

Competitive dynamics in two species of marine phytoplankton under non-equilibrium conditions

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ABSTRACT: Although mathematical models suggest that competition between primary producers in response to dynamical changes in the availability of a limiting nutrient is non-linear, experimental data supporting this basic hypothesis are sparse. Using continuous culture systems with nitrate as a single limiting nutrient, we present results of competition experiments between 2 species of marine phytoplankton, a diatom *Thalassiosira pseudonana* and a coccolithophore *Coccolithus braarudii*. These 2 organisms of similar size represent biogeochemically and ecologically distinct functional groups. Consistent with classical resource competition theory, under steady-state nitrate limitation (i.e. continuous flow chemostats), the diatom was outcompeted by the coccolithophore. However, when pulses of nitrate were provided to the chemostats (i.e. non-equilibrium, dynamical conditions) the diatom outcompeted the coccolithophore. The rate of exclusion was a linear function of the frequency of nitrate pulses. These results experimentally demonstrate that dynamical nutrient supply allows co-existence of 2 primary producers competing for a single limiting nutrient and may help us to understand phytoplankton succession in the ocean.

KEY WORDS: Ecological succession · Marine phytoplankton · Competition · Nutrient perturbation · Domain shift · Ocean turbulence · Climate change

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INTRODUCTION

Diatoms and coccolithophorids largely dominate the biologically-mediated, net exchange of carbon dioxide between the atmosphere and the ocean (Smetacek 1999, Falkowski 2002). Owing to their large range of cell sizes and mineral skeletons, these 2 phytoplankton functional groups (sensu Falkowski et al. 2003) are responsible for a large fraction of export flux and burial of biogenic carbon in the ocean interior; i.e. the so-called 'biological pump' (Volk & Hoffert 1985, Smetacek 1999, Iglesias-Rodríguez et al. 2002). However, their roles in the carbon cycle are strikingly different. Diatoms constitute a classic sink for carbon dioxide through the process of photosynthesis. However, by precipitating calcium carbonate,

coccolithophorids decrease the alkalinity of seawater, release carbon dioxide, and hence, decrease the efficiency of the biological pump (Holligan & Robertson 1996, Treguer & Pondaven 2000). This biogeochemical control may have contributed significantly to the regulation of Earth's climate, probably since the beginning of the Cenozoic (~65 million years ago) (Holligan & Robertson 1996, Falkowski 2004). Understanding the mechanisms that govern the assembly and temporal distribution patterns of these 2 phytoplankton functional groups has increasingly become the focus of marine microbial ecologists and biogeochemists (Archer et al. 2000, Boyd & Doney 2002; Hood et al. 2006).

Nutrients can control phytoplankton community composition through interspecific competition regulated

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either by (1) the compositional ratio or stoichiometry of nutrients (the so-called 'resource ratio hypothesis') or (2) the kinetics of nutrient input and uptake (the so-called 'dynamical nutrient supply hypothesis'). For instance, the correlation between the classical spring-summer succession of phytoplankton in the coastal ocean and the silica to nitrate supply ratio has been well documented, initially favoring diatoms when silica is replete and other non-siliceous phytoplankton taxa such as coccolithophores and small flagellates later when silica becomes depleted (Smayda 1989, Sieracki et al. 1993). The resource-ratio hypothesis predicts the replacement and/or coexistence of species as a function of the ratio of limiting nutrients and has been well demonstrated under laboratory controlled conditions and in field experiments (Tilman 1982, Tilman et al. 1982, Sommer 1983, Grover 1989, Egge & Aksnes 1992, Sommer 1993, Miller et al. 2005). In contrast, the dynamical nutrient supply hypothesis, which postulates that the timing of delivery of nutrients provides an ecological selection pressure, has received far less experimental support (Sommer 1985, Grover 1991a, Ducobu et al. 1998).

The role of nutrient supply dynamics was proposed originally by Hutchinson (1961, p. 137) to potentially explain the 'paradox of the plankton' in which he posed, 'The problem that is presented by the phytoplankton is essentially how it is possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sorts of materials.' Subsequently, Ramon Margalef in his (now famous) 'mandala' suggested that different combinations of nutrients and turbulence select for phytoplankton taxa according to species-specific functional traits and survival strategies (Margalef 1978, Smayda & Reynolds 2001). Based on the conceptual foundations of the 'mandala', resource-based competition models suggested that, owing to the rapid uptake of nutrients by diatoms, increased ocean turbulence (i.e. higher frequency of nutrient 'pulses') potentially would increase their ecological and evolutionary success at the expense of slow-growing coccolithophorids (Tozzi et al. 2004). Consistent with these theoretical predictions, we found that the onset or breakdown of stratification dramatically increases or decreases, respectively, the coccolithophorid to diatom biomass ratio in historical data sets from the ocean (Cermeño et al. 2008). Thus, both models and empirical evidence led to the hypothesis that enhanced ocean turbulence increases the rate of taxonomic shift from coccolithophore- to diatom-dominated assemblages (and vice versa) with important implications for understanding community dynamics and ocean biogeochemistry (Tozzi et al. 2004, Falkowski & Oliver 2007, Cermeño et al. 2008). Here, we explore the dynamical nutrient supply hypothesis

in a laboratory-simulated microcosm containing a diatom and a coccolithophore.

Competition for a single limiting nutrient: steady-state vs. non-steady-state nitrate inputs

Classical competition theory dictates that the success of an individual phytoplankton taxa reflects the intra-cellular nutrient quota and uptake rate where the organism with the lower requirement for the limiting nutrient is at a competitive advantage (Stewart & Levin 1973, Tilman 1977, Grover 1990). Several physiological traits characterize nutrient uptake kinetics and population growth, and their values directly affect the performance and competitive abilities of phytoplankton taxa.

The growth rate (μ) of a species is a saturating function of cell quota (Q , $\mu\text{mol nitrate cell}^{-1}$):

$$\mu = \mu_{\max} \left(\frac{1 - \frac{Q_{\min}}{Q}}{1 - \frac{Q_{\min}}{Q_{\max}}} \right) \quad (1)$$

As growth rate approaches zero, Q approaches a non-zero, minimum quota (Q_{\min}), and when growth asymptotically approaches a maximum, Q approaches its maximum potential (Q_{\max}). This 'Droop' model assumes that algal yield (i.e. the efficiency of algae to convert nutrients into biomass) depends on the physiological state of the population, which is primarily determined by cell quotas (Droop 1973, Grover 1991b).

The rate of change of population abundance (N) is affected by the growth rate of the population and its mortality rate (m):

$$\frac{dN}{dt} = \mu(Q)N - mN \quad (2)$$

Nutrient uptake rate (ρ , $\mu\text{mol nutrient cell}^{-1} \text{ d}^{-1}$) is an increasing function of external nutrient concentration (R , $\mu\text{mol l}^{-1}$) and can be parameterized by a hyperbolic function that is constrained by a maximum nutrient uptake rate (ρ_{\max}) and half saturation constant (K , $\mu\text{mol l}^{-1}$) for the limiting nutrient:

$$\rho = \left(\frac{\rho_{\max}R}{K+R} \right) \quad (3)$$

If the nutrient concentration in the bulk media is elevated to levels that are saturating for the uptake system, then the maximal growth rate becomes constrained by an upper bound of Q . Under these circumstances, the maximum growth rate depends on the efficiency of the photosynthetic machinery to convert nutrients into biomass with a fixed elemental stoichiometry (Falkowski & Raven 1997).

The rate of change of Q is dependent upon the nutrient uptake rate, the maximal growth rate and the difference between the instantaneous cell quota and the minimal cell quota. The larger this difference, the slower the rate of change of the cell quota:

$$\frac{dQ}{dt} = \rho - \mu_{\max}(Q - Q_{\min}) \quad (4)$$

The rate of change of R is a function of the dilution rate (D) and the total uptake by the organisms:

$$\frac{dR}{dt} = D(R^0 - R) - N\rho \quad (5)$$

where R^0 is the initial nutrient concentration entering the system.

According to this set of equations, the species-specific nutrient requirement at equilibrium (R^*) can be calculated for each species growing alone and in the absence of competitors as:

$$R^* = \frac{K \mu_{\max} Q_{\max} Q_{\min} m}{\mu_{\max} Q_{\max} (\rho_{\max} - Q_{\min} m) - \rho_{\max} m (Q_{\max} - Q_{\min})} \quad (6)$$

such that a species with the lowest R^* is predicted to win in a competition for a given single limiting nutrient (Tilman 1977, Grover 1991b). Following this logic, Tilman (1977) demonstrated that the outcome of competition for silicate among planktonic diatoms closely follows the R^* rule at steady state. However, the prediction given by R^* may change if the system does not reach equilibrium, as in the case of variable resource supply regimes (Grover 1991b, Miller et al. 2005).

High maximum nutrient uptake rates (i.e. 'luxury' uptake) and storage capabilities by diatoms are advantageous physiological traits under non-equilibrium conditions (Grover 1988, Legovic & Cruzado 1997, Litchman et al. 2007). Intracellular storage capacity introduces a time lag between the exhaustion of the external nutrient concentration and the actual nutrient limitation of growth, thereby extending the range of environmental conditions and allowing for maximal growth (Droop 1973, Grover 1991b). Furthermore, by confining acquired nutrients to a storage vacuole, cells potentially enhance the nutrient gradient across the plasmalemma (Raven 1997).

When a single limiting nutrient is temporally variable, the outcome of competition is controlled by temporal changes in the nature and strength of competitive interactions. In the steady state, continuous nutrient limitation is predicted to favor coccolithophores by virtue of their lower half-saturation constants for nutrient uptake and small intracellular quotas (Tozzi et al. 2004). Conversely, intermittent (non-equilibrium) nutrient supply potentially allows diatoms with higher maximum uptake rates to con-

sume and store the bulk of the nutrients rapidly, thereby permitting them to attain higher growth rates for several generations, while denying the coccolithophore access to the limiting resource (Tozzi et al. 2004, Litchman et al. 2007). The effect on external nutrient concentrations is even more dramatic, taking into account that fast growing cells possess high nutritional requirements (i.e. low algal yield) (Aksnes & Egge 1991), further destabilizing the competitive equilibrium towards the exclusion of slow-growing species.

To test this hypothesis, we designed a suite of experiments in which competition was based on a single limiting nutrient, nitrate, which is required by both the diatom and the coccolithophore. Here, we present the response of these 2 phytoplankton in laboratory-simulated dynamic ecosystems in which the limiting nutrient is provided as pulses at different frequencies. The experimental results clearly demonstrate that coexistence of these 2 species, each competing for the same limiting nutrient, can be maintained under dynamic conditions but not under continuous nutrient limitation.

MATERIALS AND METHODS

The 2 strains used, the diatom *Thalassiosira pseudonana* (CCMP-1335) and the coccolithophore *Coccolithus braarudii* (RCC-1201; Roscoff), have relatively similar cell volumes, averaging 180 and 520 μm^3 , respectively, thereby minimizing allometric effects on metabolic rates. Cultures were grown in monospecific continuous culture systems using 1 l temperature controlled culture vessels with nitrate limited-f/2 + Si medium using pre-filtered and autoclaved natural seawater. Nitrate limitation was obtained by diluting the nitrate stock 10-fold, thereby giving a final concentration in the medium of 88.2 $\mu\text{mol l}^{-1}$ and a N/P molar ratio of ~2.5 for the inflow media. The final nutrient concentrations of N, P and Si in the medium were 88.2, 36.2 and 106 $\mu\text{mol l}^{-1}$, respectively. High-precision metering pumps (Q pumps, Fluid Metering) delivered media to the culture vessel with a balancing overflow to maintain a constant volume in the chemostats. In the steady state, the growth rate of the cells is equal to the dilution rate. The dilution rate was set to 0.25 d^{-1} , which is in the range of average growth rates experienced by field populations of algae in oligotrophic, near steady-state ecosystems, and represents the mortality rate in the theoretical model above (i.e. the dilution rate of our continuous culture systems). The cultures were illuminated continuously (24 h) by a bank of 8 cool white plus fluorescent lamps (30W, Phillips,) emitting a photon flux of ~200 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. We used a continuous light regime instead of a light:dark

cycle, as the progressive accumulation of nutrients overnight would bias the competitive interactions in favor of the faster growing diatom (Stolte & Riegman 1995). Temperature in the cultures was maintained at $18 \pm 1^\circ\text{C}$ by circulating water through the vessel jackets using a temperature controlled water bath. The cultures were stirred and aerated through $0.2 \mu\text{m}$ filters throughout the experiments with magnetic stirrers and air pumps. The culture systems, from reservoirs to outflow flasks, were sterilized by autoclaving. Before starting nutrient uptake kinetics and competition experiments, independent monospecific cultures were grown for at least 1 wk in nitrate-limited continuous cultures.

Maximum nutrient uptake rates and half saturation constants were determined on monospecific cultures grown at steady-state. The nitrate concentration in the bulk media was measured at different time intervals in a series of aliquots to which different concentrations of nitrate were initially added ($0.25, 0.5, 1, 2.5, 5, 10, 25 \mu\text{mol l}^{-1}$). Nitrate concentrations were measured colorimetrically on seawater samples filtered through $0.45 \mu\text{m}$ pore size polycarbonate syringe filters, following the methods described in Grasshoff (1976). Cell-specific maximum nitrate uptake rates and half saturation constants were then calculated by fitting a hyperbolic function to the data. Data correspond to 2 different sets of independent measurements from which a single nitrate uptake kinetic curve was generated for each population.

Competition for nitrate between the diatom *Thalassiosira pseudonana* and the coccolithophore *Coccolithus braarudii* was simulated in mixed-population continuous culture systems. The culture conditions were similar to those specified above for monospecific cultures with the exception that the inflow media was supplemented with discrete pulses of nitrate ($7 \mu\text{mol l}^{-1}$) from a sterile stock solution ($883 \mu\text{mol l}^{-1}$) at predetermined intervals ($0.5, 1, 2$ and 5 pulses d^{-1}). The experimental matrix physically consisted of a nutrient reservoir connected to 3 digitally controlled solenoid valves where nutrients were directed to each of 3 culture vessels operating in parallel under identical light and temperature conditions. The nitrate pulses were added with a pipette through a septum in the top of the growth chamber. This procedure allowed us to rapidly increase the nitrate concentration in the bulk media without materially altering the dilution rate of the system; a total 8 ml of stock solution was added to the growth chamber in each pulse, which amounted to a 0.8% change in culture volume.

In the competition experiments, the initial phase was set up as a continuous culture system that was shifted to a pulsed regime in the second phase of the experiments. In all of the experiments, silica availability

exceeded the growth demands of the diatom, and trace metals were not limiting. The cells were counted and sized daily using a Multisizer III Coulter Counter (Beckman Coulter). Population densities and cell size on fresh samples were further verified by quantitative microscopy using an inverted microscope (Utermöhl's method). Key photosynthetic parameters (F_v/F_m : variable/maximum fluorescence; σ_{PSII} : absorption cross section of photosystem II; τ : turnover rate of photosystem II) indicative of the photosynthetic energy conversion efficiency of the entire assemblage were determined using a custom-built FiRE Fluorometer (Gorbunov et al. 1999).

In theory, the outcome of competition is independent of the initial population densities. However, these initial densities can control the dynamical transition from coccolithophores to diatoms because this transition is largely dependent on the kinetics of nutrient uptake of each individual population. In order to avoid this effect, our competition experiments were set up as invasion scenarios, such that the coccolithophore had roughly the same population density in all nutrient supply treatments and the coccolithophore to diatom (C/D) ratio was ~ 8 to 10 .

RESULTS

Steady-state vs. non-steady-state nutrient supply dynamics

Analyses of the uptake kinetics for nitrate in monospecific cultures of *Thalassiosira pseudonana* and *Coccolithus braarudii* grown at steady-state yielded maximum uptake rates and half saturation constants ($\pm \text{SE}$) of $2.18 \times 10^{-4} \pm 0.21 \times 10^{-4} \mu\text{mol cell}^{-1} \text{d}^{-1}$ and $3.75 \pm 1.1 \mu\text{mol l}^{-1}$, respectively, for the diatom and $1.38 \times 10^{-4} \pm 0.18 \times 10^{-4} \mu\text{mol cell}^{-1} \text{d}^{-1}$ and $1.06 \pm 0.5 \mu\text{mol l}^{-1}$ for the coccolithophorid (Fig. 1A). A *t*-test comparing non-linear regressions showed that the differences in the best-fit values of maximum nitrate uptake rates were statistically significant (*t*-test, $t_{44} = 2.89$, $p < 0.01$). Differences in half-saturation constants were also significant (*t*-test, $t_{44} = 2.29$, $p < 0.05$). Using these nutrient uptake kinetic parameters as reference, we conducted a simulation to examine the variability in R^* , the competition parameter, for each of our model species by assigning different potential values to their maximum growth rate and minimum and maximum cell quotas (Fig. 1B). We assumed increased nutrient requirements at higher maximum growth rate (Aksnes & Egge 1991), which is a reasonable assumption as fast growing species typically possess higher minimum quotas and lower algal yields. The mortality rate was 0.25 d^{-1} (i.e. the dilution rate). The analysis suggested that the

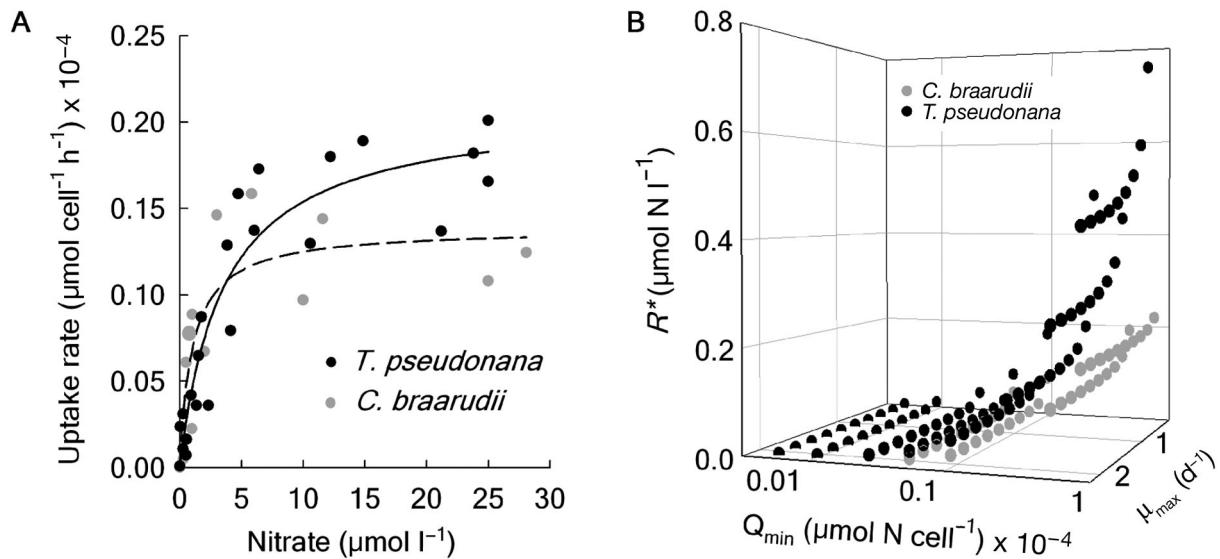


Fig. 1. *Thalassiosira pseudonana* and *Coccolithus braarudii*. (A) Nutrient uptake kinetics for the diatom *T. pseudonana* and the coccolithophore *C. braarudii*. The data are combined from 2 independent experiments on cells that had been acclimated in nitrate limited chemostats for a week. (B) Sensitivity of the competition parameter (R^*) to simulated values of maximum growth rate (μ_{\max}) and cell quotas (Q_{\min}). In all cases, the maximum cell quotas were assumed to be one-order of magnitude higher than the minimum cell quotas

coccolithophore, which had a lower R^* for the whole range of assigned values, would outcompete the diatom under continuous nitrate limitation. For example, assuming a minimum quota of $0.5 \times 10^{-4} \mu\text{mol cell}^{-1}$ and a maximum growth rate of 1.5 d^{-1} , the model predicted an R^* of 0.3 for the diatom and of 0.1 for the coccolithophore.

We initially introduced the 2 species at equal population densities in 2 independent cultures operating in parallel under similar temperature, light and nutrient supply regimes and began diluting with the inflow media. In both experiments, the nitrate in the outflow media became undetectable at ~Day 14, and the data shown in Fig. 2 begin from this period, when the 2 species were in approximately equal abundance and the system had been operating in a steady-state mode (i.e. continuous nitrate limitation). According to Eq. (6) each population will reduce nitrate concentration in the bulk media to levels predicted by their R^* , and thus the species with the lowest requirements for nitrate should competitively displace the other. Consistent with this, continuous nitrate limitation in our experimental microcosms (i.e. the classical chemostat-like system) led to a rapid decrease in the abundance of *Thalassiosira pseudonana* and a simultaneous increase of *Coccolithus braarudii* (Fig. 2). The pattern was characterized by a progressive increase of the C/D ratio at a rate of $0.05 \log \text{ units d}^{-1}$. F_v/F_m ranged from ~0.35 to 0.55 (Fig. 3). Despite an elevated silicate to nitrate ratio of the inflow media (>1.2), diatoms were excluded by the coccolithophore population under continuous

nitrate limitation, which indicated that the availability of silicate per se is a necessary but insufficient condition for the increased population density of the diatom.

To avoid competitive exclusion of *Thalassiosira pseudonana*, steady-state nitrate supply conditions were maintained until the abundance of coccolithophorids exceeded ~10-fold the density of the diatom (~Day 22). Thereafter, population dynamics were altered by providing pulses of nitrate ($7 \mu\text{mol l}^{-1}$ twice per day). Assuming minimum intracellular nitrate quotas within the range of values used in our simulation analysis and equal resource partitioning (though unrealistic, this assumption is used for heuristic purposes), each nitrate pulse would have doubled or tripled cell quota. Indeed, deviation from steady-state nitrate supply rapidly switched the outcome of competitive interactions, allowing diatoms to dominate the assemblage (Fig. 2). F_v/F_m increased to values above 0.5 (Fig. 3). Although initially nitrate pulses increased the density of both the diatom and coccolithophore populations, the C/D ratio showed a sharp and synchronous decrease, which highlights the competitive ability of the diatom under pulsed nutrient conditions (i.e. the population abundance of *T. pseudonana* increased much faster than that of *Coccolithus braarudii*) (Fig. 2). While nitrate was undetectable in the outflow media, phosphate and silicate were always in excess (Fig. 2). By ~Day 50, the C/D ratio fell to values as low as 0.2, but the assemblage was still photosynthetically active, as indicated by F_v/F_m values of ~0.4 (Fig. 3). These results were consistent in 2 independent cultures.

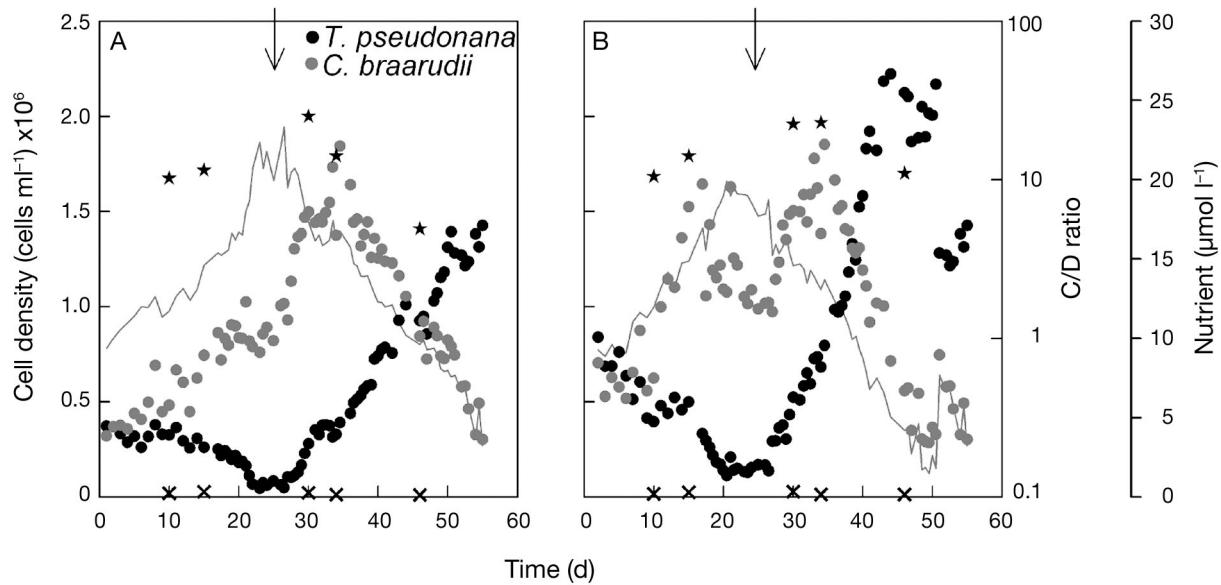


Fig. 2. *Thalassiosira pseudonana* and *Coccolithus braarudii*. Population dynamics of *T. pseudonanana* (●) and *C. braarudii* (●) competing under nitrate-limiting and nitrate-pulsing conditions. The grey line represents the coccolithophores:diatoms (C/D) ratio on a logarithmic scale. (A) and (B) correspond to 2 experiments conducted independently of each other in parallel under similar nutrient, light and temperature regimes (true replicates). Nitrate concentrations on the outflow media (x) were below detection throughout the experimental time shown, whereas silicate concentrations were by far in excess (★). The arrows mark the beginning of the nutrient pulsing (2 pulses d⁻¹) on Day 22

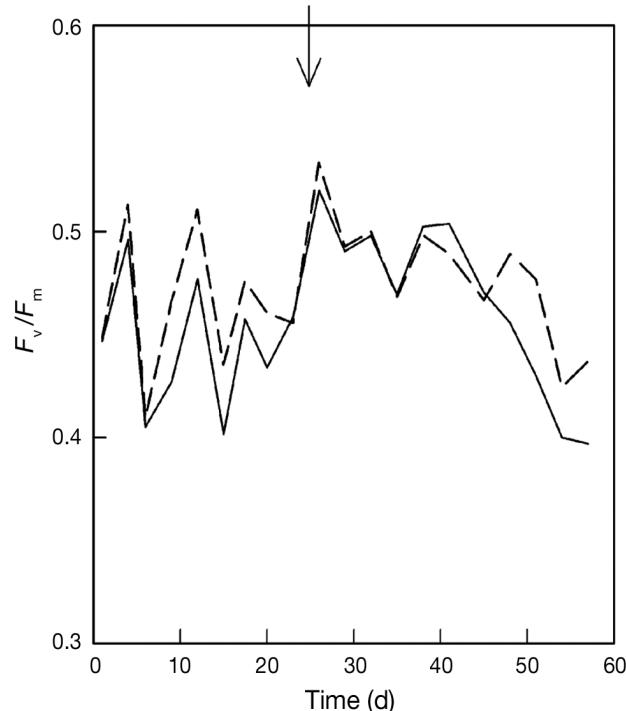


Fig. 3. Temporal changes in variable/maximum fluorescence (F_v/F_m) of mixed culture assemblages. The temporal evolution of F_v/F_m was averaged every 3 d for the experiments plotted in Fig. 2A (solid line) and Fig. 2B (dashed line). The arrow marks the beginning of the nutrient pulsing (2 pulses d⁻¹) on Day 22

Quantifying the rate of competitive exclusion

In most aquatic ecosystems, nutrient supplies are not constant over long periods of time and under certain circumstances (e.g. storms or internal waves) nutrient-limited phytoplankton can be transiently supplied with pulses of relatively high nutrient concentrations. In our experiment, we successfully manipulated selection of a coccolithophore and a diatom by simply adjusting the dynamics of nutrient supply (Fig. 2). However, the extent to which the dynamics and magnitude of nitrate inputs control the rate of competitive exclusion remained unknown. To explore this question, we conducted a suite of competition experiments in which the inflow media was supplemented with pulses of nitrate at 3 pre-determined time intervals: 0.5, 1 and 5 pulses d⁻¹. These perturbation frequencies were chosen to simulate typical oceanographic events, such as upwelling pulses, internal or tidal waves and vigorous mixing associated with transient storms, phenomena which appear to alter population dynamics and community composition in nature (Walsh et al. 1978).

Competition experiments were set up as invasion scenarios (Tilman & Sterner 1984). Initial population densities of the coccolithophore, the resident species, were ~8-fold higher than those of the diatom, the invader species (Fig. 4). Nitrate pulses increased the abundance of diatoms at the expense of the cocco-

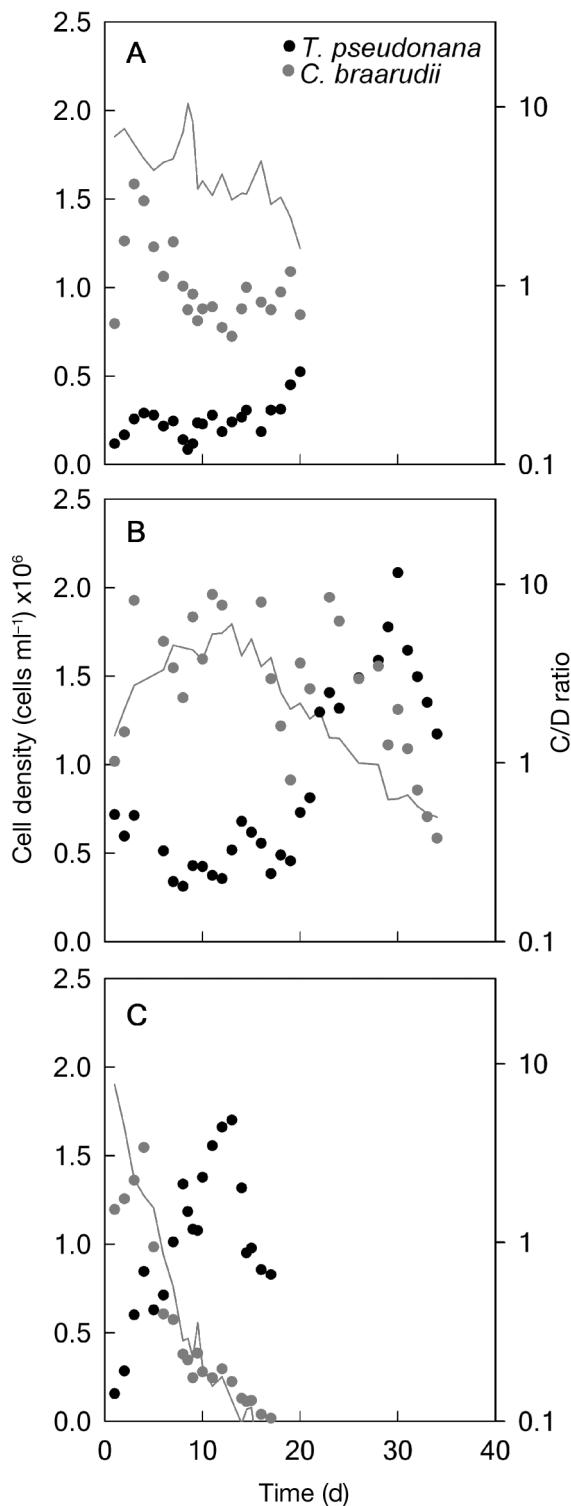


Fig. 4. *Thalassiosira pseudonana* and *Coccolithus braarudii*. Population dynamics of *T. pseudonana* (●) and *C. braarudii* (◎) and the coccolithophore:diatom (C/D) ratio (grey line) at frequencies of (A) 0.5, (B) 1 and (C) 5 nitrate pulses d⁻¹. These experiments were conducted in chemostat systems by adding pulses of nitrate at predetermined time intervals. C/D ratio is shown on a logarithmic scale

lithophore (i.e. the C/D ratio progressively declined over time), yet their population dynamics were markedly different among treatments (Fig. 4). Whereas the C/D ratio slightly decreased at 0.5 pulses of nitrate d⁻¹, the rate of coccolithophore exclusion increased rapidly with the frequency of pulses and nitrate load (Fig. 5A). From the time series of population densities (Figs. 2 & 4), we calculated the rate of competitive exclusion. The response variable, C/D ratio (t) = $N1(t)/N2(t)$, where $N1$ and $N2$ are the densities of *Coccolithus braarudii* and *Thalassiosira pseudonana*, respectively, on day t , was regressed on a logarithmic scale against t . Then, the rate of competitive exclusion was calculated as the slope of the regression model between the log C/D ratio and time (Fig. 5A). Regardless of the perturbation frequency, *T. pseudonana* competitively displaced *C. braarudii*. The temporal decay of the log C/D ratio conformed consistently to a simple linear regression model (Fig. 5A). Plotting all treatments together, the analysis revealed that the rate of exclusion of *C. braarudii* increased linearly with the availability of nitrate (Fig. 5B). Higher frequencies of nitrate input accelerated exclusion, but only under an extreme condition of 5 pulses d⁻¹ was *C. braarudii* completely excluded by Day 18. In the other treatments, the C/D ratio showed a progressive decay, while the slower rate of competitive displacement precluded reaching complete exclusion of the coccolithophore over the time course of the experiments (~Day 30). Interestingly, including rates of exclusion of *T. pseudonana* under continuous nitrate limitation (i.e. derived from grey lines on Fig. 5A), the dataset conformed to a non-linear model that predicts coexistence at some range between >0 and 0.5 pulses d⁻¹.

DISCUSSION

Our experimental results strongly suggest that the temporal supply of a single limiting nutrient can control the relative population densities of 2 species of phytoplankton. Built upon steady-state relationships, theoretical models and experimental analyses of resource competition have demonstrated that (1) among species competing for a single limiting resource only one species can persist, (2) species limited by different resources can coexist at equilibrium, and (3) the relative abundance of coexisting species is regulated by the ratio of limiting resources (Tilman 1982). For decades, these competition models have provided a fundamental basis to link algal physiology and community ecology, yet, are based upon the unrealistic assumption of environmental homogeneity.

Non-equilibrium competition theories have significantly improved our understanding of community

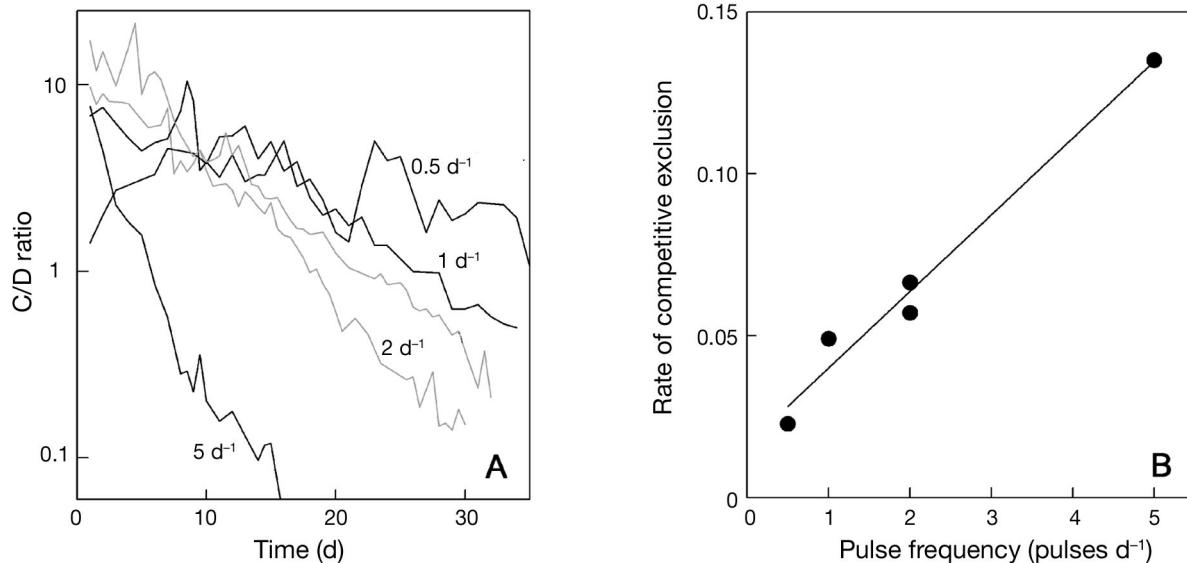


Fig. 5. (A) Changes in the coccolithophore:diatom (C/D) ratio over time at different frequencies of nitrate pulses. The temporal evolution of the C/D ratio from experiments shown in Fig. 2 (gray lines) and Fig. 4 (black lines) is shown on a logarithmic scale. The slope of the linear relationship between log C/D ratio and time represents the rate of competitive exclusion. For the 1 pulse d⁻¹ treatment, the slope was calculated from the time at which the C/D ratio began to decrease. (B) Relationship between the rate of competitive exclusion and the rate of nitrate supply (frequency of nitrate pulses). The rate of competitive exclusion, calculated from the linear regression model of log C/D ratio over time, is negative and was multiplied by -1 for this illustration. The data fit to a linear regression model: $y = 0.017 + 0.024x$, $R^2 = 0.97$, $p = 0.002$

dynamics (and biodiversity) by incorporating parameterizations that reproduce the variable efficiency of resource consumers in response to environmental stochasticity (Richerson et al. 1970, Grover 1991b). For instance, the observation that microbial plankton communities harbor more species than limiting resources, the so-called 'Paradox of the Plankton' (Hutchinson 1961), has been explained by the non-linearity of resource consumers in an ever changing scenario. The extent to which marine phytoplankton at cell densities typical of natural systems physically interact with each other is controversial (Siegel 1998). However, temporal variations in resource supply can alter the growth rate, resulting in niche segregation over time and the ensuing potential of low-density species to temporarily increase their population size. These non-equilibrium models provide a theoretical solution for the co-existence of species competing for the same limiting resource (Grover 1991b, Sommer 1993) and therefore offer a more realistic framework to approach the study of biotic interactions and community dynamics.

To the extent that microcosm experiments can inform us about natural aquatic ecosystems, we suggest that competition between marine diatoms and coccolithophores is particularly relevant to ocean biology and biogeochemistry (Smetacek 1999, Falkowski 2004). We were unable to experimentally simulate conditions that promote long-term coexistence of both populations. Either at equilibrium or under nutrient pulsing

conditions, our laboratory-simulated ecosystem deviated towards the dominance of the coccolithophore or the diatom, respectively. However, we found an increasing linear relationship between the frequency of nitrate pulses and the rate of competitive exclusion, implying a longer coexistence at low perturbation frequencies. Consistent with our analysis of data, earlier competition experiments on green algae and photosynthetic prokaryotes have shown an increased rate of competitive exclusion with decreasing pulse period (Grover 1991a, Ducobu et al. 1998). These results indicate that nutrient supply dynamics can alter the strength of competitive interactions and hence control the temporal progression of the ecological succession and domain shifts of phytoplankton populations in the ocean.

The classical succession from diatom dominance to the abundance of non-siliceous taxa (or vice versa) traditionally has been explained as a result of changes in the ratio between the supply of silicate, an absolute requirement for diatoms, and other essential nutrients such as nitrogen, phosphorous or iron. While that chemical supply scenario may also lead to phytoplankton succession, our experimental results strongly suggest that it can also be a predictable manifestation of physically-forced variable nutrient supply regimes. An alternative possibility is that allelopathic effects, the production of substances that inhibit growth of neighboring species, could have influenced population

dynamics in our experimental microcosms in a manner similar to that predicted by nutrient competition theory. However, though allelopathic interactions may contribute to regulating population dynamics, such an endogenous control is unlikely to influence the dynamical succession and domain shifts of phytoplankton species in the ocean.

The hypothesized role of nutrient supply dynamics in delaying, diverting or resetting phytoplankton succession, phenologies and domain shifts can also be viewed within the framework of the classical Connell's intermediate disturbance hypothesis (Connell 1978, Sommer 1995). Low disturbance frequencies promote competitive exclusion, while high disturbance selects for a few species tolerant to stress. Ocean stratification and the ensuing reduction of nutrient supply during, for instance, upwelling relaxation or the spring-summer transition in temperate seas would favor the dominance of taxa that take up nutrients slowly, such as coccolithophores or autotrophic prokaryotes. Conversely, high disturbance frequencies characteristic of coastal upwelling systems or open ocean regions with high submesoscale activity would select for a few 'opportunistic' groups such as diatoms that are adapted to such stressful conditions. These 2 extreme scenarios serve to illustrate the role of nutrient input dynamics in controlling the temporal progression of phytoplankton succession through an array of intermediate conditions. Although we emphasize here the importance of bottom-up controls, a number of mechanisms including predation, allelopathic interactions, dispersal, light intensity or temperature may further influence the dynamics of phytoplankton communities in nature (Reynolds 2006, Strom 2008).

The proposition that variable nutrient supplies can dominate the dynamics of phytoplankton communities implies the need for experimental approaches and modeling analyses at high spatial resolutions. Much evidence suggests the importance of vertical flux of mass associated with ocean fluid dynamics at scales of dozens of meters to hundreds of kilometers (sub- and mesoscale features) (Mahadevan et al. 2010, d'Ovidio et al. 2010). These oceanic features can play an important role in controlling community dynamics and shaping global patterns of phytoplankton distributions. Ocean biology models traditionally have given a larger importance to the effect of nutrient ratios, which can be easily inferred from property plots and isopycnal mixing lines. Instead, assessing the importance of variable nutrient supplies in ocean biology and biogeochemistry requires an understanding of mesoscale and submesoscale physical processes, which presents a new challenge for ocean biology modelers because these processes are not fully resolved in general circulation models at present.

The resource-ratio (chemical forcing) and the variable nutrient supply (physical forcing) theories offer alternative, though not mutually exclusive mechanisms, to explain phytoplankton succession and domain shifts in the ocean. Whereas the resource ratio theory is compatible with deviations of the nutrient supply ratios from the classical Redfield elemental stoichiometry, the variable nutrient supply theory conceptualized by Margalef is compatible with a temporal version of the Connell's intermediate disturbance hypothesis in which community dynamics rapidly respond to variations in the frequency of perturbations. Contemporaneous trends in climate warming may potentially alter upper ocean physics and nutrient supply regimes over different temporal and spatial scales giving rise to variations in the patterns of phytoplankton succession, phenologies and domain shifts.

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