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Non-linear dynamics of nitrogen isotopic signature based on biological kinetic model of uptake and assimilation of ammonium, nitrate and urea by a marine diatom

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ABSTRACT

Non-linear dynamics of nitrogen isotopic signature during uptake and assimilation of ammonium, nitrate and urea by culture of *Thalassiosira pseudonana* was described mathematically. Experimental data of the laboratory batch reactors were taken from Waser et al. (1998a,b). The measured dynamics of nitrogen isotopic signature was the consequence of biological kinetic model based on the modified Monod function, which additionally took into account that during substrate uptake isotopic fractionation terminates when the N-substrate concentration decreases below a certain threshold level. Contrasting to traditional description, in our approach, kinetic isotopic fractionation factor α means a constant ratio between specific maximum substrate uptake rates of light and heavy isotopes. In this case, the factor ε commonly used to describe isotopic enrichment is not supposed to be constant and its values depend on threshold concentrations of N-substrate. The lower was the threshold concentration, the higher was the maximum value of ε when N-substrate became exhausted. The model described complex substrate kinetics in N-sufficient and N-deficient conditions reasonably well using the kinetic coefficients preliminary obtained for the individual ammonium, nitrate and urea. In N-sufficient conditions, uptake of urea and nitrate started after depletion of ammonium because high ammonium concentration strongly inhibited nitrite and urea uptake and assimilation. In N-deficient conditions, inhibition of urea and nitrate uptake by ammonium was significantly less pronounced. In accordance with the model, at low initial ammonium concentration a decrease of nitrogen isotopic fractionation occurs.

1. Introduction

Nitrogen uptake and assimilation by phytoplankton are very important processes in the biogeochemical cycle of N and C in the ocean. Diatoms are responsible for about 20% of global carbon fixation (Ambrust et al., 2004). Stable isotopes are often used as natural labels to quantify the uptake of individual components from a mixture of substrates (Philips and Koch, 2002). The dynamics of isotopic incorporation, isotope mixing models, problem of routing and trophic discrimination factors remain central to isotopic ecology (Martinez del Rio et al., 2009; Navarro et al., 2011; Fry, 2012; Fry and Quinones, 1994). Dynamic isotopic equations combined with traditional ecological models may provide a new insight into internal relationships in ecosystems. For example, Cole et al. (2002) evaluated carbon flow using a model based on ambient and manipulated levels of ^{13}C in a lake along with information on key rate processes. Somes et al. (2010) incorporated nitrogen isotope fractionation equation into a model of marine ecosystem processes to develop a three-dimensional ocean climate model.

Rayleigh's distillation equation for gas mixture diffusion (1896) is commonly used for calculation of isotope fractionation:

$$\frac{R_t}{R_0} = f^{(1/\alpha - 1)} \quad (1)$$

where R_0 and R_t are isotope ratios of the substrate at the beginning and at a subsequent time point t, respectively; $f = C_t/C_0$ is the unreacted fraction of the substrate with C_0 and C_t being the concentrations of the substrate at the beginning and at the end of the time interval t, respectively; α is the kinetic fractionation coefficient between substrate and product in a closed and perfectly mixed system. Rayleigh's equation is deduced from the first-order kinetics of substrate transformation with $\alpha = k_{light}/k_{heavy}$ where k_{light} and k_{heavy} are the first-order kinetic coefficients for light and heavy

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substrates, respectively. Brodsky (1952) stated that isotopic fractionation should terminate when substrate becomes exhausted.

The degree of isotopic fractionation can be also quantified with more distinctive enrichment factor $\varepsilon = (\alpha - 1) \times 1000‰$ because α-values usually deviate only slightly from 1. Similar to Mariotti et al. (1981), the following equation can be written for nitrogen isotopic signature of biomass as a product:

$$\delta^{15}N_{PN} = \delta^{15}N_{DN_0} - \varepsilon \times \frac{f}{1-f} \times \ln f = \delta^{15}N_{DN_0} - \varepsilon \times F \quad (2)$$

where subscripts PN and DN_0 mean the current particulate nitrogen contained in phytoplankton and the dissolved substrate nitrogen for $t = 0$, respectively. A value of F tends towards -1 when the unreacted fraction of the substrate f tends towards 1. It gives $\delta^{15}N_{PN_0} = \delta^{15}N_{DN_0} + \varepsilon$ where $\delta^{15}N_{PN_0}$ is the initial nitrogen isotopic signature of biomass.

To describe biodegradation process van Breukelen and Prommer (2008) suggested using Monod-type degradation reactions instead of pure first-order kinetics. Monod function with kinetic coefficients such as maximum specific substrate consumption rate and half-saturation coefficient is commonly used in complex ecological models (Buitenhuis et al., 2010). Monod kinetics represents a simplified approach assuming that product formation is linked to biomass growth. The Monod equation has the same form as the well-known Michaelis–Menten equation, but differs in that it is empirical while the latter is based on theoretical considerations of transition state theory. The Monod-type biological kinetics combined with a description of dynamic change in $\delta^{13}C$ was used to estimate relative input of acetoclastic and hydrogenotrophic methanogenesis in boreal peatland ecosystems (Vavilin, 2012).

The purpose of this study was to describe dynamic changes in $\delta^{15}N$ using the modified Monod kinetics during batch uptake and assimilation of individual ammonium, nitrate and urea and mixtures of these substrates by phytoplankton culture of *Thalassiosira pseudonana*. It was shown that the measured dynamics of nitrogen isotopic signature was a consequence of biological kinetics.

2. Materials and methods

2.1. Description of Waser's batch experiments

The detailed experimental procedure was described in previous literature (Waser et al., 1998a,b). In brief, *T. pseudonana* (Clone 3H) was grown in 2-l flasks on artificial seawater supplemented with bicarbonate ($NaHCO_3$) to prevent carbon limitation, with ammonium, nitrate and urea supplied as single N-substrates or as a mixture of all three. In experiments with single N-substrates, *Thalassiosira* cells were acclimated to the particular substrate for a period of at least 1 week during which they have been growing exponentially for at least 8–10 generations. Incubations were done under stirred conditions, at $18.0 \pm 0.5\,^{\circ}C$, in continuous light.

In the experiment with a mixture of N-substrates added to a starved high-density culture, cells were incubated under above-described conditions for a period of 40 days during which the culture growth stopped because of the depletion of all available nitrogen from the media. Then new N-substrate was supplemented to the media. Particulate nitrogen (PN) samples were collected by vacuum filtration and analysed by mass spectrometer. The precision of each PN analysis was 1–2%. Ammonium was determined by a standard colorimetric method and urea by the diacetyl monoxime method. Nitrate was monitored by UV absorption. The precision was 3.3%. N isotope abundance was determined with a VG PRISM mass spectrometer. The precision of $\delta^{15}N$ measurements was 0.17‰.

2.2. Model

2.2.1. Uptake of individual N-substrates

In the model, both nitrogen uptake and its assimilation by algae were considered as one step. The following equations were used to describe biomass growth of *T. pseudonana* expressed as PN increase:

$$\frac{dDN}{dt} = -\rho_{DN}(DN, PN) = -\rho_m PN \frac{DN - DN_{\min}}{K_{DN} + DN - DN_{\min}}$$
$$\frac{dPN}{dt} = \rho_{PN}(DN, PN) = \rho_m PN \frac{DN - DN_{\min}}{K_{DN} + DN - DN_{\min}} \quad (3)$$

where DN is the dissolved nitrogen concentration in the particular N-substrate; $DN_{\min}$ means the minimal N-substrate concentration below which biomass growth could not occur because, unlike in chemical reaction kinetics, biological growth ceases (Bodegom, 2007); PN is the particulate nitrogen concentration in algae biomass; ρ_m is the maximum specific rate of nitrogen uptake and accumulation in biomass; K_{DN} is the half-saturation constant.

At $DN_{\min} \ll K_{DN}$ the system (3) in the particular cases can be written as

1. Logarithmic growth of algae biomass ($DN \gg K_{DN}$)

$$\frac{dDN}{dt} = -\rho_{DN}(DN, PN) = -\rho_m PN$$
$$\frac{dPN}{dt} = \rho_{PN}(DN, PN) = \rho_m PN \quad (4)$$

2. Zero-order substrate kinetics ($DN_0 \ll PN_0$, $DN \gg K_{DN}$)

$$\frac{dDN}{dt} = -\rho_{DN}(DN, PN) = -\rho_m PN_0$$
$$\frac{dPN}{dt} = \rho_{PN}(DN, PN) = \rho_m PN_0 \quad (5)$$

3. First-order substrate kinetics ($DN_0 \ll PN_0$, $DN \ll K_{DN}$)

$$\frac{dDN}{dt} = -\rho_{DN}(DN, PN) = -\frac{\rho_m}{K_{DN}} PN_0 DN = -k_1 DN$$
$$\frac{dPN}{dt} = \rho_{PN}(DN, PN) = \frac{\rho_m}{K_{DN}} PN_0 DN = k_1 DN \quad (6)$$

Recently, Vavilin and Rytov (2013) used the modified Monod function presented in (3) to describe the non-linear dynamics of carbon and hydrogen isotopic signatures during nitrite-dependent methane oxidation.

To describe the dynamics of heavy (^{15}N) substrate DN and product PN concentrations the following equations were added to the system (3):

$$\frac{dDN^h}{dt} = -\frac{DN^h}{DN} \frac{1}{\alpha_N} \frac{dDN}{dt}$$
$$\frac{dPN^h}{dt} = -\frac{dDN^h}{dt} \quad (7)$$

where DN^h/DN is the fraction of heavy isotopes in the dissolved N-substrate, α_N is the kinetic fractionation coefficient of stable nitrogen isotopes between the substrate and biomass. From the systems (3 and 5), it can be written as

$$\frac{dDN^h/dt}{dDN^l/dt} \approx \frac{dDN^h/dt}{dDN/dt} = \frac{1}{\alpha_N} \frac{DN^h}{DN} \quad (8)$$

where DN^l is the concentration of light substrate and $DN = DN^l + DN^h \approx DN^l$. In our approach, the isotopic fractionation factor is equal to

$$\alpha = \frac{\rho_m^{\text{light}}}{\rho_m^{\text{heavy}}} \quad (9)$$