

Linking limitation to species composition: importance of inter- and intra-specific variation in grazing resistance

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Received: 16 May 2007 / Accepted: 7 December 2007
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Abstract Short-term responses of producers highlight that key nutrients (e.g., N, P)—or combinations of these nutrients—limit primary production in aquatic and terrestrial ecosystems. These discoveries continue to provide highly valuable insights, but it remains important to ask whether nutrients always predominantly limit producers despite wide variation in nutrient supply and herbivory among systems. After all, predictions from simple food chain models (derived here) readily predict that limitation by grazers can exceed that by nutrients, given sufficient enrichment. However, shifts in composition of producers and/or increasing dominance of invulnerable stages of a producer can, in theory, reduce grazer limitation and retain primacy of nutrient limitation along nutrient supply gradients. We observed both mechanisms (inter- and intra-species variation in vulnerability to herbivory)

working in a two-part mesocosm experiment. We incubated diverse benthic algal assemblages for several months either in the presence or absence of benthic macro-grazers in mesocosms that spread a broad range of nutrient supply. We then conducted short-term assays of nutrient and grazer limitation on these communities. In the “historically grazed” assemblages, we found shifts from more edible, better competitors to more resistant producers over enrichment gradients (as anticipated by the food web model built with a tradeoff in resistance vs. competitive abilities). However, contrary to our expectations, “historically ungrazed” assemblages became dominated by producers with vulnerable juvenile forms but inedible adult forms (long filaments). Consequently, we observed higher resource limitation rather than grazer limitation over this nutrient supply gradient in both “historically grazed” (expected) and “historically ungrazed” (not initially expected). Thus, via multiple, general mechanisms involving resistance to grazing (changes in species composition or variation in stage-structured vulnerability), producer assemblages should remain more strongly or as strongly limited by nutrients than grazers, even over large enrichment gradients.

Communicated by Libby Marschall.

Electronic supplementary material The online version of this article (doi:[10.1007/s00442-007-0948-z](https://doi.org/10.1007/s00442-007-0948-z)) contains supplementary material, which is available to authorized users.

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Keywords Benthic algae · Bottom-up/top-down · Competition–resistance tradeoff · Food web · Nutrient enrichment

Introduction

Which factors limit primary production? The answers to this question, determined directly by (typically) short-term growth assays and whole-ecosystem nutrient additions (Schindler 1977; Hecky and Kilham 1988; Elser et al.

1990), have historically yielded great victories for basic and applied ecology. They also continue to produce surprising contemporary results (e.g., Harpole and Tilman 2007). In lake and terrestrial ecosystems, these limitation assays have established P, N, other nutrients, or some combination as key nutrient resources limiting both algal producers and plants (Hecky and Kilham 1988; Elser et al. 1990, 2008; Howarth and Marino 2006; Smith 2006; Harpole and Tilman 2007; Harpole et al. 2008). In the open ocean, similar experiments indicated that iron can limit producers (Downing et al. 1999). Thus, limitation assays have and continue to enable ecologists to discover key drivers of assemblages that pivotally influence energy and nutrient transfer in aquatic and terrestrial food webs. Furthermore, these assays can inform strategies that address and prevent eutrophication and plans to sequester C (Howarth and Marino 2006; Smith 2006; Elser et al. 2008).

Despite the importance of these results, such findings should prompt two interrelated questions. First, why are these nutrient resources, rather than grazers, targeted as limiting factors? In some systems, grazers can exert a profound influence on the biomass of producers (Chase et al. 2000a), e.g., the large zooplankton grazer *Daphnia* in freshwater systems (Carpenter and Kitchell 1993; Tessier et al. 2001). Systems receiving intense herbivory might experience greater limitation by grazing than by nutrient resources. Therefore, perhaps the grazers, not nutrient resources, most strongly determine limitation. Second, should resource limitation remain strong over gradients of enrichment? One might expect that eutrophic systems could support larger grazer populations and, hence, promote more acute grazer limitation. A simple food chain model readily captures such intuition (Oksanen et al. 1981; Osenberg and Mittelbach 1996; Grover 1997; see below). Over a gradient of nutrient supply, food chains (with stable dynamics) should experience increasing grazer biomass and freely available nutrients but constant plant biomass. In highly enriched environments, grazers can provide much stronger limitation than resources. Alternatively, eutrophication might shift limitation away from nutrient resources and towards light if higher producer biomass exacerbates self-shading.

In response to these two questions, one might predict that nutrient limitation remains prominent despite grazing and eutrophication because of shifts in species composition of producers and/or stage-structured vulnerability to grazers. For instance, food web models that incorporate the “keystone-predation” tradeoff between the ability to compete for nutrients (R^*) and vulnerability to herbivory forecast shifts in composition towards more resistant assemblages of producers (Holt et al. 1994; Leibold 1996; Grover 1997; Chase et al. 2000a, b). Long-term shifts in

composition following this tradeoff should then promote increases in aggregate producer biomass, decreased (per capita) resource availability, and depressed grazer abundance (Leibold et al. 1997; Chase et al. 2000a). Perhaps not surprisingly then, resource limitation should remain pre-eminent (and grazer limitation small) in food webs showing keystone-predation-mediated shifts in species composition (Osenberg and Mittelbach 1996; Darcy-Hall 2006; see below for a formalization). Such theoretical results may be general because a variety of systems exhibit competition–resistance tradeoffs (Lubchenco and Cubitt 1980; Huntly 1987; Bohannan and Lenski 2000; Chase et al. 2000b; Steiner 2003). However, other essentially related mechanisms may also promote consistently strong resource limitation. For instance, producers with large, invulnerable adult stages (Werner and Gilliam 1984; Chase 1999; De Roos et al. 2003) might also reduce the impact of grazing with enrichment. Adult stages of such a producer become more abundant with enrichment, and these adult stages can depress resources (directly) and grazer biomass (indirectly). As we show, a model with such stage structure predicts consistently high resource limitation and low grazer limitation over enrichment gradients. Thus, limitation (a short-term response) by nutrients may remain paramount due to multiple, long-term mechanisms involving reduced vulnerability of species or life stages.

In this study, we ultimately argue for the likelihood of both mechanisms. In a two-stage outdoors’ mesocosm experiment, we measured resource and grazer limitation in benthic algal communities that had developed over many generations without benthic macrograzers (snails and amphipods). Initially, we predicted that the “historically ungrazed” communities would respond in the long- and short-term like our model-derived predictions for a food chain. These producer assemblages were naive to grazing and therefore should have been dominated by vulnerable, superior nutrient competitors. Similarly, communities that experienced “historical grazing” should have behaved as anticipated by the food web model because species composition should shift from superior nutrient competitors and towards more resistant species with enrichment. The experiment produced predicted results for the historically grazed, food web communities (higher resource limitation, weaker grazer limitation); however, the historically ungrazed treatments also showed higher resource limitation at intermediate enrichment, but not higher grazer limitation. Without historical grazing, these communities became dominated by species (*Oedogonium* spp.) with relatively invulnerable adult stages. Hence, despite variation in historical grazing pressure and enrichment, these communities demonstrated stronger resource limitation and weaker grazer limitation overall—but due to different long-term mechanisms.

Theoretical predictions

The model and long-term predictions (equilibria)

We used a one resource–two plants–one grazer model (Grover 1997) to derive predictions for resource and grazer limitation in food chains and webs in which two producers display the competition–resistance (keystone-predation) tradeoff (e.g., the diamond model). Adding stage structure to the food chain (following Chase 1999) offered the pertinent contrast. All three chemostat models represent change in grazers, producers, and a nutrient resource as the balance between gains and losses. The food web model, which subsumes the chain and the diamond, is (see also Table 1):

$$\frac{dG}{dt} = G \left(\sum_j e_j f_j N_j - d - a \right) \tag{1a}$$

$$\frac{dN_j}{dt} = N_j \left(\frac{u_j R}{h_j + R} - f_j G - m_j - a \right) \tag{1b}$$

Table 1 Explanation of variables and parameters in the food web model

Symbol	Units	Description
State variables		
N_j^a	$\mu\text{g C l}^{-1}$	Producer C (biomass)
G	$\mu\text{g C l}^{-1}$	Grazer C (biomass)
R	$\mu\text{g P l}^{-1}$	Dissolved concentration of nutrient (P)
t	day	Time
Parameters		
a	day^{-1}	Dilution (flushing) rate
d	day^{-1}	Loss rate of grazer
e_j^a	–	Conversion efficiency on producer j
f_j^a	$(\mu\text{g C l}^{-1})^{-1} \text{day}^{-1}$	Feeding rate of grazer on producer j
h	$\mu\text{g P l}^{-1}$	Half saturation constant
m_j^a	day^{-1}	Additional, density-independent losses of producer j (not from flushing or grazing)
q	$\mu\text{g P } \mu\text{g}^{-1} \text{ C}$	Nutrient content per unit C, grazer
Q_j^a	$\mu\text{g P } \mu\text{g}^{-1} \text{ C}$	Nutrient content per unit C, producer
s	$\mu\text{g P l}^{-1}$	Concentration of P supply
u_j^a	day^{-1}	Growth rate at infinite quota

^a Note, the following subscripts are used in the text: 1 for the superior resource competitor, 2 for the inferior resource competitor, J for juvenile stages, A for adult stages

$$\begin{aligned} \frac{dR}{dt} = & a(S - R) - \sum_j \left(\frac{u_j R}{h_j + R} \right) Q_j N_j + \sum_j m_j Q_j N_j \\ & + G \left(\sum_j (Q_j - e_j q) f_j N_j + dq \right). \end{aligned} \tag{1c}$$

Growth of grazers, G (Eq. 1a) increases with consumption of producers, N_j , at linear feeding rate f_j and with conversion efficiency e_j , but they decrease at density-independent mortality rate d and dilution rate a . Production of the two plants reflects gains from uptake of nutrients, R , following Monod kinetics (where u_j is maximal growth rate and h_j is the half-saturation constant). Losses arise due to grazing ($f_j G$), other density-independent sources (m_j), and dilution. Finally, freely available resources are input (due to dilution) at concentration S and flushed at rate a , taken up by plants with nutrient:C content Q_j , recycled from dead plants ($\sum m_j Q_j N_j$), excreted by grazers after accounting for stoichiometry of the interaction (where q is the nutrient C content of producers, assuming $Q_j > e_j q$), and recycled from dead grazers (dqG).

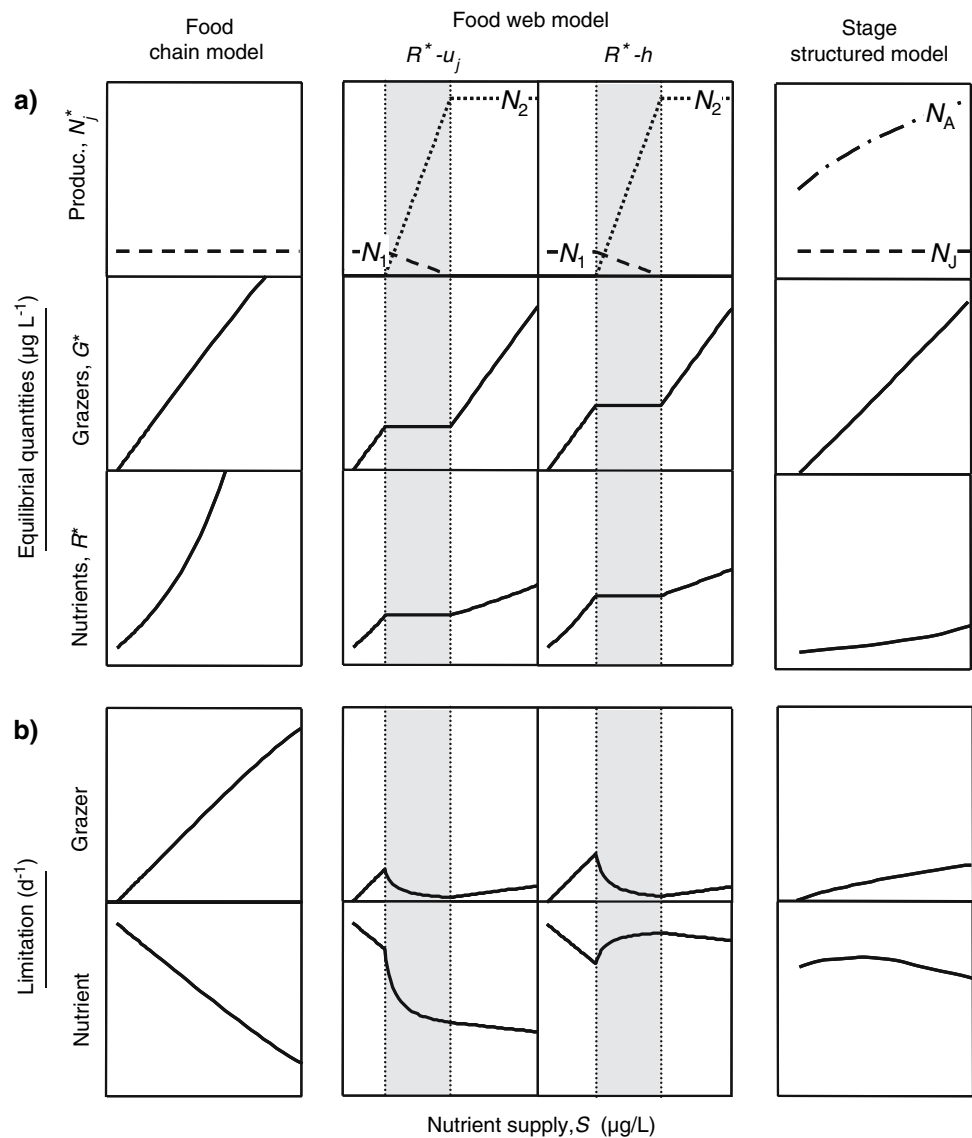
To implement the keystone-predation tradeoff, we assume that the superior-but-vulnerable competitor, N_1 , has a lower grazer-free resource requirement R_j^* than its rival, N_2 , where this synthetic minimal requirement trait is:

$$R_j^* = \frac{h_j(a + m_j)}{u_j - (a + m_j)}. \tag{2}$$

This competitive superiority (in the absence of grazers) can be achieved through (at least) two physiological pathways: N_1 has higher maximal growth rate ($u_1 > u_2$, the $R_j^* - u_j$ pathway) or lower half-saturation constant ($h_1 < h_2$, the $R_j^* - h_j$ pathway). However, the inferior competitor enjoys lower grazing rates, $f_1 > f_2$. Given this tradeoff among R_j^* and f_j traits, this model predicts shifts in producer composition over a gradient of resource supply that sandwich an intervening window of grazer-mediated coexistence (Fig. 1a). In other words, in the absence of the inferior-but-less-vulnerable competitor (N_2), the superior competitor (N_1) would persist with the grazer. However, with sufficient enrichment (which indirectly supports higher grazing intensity, $f_j G$), the competitive hierarchy among producers eventually switches. In fact, N_2 eventually displaces its rival. During the intervening window, grazer biomass and freely available nutrients remain constant. Otherwise, they increase with nutrient enrichment, but at shallower slopes than seen in the food chain with only the superior competitor (Fig. 1a).

The stage-structured model follows a similar structure (following Chase 1999):

Fig. 1 **a** Equilibril (long-term) and **b** limitation (short-term) predictions from several models over a gradient of nutrient (resource) supply: a food chain model containing a highly vulnerable species; a two-producer food web model incorporating the competition–resistance tradeoff among producer species (where N_1 is the superior competitor but more vulnerable species and N_2 is the inferior competitor but less vulnerable species, and species coexistence occurs at intermediate enrichment) (Grover 1997; gray areas); and a stage-structured model in which the grazer can only consume highly edible juvenile (J) stages, not adults (A ; Chase 1999). Equilibril quantities include biomass of producers, grazers (both $\mu\text{g C l}^{-1}$), and free nutrients ($\mu\text{g P l}^{-1}$); limitation metrics reflect removal of grazers or addition of a limiting nutrient. In the food web model, two different pathways to low R^* are presented: one involves uptake rate of free nutrients (u), the other involves half-saturation kinetics of that nutrient uptake (h ; Table 1). d^{-1} Day $^{-1}$



$$\frac{dG}{dt} = G(efN_J - d - a) \tag{3a}$$

$$\frac{dN_J}{dt} = u_A \left(\frac{R}{h_A + R} \right) N_A - u_J \left(\frac{R}{h_J + R} \right) N_J - (m_J + a + fG)N_J \tag{3b}$$

$$\frac{dN_A}{dt} = u_J \left(\frac{R}{h_J + R} \right) N_J - (m_A + a)N_A \tag{3c}$$

$$\frac{dR}{dt} = a(S - R) - \sum_{j=J,A} \left(\frac{u_j R}{h_j + R} - m_j \right) Q_j N_j + G((Q_J - eq)fN_J + dq) \tag{3d}$$

where grazers (G) consume only juvenile stages of the producer (N_j); juvenile and adult (N_A) stages of the producer reproduce or recruit from juvenile stages according

to Monod kinetics (but could have different maximal growth rates, u_j or u_A , and half-saturation constants, h_j or h_A , respectively); and dynamics of freely available nutrients (R) follow a similar reasoning as summarized above (Eq. 1c). This model predicts that juvenile biomass should remain constant (at the grazer’s minimal resource requirement) but adult producers and grazers should increase with enrichment (Fig. 1a). Furthermore, freely available resources should remain low (since large populations of adult producers readily exploit these nutrients).

Short-term predictions (limitation indices)

Given the well-known long-term (equilibril) behavior of these food chain, diamond, and stage-structured models, we then examined their short-term dynamics. Following

Osenberg and Mittelbach (1996), we represented limitation as the direct, per unit response of (equilibrium) biomass of the producer trophic level, N_T ($1/N_T \cdot dN_T/dt$), to nutrient addition or grazer removal:

$$\text{Lim}_R = \left. \frac{dN_T}{N_T dt} \right|_{N_T=N_T^*, R=\hat{R}, G=G^*} \quad (4a)$$

$$\text{Lim}_G = \left. \frac{dN_T}{N_T dt} \right|_{N_T=N_T^*, R=R^*, G=0} \quad (4b)$$

where N_T consists only of the superior competitor (N_1) in the food chain, the sum of coexisting producers in the food web (i.e., $N_T = N_1 + N_2$), or the sum of juvenile and adult producers in the stage-structured variant (i.e., $N_T = N_J + N_A$). The equation for resource limitation (Lim_R) evaluates the per unit response of this trophic level to a large pulse of resources, \hat{R} , while grazer biomass remains unchanged. This uptake-saturating pulse essentially allows nutrient kinetics to reach their maximal rate [i.e., $u(R^*) = u$]. Therefore, resource limitation will be higher in situations where growth kinetics of producers were under-saturated before the pulse, i.e., $u(R^*) \ll u$. Then, the equation for grazer limitation (Lim_G) characterizes immediate response of producers (N_T^* , consuming R^* nutrients) to removal of grazers (i.e., after G is set to 0). Grazer limitation can become substantial if herbivory inflicts high levels of per capita mortality.

These limitation indices revealed fundamental differences between the food chain with the simple, highly edible producer versus the other two scenarios (Fig. 1b). In the food chain, grazer limitation elevates while nutrient limitation declines with enrichment. In fact, relative ranking of these factors should even switch: with enough enrichment, limitation by grazing can exceed that by resources. This finding makes sense since increasing grazers maintain producer biomass at a fixed level (the grazer's own minimal resource requirement). That low level of producer biomass already enjoys high levels of ambient nutrients once grazing pressure is eliminated—perhaps even high enough to nearly saturate Monod kinetics, $u(R)$. Hence, high levels of R^* in enriched chains produces low resource limitation of producers experiencing high mortality. However, removal of this mortality source can catalyze rapid producer growth.

In contrast, species turnover or stage-structured vulnerability can keep resource limitation high and grazer limitation depressed with enrichment (Fig. 1b). In the food web, the mechanism involved is species turnover. Essentially, long-term shifts toward less vulnerable species reduce grazer biomass (compared to that achieved in the food chain) and also freely available resources. Therefore, for this producer trophic level, grazer removal yields lower instantaneous growth (i.e., lower grazer limitation) and

resource addition can further elevate producer growth (i.e., higher resource limitation if ambient R^* does not already saturate Monod growth kinetics). Note that the shape of the nutrient-limitation curve over nutrient supply can look more concave-up (through the $R_j^* - u_j$ pathway to competitive superiority) or concave down (the $R_j^* - h_j$ pathway). Another concave-down resource limitation curve arose in the case with stage-structured producers (Fig. 1b). Here, resource limitation remained high while grazer limitation stayed low with enrichment. At some level, the mechanism at work is essentially the same as in the keystone-predation food web: invulnerable adult stages keep freely available resources low and indirectly yield lower grazer biomass (compared to the food chain with an entirely edible producer). Therefore, grazer removal should not produce huge response of stage-structured producers (i.e., lower grazer limitation), but nutrient addition can readily stimulate producer growth (i.e., higher resource limitation).

Returning to the experiment, these theoretical results yielded several predictions for resource and grazer limitation over enrichment gradients. If grazing catalyzed long-term shifts in species composition, then the short-term response of historically grazed communities should respond according to predictions for the food web—that is, grazer limitation should remain lower and resource limitation higher with elevations in nutrient supply. Meanwhile, historically ungrazed communities should respond according to predictions for food chains. More specifically, grazer limitation should increase and perhaps surpass decreasing nutrient limitation with enrichment. However, this prediction relies sensitively on competitive superiority of vulnerable producers. If stage-structured producers could achieve competitive dominance (i.e., if large, invulnerable adult stages shade other species), then limitation in historically grazed and ungrazed assemblages might respond similarly in short-term limitation assays.

Materials and methods

We conducted the experiment outdoors in 1,000-l cattle tank mesocosms during summer 2003 at Michigan State University's W. K. Kellogg Biological Station (Hickory Corners, Mich.). These mesocosms are large relative to the size of algae and grazers and mimic pond environments (Leibold et al. 1997; Hall et al. 2005). Furthermore, a large subset of lentic benthic algae can persist in these systems (T. L. Darcy-Hall, unpublished data). The experiment was conducted in two phases: an initial, 3-month community establishment period (phase 1) followed by 1-week limitation assays (phase 2).

Phase 1: community establishment

Initial grazed and ungrazed algal communities were established in May 2003, using a simple experimental design. Four levels of nutrient supply were crossed with the presence/absence of a benthic grazer community, yielding eight treatments replicated 4 times (phase 1). Mesocosms were acid-washed, filled with well water, and covered with a 1-mm-mesh lid, composed of window screening. Fourteen terracotta clay flowerpots (7.6 cm diameter) served as algal substrates in each mesocosm. Each pot was silicon-sealed, upside-down, to the bottom of a plastic petri dish and was plugged with a rubber stopper. Additions of N (NH_4NO_3) and P (KH_2PO_4) to the water column established productivity treatments. Target nutrient concentrations were 10 (LOW), 30 (MED), 75 (HIGH), and 200 (XHIGH) $\mu\text{g l}^{-1}$ total P (TP), with N at a 50:1 molar ratio to ensure P limitation. These enrichment levels encompassed natural variation in TP and TN of local lakes and ponds (Hall et al. 2005; Darcy-Hall 2006) and were maintained with nutrient additions every 5 days. We introduced an assemblage of benthic grazers, including gastropods (*Physa*, *Helisoma*, *Gyraulus* spp.) and amphipods (*Hyallela azteca*, *Gammarus* sp.) to grazed mesocosms along the enrichment gradient in densities proportional to those expected across a similar enrichment gradient in nature (summed densities: 0.09, 0.24, 0.63, 1.64 g dry mass m^{-2} for the 10–200 $\mu\text{g l}^{-1}$ TP treatments, respectively; T. L. Darcy-Hall, unpublished data). Grazers could then reproduce throughout summer. To inoculate mesocosms, algae were scraped from several clay pots established in the littoral zone of seven lakes spanning a wide gradient of TP (13.5–77.5 $\mu\text{g l}^{-1}$) and TN (208.9–1,869 $\mu\text{g l}^{-1}$) and were mixed with deionized water. Resulting slurries were sieved (125 μm) to remove macrograzers, combined and mixed in a carboy, and distributed in 100-ml aliquots to each mesocosm. Algal composition among lakes was expected to vary through time, so this inoculation process was repeated twice a month during phase 1 to allow frequent colonization opportunities for a diverse algal assemblage.

Benthic algal species established across these environments for 15 weeks (~ 100 algal generations) prior to the initiation of limitation assays (phase 2). One week prior to phase 2, communities from two clay pots per mesocosm were subsampled (as described above) to measure pre-phase 2 species composition (after preservation in 10% formalin) and chlorophyll *a* (chl *a*), using cold ethanol extractions and narrow-band fluorometry (sensu Welschmeyer 1994). We also measured TP and TN using spectrophotometry and standard methods (APHA 1980; Prepas and Rigler 1982; Crumpton et al. 1992; Bachmann and Canfield 1996). We measured net ecosystem

productivity [diel dissolved oxygen (DO), $\text{mg l}^{-1} \text{h}^{-1}$] to verify that nutrient treatments created a productivity gradient. Net ecosystem productivity was calculated as the difference in DO between dawn and dusk measurements (using an YSI-55 oxygen meter; Yellow Springs, Ohio).

After sampling, the remaining 12 clay pots from each ungrazed mesocosm were shifted into a grazed mesocosm within the same nutrient supply treatment. For example, pots from an ungrazed, LOW mesocosm were shifted into a randomly chosen grazed, LOW mesocosm. Ultimately, 16 mesocosms (four at each nutrient supply level) containing both grazed and ungrazed sets of algal communities were used in the limitation assays. Resident clay pots in grazed mesocosms were lifted out of the water and relocated within the same tank. Grazer limitation by definition measures the effect of the removal of grazers on community per unit growth. Therefore, ungrazed algal communities required a period of grazing prior to the onset of limitation assays. From pilot experiments, 1 week was found to be sufficient for grazers to measurably affect biomass but not community composition (T. L. Darcy-Hall, unpublished data).

Phase 2: limitation assays

Seven-day limitation assays were initiated in half of the 16 mesocosms on 26 August 2003, while the other half began 2 days later. Previous experiments have shown that 2–7 days is sufficient to measure an algal response while avoiding indirect feedbacks from higher trophic levels (Downing et al. 1999). Within each mesocosm, two limitation assays took place: one each within the ungrazed and grazed sets of clay pots. Each assay included four, randomly assigned treatments, with three replicates each: nutrient additions alone, grazer removals alone, nutrient additions and grazer removals, and controls (no nutrients added and grazers present) (phase 2). Note that these treatments were not statistically analyzed, they were applied only to produce values of resource and grazer limitation that were analyzed (see below). All nutrient additions involved removing 25 ml of water from the clay pot (via the top hole) and either returning it (to controls and grazer removal treatments) or replacing it with 25 ml of a saturated nutrient solution (NH_4NO_3 and KH_2PO_4 in a 2:1 molar N:P ratio). The nutrient solution, $\sim 1,500$ times more concentrated than the XHIGH treatment, ensured saturation of algal nutrient uptake. N was added at a low ratio to avoid toxic effects for algae and grazers. The porous clay pot surface readily diffused nutrients over time and thus provided nutrients directly to attached algae. Grazers were manually removed from all clay pots and returned to all but grazer-removal treatments. Care was taken to evenly

redistribute grazers among control and nutrient addition pots within a mesocosm. After treatment application, each pot was placed in an overturned 1-l clear, plastic container with two, 105- μm mesh windows that retained herbivores. These containers served several purposes. First, they reduced phytoplankton immigration from the water column, which might have confounded measurements of benthic algal chl *a*. Second, they minimized nutrient flow from the nutrient addition clay pots to the control pots.

After 1 week, communities from each clay pot were harvested and subsampled (as described above) for algal chl *a* and species composition. The chl *a* data were used to calculate values of resource (nutrient) and grazer limitation, using an empirical analogue to Eq. 4:

$$\begin{aligned} \text{Lim}_R &= [\ln(\text{NAGR}) - \ln(\text{GR})]/t \\ \text{Lim}_G &= [\ln(\text{GR}) - \ln(C)]/t \end{aligned} \quad (5)$$

where t is the assay duration in days, and C , GR , and $NAGR$ are chl *a* measurements of control, grazer removal, and nutrient addition plus grazer removal treatments, respectively. Resource limitation could also be measured as the difference between nutrient additions (NA) and controls. However, NA treatments resulted in higher grazer mortality than controls, potentially biasing the calculation of resource limitation. To avoid this bias, we calculated resource limitation in the absence of grazers. Positive values of limitation indicated that community biomass increased with removal of the limiting factor, while negative values indicated that biomass decreased.

Algal enumeration and statistical analyses

Benthic algal communities were enumerated and identified using established methods (Lowe and Pan 1996). Semi-permanent wet mounts were prepared and examined at 1,250 \times magnification on a compound microscope. Three hundred natural units (e.g., a cell, filament, or colony) were identified to the species level (when possible) in each sample. In most cases, a second slide was prepared with acid-cleaned diatoms mounted in Naphrax to identify diatom species. Fifty organisms per species were measured for biovolume calculations, using published geometric forms and volume equations (Hillebrand et al. 1999). All algal composition data are in units of relative biovolume (arc-sine-square root transformed prior to analyses).

Treatment effects on log-transformed values of TN, TP, chl *a*, and DO, were initially analyzed with multivariate ANOVA (MANOVA) (Systat version 8), followed by two-way ANOVA (Systat version 8), using nutrient supply and grazer presence/absence as treatment factors. Shifts in algal composition in phase 1 were analyzed using several methods. First, we used a permutational MANOVA model

(perMANOVA; McArdle and Anderson 2001; Anderson 2001, 2005) based on Bray–Curtis distance and 9,999 randomizations of each factor, to test whether nutrients and grazers significantly affected algal composition. The perMANOVA partitions variation akin to traditional MANOVA but computes significance tests with permutations (relaxing assumptions of multivariate normality) of species data to provide F -statistics and P -values for each treatment, their interaction, post hoc treatment comparisons, and slices of significant interactions (i.e., post hoc comparisons of a treatment at each level of the other treatment; Anderson 2001).

Additionally, we used a canonical analysis of principal coordinates (CAP; Anderson 2002; Anderson and Robinson 2003; Anderson and Willis 2003) to characterize species composition data (ordination) and individual species responses (correlations) to experimental treatments. Unlike most ordination-based methods, CAP accounts for correlation structure between species (Anderson and Willis 2003). Here, CAP combined principal coordinates analysis (based on Bray–Curtis distances) with canonical correlation analysis constrained by the phase-1 ANOVA design. CAP produced canonical axes representative of these treatments, the strength of which was measured as squared canonical correlation coefficients (δ^2 , analogous to r^2). With CAP scores, we summarized algal response using correlations between species and canonical axes.

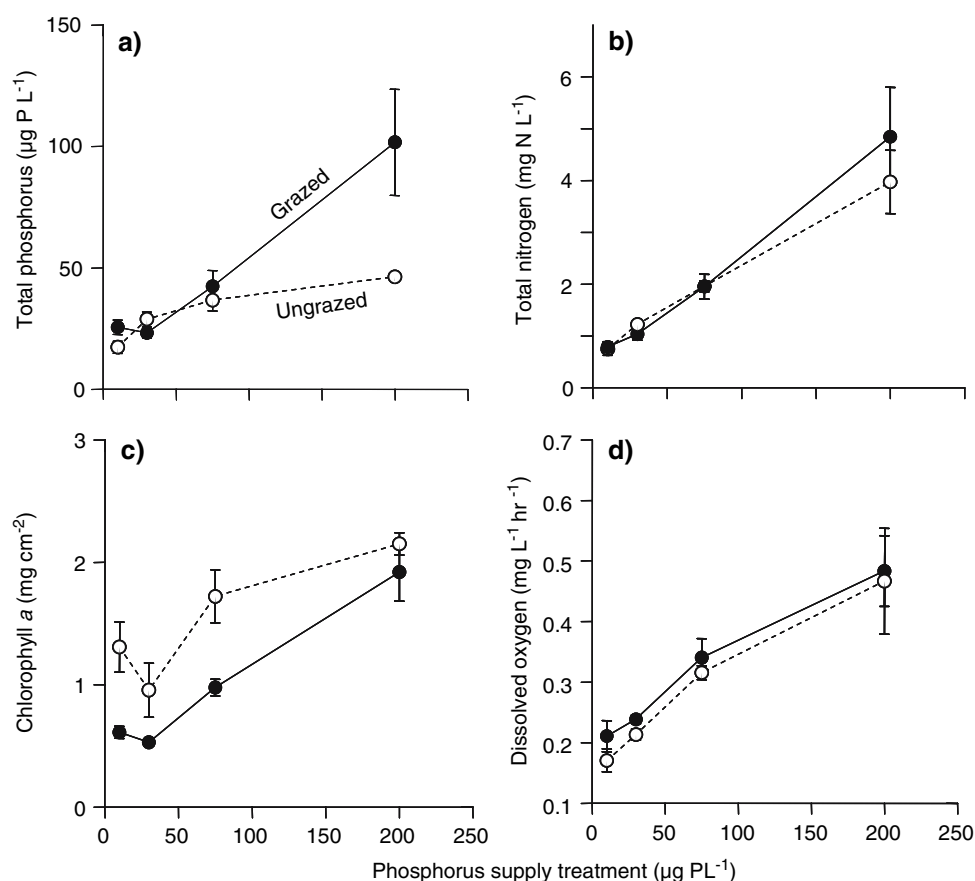
Finally, limitation of historically grazed and ungrazed communities was analyzed using split-plot ANOVA (proc mixed; SAS, version 9.1). Nutrient supply (four levels) was considered the whole-plot treatment (i.e., the mesocosm was the whole-plot experimental unit) and grazing history (ungrazed or grazed) was used as the within-plot factor [i.e., the ungrazed and grazed sets of clay pots (12 each) were the within-plot experimental units; Fig. 2]. Both nutrient supply and grazing history were considered fixed factors. Slices of significant interaction terms were conducted in SAS version 9.1 (SAS 2003) using the Satterthwaite procedure.

Results

Phase 1: algal community composition

Nutrient supply treatments created a productivity gradient (Fig. 2), with higher nutrient supply resulting in increased water column TP (Fig. 2a) and TN (Fig. 2b), higher chl *a* on clay pots (Fig. 2c) and higher net ecosystem productivity (DO; Fig. 2d). There was a significant interaction between nutrient supply and grazer history on these factors when analyzed together (MANOVA; Pillai's trace = 0.828, $F_{12,69} = 2.191$, $P = 0.021$). In separate ANOVAs,

Fig. 2 Means \pm 1 SE of **a** water column total P, **b** total N, **c** chlorophyll *a* of periphyton on terracotta clay flowerpots, and **d** dissolved oxygen in mesocosms after 3 months of nutrient additions (nutrient supply treatment) in historically ungrazed and historically grazed mesocosms



differences in TP and chl *a* across nutrient supply treatments depended upon the presence of grazers (interaction terms: $F_{TP:3,24} = 6.7$, $P = 0.002$; $F_{chl:3,24} = 3.3$, $P = 0.038$), while TN and DO were affected only by nutrient supply ($F_{TN:3,24} = 92.7$, $P < 0.0001$, $F_{DO:3,24} = 44.48$, $P < 0.0001$).

Benthic algal species composition was strongly affected by nutrient supply and grazers (Fig. 3a). The first and second CAP axes (Fig. 3a) represent variation due to nutrient supply ($\delta^2_1 = 0.82$) and grazers ($\delta^2_2 = 0.46$), respectively, while the third and higher CAP axes correlated very weakly with both treatments. Thus, lower nutrient supply mesocosms loaded negatively along the first CAP axis, while those with higher nutrient supply loaded positively (Fig. 3a). Mesocosms containing grazers tended to be negative along the second CAP axis, while ungrazed communities loaded positively (Fig. 3a). A perMANOVA on algal composition revealed a significant nutrient supply \times grazer interaction ($F_{3,24} = 1.76$, $P = 0.027$). Slices of the interaction indicated a significant difference between grazed and ungrazed communities in LOW and XHIGH treatments (see Table S1 in electronic supplementary material). Among ungrazed treatments, XHIGH community composition was significantly

different from all other nutrient supply treatments. Among grazed treatments, XHIGH community composition differed significantly from LOW and MED communities while HIGH ones marginally differed from those in LOW treatments (Table S1).

Algal composition of major taxonomic groups also exhibited dramatic changes in relative biovolume across treatments (Fig. 3b). The most striking impact of grazers was the reduction in biovolume of filamentous chlorophytes, regardless of nutrient supply (Fig. 3b). Understory species that benefited from removal of the filamentous overstory differed along the productivity gradient. At low productivity, the relative biovolume of several diatoms increased with grazing (Fig. 3b, Large Diatoms). In contrast, in XHIGH treatments, an average of half of algal biovolume was comprised of grazer-resistant basal cells of *Stigeoclonium* sp. (Fig. 3b, Large Colonial Chlorophytes). In the online Appendix of the electronic supplementary material, we provide further information on this species composition data, broken into coarse metrics (richness, diversity, and two evenness indices, following Smith and Wilson 1996; Fig. S1), and we present species-specific correlations with nutrients (first CAP axis) and grazers (second CAP axis; Fig. S2).

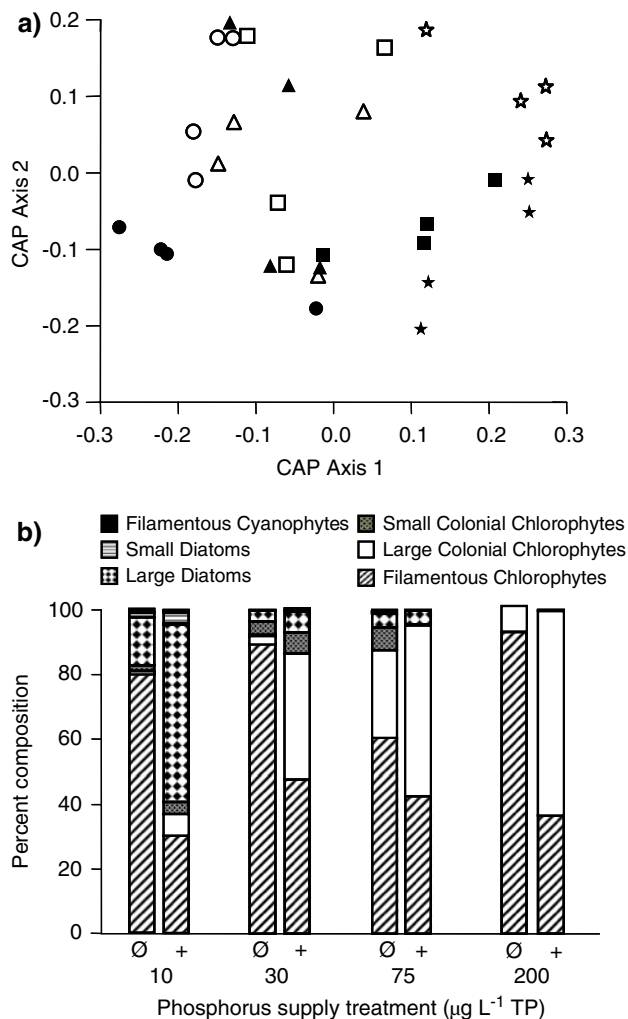


Fig. 3 **a** Ordination of experimental benthic algal communities [historically ungrazed (*open symbols*); historically grazed (*filled symbols*)] in response to various P supply concentrations (*CAP Axis 1*) and the presence/absence of herbivores (*CAP Axis 2*). Total P nutrient supply concentrations ($\mu\text{g l}^{-1}$) were low (10; *circles*), medium (30; *triangles*), high (75; *squares*) or extra high (*stars*). **b** Benthic algal composition divided into broad morphological and taxonomic groups for ungrazed (*diameter symbol*) and grazed (*plus symbol*) communities at each nutrient supply level. Here, we group *Stigeoclonium* basal cells as colonial (vs. filamentous) chlorophytes because we only found their prostrate growth form

Phase 2: limitation assays

Algal communities that developed under different grazer and nutrient environments exhibited different degrees of nutrient and grazer limitation; however, the extent of these differences varied with enrichment. Nutrient limitation exceeded grazer limitation in both historically grazed (one-tailed paired *t*-test; $P < 0.0001$, $df = 15$; Fig. 4a) and historically ungrazed communities ($P = 0.012$, $df = 15$; Fig. 4b). In these latter communities, this difference mostly appeared at intermediate nutrient supply, whereas at high

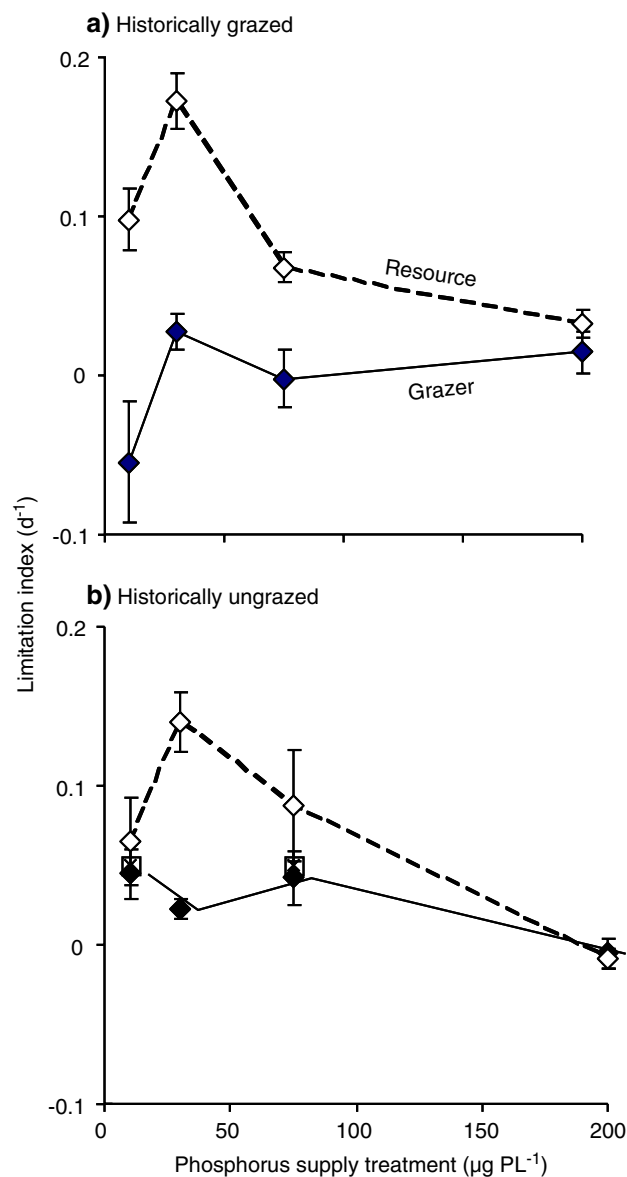


Fig. 4 Resource and grazer limitation (means ± 1 SE) in **a** historically grazed and **b** historically ungrazed communities of benthic algae, along the experimental P supply gradient of 0–200 $\mu\text{g P l}^{-1}$

levels of enrichment the two limitation indices converged (Fig. 4b). Note, however, that grazer limitation did not exceed nutrient limitation at high nutrient supply. Also, looking solely at nutrient limitation, we found a significant effect of nutrient supply (ANOVA: $F_{3,12} = 11.44$, $P = 0.0008$), and nutrient limitation in historically grazed algal communities was generally higher than in historically ungrazed communities (ANOVA grazing history effect; $F_{1,12} = 4.39$, $P = 0.058$; Fig. 4). A nutrient supply \times grazing history interaction (ANOVA, $F_{3,12} = 6.54$, $P = 0.007$) indicated that grazer limitation in historically ungrazed algal communities was higher than in historically grazed communities, especially at low (slice, $F_{1,12} = 4.70$,

$P = 0.0005$) and high productivities (slice, $F_{1,12} = 2.10$, $P = 0.058$). We even detected negative values of grazer limitation (meaning that grazer removal decreased algal biomass; Fig. 4).

Discussion

Relatively short-term growth assays have yielded considerable insight into factors limiting primary production. However, typical limitation assays ignore relative strengths of grazing versus resource limitation and lack strong conceptual connections to long-term outcomes of species' interactions (Elser et al. 1990; Osenberg and Mittelbach 1996; Downing et al. 1999). Here, we tackled both gaps by showing theoretically that short-term limitation intimately links to long-term (compositional) outcomes of species interactions. To do so, we compared limitation indices derived from a food chain, a food web, and a stage-structured model of resource competition (R^*) and grazing (Holt et al. 1994; Leibold 1996; Grover 1997; Chase 1999). When it incorporated a competition–resistance tradeoff, the food web model offers the well-known prediction that producer assemblages should shift towards increasing resistance to herbivory along enrichment gradients. This compositional shift, in turn, reduces abundance of grazers and hence grazer limitation relative to a food chain with a vulnerable producer. Additionally, increasingly resistant producer assemblages should draw down freely available resources in the presence of grazers. Consequently, they likely remain more limited by resources than does a vulnerable producer in a food chain over enrichment gradients. Thus, the food web model provided an explanation for consistently high levels of nutrient limitation seen in previous limitation assays (Hecky and Kilham 1988; Elser et al. 1990; Downing et al. 1999; Darcy-Hall 2006). However, theory also predicts that communities dominated by producers with stage-structured vulnerability (Chase 1999) might behave similarly, but for slightly different reasons. If adult stages remain invulnerable to grazing (Werner and Gilliam 1984; Chase 1999; De Roos et al. 2003), they essentially act as the less vulnerable species in the food web. Abundance of these adults increases over enrichment gradients, depressing grazer abundance but also resource availability. Thus, resource limitation should remain strong while grazer limitation stays weak with nutrient supply in such stage-structured assemblages.

Algal compositional shifts in our “historically grazed” treatments resembled long-term predictions of the food web model incorporating a competition–resistance tradeoff. These communities received grazing from benthic macrograzers (snails, amphipods) throughout the growing season and were dominated by understory diatoms at low

levels of enrichment. These diatoms are typically highly edible (vulnerable) but good resource competitors. Meanwhile, more grazer-resistant species (e.g., *Stigeoclonium* basal cells) also benefited from herbivory, especially in enriched systems. *Stigeoclonium* basal cells adhere tightly to their substrate and thus structurally resist grazing (e.g., McCormick and Stevenson 1991). These results echo those from other experiments that documented a competition–resistance tradeoff in benthic algae (Rosemond et al. 1993; Rosemond and Brawley 1996; Graham and Vinebrooke 1998; Hillebrand et al. 2002). In contrast, historically ungrazed communities were dominated by filamentous chlorophytes. This result seemed perplexing initially because fully grown filaments are often considered grazer resistant (Dudley and D'Antonio 1991; Steinman et al. 1992). Such filaments should not have dominated historically ungrazed systems, but perhaps should have dominated grazed ones. A closer look at these species' biology provides answers. Grazers consume early developmental stages of these filamentous species (DeNicola et al. 1990; Dudley and D'Antonio 1991) and thus prevent establishment in heavily grazed environments. However, once they establish in ungrazed systems, adult stages can shade (outcompete) understory species. In fact, understory species typically benefit from removal of overstory filaments (Steinman 1996).

These long-term compositional differences, driven by variation in nutrient enrichment and historical grazing pressure, produced short-term limitation results similar to those predicted by the food web and stage-structured models, not the simple food chain. For instance, nutrient limitation remained higher than grazer limitation with enrichment in both types of communities. This pattern also emerged in several field systems, including benthic algae in small lakes (Osenberg and Mittelbach 1996; Darcy-Hall 2006). Theory derived here showed that a unimodal-like curve for resource limitation could emerge over some portion of enrichment gradient in a food web where variation in resource competition is driven by certain aspects of resource kinetics (half saturation constants rather than maximal growth rates). A unimodal-like curve also readily appears when producers have invulnerable adult stages. Grazer limitation remained low over enrichment gradients. This result was initially not anticipated in the historically ungrazed treatments because we assumed that they would behave like chains with edible producers (Grover 1997). However, theory indicates that stage-structured vulnerability should keep grazer limitation consistently low. Thus, through different mechanisms, these communities remained limited more by or similarly by nutrients than by grazers over a broad enrichment gradient.

Despite these qualitative matches between theory and data, all three models considered could not predict a final

result. In historically grazed mesocosms, we found negative grazer limitation at low nutrient supply, meaning that grazer removal actually decreased per unit growth of producers. This result is not completely unanticipated based on past findings (Sterner 1986; McCormick and Stevenson 1991; de Mazancourt et al. 1998), but the three models considered here lack mechanisms which could produce it. In general, herbivory can stimulate primary productivity in systems with high losses of nutrients to detrital pathways or with grazers that translocate nutrients or that promote high tolerance of species to grazing (Augustine and McNaughton 1988; de Mazancourt et al. 1998; Chase et al. 2000a). Such effects, if they were present in our experiment, should have indeed occurred in lower nutrient, historically grazed communities. Additionally, benthic grazers can also remove epiphytic or overstory competitors and reduce physical barriers between nutrients and producers (McCormick and Stevenson 1991; Steinman 1996). Such details are pertinent to benthic algae but extend beyond assumptions of the generic models explored here.

Given these limitations, the coupling of models and experiments provided answers to questions concerning persistently high resource limitation of producer assemblages over gradients of nutrient enrichment (Hecky and Kilham 1988; Elser et al. 1990; Osenberg and Mittelbach 1996; Downing et al. 1999; Darcy-Hall 2006). Such patterns readily emerge in systems containing producers which vary in vulnerability to grazing, either among species or among life history stages (Grover 1997; Chase 1999; Strauss and Agrawal 1999; Bohannan and Lenski 2000; Chase et al. 2000a, b). However, it is not clear if similar patterns would arise in systems in which producers show a tolerance strategy, rather than a resistance strategy, to herbivory (Grover 1997; Chase et al. 2000a). Still, it is important to remember that grazers provide key components of these limitation patterns documented here because they can drive predictable, long-term shifts in species composition or influence dominance by invulnerable, adult stages along environmental gradients (Holt et al. 1994; Leibold 1996; Grover 1997). Perhaps emerging evidence for broad co-limitation of producer assemblages (Elser et al. 2008; Harpole and Tilman 2007) even indirectly reflects grazing, at least to some degree.

Acknowledgements T. L. D.-H. was supported by an MSU University Distinguished Fellowship, an NSF pre-doctoral fellowship, an NSF RTG (research training grant) to KBS (DBI-9602252), and a George H. Lauff Research Award. S. R. H. was supported by NSF OCE 02-35039 to C. Cáceres and funds from Indiana University. We thank G. Mittelbach, O. Sarnelle, A. Tessier, K. Gross and R. J. Stevenson for comments on various versions of the manuscript, P. Geddes for help in the field and K. Manoylova for aid in algal identification. This is KBS contribution no. 1454.

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