

Herbivory enhances the diversity of primary producers in pond ecosystems

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Abstract. Diversity of primary producer is often surprisingly high, despite few limiting factors such as nutrients and light to facilitate species coexistence. In theory, the presence of herbivores could increase the diversity of primary producers, resolving this “paradox of the plankton.” Little experimental evidence supports this natural enemies hypothesis, but previous tests suffer from several deficiencies. Previous experiments often did not allow for multigeneration effects; utilized low diversity assemblages of herbivores; and limited opportunities for new primary producer and herbivore species to colonize and undergo species sorting that favors some species over others. Using pond plankton, we designed a mesocosm experiment that overcame these problems by allowing more time for interactions over multiple generations, openness to allow new colonists, and manipulated higher diversity of primary producers and grazers than have previous studies. With this design, the presence of zooplankton grazers doubled phytoplankton richness. The additional phytoplankton species in grazed mesocosms were larger, and therefore likely more grazer resistant. Furthermore, phytoplankton richness in grazed mesocosms was similar to that observed in natural ponds whereas it was much lower in mesocosms without grazers. However, stoichiometric imbalance caused by variation in nitrogen : phosphorus ratios and light supply did not alter phytoplankton richness. Therefore, grazers enhanced primary producer richness more strongly than ratios of nutrient supply (even though both grazing and ratios of resource supply altered composition of primary producer assemblages). Taken together, these experimental and field data show that grazing from a diverse assemblage of herbivores greatly elevated richness of phytoplankton producers in pond ecosystems.

Key words: biodiversity; grazers; herbivory; nitrogen:phosphorus:light; paradox of the plankton; phytoplankton; species richness; stoichiometric imbalance.

INTRODUCTION

The high species richness of many primary producer assemblages has long puzzled ecologists. According to Gause’s axiom (Hardin 1960), the number of coexisting species in primary producer communities should not exceed the number of limiting factors, such as nutrients and light. Hutchinson’s “Paradox of the Plankton” (Hutchinson 1961) showcased the limits of this prediction for phytoplankton: at a given time point, phytoplankton richness in natural ponds and small lakes typically ranges between 12 and 80 species (Smith et al. 2005). However, experiments rarely reveal the presence of more than three to four resource-based limiting factors. Thus, other mechanisms must enhance primary producer diversity in natural communities. Hutchinson sought to resolve this problem by challenging the assumptions of the equilibrium-based competition theory: non-equilibrium environments and spatial heterogeneity might also promote coexistence (see also Tilman 1982, Chesson

2000, Huisman and Weissing 1999). However, even Hutchinson admitted that these factors might not sufficiently solve this conundrum. At least 12 other hypotheses propose to resolve his paradox (Wilson 1990, Roy and Chattopadhyay 2007). Many of these mechanisms, including natural enemies, have received some empirical support. Yet, their relative ability to explain richness of phytoplankton and other primary producer assemblages in nature (e.g., tropical forests; Hubbell 2001) remains unresolved.

Could natural enemies resolve this paradox? Classic experiments (Lubchenco 1978, Paine 1996) and models of predator-mediated coexistence (e.g., Holt et al. 1994, Leibold 1996, Thingstad 2000) suggest that natural enemies could diversify assemblages of their resources. Enemy-induced mortality allows more resistant prey to coexist with otherwise superior but more edible competitors. If so, individual herbivore species can each potentially serve as different “limiting factors”; coupled with limiting abiotic resources, herbivores may enable coexistence of a great number of primary producer species. However, few other experiments have captured such diversity-enhancing effects of natural enemies. In fact,

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subsequent research has shown contradictory effects of herbivores on primary producer richness: herbivores in freshwater ecosystems more often suppress than enhance richness (Proulx and Mazumder 1998, Hillebrand et al. 2007). Furthermore, in those freshwater experiments, grazers rarely enhanced primary producer richness more than 30%, an effect size insufficient to explain primary producer richness in nature. Taken at face value, these results challenge the hypothesis that natural enemies can resolve Hutchinson's paradox.

We contend, however, that previous experiments have lacked several critical elements needed to rigorously test the natural enemies hypothesis. First, most experiments do not run long enough to reveal density-dependent feedbacks between herbivores and primary producers. Instead, they often fix herbivore density, or only track a few primary producer generations. Such short-duration experiments likely underestimate long-term effects of grazing on primary producer richness that may include favoring more-resistant species by suppressing edible ones for example (Leibold 1996). Second, the majority of experiments manipulated a subset (often only a single species or genus) of the herbivore community. Such limited manipulations should only greatly enhance prey richness if the focal herbivore is a keystone species (such as the seastar *Pisaster*; Paine 1996). Thus, they cannot evaluate the richness-enhancing effect of an entire herbivore assemblage. Finally, almost all experiments use relatively isolated, or even enclosed, systems (McCauley and Briand 1979, Proulx et al. 1996, Sarnelle 2005). Without opportunities for colonization, closed species pools of primary producers cannot fully respond to long-term density-dependent feedbacks involving inter-specific interactions. This final design limitation is critical: if species from a regional pool can invade, more herbivore-resistant primary producers can potentially enter and persist in grazed habitats. Conversely, grazer removal could allow invasion of superior resource competitors, enabling displacement of other primary producer species, thereby depressing richness (Leibold et al. 1997, 2004, Olff and Ritchie 1998). Colonization of new primary producer species, then, might accelerate restructuring through species sorting (Leibold et al. 2004) and reveal the diversity-enhancing effects of grazing.

Our experiment and field survey overcame or reduced many of these limitations. In pond mesocosms, we subjected diverse assemblages of algal producers to manipulations of herbivorous zooplankton taxa. We enhanced colonization of new species with fortnightly additions of microbes and phytoplankton (inoculated into all mesocosms) and of zooplankton (in "grazer" and "predator" treatments). Furthermore, we continued the experiment for approximately 65 algal generations, 20 herbivore generations, and two predator generations, longer than most other studies done in freshwater plankton (Hillebrand et al. 2007). Additionally, we evaluated how a key predator (*Notonecta*) might modify or constrain the diversity-enhancing effects of zooplankton herbivory.

Finally, we altered inorganic nutrient supply levels, light supply levels, and resource supply ratios to these experimental food webs. These resource-supply factors could also regulate primary producer richness by creating "stoichiometric imbalances" (Tilman 1982, Cardinale et al. 2009, Brauer et al. 2012): all else equal, imbalanced resource supplies should depress primary producer richness. We compared these stoichiometric-imbalance effects on primary producer richness with those of grazers. We then refined our interpretation of these grazing vs. nutrient-supply effects on richness by contrasting them with measures of turnover and compositional changes. Finally, we surveyed phytoplankton and zooplankton richness in natural ponds, providing a nature-based comparison with diversity trends from our experiment.

METHODS

Mesocosm experiment

Our experimental design has been described more extensively elsewhere (Hall et al. 2004, 2005, 2006, 2007). Briefly, we imposed a fully factorial experimental design to 121 300-L mesocosms (i.e., "cattle tanks") that mimic natural ponds (3–4 replicates of each of 36 treatment combinations after accounting for occasional contamination). First, we altered light availability ("light" treatment) using 1-mm² fiberglass window screen only (to prevent colonization by insects) or screen plus 90% shade cloth (resembling heavy canopy cover; Hall et al. 2007). Second, we imposed a "nutrient" treatment composed of a low nutrient supply rate mimicking mesotrophic pond conditions and a high nutrient supply rate mirroring eutrophic pond conditions. Third, in a "N:P ratio" treatment, we manipulated the relative supply ratios of nitrogen (N) to phosphorus (P) to produce a relatively balanced N:P supply ratio (14:1 by mass), bracketed by more extreme (unbalanced) N:P ratios designed to induce phosphorus limitation (50:1) and nitrogen limitation (5:1). The low nutrient supply rate treatments were designed to create target total nutrient concentrations of 370.4 µg N-NO₃/L and 26.5 µg P-PO₄/L (14:1), 700 and 14 (50:1), and 221.4 and 44.3 (5:1; mesotrophic), respectively. The high nutrient treatment started with 10 times these levels (eutrophic). Throughout the experiment, we periodically added N and P to offset nutrient losses to sediments, assuming a 5% exponential loss per day. Fourth, we altered trophic ("food web") structure. We created a "producer only" treatment (potentially containing micrograzers such as ciliates and rotifers, but eliminating crustaceans); a phytoplankton plus zooplankton "+ grazer" treatment; and a phytoplankton plus zooplankton plus notonectid "+ predator" treatment. Notonectids are one of the few easily manipulated predators that can complete their life cycle in our mesocosms and are known to have important top-down effects in ponds (Arner et al. 1998, Howeth and Leibold

2010). These latter two treatments were both “grazed” by herbivores.

Our design enhanced the role of multigeneration ecological feedbacks that can influence algal richness. To address concerns about species pool and colonization, we added diverse assemblages of phytoplankton and crustacean zooplankton. We inoculated each mesocosm with small volumes of diverse assemblages of algae and zooplankton collected from 12 nearby ponds (Barry and Kalamazoo counties, Michigan, USA), both at the beginning of the experiment and then again at fortnightly intervals throughout its duration. We introduced phytoplankton separately from zooplankton by narcotizing the macrozooplankton with CO₂, decanting the supernatant algae, and double-filtering (30 µm) it as a final step. We simultaneously introduced crustacean macrozooplankton into the “grazer” treatments using animals obtained from the same ponds, filtering them on a 70 µm screen, rinsing twice with filtered water to remove adherent algae. We then re-inoculated grazers fortnightly as well. In the “predator” treatments, we added and re-inoculated macrozooplankton as well as 12 adult backswimmers (*Notonecta undulata*) at the beginning of the experiment. Each cattle tank also received 30 *Physa* sp. snails and 30 bullfrog (*Rana catesbiana*) tadpoles at the experiment’s start to control periphyton growth on mesocosm walls. Levels of re-inoculation were selected to accelerate community assembly by providing colonists that would be reliably detected only if they underwent subsequent population growth. They may approximate immigration among nearby ponds due to dispersal of resting stages by wind or phoresy or connections through water flow although this is hard to know precisely (Caceres and Soluk 2002, Louette and De Meester 2005).

We sampled the mesocosms at the end of summer (September) to characterize zooplankton and phytoplankton communities (Hall et al. 2004, 2005, 2007). This duration permitted 12 weeks of species interactions. We collected 13 subsamples (7.5 L) using PVC pipes to account for spatial variation, reserving small aliquots for nutrient analysis. Phytoplankton communities were evaluated from glutaraldehyde-preserved samples (using the Utermohl method by Phycotech, St. Joseph, Michigan, USA). Crustacean zooplankton were preserved in sucrose Lugol’s solution and identified microscopically. Taxonomic richness of both phytoplankton and crustacean macrozooplankton were calculated using the Chao-1 unbiased estimator (Chao et al. 2005). To evaluate size-based hypotheses for grazer-based responses, we obtained taxon-specific phytoplankton biovolumes from various literature sources (see Hall et al. 2007).

We also characterized nutrient environments to quantify stoichiometric imbalance. We measured both total nitrogen (TN) and phosphorus (TP) using standard chemistry (described in Hall et al. 2005), and then calculated an index of stoichiometric imbalance (Cardinale et al. 2009). This index, θ , expresses N:P imbalance as an

angle, with a minimum of 0° indicating that N and P are balanced relative to their variation among mesocosms: both N and P are similarly high or low in supply. This imbalance angle is highest (90°) when one nutrient is supplied in excess relative to another. Importantly, calculation of the θ index is independent from overall nutrient supply (as described by Cardinale et al. 2009; see Appendix S1).

Pond survey

We also collected a similar data set from a companion survey of 70 natural ponds nearby (Leibold 1999, Hall et al. 2005, 2007). Small ponds (56–17500 m²; mean, 680 m²), located in Michigan (Kalamazoo, Barry, and Mason counties), were visited during July–August in 1993 and 2000. We collected 35-L samples to count zooplankton and reserved small aliquots for phytoplankton (250 mL) and nutrient analyses. Phytoplankton, zooplankton, and nutrient samples were handled identically to those collected in the experiment. We also estimated relative light supply by measuring canopy openness with three hemispherical images per pond (only for the 2000 data), analyzed with GLA 2.0 software (see Hall et al. 2007 for more details).

Data analysis

In the mesocosm experiment, we evaluated treatment effects using ANOVA and compared richness of several treatments using *t* tests. We hypothesized that herbivory should enable invasion by larger phytoplankton species, assuming that large size correlated with higher grazing resistance but lower competitive ability for nutrients. Consequently, we compared mean size of phytoplankton species found only with grazers to those common to all three treatments. This calculation of mean size of these groups of phytoplankton species was weighted by occurrence (i.e., mean weighted size of species present in algae-only tanks is the sum of biovolume of each species times number of tanks in which it occurred all divided by the number of algae only tanks). We bootstrapped samples to calculate 95% confidence intervals for each mean size estimate. To test the stoichiometric imbalance hypothesis, we fit quadratic regressions between log₁₀ (TN:TP ratio) and phytoplankton taxon richness in tanks and ponds. We anticipated a significant, negative quadratic term, indicating a unimodal N:P vs. richness relationship. Furthermore, we expected negative relationships between stoichiometric N:P imbalance, θ , and primary producer richness, tested with linear regressions.

The core argument here centers on species richness. However, we also quantified and visualized changes in composition of phytoplankton to the main grazing and nutrient manipulations with redundancy analysis (RDA; using species-level biomass, Hellinger distance, and the vegan package in R; Legendre and Gallagher 2001, Oksanen et al. 2015). Additionally, we examined patterns

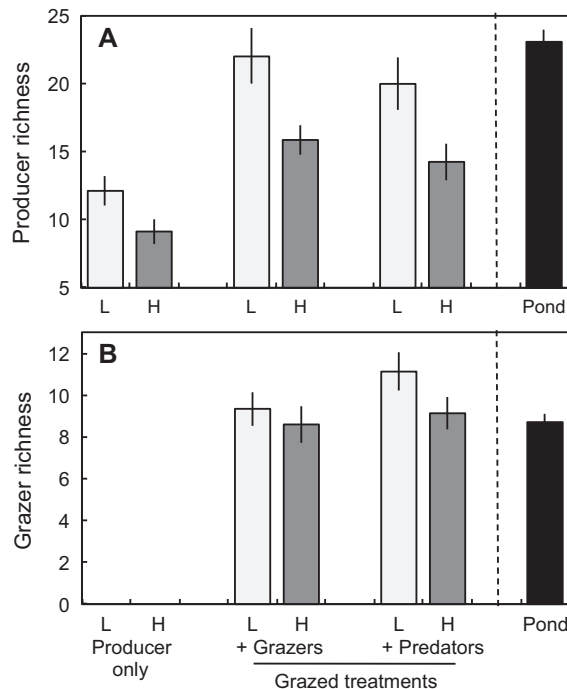


FIG. 1. Richness (mean \pm SE) of (A) algal producers and (B) zooplankton grazers in a mesocosm experiment and a survey of Michigan, USA, ponds. Three different food web manipulations are shown, including mesocosms containing only primary producers, as well as two grazed treatments: those with zooplankton grazers added (+ Grazers), and those with both grazers and predators added (+ Predators). Additionally, manipulations of overall nutrient supply rate are shown for the two nutrient level treatments: low supply rate (L) and high supply rate (H). Black bars show data from natural ponds in Michigan.

of beta-diversity (pairwise quantification of differences in composition between communities) with the main grazing and nutrient treatments. We partitioned beta-diversity into distinct components related to species richness vs. species turnover (using the betadiv R function; Podani and Schmera 2011, Legendre and De Caceres 2013) and evaluated the significance of those partitions using the adonis function in vegan (Oksanen et al. 2015).

RESULTS

Our experiment, supplemented by the field survey, produced three lines of evidence suggesting that herbivory increased phytoplankton richness. First, grazers almost doubled the species richness of primary producers in the experiment (ANOVA $F_{2,84} = 19.0$, $P < 0.0001$; Fig. 1, Appendix S1: Table S1). This diversity enhancement by grazing was not affected by the nutrient and light treatments (i.e., no interactions arose involving those resources and grazing; Appendix S1: Table S1). Furthermore, a post-hoc, pairwise comparison showed that phytoplankton richness did not differ between the two “grazed” treatments (with or without notonectid predators, Fig. 1,

ANOVA contrast $F_{1,84} = 0.38$, $P = 0.63$). Thus, enhancement of phytoplankton richness by grazers remained potent even when notonectids strongly suppressed a dominant grazer, *Daphnia pulex*, and provoked a trophic cascade by indirectly enhancing primary producer biomass (Appendix S1: Fig. S1). Notably, the mean increase in primary producer richness in grazed treatments was roughly equal to the mean number of crustacean grazer taxa (Fig. 1). Higher nutrient supply levels also decreased primary producer richness (through a main effect; ANOVA $F_{1,84} = 17.3$, $P < 0.0001$, Fig. 1). However, the nutrient supply rate (eutrophication) did not interact with the diversity-enhancing effects of grazing (i.e., there were no significant statistical interactions involving nutrient supply levels and food web structure, either two level or three level; Appendix S1: Table S1).

Second, richness of primary producer and crustacean grazer assemblages in the pond survey resembled that seen in the grazed treatments, but differed from that in the ungrazed (producer-only) treatments. Primary producer richness in natural local ponds did not differ significantly from that in our low-nutrient, grazed treatments (t test, assuming unequal variances, $t = -1.23$, $df = 62.8$, $P = 0.223$), although natural ponds were more diverse than our very eutrophic, grazed treatments ($t = -6.34$, $df = 105.1$, $P < 0.001$; Fig. 1). Ungrazed (producer-only) treatments had much lower mean richness of primary producers than that seen in nature ($t = 13.15$, $df = 132$, $P < 0.000001$; Fig. 1). Thus, primary producer richness in mesocosms rivaled that observed in natural ponds only when the experimental phytoplankton assemblages were grazed by macrozooplankton.

Third, we found that richness changes were almost entirely due to the addition of new, larger, phytoplankton species in the presence of grazers. Grazers largely enhanced richness of primary producers by enabling larger phytoplankton species to successfully persist with primary producers found in grazer-free treatments. The average body size of primary producer species common across both grazed and ungrazed systems was smaller than those species that were exclusively found only in grazed systems (Fig. 2). We observed too few species that were exclusive to ungrazed treatments to make meaningful conclusions about their size.

In contrast, we found little to no evidence that stoichiometric imbalance affected primary producer richness. Large manipulations of nutrient supply ratios (nitrogen [N]:phosphorus [P] ratios) did not change primary producer richness, neither overall or at low or high nutrient level (Fig. 3A, Appendix S1: Table S1). TN:TP ratios measured in the mesocosms were even more variable than our target ratios due to unbalanced losses from the water column by TP and TN (especially due to N accumulation in the water in the high TN:TP treatments; Fig. 3B). Despite this extremely large gradient in TN:TP ratio, we found no evidence for a unimodal relation between primary producer richness and TN:TP water levels (in tanks: Fig. 3B; in ponds: Fig. 3C; these insignificant

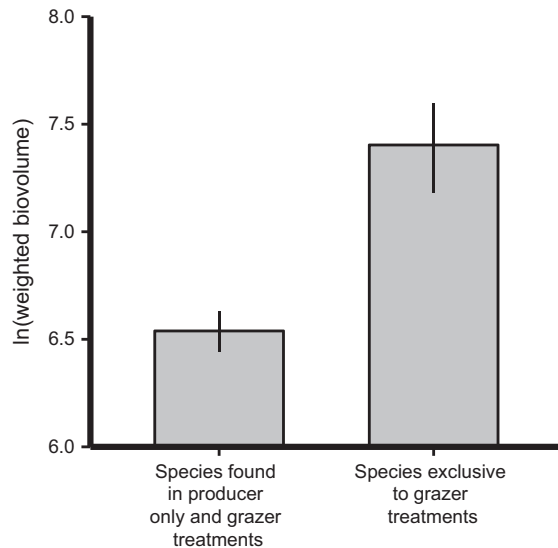


FIG. 2. Phytoplankton species found exclusively in grazed treatments were larger than species found in both producer-only and grazed treatments. Larger phytoplankton species tend to be inferior resource competitors (Burns 1968, Litchman and Klausmeier 2008); thus, we infer that the new species entering the pooled grazed treatments contained phytoplankton that were on average poorer competitors. In these calculations, the mean sizes (biovolume; measured as μm^3) of these species were weighted by their occurrence (i.e., by number of mesocosms in which each species was found). The 95% confidence intervals, found by bootstrapping mesocosms, do not overlap.

results held when separating the low and high nutrient level treatments). Furthermore, our index of stoichiometric imbalance did not correlate negatively with primary producer richness in tanks (either without grazing or with grazing; Fig. 3D) or in ponds (Fig. 3E). No correlation emerged at low nutrient supply in the tanks (as predicted by Brauer et al. 2012). Similarly, neither experimental shading (not shown; Appendix S1: Table S1) nor canopy openness measured in the natural ponds (Spearman's $r = -0.09$, $P = 0.56$; not shown) significantly influenced primary producer richness.

These richness effects do not preclude strong compositional shifts across the nutrient supply gradient. Indeed, the RDA analyses show large compositional shifts of primary producer assemblages. In the associated bi-plot, the first major axis most strongly reflected presence-absence of grazers (with grazed treatments [triangles] appearing to the left and producer-only [circles] grouping to the right; Fig. 4; $R^2 = 0.83$, $P < 0.001$). However, nutrient supply ($R^2 = 0.37$, $P = 0.001$), N:P ratio (denoted by the colored symbols: $R^2 = 0.19$, $P = 0.050$) and shading ($R^2 = 0.17$, $P = 0.077$) had smaller (and sometimes marginally significant), but still notable, effects on primary producer composition (loading mostly with the second RDA axis; Fig. 4). Thus, such broad resource supply gradients did influence composition if not richness. Yet, a companion analysis of presence-absence data confirmed the grazer-richness connection in a beta-diversity context

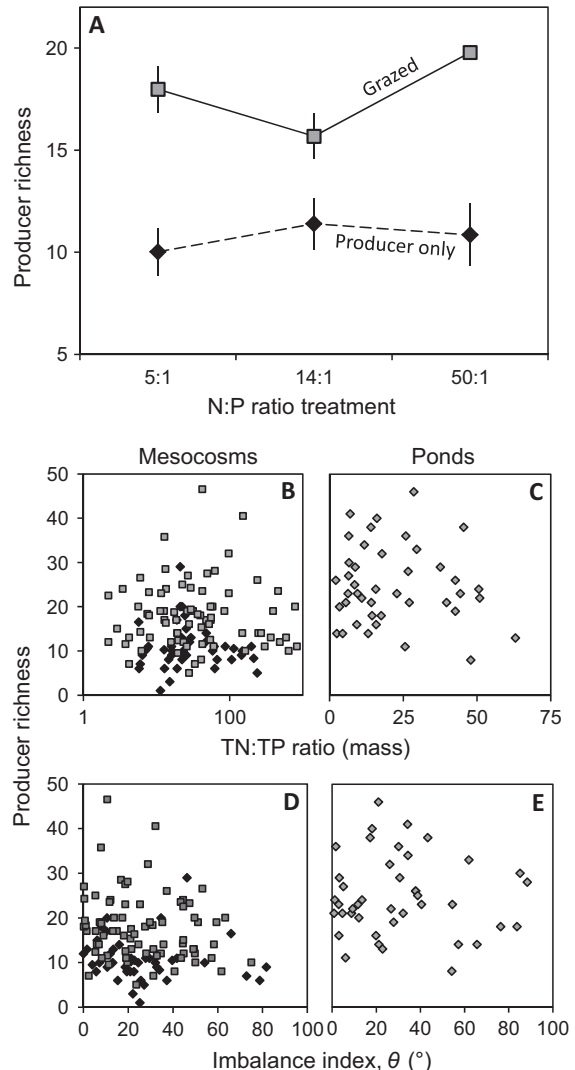


FIG. 3. Test of the "stoichiometric imbalance" hypothesis in the mesocosm experiment and natural ponds. (A) In the mesocosm experiment, there was no relationship between nitrogen:phosphorus (N:P) supply treatment (i.e., ratio of nutrient loading) and the index of producer richness, for either producer-only (black diamond, dashed lines) or grazed treatments (+ grazers and + predators; gray squares, solid lines; see also Appendix S1: Table S1). (B–E) The same conclusion arose examining actual total N:total P ratios, in mesocosms and natural ponds (shading identical with panel A). (B) In the mesocosms, $\ln(\text{TN}:\text{TP})$ ratio did not correlate with producer richness (but quadratic terms were marginally significant; $P = 0.059$ for producer-only and $P = 0.083$ for grazed systems). (C) No relationship arose between the more constrained range of TN:TP in ponds as well (but the quadratic term was only significant at the $P = 0.073$). However, the imbalance index, θ (where large values indicate large imbalances; Cardinale et al. 2009) calculated from log-transformed TN and TP showed no relationship with producer richness in (D) mesocosms or (E) ponds.

(i.e., using a completely different analysis, we arrive at the same conclusion). Only grazers (and nutrient supply) affected the richness component of beta-diversity (Appendix S1: Table S2). In contrast, almost all the

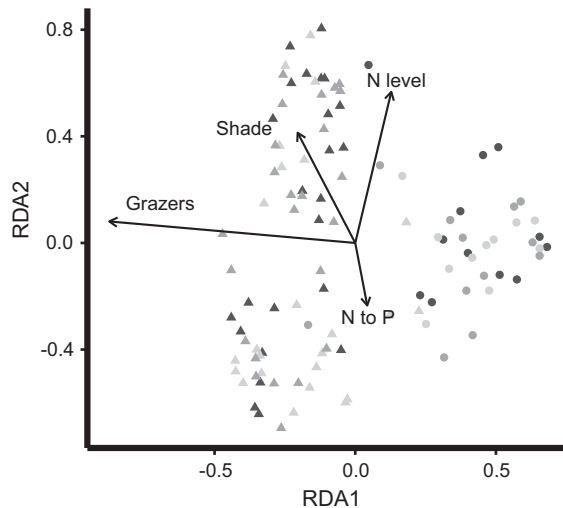


FIG. 4. Redundancy analysis of phytoplankton biomass composition in experimental mesocosms. Symbols represent distributions of mesocosms in the analysis. Vectors represent the influence of main treatments in our experiment (grazing, “Grazers”; nutrient supply, “N level”; shading, “shade”). Triangles represent grazed mesocosms, whereas circles represent ungrazed treatments. The degree of shading in these symbols is proportional to the N:P ratio treatment (“N:P”: 50:1 [black], 14:1 [dark gray], or 5:1 [light gray] by mass). Notonectid predators had no effect on composition; hence, that vector is not shown.

treatments affected the turnover component of beta diversity (i.e., the component that is independent of species richness, Appendix S1: Table S3). These results confirm our analysis of species richness alone (Fig. 1): grazing increases richness while eutrophication lowers it. Yet, they also indicate that gradients of resource supply also affected species composition.

DISCUSSION

The observed richness of most natural primary producer assemblages is much higher than predicted by equilibrium competition theory (Hardin 1960, Hutchinson 1961). During the past five decades this beguiling “paradox of the plankton” (Hutchinson 1961) has stimulated a diverse suite of experimental tests of various models aiming to address it. However, none can claim to have successfully resolved the paradox (Wilson 1990, Roy and Chattopadhyay 2007). Among the various competing hypotheses, the natural enemies hypothesis seemed posed to resolve the paradox, based on key early experiments (Lubchenco 1978, Paine 1966). In those experiments, predators reduced density of dominant competitor-prey, facilitating coexistence of less vulnerable but inferior competitors. Yet, a meta-analysis of experiments manipulating herbivores, at first glance, dooms the natural enemies hypothesis (Hillebrand et al. 2007): in their meta-analysis, grazers simply did not enhance richness of their prey enough to resolve the paradox, particularly in freshwater systems (even in planktonic ones).

Despite these previous results, we show here that the presence of an assemblage of crustacean zooplankton herbivores nearly doubled the richness of phytoplankton producers in pond mesocosms. Moreover, phytoplankton richness in these mesocosms rivaled that found in natural ponds, but only in grazed environments that were not hyper eutrophic. Furthermore, notonectid predators did not change richness either of phytoplankton (as seen elsewhere, e.g., O’Connor et al. 2013) or of zooplankton (as seen in other field surveys, e.g., with *Bythotrephes*; Walseng et al. 2015). Thus, herbivores alone increased phytoplankton richness, and an important predator did not mute this effect. Based on these findings, we propose that grazing by natural enemies offers a potent resolution to the “paradox of the plankton.”

Without grazing by large zooplankton, phytoplankton producer communities in our experimental mesocosms supported about eight species in eutrophic conditions to 12 species in mesotrophic ones. This species richness exceeds that predicted by known, resource-based limiting factors. While primary producers may experience more diversity-enhancing co-limitation by resources than tradition holds (Harpole et al. 2011, Brauer et al. 2012), resource limitation alone does not likely explain this higher diversity. In principle, richness in our grazing-free mesocosms might be maintained a suite of mechanisms. These might include (1) intransitive competition among primary producers for multiple resources (Huisman and Weissing 1999, Kerr et al. 2006); (2) joint limitation by nutrients and light (Brauer et al. 2012); (3) pulsed variation in nutrient supply (given our weekly nutrient additions), and/or temperature and light fluctuations (with movement of weather fronts to the outdoors array; Grover 1997); (4) grazing by small enemies that passed through our sieves, such as heterotrophic algae, protists, and small rotifer grazers, pathogens, etc. (Ibelings et al. 2004); (5) and other factors. That said, phytoplankton richness did not decline in low light, high nutrient environments (where superior light competitors might have suppressed others: Brauer et al. 2012). Furthermore, even if all of these mechanisms operated simultaneously, they could account for only half of the phytoplankton richness observed in mesotrophic, grazed treatments and in natural ponds. Addition of a diverse assemblage of large grazers supported the other half.

Several important facets of this grazer effect merit note. First, the increase in primary producer richness did not hinge on the presence of *Daphnia*, a critical grazer in freshwater ponds and lakes (Leibold 1989, Ives et al. 1999). In grazed environments, *Daphnia* was essentially eliminated at low nutrient supply by poor food quality and also by notonectid predators (as shown in Hall et al. 2004). Although notonectids elicited trophic cascades (see Appendix S1: Fig. S1), they did not alter phytoplankton richness. Second, grazer assemblages allowed algal species with larger body size to persist with smaller ones. This result is consistent with grazer-enhanced success of larger,

more resistant, primary producer taxa (Leibold 1996, Duffy et al. 2007). In theory, large algae resist grazing but are inferior competitors, but detecting such trade-offs can prove complicated (as illustrated by Edwards et al. 2011). Furthermore, primary producer species could show tradeoffs in their degree of resistance to different grazers (e.g., Hulot and Loreau 2006), and grazers could possibly enhance primary producer richness by other mechanisms entirely (e.g., by altering ratios or heterogeneity of nutrient regeneration [Andersen 1997, Daufresne and Loreau 2001, Grover 2002] or by creating flow through herbivore feeding and swimming activities [Kjørboe et al. 2014]). Given the breadth of possible contributing factors, pinpointing exact mechanism(s) by which grazers enhanced algal richness exceeded the scope of this paper. Third, the number of new algal species supported by grazers is approximately the same as the number of co-occurring crustacean species. This finding prompts some key questions. Could each grazer have served as a “limiting factor” for phytoplankton? What enabled coexistence of these grazers? Fourth, the presence of grazers boosted primary producer richness to levels rivaling that seen in natural ponds. Ponds must offer a more diverse habitat than cattle tanks. Nonetheless, the ponds (on average) did not support more phytoplankton species than seen in our mesotrophic, grazed mesocosms.

The diversity-enhancing effect of grazers prompts introspection into previous work. Why did so few past experiments reveal diversity enhancement by primary producers, particularly in freshwater systems (Hillebrand et al. 2007)? One possibility is that our design has better addressed key limitations of past experiments by facilitating long-term feedbacks among open, diverse assemblages of both primary producers and grazers. These three components (time, openness, diversity) may prove essential to show grazing effects on primary producer assemblages. This hypothesis could be tested in future experiments that explicitly manipulate duration, openness of species pools, and diversity of grazing assemblages. Alternatively, conclusions in the Hillebrand et al. (2007) meta-analysis may reflect the dominance of benthic systems in their data set: 83% of the freshwater studies analyzed involved benthic algae. Grazing on benthic algae may differ from grazing on plankton because of the prevalence of epiphytic algae that grow on filamentous algae or macrophytes and biofilms in the benthos. For instance, grazers can remove diversity-enhancing spatial structure where algae attach to each other in complicated manners (Steinman 1996). Instead, grazers likely reduce assemblages to more grazer-resistant, simpler growth forms (commonly seen with *Stigeoclonium*; Rosemond et al. 1993, Darcy-Hall and Hall 2008). Thus, herbivores can either enhance or reduce richness in benthic systems (Steinman 1996). Therefore, the benthic-weighted meta-analysis (Hillebrand et al. 2007) may have greatly under-represented the diversity-enhancing effects of grazers in the phytoplankton of freshwater systems.

Grazers enhanced richness of primary producer assemblages, but large variation in the supply of resources did not. More specifically, we saw no enhancement or depression of phytoplankton richness along very broad gradients of nitrogen:phosphorus supply ratios in the tanks or the ponds (as in Korhonen et al. 2011). Stoichiometric imbalance (calculated following Cardinale et al. (2009)) did not correlate with primary producer richness, with or without grazers. This result seems surprising, given that niche theory predicts that primary producer richness can peak at intermediate N:P supply ratios (Tilman 1982, Cardinale et al. 2009), at least in more oligotrophic conditions (Brauer et al. 2012). However, composition of primary producer assemblages did shift along these N:P gradients (as commonly seen: Smith 1983, Agawin et al. 2004). Furthermore, nutrient enrichment (eutrophication) itself did depress richness, a common result seen in diverse ecosystems (e.g., Stevens et al. 2004, Harpole and Tilman 2007, Hillebrand et al. 2007). However, here eutrophic conditions likely did not depress richness due to light limitation (as predicted by Brauer et al. 2012), since a major manipulation of incident light with shade cloth did not depress primary producer richness. Furthermore, we found no relationship of richness to characteristics of the light environment in the experiment (extinction coefficient, light at the bottom of the mesocosm; not shown). Thus, we conclude that grazers (and eutrophication), rather than stoichiometric imbalances (Cardinale et al. 2009) or light limitation (Brauer et al. 2012), most strongly shaped primary producer richness in our experiment.

Why do so many primary producer species coexist when simple models predict otherwise? We infer from this experiment and the pond survey that the maintenance of high richness of primary producers in plankton likely hinges upon grazing. Hence, grazing by natural enemies can help to resolve the paradox, provided that density-dependent feedbacks operate sufficiently long in ecological time in habitats open to colonization. A diverse assemblage of crustacean grazers doubled the richness of algal producers in our experiment. Hence, algal richness greatly exceeded that which should be supported by other limiting factors (e.g., nutrients and light) alone. In other systems, physical, chemical, and biological factors may modify the diversity-enhancing effects of herbivory. Nevertheless, our experimental results show that herbivory can help resolve Hutchinson's Paradox, at least in the freshwater plankton.

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DATA AVAILABILITY

Data associated with this paper are available in Dryad: <https://doi.org/10.5061/dryad.560ch>