Latitudinal variation in nutrient stoichiometry and chlorophyll-nutrient relationships in lakes: A global study

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With 5 figures and 5 tables

Abstract: We present analysis of variations in relationships between nitrogen (N), phosphorus (P) and chlorophyll-*a* (chl-*a*) in lakes along a gradient of latitude inclusive of tropical, temperate and polar regions. Total nitrogen (TN), total phosphorus (TP), chl-*a*, latitude and depth data were collated for 1316 lakes situated between 70 °S and 83 °N. Latitudinal variation was then analysed for three empirical measures of phytoplankton nutrient limitation and/or nutrient assimilation. Lastly, chl-*a* near-maxima conditional on TN and TP abundance were empirically defined for this global dataset using quantile regression. Mean TN:TP increases with distance from the equator. This relationship is independent of variation in either lake depth or trophic state, reflecting latitudinal variation in nutrient cycling processes and/or nutrient sources. There is a negative linear relationship between latitude and chl-*a*:TN which similarly suggests that N is less abundant relative to phytoplankton growth requirements at lower latitudes. Relative to temperate lakes, the statistical capability of TN and TP to predict chl-*a* is poor for both tropical and polar lakes, reflecting latitudinal variation in lake ecosystem functioning and the subsequent potential unsuitability of applying relationships derived for temperate lakes elsewhere. Chl-*a* near-maxima correspond to chl-*a*:TN and chl-*a*:TP yields of 0.046:1 and 0.87:1 respectively, although some observations greatly exceed near-maxima, suggesting possible physiologically plastic phytoplankton responses in these exceptional cases. Deficiencies in understanding the mechanisms that drive variation in macro-nutrient stoichiometry and phytoplankton biomass-nutrient relationships across large spatial scales necessitates further landscape-scale research on this topic, particularly in the tropics.

Key words: eutrophication, nitrogen, nutrient limitation, phosphorus, phytoplankton, polar, quantile regression, tropical.

Introduction

As primary producers, phytoplankton are a fundamental component of lake ecosystems, occupying a pivotal role in the global carbon cycle and providing resources to support higher trophic levels (Reynolds 2006). Following anthropogenic perturbation, however, phytoplankton can become excessively abundant, resulting in adverse ecological and economic impacts (Smith 2003). Resource limitation theory states that the yield of phytoplankton biomass is controlled by the availability of the factor (e.g. an essential macronutrient) most deficient in relation to algal growth requirements (von Liebig 1885, Klausmeier et al. 2004). This theory forms the basis of bottom-up models that have been used for decades to quantitatively predict phytoplank-

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ton biomass (usually inferred from chlorophyll-*a* (chl-*a*) concentration), based on the abundance of the essential nutrients nitrogen (N) and phosphorus (P) (e.g. Sakamoto 1966, Canfield & Bachmann 1981, OECD 1982, Phillips et al. 2008). Researchers typically focus on the mean response of dependent variables in such models, although in common with many other ecological data, the law of limiting factors (von Liebig 1885) imposes a 'factor ceiling' to data distributions (Thomson et al. 1996). Examining other portions of data distributions can therefore also provide insight to ecological relationships (Scharf et al. 1998); however, such an approach has so far been applied sparingly to limnological datasets (e.g. Kaiser et al. 1994, Jones et al. 2011).

Understanding how variation in nutrient abundance affects phytoplankton biomass has relevance to applied ecology and recent debate has focused on the relative importance of N and P in limiting phytoplankton biomass and the subsequent implications for developing effective policies to control eutrophication (Moss et al. 2012). As in this study, applied scientists are typically concerned with understanding the factors that regulate phytoplankton standing crop, as opposed to the factors that limit the instantaneous growth rate of individual species which may vary within a single assemblage (Hecky & Kilham 1988, Reynolds 2006). To date, empirical analyses of chl-*a*-nutrient relationships have predominantly (and often exclusively) considered lakes at northern temperate latitudes (e.g. OECD 1982). These analyses frequently show P to be a better predictor of chl-*a* than N, supporting the view that P is the principal nutrient that limits the production of phytoplankton biomass in lakes, with N occupying a secondary role (Sterner 2008). This view is divergent from the results of a large-scale meta-analysis of nutrient enrichment experiments which found that phytoplankton in lakes are equally responsive to N and P additions (Elser 2007).

Results of enrichment experiments suggest that there may be a latitudinal influence on chl-*a*-nutrient relationships, as bioassays suggest that N limitation may be more prevalent in tropical than temperate lakes (Hecky & Kilham 1988, Elser et al. 2007). Several hypotheses have been suggested to explain why chl-*a*-nutrient relationships might vary with latitude, including correlation between latitude and: nutrient inputs (Schindler 1978), light limitation (Canfield & Bachmann 1981), temperature-dependent metabolic enzyme activity (Markager et al. 1999), denitrification rate (Lewis 1996, 2002), rate of P weathering (Lewis 1996), stratification processes (Lewis 1990) and top-

down control (Flanagan et al. 2003). Despite this, only a small number of studies have explicitly compared chl-*a*-nutrient relationships between temperate and non-temperate lakes (e.g. Jones et al. 2000, Flanagan et al. 2003, Huszar et al. 2006). Such comparisons have yielded discordant results, for example Jones et al. (2000) showed that the chl-*a*-total phosphorus (TP) relationship in 13 reservoirs in Thailand (c. 13–16° N) matches that of temperate systems, a finding that is contrary to that of Huszar et al. (2006) who studied 192 tropical lakes and found the chl-*a*-TP relationship to be markedly poorer compared to typical temperate models. Evidently, understanding of how phytoplankton biomass-nutrient relationships vary with latitude is lacking, and as yet, no study has sought to determine how latitude influences chl-*a*-nutrient relationships in lakes along a global latitudinal gradient.

Here, we investigate relationships between latitude, chl-*a*, total nitrogen (TN) and TP using data from a large sample of lakes. To the best of our knowledge, the sample is the largest used to date to examine these relationships and comprises lakes situated along a latitudinal gradient that includes tropical, temperate and polar regions. As is inevitable with such a broad-scale study, individual measurements may not necessarily be representative of lakes within a specific latitudinal range. However, by examining relationships *between* variables along a global latitudinal cline, we seek to address the following research questions:

1. Does relative nutrient abundance (TN:TP) in lakes vary with latitude?
2. How do phytoplankton biomass-nutrient relationships differ between latitudinal zones?
3. Is there evidence of latitudinal variation in nutrient limitation of phytoplankton biomass in lakes?

Methods

Data collection

We focused analysis on latitudinal variation in three empirical measures of either phytoplankton nutrient limitation or nutrient assimilation (Table 1). Matched concentrations (mg m^{-3}) of chl-*a*, TN and TP were obtained for unfiltered water samples taken from 1317 lakes. Data were obtained for lakes in over 30 countries, situated from 69.5 ° S –83.0 ° N. In addition, latitude (to the nearest 0.1 degree) and data for lake depth (z_{max} or z_{mean}) were obtained for each of the lakes in the sample. Where possible, mean concentrations relating to a number of samples collected over several years were obtained for trophic state variables, however, in some instances values related to one or more samples taken during a single day. This is particularly the case for polar lakes, many of which are difficult to access and only

Table 1. Summary of empirical measures used in this study to quantify nutrient limitation of phytoplankton biomass and/or chlorophyll-*a* (chl-*a*)-nutrient relationships in lakes.

Empirical measure	Variable	Rationale
Mass ratio of total nitrogen (TN) to total phosphorus (TP)	TN:TP	An indicator of the relative availability of each respective nutrient, widely used to infer whether the accumulation of phytoplankton biomass in a lake is likely to be limited by N or P, or, whether the stoichiometry of the two nutrients is broadly consistent with phytoplankton growth requirements (Hecky & Kilham 1988). The predictive power of this indicator is moderate and strongly dependent on the refractory portion of the TN pool (Ptacnik et al. 2010).
Proportion of variation in mean chl-*a* attributed to variation in TN or TP	Standardised regression coefficients (β^*)	Linear regression statistics are widely used to describe the relationship between algal biomass and nutrient concentrations (Kaiser et al. 1994). Critical interpretation of relationships is necessary due to frequent covariation between N and P and non-independence of variables (Lewis & Wurtsbaugh 2008).
Yield of chl-*a* per mass unit of TN or TP	chl-*a*:TN, chl-*a*:TP	Chl-*a* yield is used as an indicator of nutrient assimilation by phytoplankton, reflecting both bottom-up and top-down processes (Stauffer 1993).

ice-free for a short period of the year. Data sources comprised published papers as well as national or regional databases of lake monitoring data (Table 2).

Lake classification

Lakes were assigned to four latitudinal categories: tropical (0–23.5° N/S), low-temperate (23.6–44.5° N/S), high-temperate (44.6–66.5° N/S) and polar (66.6–90° N/S). Boundaries of 'tropical' and 'polar' categories reflect the approximate axial tilt of the Earth, while the temperate zone was sub-divided to ensure that the four categories were approximately equal in ° N/S. We also assigned lakes to two categories based on depth: deep and shallow. For 1202 (91 %) of the lakes in the sample, we used the criterion of $z_{mean} \leq 3.0$ m to define shallow lakes (see Padisák & Reynolds 2003), or when z_{mean} was unknown, we defined lakes where $z_{max} \leq 10$ m as shallow. Data for the remaining 114 lakes (9 %) were obtained from several studies of 'ponds' and 'lakes' undertaken in the Arctic. 'Ponds' were defined as sites which completely froze to the bottom in winter whereas 'lakes' did not (e.g. see Michelutti et al. 2002). For the purpose of our study, we defined 'ponds' as shallow and 'lakes' as deep. Lakes were assigned to the following trophic state categories based on chl-*a* concentration (mg m^{-3}) defined in the OECD (1982) fixed boundary system: ultra-oligotrophic (chl-*a* ≤ 1.0), oligotrophic ($1.0 <$ chl-*a* ≤ 2.5), mesotrophic ($2.5 <$ chl-*a* ≤ 8.0), eutrophic ($8.0 <$ chl-*a* ≤ 25.0) and hypertrophic (chl-*a* > 25.0). Only one polar lake was in the eutrophic category and no polar lake was classified as hypertrophic.

Data analysis

The combined dataset was checked for outliers and one lake with an anomalously high TP concentration (> 9000 mg m^{-3}) was removed, resulting in a total sample size of 1316 lakes. Data for all trophic state parameters were logarithmically (base 10) transformed to achieve normal distributions prior to analysis using parametric tests. To conform with typical analytical detection limits, minimum chl-*a*, TP and TN concentrations were set at 0.1, 0.1 and 1.0 mg m^{-3}, respectively, and all concentrations below these values were therefore adjusted to the respective minima prior to transformation. The distribution of in-lake TN:TP displayed high kurtosis and was skewed to the right, so consequently, this variable was $\log_{10}$ transformed to achieve normal distribution. We then used least squares linear regression to analyse the relationship between $\log_{10}$ (TN:TP) and latitude. As TN:TP has been shown to negatively correlate with trophic state (Downing & McCauley 1992) and can be influenced by lake depth (Hamilton & Mitchell 1997), we analysed the residuals in the regression of $\log_{10}$ (TN:TP) on latitude and also used factorial Analysis of Variance (ANOVA) to quantify the potentially interactive effects of latitude, lake depth and trophic state on TN:TP. Eutrophic and hypertrophic lakes were not included in the ANOVA because both deep and shallow polar lakes were not represented in these trophic state categories. We used least squares linear regression to quantify linear variation in standardised TN and TP data (z scores) along the latitudinal gradient. The z scores were calculated by subtracting the mean and dividing by the standard deviation to generate descriptors with a common scale, thereby permitting direct comparison of regression slopes.

The effect of latitude on chl-*a*-nutrient relationships was investigated by using linear least squares regression to examine the relationship between $\log_{10}$ chl-*a* and both $\log_{10}$ TN and $\log_{10}$ TP in each of the four latitudinal categories. As chl-*a*-nutrient relationships can vary between deep and shallow lakes due to variation in the water column light climate (Nixdorf & Deneke 1997), we derived separate models for deep and shallow lakes, as well as for the combined (deep and shallow) sample. For each regression, we calculated the standardised regression coefficient which is the regression slope obtained when variables have been standardised. Standardised coefficients are numerically equivalent to a Pearson's correlation coefficient (r) and provide a measure of the magnitude of variance in the dependent variable (i.e. $\log_{10}$ chl-*a*) that is described by the independent variable (i.e. $\log_{10}$ TN or $\log_{10}$ TP).

The bivariate distributions of chl-*a* and both TN and TP data displayed distinct upper boundaries, typical of 'factor

Table 2. Data sources used in the study.

Geographic region / country	n	Details	Data source
Antarctica	2	Mean of two samples collected from the centre of the lakes at mid depth in December 2003 (on ice cover) and January 2004 (after ice cover had completely disappeared).	Kudoh et al. (2009)
Brazil	20	Mean of depth integrated samples from the euphotic zone collected in Nov 1999, Dec 1999 and Jan 2000.	Trevisan & Forsberg (2007)
Canada	29	Depth integrated sample collected from the euphotic zone in summer 2007.	Murphy et al. (2010)
Canada	54	One sample collected in July 2003 from 0.15 m depth.	Keatley et al. (2007)
Canada	38	One sample collected in July 1998.	Michelutti et al. (2002)
Canada	22	One sample collected in July 2000 from 0.30 m depth.	Lim & Douglas (2003)
Central and South America (Argentina, Brazil, Columbia, Ecuador, Mexico, Venezuela, Puerto Rico, USA (n = 1))	22	Mean of samples collected monthly over at least a year in the 1970s and 1980s.	Salas & Martino (1991)
Europe – Belgium	3	Mean data from 1965–2007. Only data that do not violate European Environment Agency Quality Assurance checks chosen.	European Environment Agency (2009)
Europe – Croatia	6		
Europe – Denmark	20		
Europe – Estonia	8		
Europe – Finland	188		
Europe – France	4		
Europe – Germany	19		
Europe – Great Britain	1		
Europe – Hungary	8		
Europe – Iceland	1		
Europe – Ireland	3		
Europe – Italy	109		
Europe – Latvia	39		
Europe – Lithuania	27		
Europe – Netherlands	4		
Europe – Slovenia	5		
Europe – Sweden	154		
Europe – Switzerland	9		
Europe – The former Yugoslav Republic of Macedonia	2		
Japan	3	Mean concentrations of samples taken from 13–15 locations in each lake on one occasion at a depth of 0.5 m.	Takamura et al. (2003)
New Zealand	121	Samples collected either from the lake surface, or from integrated depths in the surface mixed layer, at monthly or quarterly intervals during the period of 2004 to 2006.	New Zealand Ministry for the Environment and Regional Councils (pers. comm.)
South America (Brazil, Argentina, Uruguay)	83	Sampled once between November 2004 and March 2006 by the same team who collected integrated water samples at 20 random points in each lake. Two L of each integrated sample were gathered in a bulk sample totalling 40 L.	Kosten et al. (2009)
Sri Lanka	25	One sample collected during January – February 1997.	Silva & Schiemer (2000)
Thailand	13	Mean concentrations from samples collected in the monsoon season of 1988 and the pre monsoon season of 1991. Samples from < 4 m depth.	Jones et al. (2000)
USA (Alaska)	13	Summer means from 1992, 1993 and 1995. Samples taken from 2 m depth.	Laperriere et al. (2003a)
USA (Alaska)	15	Mean of three samples collected over summer (May – September) 1993–1994. Samples were depth integrated to a depth of twice the Secchi depth or to within 1 m of the bottom in shallow, clear lakes.	Laperriere et al. (2003b)
USA (Iowa, Kansas, Missouri, Nebraska)	247	Summer median values.	Central Plains Center for BioAssessment

ceiling' distributions characteristic of ecological datasets in which the dependent variable is subject to limitation by the independent variable (Thomson et al. 1996). We interpreted the upper boundaries as representing conditional chl-*a* maxima at a given TN or TP concentration, under conditions whereby phytoplankton biomass accumulation is solely limited by N or P abundance, respectively. Consequently, the slopes of the upper boundaries are an approximation of the maximum mass of chl-*a* per mass unit of TN or TP observed in the global dataset. Conditional maxima in the chl-*a* = *f*(TN) and chl-*a* = *f*(TP) relationships were estimated using quantile regression (Koenker & Basset 1978). Quantile regression is a semi-parametric technique that can be used to estimate rates of change in *all* parts of the distribution of a dependent variable, unlike ordinary least squares regression which estimates variation only in the mean of a dependent variable (y), conditional on values of an independent variable (x) (Cade & Noon 2003). Accordingly, we estimated conditional maxima by using the linear equation for the 95th quantile of observed chl-*a*, calculated by minimisation of the following quantity (Scharf et al. 1998):

$$\sum_i \left| y_i - \beta_i x_{j+c} \right| h_i \qquad (1)$$

with

$$h_i = \begin{cases} \tau, & [y_i - (\beta_j x_{ij}) + c] \geq \tau \\ (\tau - 1), & [y_i - (\beta_j x_{ij}) + c] < \tau \end{cases}$$

where *y* is chl-*a* concentration (mg m^{-3}) in a given lake *i*, β_j is the coefficient of the *x* term (TN or TP), x_{ij} is TN or TP concentration (mg m^{-3}), *c* is a constant and τ is the quantile value (0.95). Minimisation was performed using the Solver add-in to Microsoft Excel 2007. All other statistical analyses were undertaken using Statistica (Version 8.0; Statsoft, Tulsa, USA) and a significance level of $p < 0.05$ was assumed for all tests.

Results

### Latitudinal variation in TN:TP

The magnitude of trophic state variables varied markedly between the four latitudinal categories. Median concentrations of TN, TP and chl-*a* were all lowest in the polar category and highest in the low-temperate category (Table 3). Regression of $\log_{10}$ (TN:TP) on latitude (° N/S) yielded a significant positive relationship between the two variables ($r^2 = 0.34$, $p < 0.001$) (Fig. 1a). The relationship was also significant when only northern hemisphere (n = 1076, $r^2 = 0.33$, $p < 0.001$) or southern hemisphere lakes (n = 240, $r^2 = 0.05$, $p < 0.001$) were analysed, and was also significant when only temperate lakes were included in the regression (n = 1054, $r^2 = 0.19$, $p < 0.001$). Regression analyses of $\log_{10}$ transformed and standardised TN and TP on latitude showed that latitudinal variation in $\log_{10}$ (TN:TP) is a function of a proportionally greater decreasing trend in TP, relative to that for TN (Fig. 1b, 1 c). To test whether the relationship between TN:TP and latitude was a spurious result of co-variation between latitude and trophic state (i.e. proportionally

Table 3. Summary statistics for the lakes included in this study. Median values of variables are presented. See text for definition of depth and latitude categories.

Lake group	n	Deep (n)	Shallow (n)	Chl-*a* (mg m^{-3})	TN (mg m^{-3})	TP (mg m^{-3})	TN:TP (mass)	Chl-*a*:TN (mass)	Chl-*a*:TP (mass)
Entire dataset	1316	802	514	5.9	620.5	27.1	24.9	0.010	0.26
Polar	144	78	66	0.6	232.0	4.8	52.9	0.009	0.32
High-temperate	616	450	166	5.1	567.5	18.7	31.6	0.009	0.31
Low-temperate	438	207	231	13.2	1148.0	81.9	15.5	0.014	0.21
Tropical	118	67	51	12.8	462.5	42.3	9.8	0.024	0.28

Table 4. Summary of factorial ANOVA to examine the higher order interactive effects of lake depth (deep or shallow), trophic state (ultra-oligotrophic, oligotrophic and mesotrophic) and latitude (tropical, low-temperate, high-temperate and polar) on $\log_{10}$ (TN:TP) in 768 lakes. Significant ($p < 0.05$) *p*-values are shown in bold. Note that eutrophic and hypertrophic lakes are excluded from the ANOVA because not all categorical variables are represented at these trophic states. See text for definition of categorical variables.

|	Sum of squares	d.f	Mean of squares	*F*	*p*
Depth	0.0001	1	0.001	0.01	0.93
Latitude	22.75	3	7.58	64.08	**< 0.001**
Trophic state	0.05	2	0.25	0.21	0.81
Depth × latitude	6.39	3	2.13	18.01	**< 0.001**
Depth × trophic state	0.25	2	0.13	1.07	0.35
Latitude × trophic state	2.40	6	0.40	3.38	**< 0.01**
Depth × latitude × trophic state	1.96	6	0.33	2.76	**0.01**
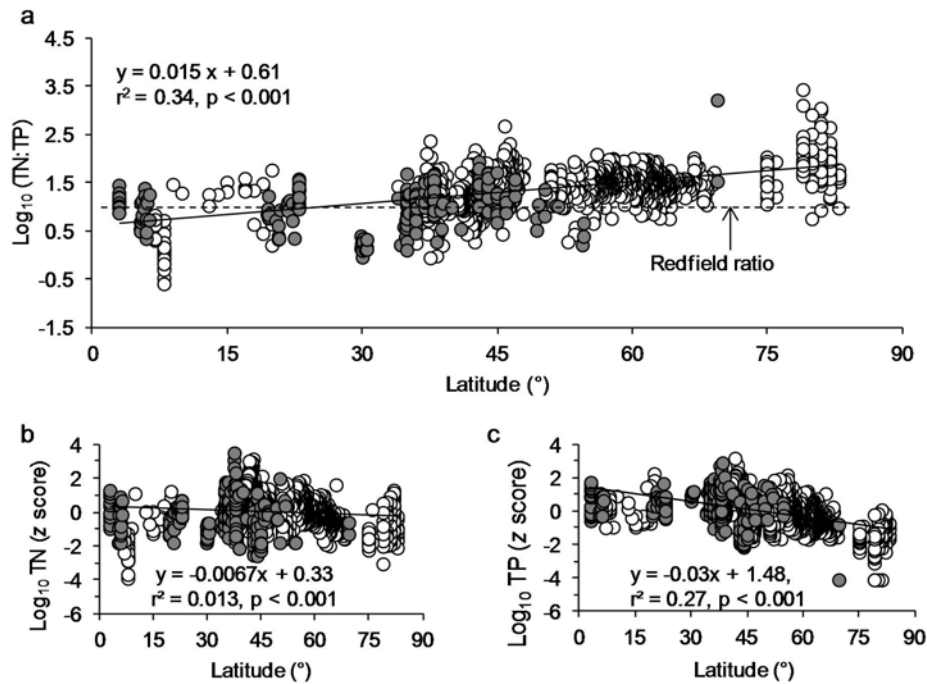
Fig. 1. Relationships between latitude and **(a)** TN:TP (by mass), **(b)** TN and **(c)** TP for 1316 lakes. Standardised TN and TP observations (z scores) are presented to allow comparison of slopes. The linear equation, r^2 and p statistic relate to the average trend in the data shown by the solid line. Open circles denote northern hemisphere lakes; shaded circles denote southern hemisphere lakes. Dependent variables are $\log_{10}$ transformed.
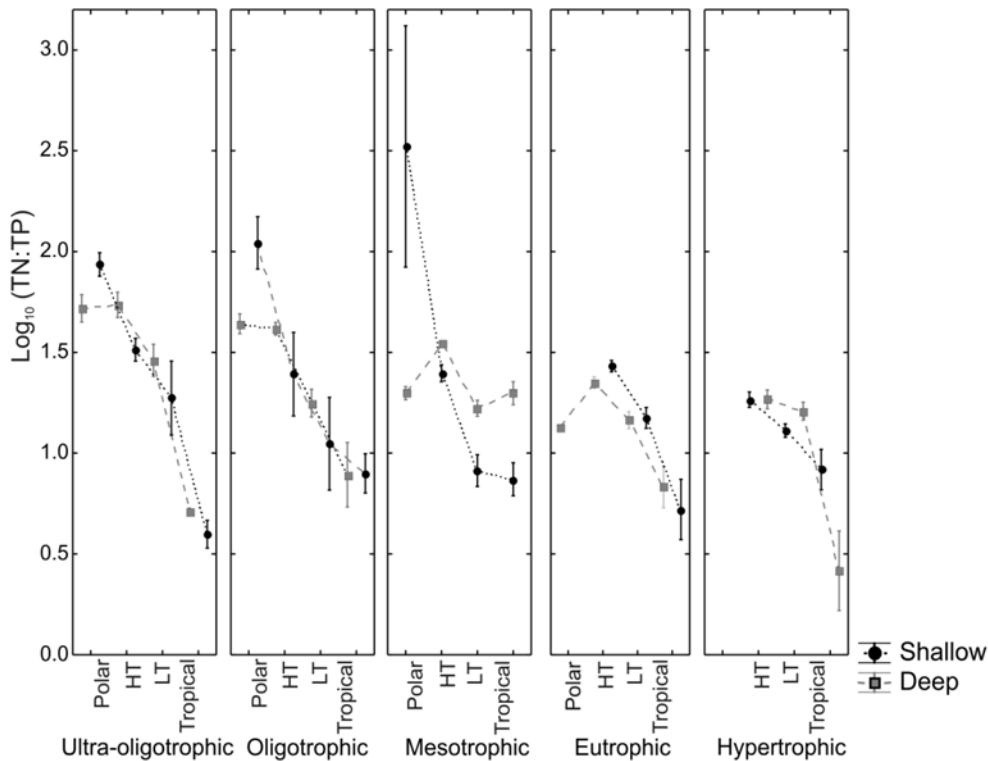
Fig. 2. Interactive effects of the categorical variables depth, trophic state and latitude on $\log_{10}$ (TN:TP) (by mass) in 1316 lakes. Vertical bars denote standard error, except where $n = 1$ (deep polar eutrophic). Temperate lakes were classified as either 'high-temperate' (HT) or 'low-temperate' (LT). See text for definition of categorical variables.

Table 5. Linear regression equations to predict chlorophyll-*a* (chl-*a*) concentration from TN and TP concentrations for selected categories of lakes in the sample of 1316 lakes. Regressions which are not statistically significant ($p > 0.05$) are denoted by 'n.s.'. Definitions of lake categories are provided in the text.

Lake category		Log$_{10}$ chl-*a* on log$_{10}$ TN			Log$_{10}$ chl-*a* on log$_{10}$ TP		
Latitude	Depth	Slope	Intercept	r^2	Slope	Intercept	r^2
Tropical	Deep	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Shallow	0.45	−0.16	0.14	1.01	−0.80	0.26
	All	n.s.	n.s.	n.s.	0.52	0.12	0.13
Low-temperate	Deep	0.71	−1.20	0.39	0.72	−0.34	0.48
	Shallow	0.80	−1.20	0.39	0.79	−0.32	0.35
	All	0.84	−1.45	0.46	0.81	−0.42	0.50
High-temperate	Deep	0.90	−1.81	0.34	0.74	−0.26	0.49
	Shallow	1.25	−2.68	0.50	1.09	−0.72	0.58
	All	1.06	−2.24	0.42	0.86	−0.39	0.54
Polar	Deep	0.60	−1.58	.09	0.48	−0.51	0.17
	Shallow	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	All	n.s.	n.s.	n.s.	0.17	−0.34	0.03
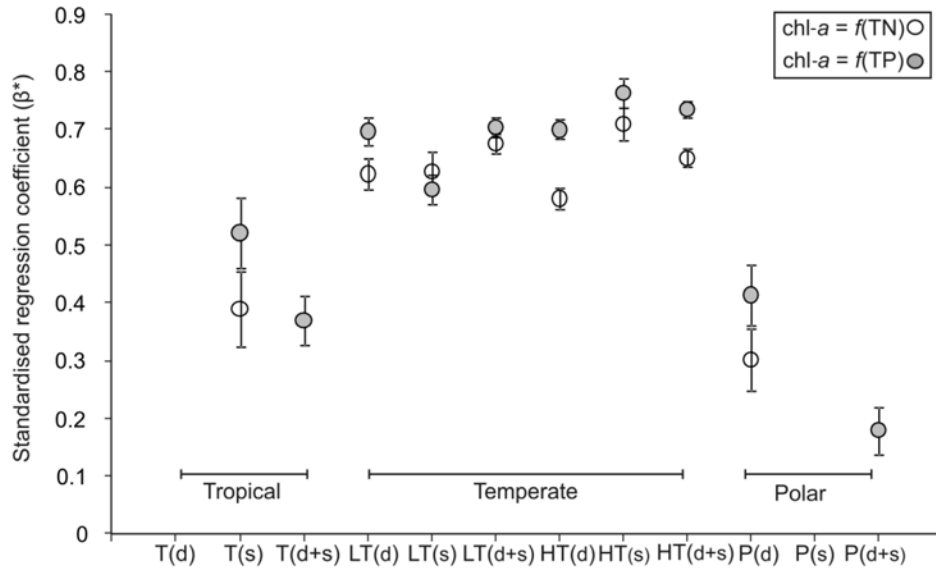
Fig. 3. Standardised coefficients of linear regression models to predict chlorophyll-*a* (chl-*a*) from TN and TP concentrations in tropical (T), low-temperate (LT), high-temperate (HT) and polar (P) lakes. Separate coefficients are calculated for deep (d), shallow (s) and combined deep and shallow (d + s) lakes and only coefficients for regressions that are statistically significant ($p < 0.05$) are presented. Vertical lines denote ± 1 standard error. See text for definition of categorical variables.

more oligotrophic lakes at higher latitudes and more eutrophic lakes at lower latitudes), we regressed the residuals from the regression of log$_{10}$ (TN:TP) on log$_{10}$ chl-*a* (slope = -0.28, $r^2 = 0.18$, $p < 0.001$) against latitude (° N/S). We found a highly significant positive relationship between the two variables in the latter regression ($r^2 = 0.19$, $p < 0.001$) indicating that the relationship between TN:TP and latitude does not simply reflect underlying variation in lake trophic state (Downing & McCauley 1992).

The results of a multi-factorial ANOVA of the effect of latitude, depth and trophic state on TN:TP supported this conclusion (Table 4). Differences in mean TN:TP were highly significant between latitudinal categories but not between categories based on depth or trophic state, and furthermore, interaction effects between latitude and the other categorical variables were significant (Table 4). Although eutrophic and hypertrophic lakes could not be included in the ANOVA due to inadequate representation of polar lakes at these

trophic states, mean TN:TP followed the same general trend at these trophic states: lowest in tropical lakes and highest in high-temperate lakes (Fig. 2).

Latitudinal variation in chl-*a*-nutrient relationships

Standardised coefficients (β*) of regression models to predict chl-*a* from TN or TP varied with latitude (Fig. 3). The coefficients were highest for temperate lake categories (β* = 0.58−0.76), indicating that both N and P concentrations explained more variation in chl-*a* concentration at temperate latitudes than at tropical or polar latitudes. Temperate lake models subsequently had higher coefficients of determination (r^2) (Table 5), as well as lower standard error (Fig. 3), than those for either tropical or polar lakes. Regression of $\log_{10}$ chl-*a* on $\log_{10}$ TN was not significant for the combined dataset of deep and shallow lakes in both tropical and polar regions and, furthermore, regressions of $\log_{10}$ chl-*a* on either $\log_{10}$ TN or $\log_{10}$ TP were

not significant for both deep tropical lakes and shallow polar lakes. Where significant relationships were established, standardised coefficients of regression models to predict chl-*a* from TP were higher than for those to predict chl-*a* from TN, with the sole exception of shallow low-temperate lakes, where the standardised coefficient for the chl-*a* = *f*(TN) regression was higher than for the chl-*a* = *f*(TP) regression (but within the range of standard error).

Approximated conditional maxima (defined as 'near-maxima') in the chl-*a*-TN (chl-$a_{\approx max\ (TN)}$) and chl-*a*-TP (chl-$a_{\approx max\ (TP)}$) relationships were determined using equations (2) and (3) respectively, derived using quantile regression (τ = 0.95) (Fig. 4):

$$\text{chl-}a_{\approx max\ (TN)} = 0.046\ TN + 4.14 \tag{2}$$

$$\text{chl-}a_{\approx max\ (TP)} = 0.87\ TP - 0.42 \tag{3}$$

Maximum yields of chl-*a* to TN [(chl-*a*:TN)$_{\tau = 0.95}$] and chl-*a* to TP [(chl-*a*:TP)$_{\tau = 0.95}$] were therefore approximated as 0.046:1 and 0.87:1 respectively. Regression
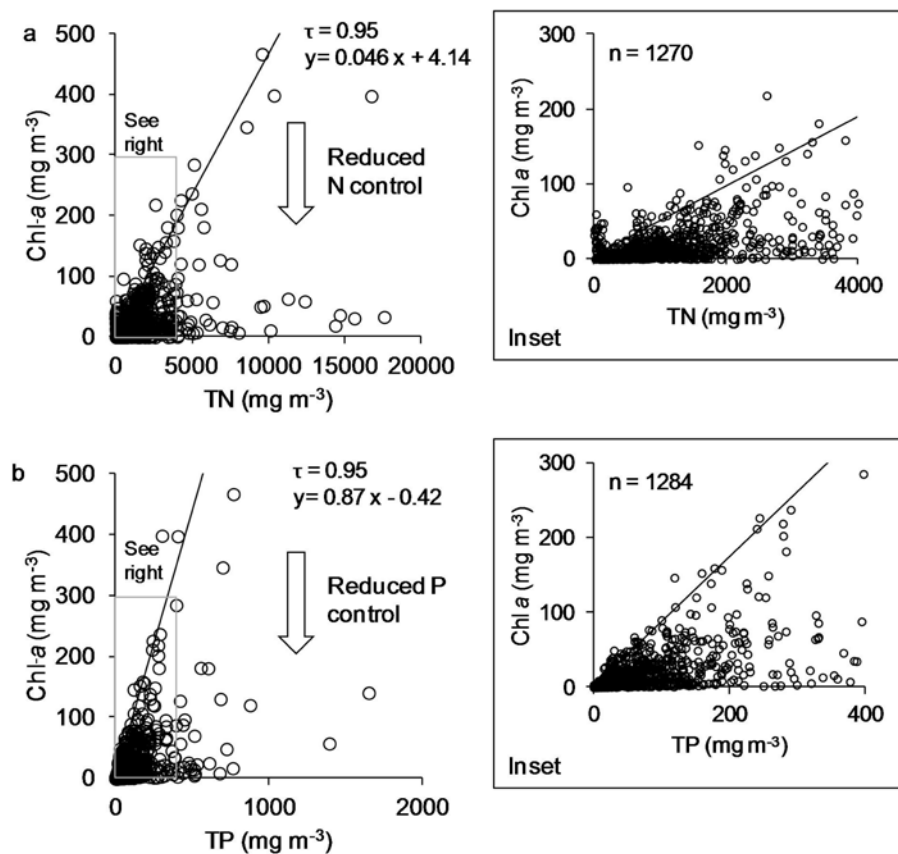
Fig. 4. Relationships between concentrations of chlorophyll-*a* (chl-*a*) and TN **(a)** and TP **(b)** for 1316 lakes. The upper boundary of chl-*a* concentration conditional on N or P abundance is estimated using quantile regression (τ = 0.95; solid line). Resource limitation theory implies that other unquantified factors contribute to limiting phytoplankton biomass accumulation in lakes represented by data points that lie below these conditional near-maxima. For clarity, one extreme data point (chl-*a* > 1000 mg m^{-3}) is omitted from the plots but was included in the regression analysis.
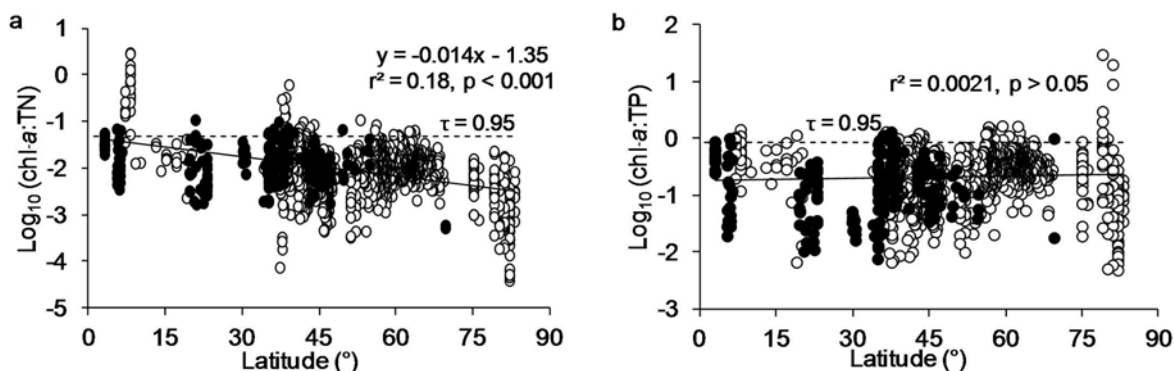
Fig. 5. Relationships between latitude and the ratio of chlorophyll-*a* (chl-*a*) concentration to TN **(a)** and TP **(b)** respectively for 1316 lakes. The linear equation, r^2 and *p* statistic relate to the average trend in the data shown by the solid line. The dashed line indicates approximate maximum yields of chl-*a* per mass unit of TN [(chl-*a*:TN)$_{\tau=0.95}$] and per mass unit of TP [(chl-*a*:TP)$_{\tau=0.95}$] estimated from quantile regression (see Fig. 4). Open circles denote northern hemisphere lakes; shaded circles denote southern hemisphere lakes. Dependent variables are log$_{10}$ transformed.

of log$_{10}$ (chl-*a*:TN) on latitude yielded a significant negative relationship ($r^2 = 0.18$, $p < 0.001$; Fig. 5a), regression of log$_{10}$ (chl-*a*:TP) on latitude did not yield a significant relationship (Fig. 5b).

Discussion

N and P stoichiometry

Our analysis shows that in-lake TN:TP increases with increasing distance from the equator, providing the most extensive example to date of "big-scale stoichiometry" (cf. Sterner & Elser 2002) along a spatial gradient in lentic ecosystems. This trend is independent of underlying co-variation between latitude and either trophic state or lake depth in our sample. Although the wide distribution of observed log$_{10}$ (TN:TP) around the regression line (Fig. 1a) indicates that factors other than latitude exert a strong influence on nutrient stoichiometry in lakes (i.e. local lake and catchment characteristics) (Downing & McCauley 1992, Hessen et al. 2009, Kosten et al. 2009), the latitudinal trend we report mandates consideration of wider-scale drivers of nutrient stoichiometry in lakes.

Our findings provide evidence for global-scale latitudinal variation in nutrient cycling processes and/or nutrient sources; either one or both of these factors could account for the latitudinal trend in TN:TP. With respect to nutrient cycling, a number of hypotheses can be proposed to explain why various processes (e.g. denitrification, N fixation, P resuspension, P weathering) might vary with latitude. Of these, latitudinal variation in denitrification is likely the best developed, and modelling of hypothetical lakes has shown that

there is a steep latitudinal gradient in the denitrification potential of lake hypolimnia driven by variation in hypolimnetic oxygen-holding capacity (Lewis 1996, 2002). Thus, more frequent de-oxygenation of bottom waters, in association with higher temperatures, may result in relatively higher denitrification rates in tropical lakes, thereby potentially causing the latitudinal trend in TN:TP observed. High abundance of leguminous terrestrial vegetation and N-fixing trees (e.g. *Alnus*) in both taiga and tundra biomes has also been linked to relative increased TN (and thus increased TN:TP) in high-latitude lakes (Laperriere et al. 2003).

It is also possible that latitudinal variation in nutrient sources could explain or at least partially account for the observed variation in TN:TP. Lake nutrient sources can vary widely in N:P (Downing & McCauley 1992) and therefore latitudinal variation in such sources could cause a commensurate trend in in-lake TN:TP. On average, TP concentrations in our sample decrease at a greater rate with increasing latitude than TN concentrations (Fig. 1b, 1 c); therefore, the occurrence of either relatively higher TP loads to lakes at low latitudes or relatively higher TN loads at high latitudes (or a combination of both) could at least partly explain the global trend in TN:TP. Nitrogen enrichment due to relatively higher levels of atmospheric N deposition at high latitudes in the Northern Hemisphere is one hypothesis that warrants particular consideration, as spatial variation in this N source has been shown to influence geographic patterns of nutrient limitation (Bergström & Jansson 2006, Elser et al. 2009), and northern hemisphere lakes are disproportionately represented in the dataset (although not necessarily when the global distribution of lakes is

considered) (Downing et al. 2006, Lewis 2011). Furthermore, the fact that the trend is strongest when only northern hemisphere lakes are analysed lends support to this hypothesis, although the existence of a significant (yet much weaker) positive relationship between latitude and TN:TP in southern hemisphere lakes implies that other mechanisms at least contribute to the trend. Other potential source-related explanations for the trend observed include latitudinal differences in: natural soil P abundance (Walker & Syers 1976), the relative proportions of arable versus pastoral agriculture (Arbuckle & Downing 2001), and, inputs of excreta and vegetation clearance rates (Moss et al. 2012).

Characterising the main drivers of this variation presents a challenge to researchers. Greater application of global-scale process-based modelling approaches (e.g. Lewis 2011) could potentially help to address this by elucidating how variables relating to physical factors (e.g. climate, geology, soils, altitude), anthropogenic pressures (e.g. agricultural land use) and ecosystem structure (e.g. piscivore abundance) interact to influence nutrient cycling and lake trophic state across large spatial scales. Such an approach could provide broad-scale understanding detached from any bias resulting from collation and analysis of uncoordinated datasets which predominantly relate to anthropogenically disturbed systems.

Chl-*a*-nutrient relationships

The marked latitudinal variation in chl-*a*-nutrient relationships highlights broad disparities in both ecosystem structure and function between lakes at different latitudes. The weak relationships between nutrients and chl-*a* in tropical, relative to temperate lakes (Table 5; Fig. 3), implies that factors other than N or P are relatively more important in controlling lentic phytoplankton productivity at tropical latitudes. This finding is in accordance with Huszar et al. (2006) who similarly found that TP was a poorer predictor of chl-*a* in tropical regions than in temperate regions, a result that they attributed to either greater prevalence of light limitation in tropical lakes or differences in food web interactions (*not* greater prevalence of N limitation). Certainly, higher turbidity in tropical lakes could at least partly explain this variation; it is well established that elevated inorganic suspended sediment concentration can decouple chl-*a*-nutrient relationships (Schindler 1978, Canfield & Bachmann 1981) and elevated turbidity can occur in tropical lakes both due to enhanced erosion during the wet season (e.g.

McCullough & Barber 2007) and following water loss in the dry season (e.g. Dejenie et al. 2008).

Chlorophyll-*a*-nutrient relationships are also markedly weaker in polar lakes compared to those in temperate lakes. We note, however, that the weaker relationships are likely to partly reflect the very low trophic status of the polar lakes; the small gradients in TN and TP that are present when concentrations of determinants are at or close to detection limits are not conducive to establishing strong relationships with chl-*a* (Michelutti et al. 2002). Similarly, unusually high chl-*a*:TP in lakes situated at 80–81° N (Fig. 5b) is likely an artefact of very low (< 1 mg m^{-3}) TP. Nevertheless, more substantive explanations may account for relatively weak chl-*a*-nutrient relationships in polar lakes. Firstly, we discount light limitation as all data for polar lakes are based on samples collected during summer months, when photosynthetically active radiation flux is comparable to other latitudes (Campbell & Aarup 1989). There are, however, numerous structural characteristics of polar lake ecosystems that may decouple water column chl-*a*-nutrient relationships. Notably, these include: strong top-down controls due to relatively high abundance of herbivorous zooplankton (e.g. Christoffersen et al. 2008), frequent occurrence of dominance by benthic, rather than pelagic, primary producers due to often high abundance of benthic algal mats in polar lakes (*ibid*), and, potentially, frequent macrophyte dominance (Teissier et al. 2012) in shallow unproductive polar lakes during summer. Low temperatures may also constrain phytoplankton biomass accumulation at high latitudes as phytoplankton primary productivity correlates positively with water temperature (Faithfull et al. 2011). Variation in ecosystem structure and temperature limitation could therefore account for the weaker chl-*a*-nutrient relationships for polar lakes compared to temperate lakes.

Other studies have highlighted the existence of upper asymptotes in nutrient-chl-*a* scatterplots due to reduction of euphotic depth at high chl-*a* concentration (Schindler 1978, Phillips et al. 2008, Jones et al. 2011). Fig. 4 highlights that there is little evidence, however, that such asymptotes are absolute. Phillips et al. (2008) report inflection points at TP $= 100$ mg m^{-3} and TN $= 1700$ mg m^{-3}, and although there seems to be a reduction in slope at these points, exceedances of chl-*a* near-maxima at twice these concentrations reflect the extreme chl-*a* concentrations that are possible, presumably in the presence of dense buoyant algal scums. Also, our results emphasise that chl-*a* seldom reaches near-maxima conditional on N and P abundance. We interpret the near-maxima as rep-

resenting chl-*a* concentrations achieved during phytoplankton blooms; blooms typically arise only when a phytoplankton assemblage comprises certain species, and, regulating factors such as temperature and light intensity are present at demand-saturating levels over timescales that correspond to multiple generation times (Reynolds 2006). Clearly, such conditions are the exception. Furthermore, Jones et al. (2011) have highlighted significant regional differences in TP-conditional chl-*a* maxima which they attributed to differences in climate and mineral turbidity. Variability in nutrient bioavailability between lakes would also influence lake-specific chl-*a* near-maxima and therefore we interpret the near-maxima that we have defined as indicative of potential limits that will likely not be reached in many lakes.

Also of note, is that some data points in Fig. 4 exceed the near-maxima, reflecting our use of the 95th percentile as a compromise between identifying the uppermost boundary and minimising any bias that may result due to analytical error present in the data. Approximately, 4 % of lakes exceed the near-maxima in Fig. 4 and, in particular, measured chl-*a* in a number of lakes substantially exceeds chl-$a_{\approx max\ (TN)}$. The average ratio of observed chl-*a* (chl-a_{obs}) to chl-$a_{\approx max\ (TN)}$ is 2.82 in lakes where this ratio > 1, whereas average chl-a_{obs}:chl-$a_{\approx max\ (TP)}$ is 1.45 in lakes where the ratio > 1. Interestingly, a substantial portion (43 %) of the 53 lakes for which chl-a_{obs}:chl-$a_{\approx max\ (TN)}$ > 1 comprises a group of reservoirs in Sri Lanka. Amongst these reservoirs there is the largest chl-a_{obs}:chl-$a_{\approx max\ (TN)}$ (10.18) and they cluster in the upper left-hand portion of Fig. 5a at 7–8° latitude. These reservoirs are characterised by low dissolved inorganic N (typically < 100 mg m^{-3}), very low TN:TP (mean = 1.9:1), extremely high chl-*a*:TN and dominance of the diatom *Aulacoseira granulata* (Silva & Schiemer 2000). *A. granulata* is a bloom-forming species that readily forms aggregates (Reynolds 2006) and has been determined to have an atypically low ratio of carbon to chl-*a* (mean = 26:1, range 10:1–57:1; Yacobi & Zohary 2010). Thus, it seems that observations may significantly exceed the empirical maxima under exceptional conditions and, in particular, some variability in chl-*a*-nutrient relationships reflects imprecision of chl-*a* as a surrogate for phytoplankton biomass due to influence by factors such as light intensity and the presence of accessory pigments (Reynolds 2006).

N and P limitation

The Redfield ratio of 7.2:1 by mass (16:1 by moles) is widely used as an indicator of the N:P ratio below which N is likely to limit phytoplankton biomass accumulation (rather than P alone or both N and P together) (Redfield et al. 1963, Klausmeier et al. 2004, Ptacnik et al. 2010). Based on the equation for the linear trend in the data (Fig. 1a), average TN:TP is predicted to be below this threshold at latitudes of approximately < 16 °N/S which correspond to the mid-tropics. This latitudinal trend in TN:TP is commensurate with the observed increase in chl-*a*:TN towards the equator and tendency towards the chl-*a*:TN near-maximum in the tropics (Fig. 5a).

Together, the latitudinal trends in TN:TP and chl-*a*:TN suggest that N limitation will be more prevalent in lakes at low than at high latitudes, consistent with experimental observations (Hecky & Kilham 1988, Elser et al. 2007). This conclusion cannot, however, be readily reconciled with the results of regression analysis between chl-*a* and nutrients for the different latitudinal categories of lakes (Table 5; Fig. 3). Although there are contradictions between substantive theory of limiting factors and the use of standard regression techniques to characterise nutrient limitation (Kaiser et al. 1994), the finding that TN was markedly inferior as a predictor of chl-*a* in tropical lakes compared to temperate lakes (Fig. 3) is inconsistent with that expected if N were more often a limiting nutrient in the tropics than at temperate latitudes. Our results therefore suggest that, while N is less abundant relative to phytoplankton growth requirements at lower latitudes, macronutrient limitation of phytoplankton biomass (by N or P) is less prevalent in the tropics overall (relative to temperate lakes) due to decoupling of chl-*a*-nutrient relationships by factors not quantified.

This last conclusion differs from those made in studies that analysed results of nutrient enrichment experiments and found that N limitation is more prevalent overall in tropical than temperate lakes (Hecky & Kilham 1988, Elser et al. 2007). To potentially explain this disparity, we note that the overwhelming majority of nutrient enrichment experiments are undertaken at the sub-ecosystem scale, i.e. using mesocosms or laboratory culture vessels. Such designs invariably simplify natural systems (Hecky & Kilham 1988) and potentially reduce the likelihood of limitation by factors other than nutrients, e.g. due to exclusion of zooplankton or maintenance of artificially high ambient light levels. Thus, it is possible that relative deficiency in N at low latitudes means that nutrient enrichment experiments using tropical lake waters have an enhanced likelihood of yielding significant growth responses to added N, even though nutrient limitation of

phytoplankton biomass in natural systems over longer time scales is secondary to other forms of limitation that are otherwise suppressed under typical experimental conditions.

An important caveat concerns the use of total nutrient pools (rather than the more labile dissolved fraction) in our analysis. The bioavailability of TN in particular is uncertain; it comprises both labile inorganic N forms, as well as organic forms which have been shown to vary in bioavailability depending on their source (Seitzinger et al. 2002). Any latitudinal bias in TN composition in our sample could therefore compromise the utility of empirical parameters derived using TN for assessing broad spatial trends in phytoplankton nutrient limitation (Ptacnik et al. 2010). Clearly, analysis of only three trophic state variables cannot provide comprehensive understanding of how lake ecosystems function, and, while our study has highlighted global-scale latitudinal trends in the stoichiometry of lentic ecosystems, more work is necessary to develop better understanding of the causes and ecological implications of these trends.

In general, our analysis supports the view that P is more likely than N to limit phytoplankton biomass in lakes (Schindler 1977). Regressions to predict chl-a from TP were significant for more of the lake categories than those with TN as the predictor variable. Furthermore, the models based on TP generally had higher standardised coefficients than those based on TN (Fig. 3), indicating that TP usually accounted for a greater proportion of the variation in chl-a concentration than TN. Shallow low-temperate lakes were an exception to this, however, as the standardised coefficient for the chl-$a = f$(TN) regression was marginally higher (but within the distribution of standard error) than for the chl-$a = f$(TP) regression. This may reflect the fact that this category contained 24 % of lakes from New Zealand where lacustrine N limitation has been shown to be more prevalent than in northern temperate regions (Abell et al. 2010). The majority (57 %) of the lakes in this category were in the southern USA, however, and this propensity for geographic variation in chl-a-nutrient relationships highlights the importance of exercising caution when applying relationships established in one locality to lakes elsewhere, particularly in the presence of latitudinal gradients. In particular, it should not be assumed that chl-a-nutrient models developed using temperate datasets will be applicable to tropical lakes and the relative paucity of observations and increasing anthropogenic pressures on water resources in the tropics necessitates enhanced research efforts focused on these systems.

As Moss et al. (2012) note, the concept that there is one predominant limiting nutrient in undisturbed lakes is likely invalid, as evolution would redress any such imbalance in selection pressure. The greater relative tendency for P limitation implied by our analysis may therefore reflect proportionally greater prevalence of N pollution in anthropogenically disturbed ecosystems and is not necessarily a fundamental reflection of natural systems.

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