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Competition for nutrients and light: testing advances in resource competition with a natural phytoplankton community

AMANDA BURSON,¹ MAAYKE STOMP,^{1,†} EMMA GREENWELL,¹ JULIA GROSSE,^{2,3} AND JEF HUISMAN^{1,4}

¹Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

²Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research (NIOZ), Den Burg/Texel, The Netherlands

Abstract. A key challenge in ecology is to understand how nutrients and light affect the biodiversity and community structure of phytoplankton and plant communities. According to resource competition models, ratios of limiting nutrients are major determinants of species composition. At high nutrient levels, however, species interactions may shift to competition for light, which might make nutrient ratios less relevant. The “nutrient-load hypothesis” merges these two perspectives, by extending the classic model of competition for two nutrients to include competition for light. Here, we test five key predictions of the nutrient-load hypothesis using multispecies competition experiments. A marine phytoplankton community sampled from the North Sea was inoculated in laboratory chemostats provided with different nitrogen (N) and phosphorus (P) loads to induce either single resource limitation or co-limitation of N, P, and light. Four of the five predictions were validated by the experiments. In particular, different resource limitations favored the dominance of different species. Increasing nutrient loads caused changes in phytoplankton species composition, even if the N:P ratio of the nutrient loads remained constant, by shifting the species interactions from competition for nutrients to competition for light. In all treatments, small species became dominant whereas larger species were competitively excluded, supporting the common view that small cell size provides a competitive advantage under resource-limited conditions. Contrary to expectation, all treatments led to coexistence of diatoms, cyanobacteria and green algae, resulting in a higher diversity of species than predicted by theory. Because the coexisting species comprised three phyla with different photosynthetic pigments, we speculate that niche differentiation in the light spectrum might play a role. Our results show that mechanistic resource competition models that integrate nutrient-based and light-based approaches provide an important step forward to understand and predict how changing nutrient loads affect community composition.

Key words: biodiversity; light; nitrogen; North Sea; nutrient load; phosphorus; phytoplankton; resource competition.

INTRODUCTION

Hutchinson’s (1961) “paradox of the plankton” raised a fundamental question in ecology: how can we explain coexistence of a rich diversity of species in simple unstructured habitats with only a handful of limiting resources? One possible answer is provided by resource competition theory, an influential theory explaining how competition for limiting nutrients affects biodiversity and community structure (Tilman 1982, Grover 1997). Theoretical studies have developed the mathematical underpinnings of this theory (León and Tumpson 1975, Tilman 1982, Huisman and Weissing 2001, Yoshiyama et al. 2009). The theory has been confirmed by laboratory competition experiments (Tilman 1977, Sommer 1985, Van Donk and Kilham 1990), applied to both freshwater and marine plankton (Sommer 1993, 1994, Edwards et al. 2011), and extended to terrestrial plant communities (Tilman 1988, Wedin and Tilman 1993, Harpole and Tilman 2007).

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³Present address: Department of Biological Oceanography, GEOMAR Helmholtz Centre of Ocean Research Kiel, Kiel, Germany.

⁴Corresponding Author. E-mail: j.huisman@uva.nl

[†]Deceased

Yet, there are some tensions between the predictions of resource competition models and real-world observations that have led to considerable debate. One of the points of debate concerns the role of nutrient ratios versus absolute nutrient loads in determining the species composition. Resource competition theory has advanced the “resource-ratio hypothesis”, which predicts that the outcome of competition depends on the ratios at which limiting resources are supplied (Tilman 1985). For instance, cyanobacterial dominance in aquatic ecosystems has been associated with low nitrogen-to-phosphorus ratios (Smith 1983, Havens et al. 2003, Vrede et al. 2009). However, other studies have emphasized high nutrient loads, rather than low nitrogen-to-phosphorus ratios, as major triggers for an increased frequency, intensity, and duration of cyanobacterial blooms (Reynolds 1998, Downing et al. 2001, McCarthy et al. 2009). Furthermore, enrichment with excessive amounts of nutrients is commonly seen as one of the main causes of diversity loss in terrestrial plant communities, as nutrient addition shifts the species interactions from competition for nutrients to competition for light (Stevens et al. 2004, Harpole and Tilman 2007, Hautier et al. 2009, Harpole et al. 2016). Hence, it appears that not only nutrient ratios but also absolute nutrient levels can have a major impact on the species composition of natural communities.

One possible way out of this debate is to incorporate light as a limiting resource in resource competition models (Tilman 1985, Huisman and Weissing 1994). Competition for light is fundamentally different from competition for nutrients, because of the unidirectional nature of the light gradient (Weiner 1990, Huisman et al. 1999, Hautier et al. 2009). Resource competition models that take the vertical light gradient into account predict that nutrient enrichment causes increased shading among species, resulting in a lower light availability further down the gradient and hence competition for light (Huisman and Weissing 1995, Passarge et al. 2006, Yoshiyama et al. 2009). As a next step, Brauer et al. (2012) developed the “nutrient-load hypothesis.” Their model extends the classic resource competition model for two nutrients (Tilman 1982) by incorporating light (Huisman and Weissing 1994, 1995) as a third resource. Similar to the classic resource competition models (Fig. 1A), the nutrient-load hypothesis predicts that nutrient ratios determine the species composition in nutrient-poor environments (Fig. 1B). In such environments, strong nutrient competitors will be dominant, while competition for light is negligible because nutrient limitation prevents the build-up of sufficient biomass for the species to affect each other through shading. However, the nutrient-load hypothesis predicts that increasing nutrient loads will also cause changes in species composition if the N:P ratio of the nutrient loads remains constant (Fig. 1B). In eutrophic environments, biomass accumulates, the species interact through mutual shading, and the best light competitor is expected to prevail. Highest species diversity is predicted at intermediate nutrient levels where co-limitation by two nutrients and light provides ample opportunities for species coexistence (Brauer et al. 2012, Harpole et al. 2016).

In this paper, we perform controlled experiments specifically designed to investigate this three-way interaction of

competition for two nutrients and light. A common approach in tests of resource competition theory is to select a limited number of isolated species that are grown in monoculture and competition experiments (e.g., Tilman 1977, Huisman et al. 1999, Passarge et al. 2006, Ji et al. 2017). This approach has the main advantage that the relevant species traits can be measured in monoculture, which enables independent prediction of the outcome of competition prior to the actual competition experiments. However, these experiments do not mimic the high biodiversity and potential complexity of natural communities. Many natural phytoplankton and plant communities consist of dozens and sometimes even hundreds of different species. Resource competition models predict that multispecies competition for three or more limiting resources will not always result in stable communities, but can also produce species oscillations and chaos if the competitive abilities of the species form intransitive loops (Huisman and Weissing 1999, 2001). Hence, an interesting alternative approach to test predictions of resource competition theory, specifically in case of three or more limiting resources, is to incorporate the high species richness of natural communities in the experiments.

We inoculated a diverse phytoplankton community sampled from the North Sea into laboratory chemostats provided with different N and P concentrations to mimic different N and P loads. The following predictions were investigated (Fig. 1B, see also Brauer et al. 2012):

- (1) Low N loads lead to N limitation, low P loads to P limitation, combined low N and P loads to N + P co-limitation, combined intermediate N and P loads to co-limitation by nutrients and light, and combined high N and P loads to light limitation.
- (2) N limitation, P limitation and light limitation favor the dominance of different phytoplankton species.

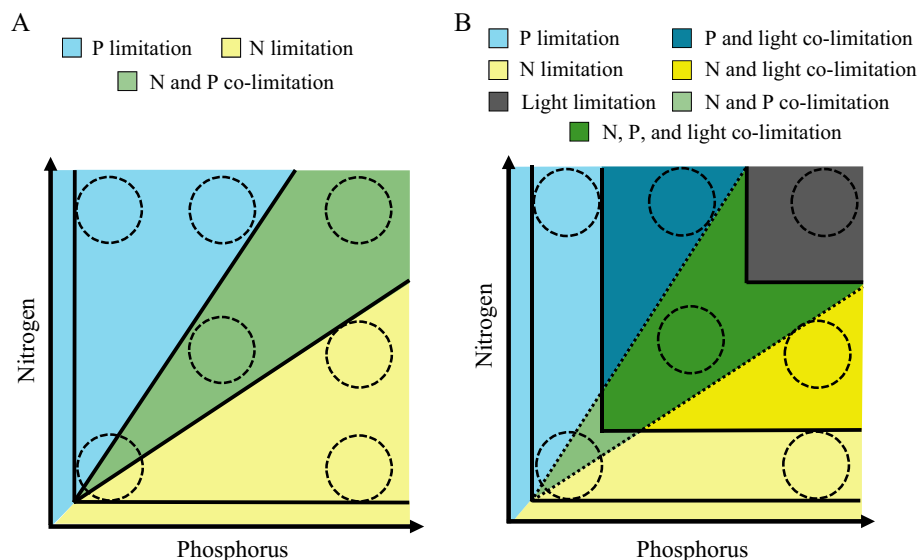


FIG. 1. Conceptual models of resource limitation as function of the nitrogen and phosphorus loads. (A) The classic resource-ratio hypothesis predicts that the pattern of resource limitation depends on the ratio of the nitrogen and phosphorus loads. (B) The nutrient-load hypothesis incorporates light as a third limiting resource, and consequently the resource limitation pattern depends not only on the ratio but also on the absolute values of the nitrogen and phosphorus loads. Both models predict that different resource limitations will favor the dominance of different species. Dashed circles represent the targeted resource limitations of the seven competition experiments.

- (3) Co-limitation by multiple resources enables species coexistence, whereas single resource limitation leads to competitive exclusion.
- (4) Co-limitation can lead to stable coexistence, alternative stable states, species oscillations, or chaos depending on the traits of the competing species.
- (5) Changes in nutrient loads will cause changes in species composition, even if the N:P ratio of the nutrient loads remains constant.

All competition experiments were seeded with the same mixture of natural phytoplankton and hence they all started from the same community composition.

METHODS

Collection of natural community

Natural phytoplankton communities were sampled at eight stations along a 450-km transect in the North Sea, during a research cruise with the *R.V. Pelagia* from 15 to 25 March 2013 (see Burson et al. 2016 for details of cruise route). The transect captured the full range from P-limited phytoplankton in coastal waters, via N and P co-limitation further offshore, to N-limited phytoplankton communities in the central North Sea (Burson et al. 2016). At each station, a 20-L carboy was rinsed and filled with water collected at 7 m depth using a sampling rosette equipped with 24 Niskin bottles. Water was passed through an 80- μm mesh to remove large zooplankton, and then gently bubbled for 30 min with N_2 gas and 30 min with CO_2 to eliminate smaller grazers via oxygen displacement while ensuring sufficient inorganic carbon availability for phytoplankton photosynthesis. The carboys were kept at 4°C, until initiation of the chemostat experiments at the University of Amsterdam 2 d after the cruise ended. Prior to being added to the chemostats, 2 L of water from each station were combined, resulting in a single inoculum containing a mixture of phytoplankton species from all eight stations. Hence, all chemostat experiments were inoculated at the same day with the same initial community composition.

Laboratory experiments

The experiments were conducted in flat-walled chemostats, specifically built to study the population dynamics of phytoplankton species (Huisman et al. 1999, Stomp et al. 2004, Passarge et al. 2006). The chemostats allowed full control of light conditions, temperature, pCO_2 in the gas flow, and nutrient concentrations in the mineral medium. The working volume of the chemostat vessels was 1.7 L. To initiate experiments, each chemostat was provided with 0.5 L of the North Sea inoculum and filled up with mineral medium with different nitrate and phosphate concentrations.

In total, we ran seven competition experiments according to the design laid out in Fig. 1B. The nitrate concentration in the mineral medium was either low (LN = 64 $\mu\text{mol/L}$), medium (MN = 160 $\mu\text{mol/L}$), or high (HN = 2,000 $\mu\text{mol/L}$). Likewise, the phosphate concentration in the mineral medium was low (LP = 4 $\mu\text{mol/L}$), medium (MP = 10 $\mu\text{mol/L}$), or high (HP = 125 $\mu\text{mol/L}$). All other macro- and micronutrients were

provided at high non-limiting concentrations at a salinity of 35 psu (Appendix S1: Table S1). With this design, we aimed for N limitation in the LN:HP experiment, P limitation in the HN:LP experiment, light limitation in the HN:HP experiment, and different co-limitations in the other experiments to test Predictions 1–4. Furthermore, the N:P ratios in the mineral medium of the LN:LP experiment, MN:MP experiment, and HN:HP experiment were all equal to the Redfield ratio of 16:1 to enable testing of Prediction 5.

The front surfaces of the flat chemostat vessels were lit with a constant incident irradiance (I_{in}) of 40 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, provided by white fluorescent tubes (Philips PL-L 24W/840/4P, Philips Lighting, Eindhoven, The Netherlands). This irradiance level is comparable to the irradiance of 30–100 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ that we measured at 5–10 m depth when collecting the samples during the North Sea cruise. The chemostat vessels had an optical path length (“mixing depth”) of 5 cm. Irradiance passing through the chemostats (I_{out}) was measured daily with a LI-COR LI-250 quantum photometer (LI-COR Biosciences, Lincoln, Nebraska, USA) placed at 10 evenly distributed positions at the back surface of the chemostat vessel.

Inorganic carbon was added as sodium bicarbonate (0.5 mmol/L) in the mineral medium and as CO_2 in filtered air, which was bubbled through the chemostats at a flow rate of 80 L/h using Brooks instrument pressure flow systems (Hartford, Pennsylvania, USA). Concentrations of CO_2 in the air flow were adjusted as biomass increased to maintain a pH of 8.2, which was checked daily with a SCHOTT pH meter (SCHOTT AG, Mainz, Germany). Bubbling of the chemostats further ensured homogeneous mixing of the phytoplankton community, while daily scraping with a magnetic stir bar minimized wall growth. Temperature was maintained at 18°C using cooling plates connected to a thermocryostat, and dilution rates of the chemostats were set at 0.2 d^{-1} . The chemostat experiments continued until the biovolume of the total phytoplankton community remained stable for at least 5 d.

Phytoplankton and nutrient analysis

Samples were taken every other day for phytoplankton counts and nutrient analysis. Phytoplankton samples (45 mL) were transferred to 50-mL centrifuge tubes, preserved with 1% Lugol’s iodine, and stored in the dark at 15°C until microscopic analysis. Furthermore, 4.5-mL samples were preserved with 0.5 mL formaldehyde (18% v/v)-hexamine (10% w/v) solution in 5-mL cryogenic vials. These samples were placed in 4°C for 30 min, flash frozen in liquid nitrogen, and stored at -80°C until analysis via flow cytometry. Nutrient samples (15 mL) were gently filtered over a 0.22 μm polycarbonate filter into 20 mL polyethylene vials, and stored in the dark at -20°C until analysis.

Phytoplankton larger than 3 μm were identified to genus level and counted with an inverted microscope (DM IRB, Leica Microsystems, Wetzlar, Germany) using 1 mL gridded Sedgewick Rafter counting chambers. We counted the entire chamber or 200 cells per species depending on cell concentrations. Smaller phytoplankton cells ($<3 \mu\text{m}$) were counted using an Accuri C6 flow cytometer (BD Biosciences, San Jose, California, USA) equipped with a blue laser (488 nm) and red laser (640 nm). The flow cytometer counts were

dominated by two *Synechococcus* clusters, which partially overlapped, and were therefore grouped as *Synechococcus* spp. Biovolumes of the phytoplankton were calculated from cellular dimensions and geometry according to Hillebrand et al. (1999). Nutrients were analyzed using standard colorimetric methods for nitrate and nitrite (Grasshoff et al. 1983), ammonium (Helder and de Vries 1979), and dissolved inorganic phosphorus (DIP; Murphy and Riley 1962). Dissolved inorganic nitrogen (DIN) was defined as the sum of nitrate, nitrite, and ammonium. The dominant species in the experiments were isolated, and their light absorption spectra were measured at a 0.4 nm resolution using an AMINCO DW-2000 double-beam spectrophotometer (Olis, Bogart, Georgia, USA).

RESULTS

Nutrient and light conditions

Total phytoplankton in the competition experiments gradually increased, while DIN and DIP concentrations and irradiance passing through the chemostats (I_{out}) decreased, until steady-state conditions were reached after 20–75 d depending on the experiment (Appendix S1: Fig. S1).

In the HN:LP experiment the DIP concentration was depleted to 0.5 $\mu\text{mol/L}$ at steady state, while DIN levels remained high at 1,190 $\mu\text{mol/L}$ (Fig. 2A; Appendix S1: Table S2). Consequently, the steady-state DIN:DIP ratio was 2,380, far above the Redfield ratio of 16:1. The low phosphate concentration in the mineral medium supplied to this experiment kept the total biovolume low, and hence light passing through the chemostat stayed high at $I_{out} = 23 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2B). Nutrient dynamics in the HN:MP experiment were similar to the HN:LP experiment, resulting again in a high DIN:DIP ratio well above the Redfield ratio (Fig. 2A). However, the higher phosphate concentration in the mineral medium supplied to the HN:MP experiment led to a higher biovolume, which reduced I_{out} to lower values (Fig. 2B).

Conversely, in the LN:HP experiment the DIP concentration remained high at 46 $\mu\text{mol/L}$ at steady state, whereas the DIN concentration was depleted to 2 $\mu\text{mol/L}$ (Fig. 2A). As a result, the DIN:DIP ratio was only 0.04, far below the Redfield ratio. The nutrient dynamics in the MN:HP experiment followed a similar pattern as in the LN:HP experiment, resulting again in a DIN:DIP ratio far below the Redfield ratio. In line with expectation, the biovolume was higher and hence I_{out} was lower in the MN:HP than in the LN:HP experiment (Fig. 2B).

In the HN:HP experiment, the steady-state DIN and DIP concentrations remained high at 181 and 29 $\mu\text{mol/L}$, respectively (Fig. 2A; Appendix S1: Table S2). However, the dense phytoplankton biovolume that developed in this experiment absorbed almost all incident light, reducing I_{out} to 0.4 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the lowest of all competition experiments (Fig. 2B).

In the LN:LP and MN:MP experiments, DIN concentrations were depleted to <4 $\mu\text{mol/L}$ and DIP concentrations to <3 $\mu\text{mol/L}$ at steady state (Fig. 2A). This resulted in DIN:DIP ratios of 2 and 1 in the LN:LP and MN:MP experiment, respectively. The higher nitrate and phosphate concentrations

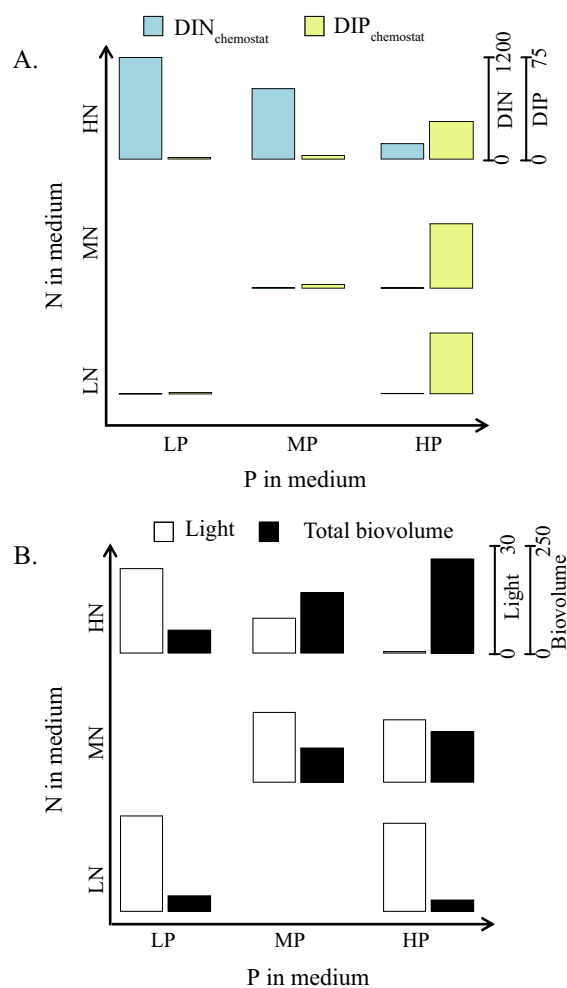


FIG. 2. Nutrient and light conditions reached at steady state in the competition experiments. (A) DIN and DIP concentrations (in $\mu\text{mol/L}$) measured at steady state. (B) Light transmission (I_{out} , in $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and total biovolume (mm^3/L) measured at steady state. The axes represent the nitrogen and phosphorus concentrations in the mineral medium supplied to the competition experiments (LN, low nitrogen; MN, medium nitrogen; HN, high nitrogen; LP, low phosphorus; MP, medium phosphorus; HP, high phosphorus).

in the mineral medium supplied to the MN:MP experiment yielded a higher phytoplankton biovolume, and hence lower I_{out} , than in the LN:LP experiment (Fig. 2B).

Initial community composition

The initial phytoplankton community consisted of 19 taxonomic groups, most of them identified to the genus level (Fig. 3). The community comprised a wide range of cell sizes, from small picocyanobacteria of the genus *Synechococcus* to large diatoms of the genus *Coscinodiscus* (Table 1). The total biovolume of the community at the start of each experiment was 0.61 mm^3/L . The dominant genera in terms of their contribution to total biovolume were the diatoms *Coscinodiscus* spp. at 20%, the green algae *Chlorella* spp. at 18%, the cyanobacteria *Synechococcus* spp. at 17% and the dinoflagellates *Gymnodinium* spp. at 11%. In terms

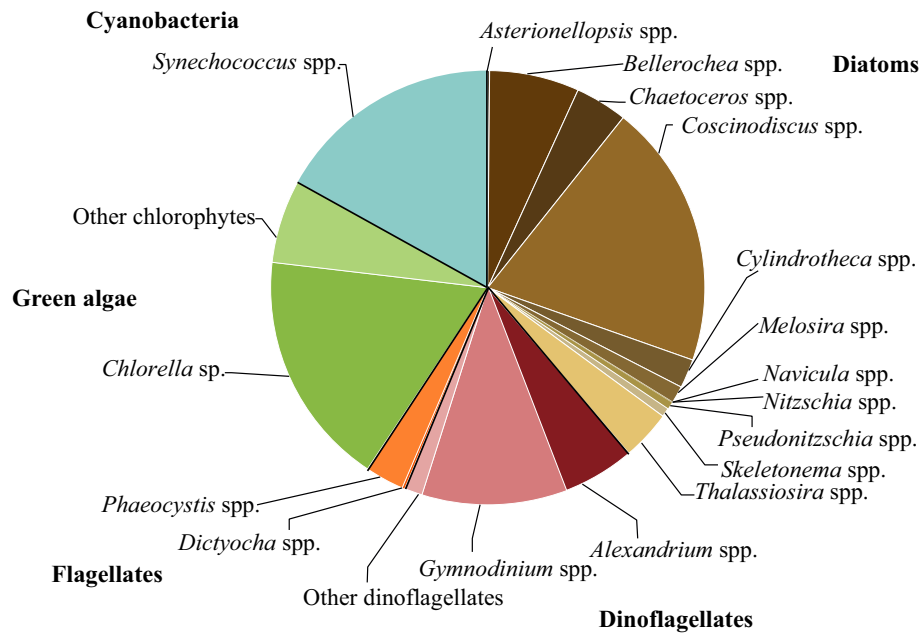


FIG. 3. Initial phytoplankton community composition. The phytoplankton mixture was sampled from the North Sea, and provided the starting point for the competition experiments. The community composition is displayed as the relative contributions of the different taxa to the total biovolume.

TABLE 1. Cellular biovolumes (μm^3) of the species present in the natural phytoplankton community used in our experiments, organized by functional group.

Species	Cell biovolume† (μm^3)
Diatoms	
<i>Nitzschia pusilla</i>	190 ± 27
<i>Nitzschia agnita</i>	300 ± 53
<i>Cylindrotheca</i> spp.	513 ± 105
<i>Skeletonema</i> spp.	827 ± 168
<i>Navicula</i> spp.	1,590 ± 360
<i>Asterionellopsis</i> spp.	2,754 ± 912
<i>Pseudo-nitzschia</i>	3,060 ± 695
<i>Chaetoceros</i> spp.	3,605 ± 887
<i>Thalassiosira</i> spp.	3,770 ± 808
<i>Melosira</i> spp.	10,179 ± 2,553
<i>Coscinodiscus</i> spp.	259,770 ± 67,180
Dinoflagellates	
Other dinoflagellates	754 ± 411
<i>Gymnodinium</i> spp.	3,142 ± 1,141
<i>Alexandrium</i> spp.	16,607 ± 1,002
Flagellates	
<i>Phaeocystis</i> spp.	34 ± 10
<i>Dictyocha</i> spp.	11,219 ± 1,059
Green algae	
<i>Chlorella marina</i>	22 ± 8
Other green algae	180 ± 15
Cyanobacteria	
<i>Synechococcus</i> spp.	1.7 ± 0.6

†Data show mean ± SD of 20 cells per species.

of broader functional groups, diatoms dominated biovolume with 39%, followed by green algae with 24%, then dinoflagellates and cyanobacteria with 18 and 17% respectively, while other flagellates were less abundant.

Competition dynamics

Initially, all species increased in biovolume (Fig. 4), except the flagellates *Dictyocha* spp. and *Phaeocystis* spp. and the category “other green algae.” This indicates that the laboratory chemostats provided suitable growth conditions for the great majority of species in this natural community mixture. After 1–3 weeks, one or more resources were depleted in the competition experiments and many species started to decline. In several treatments, the diatoms *Navicula* spp. and/or *Coscinodiscus* spp. persisted one or two weeks longer, but ultimately also declined. In the end, 15 of the 19 taxa were competitively excluded from all competition experiments by a common set of four species consisting of the diatoms *Nitzschia pusilla* and *N. agnita*, the green alga *Chlorella marina* and the picocyanobacterium *Synechococcus* spp. In the HN:HP experiment, only three species survived as it also lost *N. pusilla* (Fig. 4C). We note that the diatoms *N. agnita* and *N. pusilla* were the two smallest diatom species in the multispecies community, and the green alga *C. marina* and picocyanobacteria of the *Synechococcus* genus were even smaller (Table 1). In all experiments, the surviving species maintained a stable coexistence for several weeks (Fig. 4).

Relative abundances of the species

Relative abundances of the species at steady state differed between competition experiments (Fig. 5). The diatom *N. pusilla* dominated the HN:LP experiment, was co-dominant in the LN:LP experiment, rare in the HN:MP experiment, and absent from the HN:HP experiment. The relative abundance of *N. pusilla* did not increase or decrease with the N:P ratio of the mineral medium, but decreased

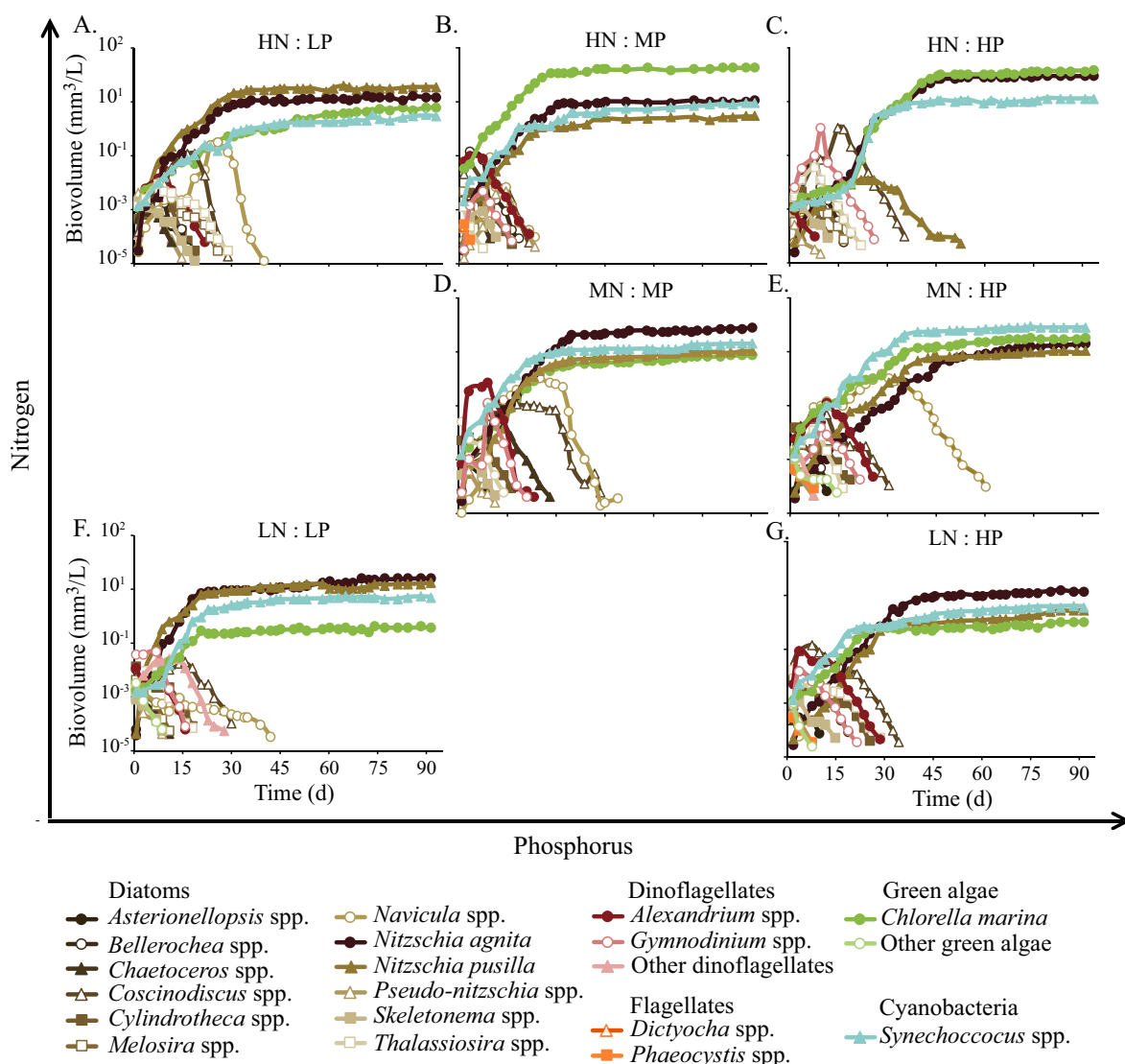


FIG. 4. Time series of phytoplankton species in the competition experiments. The seven experiments in panels A–G were performed at the targeted resource limitations indicated in Fig. 1. Time points of *Nitzschia agnita* in panels C and F and of *Chlorella marina* in panels D and E were slightly jittered to avoid overlap with the time series of other species in the same panel.

significantly with the total phytoplankton biovolume in the competition experiments (Table 2; Appendix S1: Fig. S2).

The diatom *N. agnita* dominated the LN:HP and MN:MP experiments, and was co-dominant in the LN:LP and HN:HP experiments (Fig. 5). The relative abundance of *N. agnita* did not show a significant relationship with the N:P ratio or total biovolume (Table 2).

The green alga *C. marina* dominated in the HN:MP and HN:HP experiments, and was very rare in the LN:LP experiment (Fig. 5). It was not significantly related to the N:P ratio, but increased significantly with the total biovolume in the competition experiments (Table 2; Appendix S1: Fig. S2).

The cyanobacterium *Synechococcus* spp. dominated in the MN:HP experiment, and comprised 5–17% in the other competition experiments (Fig. 5). Its relative abundance decreased significantly with the N:P ratio of the mineral medium, and was not related to the total biovolume (Table 2; Appendix S1: Fig. S2).

DISCUSSION

We can now evaluate the five predictions derived from the nutrient-load hypothesis using the results of the competition experiments.

Prediction 1: Low N loads lead to N limitation, low P loads to P limitation, combined low N and P loads to N+P co-limitation, combined intermediate N and P loads to co-limitation by nutrients and light, and combined high N and P loads to light limitation.—This prediction was offered predominantly to ensure that the experimental design in this study was indeed suitable to test the nutrient-load hypothesis. As is clearly displayed by the steady-state nutrient concentrations and light levels (Fig. 2), N was the only resource depleted in the LN:HP experiment and P was the only resource depleted in the HN:LP competition experiment. Hence, these experiments led to N and P limitation, respectively. These patterns are

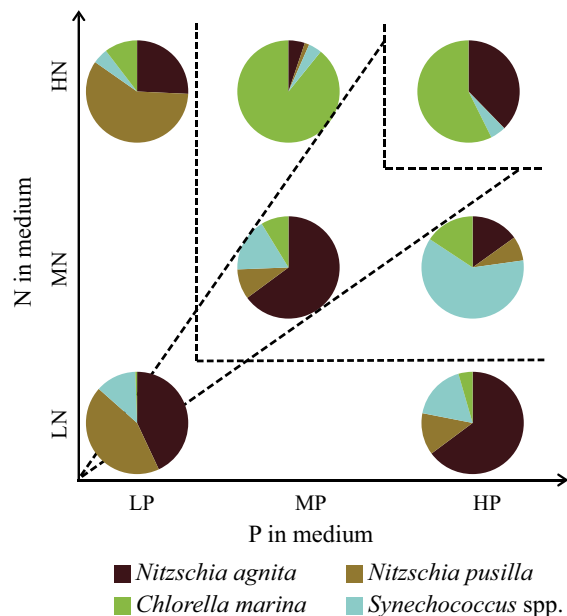


FIG. 5. Phytoplankton community composition at steady state in the competition experiments. The community composition is displayed as the relative contributions of the different taxa to the total biovolume. The axes represent the nitrogen and phosphorus concentrations in the mineral medium supplied to the competition experiments. Dashed lines delineate the targeted resource limitation pattern presented in Fig. 1B.

TABLE 2. Slopes of linear regression between the steady-state relative abundances of the coexisting species and the N:P ratio of the mineral medium or the total biovolume in the competition experiments.

Species	N:P _{medium}	Total biovolume
<i>N. pusilla</i>	n.s.	-0.0090*
<i>N. agnita</i>	n.s.	n.s.
<i>C. marina</i>	n.s.	+0.0067*
<i>Synechococcus</i> spp.	-0.3062*	n.s.

Notes: Regression is based on $\log(y) = a \log(\text{N:P}_{\text{medium}}) + b$ and $\log(y) = a \text{Biovolume} + b$, where y is the relative abundance of the species concerned and biovolume is expressed in mm^3/L . The regressions are shown in Appendix S1: Fig. S2. * $P < 0.05$, n.s. = not significant.

further confirmed by the cellular C:N:P ratios and biochemical composition of the cells in our experiments (reported in Grosse et al. 2017).

Our design aimed at N + P co-limitation in the LN:LP and MN:MP competition experiments. Indeed, both DIN and DIP concentrations were depleted, but the resulting DIN:DIP ratios were below the Redfield ratio, which may suggest that N was more limiting than P in these experiments. However, cellular C:N:P ratios and biochemical composition of the cells in the LN:LP and MN:MP experiments were not reflective of N limitation but were more balanced (Grosse et al. 2017), supporting the expectation of N + P co-limitation.

In the HN:MP and MN:HP competition experiments depletion of the expected inorganic nutrient was accompanied by a marked reduction in the measured irradiance I_{out}

in comparison to the HN:LP and LN:HP experiments, indicative of co-limitation by P and light in the HN:MP experiment and co-limitation by N and light in the MN:HP experiment.

The HN:HP experiment showed clear light limitation with extremely low steady-state I_{out} values, while DIN and DIP concentrations were not depleted.

Hence, Prediction 1 is supported by the experiments.

Prediction 2: N limitation, P limitation and light limitation favor the dominance of different phytoplankton species.—This prediction is confirmed by our experiments. Although total exclusion did not occur, the diatom *N. pusilla* dominated under P limitation in the HN:LP experiment, and therefore appears to have been the superior P competitor. The diatom *N. agnita* dominated under N limitation in the LN:HP experiment, which indicates that it was the superior N competitor. The green alga *C. marina* dominated under light-limited conditions in the HN:HP experiment, and thus was the superior light competitor. Hence, different species became dominant under different resource limitations, indicative of trade-offs between competitive abilities for these resources (Litchman et al. 2007, Edwards et al. 2011).

Prediction 3: Co-limitation by multiple resources enables species coexistence, whereas single resource limitation leads to competitive exclusion.—This prediction was not confirmed by our experiments. Stable coexistence of four species occurred in almost all chemostats. Even under single nutrient limitation in the LN:HP and HN:LP experiments, we found species coexistence rather than competitive exclusion. Light-limited conditions in the HN:HP experiment resulted in a slightly lower diversity, as the diatom *N. pusilla* was competitively excluded. Yet, three species remained in this experiment, whereas theory predicts that the best competitor for light should exclude all other species. Equilibrium coexistence of a larger number of species than limiting resources contradicts the principle of competitive exclusion (Hutchinson 1961, Levin 1970, Armstrong and McGehee 1980). However, similar results have been found in several previous competition experiments using natural phytoplankton communities as inoculum (Sommer 1983, 1985, Kilham 1986).

One potential explanation for our results might be that the species are similar in their competitive abilities, which can result in a high biodiversity through neutral coexistence (Bell 2001, Hubbell 2001). However, competitive exclusion of *N. pusilla* in the HN:HP experiment indicates that this species is an inferior competitor for light in comparison to the other three species. Thus, neutral coexistence is not a sufficient explanation for the coexistence of all four species in the competition experiments. Furthermore, if the remaining three species would be neutral competitors, environmental stochasticity in combination with random drift would most likely lead to erratic variation in their relative abundances. Although we did not perturb the experiments to investigate the stability of the species composition, the smoothness of the time series (Fig. 4) points at stable species coexistence rather than drifting species abundances associated with neutral coexistence.

Another possible explanation for species coexistence is that light does not represent a single resource, but an entire

spectrum of resources. In a series of theoretical, experimental and field studies, Stomp et al. (2004, 2007a, b) developed a competition model that takes the underwater light spectrum and light absorption spectra of competing phytoplankton species into account. The model was tested in well-mixed chemostat experiments with a green picocyanobacterium containing phycocyanin and a red picocyanobacterium containing phycoerythrin. Both the mathematical model and the competition experiments showed that the green picocyanobacterium won in red light, the red picocyanobacterium won in green light, and the two cyanobacteria coexisted at stable equilibrium in white light (Stomp et al. 2004). Additional experiments with a third species capable of adapting its pigment composition through complementary chromatic adaptation also showed stable coexistence with either the red or the green species by investing in the pigment not utilized by its competitor. Hence, these studies demonstrated that the light spectrum offers an important template for niche differentiation in phytoplankton communities, enabling stable coexistence of species utilizing different parts of the light spectrum.

In our HN:HP experiment, where light was limiting, we found stable coexistence of a green alga, diatom, and cyanobacterium. These three taxa differ in their pigment composition. All three taxa contain the ubiquitous pigment chlorophyll *a*, with which they absorb in the blue and red colors (440 nm and 680 nm) of the light spectrum (Fig. 6). In addition, green algae contain chlorophyll *b*, which has absorption peaks in the blue-green and red parts of the spectrum at 475 and 650 nm, respectively, adjacent to the chlorophyll *a* peaks. Diatoms lack chlorophyll *b* but instead contain chlorophyll *c* and the accessory pigment fucoxanthin, which causes a green shoulder in the absorption spectrum at 500–530 nm. The cyanobacterium in this study contained the accessory pigment phycocyanin, which has a distinct absorption peak in the orange region at 630 nm. Hence, green algae, diatoms, and cyanobacteria exploit different parts of the light spectrum in different ways (Fig. 6), which may have enabled their stable coexistence in our experiments.

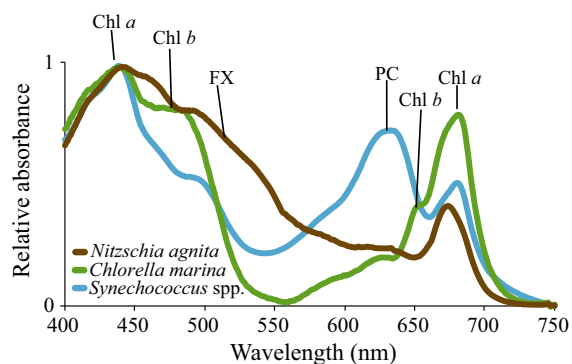


FIG. 6. Light absorption spectra of the diatom *Nitzschia agnita*, the green alga *Chlorella marina*, and the cyanobacterium *Synechococcus* spp. *N. pusilla* had the same absorption spectrum as *N. agnita*. Absorption peaks of several characteristic pigments are indicated. Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; FX, fucoxanthin; PC, phycocyanin. The spectra were obtained under nutrient replete conditions and are normalized to minimum absorbance at 750 nm and maximum absorbance at 440 nm.

Competitive displacement of one of the two diatom species (*N. pusilla*) from the HN:HP experiment is consistent with this explanation. The diatom species *N. pusilla* and *N. agnita* have a similar pigmentation, and hence use the same wavelengths. Given this lack of niche differentiation, one would expect that, under light-limited conditions, one of these two diatom species (i.e., the better light competitor) will competitively displace the other species (Stomp et al. 2004, 2007a). This is further supported by the HN:MP experiment, where light was co-limiting and *N. pusilla* was still rare in steady state. Under nutrient-limited conditions (in the HN:LP, LN:LP, LN:HP experiments), *N. pusilla* was present in higher abundances. In all, this suggests that *N. pusilla* is an inferior competitor for light, but can coexist with the other species under nutrient-limited conditions because it is a strong nutrient competitor.

A third explanation for multispecies coexistence might be that the superior competitors were suppressed by predators or parasites in a density-dependent way. Zooplankton were successfully excluded from the chemostats, as confirmed by microscopic inspection of experimental samples, by filtering the North Sea samples through an 80- μ m plankton net prior to inoculation in the chemostats and by removing oxygen in the chemostats through bubbling with N_2 and CO_2 . However, viruses and probably also many pathogenic bacteria cannot be excluded by this approach. According to the “killing the winner” hypothesis, viruses promote species coexistence by suppressing the most competitive host species, thus allowing the persistence of inferior resource competitors (Thingstad 2000, Maslov and Sneppen 2017). Although we cannot exclude the possibility that viruses or pathogenic bacteria played a role in the competition experiments, we would have expected dynamic fluctuations or sudden collapses of infected phytoplankton populations rather than a stable community composition. Hence, viruses or pathogens are an unlikely explanation for the stable coexistence observed in our experiments.

Prediction 4: Co-limitation can lead to stable coexistence, alternative stable states, species oscillations or chaos depending on the traits of the competing species.—In all experiments, the community composition converged toward a stable coexistence equilibrium (Fig. 4). Resource competition models can predict alternative stable states in which the winner(s) of competition depend on the initial species abundances (Tilman 1982, Brauer et al. 2012). We did not test for alternative stable states in this study as we inoculated each competition experiment with the same initial community composition. However, stable coexistence of the same three or four species in all seven competition experiments is an unlikely result if alternative stable states were present.

Models of multispecies competition for three or more limiting resources can also produce intransitive interactions, for instance if species A is a better competitor for N but becomes limited by P, species B is a better competitor for P but becomes limited by light, and species C is a better competitor for light but becomes limited by N (Huisman and Weissing 1999, 2001). Intransitive interactions can induce oscillations and chaos, and these non-equilibrium conditions may enable coexistence of a large number of species (Huisman and Weissing 1999). However, the species abundances in our chemostat

experiments did not display oscillations or chaos, but converged to stable equilibrium. If intransitive interactions lead to stable equilibrium, resource competition theory predicts that the number of species cannot exceed the number of limiting resources (Huisman and Weissing 2001). Therefore, intransitive interactions do not explain the stable coexistence of four species on three limiting resources observed in our experiments.

Prediction 5: Changes in nutrient loads will cause changes in species composition, even if the N:P ratio of the nutrient loads remains constant.—This prediction differentiates between the classic resource competition models with two limiting nutrients (Tilman 1982) and the nutrient-load hypothesis of Brauer et al. (2012) where light is added as a third resource. The prediction is clearly confirmed by our experiments, as can be seen by comparison of the results of the LN:LP, MN:MP, and HN:HP experiments. All three experiments used nutrient loads with the same N:P ratio of 16:1. As the nutrient loads increased, biomass accumulated, light levels were reduced, and clear changes in phytoplankton community structure occurred despite the consistent N:P ratio in the supplied mineral medium. Most striking is the co-dominance of the diatom *N. pusilla* in the LN:LP experiment and its competitive exclusion in the HN:HP experiment, while the green alga *C. marina* exhibits the opposite transition from very rare in the LN:LP experiment to dominant in the HN:HP experiment.

Comparison with natural phytoplankton communities

At the level of functional groups, the stable coexistence of diatoms, green algae, and cyanobacteria in the experiments is in good agreement with the composition of the natural phytoplankton community where diatoms, green algae, and cyanobacteria contributed 80% of the total phytoplankton biovolume (Fig. 3). Our experimental results indicate that changes in N and P loads may cause shifts in the relative abundances of these functional groups, which is consistent with field observations of the coastal North Sea where changing nutrient loads led to major shifts in phytoplankton community structure (Philippart et al. 2000, Burson et al. 2016). Dinoflagellates and other flagellates, which are also important components of the phytoplankton community in the North Sea, did not persist in the competition experiments. Possibly, they were sensitive to the vigorous mixing applied to the chemostats (Van de Waal et al. 2014), although several dinoflagellate species increased during the first one or two weeks of the experiments (e.g., *Alexandrium* and *Gymnodinium* spp.; Fig. 4).

Interestingly, small species became dominant in all experimental treatments, while larger species were competitively displaced. For instance, *N. pusilla* and *N. agnita* were the two smallest diatom species in our experimental community. These results support the common understanding that small cell size offers several competitive advantages under resource-limited conditions (Raven 1998, Litchman et al. 2010, Marañón 2015). Small cells are considered to be stronger competitors for nutrients because of their higher surface-to-volume ratio and thinner diffusion boundary layer (Grover 1989, Kiørboe 1993, Edwards et al. 2011). Furthermore,

small phytoplankton species can use light more efficiently than larger species (Fujiki and Taguchi 2002, Key et al. 2010, Schwaderer et al. 2011). For instance, small cells are less affected by the “package effect” caused by self-shading of photosynthetic pigments (Kirk 1994, Finkel 2001).

Although our experiments show that competition for nutrients and light enables stable coexistence of phytoplankton species, the species diversity in the experiments was lower than the diversity of the natural phytoplankton community. This indicates that other diversifying factors must also be at play in the coastal waters from which this community was retrieved. For instance, high diversity can be promoted by environmental fluctuations (Flöder and Sommer 1999, Litchman and Klausmeier 2001, Scheffer et al. 2003), reduced turbulent mixing (Bracco et al. 2000, Huisman et al. 2006, Yoshiyama et al. 2009), selective grazing (McCauley and Briand 1979, Sarnelle 2005, Weis and Post 2013), and viral and pathogen infections (Thingstad 2000, Brussaard 2004). Indeed, tides and weather variability are known to cause pronounced phytoplankton fluctuations at different time scales in the North Sea (Blauw et al. 2012, 2018). Ultimately, integration of resource competition theory with these other environmental factors will be required to fully explain the high biodiversity of natural ecosystems.

CONCLUSIONS

Four of the five predictions are supported by our experimental results. These results illustrate that a further integration of nutrient-based and light-based approaches contributes to improved understanding and prediction of how changes in nutrient loads will affect the species composition of natural communities. One prediction (Prediction 3) is not supported, we found a larger number of coexisting species than expected by classic resource competition theory. Further refinements of models will be required to explain the multispecies coexistence observed in our experiments, e.g., through incorporation of the underwater light spectrum (Stomp et al. 2004). Yet, the species diversity in the experiments did not rival the high biodiversity of natural plankton communities, indicating that the paradox of the plankton remains an interesting enigma.

In recent decades, many freshwater, marine, and terrestrial ecosystems have experienced major changes in nutrient loads due to eutrophication and subsequent de-eutrophication efforts (e.g., Jeppesen et al. 2005, Hautier et al. 2009, Grizzetti et al. 2012, Glibert 2017). These changes in nitrogen and phosphorus loads may cause large shifts in resource limitation patterns, with major changes in biodiversity, productivity, and community structure as a result (Conley et al. 2009, Burson et al. 2016, Harpole et al. 2016). The development of a comprehensive theoretical framework to understand and predict how changes in nutrient loads affect the species composition of natural communities may therefore offer a conceptual advance that is not only of academic interest but may also help to support sound stewardship of our natural environment.

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