

Optimization-based model of multnutrient uptake kinetics

S. Lan Smith¹ and Yasuhiro Yamanaka

Ecosystem Change Research Program, Frontier Research Center for Global Change, JAMSTEC, Yokohama, Japan

Abstract

We present a new, optimization-based model for uptake kinetics of multiple nutrients, which has the same number of parameters (two for each nutrient) as the Michaelis–Menten model. We fit this model and an existing inhibition-based model to data from chemostat experiments at various flow rates (under extreme limitation by both nitrogen [N] and phosphorus [P]) and compared these models and the Michaelis–Menten model to an independent data set for the same species in a chemostat at various N:P input ratios (at constant flow rate). Our model fit the data well, with a slightly higher square error than the much more complex inhibition model. We also successfully applied our model to a data set for a different species under various degrees of vitamin B12- and P-limitation. Our model agrees with measured cell quotas of nonlimiting nutrients when supply ratios differ greatly from the optimal ratio for phytoplankton, whereas the Michaelis–Menten model greatly overestimates the uptake of nonlimiting nutrients at these extreme nutrient supply ratios. The key to our model's success is the optimization of uptake for the limiting nutrient, which results in distinct behavior for limiting versus nonlimiting nutrients, without additional parameters; phytoplankton allocate their internal resources (nitrogen) to optimize uptake of the limiting nutrient, but not in response to changes in ambient nutrient ratios.

For ambient nutrient ratios that are very different from the optimal ratio of phytoplankton, straightforward application of separate Michaelis–Menten equations for multiple nutrients greatly overestimates the uptake rates of nonlimiting nutrients compared with data from chemostat experiments (Droop 1974; Rhee 1974). Uptake of the same nutrient is faster when it is limiting than when it is nonlimiting (Rhee 1974; Gotham and Rhee 1981*a,b*). Droop (1974) developed a parameterization for uptake of nonlimiting nutrients to match his observations, and Gotham and Rhee (1981*a,b*) developed an inhibition-based model in which the maximum uptake rate of a nutrient is a decreasing function of its cell quota (internal concentration). Both of these approaches yield more accurate uptake rates for nonlimiting nutrients, but both add parameters, which must be determined separately for various nutrients and even for the same nutrient with different ratios of ambient nutrient concentrations.

We present a new optimization-based model for uptake kinetics of multiple nutrients. Our uptake model is an extension of the single-nutrient optimal-uptake equation of Pahlow (2005), which is itself an extension of the affinity-based uptake model of Aksnes and Egge (1991).

For this study we embedded both our uptake model and the inhibition model of Gotham and Rhee (1981*a,b*) into a model of phytoplankton growth on multiple nutrients (Legovic and Cruzado 1997; Klausmeier et al. 2004) and applied the resulting models to simulate chemostat experiments. We fit both models to data from chemostat experiments at extreme nitrogen:phosphorus (N:P) input ratios at various flow rates. We also compared both models to an independent data set for the same species in

a chemostat over a range of less extreme N:P ratios at fixed flow rate. We compared our model and that of Droop (1974) to a data set for a different species under various degrees of limitation by P and vitamin B12, at various flow rates (Droop 1974). Thus, we quantitatively compared the models' agreement with data under various conditions of limitation by N, P, and vitamin B12.

Phytoplankton model

To describe changes in phytoplankton composition requires a model of their biomass and internal nutrient concentrations as well as their uptake rates. Legovic and Cruzado (1997) presented such a model for two nutrients; Klausmeier et al. (2004) further analyzed their model and compared it to data from chemostat experiments. This model (hereafter, the MM model) combines the quota model for growth (Caperon 1968; Droop 1968), the Michaelis–Menten model for uptake kinetics, and Liebig's Law of the minimum for a reactor (Fig. 1) with nutrient supply rate a , with nutrient concentrations $S_{in,1}$ and $S_{in,2}$ in the inflow, and assuming constant, density-independent mortality rate $m \geq a$.

The quota model (Caperon 1968; Droop 1968) describes growth rate, μ , as:

$$\mu = \mu_{\infty} \left(1 - \frac{Q_{0,lim}}{Q_{lim}} \right) \quad (1)$$

where Q_{lim} and $Q_{0,lim}$ are, respectively, the cell quota (ratio of internal concentration to biomass) and minimum cell quota for the limiting nutrient and where μ_{∞} represents the growth rate at infinite cell quota. According to Liebig's Law of the minimum, the limiting nutrient is the one for which $Q_{lim}/Q_{0,lim}$ is the minimum of all values of $(Q_i/Q_{0,i})$.

According to the Michaelis–Menten model, uptake rate v_i of nutrient i with concentration S_i is

¹ Corresponding author (lanimal@jamstec.go.jp).

Acknowledgments

We thank Eitaro Wada and Markus Pahlow for helpful discussions and reviewing of the manuscript and the editor and two anonymous reviewers for their constructive comments.

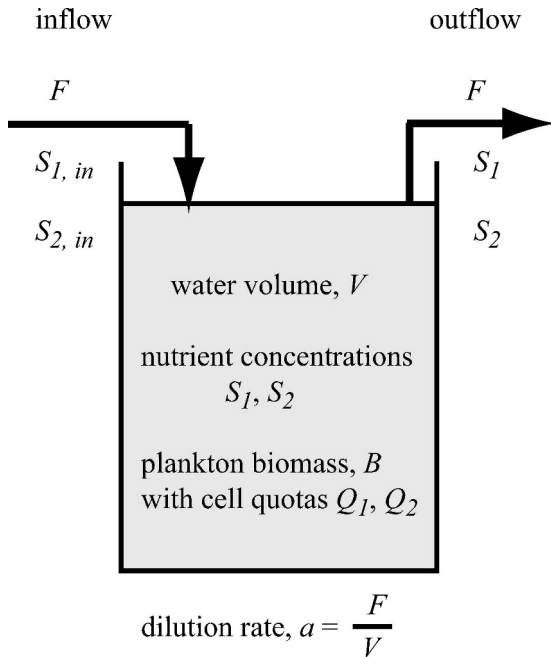


Fig. 1. Schematic of the reactor used for chemostat experiments. When run to steady state, the growth rate, μ , must equal the dilution rate, a .

$$v_i(S_i) = \frac{V_{\max,i} S_i}{K_i + S_i} \quad (2)$$

where $V_{\max,i}$ is its maximum uptake rate and K_i is its half-saturation constant.

The model solves for the concentrations of two nutrients (S_1 and S_2 , moles per unit volume of water), their respective cell quotas (Q_1 and Q_2 , moles per cell), and the biomass of phytoplankton (B , cells per unit volume of water), via the following differential equations.

$$\frac{dS_i}{dt} = a(S_{in,i} - S_i) - v_i(S_i)B \quad (3)$$

where $S_{in,i}$ is the input concentration (in the inflow) of S_i ($i = 1, 2$). For cell quotas,

$$\frac{dQ_i}{dt} = v_i(S_i) - \mu_{\infty} \min\left(1 - \frac{Q_{0,1}}{Q_1}, 1 - \frac{Q_{0,2}}{Q_2}\right) Q_i \quad (4)$$

where the expression “min” denotes taking the minimum of the two limitation terms in parentheses, according to Liebig’s Law of the minimum. The second term in this equation is $\mu_{\infty} Q_i$, and it is therefore required that at steady state, $\mu Q_i = v_i(S_i)$. For biomass,

$$\frac{dB}{dt} = \mu_{\infty} \min\left(1 - \frac{Q_{0,1}}{Q_1}, 1 - \frac{Q_{0,2}}{Q_2}\right) B - mB \quad (5)$$

Inhibition model

Gotham and Rhee (1981a,b) developed a model (hereafter, the GR model) in which uptake of a nutrient, i

(limiting or not), is inhibited by its own cell quota, Q_i , so that the maximum uptake rate, $V_{\max,i}$ (for use in Eq. 2) is a decreasing function of Q_i , thus:

$$V_{\max,i} = \frac{V_{inh} K_{inh} - C_{inh}(Q_i - Q_{0,i})}{K_{inh} + Q_i - Q_{0,i}} \quad (6)$$

in which V_{inh} , K_{inh} , and C_{inh} are, respectively, the maximum possible uptake rate, a half-saturation constant for inhibition, and an inhibition factor in terms of cell quota. Gotham and Rhee fitted this equation to experimental data, yielding good fits for several species under both N- and P- limitation.

Optimal uptake model: SPONGE

Aksnes and Egge (1991) presented an affinity-based model of nutrient uptake, based on an analysis of the timescales required for nutrient ions to encounter uptake sites on the cell surface and to be “handled” (assimilated into organic matter) by those sites. Uptake v of substrate S is

$$v(S) = \frac{1}{(V_{\max})^{-1} + (AS)^{-1}} \quad (7)$$

where the maximum uptake rate, V_{\max} , and the affinity, A , depend on the number of uptake sites, their surface area, the handling time, etc. Aksnes and Egge showed that under certain assumptions, such that V_{\max} and A are constants, their model reduces to the Michaelis–Menten model. This can be seen by rearranging the above equation to yield

$$v(S) = \frac{V_{\max} S}{\left(\frac{V_{\max}}{A}\right) + S} \quad (8)$$

which is equivalent to the Michaelis–Menten equation (Eq. 2) with half-saturation constant, $K = V_{\max}/A$.

Pahlow (2005) extended this model by separating the uptake sites into those on the surface and internal enzymes. The key idea of his optimization for nutrient uptake is that phytoplankton allocate a portion of their internal nitrogen between surface uptake sites, which determine the encounter timescale, versus internal enzymes, which “handle” (or assimilate) the nutrients once encountered (necessary to clear the uptake sites so that they can take up more nutrients: Pahlow [2005] termed this turnover). Both the uptake sites and enzymes, being mostly made of protein, would require significant nitrogen. This internal pool of nitrogen available for uptake hardware was assumed to be some part of the subsistence cell quota, $Q_{0,N}$.

Because Pahlow (2005) did not present a derivation of his uptake equation, we present one here, which closely resembles that of Aksnes and Egge (1991). As in the model of Aksnes and Egge (1991), the number of moles of nutrient ions encountered (per cell) in time T_1 can be expressed as $m = T_1 n_s a v_{mt} S$, where n_s is the number of surface uptake sites per cell for the nutrient considered, a is the surface area of each uptake site, v_{mt} is a mass transfer coefficient (volume per surface area per unit time), and S is the ambient

concentration of nutrient (moles per volume). The “handling” time for assimilating these m moles of nutrient ions into organic matter is $T_2 = m(n_e r_e)^{-1}$, where n_e is the number of internal enzymes per cell for processing this nutrient and r_e is the rate of processing (moles per enzyme per time). The uptake rate (moles of nutrient per cell per time) can then be expressed as $v = m/(T_1 + T_2)$, which yields

$$v(S) = \frac{m}{m(n_s a v_m S)^{-1} + m(n_e r_e)^{-1}} \quad (9)$$

For fixed values of n_s and n_e , Eq. 9 can be rearranged by dividing both numerator and denominator by m and introducing the substitutions $A = n_s a v_m$ and $V_{\max} = n_e r_e$, to yield Eq. 7, the equation of Aksnes and Egge (1991). [Aksnes and Egge (1991) further assumed that $n_e = n_s = n$ (constant), so that their constant “handling time per ion,” h , is equivalent to $(r_e)^{-1}$ here. In the model of Pahlow (2005), handling time per ion is $(n_e r_e)^{-1}$, which varies inversely with n_e .]

The model assumes that the pool of nitrogen available for uptake hardware is allocated exclusively between surface uptake sites and internal enzymes (for assimilating nutrients). Thus, if all of this nitrogen pool for uptake were allocated to surface sites, the maximum potential number of surface sites per cell would be $n_{s,0}$, and no nitrogen would be left for internal enzymes ($n_e = 0$). Alternatively, the maximum potential number of internal enzymes, $n_{e,0}$, could be achieved only if all of this pool of nitrogen was allocated to such enzymes, leaving none for surface sites $n_s = 0$. Then the actual number per cell of surface sites can (ideally) be expressed in terms of the fraction, f_A , of this pool of internal nitrogen that is allocated to surface sites, with the remainder, $(1 - f_A)$, allocated to internal enzymes, thus: $n_s = f_A n_{s,0}$ and $n_e = (1 - f_A) n_{e,0}$. The optimal value of f_A must therefore be greater than zero and less than one. Substituting into Eq. 9 and dividing out the m in that equation yields

$$v(S) = \frac{1}{(f_A n_{s,0} a v_m S)^{-1} + [(1 - f_A) n_{e,0} r_e]^{-1}} \quad (10)$$

which can be rearranged by introducing the substitutions $A_0 = n_{s,0} a v_m$ and $V_0 = n_{e,0} r_e$, to yield the uptake equation presented by Pahlow (2005):

$$v(S) = \frac{1}{(f_A A_0 S)^{-1} + [(1 - f_A) V_0]^{-1}} \quad (11)$$

The affinity (maximum possible clearance rate), A , and maximum uptake rate, V_{\max} , can be expressed in terms of the allocation of internal nitrogen as

$$A = f_A A_0 \quad (12)$$

$$V_{\max} = (1 - f_A) V_0 \quad (13)$$

where A_0 and V_0 are, respectively, the potential affinity and potential maximum uptake rate for substrate S . These

potential values depend only on the total amount of nitrogen available to be allocated for uptake machinery of that nutrient, whereas the values of the actual affinity and maximum uptake rate (A and V_{\max}) also depend on how that pool of nitrogen is allocated between them.

For any fixed value of f_A , Eq. 11 is equivalent to the Michaelis–Menten equation, with $V_{\max} = (1 - f_A) V_0$ and $K = [(1 - f_A)/f_A] V_0/A_0$. Finding the optimal allocation of resources for uptake implies optimizing $v(S)$ in terms of f_A (setting $dv(S)/df_A = 0$ and solving for f_A), to yield

$$f_A = \frac{1}{\sqrt{\frac{A_0 S}{V_0} + 1}} \quad (14)$$

Thus, the optimal uptake model varies the values of A and V_{\max} to maximize uptake, with the constraint that a fixed amount of nitrogen (or whatever resource) is available for this purpose. This is equivalent to a Michaelis–Menten equation with varying K and V_{\max} . This allocation of internal nitrogen (breaking down and synthesizing enzymes) is assumed to be instantaneous, although in reality it must require some time and energy. Still, phytoplankton typically require only several hours to minutes to acclimate their uptake rates and cell quotas to environmental conditions (Harrison and Morel 1986; Morel 1987). Therefore, the assumption of instantaneous acclimation is reasonable for timescales of at least a day, and, indeed, Pahlow (2005) successfully applied his model to a detailed data set for a batch incubation with measurements every 12 h.

Substituting Eq. 14 into Eq. 11 yields the single-nutrient optimal uptake equation of Pahlow (2005):

$$v_{opt}(S) = \frac{A_0 S}{\left(\sqrt{\frac{A_0 S}{V_0} + 1}\right)^2} \quad (15)$$

For any value of f_A , say f'_A , there is one substrate concentration, S' , at which Eq. 14 yields $f_A(S') = f'_A$. At this substrate concentration, the optimal uptake model yields the same uptake rate as the Michaelis–Menten equation with $V_{\max} = (1 - f'_A) V_0$ and $K = [(1 - f'_A)/f'_A] V_0/A_0$. Thus, one can choose parameters such that the two models will yield the same uptake rate for any value of f'_A (with corresponding value S'). Once the parameters are fixed, however, at any other concentration, $S \neq S'$, the optimal uptake equation will yield a greater uptake rate than the Michaelis–Menten equation (with fixed K and V_{\max}). Thus, for any parameter set (A_0 , V_0), the optimal uptake model will allow a greater uptake rate than the Michaelis–Menten model with parameter set ($K = [(1 - f'_A)/f'_A] V_0/A_0$, $V_{\max} = (1 - f'_A) V_0$) for any substrate concentration S except S' , at which the two models will yield the same uptake rate. Furthermore, the difference between the two models will increase with the difference between S and S' (i.e., the greater the variability in nutrient

concentration, the greater the difference between the two uptake models).

Multielement optimal uptake—Assuming separate sets of uptake sites and internal enzymes for each nutrient, there are many possible allocations of internal resources. We considered two possible strategies by which phytoplankton might optimize their uptake hardware.

First, the most efficient allocation of internal nitrogen (or of another resource) would consist of optimizing uptake for the limiting nutrient, with only the minimum required allocation for each nonlimiting nutrient. For any nonlimiting nutrient, the minimum required allocation would be that required to keep its internal concentration (cell quota) from becoming so low that it became the limiting nutrient. This would result in phytoplankton maintaining their optimal ratios of nonlimiting to limiting nutrient (the ratio that defines the co-limitation point). Although this optimal ratio may vary with growth rate (Terry et al. 1985), such variation could not account for the much wider variations in phytoplankton composition as a function of nutrient supply ratio (even at constant growth rate). Nonlimiting nutrients are taken up in excess of cellular requirements, even at steady state (Droop 1974; Rhee 1974, 1978). This optimization strategy must therefore be rejected.

Second, we considered a simpler strategy, in which phytoplankton allocate internal nitrogen between surface sites and internal enzymes in the same proportion for all nutrients. This strategy is less efficient, but it allows for variations in ratios of internal nutrient concentrations. Uptake of the limiting nutrient is optimized according to the single-nutrient optimal-uptake model (Eq. 14), and the same allocation (f_A) determined by the external concentration of limiting nutrient is assumed to apply for all nutrients, i :

$$f_{A,i} = f_{A,\text{lim}} \forall i \quad (16)$$

This means that for any nutrient the same fraction of internal resources (i.e., nitrogen) is allocated to surface uptake sites versus internal enzymes. For simplicity, we have assumed that the total amount of internal resources available for uptake of each nutrient (for both surface uptake sites and internal enzymes) is fixed, although it may vary with cellular state, as in the photoacclimation model of Pahlow (2005), which made V_0 a function of cell quota and chlorophyll content. Therefore, in our model each nutrient has its own fixed parameters (A_0 , V_0) in Eq. 11.

In this strategy, phytoplankton adjust their uptake hardware for all nutrients in response to changes in the concentration of the limiting nutrient, but not in response to changes in concentrations of nonlimiting nutrients. Our motivation for choosing this strategy was that it seems consistent with evolution in an ocean with variable nutrient concentrations (in the euphotic zone) but with relatively constant stoichiometry of nutrient supply (as is the case if the vast majority of nutrient supply comes from the aphotic zone of the ocean, which has relatively constant stoichiometry). This would not have required constant nutrient ratios over evolutionary timescales, but only that nutrient

ratios varied little over most of the time when significant concentrations of nutrients were available to support rapid growth (i.e., soon after upwelling events). In such an ocean, it would be advantageous to adapt to frequent changes in concentration of limiting nutrient, but there would be less benefit in adapting to changes in nutrient ratios. Nutrient ratios in the euphotic zone do vary, but significant deviations from Redfield ratios tend to occur after nutrients have been mostly depleted (and, hence, after most nutrient uptake and growth has occurred). We call our model the Simple Phytoplankton Optimal Nutrient Gathering Equations (SPONGE) model. For validation, we quantitatively compared the model to data from chemostat experiments (and to other models).

As in Pahlow (2005), we assume that acclimation (allocation of internal nitrogen for uptake hardware) is instantaneous. Our model should therefore only be applicable at timescales longer than the time required for acclimation, which is reported to require from hours to minutes (Harrison and Morel 1986; Morel 1987). The allocation of internal nitrogen for acclimation also requires energy and other resources (notably phosphorus for energy transfer). In applying the model, we assume that energy and other resources are sufficient to allow rapid acclimation.

Solution

We solved the phytoplankton model at steady state, with the assumption of a perfect chemostat ($m = \mu = a$). The solution is mainly the same as in that in the model of Klausmeier et al. (2004), and therefore we only outline it briefly, emphasizing the differences for the different uptake models.

The limiting nutrient is the one for which Q/Q_0 is lower. Its cell quota is obtained by setting $dB/dt = 0$, and it depends only on the flow rate (and parameters μ_∞ and $Q_{0,\text{lim}}$). The concentration of limiting nutrient is determined by setting $dQ_{\text{lim}}/dt = 0$, which yields

$$v_{\text{lim}}(S_{\text{lim}}) = \mu_\infty \left(1 - \frac{Q_{0,\text{lim}}}{Q_{\text{lim}}} \right) Q_{\text{lim}} \quad (17)$$

which, from Eq. 1, is equivalent to $v_{\text{lim}}(S_{\text{lim}}) = \mu Q_{\text{lim}}$. Furthermore, at steady state, $\mu = a$. With Michaelis-Menten kinetics (Eq. 2 for v_{lim}), this equation can be solved analytically (Klausmeier et al. 2004). Equation 17 can be solved analytically for the inhibition model as well, because uptake of the limiting nutrient is still described by Eq. 2. When applying our SPONGE model, uptake of limiting nutrient is described by Eq. 15, and Eq. 17 can be solved analytically, thus:

$$S_{\text{lim}} = \frac{V_0}{A_0 \left(\sqrt{\frac{V_0}{\mu Q_{\text{lim}}} - 1} \right)^2} \quad (18)$$

Biomass is obtained by setting $dS_{\text{lim}}/dt = 0$, yielding a straightforward equation in terms of Q_{lim} and S_{lim} . The

concentration of the nonlimiting nutrient is obtained by setting $dS_{non}/dt = 0$ to yield

$$v_{non}(S_{non}) = a(S_{in,non} - S_{non})/B \quad (19)$$

Combined with the expressions for $v(S_{non})$, this yields a quadratic equation in S_{non} both for the case of Michaelis–Menten kinetics (Eq. 2) and for our SPONGE model (Eq. 11). Once S_{non} is known, Q_{non} is readily obtained by setting $dQ_{non}/dt = 0$, which yields $\mu Q_{non} = v(S_{non})$. For the inhibition model, the nonlinear dependence of $V_{max,non}$ on Q_{non} (Eq. 6) results in a cubic equation in Q_{non} , and we therefore solved Eq. 19 numerically.

Simulations of chemostat experiments

We applied the model to chemostat experiments with *Scenedesmus* sp. for a range of input ratios at constant flow rate (Rhee 1978), as in Klausmeier et al. (2004), and at extreme nutrient input ratios at various flow rates (Rhee 1974). When applying the GR model, Eq. 6 was used to calculate the values of V_{max} for Eq. 2. When applying our SPONGE model, we replaced Eq. 2 with Eq. 15 for the limiting nutrient and with Eq. 11 for the nonlimiting nutrient.

As initial guesses, we used the parameter values of Klausmeier et al. (2004), except for the values of minimum cell quota, which we fit simultaneously to the data from both Rhee studies (1974, 1978). For the GR model, we used the parameter values of Gotham and Rhee (1981a,b) as initial guesses and then fit the uptake parameters to the data of Rhee (1974). For the SPONGE model, we chose initial guesses for the four parameter values (A_0 and V_0 for both N and P) based on the Michaelis–Menten uptake parameters of Klausmeier et al. (2004), by choosing values of f_A (as described above). Then we fit the phytoplankton model with our SPONGE model for uptake kinetics to the data of Rhee (1974). For the Droop model, we took the parameter values of Droop (1974) as initial guesses and estimated SPONGE parameters based on these. Then we fit uptake parameters for both the Droop and SPONGE models to the data of Droop (1974).

To fit the uptake parameters, we used the Monte Carlo Markov Chain, as applied by Hargreaves and Annan (2002) and Smith et al. (2007). The sum of squared errors (cost function) was calculated using the reciprocal of the standard deviation of each nutrient's respective measured cell quota ($1/\sigma_Q$) as the weight for data of the cell quota of that nutrient. The standard deviations were calculated from the data of Rhee (1978), counting data for each nutrient only when it was the limiting nutrient (when its cell quota would ideally be constant for the constant flow rate in those experiments). In the summations for square errors, only data for nonlimiting nutrients were counted, because the quota of limiting nutrient does not depend on the uptake parameters. As in Smith et al. (2007), we performed paired assimilations, one starting at the initial guess parameter values and one at twice those values. A successful fit required that both assimilations converge (statistically) to the same solution. All three models (MM,

GR, and SPONGE) were fit only to the data of Rhee (1974) at extreme N:P ratios. We applied the same methodology to fit both our model and the Droop model (uptake parameters and complexation parameters) to the data of Droop (1974), which included biomass and nutrient concentrations (for which we chose weights for each data type based on the precision of the reported values).

Results

Fits of both the GR and SPONGE models to the data at extreme N:P ratios (Rhee 1974) yielded lower total errors than the initial guesses for both models (Fig. 2; Tables 1, 2), although the difference between the initial guesses and best-fits was much greater for the GR model. For the SPONGE model, we were able to constrain all four parameters ($A_{0,N}$, $A_{0,P}$, $V_{0,N}$, and $V_{0,P}$). For the GR model, it was only possible to constrain the six parameters that determine the values of $V_{max,N}$ and $V_{max,P}$ (Eq. 6); the half-saturation constants were left at their initial guess values. Even with its best-fit solution, the MM model greatly overestimates the cell quota of the nonlimiting nutrient under both P-limitation (Fig. 2A) and N-limitation (Fig. 2B). This is because the MM model contains no mechanism for distinguishing the behavior of a nutrient when it is limiting or not, which is the key behavior here (Rhee 1974). Total square error for this data set was 192 for the GR model, 1.63×10^5 for the MM model, and 2,140 for the SPONGE model.

The GR model fit the data under N-limitation much better, but the SPONGE model fit the data under P-limitation better (Table 2). With the SPONGE model, the plots of cell quota of nonlimiting nutrient versus flow rate were U-shaped (concave up) for all three input N:P ratios (Fig. 2), but with the GR model, cell quota of nonlimiting nutrient decreased monotonically with increasing flow rate (albeit with a very small slope in the N-limited cases). In the case of P-limitation, the data show such a U shape (Fig. 2A), although there is some scatter in the data. In the N-limited cases, the pattern is not clear because of scatter in the data and because the ranges of flow rates measured at the two input ratios barely overlap (Fig. 2B).

For these same best-fit parameter sets we also compared all three models to the data of Rhee (1978) for a range of less extreme N:P ratios at a constant, moderate flow rate of 0.59 d^{-1} (Fig. 3). As found for the MM model (Klausmeier et al. 2004), biomass from both the GR and SPONGE models agreed well with the data (results not shown), with slightly higher biomass for the SPONGE model. In this set of experiments, the phytoplankton were completely flexible in their composition, with the N:P ratio of biomass equal to the N:P ratio of input to the chemostat (Fig. 3A), within experimental error. This is because the phytoplankton consume essentially all nutrients and therefore must have the same N:P ratio as the input. (Because they consume essentially all the nutrients, and because biomass is determined by uptake of the limiting nutrient, biomass must be very similar for the two models.) In the terminology of Klausmeier et al. (2004), they are “supply limited,” meaning that the rate of supply of

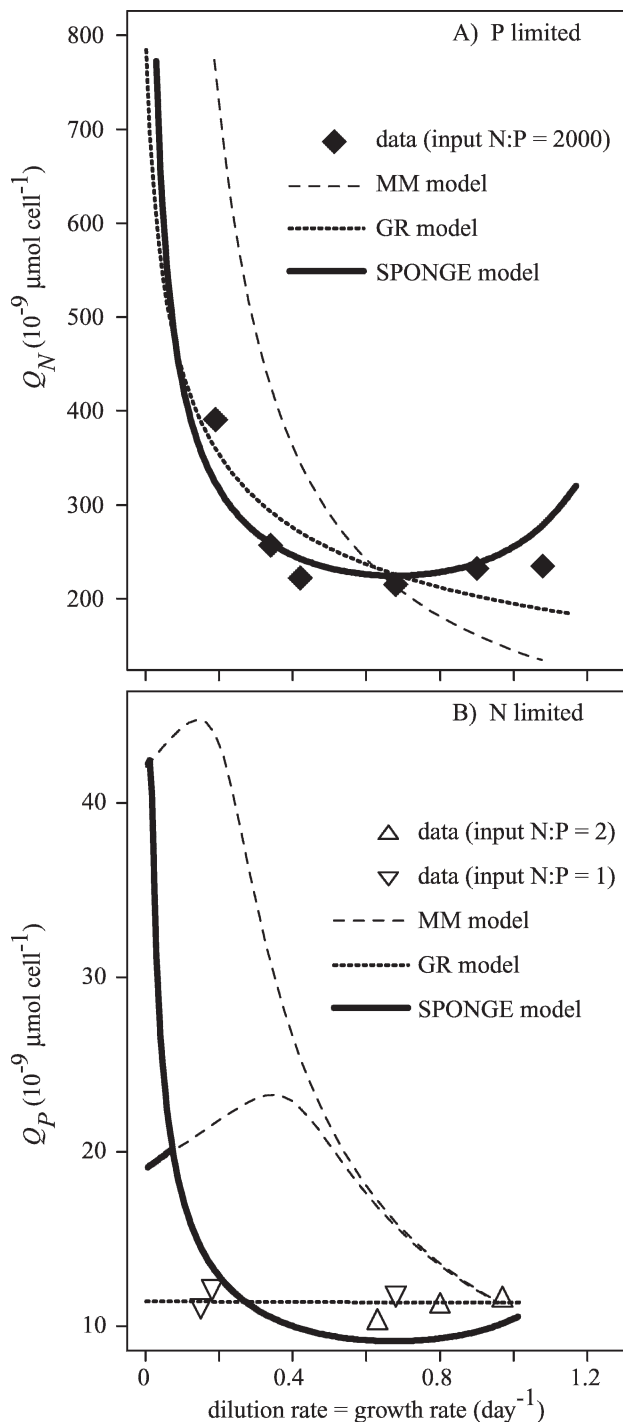


Fig. 2. Cell quota of nonlimiting nutrient versus flow rate for the chemostat experiments of Rhee (1974) at extreme N:P input ratios. (A) Input N:P = 2,000 (P-limitation). (B) Input N:P = 1 or 2 (N-limitation). Lines are models with different uptake kinetics (MM = Michaelis–Menten; GR = Gotham and Rhee [1981*a,b*], and Simple Phytoplankton Optimal Nutrient Gathering Equations [SPONGE] model), each with best-fit values for uptake parameters, fit only to the data of Rhee (1974).

nutrients, rather than the uptake kinetics, limits the uptake rate.

The MM model allows the fastest uptake rates and hence comes closest to having the biomass N:P = input N:P. With these best-fit parameter sets, fit only to the data of Rhee (1974), the GR model fit the data over a range of N:P ratios at constant flow rate (Rhee 1978) best (Fig. 3). Total square error, counting only the data of Rhee (1978), was 1,320 for the GR model, 1,920 for the MM model, and 1,840 for the SPONGE model (Table 2). The MM model had the highest square error, even though it allows the plankton to be essentially completely “supply limited” (Fig. 3A), whereas both the GR and SPONGE models take up less nonlimiting nutrient at extreme N:P ratios. Both the GR and SPONGE models underestimated the P cell quota at the lowest N:P ratios, the latter more severely. However, the lower square error for these two models that are not perfectly “supply limited” and the evident scatter in the data indicate that these differences may not be significant.

We also fit both the SPONGE and GR models simultaneously to the two data sets (Rhee 1974, 1978). This yielded only slightly better overall fits for each model (results not shown), with a similar mismatch between the data and both models for the experiments of Rhee (1978).

As Klausmeier et al. (2004) found for the GR model, our SPONGE model can easily be tuned to match the data of Rhee (1978) over a range of less extreme N:P ratios (results not shown). This requires only that one choose the parameters such that the potential uptake rate exceeds the supply rate for both nutrients at the given (fixed) flow rate, so that the phytoplankton will be “supply limited” over the range of N:P ratios examined. The data from Rhee’s 1974 study, at extreme N:P ratios at various flow rates, constrain the uptake parameters much more tightly than do the data from the 1978 Rhee study. Despite the great difference between the MM model and the other two models for the former data set, the MM model agrees well with the latter data set, for which the differences between models are much smaller than for the former.

We also obtained good fits to the data of Droop (1974) for uptake of vitamin B12 and P as nonlimiting nutrients (Fig. 4), with both a modified version of the model developed in that study (hereafter, Droop model; see Table 3) and our SPONGE model. However, these experiments were complicated by dissolved organic matter (DOM) production and complexation of nutrients. Accounting for this required two additional parameters for each nutrient (Droop 1974), and the Droop model further added one more parameter ($R_{\max,i}$; Table 3) to describe uptake of each nonlimiting nutrient i . We modified the representation of excretion by applying a specific rate of excretion for each nutrient considered, instead of the minimum concentration for uptake applied by Droop (1974). This modification yielded better fits to the data (without increasing the number of model parameters) for both the Droop model and our SPONGE model. As in Droop (1974), we were not able to uniquely constrain values for all (10) uptake parameters in the Droop model; we were able to constrain values for nine parameters,

Table 1. Values of parameters in the phytoplankton model. Minimum cell quotas were fit to the data of Rhee (1974, 1978). Values for uptake parameters are best-fits to the data of Rhee (1974), except for the half-saturation constants for the Gotham and Rhee (GR) model, which are initial guesses because their values could not be constrained. For the best-fit values, standard deviations (SDs) were calculated over the latter half of the ensembles from the Monte Carlo Markov Chain assimilations, only for parameters that were fit to data.

Parameter	Description	Value	SD
a	Flow (dilution) rate of chemostat (d ⁻¹)	Variable	
μ_∞	Growth rate at infinite cell quota (d ⁻¹)	1.35	
$Q_{\min,P}$	Minimum (min.) cell quota of phosphorus (P) (10 ⁻⁹ μmol cell ⁻¹)	1.56	
$Q_{\min,N}$	Minimum cell quota of nitrogen (N) (10 ⁻⁹ μmol cell ⁻¹)	42.0	
m	Mortality rate (d ⁻¹)	a	
Uptake parameters for Michaelis–Menten (MM) model			
$V_{\max,P}$	Maximum (max.) uptake rate of P (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	11.4	0.0274
$V_{\max,N}$	Max. uptake rate of N (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	145	0.0116
K_P	Half-saturation constant for P (μmol L ⁻¹)	3.94×10 ⁻³	2.89×10 ⁻⁵
K_N	Half-saturation constant for N (μmol L ⁻¹)	0.368	0.0430
Uptake parameters for GR model (Gotham and Rhee 1981 <i>a,b</i>)			
$V_{inh,P}$	Max. uptake rate for P (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	1,970	600
$V_{inh,N}$	Max. uptake rate for N (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	33,200	7,870
$K_{inh,P}$	Half-saturation constant for P inhibition (10 ⁻⁹ μmol cell ⁻¹)	24.3	8.76
$K_{inh,N}$	Half-saturation constant for N inhibition (10 ⁻⁹ μmol cell ⁻¹)	1.13	2.02
$C_{inh,P}$	Feedback parameter (param.) for P inhibition (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	4,840	676
$C_{inh,N}$	Feedback param. for N inhibition (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	49.2	18.2
K_P	Half-saturation constant for P (μmol L ⁻¹)	0.2	
K_N	Half-saturation constant for N (μmol L ⁻¹)	5.6	
Uptake parameters for the Simple Phytoplankton Optimal Nutrient Gathering Equations (SPONGE) model			
$V_{0,P}$	Max. potential uptake rate of P (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	1.70	0.148
$V_{0,N}$	Max. potential uptake rate of N (10 ⁻⁹ μmol cell ⁻¹ d ⁻¹)	43.1	6.74
$A_{0,P}$	Max. potential affinity for P (10 ⁻⁹ L cell ⁻¹ d ⁻¹)	9.92×10 ⁵	19.6×10 ⁹
$A_{0,N}$	Max. potential affinity for N (10 ⁻⁹ L cell ⁻¹ d ⁻¹)	56.1	4.56×10 ⁸

leaving $R_{\max,B}$ fixed at its value from Droop (1974). For the SPONGE model, we were able to constrain all (eight) uptake parameters.

With the best fits, total square error (a weighted sum counting biomass, nutrient concentrations, and cell quota of nonlimiting nutrient) for the Droop model was approximately 1.5 times that for the SPONGE model (Table 4). Counting only the cell quotas of nonlimiting nutrient, the square error for the Droop model was approximately four times that for the SPONGE model.

The SPONGE model also agreed better with the data for biomass (Table 4). For experiments II and IV (vitamin B12–limited), the Droop model, which uses Michaelis–Menten kinetics for uptake of limiting nutrient, tended to slightly overestimate biomass at low flow rates and to underestimate it at high flow rates (Fig. 5). The SPONGE model also agreed better with the concentrations of nutrients (Fig. 5), although the differences between models were less consistent than for the other data. Because of the complications from DOM production and complexation,

Table 2. Square errors, counting only data for cell quota of nonlimiting nutrient for each model with respect to the data of Rhee (1974, 1978). Each model was fit only to the data of Rhee (1974). Data for the cell quota of each nutrient were weighted by the reciprocal of their respective standard deviation, σ_Q^{-1} , calculated from the data of Rhee (1978) only when the respective nutrient was limiting, to yield $\sigma_{QP} = 0.11 \times 10^{-9}$ and $\sigma_{QN} = 10.9 \times 10^{-9} \mu\text{mol cell}^{-1}$.

Data set	GR model		MM model		SPONGE model	
	IG	Best	IG	Best	IG	Best
Rhee (1974) (extreme N:P ratios)						
P-limited	277	64.9	2.48×10^4	1,660	97.9	59.2
N-limited	1,809†	127	1.87×10^5	1.61×10^5	2,213	2,083
Both	2,090	192	2.12×10^5	1.63×10^5	2,310	2,140
Rhee (1978) (variable N:P ratios)						
P-limited	53.6	13.0	10.2	10.2	10.8	19.4
N-limited	1,330	1,302	1,660	1,907	2,725	1,821
Both	1,380	1,320	1,670	1,920	2,740	1,840
Both data sets						
P-limited	331	77.9	2.48×10^4	1,670	109	78.6
N-limited	3,139	1,429	1.89×10^5	1.63×10^5	4,938	3,904
Both	4,470	1,510	2.14×10^5	1.65×10^5	5,050	3,980

* GR, Gotham and Rhee [model]; MM, Michaelis–Menten [model]; SPONGE, Simple Phytoplankton Optimal Nutrient Gathering Equations [model]; IG, initial guess; Best, best-fit; N, nitrogen; P, phosphorus.

† Excluding the data point at flow rate = 0.97 d^{-1} , for which the initial guess values of uptake parameters did not allow a solution, because uptake of limiting nutrient was insufficient.

this data set provides a less rigorous test of the uptake models than do those of Rhee (1974, 1978). Still, the SPONGE model performed better than the model of Droop (1974), even though the latter includes one more parameter for each nonlimiting nutrient.

Discussion

Our new model for uptake kinetics of multiple nutrients is based on the premise that phytoplankton optimize their allocation of fixed internal resources for uptake hardware to adapt to changes in ambient concentration of limiting nutrient, without regard to changes in ratios of nutrient concentrations. This differs significantly from the assumption that uptake rate of a given nutrient is controlled by feedback from its own internal nutrient concentration (cell quota). Such feedback processes may indeed be one means by which phytoplankton control the allocation of their internal resources, but the difference in modeling approach is important, yielding a much simpler model in our case.

We have not yet addressed the complication of nutrients taken up in more than one chemical form (e.g., nitrate and ammonium). Armstrong (1999) developed an optimization-based model specifically for the iron–light–ammonium co-limitation of nitrate uptake, based on optimal allocation of cellular resources and Michaelis–Menten kinetics. This indicates a way to extend the SPONGE model for nitrate and ammonium. Optimization-based approaches have also yielded simpler models of photoacclimation (Pahlow 2005; Armstrong 2006) that capture its complex dynamics at least as well as earlier, more complex models. Optimization-based modeling can yield simpler models that are applicable over a wider range of conditions, but validation is still critical, because (as in this study) organisms are generally

found to be less than perfectly adapted (Rose and Lauder 1996).

As expected, the GR model, with four more parameters (two more for each nutrient), fits the data better than our SPONGE model. Together with its simplicity, the good agreement of our model with the data under N-, P-, and vitamin B12-limitation indicates that it may be mechanistically correct. Our model is consistent with the idea that evolution in the ocean should favor the ability to acclimate to variations in nutrient concentrations in times of relatively rapid growth (i.e., after upwelling events) and the idea that there should be less evolutionary advantage in the ability to acclimate to variations in nutrient ratios, which occur mainly when nutrient concentrations, and hence growth rates, are low. However, we have no proof that this is the case. Likewise, Gotham and Rhee (1981a) stated that "... the empirical form ... [of their inhibition model] ... is not sufficient evidence to invoke a specific physiological mechanism...."

Our model does not agree perfectly with both the data at extreme N:P ratios (Rhee 1974) and that at less extreme ratios (Rhee 1978). The significant scatter in the data, especially in the latter data set, indicates that this difference is not so important, however. It is also possible that this disagreement results from differences in experimental conditions or some genetic difference in the strain of *Scenedesmus* sp. used in the two sets of experiments. Droop (1974) observed distinctly different growth patterns for *Monochrysis* sp. in chemostats at low ($<0.45 \text{ d}^{-1}$) versus higher flow rates. He was not able to determine whether this difference was an induced adaptation or the result of a genetic inhomogeneity in the clone of phytoplankton cultured. It is impossible to conclude anything based on this small disagreement between our model and the data of

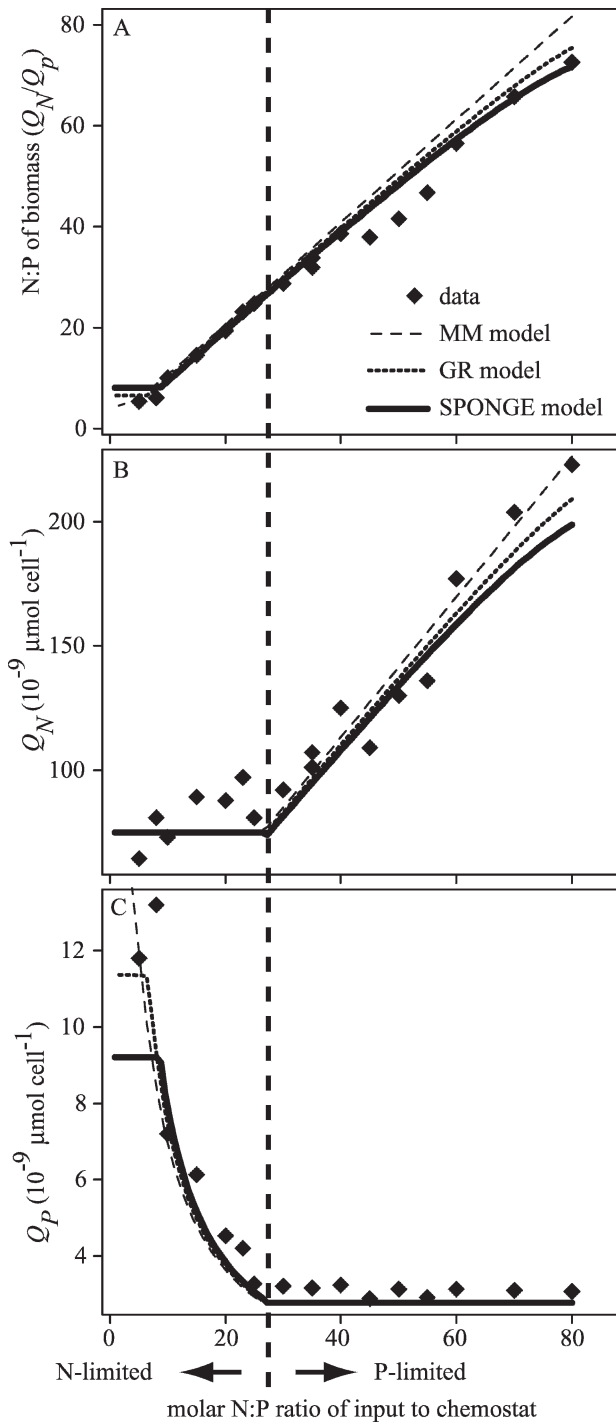


Fig. 3. (A) Molar N:P ratio of biomass and cell quotas of (B) N and (C) P versus N:P ratio of input to the chemostat for the experiments of Rhee (1978) at constant flow rate (0.59 d^{-1}). Lines are models (same as in Fig. 2). The phytoplankton switch from N-limitation to P-limitation at their optimal N:P ratio of 27:1 (dashed vertical line).

Rhee (1978) for this one species. Our model needs to be further validated with more data for multiple species.

Plasticity of phytoplankton composition—Because at steady state in a chemostat the uptake rate of any nutrient

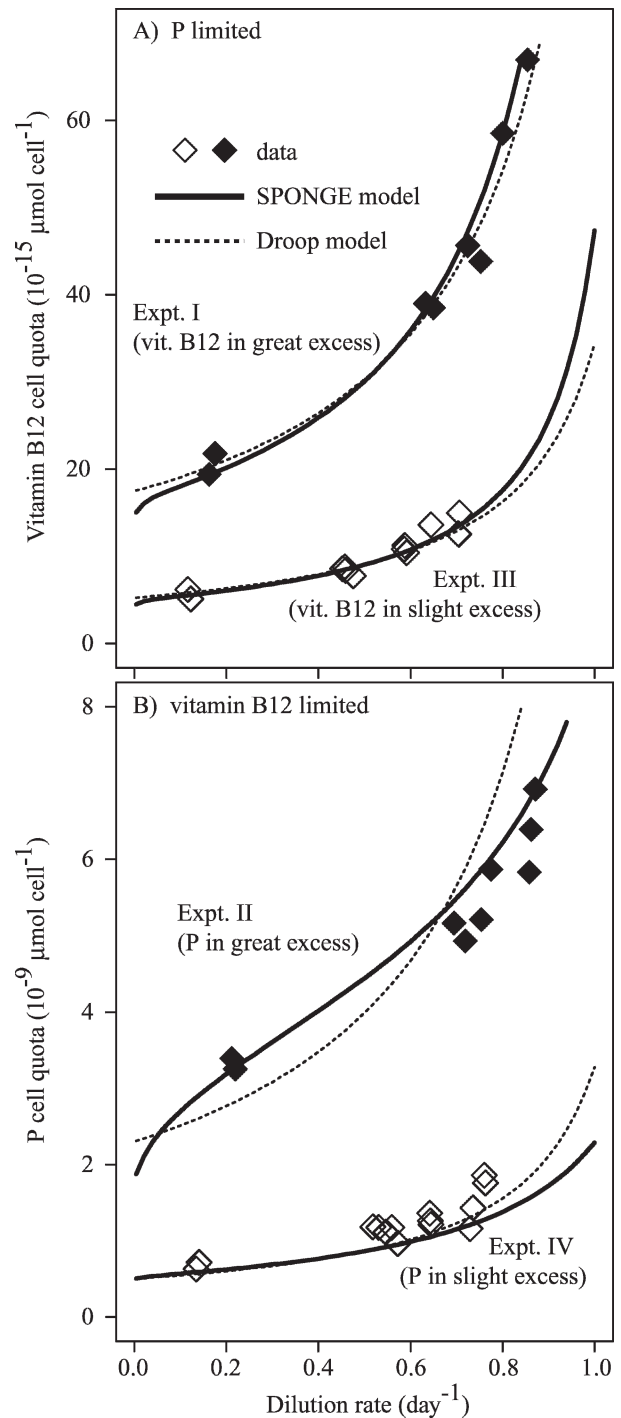


Fig. 4. Data for cell quotas of nonlimiting nutrients in the experiments of Droop (1974), under both vitamin B12- and P-limitation, with different degrees of excess for each nutrient. Lines are models with different uptake kinetics (Droop = Droop [1974], as modified here, and Simple Phytoplankton Optimal Nutrient Gathering Equations [SPONGE]), each with best-fit values for uptake and nutrient complexation parameters (Table 3).

must equal its cell quota times the growth rate (=dilution rate), $v = \mu Q$, the ratio of uptake rates of any two nutrients must equal the ratio of their cell quotas: $v_1/v_2 = Q_1/Q_2$. At low flow rates, the measured N:P of biomass (ratio of

Table 3. Parameters in the phytoplankton model of Droop (1974) and our Simple Phytoplankton Optimal Nutrient Gathering Equations (SPONGE) model (both with our modified treatment of excretion). Uptake parameters for each were fit to the same data for *Monoschrysis lutheri* (Droop 1974). In the Droop model, Michaelis–Menten kinetics are applied for the limiting nutrient, and the uptake rate of nonlimiting nutrient is a multiple, R_n , of that for the limiting nutrient.

Parameter	Description	Value
μ_∞	Growth rate at infinite cell quota (d^{-1})	1.18
μ	Growth rate (d^{-1})	
$Q_{\min,P}$	Minimum (min.) cell quota of phosphorus (P) ($10^{-9} \mu\text{mol cell}^{-1}$)	0.37
$Q_{\min,B}$	Min. cell quota of vitamin B12 ($10^{-15} \mu\text{mol cell}^{-1}$)	2.45
B	Biomass (cells L^{-1})	
S_i	Free dissolved concentration of nutrient i	
β_i	Ratio of complexed to free dissolved concentration of i , $\beta_i = (\beta \mu^{-1} \tau_i / \kappa_i)$	
T_i	Total dissolved concentration of nutrient i , $T_i = S_i(1 + \beta_i)$	
Uptake parameters for Droop model		
$V_{\max P}$	Maximum (max.) uptake rate of P ($10^{-9} \mu\text{mol cell}^{-1} \text{d}^{-1}$)	1,760
$V_{\max B}$	Max. uptake rate of vitamin B12 ($10^{-15} \mu\text{mol cell}^{-1} \text{d}^{-1}$)	280
K_P	Half-saturation constant for P ($\mu\text{mol L}^{-1}$)	0.184
K_B	Half-saturation constant for vitamin B12 ($\mu\text{mol L}^{-1}$)	2.30×10^{-5}
τ_P / κ_P	Complexation factor for P ($\text{L cell}^{-1} \text{d}^{-1}$)	9.73×10^{-7}
τ_B / κ_B	Complexation factor for vitamin B12 ($\text{L cell}^{-1} \text{d}^{-1}$)	1.68×10^{-10}
v_L	Uptake rate of limiting nutrient $L = V_{\max, L} S_L / (K_L + S_L)$	
r_P	Specific rate of excretion of P (d^{-1})	0.0931
r_B	Specific rate of excretion of vitamin B12 (d^{-1})	0.910
R_n	Luxury uptake ratio, $R_n^{-1} = \left(1 - R_{\max,n}^{-1}\right) (S_{in,L} / S_{in,n}) (Q_{\min,n} / Q_{\min,L}) + R_{\max,n}^{-1}$	
$R_{\max,P}$	Max. ratio for luxury uptake of P	10.4
$R_{\max,B}$	Max. ratio for luxury uptake of vitamin B12	∞
Uptake parameters for SPONGE model		
$V_{0,P}$	Max. potential uptake rate of P ($10^{-9} \mu\text{mol cell}^{-1} \text{d}^{-1}$)	7,916
$V_{0,B}$	Max. potential uptake rate of vitamin B12 ($10^{-15} \mu\text{mol cell}^{-1} \text{d}^{-1}$)	1.23×10^6
$A_{0,P}$	Max. potential affinity for P ($10^{-9} \text{L cell}^{-1} \text{d}^{-1}$)	16.1
$A_{0,B}$	Max. potential affinity for vitamin B12 ($10^{-15} \text{L cell}^{-1} \text{d}^{-1}$)	4.54×10^5
τ_P / κ_P	Complexation factor for P ($\text{L cell}^{-1} \text{d}^{-1}$)	1.06×10^{-10}
τ_B / κ_B	Complexation factor for vitamin B12 ($\text{L cell}^{-1} \text{d}^{-1}$)	3.88×10^{-5}
r_P	Specific rate of excretion of P (d^{-1})	1.92
r_B	Specific rate of excretion of vitamin B12 (d^{-1})	0.190

measured N and P cell quotas) is closest to that of the input (Fig. 6), as expected when phytoplankton consume nearly all available nutrients (Klausmeier et al. 2004). It never equals the input ratio, however. This may be because the lowest flow rate measured is not low enough for the phytoplankton to be completely “supply limited” at these extreme nutrient ratios. Alternatively, there may be a limit to the plasticity of phytoplankton composition. In a study of field observations and mesocosm and laboratory experiments, Hall et al. (2005) found that the N:P ratio of phytoplankton biomass generally did not closely track the N:P ratio of nutrient supply. They showed that besides the effect of growth rate, limits on the ability of phytoplankton to store nutrients (imposing maximum cell quotas in models) could also account for that finding.

In both the MM and SPONGE models, the N:P of biomass approaches the input N:P ratio as flow rate approaches zero (Fig. 6), which is theoretically appealing. In the inhibition model, however, it does not, because of the strong negative feedback between cell quota and uptake rate of nonlimiting nutrient. In the SPONGE model, the N:P of biomass is independent of the input ratio in the N-

limited experiments for all but very low flow rates, where it diverges to match the distinct input ratios.

As is the case for the MM model (Klausmeier et al. 2004), for our SPONGE model the uptake of nonlimiting nutrient becomes independent of the input ratio when its uptake is saturated. In our model, this happens at a lower flow rate (and, therefore, at a lower concentration of nonlimiting nutrient). This is because the optimization (Eq. 14) varies the uptake parameters according to the concentration of limiting nutrient, S_{lim} , which also increases with flow rate (shown for the case of N-limitation in Fig. 7A). The affinities for uptake of both limiting and nonlimiting nutrient are proportional to f_A (Eq. 12). They both decrease with flow rate, because of the inverse relationship between concentration of limiting nutrient and f_A (Eq. 14; Fig. 7B). The maximum uptake rates of both limiting and nonlimiting nutrients are proportional to $1 - f_A$ (Eq. 13) and therefore increase with flow rate (Fig. 7C). Because uptake is optimized for the limiting nutrient, its uptake rate increases more steeply with flow rate. Over the whole range of flow rates, the uptake of nonlimiting nutrient (in this case P) is

Table 4. Square errors for fits of the Droop model and the Simple Phytoplankton Optimal Nutrient Gathering Equations (SPONGE) model (both with our modified treatment of excretion) to the data of Droop (1974). For fitting, errors were calculated including data for biomass, nutrient concentrations, and cell quotas of nonlimiting nutrient from all four experiments. To calculate square errors, each data type was weighted by the reciprocal of its respective standard deviation, σ^{-1} , which we estimated as 1.0×10^8 cells L^{-1} for biomass, 7.4×10^{-10} $\mu\text{mol cell}^{-1}$ for phosphorus (P) quota, 4.9×10^{-15} $\mu\text{mol cell}^{-1}$ for vitamin B12 quota, 2.0×10^{-7} mol L^{-1} for total dissolved P concentration, and 1.0×10^{-12} mol L^{-1} for total dissolved vitamin B12 concentration.*

Data set	Droop model		SPONGE model	
	IG	Best	IG	Best
Error counting only cell quota of nonlimiting nutrient				
P-limited (experiments I and III)	2.72	2.72	214	3.39
Vitamin B12-limited (experiments II and IV)	44.8	34.9	74.8	5.71
All four experiments	47.5	37.6	289	9.10
Error counting only biomass				
P-limited (experiments I and III)	124	56.4	125	54.9
Vitamin B12-limited (experiments II and IV)	146	47.4	69	31.0
All four experiments	270	104	194	86.0
Error counting only concentration of phosphate				
P-limited (experiments I and III)	8.04	3.94	3.26	5.64
Vitamin B12-limited (experiments II and IV)	756	83.1	1,067	60.0
All four experiments	765	87.0	1,070	65.7
Error counting only concentration of vitamin B12				
P-limited (experiments I and III)	507	18.6	6,627	17.7
Vitamin B12-limited (experiments II and IV)	49.8	4.57	3.82	0.927
All four experiments	557	23.1	6,631	18.7
Error counting all data types				
P-limited (experiments I and III)	642	81.6	6,970	81.7
Vitamin B12-limited (experiments II and IV)	997	170	1,215	97.7
All four experiments	1,640	252	8,185	179

* IG, initial guess; Best, best-fit.

nearly saturated, whereas that of the limiting nutrient is less saturated (compare Fig. 7C,D). For the limiting nutrient, both the maximum uptake rate and the degree of saturation of the uptake expression (Eq. 11) increase with flow rate. Therefore, the ratio of uptake rates, v_{lim}/v_{non} , increases with flow rate (Fig. 7E), and this increase occurs at lower flow rates than for the MM model (Fig. 6B).

Critical flow rate—The models also differ in their critical flow rate, a_c , the maximum possible flow rate that can be sustained in a chemostat. Legovic and Cruzado (1997) showed that for their model with Michaelis–Menten uptake kinetics, a_c depends on the maximum uptake rate of limiting nutrient, $V_{max,lim}$, and its inflow concentration, $S_{in,lim}$:

$$a_c = \left[\frac{Q_{0,lim}(K_{s,lim}/S_{in,lim} + 1)}{V_{max,lim}} + \frac{1}{\mu_{\infty}} \right]^{-1} \quad (20)$$

which, together with Eq. 2 at $S_{in,lim}$, yields

$$a_c = \left[\frac{Q_{0,lim}}{v_{lim}(S_{in,lim})} + \frac{1}{\mu_{\infty}} \right]^{-1} \quad (21)$$

Thus, the critical flow rate increases with $S_{in,lim}$. This

equation requires that, for the uptake parameters specified, $S_{in,lim}$ is sufficient to sustain growth rate $\mu \leq a_c$.

Pahlow's optimization for uptake of the limiting nutrient (Eq. 15) yields a greater uptake rate, v_{lim} , than does a Michaelis–Menten equation with corresponding parameters at any concentration except one (the “matching concentration”). Thus, the same $S_{in,lim}$ will sustain higher growth rates with the SPONGE model, unless the uptake parameters are such that the two models match at that concentration. Therefore a_c is, in general, greater with optimal uptake kinetics than with Michaelis–Menten kinetics, if parameters for the SPONGE model are chosen to “match” the parameters of the MM model. However, when parameters for the SPONGE model are chosen independently (as in our fits), the critical flow rate can be lower. With the best-fit parameter values for our SPONGE model, the critical flow rates were lower (0.92 d^{-1} and 1.01 d^{-1} for the cases with $S_{in,N}/S_{in,P} = 1$ and 2, respectively) than with the initial guesses.

Possible experimental validation—Directly validating the optimization mechanism in our model would require identifying and quantifying uptake sites on the cell surface and internal enzymes. Besides this, our model can also be tested using chemostat experiments. Experiments at very

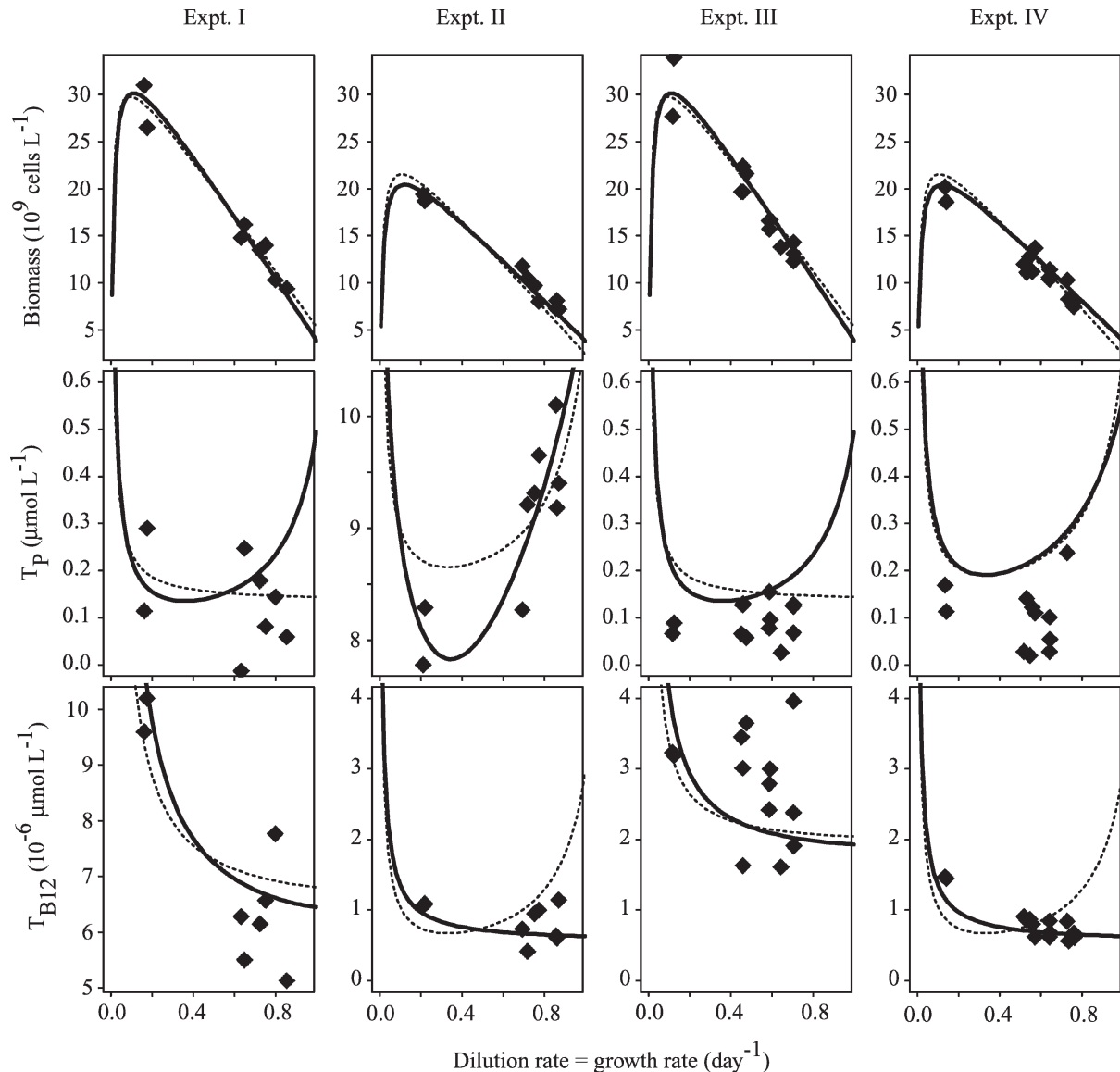


Fig. 5. Data (diamonds) for biomass and total dissolved concentrations of P and vitamin B12 (T_P and T_{B12} , respectively) from the experiments of Droop (1974). Plots are organized by data type (rows) and by experiment (columns). Lines are the same models as in Fig. 4: (thin dotted lines) Droop = Droop (1974), as modified here, and (thick solid lines) Simple Phytoplankton Optimal Nutrient Gathering Equations (SPONGE), each with best-fit values for uptake and nutrient complexation parameters (Table 3).

low flow rates and different input ratios of nutrients could reveal whether phytoplankton composition does in fact approach the input ratio, as predicted by our model but not by the GR model. Experiments at very high flow rates could test the dependence of critical flow rate on uptake parameters, although the differences may be small and the experiments difficult. Care must be taken to minimize complications from DOM and nutrient complexation and to plan experiments so that the data can provide constraints on rate expressions for nutrient uptake. The latter implies that researchers make certain that the uptake of nutrients is limited by the kinetics, as in the experiments of Rhee (1974) and Droop (1974), rather than by the rate of nutrient supply, as in the experiments of Rhee (1978).

Measurements of biomass may provide the easiest test. With our best-fit parameter values for the experiments of Rhee (1974), the difference between our model and the GR model increased with flow rate. For the experiment with input N:P ratio = 1, at $a = 0.90 d^{-1}$, biomass in our SPONGE model versus the GR model was 1.1×10^7 versus 6.1×10^7 cells L^{-1} , respectively. Measurements of biomass would therefore be useful for testing these models, but unfortunately there were no biomass measurements for these experiments. For the experiments of Droop (1974), the differences in biomass between the Droop and SPONGE models were less dramatic (Fig. 5), but the SPONGE model did agree better with the data for both biomass and cell quotas (Table 4), especially for the experiments in which vitamin B12 was limiting.

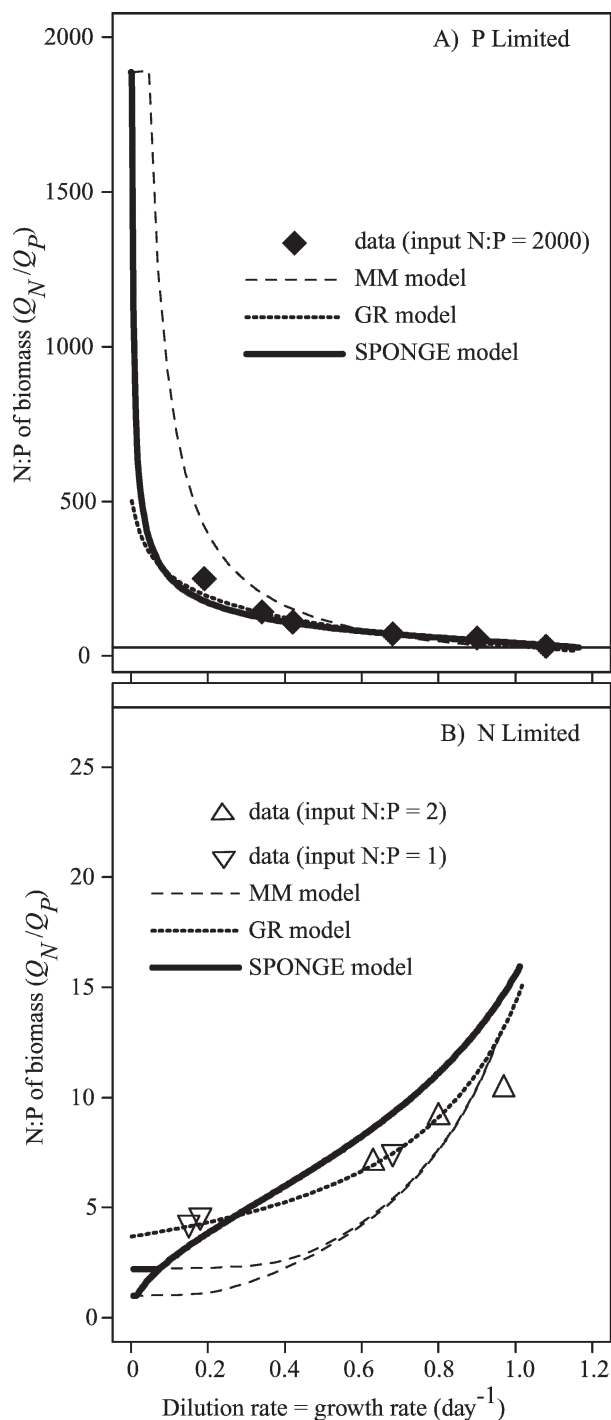


Fig. 6. Molar N:P ratio of biomass versus flow rate for the chemostat experiments at extreme N:P input ratios under (A) P-limitation (input N:P = 2,000) and (B) N-limitation (two cases, with input N:P = 1 and 2). All models (same as Fig. 2) are with best-fit parameter values. Solid horizontal lines show the optimal N:P ratio for this species (27:1).

Although we do not have direct proof for the assumed optimization of uptake hardware, based on our results we propose the hypothesis that phytoplankton do optimize their uptake hardware for changes in concentration of

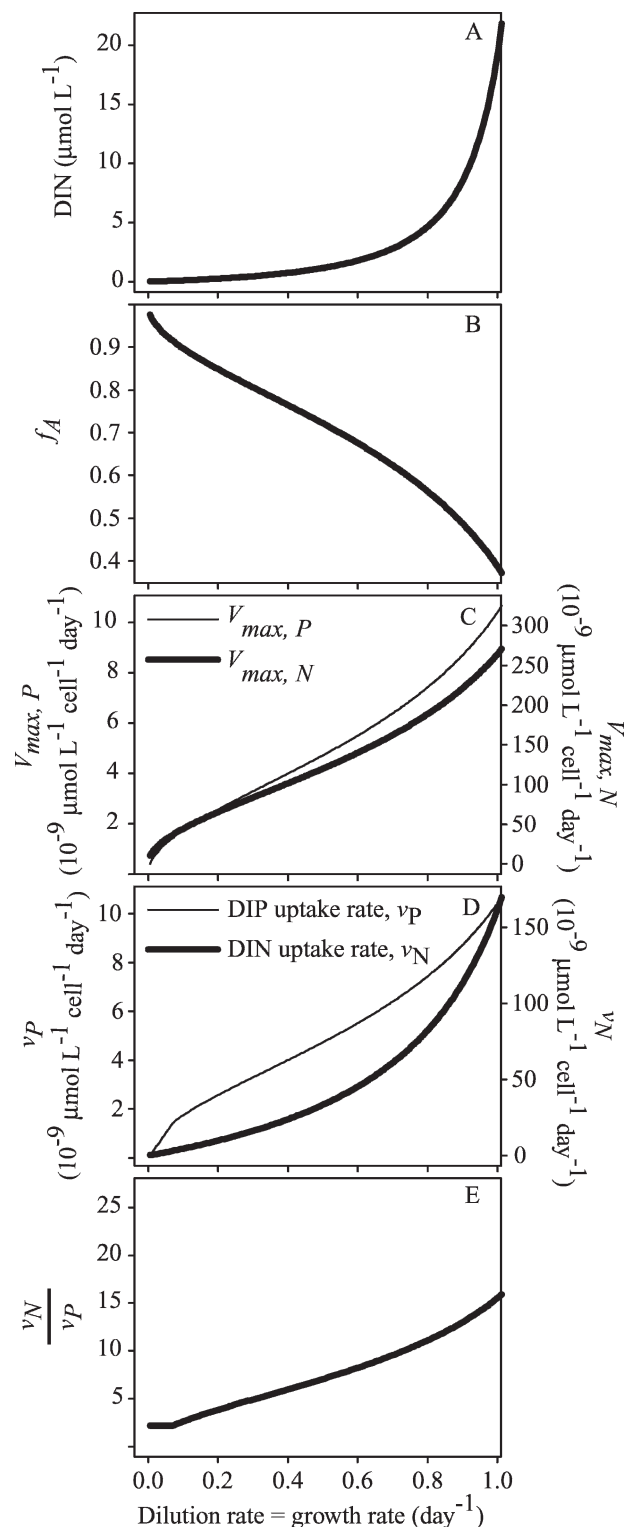


Fig. 7. For the SPONGE model applied to the N-limited chemostat experiments at input N:P ratio = 2 (Rhee 1974). (A) Dissolved inorganic nitrogen (DIN) concentration, (B) allocation of internal nitrogen to surface uptake sites, f_A , (C) maximum uptake rates of DIN and dissolved inorganic phosphorus (DIP), (D) actual uptake rates of DIN and DIP, and (E) ratio of DIN:DIP uptake rates.

limiting nutrient, but not for changes in ratios of nutrient concentrations. Differences between our model's predictions and those of other models indicate several means of testing this hypothesis with experiments.

Quantitative comparisons show that our SPONGE model agrees well with data from both N- and P-limited chemostat experiments at extreme nutrient ratios and at ratios more typical of natural environments. Our model also agrees well with the data of Droop (1974) for a different species under various degrees of both vitamin B12- and P-limitation. Although it agrees with data for these nutrients, there is reason to believe that uptake of trace metals and other micronutrients may differ, based on genetic differences among species and on the relative abundances of nutrients in phytoplankton versus seawater (Quigg et al. 2003). For multinutrient applications, at least with macronutrients and possibly with others, we propose this model as a more versatile alternative to Michaelis-Menten uptake kinetics.

References

- AKSNES, D. L., AND J. K. EGGE. 1991. A theoretical model for nutrient uptake in phytoplankton. *Mar. Ecol. Prog. Ser.* **70**: 65–72.
- ARMSTRONG, R. A. 1999. An optimization-based model of iron-light-ammonium colimitation of nitrate uptake. *Limnol. Oceanogr.* **44**: 1436–1446.
- . 2006. Optimality-based modeling of nitrogen allocation and photoacclimation in photosynthesis. *Deep-Sea Res. II* **53**: 513–531.
- CAPERON, J. 1968. Population growth response of *isochrysis galbana* to nitrate variation at limiting concentrations. *Ecology* **49**: 866–872.
- DROOP, M. R. 1968. Vitamin B12 and marine ecology. 4. The kinetics of uptake, growth and inhibition of *Monochrysis lutheri*. *J. Mar. Biol. Assoc. UK* **48**: 689–733.
- . 1974. The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Assoc. UK* **54**: 825–855.
- GOTHAM, I. J., AND G.-Y. RHEE. 1981a. Comparative kinetic studies of nitrate-limited growth and nitrate uptake in phytoplankton in continuous culture. *J. Phycol.* **17**: 309–314.
- , AND ———. 1981b. Comparative kinetic studies of phosphate-limited growth and phosphate uptake in phytoplankton in continuous culture. *J. Phycol.* **17**: 257–265.
- HALL, S. R., V. H. SMITH, D. A. LYTLE, AND M. A. LEIBOLD. 2005. Constraints on primary producer N:P stoichiometry along N:P supply ratio gradients. *Ecology* **86**: 1894–1904.
- HARGREAVES, J. C., AND J. D. ANNAN. 2002. Assimilation of paleodata in a simple earth system model. *Clim. Dyn.* **19**: 371–381.
- HARRISON, G., AND E. M. M. MOREL. 1986. Response of the marine diatom *Thalassiosira weissflogii* to iron stress. *Limnol. Oceanogr.* **31**: 989–997.
- KLAUSMEIER, C. A., E. LITCHMAN, AND S. A. LEVIN. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. *Limnol. Oceanogr.* **49**: 1463–1470.
- LEGOVIC, T., AND A. CRUZADO. 1997. A model of phytoplankton growth on multiple nutrients based on the Michaelis-Menten-Monod uptake, Droop's growth and Liebig's Law. *Ecol. Model.* **99**: 19–31.
- MOREL, F. M. M. 1987. Kinetics of nutrient uptake and growth in phytoplankton. *J. Phycol.* **23**: 137–150.
- PAHLOW, M. 2005. Linking chlorophyll-nutrient dynamics to the Redfield N:C ratio with a model of optimal phytoplankton growth. *Mar. Ecol. Prog. Ser.* **287**: 33–43.
- QUIGG, A., AND OTHERS. 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature* **425**: 291–294.
- RHEE, G.-Y. 1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. *J. Phycol.* **10**: 470–475.
- . 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.* **23**: 10–25.
- ROSE, M. R., AND G. V. LAUDER. 1996. *Adaptation*. Academic Press.
- SMITH, S. L., B. E. CASARETO, M. P. NIRLAULA, Y. SUZUKI, J. C. HARGREAVES, J. D. ANNAN, AND Y. YAMANAKA. 2007. Examining the regeneration of nitrogen by assimilating data from incubations into a multi-element ecosystem model. *J. Mar. Syst.* **64**: 135–152.
- TERRY, K. L., E. A. LAWS, AND D. J. BURNS. 1985. Growth rate variation in the N:P requirement ratio of phytoplankton. *J. Phycol.* **21**: 323–329.

Received: 24 May 2006
Accepted: 18 February 2007
Amended: 4 January 2007