN and TP pressure-response relationship is best modelled in a piecewise fashion, where phytoplankton biomass is a function of TN for low N : P lakes and TP for high N : P lakes. Furthermore, they show that ignoring the influence of nitrogen, or including it as a predictor in a standard regression model, results in TP targets that are too high. This is because the effects of N and P are non-additive and Liebig like, whereas standard multiple regression models assume additive or multiplicative effects of TN and TP.

We analysed a large database of lake water quality measurements to determine the frequency and seasonal patterns of nitrogen and phosphorus limitation for phytoplankton development in lowland German lakes of different types (e.g. mixing behaviour). We also estimated target concentrations for both TN and TP to achieve good ecological status according to WFD assessment criteria.

Methods

Outline of methods

We used the piecewise model of Dolman & Wiedner (2015), with data from 369 natural lakes in the north eastern lowland area of Germany, to estimate the N : P ratio (mass) at which phytoplankton biomass switches between being better predicted by TN or TP. We then used this ratio, together with absolute concentrations of dissolved inorganic nitrogen (DIN) and phosphorus (DIP), to predict the limiting nutrient at each lake in each month of the vegetation period. This allowed us to examine the seasonal and lake-type-specific patterns of N and P limitation for a large regional sample of lakes. We then used the estimated ratio to divide the data into primarily N and primarily P limited lakes and separately estimated target concentrations for TN and TP respectively from these groups. We compared targets estimated using phytoplankton biovolume as a simple index of ecological status, with targets estimated using the Phyto-See-Index (PSI), a complex multimetric index of ecological status developed for WFD assessment in Germany (Mischke *et al.*, 2008).

Data and data processing

We compiled water quality data for 369 natural lakes from the north eastern lowland area of Germany. These data came from multiple German state government offices and research institutes (Table S1) and were collected for water quality monitoring and the assessment of ecological status based on phytoplankton according to the European Water Framework Directive (EC 2000). Mixed water samples were usually collected from the deepest point of a lake. When fully mixed the water column was sampled down to approximately 0.5 m above the lake bottom for shallow lakes and down to the mean depth for deep lakes. During periods of stratification samples were taken from either the euphotic zone or the epilimnion, whichever extended deeper. Chemical and biological analyses of the samples were carried out according to ISO and EN standard methods (DEV 1981). Chlorophyll *a* was estimated by spectrometer after 90% ethanol extraction. Phytoplankton biovolumes were estimated following the national analysis instruction (DIN EN 16695 2013; Nixdorf *et al.*, 2014) by applying the most similar geometrical shape on the cell count results of each taxon (EN 15204:2006; Utermöhl, 1958).

For WFD assessment, lowland German lakes have been classified into seven types (Table 1) according to their mean depth, stratification behaviour, catchment size relative to volume (VQ) and hydraulic residence time (Mathes, Plambeck & Schaumburg, 2002). This classification system was designed for lakes greater than 0.5 km^2 (50 ha) but, in order to increase our sample size, we added 36 lakes smaller than 0.5 km^2 (50 ha) to their appropriate category given their other characteristics. The German type 11.2 covers the common European type L-CB2 for very shallow lowland lakes and German types 10.1 and 13 cover European type L-CB 1 (see EC 2008). The mean depths of these lakes range from <1 m to 29 m, with median mean depths of 7.5 m for the stratified lakes and 2.8 m for the polymictic. Overall they are a eutrophic set of lakes; using Carlson's trophic state ranges for total phosphorus (Carlson, 1977; Carlson & Simpson, 1996) of 12, 24, 96 and 384 + $\mu g\ L^{-1}$ TP, only 4% of the stratified lakes would be classed as oligotrophic, with 23%, 67% and 6% mesotrophic, eutrophic and hypereutrophic respectively. No polymictic lakes would be oligotrophic and only 4% mesotrophic, with 46% and 50% eutrophic and hypereutrophic. A summary of the limnological characteristics of these lakes is provided in Table S2.

Predicting biovolume from TN, TP or both

To create a data set with which to compare alternative pressure-response models and to estimate the N : P ratio at which phytoplankton biomass switches between being better predicted by TN and TP, we aggregated the data in the sequence given below.

Sequence 1.

1. Select samples taken during the vegetation period (April–October).
2. Calculate the mean monthly values of TN, TP, DIN, DIP, phytoplankton biovolume, $\log_{10}$ TN : TP, $\log_{10}$ DIN : DIP, $\log_{10}$ DIN : TP for each year at each lake from the samples selected in step 1.
3. Calculate the vegetation period means for each year at each lake from the monthly means calculated in step 2.
4. Take the mean over years for each lake to arrive at a single mean value of each variable for each lake.
5. Back-transform the mean of the nutrient ratios TN : TP, DIN : DIP and DIN : TP.

Table 1 Classification of German lowland lakes into seven lake types, together with approximate good-moderate ecological status boundaries for phytoplankton biovolume and chlorophyll *a* concentration (mean during the vegetation period, April–October). VQ is the volume quotient, catchment area (km^2) divided by lake volume (10^6 m^3); RT is the residence time (days). Good–Moderate boundaries are according to Mischke *et al.* (2008).

Lake Type	No. lakes (<50 ha)	Depth or stratification type	Catchment influence	Good-Moderate boundaries: Biovolume (mm^3 L^{-1})	Chlorophyll *a* (μg L^{-1})
11.2	90 (23)	Polymictic < 3 m	Medium (VQ > 1.5 m^{-1})	9.0	24.8
11.1	68 (11)	Polymictic ≥ 3 m	Medium (VQ > 1.5 m^{-1})	6.0	17.8
12	46 (0)	Riverine	Riverine (RT < 30 d)	8.9	32
14	5 (0)	Polymictic > 3 m	Small (VQ ≤ 1.5 m^{-1})	4.4	13.2
13	58 (2)	Stratified	Small (VQ ≤ 1.5 m^{-1})	1.7	8.6
10.1	77 (0)	Stratified	Medium (VQ > 1.5 m^{-1})	3.3	12
10.2	34 (0)	Stratified	Large (VQ > 15 m^{-1})	3.3	12

6. Exclude lakes where the mean DIN concentration was above 140 μg L^{-1} AND mean DIP was above 25 μg L^{-1} (means from step 4).

The nutrient ratios, TN : TP, DIN : DIP and DIN : TP (mass), were log-transformed before aggregation because ratios between two positive variables naturally have highly skewed distributions. In step 6, we excluded data from 80 lakes where both their mean DIN and mean DIP concentrations were high and therefore phytoplankton biovolume was unlikely to be limited by either N or P.

To the lake means resulting from sequence 1 we fitted and compared a set of regression models described in Dolman & Wiedner (2015) to:

a). Test whether biovolume is better predicted from TN when a lake has low N availability relative to P, and from TP when P is scarce relative to N, with relative N and P availability measured by one of three N : P ratios: TN : TP, DIN : DIP or DIN : TP;
b). Determine which of three N : P ratios better discriminates between TN and TP as alternative predictors of biovolume; and
c). Estimate the value of this ratio that best splits lakes into TN predicted versus TP predicted groups.

Details of the models are given in Table 2. Models 1a and 1b each predict biovolume from the concentration of a single nutrient, TN or TP respectively. Model 2 predicts the biovolume of each lake from both TN and TP. Models 3a-c are piecewise functions that predict biovolume from either TN or TP according to the whether the value of an N : P ratio is above or below the critical ratio b_R. N_i/P_i can represent the TN : TP (3a), DIN : DIP (3b) or DIN : TP (3c) ratios.

For each model described above, two versions were fitted: one where only the intercept parameter varies between lake types, and one where the slope also varies between lake types, denoted by the additional subscript (LT). Models 1 and 2 are standard linear regression models and were fit with the function *lm* from the R language (R Core Team, 2012). For a given value of parameter b_R, models 3a–c can also be written as linear models and fit with *lm*. For each of the three N : P ratios we used a simple line search method to find the optimum value, b_R, at which to split lakes into low and high N : P groups.

Seasonal and lake-type-specific patterns of limitation

To quantify seasonal lake-type-specific limitation patterns we classified each lake-month into one of three limitation categories and then calculated for each month and lake–type the proportion of N, P or neither N nor P limited lakes for each.

a). Likely N limited: (DIN < 100 μg L^{-1} AND DIN : TP < 1.6).
b). Likely P limited: (DIP < 10 μg L^{-1} AND DIN : TP ≥ 1.6).
c). Neither N nor P limited: Everything else
   i. (DIN > 100 μg L^{-1} AND DIP > 10 μg L^{-1}) OR
   ii. (DIN < 100 μg L^{-1} AND DIN : TP ≥ 1.6 AND DIP > 10 μg L^{-1}) OR
   iii. (DIP < 10 μg L^{-1} AND DIN > 100 μg L^{-1} AND DIN : TP < 1.6)

We used absolute thresholds of 100 μg L^{-1} for DIN and 10 μg L^{-1} for SRP suggested by Maberly *et al.* (2002) as prerequisites for N and P limitation respectively. In nutrient addition experiments, positive responses to nitrogen and phosphorus additions were rarely observed when initial concentrations of DIN or DIP were above these thresholds (Morris & Lewis, 1988; Ptacnik, Andersen & Tamminen, 2010; Kolzau *et al.*, 2014). On their own, low DIN or DIP concentrations are an unreliable indicator of N or P limitation respectively

Table 2 Alternative pressure-response regression models. B_i, TP_i and TN_i are variables representing the mean biovolume of phytoplankton, mean TN and mean TP concentration in lake i respectively; b_{LT} represents a vector of intercept parameters, one for each lake type (LT); $b_{TN(LT)}$ and $b_{TP(LT)}$ are fitted parameters for the slope of the relationship between $\log_{10}$ Biovolume and $\log_{10}$ TN (model 1a) or $\log_{10}$ TP (model 1b); ε is a normally distributed random error term.

Model	Function	Model type
1a	$\log(B_i) = b_{\mathrm{LT}} + b_{\mathrm{TN(LT)}} \cdot \log(\mathrm{TN}_i) + \varepsilon$	Single linear
1b	$\log(B_i) = b_{\mathrm{LT}} + b_{\mathrm{TP(LT)}} \cdot \log(\mathrm{TP}_i) + \varepsilon$	Single linear
2	$\log(B_i) = b_{\mathrm{LT}} + b_{\mathrm{TN(LT)}} \cdot \log(\mathrm{TN}_i) + b_{\mathrm{TP(LT)}} \cdot \log(\mathrm{TP}_i) + \varepsilon$	Multiple linear
3a,b,c	$\log(B_i) = \begin{cases} b_{\mathrm{LT\_N}} + b_{\mathrm{TN(LT)}} \cdot \log(\mathrm{TN}_i) + \varepsilon, \mathrm{N}_i/\mathrm{P}_i < b_{\mathrm{R}} \\ b_{\mathrm{LT\_P}} + b_{\mathrm{TP(LT)}} \cdot \log(\mathrm{TP}_i) + \varepsilon, \mathrm{N}_i/\mathrm{P}_i \geq b_{\mathrm{R}} \end{cases}$	Piecewise

as phytoplankton can have considerable internal stores of nutrients (Reynolds, 2006). Several previous studies have found the DIN : TP ratio to be a good indicator of limitation status (Morris & Lewis, 1988; Axler, Rose & Tikkanen, 1994; Bergström, 2010; Ptacnik *et al.*, 2010; Kolzau *et al.*, 2014). We used the DIN : TP ratio with a value of 1.6 (mass ratio, see results) to discriminate between N and P limitation when concentrations of both DIN and DIP were low, and additionally to exclude limitation when for example DIP concentrations were low (<10 μg L^{-1}) but the DIN : TP ratio was high.

Target concentrations for TN and TP to achieve a good ecological status

The ecological status of German lakes are assessed for WFD reporting using the Phyto-See-Index (PSI) a multimetric index that combines measurements of chlorophyll *a* concentration, phytoplankton biovolume and taxonomic composition (Mischke *et al.*, 2008). Therefore, to derive target nutrient concentrations, we estimated the pressure-response relationships for PSI in addition to biovolume. PSI was calculated for individual lake-years using the software PhytoSee version 5.0 (available on request; an updated version PhytoSee 6.0 is available here: http://www.igb-berlin.de/datenbanken.html). To obtain separate N limited and P limited data sets, we aggregated the data in the following sequence.

Sequence 2.

1. Repeat steps 1–3 from sequence 1 above.
2. Split the data into separate N and P limited sets of lake–years according to the criteria below; the same lake can appear in both the N and P limited sets if data from different years indicate a switch in limitation type.
   a. Likely N limited: (Mean DIN < 140 μg L^{-1} AND Mean DIN : TP < 1.6).
   b. Likely P limited: (Mean DIP < 25 μg L^{-1} AND Mean DIN : TP ≥ 1.6).
   c. Neither N nor P limited:
      i. (Mean DIN > 140 μg L^{-1} AND Mean DIP > 25 μg L^{-1}) OR
      ii. (Mean DIN < 140 μg L^{-1} AND Mean DIN : TP ≥ 1.6 AND Mean DIP > 25 μg L^{-1}) OR
      iii. (Mean DIP < 25 μg L^{-1} AND Mean DIN > 140 μg L^{-1} AND Mean DIN : TP < 1.6)
3. Treating the N and P limited sets separately, calculate single lake means for each variable.
4. From the N limited set, exclude lakes where mean TN > 2500 μg L^{-1}; for P limited lakes where mean TP > 300 μg L^{-1}.

We used higher absolute thresholds for DIN and DIP to infer limitation status for whole seasons than to infer limitation for individual samples.

To the N limited data set, we fitted regression models predicting PSI and $\log_{10}$ biovolume from $\log_{10}$ TN. Despite the presence of measurement uncertainty in mean TN (and TP) concentrations, we used ordinary least squares (OLS) and not major-, or reduced-major-axis regression, because the uncertainty in measured ecological status (biovolume or PSI) was much larger than that for nutrients, and so any resulting bias in slopes would be small (McArdle, 1988). Regression models contained separate intercept parameters for each lake type, but a single slope parameter. Models with different slopes for each lake type were tested but had higher AIC values (Akaike's

Information Criterion, Burnham & Anderson, 2004); we used the simpler single slope models to reduce variance in the predictions. Because we were interested in estimating pressure-response relationships when TN concentrations are low, we excluded lakes where the mean TN concentration exceeded 2500 μg L^{-1}. To the P limited set we fit the equivalent models for TP, excluding lakes where mean TP was above 300 μg L^{-1}.

We used these fitted models to calculate, for each lake type, the nutrient concentration where the expected PSI (or biovolume) crossed the boundary between good and moderate status. This is illustrated in Fig. 1 for a single lake type and nutrient; plots for each lake type and nutrient are given in Figs S1–S4. For biovolume, we used the boundary values adopted by the German working group on water issues of the federal states and the federal government represented by the federal environment ministry LAWA (Mischke *et al.*, 2008). These were developed in accordance with the WFD (EC 2008), and following an intercalibration exercise between member states (EC 2008; Poikane *et al.*, 2011). The German PSI is scaled so that a value of 2.5 lies on the boundary between good and moderate status.

To obtain confidence intervals for these target values we sampled from the variance covariance matrix of the parameters in the fitted model. In the case of lake type 14 (polymictic lakes with a mean depth ≥ 3 m and small catchment relative to lake volume, VQ < 1.5), for which there were very few lakes, we used the boundary values for type 14 together with parameter values estimated for lake type 11.1 (polymictic lakes with mean depth > 3 m but a moderate catchment relative to volume, VQ > 1.5). These targets correspond to the nutrient concentration at which 50% of lakes are expected to be in a good ecological status, when that nutrient is limiting.

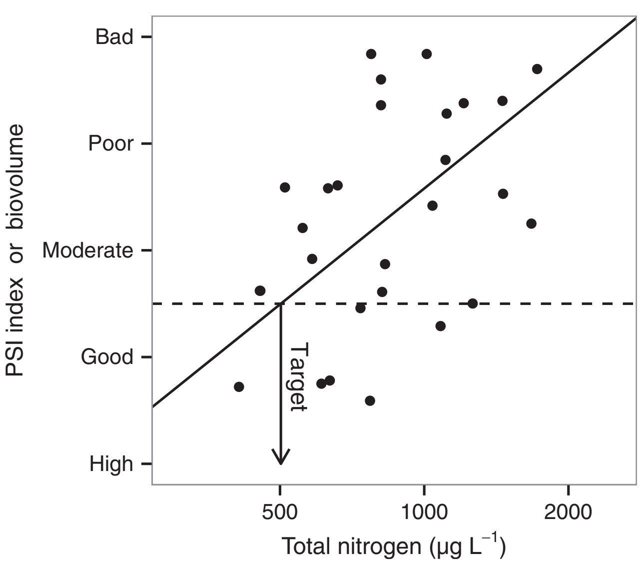

Fig. 1 Example of TN target estimation procedure. For each lake type, a regression model with $\log_{10}$ TN predicting $\log_{10}$ biomass, or PSI, was used to estimate an appropriate target concentration for TN. The target corresponds to the TN concentration at which the regression line crosses the boundary between Good and Moderate status. For TN targets, only low DIN : TP ratio lakes were used, that is, those for which N availability is therefore expected to influence phytoplankton biomass development.

Results

Predicting biovolume from TN, TP or both

When a single nutrient was used (models 1a and 1b), TP was the better predictor of phytoplankton biovolume in terms of R^2 and was the better fitting in terms of AIC (Table 3). Using both TN and TP as predictors for all lakes (model 2) made only a slight improvement to R^2 and AIC values were similar. In contrast, piecewise models (models 3a–c), all performed considerably better than standard single or dual predictor regression models. AIC was lowest at some intermediate value for all three N : P ratios tested and the lowest (best) AIC values were achieved by model 3c, using a DIN : TP ratio of 1.6 (mass ratio) to discriminate between prediction of biovolume from TN or TP.

Lower AIC values (indicating better fit) were obtained for all models when slopes were kept the same for all lake types, that is, the simpler models performed almost as well as the more flexible models with more parameters, and therefore were preferred on the basis of parsimony.

Seasonal and lake-type-specific patterns of limitation

We found clear differences between lake types in the proportion of lakes predicted to be P, N and non-nutri-

Table 3 Goodness of fit statistics for the standard and piecewise regression models. b_R indicates the best fit value of the corresponding N : P ratio (mass) for piecewise models. dAIC is the difference in AIC between each model and the best fitting model; lower values are better.

		Varying intercepts			Varying slopes and intercepts		
	Model	b_R	dAIC	R^2	b_R	dAIC	R^2
1a	TN		101	0.44		104	0.46
1b	TP		64	0.51		70	0.52
2	TN + TP		61	0.51		76	0.53
3a	TN or TP | TN : TP	29.4	18	0.60	29.4	30	0.62
3b	TN or TP | DIN : DIP	8.4	13	0.60	8.6	31	0.61
3c	TN or TP | DIN : TP	1.6	0	0.62	1.6	14	0.63

ent limited (Fig. 2). There were also differences in the strength of seasonal shifts between these limitation categories. In stratified lakes, P limitation occurred more often than N limitation (Fig. 2), particularly in the deepest lakes with the smallest catchments relative to lake volume (type 13). Approximately 70% of lakes in this category were predicted to be P limited and this proportion was more or less constant across all months. N–limitation peaked at around 30% in July, and fewer than 20% of lakes were neither N nor P limited. For the other stratified lake types, P limitation declined from 60–70% of lakes in April–May to 40–50% in August–October, while N limitation increased to about 45% by late summer.

In polymictic lakes, N Limitation occurred more often than P limitation and the seasonal shift from P to N–limitation was much more pronounced. In non-riverine polymictic lakes, P–limitation declined strongly from 60–70% in April to 10–20% in July–September, while N limitation increased to 50–60% of lakes in the summer (June–September) period. In the riverine category we found the highest proportion of lakes that were limited by neither N nor P.

Target concentrations for TN and TP to achieve a good ecological status

Estimated target values were highest for very shallow lakes (11.2) and riverine lakes (12) and lowest for stratified lakes (10.1, 10.2) especially those with small catchment influence (13) (Fig. 3 and Table 3). This is largely a reflection of the higher boundary values allowed for shallow lakes given their naturally higher trophic status; if all lake types were 'allowed' the same biovolume of phytoplankton, shallow lakes would require the lowest target nutrient concentrations to compensate for their generally higher yields of biovolume for a given nutrient concentration (see Fig. S5), due to, for example, higher average water column light intensity (Nixdorf & Deneke, 1997). Fitted regression parameters are listed in Table S3.

PSI and biovolume derived targets were similar for most lake types, with the exception of the very shallow lakes, for which the PSI derived target was much lower (Fig. 3, Table 4). Confidence intervals for PSI targets were narrower than those for biovolume; this is a consequence of the lower between-lake variance in PSI than biovolume, which is expected from a metric that combines measurements of multiple variables. PSI targets were also less variable across lake types than those derived from biovolume.

Comparing the estimated target concentrations with the observed range of in-lake TN and TP concentrations (Fig. 4), it is clear that many lakes had concentrations far above their targets. Of those lakes above both TN and TP targets, 85% (73–95% depending on lake type) were classified as having moderate or worse ecological status according to their biology (PSI). Of those lakes with TP concentrations below their targets, 70% (60–80%) had good or very good ecological status. Very few

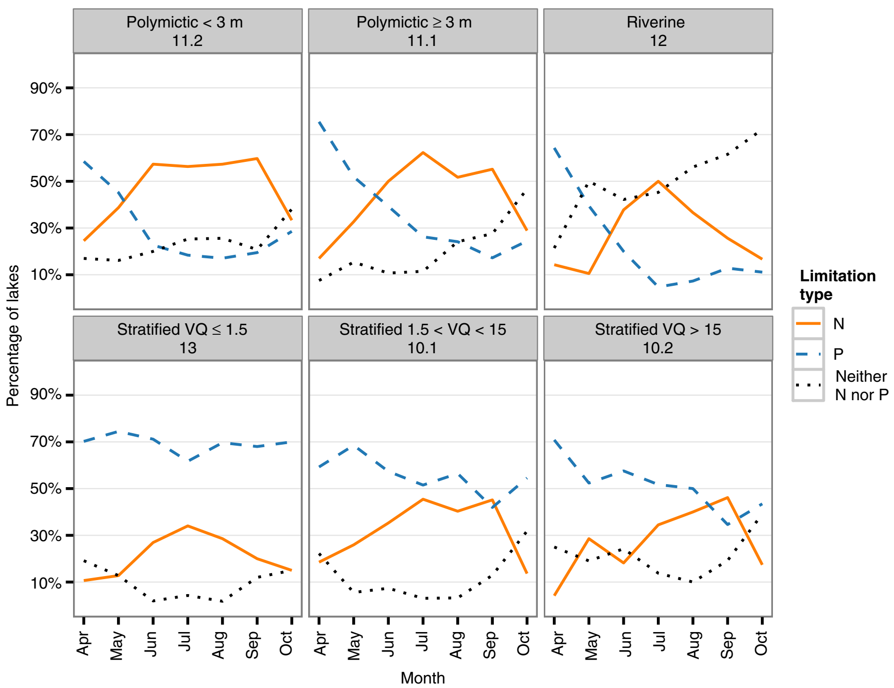

Fig. 2 Seasonal limitation patterns in different lake types. Lines show the proportion of lakes whose phytoplankton biovolume is predicted to be limited by nitrogen, phosphorus, or by neither nitrogen nor phosphorus. Groups are according to the German typology for calcareous lowland lakes. Depths (e.g. ≥3 m) refer to mean depth, the volume quotient (VQ) is the catchment area in km^2 divided by the lake volume in km^3.

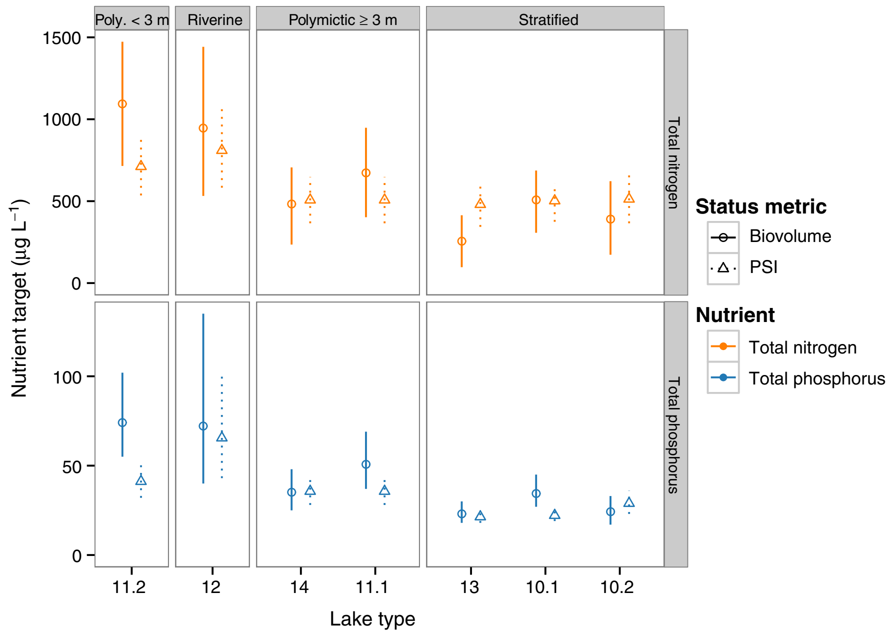

Fig. 3 Estimated target concentrations for total nitrogen and total phosphorus to achieve good ecological status for seven lowland German lake types. Vertical bars indicate 95% confidence intervals. Groups are according to the German typology for calcareous lowland lakes. Depths (e.g. ≥3 m) refer to mean depth, the volume quotient (VQ) is the catchment area in km^2 divided by the lake volume in km^3.

Table 4 Estimated target nutrient concentrations to achieve good ecological status for seven lowland German lake types.

	TN target (µg L^{-1})		TP target (µg L^{-1})	
Lake type	Biovolume	PSI	Biovolume	PSI
11.2, Poly < 3 m, VQ > 1.5	1090 (740–1470)	710 (520–880)	74 (54–103)	41 (32–51)
12, Riverine, RT < 30 d	950 (530–1440)	810 (570–1060)	72 (38–141)	66 (43–101)
14, Poly ≥ 3 m, VQ < 1.5 m^{-1}	480 (240–690)	510 (360–640)	35 (25–47)	36 (28–44)
11.1, Poly ≥ 3 m, VQ > 1.5	670 (400–920)	510 (360–640)	51 (37–69)	36 (28–44)
13, Stratified, VQ < 1.5 m^{-1}	260 (100–410)	480 (350–620)	23 (18–30)	21 (18–25)
10.1, Stratified, VQ > 1.5 m^{-1}	510 (310–690)	500 (380–610)	34 (27–45)	22 (19–26)
10.2, Stratified, VQ > 15	390 (180–630)	510 (360–680)	24 (16–34)	29 (23–36)

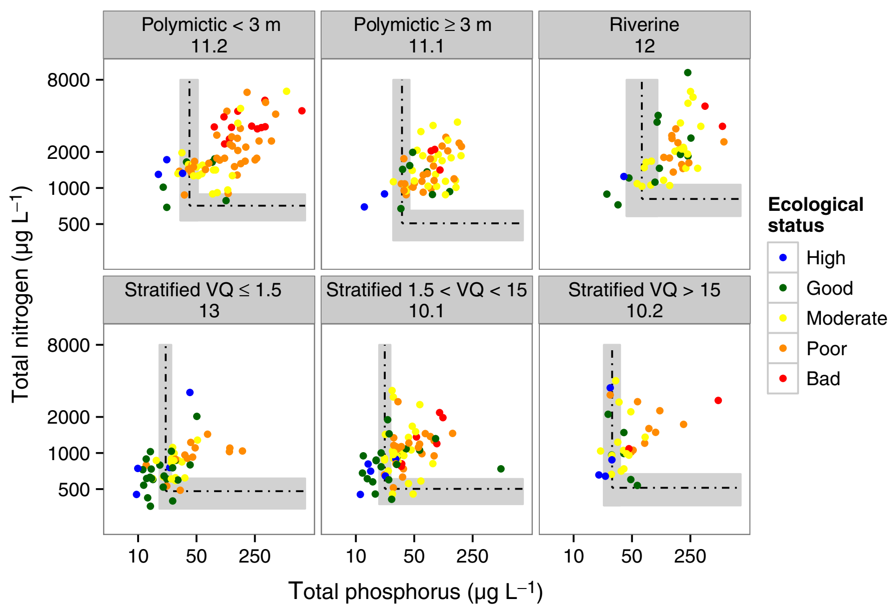

Fig. 4 A comparison of current TN and TP concentrations with estimated target concentrations. Target nutrient concentrations are shown with vertical (TP) and horizontal (TN) dotted lines, confidence intervals for these targets are indicated by the grey shaded region around each dotted line. Groups are according to the German typology for calcareous lowland lakes. Depths (e.g. ≥3 m) refer to mean depth, the volume quotient (VQ) is the catchment area in km^2 divided by the lake volume in km^3.

lakes of any type were below their TN target, and most of these were also below their TP target. The lack of lakes with low TN concentrations is the main reason why confidence intervals are wider for TN than TP targets. Nevertheless, confidence intervals, even for TN targets, are narrow relative to the distribution of in-lake nutrient concentrations. About 15% of lakes were classified as having a good or very good biological (PSI) ecological status despite being above both their TN and TP targets (Fig. 4).

Discussion

While phosphorus limits phytoplankton biovolume in many lakes, and particularly in deep lakes, when N : P ratios are low, nitrogen is a better predictor of phytoplankton biovolume than is phosphorus. We have shown this here for German lowland lakes, and previously for lakes in Florida and across the USA (Dolman & Wiedner, 2015). Phytoplankton in subsets of European lakes with TN : TP < 10 (Phillips *et al.*, 2008), and eutrophic lakes with low N : P ratios (McCauley & Downing, 1991) also have stronger relationships with TN than TP. Here, we have fitted a model that assumes very strict Liebig limitation, and this performs much better than standard regression models that assume a degree of co-limitation at all N : P ratios. At intermediate N : P ratios, strict Liebig limitation probably does not apply to multi-species phytoplankton communities (Danger *et al.*, 2008), but in lakes with N : P ratios that are far from optimal for most taxa, total phytoplankton biomass will be influenced much more strongly by the nutrient in least supply. Therefore, in cross-lake studies, the apparent importance of N and P will vary strongly with the ratios of N and P in the set of lakes studied.

There are strong seasonal patterns in the concentrations and ratios of TN and TP in lakes (Søndergaard, Jensen & Jeppesen, 2005; Kolzau *et al.*, 2014), particularly in shallow lakes where TP and TN often show opposing seasonal trends, with a summer peak in TP concentration coinciding with the lowest annual TN concentrations. As external loading of TN and TP are both highest in the wetter winter months and lowest in summer (Søndergaard *et al.*, 2005), opposing trends in concentrations are likely to be due to enhanced denitrification and sediment P release in summer, both of which are more pronounced in shallow lakes (Moss *et al.*, 2012). Indeed, here we found strong lake-type and seasonal variation in inferred limitation status, with N limitation much more common during summer and in shallow and polymictic lakes. The impact on water quality of controlling nitrogen concentrations is therefore likely to be the greatest for shallow lakes, and on phytoplankton biomass during summer rather than the spring bloom. In contrast, P control may be more effective for stratifying lakes, and particularly for oligotrophic lakes in areas of high atmospheric N deposition, which can shift otherwise N limited lakes to P limitation (Elser *et al.*, 2009; Bergström, 2010). In estimating target nutrient concentrations, we averaged within-lake seasonal variation in concentrations because, at the present time, targets are defined as annual or vegetation period means.

Nutrient targets depend on both the estimated pressure-response relationships and the good-moderate status boundary adopted for a particular lake type. For the German PSI index, which has been calibrated against the systems of other EU member states (Poikane *et al.*, 2011), these boundaries correspond to approximate chlorophyll *a* concentrations of 25, 18 and 12–8 μg L^{-1} for very shallow (<3 m), polymictic (>3 m), and stratified lakes respectively. These match quite well with thresholds estimated by Poikane *et al.* (2014) of 21–23 μg L^{-1} chlorophyll *a* for very shallow lakes (<3 m mean depth) and 10–12 μg L^{-1} for moderately deep lakes (mean depth 3–15 m) based on changes in cyanobacterial dominance, macrophyte abundance and macrophyte colonisation depth.

The pressure-response relationships themselves were estimated using a set of mainly eutrophic lakes and so we cannot be sure how well they would extrapolate to lower nutrient ranges. Some taxa can achieve very low nutrient cell quotas in oligotrophic conditions, or regulate their depth to access nutrients in the metalimnion or near the sediment (Reynolds, 2006) and so low nutrient concentrations in surface waters may not control biomass in all cases. Nevertheless, our estimates of the nutrient concentrations needed to achieve good ecological status do correspond quite well with those from other studies, despite variation in the objectives and methods. For example, shallow high–alkalinity lakes in the UK, comparable with types 14 and 11.1, have a TP target of 35 μg L^{-1} (22–46); while very shallow lakes comparable with type 11.2 have a target of 49 μg L^{-1} (33–75) (UK Environmental Standards and Conditions (Phase 2)). Similarly, Carvalho *et al.* (2013), used non-linear quantile regression to estimate targets to avoid cyanobacterial blooms. For lakes/reservoirs with high importance for water supply or recreation they suggest a target of <20 μg L^{-1} TP for a low (<10%) risk of breaching the low risk WHO threshold for recreational use.

The lake-type-specific targets calculated here are intended to indicate the typical nutrient concentrations required to achieve good ecological status for groups of lakes with similar morphology. However, the biovolume and composition of phytoplankton is influenced by many additional factors that vary between lakes, such as water temperature and flushing rate (Elliott, 2012; Rigosi *et al.*, 2014). Consequently, individual lakes may not achieve good status despite having concentrations below these targets, and conversely, some lakes will have good status at nutrient concentrations above these targets. Indeed here 30% of lakes below their TP target had only moderate status (or worse), while some 15% of lakes had good or very good status despite having concentrations above both their TP and TN targets. This could pose a problem for lake managers if these nutrient targets were adopted as supporting element boundary values, because the wording of the WFD states that the lower (worse) of biological and supporting element metrics should be used to report ecological status (EC 2000 Annex V section 1.4.2). Lakes with good (biological) status would have to be reported as failing if they were above nutrient boundaries. However, we would argue that lakes with good biological status, despite high nutrient concentrations, are nevertheless vulnerable to a rapid deterioration if other conditions such as water retention time or temperature were to change.

While the utility of limiting phosphorus inputs is widely accepted, there is less agreement about whether nitrogen can be used to control phytoplankton biomass in fresh waters. It has been argued that N_2-fixing cyanobacteria cannot be controlled via nitrogen as their biomass is not restricted by the supply of reactive nitrogen and they may replace all or a portion of the nitrogen removed in water improvement programmes (e.g. Schindler *et al.*, 2008). However, it appears that N fixation cannot in general compensate fully for nitrogen deficiency, either because N fixation rates are restricted by low light or micronutrient availability (Lewis & Wurtsbaugh, 2008; Moss *et al.*, 2012), or because elimination of N via denitrification is correlated in time and space with N fixation, so that N-deficient states are maintained or quickly regained (Halm *et al.*, 2009; Scott & Grantz, 2013; Grantz, Haggard & Scott, 2014).

Improving the water quality of lakes, rivers and other waterbodies is an environmental issue with considerable public and political backing, as evidenced by legislation such at the WFD in Europe, and by the public's willingness to pay (e.g. Meyerhoff, Boeri & Hartje, 2014). Substantial reductions in phosphorus concentrations have been achieved and have undoubtedly improved water quality (Jeppesen *et al.*, 2005). However, the focus on a controlling phosphorus alone may have limited improvement in very shallow lakes that are often N limited, and furthermore may have had a detrimental effect on coastal environments by reducing nitrogen removal rates in lake and river systems and increasing downstream export (Finlay, Small & Sterner, 2013). So while phosphorus reductions have been very beneficial, and should be continued, a broader catchment perspective should be taken and nitrogen supplies reduced in tandem.

While there is some uncertainty in the estimated target concentrations, particularly for riverine lakes, this uncertainty is small compared to the range of nutrient concentrations currently seen in these lakes. There seems to be little risk of overshooting either the nutrient targets themselves, or the desired improvement in ecological status. Were the proportion of lakes achieving good status to increase it would become easier to refine these estimates in the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Details of the research institutes and regional government offices from which the Nitrolimit data were obtained.

Table S2. Limnological characteristics of the lakes used in these analyses.

Figure S1. Lake-type-specific relationships between phytoplankton biovolume and total nitrogen for lakes predicted to be predominantly limited by nitrogen.
Figure S2. Lake-type-specific relationships between phytoplankton biovolume and total phosphorus for lakes predicted to be predominantly limited by phosphorus.
Figure S3. Lake-type-specific relationships between PSI and total nitrogen for lakes predicted to be predominantly limited by nitrogen.
Figure S4. Lake-type-specific relationships between PSI and total phosphorus for lakes predicted to be predominantly limited by phosphorus.
Figure S5. Predicted phytoplankton biovolume at fixed nutrient concentrations of 500 μg L^{-1} TN or 30 μg L^{-1} TP.

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