# Can we predict nutrient limitation in streams and rivers?

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## Abstract

1. Anthropogenic impacts on the biogeochemical cycles of nitrogen (N) and phosphorus (P) affect natural ecosystems worldwide. Modelling is required to predict where and when these key nutrients limit primary production in freshwaters.

2. We reviewed 382 nutrient-enrichment experiments to examine which factors promote limitation of microphytobenthos biomass by N or P in streams and rivers. Using regression models, we examined whether the response of microphytobenthos biomass to N and P additions could be predicted by the absolute N and P concentrations in the water, the water N:P ratio or a combination of the two.

3. The absolute N concentration in the water was the best predictor of the magnitude of the response of microphytobenthos biomass to N additions. In comparison, the N:P ratio was the best predictor of whether or not N was limiting. However, predictions were uncertain except at extreme N:P ratios <1:1 and >100:1.

4. The absolute P concentration in the water was the best predictor of the magnitude of the response of microphytobenthos biomass to P additions. Neither the absolute nor the relative N and P concentrations predicted whether or not P was limiting.

5. The absolute and the relative N and P water concentrations contribute significant and complementary insights into the responses of microphytobenthos biomass to nutrient enrichment in running waters. However, ability to predict nutrient limitation from these concentrations is constrained by substantial error in the models. In the future, the prediction ability of models of nutrient limitation might be improved by focussing on regional scales and accounting for additional factors such as light and disturbance.

# Contents

# 1 Introduction

The biogeochemical cycles of phosphorus (P) and nitrogen (N) have been altered by human activities since the industrial revolution. At present, the global amount of these elements mobilised by fossil fuel combustion (N), mining (P) and agricultural practices (N and P) has exceeded natural supplies (Galloway et al., 2004; Filippelli, 2008; Rockström et al., 2009). Because N and P are essential nutrients for primary producers, alterations in their input into the environment affect the structure and functioning of natural ecosystems (Smith et al., 1999). According to Liebig's law of the minimum (Liebig, 1840), profound effects should be expected especially in ecosystems where these elements are limiting resources (Vitousek et al., 1997). Thus, in their global analysis of N and P limitation, Elser et al. (2007) highlighted the necessity to understand 'if, where, and by how much these key nutrients limit production' to evaluate and predict the ecological consequences of alterations in their cycles.

Until recently, it was usually believed that P is the primary limiting nutrient in freshwater ecosystems (Schindler, 1977). Therefore, the management of nutrient enrichment (eutrophication) in these ecosystems often focussed on P abatement (Lewis and Wurtsbaugh, 2008). However, recent studies have shown that N can also be limiting in lakes (Elser et al., 1990; Bergström et al., 2005; Lewis and Wurtsbaugh, 2008) and rivers (Dodds and Welch, 2000; Francoeur, 2001). Moreover, a meta-analysis by Elser et al. (2007) showed that N limitation is as frequent as P limitation in streams. These findings have generated renewed interest for the identification of limiting nutrients in freshwater ecosystems and make the case for a new framework of nutrient limitation (Elser et al., 2007; Lewis and Wurtsbaugh, 2008).

To predict which nutrient is limiting, freshwater ecologists have often applied the hypothesis that there is a critical ratio between N and P supply rates that maximises the growth of primary producers (Redfield, 1958; Schanz and Juon, 1983). Of the critical ratios proposed, the best known is the Redfield ratio of 16N:1P (by atoms; Redfield, 1958), which is often used to predict nutrient limitation in freshwaters although it was derived from oceanic seston. Redfield (1958) suggested that, on average, marine algal cells contain N and P in a 16:1 ratio. Therefore, by extension from Liebig's law of the minimum, and assuming that nutrient concentrations in the water reflect supply rates, if the water N:P ratio is lower than 16, algae should be limited by N; if it is higher, they should be limited by P. In lakes, water N:P ratios can successfully predict the nutrient-limiting phytoplankton, although shifts from N to P limitation may occur at ratios different from the Redfield ratio (Morris and Lewis, 1988; Elser et al., 2009; Bergström, 2010). For example, the ratio between dissolved inorganic nitrogen (DIN) and total phosphorous (TP) in the water predicted 72% of the variation in the response of phytoplankton to nutrient-enrichment assays in 106 oligotrophic lakes across Europe and North America (Bergström, 2010). In these lakes, phytoplankton shifted from N limitation at DIN:TP mass ratios <1.5 (approximately 3 by atoms) to P limitation at DIN:TP mass ratios >3.4 (approximately 8 by atoms).

With regard to running waters, Grimm and Fisher (1986) suggested that the microphytobenthos (benthic microscopic algae and cyanobacteria; MacIntyre et al., 1996) of desert streams contains N and P in an atomic ratio of 17:1. Schanz and Juon (1983) reported N limitation of stream microphytobenthos at atomic N:P ratios <10 and P limitation at N:P ratios >20. However, the water N:P ratio has often failed to predict the nutrient-limiting microphytobenthos in streams (Francoeur et al., 1999; Wold and Hershey, 1999; Snyder et al., 2002; Tank and Dodds, 2003; Schiller et al., 2007; Elsaholi et al., 2011; but see Grimm and Fisher, 1986; Pringle, 1987; Peterson et al., 1993). There are two possible explanations for the failures. First, it has been suggested that nutrient limitation in streams reflects not only the ratio between nutrients in the water but also their absolute concentrations (Bothwell, 1985; Dodds, 2003). In this case, accurate predictions of nutrient limitation might be achieved so long as the effects of nutrient ratios and absolute concentrations are both considered. Second, Liebig's law of the minimum, originally intended for single crop species (Danger et al., 2008), may not apply to the mixed microphytobenthos assemblages that live in streams. For example, contrary to individual plant species, microphytobenthos assemblages may not be limited by a single nutrient at any time. In this case, either nutrient ratios or the joint effect of relative and absolute nutrient concentrations in the water may not identify a limiting nutrient of stream microphytobenthos.

To address these explanations, we reviewed 382 published and unpublished in situ nutrient-enrichment experiments in rivers and streams worldwide. From each study, we extracted the size of the effect of nutrient (N or P) additions on microphytobenthos biomass, the identity of the limiting nutrient according to the study authors and information on N and P concentrations in the water. We used linear and logistic regression models to assess the effects of N and P concentrations in the water (absolute, relative and both) on the response of microphytobenthos biomass to N and P experimental enrichments. We asked: do the absolute N and P concentrations

in the water allow more accurate predictions of the nutrient limiting microphytobenthos biomass (alone or with the water N:P ratio) than the N:P ratio? Or, is nutrient limitation difficult to predict regardless of which predictors are used, suggesting that single-nutrient limitation does not apply to microphytobenthos biomass in streams?

## 2 Methods

### 2.1 Data collection and extraction

Studies (i.e. papers, reports or dissertations) about in situ nutrient manipulations were searched from January to July 2011 in the online databases ISI Web of Knowledge (ISI Web of Science), Science Direct, Wiley InterScience, Springer Link and Ebsco (including Francis and Pascal). Further electronic searches were conducted using the metasearch engine Google Scholar and its tool 'Related articles'. The combinations of keywords used in the electronic searches are reported in the Supporting Information, Appendix S1. The electronic searches were complemented with an inspection of the literature cited by previous reviews on nutrient limitation in streams, paying special attention to other meta-analyses (Francoeur, 2001; Elser et al., 2007).

Studies had to meet three criteria to be included in our database. First, studies had to present results of in situ nutrient-enrichment experiments in running waters (streams and rivers). We included studies that used nutrient-diffusing substrates (NDS; Fairchild and Lowe, 1984), flow-through systems (FTS; Grimm and Fisher, 1986) or periphytometers (Matlock et al., 1999). However, manipulations involving organic substrates (such as wood) were excluded because (i) the presence of fungi on these substrates can alter the magnitude of the response of microphytobenthos biomass to nutrient additions and (ii) these manipulations were too few to be analysed separately (Tank and Dodds, 2003; Hoellein et al., 2010). Second, studies had to provide results for at least one nutrient treatment (N or P addition) and one matching control. Third, studies had to provide information about nutrient concentrations in the water, including DIN and soluble reactive P (SRP) or TP concentrations. TP may be a better indicator of the total P available to microphytobenthos than SRP because SRP largely consists of inorganic P, whereas some forms of organic P are also biologically available (Morris and Lewis, 1988). In lakes, for example, DIN:TP allows to predict nutrient limitation more accurately than DIN:SRP (Morris and Lewis, 1988; Bergström, 2010). However, only 15% of the studies reviewed provided information on TP. We did not restrict our analysis to these studies because we reasoned that any gain in accuracy owing to the use of TP might have been outweighed by the effects of the small sample size. Moreover, it is not clear that TP is a better indicator of P availability than SRP in running waters, as it apparently is in lakes. Therefore, we included studies that reported P as either SRP or TP (or both), and we analysed them separately (see below).

From each selected publication, we extracted the mean biomass of microphytobenthos for nutrient treatments (N and / or P) and controls (C). The biomass of microphytobenthos was quantified as chlorophyll *a* density (mass per unit area). A caveat with this approach is that the relationship between chlorophyll *a* and microphytobenthos biomass is influenced by variables including light intensity, nutrient concentrations, taxonomic composition and cell physiological conditions (e.g. Vörös

and Padisák, 1991). Therefore, chlorophyll *a* is only an approximate index of microphytobenthos biomass. However, it was the most commonly reported index across the studies, and ash-free dry mass, the other frequently reported index, does not distinguish microphytobenthos from other organic materials (bacteria, fungi, detritus and extracellular polysaccharides). The mean chlorophyll *a* density for each treatment, the associated standard error, the number of replicate treatments and the concentrations of DIN, SRP and, when available, TP in the water were also recorded. If, during a same experiment, chlorophyll *a* was measured at the end of the experiment and at intermediate sampling occasions (midway through, for example), we recorded only the final value. If nutrient concentrations were measured more than once, we used the average value. All nutrient concentrations were expressed in $\mu mol.L^{-1}$, and N:P ratios (either DIN:SRP or DIN:TP) were expressed in a molar basis (i.e. by atoms). Finally, the authors' conclusions about whether or not N or P was limiting, based on ANOVA-type tests, were binary-coded (1 or 0) for each experiment.

Some studies included results from multiple sites, seasons or treatments. Sites were considered independent if they were $\geq 1$ km from each other; otherwise, they were pooled to reduce spatial autocorrelation. When multiple results were available for the same site (or pool of sites within 1 km) and season, we selected one result at random. Where the nutrient treatment (control versus enrichment) was crossed with an additional treatment (e.g. fish exclosure), we disregarded the results concerning the additional manipulation, so that the results would be most comparable across studies. Therefore, each combination of study, site (or pool of sites within 1 km) and season resulted in only one observation in our database.

The final database included the results of nutrient-enrichment experiments extracted from 61 studies (see Appendix S2 for the list of references). For the analysis, the database was split into a 'SRP data set', the set of manipulations that reported P as SRP (382 observations), and a 'TP data set', the sub-set of manipulations that reported P as TP (59 observations). The experiments were conducted in 168 streams and rivers. Of these, 111 were located in North America, 32 in Oceania, 22 in Europe, 1 in Central America, 1 in South America and 1 in Asia. Almost all of the study sites were distributed within temperate latitudes, with greatest frequency between 30°and 50°either N or S (see Figure S1 for the frequency of sites by degree of latitude).

## 2.2 Statistical analyses

We identified no single best statistical approach to the analysis of our data; therefore, we used three complementary approaches to overcome the potential limitations of each. The first approach used was meta-regression. Meta-regressions differ from classic regressions in that they account for extra sources of heterogeneity, the within-study variability and the between-study variability (Gurevitch and Hedges, 1999). Therefore, they are particularly useful for regression analyses of data garnered from different studies.

Our meta-regression focussed on the estimated effect of enrichment by a nutrient $x$, either N or P, on chlorophyll a. Meta-regressions require a standardised measure of the magnitude (or size) of the effect of the factors of interest. Here, the standardised effect size was quantified as the ln response ratio $\ln(X/C)$, where $X$ is the mean

chlorophyll a density measured in a treatment $x$ and $C$ is the mean chlorophyll a density measured in the control (Hedges et al., 1999). Hereafter, we refer to the ln response ratio as LRR$x$, with $x =$ N or P. The ln response ratio is widely used for meta-analyses in ecology, including previous meta-analyses of nutrient-enrichment experiments, because it allows conclusions to be drawn from a heterogeneous sample of studies (Downing et al., 1999; Francoeur, 2001; Elser et al., 2007).

Our meta-regression approach used linear mixed models (Bolker et al., 2009) to assess the effects of water DIN, P (either SRP or TP) and DIN:P ratio (either DIN:SRP or DIN:TP) on LRR$x$. These models use random effects to account for between-study variability and lack of independence among experiments. We tested the effect size of DIN and DIN:P separately (using models M1$_{LRRx}$ and M2$_{LRRx}$ , respectively) and jointly in an additive model (M3$_{LRRx}$ ). The explanatory variables were $\log_{10} -transformed$ to reduce the influence of extreme values. The models and their parameters are further described in Table 1. Regressions were fitted with the REML (restricted maximum likelihood) method and weighted by the inverse of the estimated variance of each LRR$x$ value to account for within-study variability (Hedges et al., 1999). Three levels of nested random effects (i.e. intercepts) were specified (study, river and site) to account for correlation of variables within these hierarchical levels. Fixed effects were tested from computation of their confidence intervals (CI) using Markov chain Monte Carlo (MCMC) sampling (Bolker et al., 2009). An explanatory variable was considered to contribute significantly to a model if the confidence interval of its parameter did not include zero. Goodness of fit indexes ($R^2$) could not be used in this study, because in mixed models unidimensional indexes such as $R^2$ cannot easily account for variation from both fixed and random effects (Kreft and Leeuw, 1998). Therefore, the amount of variance unexplained was assessed by examining the residuals and the scatter of data points around the fitted regression lines.

The second approach used was quantile regression, which we included in our analysis to address the possibility that limiting factors have stronger effects on the maximum (upper quantile) responses than on the average responses considered in classic regression (Cade et al., 1999). We used an exploratory local quantile regression based on a combination of repeated running quantile (95th) and the LOESS smoother (Cleveland et al., 1992). For example, this flexible, nonparametric approach was used to analyse a meta-data set relating stream-insect richness to geographical variables by Vinson and Hawkins (2003). The explanatory variables were the same as those included in the linear mixed models (i.e. DIN, P and DIN:P), whereas the dependent variables were the upper limits (95th quantiles) of the LRR$x$ distributions.

The third approach used was mixed logistic regression (GLMM). In this approach, we modelled the probability that each nutrient was limiting (based on the binary-coded conclusions of the authors of the studies reviewed) as a function of the N:P ratio and the absolute N and P concentrations. Binary data contain less information than effect sizes. However, we included this logistic approach because it might be better suited to analyse nonlinear responses, which were expected in this study. Moreover, the logistic approach should be robust to potential influences of experimental conditions, including NDS design and nutrient concentration (e.g. Capps et al., 2011), on effect sizes. Models and parameters were similar to those

| | | Fixed effects | | | Random effects |
|---|---|---|---|---|---|
| Model | Method | DIN | P | DIN:P | Study/River/Site |
| M1$_{LRRN}$ | Linear | • | | | • |
| M2$_{LRRN}$ | Linear | | | • | • |
| M3$_{LRRN}$ | Linear | • | | • | • |
| M1$_{LRRP}$ | Linear | | • | | • |
| M2$_{LRRP}$ | Linear | | | • | • |
| M3$_{LRRP}$ | Linear | | • | • | • |
| M1$_{BRN}$ | Logistic | • | | | • |
| M2$_{BRN}$ | Logistic | | | • | • |
| M3$_{BRN}$ | Logistic | • | | • | • |
| M1$_{BRP}$ | Logistic | | • | | • |
| M2$_{BRP}$ | Logistic | | | • | • |
| M3$_{BRP}$ | Logistic | | • | • | • |

Table 1: Parameters included in our regression models, by model acronym. P refers to either SRP for the SRP data set or TP for the TP data set, which were analysed separately (see Methods)

described for the linear mixed models (Table 2). Parameter significance was tested with *Z* Wald tests.

All statistical analyses were performed using `R 2.13.0` software (R Development Core Team, 2011). Mixed models were adjusted using the `lme4` package (Bates et al., 2011).

# 3   Results

## 3.1   Linear meta-regressions

With regard to the SRP data set, water DIN had a significant, negative effect on LRRN in models M1$_{LRRN}$ and M3$_{LRRN}$ (significance indicated by 95% CI of the parameter estimate non-overlapping zero; Table 2a), indicating that the effect of N enrichment on chlorophyll a density decreased with increasing water DIN concentration (Figure 1a). The effect approached zero beyond DIN concentrations of approximately 100 µmol.L$^{-1}$ (1400 µg.L$^{-1}$ ; Figure 1a). Water DIN:SRP was a significant predictor in model M2$_{LRRN}$ but not in model M3$_{LRRN}$ (Table 2a), suggesting that DIN:SRP played at most a secondary role relative to DIN in determining LRRN (Figure 1b). Water SRP or DIN:SRP had no apparent effects on LRRP (Figure 1c,d; Table 2a).

With regard to the TP data set, water DIN had a significant, negative effect on LRRN in model M3$_{LRRN}$ (Table 2b), paralleling the results of the analysis of the SRP data set. In addition, water TP had a significant, negative effect on LRRP in models M1$_{LRRP}$ and M3$_{LRRP}$ (Figure 1e, Table 2b). The effect of TP means that the effect of P addition on chlorophyll *a* density decreased with increasing water TP concentration. Water DIN:TP had no additional effects on either LRRN or LRRP.
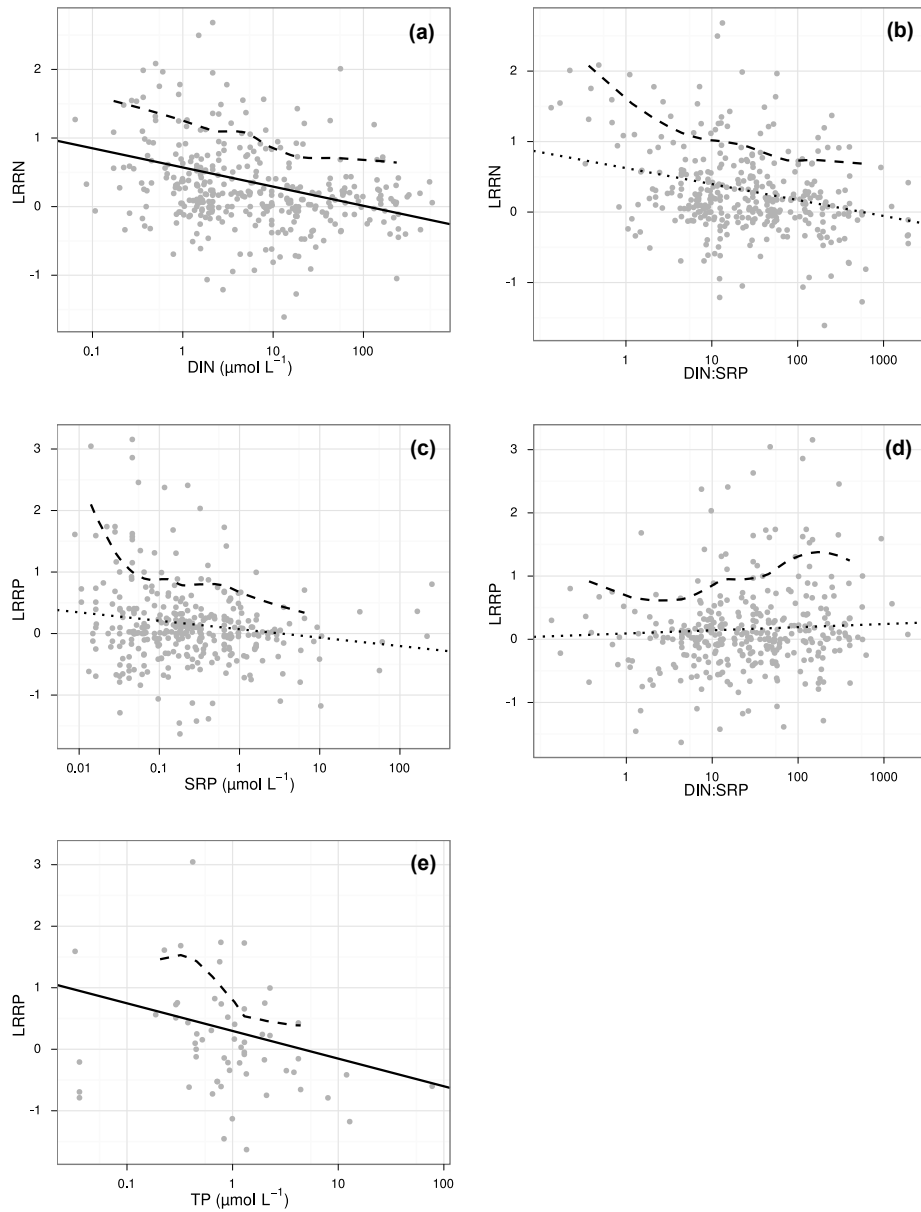
Figure 1: Results of the linear meta-regression and local quantile regression approaches. Response (ln response ratio) of microphytobenthos biomass to nitrogen addition (LRRN) as a function of DIN (a; log scale) and DIN:SRP (b; log scale). Response (ln response ratio) of microphytobenthos biomass to phosphorus addition (LRRP) as a function of SRP (c; log scale), DIN:SRP (d; log scale) and TP (e; log scale). Fitted linear mixed models are represented by solid lines if significant or by dotted lines if non-significant. Smoothed 95th quantile lines are represented by dashed lines.

## 3.2 Local quantile regressions

Patterns of the smoothed 95th quantile regression lines roughly paralleled those of the linear mixed models (Figure 1), with some deviations. With regard to the SRP data set, the 95th quantile smooth line representing the maximum effect of water DIN on LRRN showed a declining (but uneven) trend that nearly flattened above approximately 20 µmol.L$^{-1}$ DIN (Figure 1a). The 95th quantile smooth line representing the maximum effect of water SRP on LRRP showed a declining trend throughout, but the slope was less steep above an apparent breakpoint (i.e. point of abrupt change in slope) of approximately 0.1 µmol.L$^{-1}$ SRP (Figure 1c). The 95th quantile smooth line representing the maximum effect of DIN:SRP on LRRN appeared to show a breakpoint at a DIN:SRP ratio of approximately 10 (Figure 1b). With regard to the TP data set, the 95th quantile smooth line representing the maximum effect of water TP on LRRP showed a possible breakpoint of approximately 1–2 µmol.L$^{-1}$ TP (Figure 1e).

## 3.3 Logistic regressions

With regard to the SRP data set, extremely low values of water DIN:SRP (approximately $\leq$ 1:1; Figure 2a) were significantly associated with a high probability of N limitation of chlorophyll *a* biomass (indicated by the significance of the DIN:SRP parameter estimate in Table 3). The probability of N limitation decreased with increasing water DIN:SRP ratio, but did not tend to zero until beyond a ratio of approximately 100:1. In model M1$_{BRN}$ , DIN appeared to have an effect on the probability of N limitation (Table 3). However, since the effect of DIN disappeared when DIN:SRP was added to the model (model M3$_{BRN}$), it was probably spurious, driven by partial correlation between DIN and DIN:SRP (Figure 3). Neither SRP nor DIN:SRP did appear to influence the probability of P limitation (Figure 2b; Table 3).

When the logistic models M1–M3 were applied to the TP data set, parameter estimates failed to converge, presumably because the data available were insufficient.

# 4 Discussion

We draw two main conclusions from our study. First, both the absolute and the relative N and P water concentrations contribute significant, albeit complementary, insights into the responses of microphytobenthos biomass to nutrient enrichment in running waters. Second, the ability of regression models based on N and P concentrations to predict these responses is nonetheless poor. We discuss these conclusions and their implications for fundamental and applied ecology in turn.

## 4.1 Importance of N and P for predicting limitation

Our linear meta-regression approach showed that the response of microphytobenthos biomass to N enrichment is influenced by the absolute concentration of N (DIN) in the water, the response decreasing with increasing DIN concentration. This outcome was expected, because it is consistent with predictions from kinetic nutrient
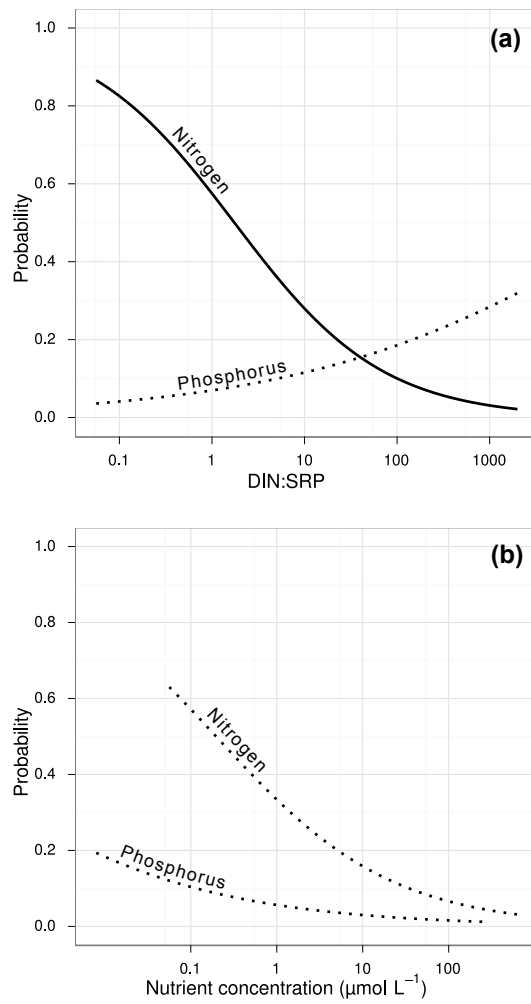
Figure 2: Results of the logistic regression approach. Probabilities that N and P were limiting microphytobenthos biomass as a function of DIN:SRP ratio (a; log scale) and absolute nutrient concentration (b; log scale). Probabilities are represented by solid lines if significant or by dotted lines if non-significant.
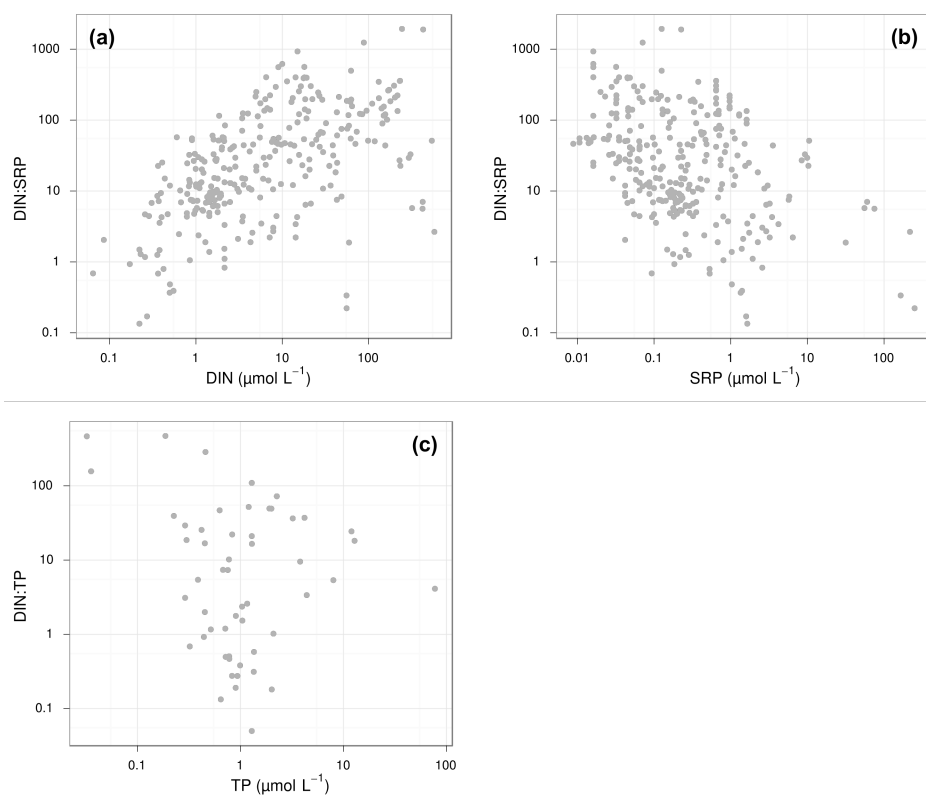
Figure 3: Relationships between the predictors used in our regression models. (a) DIN versus DIN:SRP; (b) SRP versus DIN:SRP; (c) TP versus DIN:TP.

| Model | Parameter | Coefficient | SE coefficient | CI coefficient (95%) |
|---|---|---|---|---|
| (a) | | | | |
| M1$_{LRRN}$ | Intercept | 0.570* | 0.00325 | [0.362, 0.778] |
| | DIN | -0.279* | 0.00234 | [-0.466, -0.168] |
| M2$_{LRRN}$ | Intercept | 0.624* | 0.00386 | [0.404, 0.957] |
| | DIN:SRP | -0.226* | 0.00231 | [-0.434, -0.106] |
| M3$_{LRRN}$ | Intercept | 0.642* | 0.00390 | [0.301, 0.866] |
| | DIN | -0.203* | 0.00290 | [-0.415, -0.072] |
| | DIN:SRP | -0.108 | 0.00291 | [-0.256, 0.091] |
| M1$_{LRRP}$ | Intercept | 0.071 | 0.00294 | [-0.119, 0.234] |
| | SRP | -0.137 | 0.00265 | [-0.251, 0.018] |
| M2$_{LRRP}$ | Intercept | 0.050 | 0.00421 | [-0.214, 0.276] |
| | DIN:SRP | 0.048 | 0.00244 | [-0.054, 0.211] |
| M3$_{LRRP}$ | Intercept | 0.069 | 0.00409 | [-0.244, 0.258] |
| | SRP | -0.136 | 0.00288 | [-0.253, 0.070] |
| | DIN:SRP | -0.001 | 0.00258 | [-0.094, 0.209] |
| (b) | | | | |
| M2$_{LRRN}$ | Intercept | 0.589 | 0.01436 | [-0.020, 1.319] |
| | DIN:TP | -0.234 | 0.00955 | [-0.657, 0.117] |
| M3$_{LRRN}$ | Intercept | 0.621 | 0.01430 | [0.056, 1.022] |
| | DIN | -0.307* | 0.01415 | [-0.647, -0.020] |
| | DIN:TP | 0.022 | 0.01539 | [-0.296, 0.444] |
| M1$_{LRRP}$ | Intercept | 0.297 | 0.01049 | [-0.255, 0.849] |
| | TP | -0.450* | 0.00811 | [-0.926, -0.152] |
| M2$_{LRRP}$ | Intercept | 0.086 | 0.01457 | [-0.621, 0.871] |
| | DIN:TP | 0.227 | 0.00891 | [-0.321, 0.491] |
| M3$_{LRRP}$ | Intercept | 0.114 | 0.01286 | [-0.362, 0.739] |
| | TP | -0.404* | 0.00763 | [-0.934, -0.126] |
| | DIN:TP | 0.168 | 0.00768 | [-0.192, 0.467] |

Table 2: Results of the linear meta-regression approach (linear mixed models): (a) SRP data set, (b) TP data set. *Significant effect.

theory (Borchardt, 1996), and models predicting algal growth rate from either ambient nutrient concentration (e.g. the Monod model; Droop, 1961; Caperon, 1967) or nutrient cell quotas (e.g. the Droop model; Droop, 1968). All these models predict that algae can take up nutrients up to a certain supply rate, beyond which uptake capacity is saturated. Therefore, as N concentrations in the water approach the saturation capacity, the effect of N addition on microphytobenthos biomass should tend towards zero. Our results suggest that N additions stimulated microphytobenthos biomass at water DIN concentrations up to approximately 100 µmol.L$^{-1}$. However, the trend flattened somewhat above 20 µmol.L$^{-1}$ DIN. Similarly, data from 200 temperate streams reviewed by Dodds et al. (2002) indicated that relationship between maximum chlorophyll concentrations and total N (TN) had a breakpoint of about 150 µg.L$^{-1}$ (11 µmol.L$^{-1}$), which was interpreted as evidence for a saturation effect.

Adding water DIN:SRP did not improve the linear meta-regression model to predict the size of the response of microphytobenthos biomass to N additions over

| Model | Parameter | Coefficient | SE coefficient | P-value |
|---|---|---|---|---|
| M1$_{\text{BRN}}$ | Intercept | -0.556 | 0.3150 | 0.077 |
| | DIN | -0.871* | 0.2524 | <0.001 |
| M2$_{\text{BRN}}$ | Intercept | 0.303 | 0.3837 | 0.429 |
| | DIN:SRP | -1.249* | 0.2660 | <0.001 |
| M3$_{\text{BRN}}$ | Intercept | 0.341 | 0.3757 | 0.365 |
| | DIN | -0.242 | 0.2750 | 0.379 |
| | DIN:SRP | -1.070* | 0.3016 | <0.001 |
| M1$_{\text{BRP}}$ | Intercept | -2.244* | 0.3546 | <0.001 |
| | SRP | -0.654 | 0.3367 | 0.052 |
| M2$_{\text{BRP}}$ | Intercept | -2.593* | 0.5407 | <0.001 |
| | DIN:SRP | 0.556 | 0.3107 | 0.073 |
| M3$_{\text{BRP}}$ | Intercept | -2.634* | 0.5334 | <0.001 |
| | SRP | -0.500 | 0.3633 | 0.169 |
| | DIN:SRP | 0.339 | 0.3262 | 0.298 |

Table 3: Results of the logistic regression approach. *Significant effect.

the model based on DIN alone. In comparison, our logistic regression approach showed that the DIN:SRP ratio was the best predictor of the probability that N was limiting microphytobenthosbiomass. The probability that N was limiting increased over a wide range of DIN:SRP ratios, from 100:1 to 1:1. This wide range of transition does not support the idea implied by the critical-ratio hypothesis that N limitation sets in at a precise tipping point (Redfield, 1958) or over a narrow range of N:P ratios (Schanz and Juon, 1983). However, the expectation that aquatic systems 'tip over' from N limitation to P limitation at a definite N:P ratio stems from a questionable extension of Liebig's law of the minimum.

Liebig's law suggests that autotrophs are limited only by one resource at a given time, but this law was developed for individual crops, not entire communities (Danger et al., 2008). In reality, communities of autotrophs can probably circumvent single-nutrient limitation and take advantage of additions of either N or P over a wide range of N:P ratios through at least two adjustments. First, individual autotrophs may increase acquisition and reduce losses of the scarcest nutrient to reduce its limiting effects. Second, autotroph species vary in their optimal N:P ratio, and so shifts in water N:P ratio are likely to be accompanied by shifts in relative species abundance (Rhee and Gotham, 1980; Tilman et al., 1982; Kilham and Hecky, 1988; Stelzer and Lamberti, 2001; Klausmeier et al., 2004). These adjustments should lead to greater resource acquisition and productivity at the community level if N (or P) is added over a range of N:P ratios.

However, our results show that there are constraints to these adjustments. The decline in the probability of N limitation over the 1:1 to 100:1 DIN:SRP ratio interval suggests that microphytobenthos assemblages encounter increasing difficulty in making these adjustments as the water N:P ratio increases. Likewise, N limitation at extremely low ($\leq$ 1:1) water N:P ratios suggests that under these conditions individual autotrophs can no longer adjust physiologically, and even species of algae and cyanobacteria with the lowest critical N:P ratios have difficulties sustaining growth. In theory, N-fixing cyanobacteria could grow even without N supply from the wa-

ter and should not be limited by low water N:P ratios (Elser and Urabe, 1999). However, N-fixing cyanobacteria are poor competitors for P (Suttle and Harrison, 1988) and are rare where absolute P concentrations are lower than approximately 1 µmol.L$^{-1}$ TP (Downing et al., 2001). In most streams and rivers, P concentrations are probably too low to support substantial biomass of these autotrophs.

Microphytobenthos responses to P enrichment were influenced by the absolute water P (TP) concentration. Again, the negative effect of water P concentration on the microphytobenthos response implies a saturation effect. Interestingly, however, the effect of P emerged when we used TP as an index of bioavailable P, but not when we used SRP. This difference supports the contention put forward by lake ecologists that TP may reflect more accurately the total P available to algae and cyanobacteria, because these organisms can use organically bound as well as inorganic P (Rigler, 1966; Morris and Lewis, 1988; Bergström, 2010). The possible breakpoint of 1–2 µmol.L$^{-1}$ TP identified in this study is within the values of 100 µg.L$^{-1}$ (approximately 3 µmol.L$^{-1}$) or 27 µg.L$^{-1}$ (approximately 1 µmol.L$^{-1}$), indicating breakpoints in the relationship between maximum chlorophyll *a* and TP in the data set reviewed by Dodds et al. (2002). Water DIN:SRP failed to predict P limitation, but, given the limitations of SRP as an index of bioavailable P, the lack of relationship is not surprising. Unfortunately, owing to scarcity of data, we were not able to assess whether DIN:TP is a better predictor of P limitation in streams, as it is in lakes (Morris and Lewis, 1988; Bergström, 2010).

A main question addressed in this study concerns the relative importance of the ratio between nutrients and their absolute concentrations for predicting nutrient limitation (Bothwell, 1985; Dodds, 2003). We suggest that absolute and relative concentrations are both important to microphytobenthos, but influence different aspects of it. Excluding extreme values, the water N:P ratio influences microphytobenthos species composition and, possibly, physiological mechanisms of nutrient retention and acquisition within species. In comparison, the absolute concentrations of N or P determine the potential magnitude of the effect of nutrient addition on microphytobenthos biomass. If the initial concentration of one nutrient is well below saturation, the response of microphytobenthos biomass to an addition of that nutrient is potentially large (even if the concentration of other nutrients is above saturation, see Donald et al., 2011). If the initial concentration is at or above saturation, no further response should be possible (Bothwell, 1985; Stelzer and Lamberti, 2001). According to the studies reviewed, concentrations of N and P above saturation (approximately 20 µmol.L$^{-1}$ DIN and 1– 2 µmol.L$^{-1}$ TP, see above) are unusual in streams. Therefore, additions of either N or P (or both) should likely lead to increases in microphytobenthos biomass in streams, except where other ecological factors (e.g. light or micronutrients) are more limiting. However, based on our results, we cannot disregard the possibility that DIN:SRP had an effect at least on the microphytobenthos response to N enrichment. The effect would have been difficult to detect owing to some heteroscedasticity in the data (that transformations could not remove), a correlation between DIN and DIN:SRP (Spearman correlation = 0.58) and a slightly nonlinear relationship between DIN:SRP and the microphytobenthos response. Nonetheless, any effect by DIN:SRP would have been weaker than the effect by DIN concentration we were able to detect.

## 4.2 Predictive ability of our models

Despite the importance of absolute and relative water concentrations of N and P for predicting the microphytobenthos response to N or P additions, a considerable amount of variation in our data remained unexplained. The remaining variability may arise from differences in experimental conditions (Capps et al., 2011) and variation in environmental factors including light (Hill et al., 1995), light–nutrient interaction (Rosemond, 1994; Carey et al., 2007; Elsaholi et al., 2011), grazing (Hill et al., 1992; Rosemond et al., 1993) and physical disturbance (Biggs and Close, 1989). Unfortunately, we could not assess the importance of these factors in our global meta-analysis. Environmental factors such as light or grazing were rarely reported in the studies reviewed. Experimental conditions including the duration of the manipulation and the concentration of the nutrients used were usually reported, but our models already included three covariates (random effects of river, site and study) and up to three explanatory variables. Adding further covariates would have impaired model convergence and hindered our chance to detect the effects of the explanatory variables we were most interested in studying (F. Keck, pers. observ.).

Another possible obstacle to the predictive ability of nutrient limitation models is that microphytobenthos, when relatively thick, can hinder diffusion of nutrients from the water column (Bothwell, 1985; Mulholland et al., 1994). As a result, algae and cyanobacteria within thick microphytobenthos matrices may develop nutrient limitation over a wide range of external (water column) concentrations, even above concentrations that would saturate single cells. We do not know the thickness of microphytobenthos matrices at the end of the experiments reviewed, but in some cases the matrices might have been thick enough to have become decoupled from nutrient concentrations in the water column and dependent on internal nutrient cycling. Such cases would weaken the relationship between nutrient limitation and nutrient concentrations in the water column.

Ability to predict nutrient limitation would have important applications; for example, it would enable managers to target nutrient-abatement schemes to reduce eutrophication, or foresee the ecological effects of pollution by either P or N (Lepori and Keck, 2012). However, based on the results of this study, we advocate caution in predicting which nutrient limits primary producers in running waters based on the relative and absolute concentrations of N and P in the water. The traditional approach, in which the limiting nutrient is predicted based on published critical N:P ratios, probably results in frequent errors. The global regression models presented in this paper should be more accurate, but have limited practical use. For example, the majority of the streams and rivers in our data set (67%) had water DIN:SRP ratio within the range where the ability of our models to predict the limiting nutrient is poor (100:1 to 1:1).

In all probability, stream ecosystems have differences and idiosyncrasies at the individual and catchment scale which constrain the predictive ability of nutrient limitation models. However, models developed at smaller spatial scales might yield more accurate predictions and be more useful for applied purposes. For example, models developed within ecoregions (Omernik, 1977) could reduce the influences of latitude, temperature and the regional pools of organisms, which probably add substantial variance around the relationships between nutrients and microphytobenthos in our models. Moreover, nutrient limitation models should ideally take into

account sources of variability such as light, grazers, physical disturbance and the interactions among these factors, in addition to water nutrient concentrations. Finally, we also propose that the extent of single-nutrient limitation in running waters should be investigated further. If single-nutrient limitation applies only to extreme N:P ratios, as our results suggest, it would be important to understand what controls microphytobenthos biomass across typical N:P ratios.

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# 6   Supplementary Material

Figure S1: Frequency of the nutrient manipulations included in the dataset by the latitude of the streams and rivers in which they were conducted.

Appendix S1. Strings of keywords used for electronic searches.

Appendix S2. List of studies included in the data set.

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