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An experimentally validated nitrate–ammonium–phytoplankton model including effects of starvation length and ammonium inhibition on nitrate uptake

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ABSTRACT

Nitrate and ammonium are the two most important ionic forms of inorganic nitrogen driving biomass production in marine and freshwater aquatic systems. The performance of plants and algae often changes when reared with either of these two forms of nitrogen individually, as well as when they are both present, or when cells have experienced previous periods of nitrogen starvation. Current functional responses quantifying how ambient nitrogen drives changes in population density are unable to capture interacting and transient effects of nitrate and ammonium. Hence, in this paper we formulate, calibrate, and test a new nitrate–ammonium quota model that accounts for nitrate and ammonium uptake, as well as the effects of nitrogen starvation length and ammonium-induced nitrate uptake inhibition. We fit the model with several time-series from the green alga *Chlorella* sp. reared in laboratory batch cultures under multiple initial conditions. We show that a single set of calibrated model parameters can capture time-series collected from experiments inoculated at 12 different initial concentrations of nitrate, ammonium, and biomass. The model also performed well when validated against time-series from two novel initial conditions withheld from model calibration. Our model therefore provides a framework for evaluating the potential broader ecological and environmental consequences of ambient nitrate and ammonium regimes for phytoplankton communities in nature and aquaculture.

1. Introduction

All living organisms require nitrogen (N) for the production of new biomass. While heterotrophic organisms rely exclusively on organic N from their diet, autotrophic organisms can also absorb inorganic N from the environment (Crawford et al., 2000). Ammonium and nitrate are the two most common ionic (reactive) forms of inorganic N, and their assimilation by plants and photosynthetic algae quantitatively dominates the nitrogen cycle (Gruber, 2008; Zehr and Ward, 2002). However, the way autotrophic organisms incorporate these two N differs. Ammonium is easier to assimilate because most amino acids are in the same oxidation state; in contrast, nitrate must be first reduced to ammonium by means of specialized enzymes and then assimilated (Berges, 1997; Guerrero et al., 1981; Syrett, 1981). This key difference between nitrate and ammonium assimilation leads to different assimilation kinetics in autotrophic organisms, which have far reaching implications in many areas, including understanding changes in species competition (Donald et al., 2011; Jackson et al., 1989), evaluating the effects of eutrophication (Cox et al., 2009), quantifying fluxes of the nitrogen cycle (Fowler et al., 2013), and analyzing optimal fertilization for industrial production (Michalczyk et al., 2014).

Assimilation of nitrate and ammonium is particularly important for phytoplankton, estimated to be responsible for around 30–40% of global primary productivity (Duarte and Cebrià n, 1996). Nitrate and ammonium concentrations in natural environments affect phytoplankton ecology, by selecting for different phytoplankton species (Donald et al., 2011) or modifying the risk for algal bloom formation (Dugdale et al., 2007). Because of the importance of N sources in phytoplankton ecology, numerous studies over the last 40 years have documented a range of processes

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regulating nitrate–ammonium assimilation kinetics in phytoplankton cells (Dortch, 1990; Flynn et al., 1997). First, phytoplankton cells can display different degrees of specialization toward ammonium or nitrate by presenting better kinetic parameters when reared with either source of N (here referred to as "preference"; reviewed in Dortch, 1990). Second, supplying ammonium can repress the nitrate uptake of a cell by either altering the activity of specific transport enzymes or by preventing their synthesis (Berges, 1997; L'Helguen et al., 2008; "inhibition"; Morris and Syrett, 1963; although not all species are affected, Mulholland and Lomas, 2008). Physiological studies have determined that the observed ammonium-induced inhibition is a product of the ammonium assimilation pathway, which often impairs the ability of a cell to assimilate nitrate (Rigano et al., 1979; Syrett and Morris, 1963). Third, periods of N starvation can lead to an initial delay in nitrate assimilation and cell division ("starvation"; De La Rocha et al., 2010; Dortch et al., 1982; Martinez, 1991). However, our understanding of these phenomena is incomplete; in particular, we do not know how processes of preference, starvation and inhibition can interact to simultaneously influence phytoplankton dynamics under different nitrate and ammonium concentrations. This aspect can be important in multiple fields. In nature, phytoplankton communities can be exposed to periods of N starvation with episodic and cyclic resupplies of nitrate or ammonium (Priddle et al., 1997; Young and Beardall, 2003). In aquaculture, imposing periods of N limitation can increase the quality of the final product by increasing the specific lipid content in the biomass (Griffiths et al., 2014). Finally, managing aquatic environments also involve regulating nitrate-polluting (e.g. land clearing, agriculture) and ammonium-polluting activities (e.g. human waste discharge, intensive livestock) in order to minimize risks of algal bloom formation (Domingues et al., 2011).

Some time ago, Dortch (1990) called for an improved approach for quantifying N utilization in single species to make better sense of phytoplankton dynamics in nature. Molecular methods of measuring the activity of assimilatory enzymes can provide important information about N utilization (Fan et al., 2003; Lomas, 2004), but quantifying N uptake and its conversion into producer biomass still requires monitoring total phytoplankton assimilation (either directly with isotope techniques, or indirectly from ambient N depletion) and producer population densities (Bronk et al., 2007). Typically, species-specific kinetic estimates for per-cell N uptake are calculated by dividing N consumed by cell density at successive points in time, and then fitting a saturating Michaelis–Menten functional response (Laws et al., 2011; Maguer et al., 2007; Tantanasarit et al., 2013). While convenient when analyzing rates of N utilization under constant nutrient regimes, this technique cannot tractably capture the functional relationships that govern important processes, such as interactions between nitrate and ammonium uptake and acclimatization following extended starvation periods. Furthermore, the precision of this technique is limited by the fact that each estimate for per capita uptake rate is based on only two observations at successive times. One way forward is to develop a more process-oriented framework for modeling nitrate and ammonium utilization in phytoplankton also accounting for the interactive and transient dynamics involved in this process.

In this study, we develop, calibrate, and test a model to characterize nitrate–ammonium utilization of phytoplankton populations reared in laboratory conditions, including transient effects of preference, starvation, and inhibition. The only previous models describing nitrate–ammonium utilization in phytoplankton cells (without the effect of starvation) are very detailed, explicitly characterizing the main biochemical processes that regulate the flows between multiple internal pools of different N forms (Flynn and Fasham, 1997; Flynn et al., 1997). Such a modeling approach requires estimates of biochemical rate parameters that can only be obtained from expensive and time-consuming measurements that are very rarely made in N utilization experiments. For example, the ANIM model of Flynn et al. (1997) requires estimates of the shape parameters for the size of the glutamine pool that stops NH_4 uptake (*NH4mGLN*), for the maximum size of the nitrate and ammonium internal pools assuming a maximum biomass N:C ratio (*NO3Pm*, *NH4Pm*), and for the curve characterizing glutamine suppression of nitrate–nitrite reductase synthesis (*NNiRhGLN*). Indeed, as yet, no comprehensive set of parameter estimates for any such model has been obtained for any species. Our goal here is to sacrifice the explicit characterization of the dynamics of multiple intracellular N pools, and instead to construct more tractable models whose best-fit parameter values and 95% confidence limits can be estimated from time-series of external nutrient concentrations and population size, variables that are commonly measured in phytoplankton laboratory cultures. Our results show that transient and interactive processes between nitrate and ammonium uptake play an important role determining the dynamics of our species. The present approach contributes to a more comprehensive understanding of the factors underpinning the high variation in nitrate–ammonium assimilation observed in natural and experimental systems.

2. Methods

2.1. Model

To analyze nitrate–ammonium utilization in *Chlorella* sp., we first design a process-based model derived from our current understanding of the biological processes acting on the study system (see Table 1 for parameter definitions and units). Our model extends the commonly used "Quota" model for a single nitrogen (N) source (Droop, 1975; Legovic and Cruzado, 1997), to explicitly account for two different N sources (i.e. nitrate and ammonium) and how they drive cell division. In the original Quota model, cells are assumed to assimilate a single generic N form and divide at a rate that is proportional to their internal N concentration as follows:

$$\frac{dN}{dt} = -f_N(N(t)) \times B(t) \tag{1a}$$

$$\frac{dQ}{dt} = f_N(N(t)) - \mu_{max} \times \left(1 - \frac{Q_{min}}{Q(t)}\right) \times Q(t) \tag{1b}$$

$$\frac{dB}{dt} = \mu_{max} \times \left(1 - \frac{Q_{min}}{Q(t)}\right) \times B(t) \tag{1c}$$

where $N(t)$, $Q(t)$, and $B(t)$ represent external N, internal N within each cell, and population density, respectively, as a function of time, $f_N(N(t))$ represents the functional response quantifying uptake rate as a function of medium N concentration, μ_{max} is the growth rate of a cell at infinite internal N, and Q_{min} is the threshold of internal N concentration at which no cell division occurs.Our formulation extends this framework by allowing N to be assimilated as either nitrate or ammonium. In doing so, we account for the main interactions known to regulate nitrate and ammonium utilization in phytoplankton cells (Dortch, 1990).

We use two saturating functional responses for each N type:

$$f_{NO_3}(NO_3(t), NH_4(t)) = \nu_{NO_3} \times \frac{NO_3(t)}{NO_3(t) + k_{NO_3}} \times I_{sNO_3}\left(r_{NO_3}, t\right) \times I_{inh}(NH_4(t)) \tag{2a}$$

$$f_{NH_4}(NH_4(t)) = \nu_{NH_4} \times \frac{NH_4(t)}{NH_4(t) + k_{NH_4}} \tag{2b}$$

where ν_{NO_3} and ν_{NH_4} represent the maximum feasible per-cell uptake rate, and k_{NO_3} and k_{NH_4} specify the half-saturation constants, for nitrate and ammonium, respectively. $I_{sNO_3}\left(r_{NO_3}, t\right)$ and $I_{inh}(NH_4(t))$ are indicator functions (i.e.