

# A model of flexible uptake of two essential resources

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## Abstract

Microbes require multiple essential elements that they acquire from the environment independently. Here we investigate how microbial stoichiometry and uptake rates depend on the conditions in which they grow. We modify a recent model of growth based on a multinutrient extension of the Droop model to allow a trade-off between ability to acquire two essential resources. In a static analysis, we show that the optimal allocation strategy is the one that results in colimitation by both nutrients. We then add a dynamic equation to model the physiological acclimation uptake rates in changing conditions. This dynamic model predicts that the response of organismal stoichiometry to nutrient supply ratio can vary over time. The response of organismal stoichiometry and growth rate to a nutrient pulse depends on the speed at which cells adapt their uptake rates. In a variable environment, very fast or very slow acclimation may be better strategies than intermediate speed acclimation. We suggest experimental tests of the model and avenues for future model development. © 2007 Elsevier Ltd. All rights reserved.

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## 1. Introduction

Organisms require multiple elements to live and reproduce. While phagotrophs acquire these elements in packages that contain multiple elements, osmotrophs such as phytoplankton obtain each element separately from the environment. How organisms satisfy these requirements and maximize their growth and fitness has been the subject of extensive research, in the ecological (Tilman, 1982), microbiological (Narang and Pilyugin, 2005), and mathematical biology (Schreiber and Tobiason, 2003) literatures. Tilman (1982) used graphical optimal foraging arguments to argue that organisms should consume essential resources in the exact ratio they require, leading to colimitation. Abrams (1987) supported this conclusion analytically. Schreiber and Tobiason (2003) used the techniques of adaptive dynamics (Geritz et al., 1997) to find that there is a single continuously stable strategy (CSS; an evolutiona-

rily stable strategy (ESS) that is also an evolutionary attractor) of allocation to uptake with a linear trade-off and hemiessential resources.

These preceding papers all assume that growth depends on the external concentration of the nutrients. Models where growth depends on internal nutrient concentrations are more mechanistically detailed and can account for the observation that the nutrient content of phytoplankton and bacteria can be quite variable, depending on growth rate and nutrient supply ratios (Droop, 1974; Sterner and Elser, 2002). Recently, we analyzed a simple model of microbes growing in a chemostat with multiple limiting resources (Klausmeier et al., 2004a). While stated in terms of phytoplankton, this model is equally applicable to osmotrophic bacteria (cf. Grover, 2003). The model was able to capture the effect of varying nutrient supply ratios on elemental stoichiometry across a broad range of nitrogen-to-phosphorus (N:P) supply ratios, following Rhee's classic experiments with *Scenedesmus*, where phytoplankton N:P matched supply N:P over a range from 5 to 80 by atoms. However, a recent survey of the dependence of phytoplankton N:P stoichiometry on N:P supply ratios suggests that Rhee's (1978) *Scenedesmus* cultures were unusually

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flexible in their N:P stoichiometry; typically species show markedly less flexibility (Hall et al., 2005). Moreover, our model overestimated the ability of *Scenedesmus* to match extreme N:P ratios of 1, 2.2, and 1890 (Klausmeier et al., 2004a). When amended to account for the regulation of nutrient uptake, it was better able to match laboratory data.

In that paper we modeled the regulation of nutrient uptake by letting the maximum uptake rate of a nutrient decrease with the cellular content of the same nutrient (Klausmeier et al., 2004a). This formulation is traditional (Morel, 1987; Grover, 1991; Andersen, 1997) and often fits experimental data well (Grover, 1991; Ducobu et al., 1998). However, it has some potential conceptual problems. First, it is phenomenological since the mechanism of nutrient uptake regulation is omitted. Second, cells adjust their uptake rates based only on the intracellular content of the same nutrient. This response is maladaptive when only a single resource is limiting or when multiple nutrients are superabundant: since growth rate increases with uptake rate, decreasing uptake rate decreases growth rate. Third, the response is assumed to be instantaneous, which may not be a valid approximation.

In this paper we propose an alternative model of how organisms with variable nutrient content adjust their nutrient uptake rates in response to their internal nutrient status. The model is mechanistic in that it maps the allocation to nutrient transport proteins to the kinetic parameters for nutrient uptake. In Section 2 we consider the static problem of determining the optimal allocation patterns during exponential growth (Section 2.1) and at equilibrium (Section 2.2). In Section 3 we allow the allocation to different uptake machinery to vary dynamically. Using this dynamic model, we look at how stoichiometry and uptake machinery allocation change over the course of exponential growth to equilibrium phase, how organisms respond to a pulse of a limiting resource, and how competition in a variable environment may select for either fast or slow acclimation rates. In the discussion (Section 4), we suggest a set of experiments to test and parameterize this new model. Previous models of uptake acclimation include Van den Berg et al. (2002), who used techniques of optimal control theory to investigate the optimal uptake strategy during exponential growth, and Koch (2005), who used a simulation model to investigate different regulatory mechanisms in bacteria.

## 2. Static model

In Klausmeier et al. (2004a), we investigated the following model for one species of phytoplankton with variable internal stores of two essential resources (see also Legović and Cruzado, 1997, where other aspects of this model were investigated, De Leenheer et al. (2006), where global stability was proven, and Li and Smith (in review),

where multiple species competition was studied).

$$\begin{aligned}\frac{dR_P}{dt} &= a(R_{in,P} - R_P) - f_P(R_P)B, \\ \frac{dR_N}{dt} &= a(R_{in,N} - R_N) - f_N(R_N)B, \\ \frac{dQ_P}{dt} &= f_P(R_P) - \mu_\infty \min\left(1 - \frac{Q_{min,P}}{Q_P}, 1 - \frac{Q_{min,N}}{Q_N}\right)Q_P, \\ \frac{dQ_N}{dt} &= f_N(R_N) - \mu_\infty \min\left(1 - \frac{Q_{min,P}}{Q_P}, 1 - \frac{Q_{min,N}}{Q_N}\right)Q_N, \\ \frac{dB}{dt} &= \mu_\infty \min\left(1 - \frac{Q_{min,P}}{Q_P}, 1 - \frac{Q_{min,N}}{Q_N}\right)B - mB, \quad (1)\end{aligned}$$

where  $f_i(R_i) = v_{max,i}R_i/(R_i + K_i)$ . In this model,  $R_i$  represents nutrient  $i$  available for uptake,  $Q_i$  represents the quota of nutrient  $i$  (nutrient content per cell), and  $B$  represents biomass. We label the two resources N and P, inspired by Rhee's work with nitrogen and phosphorus, but our results hold for any pair of essential resources. Nutrient supply is defined by the dilution rate  $a$ , and input concentrations  $R_{in,i}$ . Nutrient uptake follows Michaelis–Menten kinetics  $f_i(R_i)$ , with maximum uptake rates  $v_{max,i}$  and half-saturation constants  $K_i$ . Growth rate is determined by the minimum of two Droop functions (Liebig's Law of the Minimum), with growth rate  $\mu_\infty$  at infinite quotas and minimum quotas  $Q_{min,i}$ . Phytoplankton die at per capita rate  $m$ , which includes death by dilution so that  $m \geq a$ . In the case of a perfect chemostat, death due to sources other than dilution is ignored ( $m = a$ ).

In this model, at low dilution/death rates, equilibrium phytoplankton stoichiometry closely matches the nutrient supply ratio over large ranges of  $R_{in,N}/R_{in,P}$ , in agreement with Rhee's (1978) chemostat experiments. That is, under limitation by P, cells store large quantities of N, and vice versa, causing phytoplankton stoichiometry to deviate strongly from the "optimal ratio" of  $Q_{min,N}/Q_{min,P}$  that represents co-limitation in this model. However, there are limits to this flexibility that this model may not capture (Hall et al., 2005; Smith and Yamanaka, in press).

This model (Klausmeier et al., 2004a) treats the maximum uptake rates as constant, but in reality it is known that phytoplankton are physiologically plastic and that maximum uptake rates can vary widely depending on growth conditions (Rhee, 1980). Traditionally this has been modeled by assuming that the maximum uptake rate of a nutrient declines with the internal stores of the same nutrient (DiToro, 1980; Gotham and Rhee, 1981a, b; Morel, 1987; Grover, 1991; Andersen, 1997). While this approach often fits experimental data, it is not mechanistic and does not consider interactions between nutrients. Smith and Yamanaka (in press) propose a new model that does consider the interaction between two nutrients that also fits experimental data well, but does not result in optimal growth.

Here we propose an alternative, more mechanistic approach to the regulation of nutrient uptake based on optimization. We assume that there is a trade-off between

the uptake of the two resources. This could result from either a limited amount of material to allocate to uptake proteins (as in Klausmeier et al., 2004b) or from a limited amount of cell surface area for uptake proteins (Aksnes and Egge, 1991). Both cases lead to a linear trade-off between maximum uptake rates:

$$A_P + A_N = 1, \quad (2)$$

where  $A_i$  is the allocation to uptake of nutrient  $i$ , scaled to add to one. Uptake rates are

$$v_{max,i} = v'_{max,i} A_i. \quad (3)$$

Since the traits for uptake ability of the two nutrients are linked by the trade-off (2), we can consider the strategy to consist of just one trait, which we take as  $A_P$ . We now solve for the optimal allocation strategy under two contrasting ecological scenarios: nutrient-replete exponential growth and competitive equilibrium.

### 2.1. Optimal uptake during exponential growth

During exponential growth into an empty environment ( $B \approx 0$ ,  $R_N = R_{in,N}$ ,  $R_P = R_{in,P}$ ), the optimal allocation strategy is that which maximizes the exponential growth rate. To find the exponential growth rate, we let the quotas reach a quasi-steady state given by setting  $dQ_N/dt = 0$  and  $dQ_P/dt = 0$ . Substituting these quasi-equilibrium quotas into the equation for  $dB/dt$  yields

$$\frac{dB}{dt} = (\mu - m)B, \quad (4)$$

where

$$\mu = \min \left( \frac{\mu_\infty f_P(R_{in,P})}{\mu_\infty Q_{min,P} + f_P(R_{in,P})}, \frac{\mu_\infty f_N(R_{in,N})}{\mu_\infty Q_{min,N} + f_N(R_{in,N})} \right). \quad (5)$$

Under nutrient-replete conditions ( $R_i \gg K_i$ ),  $\mu$  simplifies to give the maximum gross growth rate

$$\mu = \mu_{max} = \min \left( \frac{\mu_\infty v_{max,P}}{\mu_\infty Q_{min,P} + v_{max,P}}, \frac{\mu_\infty v_{max,N}}{\mu_\infty Q_{min,N} + v_{max,N}} \right) \quad (6)$$

(Klausmeier et al., 2004a).

The first term in the minimum function of Eq. (5), the growth rate under P-limitation, is a strictly increasing function of allocation to P-uptake,  $A_P$ , whereas the second term, the growth rate under N-limitation, is a strictly decreasing function of  $A_P$ . Therefore, the optimal allocation strategy is unique and is the one that leads to equality of the terms in the minimum function (i.e. colimitation). In the case of nutrient-replete conditions ( $R_i \gg K_i$ ), the optimal allocation strategy is

$$A_P = \frac{v'_{max,N} Q_{min,P}}{v'_{max,N} Q_{min,P} + v'_{max,P} Q_{min,N}}. \quad (7)$$

Adoption of this optimal strategy leads the phytoplankton stoichiometry and the ratio of uptake rates to match the

ratio of minimum quotas

$$\frac{Q_N}{Q_P} = \frac{v_{max,N}}{v_{max,P}} = \frac{Q_{min,N}}{Q_{min,P}}. \quad (8)$$

This justifies the use of this “optimal uptake assumption” in Klausmeier et al. (2004a) and agrees with the idea that phytoplankton stoichiometry at maximum growth is optimal (Goldman et al., 1979).

### 2.2. Optimal uptake at competitive equilibrium

To find the optimal allocation strategy at competitive equilibrium, we must first augment the single-strategy model (1) to include competition between populations with different strategies. We assume that populations differ only in their allocation strategy  $A_P$  and interact solely through resource competition. Expanding the single-strategy model (1) to include competition between populations results in

$$\begin{aligned} \frac{dR_P}{dt} &= a(R_{in,P} - R_P) - f_{P,1}(R_P)B_1 - f_{P,2}(R_P)B_2, \\ \frac{dR_N}{dt} &= a(R_{in,N} - R_N) - f_{N,1}(R_N)B_1 - f_{N,2}(R_N)B_2, \\ \frac{dQ_{P,j}}{dt} &= f_{P,j}(R_P) - \mu_\infty \min \left( 1 - \frac{Q_{min,P}}{Q_{P,j}}, 1 - \frac{Q_{min,N}}{Q_{N,j}} \right) Q_{P,j}, \\ \frac{dQ_{N,j}}{dt} &= f_{N,j}(R_N) - \mu_\infty \min \left( 1 - \frac{Q_{min,P}}{Q_{P,j}}, 1 - \frac{Q_{min,N}}{Q_{N,j}} \right) Q_{N,j}, \\ \frac{dB_j}{dt} &= \mu_\infty \min \left( 1 - \frac{Q_{min,P}}{Q_{P,j}}, 1 - \frac{Q_{min,N}}{Q_{N,j}} \right) B_j - mB_j, \end{aligned} \quad (9)$$

where  $j = 1, 2$  represents the two populations.

We assert that at competitive equilibrium, the optimal uptake strategy (denoted  $A_P^*$ ) is the one that leads to colimitation, as in the case of exponential growth (Section 2.1). In contrast to the case of exponential growth, however, now the population affects the external resource concentrations. Instead of simple maximization techniques, we need to employ a game theoretical approach. The definition of optimal we use is that of a CSS: a strategy that is both uninvadable once established (an ESS) and an evolutionary attractor (also known as continuous stability) (Eshel and Motro, 1981; Geritz et al., 1997).

Both of the CSS conditions can be seen from the zero-net growth isoclines (ZNGIs) associated with all strategies (Fig. 1). A ZNGI is the set of external resource levels at which net growth is zero (Tilman, 1982), calculated after the quotas have reached a steady state (Klausmeier et al., 2004a). Each strategy has a right-angle ZNGI characteristic of essential resources (Tilman, 1982), with the corner at  $(R_P^*, R_N^*)$  defined by

$$R_i^* = \frac{Q_{min,i} m \mu_\infty K_i}{v_{max,i} (\mu_\infty - m) - Q_{min,i} m \mu_\infty} \quad (10)$$

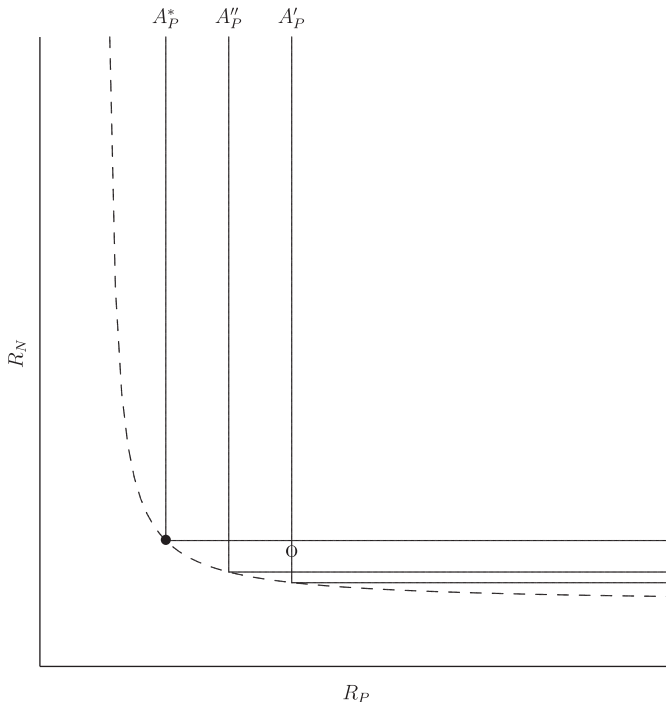


Fig. 1. The zero net growth isoclines (ZNGIs) of three strategies (solid lines) along a continuous trade-off curve (dashed line), derived after quotas have reached a quasi-steady state.  $A_P^*$  is the CSS, which leads to colimitation at equilibrium (solid dot). The strategy  $A_P'$  has less allocation to P-uptake and ends up limited by P (open dot), which allows invasion by a strategy closer to the CSS such as  $A_P''$ .

(Klausmeier et al., 2004a). At equilibrium, under limitation by P,  $\hat{R}_P = R_P^*$  and  $\hat{R}_N \geq R_N^*$ , and vice versa under limitation by N (Klausmeier et al., 2004a).

Now consider the conditions for invasion of a resident population by a rare invader. Let  $(\hat{R}_{N,res}, \hat{R}_{P,res})$  denote the equilibrium external nutrient concentrations when the resident population is at its monoculture equilibrium. When the invader's biomass is approximately zero, it has no effect on the external nutrient concentration or the other population, so we can consider the exponential growth rate of the invader in the environment set by the resident. Substituting  $(\hat{R}_{N,res}, \hat{R}_{P,res})$  into (4) and (5) and rearranging, we find that the invader's net per capita growth rate is positive only when  $R_{P,inv}^* < \hat{R}_{P,res}$  and  $R_{N,inv}^* < \hat{R}_{N,res}$ . Graphically, this means that invasion is possible only when the equilibrium external nutrient concentrations left by the resident lies above the invader's ZNGI. This agrees with results based on linear stability analysis on a more general chemostat model by Li and Smith (in review) and justifies the graphical approach we use below.

First we show that the strategy that leads to colimitation is an ESS. At equilibrium  $\hat{R}_P = R_P^*$  and  $\hat{R}_N = R_N^*$  under colimitation. Eqs. (2), (3), and (10) show that  $R_P^*$  is a decreasing function of  $A_P$  and  $R_N^*$  is an increasing function of  $A_P$ . Thus, the corners of all ZNGIs form a negative-sloped trade-off curve (the dotted line in Fig. 1). A strategy that leads to colimitation reduces resource levels to lie on

the trade-off curve, below the ZNGIs of all other strategies, which prevents invasion by any other strategy (the solid dot in Fig. 1). Thus, colimitation is an ESS.

Next we show that the strategy that leads to colimitation is an evolutionary attractor (continuously stable). Specifically, an evolutionary equilibrium is continuously stable “if a population of a nearby phenotype can be invaded by mutants that are even closer to” the evolutionary equilibrium (Geritz et al., 1998). Note that a strategy with allocation to P-uptake,  $A_P'$ , less than the optimal strategy,  $A_P^*$ , will be limited by P because the P-limited growth rate is an increasing function of  $A_P$  and the N-limited growth rate is a decreasing function of  $A_P$ . This leaves it invisable by a strategy closer to the CSS,  $A_P''$  where  $A_P^* > A_P'' > A_P'$ , since  $R_P^*(A_P'') < R_P^*(A_P')$  (Fig. 1). Likewise, a strategy that has more allocation to P-uptake than the optimal strategy will be limited by N, leaving it invisable by a strategy closer to the CSS. Therefore, the strategy that leads to colimitation is attracting, and therefore optimal in the sense of a CSS.

An easy way to find the optimal strategy is to equate the biomass under N- and P-limitation and solve for  $A_P^*$ . This works because biomass under N-limitation is a decreasing function of  $A_P$ , while biomass under P-limitation is an increasing function. This implies that there is a unique value of  $A_P$  consistent with simultaneous limitation by both resources. Using the expressions for biomass from Klausmeier et al. (2004a), one can solve

$$\begin{aligned} \hat{B}_{R_{P-lim}} &= \hat{B}_{R_{N-lim}}, \\ \frac{a(R_{in,P} - R_P^*(A_P^*))(\mu_\infty - m)}{\mu_\infty Q_{min,P}m} &= \frac{a(R_{in,N} - R_N^*(A_P^*))(\mu_\infty - m)}{\mu_\infty Q_{min,N}m} \end{aligned} \quad (11)$$

for  $A_P^*$ . The resulting expression is not informative, but can be used to plot the optimal strategy  $A_P^*$  as a function of the resource supply ratio,  $R_{in,N}/R_{in,P}$  (Fig. 2). This shows that the allocation to P-uptake is a sigmoidal function of the N:P supply ratio, with high allocation to P-uptake and correspondingly high maximum uptake rates under P-limitation, and low allocation to P-uptake and correspondingly low maximum uptake rates under N-limitation (Fig. 2). The maximum and minimum values of the maximum P-uptake rate can be computed by solving for the  $A_P^*$  at which  $R_P^*$  and  $R_N^*$  become infinite. These are

$$\begin{aligned} A_{P,min} &= \frac{Q_{min,P}m\mu_\infty}{v'_{max,P}(\mu_\infty - m)} \quad \text{and} \\ A_{P,max} &= 1 - \frac{Q_{min,N}m\mu_\infty}{v'_{max,N}(\mu_\infty - m)}. \end{aligned} \quad (12)$$

Notice that the range of flexibility in maximum uptake rates depends on the mortality rate (which includes dilution), with greater flexibility allowed at low mortality rates (Fig. 2).



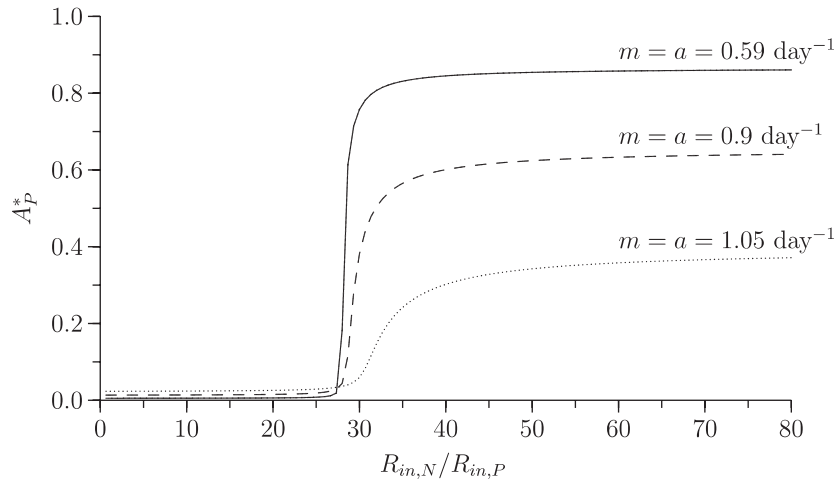


Fig. 2. Optimal allocation to P-uptake as a function of N:P supply ratio for three different mortality rates.

### 3. Dynamic model

The above analysis treats the allocation strategy, and therefore the maximum uptake rates, as fixed. In reality, phytoplankton exhibit physiological plasticity in uptake rates (Rhee, 1980). Traditionally this flexibility has been modeled by making the uptake rate of a given nutrient a decreasing function of the quota of the same nutrient (Gotham and Rhee, 1981a, b; Morel, 1987; Grover, 1991; Andersen, 1997). While it often works at equilibrium, this approach has some shortcomings. (1) The uptake rate may not adjust instantaneously, but with some time lag. (2) The uptake rate of a particular nutrient may depend also on the quota of another nutrient. To accommodate these factors, we suggest a different approach to the regulation of uptake rates, where the allocation to nutrient uptake proteins varies dynamically in response to the quotas of both nutrients. Thus, we now model physiological acclimation rather than evolutionary adaptation (later we will consider the evolution of this physiological acclimation rate). Stomp et al. (2004) recently used a similar approach to model physiological adaptation to two substitutable resources (different wavelengths of light). Without knowledge of the details of the acclimation process, we augment the static model above by assuming that allocation to P-uptake proteins increases under P-limitation and allocation to N-uptake proteins increases under N-limitation. This type of adaptive dynamics (sensu Abrams, 2005) means that, at equilibrium, the CSS of the static model will be reached, leading to colimitation and phytoplankton stoichiometry to match the optimal ratio,  $Q_{min,N}/Q_{min,P}$ . One model for the acclimation process that meets this assumption (and also ensures that allocation to  $A_P$  and  $A_N$  remain positive) is

$$\frac{dA_P}{dt} = \begin{cases} c(1 - A_P), & Q_N/Q_{min,N} > Q_P/Q_{min,P} \text{ (P-limited)}, \\ -cA_P, & Q_N/Q_{min,N} < Q_P/Q_{min,P} \text{ (N-limited)}, \\ 0, & Q_N/Q_{min,N} = Q_P/Q_{min,P} \text{ (colimited)}, \end{cases} \quad (13)$$

where  $c$  is a constant that sets the rate of acclimation. Biologically, this represents the conversion of N-uptake proteins into P-uptake proteins with first-order kinetics under P-limitation and vice versa. A similar acclimation strategy based on internal resource ratios was shown by Van den Berg et al. (2002) to be close to optimal. Other forms of this equation that give the same sign of  $dA_P/dt$  with respect to  $Q_N/Q_P$  give qualitatively similar results (unpublished results).

While Eq. (13) has a clear biological interpretation, it poses mathematical problems due to its discontinuous right-hand side. Analytically, (13) violates the assumptions that typically guarantee the existence and uniqueness of solutions. Numerically, (13) causes adaptive step size routines to get stuck at the discontinuity, resulting in extremely slow numerical solution or outright failure. Although advances have been made in the treatment of such piecewise-defined systems (De Jong et al., 2004), we instead approximate Eq. (13) by the following continuous equation:

$$\frac{dA_P}{dt} = c \left( -A_P + \frac{1}{2} + \frac{1}{\pi} \tan^{-1} \left( k \left( \frac{Q_N}{Q_{min,N}} - \frac{Q_P}{Q_{min,P}} \right) \right) \right), \quad (14)$$

where  $k$  is a shape parameter. For large  $k$ , this approximates the step function of (13). We use (14) with  $k = 10,000$  in all numerical solutions of the model; the results do not change significantly with larger  $k$  (see Supplementary Material, section Accuracy of Smooth Approximation to Discontinuous  $dA/dt$  Equation). All numerical solutions were generated with Mathematica 5.2's NDSolve command as shown in the Supplementary Material (section Figures).

The dynamics of the model depend strongly on the rate of acclimation,  $c$  (Fig. 3) (Table 1). For  $c = 0$  (Fig. 3A–D), acclimation does not occur and the dynamics are given by the static model: phytoplankton stoichiometry is set by the N:P supply at equilibrium (Klausmeier et al., 2004a). For intermediate  $c$  (Fig. 3E–H, I–L) phytoplankton

stoichiometry diverges as exponential growth ceases, but returns to match the optimal N:P as the cells adapt. For large  $c$  (Fig. 3M–P), acclimation is fast enough that phytoplankton stoichiometry never deviates much from the optimal N:P. Thus, phytoplankton stoichiometry is determined not only by the supply stoichiometry, but also the physiological response of the organisms.

For a phytoplankton species with an intermediate acclimation rate  $c$ , the relationship between phytoplankton stoichiometry and nutrient supply ratio depends on the time at which the phytoplankton are sampled. For short times after the biomass has seemingly reached equilibrium (less than 1% biomass change in a day), phytoplankton

stoichiometry matches the input stoichiometry over a large range (solid line, Fig. 4). As physiological acclimation occurs, the relationship between nutrient supply stoichiometry and phytoplankton stoichiometry becomes more constant (Fig. 4). Thus, the results of an experiment such as Rhee's (1978) may depend on the time the phytoplankton are sampled, even after every easily observable variable such as biomass has seemed to reach steady state.

The reaction of phytoplankton growth and stoichiometry to a pulse of the limiting nutrient depends on its ability to adjust its uptake machinery. A nonadaptive species (with allocation strategy set to ensure colimitation during exponential growth) shows marked growth in response to a

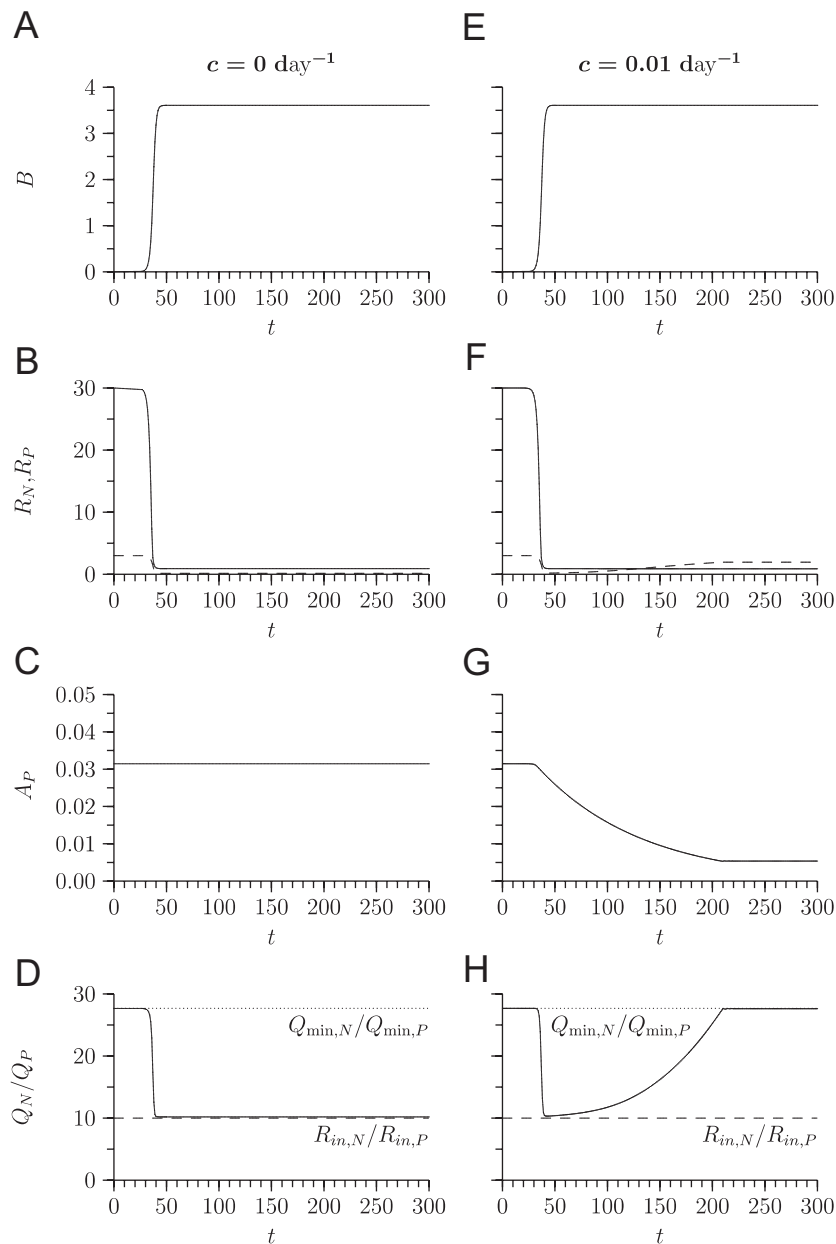


Fig. 3. Numerical solution of the dynamic model with different adaption speeds.  $R_{in,N} = 30$ , other parameters are given in Table 1. (A–D)  $c = 0$ . (E–H)  $c = 0.01 \text{ day}^{-1}$ . (I–L)  $c = 0.1 \text{ day}^{-1}$ . (M–P)  $c = 1 \text{ day}^{-1}$ . (A, E, I, M) Biomass. (B, F, J, N) Available N and P,  $R_N$  (solid line) and  $R_P$  (dashed line). (C, G, K, O) Allocation to P-uptake,  $A_P$ . (D, H, L, P) Phytoplankton stoichiometry,  $Q_N/Q_P$ , (solid line), optimal N:P (dotted line), and input N:P (dashed line).

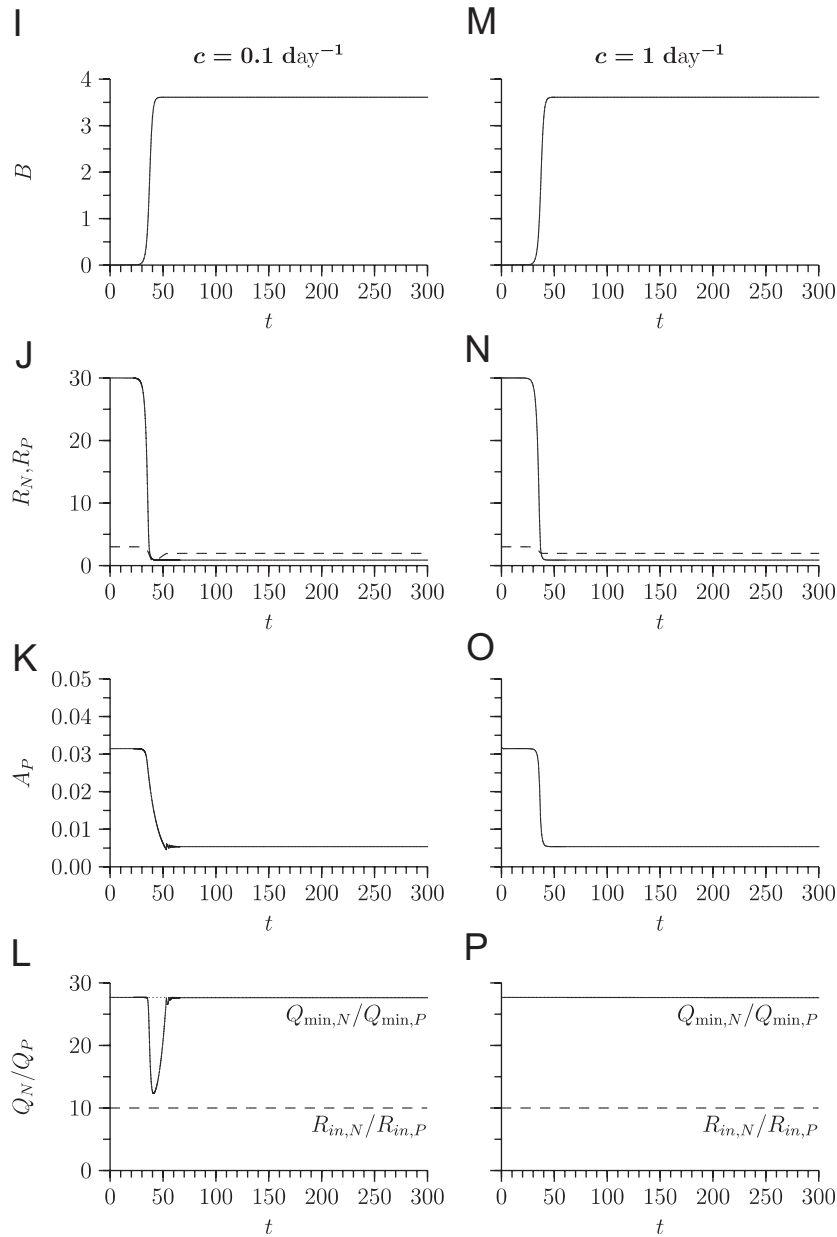


Fig. 3. (Continued)

pulse of the limiting nutrient (here  $P$ ) and its stoichiometry changes from very far from optimal, towards optimal, then back to its baseline level as the effect of the pulse wears off (Fig. 5A–D). A slowly adjusting species experiences a large change in stoichiometry, from optimal to a small N:P back to optimal, but does not grow much (Fig. 5E–H). More rapidly adapting species do grow in response to the pulse (Fig. 5I–L, M–P) with the moderately adapting species showing marked damped fluctuations in stoichiometry and uptake strategy (Fig. 5I–L) and the most rapidly adapting species showing rapid but low amplitude fluctuations in stoichiometry (Fig. 5M–P).

The fact that a species with a fixed uptake strategy can grow more after a pulse of the limiting nutrient than a

slowly adapting species (Fig. 5A–D vs. E–H) suggests that in a fluctuating environment, no or very slow acclimation may sometimes be better than slow acclimation. To verify this, we study competition between two species with different acclimation rates in a nonequilibrium environment, where, with period  $T$ , we alternate between two different supply concentrations, each for half the period.

To determine the outcome of competition, we generated a pairwise invasibility plot (PIP) (Fig. 6A), which measures the exponential growth rate of a rare phenotype introduced to a resident population on its long-term attractor (Geritz et al., 1998). We let the invader's physiological variables ( $Q_P$ ,  $Q_N$ ,  $A_P$ ) first reach an attractor before calculating its growth rate averaged over a period (Ferriere and Gatto,

Table 1

Parameters used in numerical solutions unless otherwise noted. Based on Rhee (1974), Rhee (1978) and Klausmeier et al. (2004a)

Parameter	Meaning (units)	Value
$a$	Chemostat dilution rate ( $\text{day}^{-1}$ )	0.59
$R_{in,P}$	Input P concentration ( $\mu\text{mol P L}^{-1}$ )	3
$R_{in,N}$	Input N concentration ( $\mu\text{mol N L}^{-1}$ )	15–240
$v'_{max,i}$	Maximum nutrient uptake rate per $A_i$ ( $10^{-7} \mu\text{mol nutrient cell}^{-1} \text{ day}^{-1}$ )	3.533
$K_P$	P half-saturation constant ( $\mu\text{mol P L}^{-1}$ )	0.2
$K_N$	N half-saturation constant ( $\mu\text{mol N L}^{-1}$ )	5.6
$\mu_\infty$	Growth rate at infinite quota ( $\text{day}^{-1}$ )	1.35
$m$	Mortality rate ( $\text{day}^{-1}$ )	$a$
$Q_{min,P}$	Minimum P quota ( $10^{-9} \mu\text{mol P cell}^{-1}$ )	1.64
$Q_{min,N}$	Minimum N quota ( $10^{-9} \mu\text{mol N cell}^{-1}$ )	45.4
$Q_{min,N}/Q_{min,P}$	Optimal N:P ratio ( $\text{mol N (mol P)}^{-1}$ )	27.7
$c$	Physiological adaptation rate ( $\text{day}^{-1}$ )	0–1.0
$k$	Steepness parameter (Eq. (14))	10 000

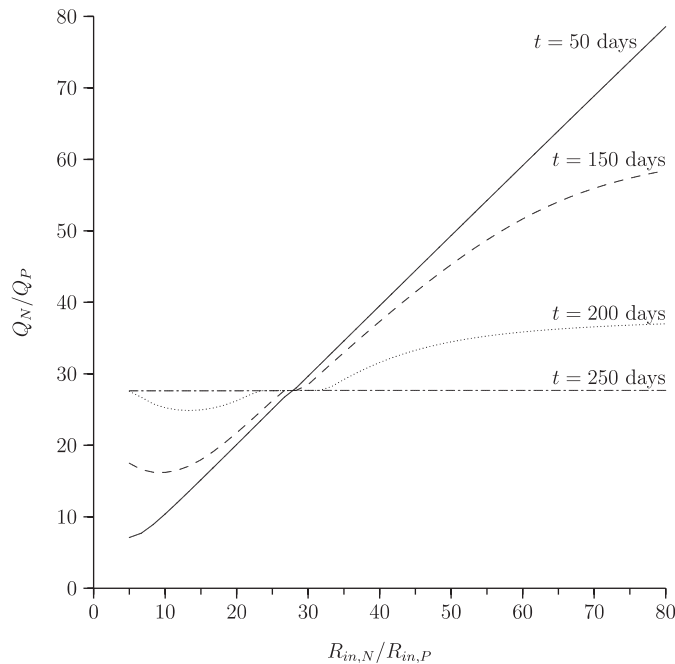


Fig. 4. Phytoplankton N:P stoichiometry,  $Q_N/Q_P$ , as a function of nutrient supply ratio,  $R_{in,N}/R_{in,P}$  at different times ( $t = 50$  days, solid line;  $t = 150$  days, dashed line;  $t = 200$  days, dotted line;  $t = 250$  days, dash-dotted line). Here  $c = 0.01 \text{ day}^{-1}$ . Biomass was effectively at equilibrium at  $t = 50$  days or earlier in all these simulations.

1995). The Mathematica code is included in the Supplementary Material. The PIP can be used in ecological and evolutionary analysis (Geritz et al., 1998). Invasibility analysis is one way to assess the ecological outcome of competition between two species (Armstrong and McGehee, 1980): when species 1 can invade a monoculture of species 2, but 2 cannot invade 1, 1 outcompetes 2 (and *vice versa*); when neither 1 nor 2 can invade a monoculture of the other, founder control occurs; and when 1 and 2 can

each invade a monoculture of the other, 1 and 2 coexist. By flipping the PIP around its  $45^\circ$ -axis and superimposing it back on itself, we exchange the role of resident and invader to show the outcome of competition between two species with different adaption speeds  $c_1$  and  $c_2$  (Fig. 6B). This shows that a slower adapting species can often outcompete a faster adapting one. Coexistence does not occur here because there are no pairs of strategies that can each invade the other. The rate of exclusion can be estimated by the negative invasion rate of the inferior competitor. In the scenario shown here, that can be as high as  $-0.00586 \text{ day}^{-1}$  for  $c_{superior} = 10^{-4}$  and  $c_{inferior} = 10^{-1.5}$ . This rate is borne out by numerical solution of two species competition, with exclusion taking on the order of 1000 days (Supplementary Material, section Two Species Competition). The outcome of mutation-limited evolution can be determined from the PIP (Geritz et al., 1998). In the examples shown here (Fig. 6A), the only evolutionarily singular strategy is an evolutionary repeller near  $c = 10^{-1.5}$ . If evolution on trait  $c$  proceeds by small, infrequent mutations, then there is runaway evolution to either faster or slower acclimation, depending on the initial trait. This outcome does not depend sensitively on the forcing period. Fig. 6C and D show PIPs with  $T = 60$  and 120 days. In both cases, the qualitative result is the same, with the trait value of the evolutionary repeller decreasing with increased period.

#### 4. Conclusions

In this paper we proposed a new model of the regulation of nutrient uptake of osmotrophs that require two essential nutrients. Assuming a trade-off between the uptake of the two nutrients, we showed that there is a unique optimal allocation strategy and that it is the one that leads to colimitation. This agrees with other models of optimal uptake of two nutrients (Tilman, 1982; Abrams, 1987; Van den Berg et al., 2002) and optimal foraging for a nutrient and light by terrestrial plants (Reynolds and Pacala, 1993) and motile phytoplankton (Klausmeier and Litchman, 2001). The fact that this result holds for such a range of models indicates that it might be thought of as a general principle.

Using a dynamic model that adjusts the allocation strategy to achieve colimitation, we showed that the relationship between organismal and nutrient supply ratios (Fig. 4) and the response to a nutrient pulse (Fig. 5) depend on the timescale of acclimation. The dependence of phytoplankton stoichiometry on physiological acclimation has important implications for the interpretation of classic chemostat studies on phytoplankton stoichiometry. Rhee (1978) grew *Scenedesmus* sp. in a series of chemostats at a fixed dilution rate with N:P supply varying from 5 to 80 and found that the phytoplankton stoichiometry matched the supply ratio across the entire range. The idea that primary producer stoichiometry is extremely flexible is a central one of ecological stoichiometry (Sterner and Elser,



2002), but a recent survey of field and laboratory studies shows that this extreme flexibility may be the exception, not the rule (Hall et al., 2005). They found that even single-species laboratory studies may not show matching of phytoplankton N:P with the supply ratio and suggested two mechanisms to account for this departure from expectation (low growth rates and limits to nutrient storage). Our model provides a third mechanism, because the relationship between nutrient supply ratio and phytoplankton stoichiometry may depend on the time, even after the biomass has apparently reached a steady state (Fig. 4).

Interestingly, a fast-adapting species with variable nutrient stores always consumes the two resources in the

same optimal ratio, making it behave as a species with a fixed stoichiometry. In this case the two extensions to fixed stoichiometry models (variable internal stores and dynamic uptake allocation) cancel out. This might explain why fixed stoichiometry models often work well (e.g. Tilman, 1976), especially at equilibrium. We also found that in a nonequilibrium environment, a slower adapting species may sometimes outcompete a faster adapting one (Fig. 6). Because it accumulates excess quantities of the nonlimiting nutrient, such a slow-adapting species may be thought of as a type of “storage specialist”, which Grover found could be competitively dominant in some fluctuating environments (Grover, 1991). Traditional models of the regulation of

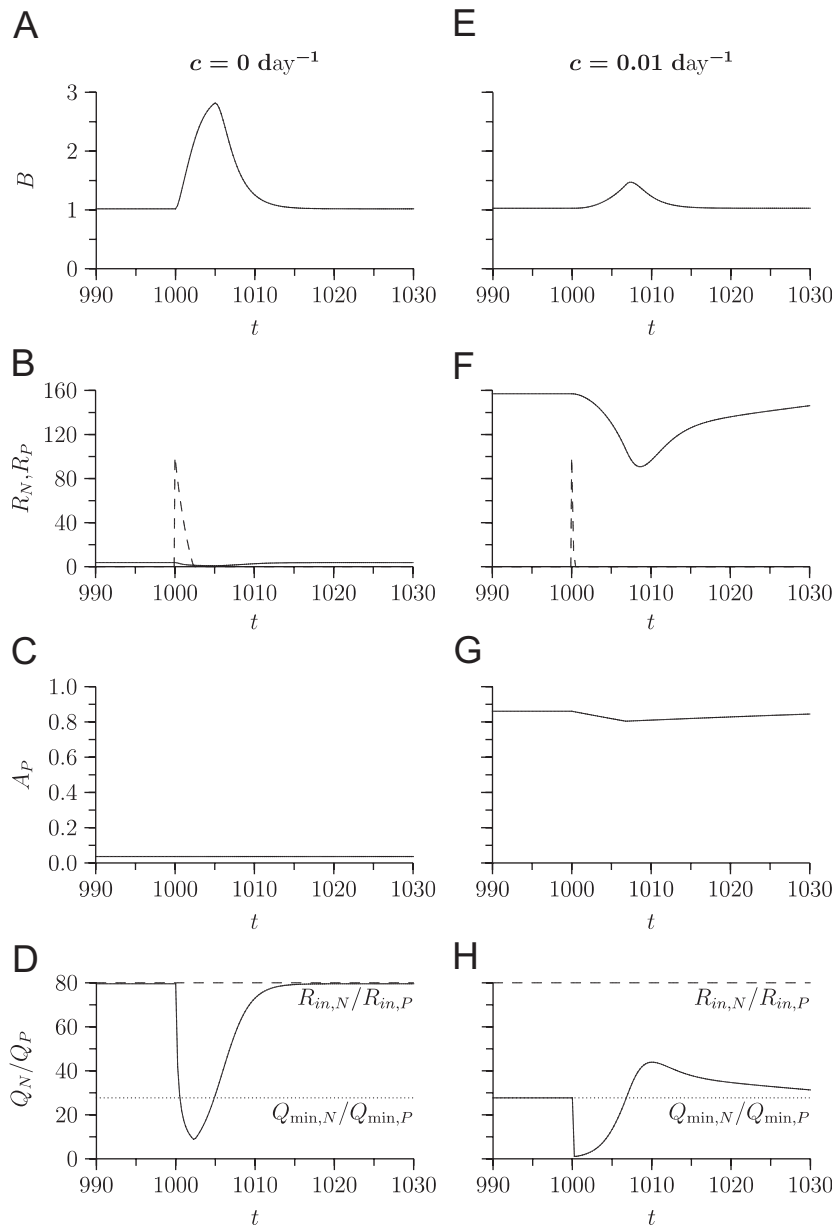


Fig. 5. Reaction of phytoplankton to a pulse of a limiting nutrient. Phytoplankton were grown to equilibrium under P-limitation ( $R_{in,N} = 240$ ,  $R_{in,P} = 3$ ), then given a  $100 \mu\text{mol L}^{-1}$  pulse of P at  $t = 1000$  days. Panels are as in Fig. 3. (A–D) Non-adaptive phytoplankton ( $c = 0$ ) with allocation strategy fixed to ensure colimitation during exponential growth. (E–H) Slowly adapting phytoplankton ( $c = 0.01 \text{ day}^{-1}$ ). (I–L) Fast adapting phytoplankton ( $c = 0.1 \text{ day}^{-1}$ ). (M–P) Very fast adapting phytoplankton ( $c = 1 \text{ day}^{-1}$ ).

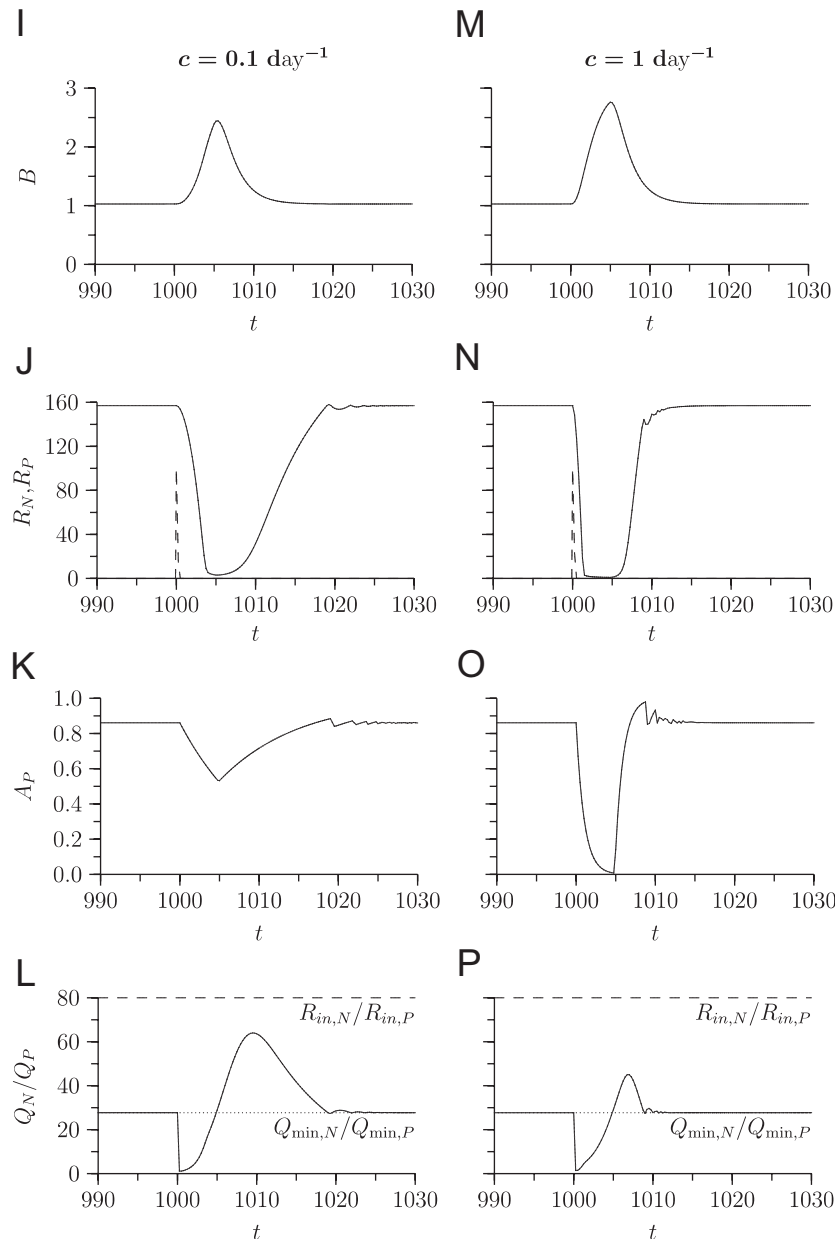


Fig. 5. (Continued)

nutrient uptake deal with only a single nutrient (Morel, 1987; Grover, 1991; Andersen, 1997). We suggest that this new approach to acclimation of uptake rates may be useful in models of multinutrient limitation.

Recent theoretical work has shown that competition for three essential resources can lead to limit cycle dynamics, which then allows the nonequilibrium coexistence of more than three species on three resources (Huisman and Weissing, 1999). One of the conditions required for these nonequilibrium dynamics to occur is that species consume most of the resource that limits them second (Huisman and Weissing, 2001). If our analysis for two resources holds for three, then the optimal uptake allocation strategy would be to consume most of the resource that limits growth most.

In this case, we expect stable coexistence of only three species, not the nonequilibrium coexistence of more (see also the optimal foraging arguments of Tilman, 1982).

The model could be experimentally tested in a few ways. A series of chemostats with varying nutrient supply ratios could be run. The first test would measure the relationship between organismal stoichiometry and nutrient supply ratio at different time intervals. The model predicts a transition from a one-to-one relationship early to a constant stoichiometry later (Fig. 4). The second test would measure the maximal uptake rate across this supply ratio gradient. The model predicts a sigmoidal relationship between maximum uptake rate and nutrient supply ratio at equilibrium.

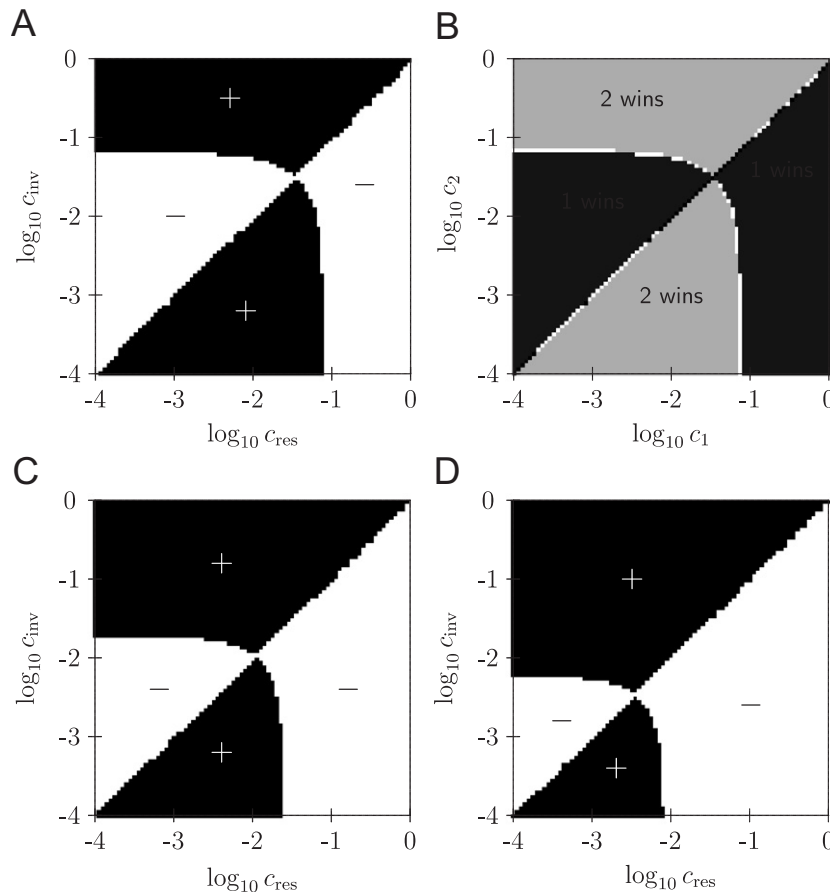


Fig. 6. Competition between species with differing acclimation rates  $c_i$  in a variable environment. In phase one,  $R_{in,N} = 240$  and  $R_{in,P} = 3$ ; in phase two,  $R_{in,N} = 15$  and  $R_{in,P} = 3$ . Period  $T = 30$  days. (A) A pairwise-invasibility plot showing whether a rare invader with acclimation rate  $c_{inv}$  can invade a resident population with acclimation rate  $c_{res}$  on its long-term attractor. Dark regions indicate that invasion is possible. (B) The outcome of competition, determined by the mutual invasibility test. (C and D) Pairwise-invasibility plots for longer periods, (C)  $T = 60$  days, (D)  $T = 120$  days.

Although this model is more explicit about the mechanisms of nutrient uptake regulation than previous models, there are still many possible modifications that could be important. First, we assume a single trade-off between nutrient uptake proteins. There could be multiple constraints operating simultaneously: limited material, cell surface, or energy to run nutrient transporters. Second, there are multiple ways in which cells can regulate uptake: either by building/destroying nutrient transporters or by activating/inactivating existing ones. These two mechanisms of regulation will operate on different time-scales. Aside from regulating uptake, cells can also dispose of excess nutrient through excretion (Koch, 2005), a process not included here. Third, there may be other cellular functions aside from nutrient uptake that could enter into a multiway trade-off with the uptake of a given nutrient, such as growth (Klausmeier et al., 2004b). Finally, the equation we used to model acclimation (Eq. (13)) is plausible, but has no empirical support to suggest it over other such equations. We have obtained similar results using other equations to model acclimation (unpublished) but quantitative results differ. In short, the microeco-

nomics of microbial growth is complex and bears further theoretical development and experimental work.

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