

CONSTRAINTS ON PRIMARY PRODUCER N:P STOICHIOMETRY ALONG N:P SUPPLY RATIO GRADIENTS

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Abstract. A current principle of ecological stoichiometry states that the nitrogen to phosphorus ratio (N:P) of primary producers should closely match that from environmental nutrient supplies. This hypothesis was tested using data from ponds in Michigan, USA, a freshwater mesocosm experiment, a synthesis of studies from diverse systems (cultures, lakes, streams, and marine and terrestrial environments), and simple dynamic models of producer growth and nutrient content. Unlike prior laboratory studies, the N:P stoichiometry of phytoplankton in Michigan ponds clustered around and below the Redfield ratio (7.2:1 by mass), despite wide variation in N:P supply ratios (2:1–63:1 by mass) and the presence of grazers. In a mesocosm experiment, the N:P stoichiometry of phytoplankton cells again deviated from a nearly 1:1 relationship with N:P supply. Phytoplankton seston exhibited lower N:P content than expected at high N:P supply ratios, and often higher N:P content than anticipated at low N:P supply ratios, regardless of herbivore presence. Similar deviations consistently occur in the N:P stoichiometry of algae and plants in the other diverse systems. The models predicted that both high loss rates (sinking, grazing) and physiological limits to nutrient storage capacity could attenuate producer stoichiometry. In the future, research should evaluate how limits to elemental plasticity of producers can influence the role of stoichiometry in structuring communities and ecosystem processes.

Key words: aquatic ecosystems; food webs; grazers; nitrogen:phosphorus ratio; N:P; primary producers; quota saturation; Redfield ratio; stoichiometry.

INTRODUCTION

Ecological stoichiometry (Sterner and Elser 2002) proposes that mismatches in elemental composition (stoichiometry) between consumers and their resources may constrain species interactions and regulate food web structure. Such mismatches may occur in aquatic ecosystems when the ratio of nitrogen to phosphorus (N:P) content of primary producers differs from that of their grazers. Furthermore, flexible N:P stoichiometry of producers may accentuate this mismatch, since the N:P content of algae can greatly exceed that of relatively homeostatic, P-rich grazers. As a result, this stoichiometric mismatch may increase nutrient limitation of herbivores and further skew nutrient cycling from grazers (Elser et al. 2000, Sterner and Elser 2002). Thus ecologists need to focus on drivers of elemental flexibility of producers, such as environmental N:P sup-

ply gradients, to gain a better understanding of stoichiometric constraints on food webs.

This focus on N and P is fundamental because these macronutrients most frequently limit primary production in aquatic ecosystems (Smith 1979, 1982, Hecky and Kilham 1988, Downing 1997). In these ecosystems, co-limitation of producer growth by these essential resources occurs infrequently, since N:P supply ratios typically deviate from optimal requirements of producers (Downing and McCauley 1992, Andersen 1997, Downing 1997). As a result, individual producer species often store nutrients supplied in excess of their cellular requirements via “luxury consumption” (Rhee 1973, Sterner and Elser 2002). This adaptation allows producers to exploit variable resource supply conditions. However, it can also create wide variation in producer elemental composition at low and high N:P supply ratios (Elser et al. 2000, Sterner and Elser 2002, Klausmeier et al. 2004).

Phytoplankton in laboratory experiments clearly illustrate the great plasticity in producer cell quotas of N and P. In chemostats, *Scenedesmus* (Rhee 1978) and *Dunaliella* (Goldman et al. 1979) can vary greatly in their N:P content at equilibrium (see Fig. 1A for Rhee’s data). Furthermore, the N:P composition of the slowly growing phytoplankton closely tracks variations in N:P supply ratio (Fig. 1A; Rhee 1978, Klausmeier et al. 2004). Thus phytoplankton at low equilibrium growth

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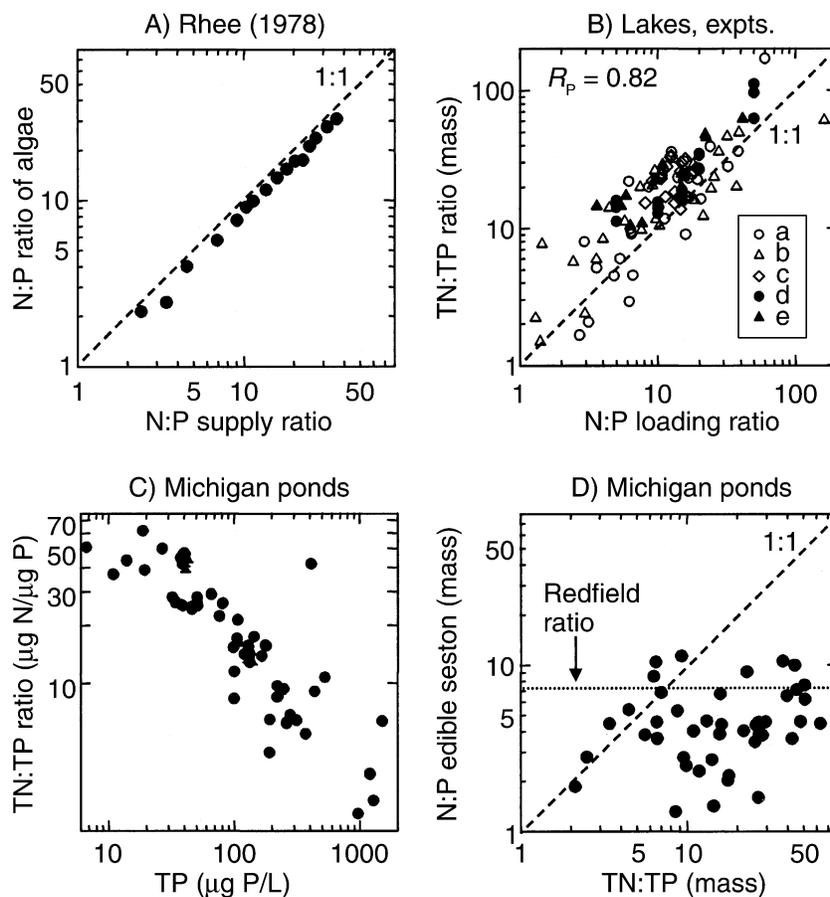


FIG. 1. (A) In Rhee's (1978) experiment, the N:P content of *Scenedesmus* spp. closely tracked N:P supply ratios. (B) In lakes and mesocosms, TN:TP was positively correlated with observed TN:TP ratios based on the following sources: a, Salas and Martino (1991); b, Baker et al. (1981); c, South Florida Water Management District, (Karl E. Havens, *personal communication*); d, V. Smith (*unpublished data*); e, Levine and Schindler (1992, 1999). (C) In Michigan ponds, TN:TP ratios ranged widely but were negatively correlated with TP. (D) Unlike *Scenedesmus* in Rhee's (1978) experiment, the N:P ratio of edible (<35 μm) algal seston in Michigan ponds did not increase with TN:TP ratio past ~ 10 $\mu\text{g N}/\mu\text{g P}$. Note the log scales throughout.

rates do not maintain N:P homeostasis and are instead "what they have available to eat" (Sterner and Elser 2002). These observations prompted Sterner and Elser (2002) to propose a key principle of ecological stoichiometry: that the N:P of phytoplankton biomass generally mirrors the N:P of the inorganic nutrient supply.

The plasticity of nutrient content seen in laboratory experiments could be accentuated in nature by large gradients of nutrient supply ratios and consumer-mediated nutrient recycling. The N:P ratio of nutrient loading to both freshwater lakes (Downing and McCauley 1992) and marine ecosystems (Downing 1997) can vary by more than two orders of magnitude among sites. If the laboratory results successfully extrapolate to nature, this variation in nutrient supply ratios should, in turn, promote wide variation in producer N:P stoichiometry. Additionally, the grazing activities of herbivorous consumers (e.g., crustacean zooplankton) can modify the nutrient content of producers. Compared to

their phytoplankton prey, zooplankton grazers maintain relative N:P homeostasis and contain more P than do algae (Elser et al. 2000, Sterner and Elser 2002). This elemental imbalance between plant and herbivore can induce P-limitation of grazers and skew the ratio of nutrients that grazers recycle (Sterner 1990, Andersen 1997, Elser and Urabe 1999, Grover 2002). Such a skew could induce a positive feedback in which P-rich grazers preferentially store P and recycle excess N. This positive feedback effect increases both the effective N:P supply ratio and the N:P content of phytoplankton (Sterner 1990, Elser and Urabe 1999, Sterner and Elser 2002). Therefore grazer-mediated nutrient recycling could push producers in nature toward even more extreme N:P content which, in turn, could further exacerbate nutrient-limited herbivory.

This study is centered on plasticity of N:P stoichiometry of producers. We tested the hypothesis that, as in laboratory experiments, the N:P stoichiometry of

primary producers in nature is tightly linked to variation in N:P supply ratios. We used data obtained from three very different sources: a survey of ponds in southwestern Michigan (USA); a seminatural pond mesocosm experiment in which we controlled N:P supply ratios, total nutrient supply, light supply, and trophic structure; and a literature survey of diverse aquatic and terrestrial systems. To make these interecosystem comparisons, we assumed that the stoichiometry of multicellular producers behaves like that in phytoplankton, as described in Sterner and Elser (2002). With these data and an examination of variations on a mathematical model, we addressed two questions: (1) Does producer stoichiometry in natural communities closely mirror variation in N:P supply ratios, as seen in phytoplankton cultures (Rhee 1978, Goldman et al. 1979)? (2) If not, which mechanism(s) may act to regulate, constrain, or accentuate variation in producer N:P stoichiometry along gradients of N:P supply ratios in natural ecosystems?

METHODS

Pond survey

During the summer of 2000, we characterized variation of the N:P composition of pond phytoplankton assemblages along broad gradients of nutrient supply. These ponds were mostly small (median surface area: 678 m²; minimum 56 m², maximum 17 500 m²) and experienced a wide gradient of incident irradiance (median: 50% forest canopy openness; minimum 9%, maximum 81%). All sites were located in southwest Michigan, USA, either proximate to the Kellogg Biological Station's Experimental Pond Facility, Bird Sanctuary, and Lux Arbor Preserve (Kalamazoo and Barry Counties); within Barry and Middleville State Game Areas (Barry County); or within Ludington State Park (Mason County). We collected water from at least four different sites in each pond, pooled the samples, and used duplicate 250 mL aliquots for water chemistry analyses. Samples were kept on ice until we returned to the lab. Spatial coordinates were noted using a Garmin GPS 12 unit. Total phosphorus in pond water was measured using the molybdate–ascorbic acid method (APHA 1980, Prepas and Rigler 1982), whereas total nitrogen concentration was analyzed using second-derivative ultraviolet spectroscopy (Crompton et al. 1992, Bachmann and Canfield 1996). To measure the N:P content of seston (algae plus detritus plus bacteria), we sieved samples through 35- μ m Nitex screening (Wildlife Supply Company, Saginaw, Michigan, USA; Cottingham 1999) and filtered two samples per tank onto precombusted, sulfuric-acid cleaned GF/F filters (Whatman plc., Florham Park, New Jersey, USA). Filters for C:N analysis were waved in sulfuric-acid fumes to remove inorganic carbonates before drying to constant weight at 60°C. Dried filters were packed into tins (Cottech Analytical Technologies, Inc., Valencia CA

91355) and were combusted at 1050°C in a Carlo-Erba CHN autoanalyzer (Carlo Erba Instruments, Milan, Italy). Samples for analysis of particulate P (PP) were frozen at –80°C and autoclaved for 2 h in persulfate. Digestate was filtered through a 1- μ m pore syringe filter and analyzed for P content using the molybdate–ascorbic acid method (APHA 1980, Prepas and Rigler 1982).

We assumed that TN:TP (total nitrogen to total phosphorus) ratios observed in pond water corresponded well with the N:P ratio at which nutrients were supplied (loading ratio). To support this assumption, we collated literature-based data from lakes and mesocosm experiments (Baker et al. 1981, Salas and Martino 1991, Levine and Schindler 1992, 1999; South Florida Water Management District, *personal communication*; V. Smith, *unpublished data*). The loading N:P and observed TN:TP ratios were highly and positively correlated on a log₁₀ scale (Fig. 1B; Pearson $R = 0.819$, $P < 0.0001$ based on 9999 randomizations, $N = 108$). However, TN:TP concentration ratios were usually higher than those based on N and P loading ratios (Fig. 1B). Analysis by ordinary least squares (OLS)-based linear regression yielded a relationship of log₁₀(TN:TP) vs. log₁₀(N:P loading ratio) with an intercept of 0.257 (lower and upper 95% confidence limits of 0.123 and 0.395, respectively) and a slope of 0.917 (lower and upper 95% confidence limits 0.798 and 1.04, respectively; $R^2 = 0.671$, $P < 0.0001$; confidence intervals were determined using 10 000 bias-corrected, semi-parametric bootstraps).

Mesocosm experiment

During the summer of 2000, we created broad gradients of nutrient supply, irradiance, and trophic structure in a series of 108 experimental pond mesocosms. The rationale for this design, a study of stoichiometrically explicit plant–herbivore dynamics, and details of the experiment are described by Hall et al. (2004). Thus only the specifics germane to N:P stoichiometry are given here. During May to June 2000, we created light–nutrient–trophic manipulations in 300-L cattle tanks. To each tank, we added silica sand substrate, well water, and inorganic nitrogen (as NaNO₃) and phosphorus (as NaH₂PO₄) to raise nutrient concentrations to three target N:P ratios (ratio treatment: 5:1, 14:1, and 50:1 by mass) at two different total supply rates (low, 1 \times ; high, 10 \times), yielding six ratio–supply treatments in total. We added nutrients weekly to maintain these six target levels, assuming a 5% per day loss rate of N and P from the water column (M. A. Leibold and V. H. Smith, *unpublished data*), until three weeks before sampling the experiment for seston stoichiometry and water chemistry. Food web manipulations (trophic treatment) involved exclusion of grazers, addition of zooplankton, and addition of 12 adults of the carnivorous insect *Notonecta undulata*. We reduced light intensities by 90% in half of the mesocosms using neutral density shade cloth (light treatment).

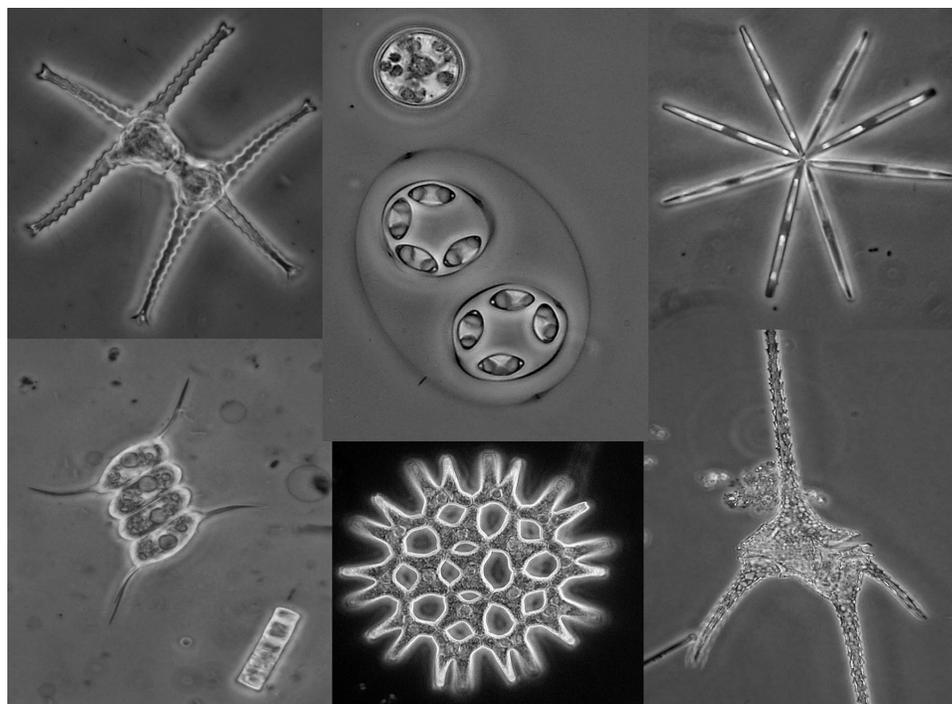


PLATE 1. We report on the stoichiometric response of phytoplankton assemblages from aquatic ecosystems. These assemblages include diverse and beautiful taxa, such as (clockwise, from top left): *Staurastrum*, *Cyclotella* (bottom) and *Oocystis* (top), *Actinastrum*, *Ceratiium*, *Pediastrum*, and *Scenedesmus*. Photo credit: R. O. Megard.

We inoculated each mesocosm with diverse assemblages of algae and zooplankton every two weeks. These inocula contributed only inconsequentially (<0.6%) to the daily loading rates of N and P in the most nutrient-poor treatments. By continuously adding these diverse assemblages, we sought to mimic how a regional species pool, not just a local species assemblage, would respond to the resource supply gradients (Leibold et al. 1997, Hall et al. 2004). Inocula were collected from local ponds along wide gradients of irradiance, nutrient concentration, and trophic structure. Each tank also received 30 *Physa* spp. snails and 30 *Rana catesbeiana* tadpoles to graze and remineralize nutrients bound in benthic algae. These grazers maintained relatively low periphyton growth on the walls of the mesocosms (Hall et al. 2004).

Final algal stoichiometry was characterized by sampling at the end of the summer (15–17 September 2000), three weeks after nutrient additions were stopped. At that time 8.5-L samples were collected using tube samplers and a defined spatial sampling regime. We sieved water through 35- μ m mesh for subsequent N:P analysis of the “edible” algal fraction (Cottingham 1999). This fine seston was concentrated onto precombusted, acid-rinsed GF/F filters and analyzed for particulate N and P, as described in the *Pond survey* section.

Literature surveys

We collected published data from diverse sources to more thoroughly explore the relationship between pri-

mary producer N:P stoichiometry and that of their nutrient supply ratios. These sources include phytoplankton from freshwater lakes and mesocosms, marine ecosystems, and single-species laboratory cultures (see Plate 1); periphyton from streams and lakes; and plants from terrestrial and wetland habitats (see Appendix A for details). Wherever possible, we obtained values for the actual N:P supply ratio directly from the methods section of each study. However, when direct measurements of the N:P supply ratio were not available, we used reported TN:TP ratios to estimate the external N:P supply ratio (which was reasonable, given results shown in Fig. 1B).

Statistical analyses

In the pond survey data, we calculated significance of the Pearson correlation (R_p) between $\log_{10}(\text{TP})$, $\log_{10}(\text{TN})$, and $\log_{10}(\text{TN:TP ratio})$ using 9999 randomizations. Spatial autocorrelation between ponds could also potentially confound this relationship. Thus we also calculated the partial Mantel statistic, a common test for spatial relationships among variables (Legendre and Legendre 1998). Using a statistic closely related to Pearson's correlation, the Mantel test measures the extent to which variation in differences in measured variables (e.g., nutrient concentration) corresponds to spatial distances among ponds. A significant partial Mantel statistic (R_M) ensured that a strong relationship existed between nutrient variables after controlling for

spatial autocorrelation. Typically comparisons between distance matrices produce positive (partial) Mantel correlations, so we computed a single-tailed test using 9999 randomizations (Legendre and Legendre 1998).

We also fit linear OLS regression models between water column TN and TP concentrations in ponds, and between TN:TP ratios and N:P content of edible pond seston. We calculated 95% bias-corrected confidence intervals on each of the regression coefficients using 10 000 semiparametric bootstraps, and we calculated the significance of the regression using 9999 randomizations. For each of light–nutrient–trophic treatments in the mesocosm experiment and for literature-based data sets, we fit linear OLS regressions between the cellular stoichiometry of primary producers (N:P) and estimates of N:P supply ratios. We used the same randomization and bootstrapping methods for these regressions as for the pond survey data set. Assuming close matching of producer stoichiometry to N:P supply ratios, we anticipated a priori that 95% confidence intervals on the intercept parameter of these regressions from the pond survey, mesocosms, and literature data sets should overlap with zero, and those of the slope parameter should overlap with one. The Rhee (1978) data (Fig. 1A) met this first expectation (intercept of -0.122 , with lower and upper 95% confidence limits of -0.902 and 0.325 , respectively). However, the slope of the Rhee (1978) data was significantly less than unity (slope of 0.857 , with lower and upper 95% confidence limits of 0.826 and 0.882 , respectively). Thus we adjusted the expected slope to 0.85 .

Theoretical analyses

We used standard chemostat models to evaluate how producer stoichiometry might deviate from ratios expected on the basis of nutrient supply ratios. These analyses focused on high rates of cell loss from the algal population and physiological limitations to nutrient storage. In both cases, we used modified versions of Klausmeier et al.'s (2004) model to track temporal changes in producer carbon, N and P quotas, and freely available (dissolved) N and P. Details of model structure and performance are presented in Appendix B. In our version of the model, primary production increased multiplicatively with light (L) following a Monod model and with nutrient quota of the plant in a saturating fashion following the Droop model. We added the potential for light limitation because we surveyed some shaded ponds and manipulated irradiance in the mesocosm experiments. However, the plant was only limited by one nutrient at a time (following Liebig's Law). Nutrients and producers were lost from the model system at a constant rate (dilution rate, a) and as a result of producer mortality at rate, m . However, nutrients sequestered in these dead producers were immediately recycled (Grover 1997), unlike in Klausmeier et al.'s model (Klausmeier et al. 2004). In chemostats in which only nutrients limited primary production, these com-

bined loss rates ($m + a$) typically correspond to per capita growth rate (turnover) of the producer at equilibrium (Goldman et al. 1979, Klausmeier et al. 2004).

In the first variant of the model, we did not impose a physiological limit on the nutrient content of producers. Thus producers could store larger quantities of nutrients than their physiology might allow. However, structural and physiological limits must eventually place upper boundaries on nutrient storage capacity of plants. Therefore, in the second model version, we explored the implications of such limits to storage by prohibiting N and P quotas from exceeding defined maximal levels (Appendix B). Thus in this analysis, further nutrient storage ceased once the producer exceeded its storage quota for that nutrient. To illustrate the potential effect of limits to storage, we considered a range of variation (2 , $1\times$, $0.5\times$, $0.25\times$) in storage capacity, derived from Andersen's (1997) review of published phytoplankton traits while holding other phytoplankton traits (parameters) constant. Here the $1\times$ case corresponded to the storage capacity of the "typical" phytoplankton species (7.5 for P, 3.9 for N).

RESULTS

Empirical analyses

The pond study revealed a broad gradient in N:P supply ratios but highly constrained producer stoichiometry. As observed in lakes and marine ecosystems (Downing and McCauley 1992, Downing 1997), the large gradient in water column TN:TP ratios in Michigan ponds were strongly and negatively correlated with the broad range of pond fertility as measured by TP (Fig. 1C; $R_p = -0.85$, $P < 0.0001$; $R_M = 0.68$, $P < 0.0001$; $N = 44$) and less strongly correlated with TN (not shown; $R_p = -0.33$, $P = 0.0304$; $R_M = 0.074$, $P = 0.0123$; $N = 44$). While a negative relationship between TP and TN:TP was expected a priori because of autocorrelation, OLS regression showed that TP increased more quickly than TN, as the slope of the OLS regression between $\log_{10}(\text{TN})$ and $\log_{10}(\text{TP})$ was substantially less than unity (0.431 , with lower and upper 95% confidence limits of 0.321 and 0.536 , respectively). Thus high-nutrient systems had lower TN:TP ratios than did low-nutrient systems. Unlike the single-species cultures of Rhee (1978), the N:P stoichiometry of edible algal seston in these ponds bore little relationship to the N:P supply ratio gradient (Fig. 1C; $R^2 = 0.05$, $P = 0.13$, $N = 44$). Instead, phytoplankton N:P ratios clustered around or below the Redfield ratio (Fig. 1C), as the intercept of the regressions of N:P supply vs. producer N:P content was greater than zero (4.07 , with lower and upper 95% confidence limits of 2.91 and 5.40 , respectively), and the confidence interval of the slope coefficient overlapped with zero (0.04 , with lower and upper confidence limits of -0.007 and 0.09 , respectively).

TABLE 1. Regression results from the 2000 mesocosm experiment on N:P content of seston (dependent variable) vs. N:P supply ratio (independent variable) (with both variables scaled arithmetically).

Treatment, by trophic level	Regression statistics			Regression parameters	
	R^2	P^\dagger	N	Intercept (95% CI) $‡$	Slope (95% CI) $‡$
Algae only					
High supply					
Full light	0.71	0.0014	9	6.80 (1.52, 12.77)	0.46 (0.28, 0.66)
Shaded	0.80	0.0016	9	8.37 (4.92, 11.91)	0.37 (0.26, 0.49)
Low supply					
Full light	0.00	0.98	9	12.81 (10.10, 16.91)	-0.0013 (-0.102, 0.118)
Shaded	0.0050	0.85	9	11.16 (8.27, 14.12)	-0.010 (-0.11, 0.09)
Grazers added, no predators					
High supply					
Full light	0.11	0.35	9	6.97 (3.69, 10.86)	0.067 (-0.047, 0.19)
Shaded	0.002	0.99	9	9.13 (0.83, 24.48)	-0.027 (-0.36, 0.53)
Low supply					
Full light	0.09	0.45	9	18.99 (11.52, 30.06)	-0.14 (-0.42, 0.18)
Shaded	0.21	0.19	9	6.38 (4.95, 7.95)	0.038 (-0.0097, 0.090)
Grazers and predators added					
High supply					
Full light	0.32	0.08	9	5.05 (2.42, 7.91)	0.099 (0.012, 0.20)
Shaded	0.19	0.245	9	3.55 (2.12, 5.40)	0.038 (-0.014, 0.094)
Low supply					
Full light	0.27	0.15	9	7.32 (4.41, 9.67)	0.080 (-0.014, 0.16)
Shaded	0.15	0.29	9	6.77 (5.04, 7.83)	0.030 (-0.017, 0.071)

† Significance values determined with 9999 randomizations using R^2 as the pivotal statistic.

$‡$ Bias-corrected, 95% confidence intervals on intercept and slope parameters were determined with 10 000 semiparametric bootstraps.

As in Michigan ponds, N:P ratios of algal seston in the experimental mesocosms also deviated strongly from the expected 1:1 relationship with N:P supply ratio (Table 1, Fig. 2). In all treatments, intercepts of the N:P supply vs. seston N:P regressions were significantly and substantially greater than zero, while the slopes from these regressions were all much less than Rhee's (1978) value of 0.85. Producers tracked the N:P supply ratio most closely (i.e., had the highest slope) at a high nutrient supply without grazers (Table 1). In most other cases, producers were more P-rich relative to N at high N:P supply ratios (50:1), and often more N-rich relative to P than expected in the low N:P supply treatments (5:1). Notably, at high nutrient supply, N:P content of producers was lower in the 50:1 supply ratio treatments with grazers than in those without. Thus skewed recycling by grazers did not apparently induce net positive feedback on producer stoichiometry. Such feedback would drive N:P content of producers even higher than their supply ratios (Fig. 2).

Similar deviations from a 1:1 relationship between N:P content of producers and their N:P supply ratios were evident in literature data representing diverse aquatic and terrestrial communities (Fig. 3). Not surprisingly, these data were noisy, as they summarized many different producer species, studies, and analytical methods (see Appendix A for more details). In all cases, regression intercepts were >3.0 , while their 95% confidence intervals never overlapped with zero (Table 2).

Additionally, the slope parameters were always <0.3 , while the 95% confidence intervals never overlapped with the expected value of 0.85 from Rhee's (1978) experiment. Thus in lakes, lake and pond mesocosms, stream and lake periphyton, terrestrial and wetland plants, and marine systems, the cellular N:P content of diverse primary producers was consistently greater than expected at low N:P supply ratios, and lower than expected at high N:P supply ratios (Fig. 3). This pattern was also evident from uni-algal laboratory cultures, indicating that Rhee's (1978) result with *Scenedesmus* was not characteristic of most phytoplankton, even those in the laboratory.

Theoretical analyses

Variations on a simple chemostat model revealed two possible mechanisms that may underlie this consistently attenuated response of producer stoichiometry to N:P supply gradients. The first mechanism involved variation in loss rates of the producer. At low loss rates, N:P content of the producer closely tracked N:P supply, even at very high supply ratios (Fig. 4A; see also Fig. 3C of Klausmeier et al. 2004). However, at high loss rate (e.g., $m + a > 0.5/\text{day}$, where m is mortality and a is dilution rate) and at high light supply ($L = 750 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the N:P content of producers deviated from N:P supply ratios. Specifically, plants contained more P relative to N than expected at high N:P supply ratios and less P relative to N than expected

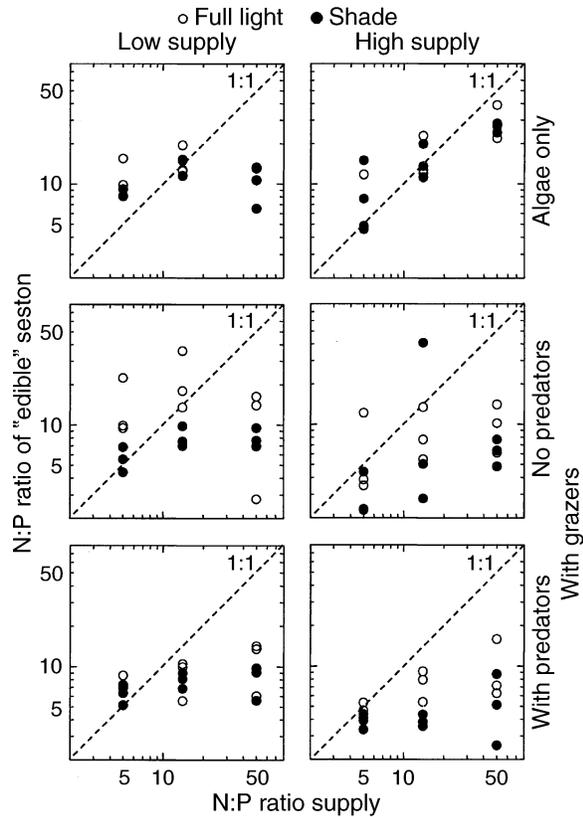


FIG. 2. The N:P ratio of algal seston in response to gradients of N:P supply ratio in a pond mesocosm experiment. Light, trophic, and nutrient supply treatments were analyzed separately (Table 1). Seston contained much more P relative to N than predicted by the 1:1 line at the 50:1 N:P supply ratio, and sometimes less P relative to N than predicted at the 5:1 N:P treatment. Axes were scaled logarithmically for visual clarity.

at low supply ratios (Fig. 4A). In addition, this pattern held if mortality (m) was low but dilution rate (a) was high (results not shown). In both cases, high loss rates promoted high realized growth rates of the producer at equilibrium. These high growth rates, in turn, drive attenuation of N:P ratios in producers because quickly growing producers “eat what they need” (Goldman et al. 1979, Klausmeier et al. 2004). However, low light supply (e.g., $L = 75 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) greatly diminished this attenuation effect (Fig. 4A). At a given loss rate, a producer’s equilibrium growth rate is lower at low irradiance than at high irradiance, due to increased degree of light limitation. Therefore even at high loss rates, strong light limitation eliminates this attenuation effect by reducing the equilibrium growth rate of the producer.

The second mechanism focused on limits to nutrient storage by plants. Here physiological constraints on cellular storage of nutrients reduced the ability of the producers to adjust their stoichiometry in response to changes in elemental supply ratios (Fig 4B). For ex-

ample, producers with intermediate to low storage capacity only tracked N:P supply at intermediate supply ratios, regardless of the irradiance regime. At more extreme N:P supply ratios, producer N:P ratios plateaued (Fig. 4B), and producers became saturated with P at the lower plateau and with N at the upper plateau. In both cases, once the producers became saturated, their N:P content ceased to respond to a more extreme N:P supply ratio. Not surprisingly, the N:P content of producers with lower nutrient storage capacity plateaued at more intermediate N:P supply ratios than did the N:P content of producers with higher storage capacity. This plateau effect, driven by physiological limits to storage, was accentuated when loss rate (m and/or a) increased (Fig. 4B; see Appendix B for a detailed explanation).

DISCUSSION

In their treatise on ecological stoichiometry, Sterner and Elser (2002) hypothesize that the N:P content of producers should closely match the ratio of N and P supplied to ecosystems. This principle stems in part from the plastic response of several phytoplankton species to wide gradients of N:P supply in laboratory experiments (Rhee 1978, Goldman et al. 1979). Given these previous findings, we had expected that producer communities in both seminatural and natural settings should also closely track changes in their N:P supply. Such tracking might exacerbate P limitation of grazers in these systems (Sterner and Elser 2002). We found instead that phytoplankton in ponds and in experimental pond mesocosms exhibited little response to wide gradients of N:P supply ratios. This attenuated response of cellular N:P stoichiometry was not restricted to shallow systems, as primary producers in diverse lake, benthic, terrestrial, and marine systems also contained less P relative to N than predicted at low N:P supply, and more P relative to N at high N:P supply. Overall these data provide powerful evidence that the cellular stoichiometry of primary producers in nature behaves much less responsively to variations in N:P supply ratios than was previously proposed.

The shallow depths of the ponds and pond mesocosms may have indirectly imposed a constraint on producer stoichiometry. The range of N:P supply ratios to Michigan ponds resembled the very broad range observed in other aquatic systems (Downing and McCauley 1992, Downing 1997). Yet phytoplankton N:P stoichiometry in these ponds and experimental pond mesocosms grouped around the Redfield ratio, showing much less variability than that reported for lakes, terrestrial plants, and oceans (Elser and Hassett 1994, Elser et al. 2000). Results from the chemostat model suggested that stoichiometry of strongly nutrient-limited producers experiencing high loss rates should respond much less to gradients in N:P supply than those experiencing low loss rates. This phenomenon occurs because high loss rates in these situations yield high

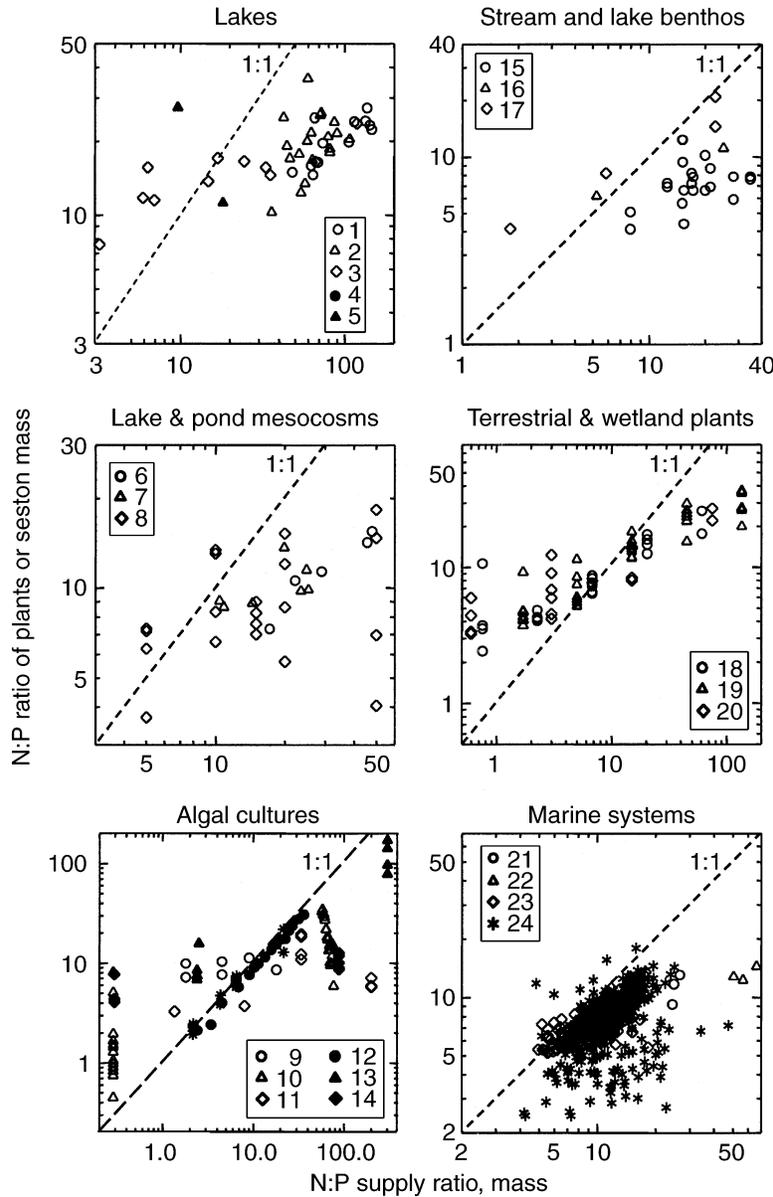


FIG. 3. Relationships between the N:P content of producers and N:P supply ratios for lakes, mesocosm experiments, single-species algal cultures, stream and lake periphyton, terrestrial and wetland plants, and marine algae (see Appendix A for reference numbers and details). Axes were scaled logarithmically for visual clarity.

growth rates (turnover) of producers at equilibrium. Furthermore, quickly growing producers are much less plastic than slowly growing producers (Klausmeier et al. 2004). This result may be relevant to field observations because phytoplankton in ponds sheltered from wind, such as the Michigan ponds, may quickly sink out of shallow water columns. Phytoplankton sink at a rate of 0.7–0.9 m/d (Fosberg 1985), and our ponds ranged from 0.1–2.0 m deep (and mesocosms were 0.25 m deep). Thus high sinking rates could promote high equilibrium growth rates (turnover) of pond phytoplankton and shunt the N:P content of producers closer to

the Redfield ratios (Fig. 4; Goldman et al. 1979, Klausmeier et al. 2004). This explanation is incomplete, however, as algae in heavily shaded Michigan ponds and shaded mesocosms also exhibited attenuated ranges of elemental ratios, contradicting model predictions at low irradiance, even at high loss rates (Fig. 4A). We infer that other mechanisms must have driven the attenuation pattern in shaded systems, although clearly further experimentation will be necessary to resolve this issue.

Physiological limits to a plant's storage capacity for nutrients may also decouple producer stoichiometry from nutrient supply ratios in field settings. At high N:

TABLE 2. Results from fits of ordinary least-squares linear regression models of N:P content of producers (dependent variable) and N:P supply ratio (independent variable, measured directly or estimated using TN:TP ratio) (with both variables scaled arithmetically) from literature data sets.

Data set	R^2	P^\dagger	N	Intercept (95% CI) ‡	Slope (95% CI) ‡
Lakes	0.29	<0.0001	44	14.03 (11.85, 17.03)	0.079 (0.045, 0.11)
Mesocosms	0.19	0.0100	33	7.53 (5.5798, 9.30)	0.10 (0.033, 0.18)
Algal cultures	0.34	<0.0001	89	3.27 (-0.97, 7.74)	0.26 (0.22, 0.32)
Periphyton	0.14	0.0353	31	5.59 (3.51, 8.32)	0.15 (0.019, 0.29)
Terrestrial plants	0.73	<0.0001	81	7.44 (6.29, 8.71)	0.19 (0.17, 0.22)
Marine systems	0.18	<0.0001	541	5.70 (5.22, 6.18)	0.21 (0.17, 0.25)

† Significance values determined with 9999 randomizations using R^2 as the pivotal statistic.

‡ Bias-corrected, 95% confidence intervals on intercept and slope parameters determined with 10 000 semiparametric bootstraps.

P supply ratios, limits to N storage in the chemostat model could constrain even a typical phytoplankton's stoichiometry (Andersen 1997), while at low N:P supply, limits to P storage yielded higher producer N:P than expected. Increased loss rates accentuated this attenuation effect irrespective of light supply. Thus limits to storage could explain why we observed the attenuation of pond producer N:P stoichiometry at low irradiance. The storage result is also important because most phytoplankton species store less P than *Scenedesmus*, the genus featured in Rhee's (1978) study and in many experiments testing stoichiometric hypotheses with grazers (Andersen 1997, Sterner and Elser 2002). Assuming that P storage capacity correlates with N storage capacity, Andersen's (1997) survey suggests that *Scenedesmus* is extremely plastic relative to other

species. Therefore in natural assemblages dominated by less stoichiometrically plastic species, limits to nutrient storage could stabilize N:P content of producer assemblages along N:P supply gradients. Finally, cell-quota saturation may constrain variation in producer stoichiometry for nutrient elements other than N and P, such as silicon in marine (Kudo 2003) and freshwater diatoms (Peterson 1979).

Herbivores may also decouple algal stoichiometry from nutrient supply ratios, regardless of the producer's nutrient storage capacity. Grazers influence producer stoichiometry by physically reducing plant biomass, which increases turnover rates, and also by recycling nutrients (Sterner 1986). Through the negative feedback effect of biomass removal, grazers might stabilize the N:P response of producers toward the Redfield ratio

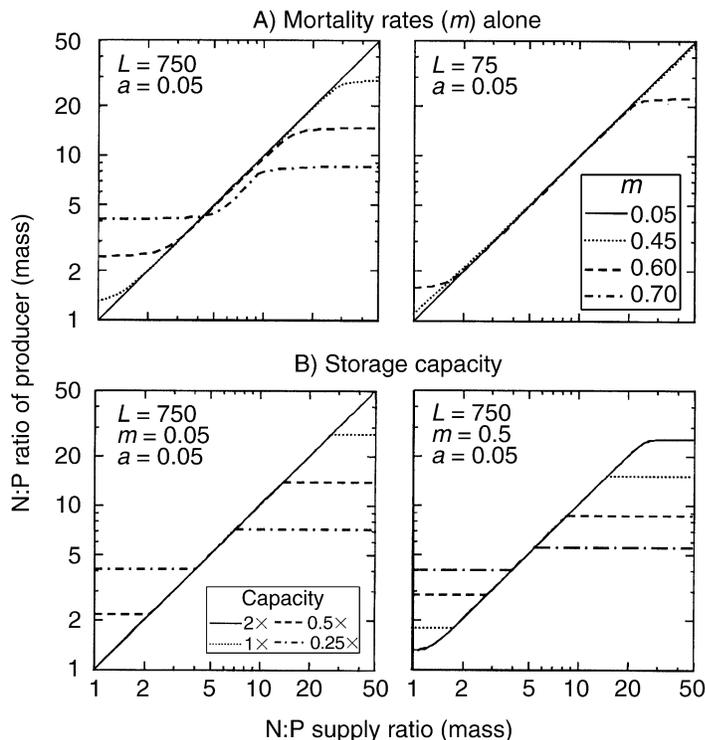


FIG. 4. Predictions of N:P content of producers over N:P supply gradients from variations on a chemostat model (Klausmeier et al. [2004]; see Appendix B for details). The dilution rate, a , is the rate of loss of nutrients and producers from the chemostat through dilution. (A) The N:P content of producers closely tracked broad gradients of N:P supply ratios at low mortality rate (m) but plateaued at low and high N:P levels as loss rate increased. This attenuation effect was pronounced at high light supply ($L = 750 \mu\text{mol of photons}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), but diminished considerably at low light ($L = 75$). (Note: At low L , the producer could not persist at $m = 0.70$.) (B) Limitations on nutrient storage also produced the plateau response. Storage capacities for N and P either increased ($2\times$) or decreased ($0.5\times$, $0.25\times$) from the "typical" (median) distributions (7.5 for P, 3.9 for N) presented in Andersen (1997:Table 3.1). As storage capacity decreased, producer N:P plateaued at more intermediate N:P supply ratios, especially as mortality rates increased. Note the log scales.

over a wide range of N:P supply ratios. In theory, the positive-feedback effect of differential nutrient recycling might counter this stabilization and push the N:P content of producers away from the Redfield ratio (Sterner 1990, Andersen 1997, Elser and Urabe 1999, Sterner and Elser 2002). Unfortunately, no fully dynamic model has evaluated the relative importance of these positive and negative feedbacks for producer N:P content, although a simplified version of Grover's (2003) model might offer an initial starting point. Regardless, results from our mesocosm experiment suggested that zooplankton grazers either had little net effect on the N:P content of phytoplankton (low nutrient supply treatments) or actually increased the P content of phytoplankton relative to N (high nutrient supply treatments; Fig. 3).

Other mechanisms potentially influence the N:P content of producers and investigators' estimates of producer stoichiometry. For instance, competition between algal species along N:P gradients (Tilman 1982, Sommer 1989, Grover 1997, Levine and Schindler 1999) could also stabilize the N:P content of producers at low N:P supply. In this scenario, N_2 fixation by cyanobacteria could elevate producer N content and thus increase the assemblage's N:P content (Smith 1983, 1986). Second, some phytoplankton in shallow lakes may be resuspended benthic algae (metaphyton; Meijer et al. 1999) that ordinarily receive nutrients from sediments (Scheffer 1998). The N:P supply from these sediments may differ from that measured in the water column. Third, the chemostat model presented here assumed a continuous nutrient supply, while the nutrient supply to many natural systems may occur episodically (e.g., Vanni et al. 2001). Such periodic supply may keep phytoplankton growing quickly, a feature that should stabilize cellular N:P ratios (Fig. 4). Finally, several of the data sets measure the N:P content of seston, which is actually a mixture of live algae, detritus and microbes. If P-rich bacteria and other microbes (Sterner and Elser 2002) occur abundantly, they could potentially reduce the N:P content of seston at high N:P supply ratios. While each alternate mechanism remains plausible in certain situations, it is unlikely that they explain the insensitivity of producer N:P stoichiometry to nutrient supply ratios observed in all of the diverse ecosystems analyzed here (Fig. 3). Instead, additional research will be required to evaluate mechanisms operating in each habitat.

Despite nuances specific to individual ecosystems, the overall message of this study remains clear. The N:P content of producers did not reflect elemental supply ratios at either high or low ratios. Thus insights from an influential experiment with laboratory phytoplankton cultures (Rhee 1978) neither adequately extrapolated to larger scales in systems with diverse assemblages of producers (and sometimes grazers) nor captured variation in the N:P stoichiometry of plants from other ecosystems. Instead, the data from various eco-

systems strongly echoed Goldman et al.'s (1979) general argument that factors such as high loss rates constrain variation in phytoplankton stoichiometry. These constraining factors include the physical structure of ecosystems, the physiological limitations of producers to store nutrients, and possibly food web architecture. As a result, ecologists who explore the implications of stoichiometric mismatches for food web architecture must also consider constraints to stoichiometric plasticity of producers.

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APPENDIX A

A table describing sources of literature data used to create Fig. 3 is available in ESA's Electronic Data Archive: *Ecological Archives* E086-100-A1.

APPENDIX B

A description and table summarizing the chemostat model used to create Fig. 4 is available in ESA's Electronic Data Archive: *Ecological Archives* E086-100-A2.