Seasonal characteristics of the nitrogen isotope biogeochemistry of settling particles in the western subarctic Pacific: A model study

Masahito Shigemitsu a,⁎, Yasuhiro Yamanaka a,b, Yutaka W. Watanabe a, Nobuhiro Maeda c, Shinichiro Noriki a

a Graduate School of Environmental Science, Hokkaido University, Kita-10, Nishi-5, Kita-Ku, Sapporo 060-0810, Japan
b Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Japan
c Environmental Engineering Department, The General Environmental Technos Co., Ltd., 1-3-5, Aizumachi, Chuo-ku, Osaka, 541-0052, Japan

ARTICLE INFO

Article history:
Received 23 April 2009
Received in revised form 28 November 2009
Accepted 27 January 2010
Available online 15 March 2010

Editor: M.L. Delaney

Keywords:
nitrogen isotope
settling particle
western subarctic Pacific
ecosystem model

ABSTRACT

We used moored time-series sediment traps to collect settling particles at station KNOT (44°N, 155°E; trap depth 770 m) in the western subarctic Pacific (WSAP) from October 1999 to May 2006. Particulate nitrogen content (PN) and isotope ratios (δ15NPN) were measured in the samples collected. The general pattern of variation in δ15NPN results showed lower values during the spring bloom periods and summer, and higher values during winter. To interpret the processes controlling such variations quantitatively and reveal some implications for paleoenvironographic use of δ15NPN, we developed an ecosystem model that included nitrogen isotopes. This model was validated with an observed data set and successfully reproduced the seasonal variations of δ15NPN. In simulations, the lower δ15NPN during the spring bloom period was caused mainly by the highest proportion of dead large phytoplankton (diatom) in PN within a year, the highest f-ratio of the year, and phytoplankton assimilation of nitrate with the lowest δ15N of the year. The lower δ15NPN in summer was due to the high relative proportion of dead non-diatom small phytoplankton and microzooplankton fecal pellet with the lowest δ15N values among all the PN components in our model. The higher δ15NPN in winter was mainly caused by the highest proportion of zooplankton components in PN, with higher δ15N values than phytoplankton components, and the enhanced δ15N values of ammonium induced by nitrification and its subsequent assimilation by phytoplankton. Our identification of nitrification as one cause of higher δ15NPN in winter is consistent with previous findings in a proximal marginal sea, the Okhotsk Sea, with an ecosystem model simpler than our model. This might indicate that the cause of higher δ15NPN in winter is common in the WSAP. In our model, we optimized the isotope effect of each process using our observational data of δ15NPN and δ15N of nitrate published elsewhere as constraints, and investigated the sensitivity of the annual flux-weighted mean of δ15NPN to the isotopic fractionation effects. As a result, we found that the isotope effects of nitrate assimilation appear to be different for non-diatom small phytoplankton and large diatom, and the annual flux-weighted mean of δ15NPN can be influenced to some extent by the isotope effect of nitrification.

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1. Introduction

The nitrogen isotopic composition of settling particulate nitrogen (δ15NPN) has been proposed as a proxy for surface-water nitrate utilization (the fraction of supplied nitrate removed by export; e.g., Altabet and Francois, 1994). The preferential assimilation of 15NO3− by phytoplankton yields organic nitrogen that is depleted in 15N relative to the nitrate from which it was formed. As a result, this assimilation causes the surface nitrate pool to become progressively enriched in 15NO3− as nitrate concentration decreases (e.g., Wu et al., 1997). In turn, phytoplankton utilize this 15N-rich nitrate, resulting in a concurrent increase in δ15N of the phytoplankton-derived organic nitrogen (e.g., Waser et al., 1998a). Thus, higher nitrate utilization yields a higher δ15NPN. This signal, generated in the surface ocean, appears to be propagated to the seafloor by settling particulate nitrogen (PN), establishing the connection between surface nitrate utilization and δ15N of sediments (e.g., Altabet and Francois, 1994). This presents the possibility of reconstructing past variations of nitrate utilization in regions where nitrate is not completely consumed by phytoplankton during the annual cycle.

The better interpretation of δ15N in sediments as a proxy of past nitrate utilization requires an understanding of the relationships between seasonal variations of both δ15NPN and surface-ocean biogeochemistry in various regions, because the δ15N of sediments is an integrated value incorporating seasonal variations in δ15NPN produced in the surface ocean. Many studies have examined these relationships using sediment-trap time-series data. Most works carried out in the open ocean report inverse seasonal relationships.
between $\delta^{15}N_{\text{PN}}$ and PN flux (Altabet et al., 1991; Voss et al., 1996; Wu et al., 1999; Altabet and Francois, 2001; Lourey et al., 2003); that is, $\delta^{15}N_{\text{PN}}$ is lower during periods of high PN flux (spring to early summer) and higher during periods of low PN flux (fall to winter).

These studies attribute the inverse relationship to the isotope fractionation effect of nitrate assimilation by phytoplankton during nitrate-replete conditions (spring to early summer) that leads to the formation of the phytoplankton-derived organic nitrogen with lower $\delta^{15}N$. Although higher $\delta^{15}N_{\text{PN}}$ in fall can be explained by increases in $\delta^{15}N$ of nitrate with the progression of nitrate consumption by phytoplankton from spring to fall, the observed relationship between maximum $\delta^{15}N_{\text{PN}}$ and minimum PN flux in winter in these studies was not explained successfully by relative nitrate utilization because the lowest $\delta^{15}N$ of nitrate during the year is thought to occur in surface waters of this season. This low $\delta^{15}N$ of nitrate generally results from strong convective mixing of subsurface waters containing nitrate with low $\delta^{15}N$ into surface waters.

To explain the maximum $\delta^{15}N_{\text{PN}}$ in winter, the following have been presented as possible causes: (1) an increase of amount of the most degraded particles with higher $\delta^{15}N$ in a year (Voss et al., 1996; Altabet and Francois, 2001); and (2) an increase of zooplankton components with higher $\delta^{15}N$ than phytoplankton (Wu et al., 1999; Altabet and Francois, 2001). The former is related to the process occurring during sinking and the latter is associated with that occurring in the surface water. Recently, Yoshikawa et al. (2005) found seasonal variations of $\delta^{15}N_{\text{PN}}$ in the Okhotsk Sea similar to those reported in the above-cited works, and used a 6-compartment ecosystem model that included nitrogen isotopes to explain the factors causing such seasonal variations of $\delta^{15}N_{\text{PN}}$. The new insights from this study suggested that the $\delta^{15}N_{\text{PN}}$ maximum in winter might result from the higher $\delta^{15}N$ of ammonium—resulting from isotope fractionation effects during nitrification—and its subsequent assimilation by phytoplankton. This explanation is intriguing because Wankel et al. (2007), on the basis of nitrogen and oxygen isotopic composition of nitrate, reported the possibility that nitrification occurs in the surface water of Monterey Bay (California, USA) and that the $\delta^{15}N_{\text{PN}}$ might increase when regenerated ammonium is consumed more by nitrification than by phytoplankton assimilation. This argument is essentially consistent with the explanation of Yoshikawa et al. (2005) for the highest $\delta^{15}N_{\text{PN}}$ occurring in winter. Thus, it would be useful to investigate whether and how nitrification affects $\delta^{15}N_{\text{PN}}$ in other regions. The results should lead to a better interpretation of $\delta^{15}N$ in marine sediments as a proxy for reconstructing past oceanic nitrogen conditions because, until now, the process of nitrification has not been considered to affect the $\delta^{15}N$ in marine sediments.

For this study, we developed a more complex ecosystem model (11 compartments and including nitrogen isotopes) than that used by Yoshikawa et al. (2005) (6 compartments) and we applied the model to observations at an open-ocean station in the western subarctic Pacific (WSAP) (Station KNOT) where the surface-ocean biogeochemistry was investigated in detail from 1998 to 2000 and the nitrate was not completely depleted even in summer, and where time-series sediment-trap samples have been collected for about 7 years, from 1999 to 2006. Fujii et al. (2002) has already applied this model, without including nitrogen isotopes, to conditions at Station KNOT and reproduced the ecosystem dynamics there. The two phytoplankton groups represented in the model (diatom and non-diatom phytoplankton) proved to be especially important for reproducing the spring diatom bloom and subsequent non-diatom phytoplankton growth. Because there are also differences in the isotope effects of nitrate and ammonium assimilation between diatom and non-diatom phytoplankton (e.g., Montoya and McCarthy, 1995), we used the 11-compartment ecosystem model in this study rather than the 6-compartment model. Our objectives are to identify the factors controlling the seasonal variations in $\delta^{15}N_{\text{PN}}$ in the WSAP and to obtain some insights into the use of $\delta^{15}N$ in sediments as a paleoceanographic proxy.

### 2. Materials and methods

#### 2.1. Sample collection and analysis

Settling particles were collected from October 1999 to May 2006 using time-series sediment traps (Nichiyu HX-10; Noriki et al., 1995) moored at Station KNOT in the WSAP (44°N, 155°E, bottom depth 5320 m; Fig. 1). The station KNOT is located near the southwestern edge of the Western Pacific Subarctic Gyre (WSG). There were 6 sediment-trap deployments: from October 1999 to May 2000, from May 2000 to June 2001, from June 2002 to May 2003, from May 2003 to May 2004, from June 2004 to May 2005, and from May 2005 to May 2006. Sediment-trap depth was approximately 770 m during all deployments. The sediment trap used in this study has 13 sampling cups that were set to collect samples on an approximately monthly basis (20–30 days). All sampling cups were filled with filtered seawater containing 10% neutral formaldehyde before deployment to prevent degradation of collected organic matter. Samples were placed in a refrigerator immediately after recovery of the sediment trap and kept in it until analysis. Swimmers were removed from the collected samples by handpicking or sieving through 1-mm mesh. The particulate sample in each cup was collected onto preweighed 0.6-μm pore-size Nuclepore® filters. The particles on the filter were freeze-dried overnight and weighed to determine sample weight and total mass flux. Dried samples were then homogenized with a mortar and pestle.

For nitrogen content and isotope analysis, a few mg of dried sample was placed in a silver capsule and acidified using small amounts of 20% HCl to remove inorganic carbon. The carbonate-free samples were dried for 1 to 2 days in a clean glass desicator containing NaOH pellets and P₂O₅ powder. The sample was then wrapped in a tin capsule. The nitrogen content and $\delta^{15}N$ were determined by the flow-injection method using a Finnigan MAT 252 mass spectrometer connected to a Fisons NA 1500 elemental analyzer. The analytical precision for replicate measurements of subsamples was within 5% for nitrogen content and within 0.2% for $\delta^{15}N$. The isotope values in this study are reported relative to N₂ in air.

#### 2.2. Model description

We used an 11-compartment marine ecosystem model (Fujii et al., 2002; Yamanaka et al., 2004) based on the North Pacific Ecosystem Model Used for Regional Oceanography (NEMURO; Kishi et al., 2007).
This ecosystem model has already been applied at Station KNOT and has successfully reconstructed the observed large seasonal biogeochemical cycles (Fuji et al., 2002). In this model, phytoplankton are categorized into diatom (PL) and non-diatom small phytoplankton (PS). Phytoplankton components in the model utilize nitrate and ammonium, produce particulate organic nitrogen (PON) during photosynthesis, and excrete dissolved organic nitrogen (DON). The diatom component of the model also utilizes silicate during photosynthesis to produce frustules of biogenic silica. Zooplankton components are categorized into microzooplankton (ZS), mesozooplankton (ZL), and predatory zooplankton (ZP). Seasonal vertical migration of mesozooplankton is taken into account in the model: the mesozooplankton migrate vertically out of the model domain, representing the surface mixed layer, at the end of August, and 20% return at the beginning of April every year. The original ecosystem model (Fuji et al., 2002) also includes calcium carbonate, calcium ion, total alkalinity, and total dissolved inorganic carbon as prognostic variables, and is combined with a one-dimensional mixed-layer model. In this study, for simplicity, we did not consider the four variables associated with carbon, and we combined the ecosystem model with a zero-dimensional model representing the upper mixed layer, instead of the one-dimensional model used by Fuji et al. (2002). All parameters used for the ecosystem model in this study are the same as those of Fuji et al. (2002). Since carbon is not a limiting factor in the model, there is no problem in not treating the four carbon variables. The details of the equations governing the ecosystem model are described by Yamanaka et al. (2004).

In this study, we added the $^{15}$N (nitrogen isotope) cycle to the nitrogen cycle in the ecosystem model and took into consideration the isotopic fractionation of each process, as shown in Table 1. The isotopic fractionation effects were optimized by using monthly mean values of $\delta^{15}$N$_{nitrate}$, which were measured in this study and a nitrate $\delta^{15}$N value measured in the surface water near the station KNOT in June 2002 (Lehmann et al., 2005) as constraints. We detail the optimization algorithm in the next section. In our model, $-^{15}$N and $+^{15}$N $+^{14}$N were concurrently proaged in a given time step. These values were converted to $\delta^{15}$N values by convention for comparing the calculated results with observations. The computation method of $^{15}$N is the same as that of Yoshikawa et al. (2005).

We set the water temperatures used in the equations for biological processes to match the observed values in the upper mixed layer during the Station KNOT time-series observation program in 1998, 1999, and 2000 (Tsurushima et al., 2002). To represent seasonal variations in the mixed layer, the mixed-layer depth and the value for water exchanges between the upper mixed layer and the layer below were changed seasonally. Boundary conditions for the nitrate concentration, nitrate $\delta^{15}$N value, and silicate concentration were fixed at 23 μmol/L, 6‰, and 40 μmol/L, respectively, based on previous measurements (Tsurushima et al., 2002; Lehmann et al., 2005). The light intensities were set to match the monthly mean values from 1999 to 2006 in the National Center for Environmental Prediction (NCEP) data set. The seasonal variations of water temperature, mixed-layer depth, light intensity, and vertical exchange coefficient that we used are shown in Fig. 2. We repeated the annual cycle for 7 iterations under these climatological forcings to obtain a quasi-steady state.

### 2.3. Optimization of each isotope fractionation effect

#### 2.3.1. The cost function

For the optimization, we defined the following cost function ($J$) as a sum of weighted least square misfits between model results and observational data:

$$J = \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{1}{2\sigma^2} (m_{ij} - d_{ij})^2,$$

where $m_{ij}$ denotes modeled monthly mean values of variables, $d_{ij}$ represents monthly mean values of observations with prescribed standard deviation $\sigma_i$, $M$ represents the number of different types of observations: (1) Monthly averaged $\delta^{15}$N$_{nitrate}$ measured in this study, and (2) $\delta^{15}$N value of nitrate measured in the station near station KNOT in June 2002 by Lehmann et al. (2005), and $N$ represents the number of observations for each type data ($N_1 = 12$, $N_2 = 1$). We set the both prescribed standard deviations at 0.2‰. For our optimization problem, we imposed constraints on the ranges of possible isotope effect values (Table 1). The isotope effect estimates are not penalized by the constraint within each isotope effect range with upper and lower limits, $p_{\text{eff}}^l$ and $p_{\text{eff}}^u$ respectively, and the constraint form is described as follows:

$$p_{\text{eff}} = \frac{1}{2} \sum_{k=1}^{14} \left( \frac{1}{(\sigma_k)^2} \left( (p_k - p_{\text{eff}}^l)^2 + (p_{\text{eff}}^u - p_{\text{eff}}^l) \right)^2 \right).$$

where $k$ denotes different isotope effects which are optimized (Table 1) and $p_{\text{eff}}^l$ is the isotope effect estimated. In this study, the
standard deviations at the limits were assumed to be 100% of their values, namely, $(\delta_{\text{PON}})^{2} = (\delta_{\text{PN}})^{2}$, $(\delta_{\text{PON}})^{2} = (\delta_{\text{PN}})^{2}$ as in Schartau et al. (2001). Each range is set based on the previously reported values with exceptions of ammonium assimilation and nitrification. For the isotope fractionation effect of ammonium assimilation by phytoplankton, several studies have suggested that at very low ambient ammonium concentrations, such as those in the open ocean, the value may become reduced because diffusion of ammonium into the cells becomes the rate-limiting process (Cifuentes et al., 1989; Montoya et al., 1991, Pennock et al., 1996). Thus, we set the lower limit values at 1. For nitrification, we also set the value at 1 because the same principle may hold. We determined the most likely isotope effect solution space by minimizing $J + f_{\text{penal}}$.

2.3.2. Optimization algorithm

We chose a simulated annealing method (e.g., Krüger, 1993; Matear, 1995) to find the optimal isotope fractionation effect of each process. Basically, we used the same procedure as that in Matear (1995). In the procedure, the following parameters are required: (1) the initial temperature ($T_{0}$), (2) the vector denoting the standard deviations of Gaussian noise which is added to the model parameters ($\sigma_{\text{PON}}$), (3) the reduction factor for $T_{0}$ after each annealing step ($d_{T}$), (4) the reduction factor for $\sigma_{\text{PON}}$ after each annealing step ($d_{\sigma}$), (5) the maximum number of perturbations per annealing step ($N_{\text{max}}$), and (6) the maximum number of acceptable perturbations required before exiting an annealing step ($N_{g}$). To calculate the initial value of $T_{0}$, 1000 perturbations were conducted by the same method as that by Matear (1995). We set the vector $\sigma_{\text{PON}}$ to the standard deviation of 1‰ and chose other parameters as follows: $d_{T} = 0.5$, $d_{\sigma} = 0.9$, $N_{\text{max}} = 2500$, $N_{g} = 550$. 600 thousand iterations were used for this optimization. The temperature ($T$) and $\sigma$ are set at $1.0 \times 10^{-8}$ °C and $2.5 \times 10^{-5}$‰, respectively once $T$ and $\sigma$ become less than those values in cooling steps. The parameters which are different from those in Matear (1995) were determined by confirming that the algorithm converges to the same value of the cost function independently of the initial guess of the isotope effects.

2.3.3. Estimation of errors of each isotope fractionation effect

We estimated the errors of the respective isotope effects generated by the simulated annealing method from 6 additional optimizations with resampled data sets as in Schartau and Oschlies (2003), in order to assess the reliability of the optimization method. The measured data which are used as constraints are regarded as one realization, with exceptions of ammonium assimilation and nitrification, we determined the most likely isotope effect at 1. For nitri...
Fig. 3. Simulated results of (a) net primary production, (b) PON flux at 770 m depth, (c) nitrate, (d) biomass of each plankton component, (e) DON, and (f) ammonium. Plankton components in d are ZP; predatory zooplankton; ZL, mesozooplankton; ZS, microzooplankton; PL, diatom; and PS, non-diatom, small phytoplankton. Dash-dotted line, ZP; dotted line, ZL; dashed line, ZS; thin solid line, PL; thick solid line, PS. Symbols in a, b and c denote field observations. Field observations are from (a) Imai et al. (2002) (open circles) and Shiomoto et al. (1998) (solid circle), (b) this study, and (c) Tsurushima et al. (2002) (open circles) and Lehmann et al. (2005) (solid circle). In b, data from 1999 to 2006 are indicated respectively by open circles (1999), open diamonds (2000), crosses (2001), plus signs (2002), open triangles (2003), solid circles (2004), solid squares (2005), and solid diamonds (2006). The data of Shiomoto et al. (1998) in a and Lehmann et al. (2005) in c were obtained not at station KNOT but within the Western Subarctic Gyre (WSG). For the datum of Lehmann et al. (2005), the uppermost one at station K1 located within the WSG is used in this study. See color version of this figure in the HTML.

Fig. 4. Simulated results for δ¹⁵N of (a) each plankton component, (b) nitrate, (c) PON at 770 m depth, (d) ammonium, and (e) DON. Plankton components in a are the same as in Fig. 3d. Symbols represent data from (b) Lehmann et al. (2005) and (c) this study. The datum of Lehmann et al. (2005) in b was obtained not at station KNOT but at station K1 within the Western Subarctic Gyre (WSG), and the uppermost one is used in this study. Symbols in c except for solid triangles are defined as in Fig. 3b. Solid triangles denote the monthly averaged observations of PON δ¹⁵N. See color version of this figure in the HTML.
The simulated primary production in our model reasonably reproduced the observed seasonal amplitude to the same extent as the previous ecosystem model coupled to the one-dimensional mixed-layer model (Fuji et al., 2002). The simulated nitrate agreed well with the observations. The modeled nitrate concentrations were the highest in late March to early April due to the strong convective mixing during winter. The high surface nitrate and silicate (not shown here) induced a spring bloom of diatom (PL) (Fig. 3d). In April to September, nitrate was consumed by diatom (PL) and non-diatom small phytoplankton (PS), and the concentration reached minimum values at the end of September. Subsequently, gradually increasing convective mixing (Fig. 2b and d) supplied nitrate from below into the surface mixed layer, and thus nitrate progressively increased. In this model, we assumed that the PON passing through the upper mixed layer instantaneously reached 770 m depth and that seven-eighths of the PON degraded before reaching that depth. We also adjusted the modeled PON flux at the depth by multiplying by a trapping efficiency of about 0.4, as estimated by Otosaka et al. (2004) at 1000 m depth of Station KNOT. Despite these ad hoc assumptions, the simulated PON fluxes were also realistically reconstructed by the model (Fig. 3b).

Actually, if we compute the correlation coefficient between monthly averages of observed and modeled data, there is a significant correlation ($r = 0.51$, $p < 0.05$). In particular, model results showing two flux maxima in spring and summer were also seen in the field observations. From this reasonable reproduction by our model of the field observations at Station KNOT, we concluded that we could use the ecosystem model including nitrogen isotopes coupled with a zero-dimensional model with as much confidence as the model of Fuji et al. (2002), which combined the ecosystem model with a one-dimensional mixed-layer model.

### 3.2.2. Nitrogen isotope dynamics

The optimized isotope effect of each process is shown in Table 1. At first glance, isotope effects for excretion and egestion by zooplankton, remineralization, and decomposition are close to their respective upper or lower bounds imposed to the optimization algorithm. One reason behind this is that the observational or experimental data for these processes are few, and the upper and lower bounds are not effectively set by the data. In this study, we set the variation range of each isotope effect as in Table 1. Furthermore, we speculate that these parameters are not independent of other parameters, particularly isotope effects of ammonium assimilation by phytoplankton and nitrification, because these isotope effects are closely associated with $\delta^{15}$N of ammonium. Thus, to constrain these parameters better, we will need the ammonium $\delta^{15}$N data. Nevertheless, holding these isotope effects within the imposed ranges, the isotope effects for nitrate assimilation by diatom (PL) and non-diatom small phytoplankton (PS), ammonium assimilation by diatom (PL), and nitrification are constrained well. For ammonium assimilation by non-diatom small phytoplankton, the error was the largest, meaning that this isotope effect is not constrained well. The uncertainty of each isotope effect is inversely proportional to the cost function’s sensitivity to the variation (Schartau et al., 2007). Actually, as we mention in Section 4.2, this isotope effect sensitivity to the modeled $\delta^{15}$N of PON was insignificant. We compared model-simulated results regarding nitrogen isotopes using the optimized isotope effects with field observations (Fig. 4). All components except for nitrate showed the lowest $\delta^{15}$N values in spring and summer, followed by gradual increases from summer to fall and the highest or higher $\delta^{15}$N values in winter. For each plankton and DON, there were also maxima in fall. The seasonal variation of nitrate $\delta^{15}$N showed values near the lower boundary value of $6.0_\circ$ from winter to spring, which then gradually increased up to $9.5_\circ$ during spring through fall. The model results reproduced the degree of nitrate $\delta^{15}$N increase in the WSG reported by Lehmann et al. (2005). The simulated $\delta^{15}$N of PON agreed well with the observed values. If we compute the correlation coefficient between monthly averages of modeled and observational data, there was a significant correlation ($r = 0.90$, $p < 0.01$). At the start of the spring bloom in April, the simulated $\delta^{15}$N values of PON decreased rapidly from 6.5$_\circ$ to 4.0$_\circ$. The low values also exist in summer. From summer to fall, $\delta^{15}$N values of PON increased gradually, and from fall to winter the $\delta^{15}$N continued to increase and then reached the maximum values. Section 4.1 is a discussion of the factors controlling such variations.

### 4. Discussion

#### 4.1. Factors controlling seasonal variations in $\delta^{15}$N of PON in our model

The $\delta^{15}$N of PON showed the following characteristic seasonal variations (Fig. 4c): (1) the minimum occurred during the spring bloom and summer, (2) a progressive increase occurred from summer to fall, and (3) the maximum occurred in winter. Here we examine the factors controlling these variations.

One possible factor affecting the seasonal variations in $\delta^{15}$N of PON is changes in the composition of PON. Previous studies (e.g., Minagawa and Wada, 1984) report the enrichment of $\delta^{15}$N with increasing trophic level in the ocean. Our model reproduced this stepwise increase in $\delta^{15}$N with trophic level (Figs 4a and 5c). The $\delta^{15}$N of dead individuals ("MT" representing mortality in Fig. 5c) of microzooplankton (ZS), which grazes on non-diatom small phytoplankton (PS), were higher than that of the non-diatom small phytoplankton (PS) component by about 3$_\circ$. Likewise, the $\delta^{15}$N of dead individuals and fecal pellets ("EG" representing egestion in Fig. 5c) of mesozooplankton (ZL) and predatory zooplankton (ZP), which graze on diatom (PL), were higher than that of the diatom (PL) component. In PON, the relative proportion of the dead diatom (PL)
component reached about 80% in spring, that of the dead non-diatom small phytoplankton (PS) and microzooplankton (ZS) fecal pellet reached about 60% in summer, and the proportion of predatory zooplankton (ZP) components reached about 80% in winter. Thus, the PON $\delta^{15}N$ minimum in spring and summer, and maximum in winter are partially accounted for by the high proportions of diatom (PL) in spring, non-diatom small phytoplankton (PS) and microzooplankton (ZS) in summer and predatory zooplankton (ZP) in winter. Another factor that can affect the variation is the seasonal change in $\delta^{15}N$ values of nutrients (nitrate and ammonium). Almost all components of PON had similar seasonal patterns of $\delta^{15}N$ variation (Fig. 5c). This indicates that the $\delta^{15}N$ of phytoplankton, which reflects the $\delta^{15}N$ of the nutrients (nitrate and ammonium), was transmitted to the higher trophic levels. To understand how the concentration and $\delta^{15}N$ of nitrate and ammonium are controlled in our model, we considered the factors affecting the seasonal variations in $\delta^{15}N$ of nitrate and ammonium.

The $\delta^{15}N$ of nitrate and ammonium are determined by processes involved in both their production and consumption. To examine the effect of each production or consumption process on the $\delta^{15}N$ values, we defined the time rate of $\delta^{15}N$ change by each process involved, $T_N$ ($\%$/d), of nitrate or ammonium following Yoshikawa et al. (2005):

$$T_N = F/C_n \times (\delta^{15}N_{f} - \delta^{15}N_{n}),$$

where $F$ and $\delta^{15}N_f$ are the flux and $\delta^{15}N$ involved in each process, respectively, and $C_n$ and $\delta^{15}N_n$ are concentration of nitrate or ammonium and their nitrogen isotopic ratios, respectively. The positive value of $T_N$ means that the process involved tends to increase $\delta^{15}N$ of nitrate or ammonium, and vice versa. The results of these simulations are shown in Fig. 6.

The $\delta^{15}N$ of nitrate increased from spring to fall as the consumption of nitrate through photosynthesis by PS and PL proceeded (Fig. 6N-b and N-d). In winter, nitrate was supplied from lower layer by strong convective mixing in the model and the concentration increased (Fig. 6N-a). Along with this increase, the $\delta^{15}N$ of nitrate approached the lower boundary condition of 6‰ (Fig. 6N-b and N-d). Thus, the $\delta^{15}N$ of nitrate in winter could not account for the winter increase in $\delta^{15}N$ of PON in our model, although the increase in nitrate $\delta^{15}N$ from spring to fall contributed to the PON $\delta^{15}N$ increase in that period. The spring minimum in $\delta^{15}N$ of PON was in part due to the simultaneous occurrence of the highest $f$-ratio (Fig. 5a) and the lowest $\delta^{15}N$ of nitrate (Fig. 4b).

The $\delta^{15}N$ of ammonium increased and ammonium concentration decreased from spring to fall in a pattern similar to the $\delta^{15}N$ of nitrate and nitrate concentration as the consumption of ammonium during photosynthesis by PS and PL proceeded, although there were some fluctuations in ammonium $\delta^{15}N$ and concentrations due to some production processes (remineralization of DON and PON, and

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Fig. 6. Left panels: simulated results for (N-a) the contribution of each process to the flux budget of nitrate, (N-b) $T_N$ for nitrate, the tendencies of nitrate $\delta^{15}N$ from each process (see Section 4.1), (N-c) the same panel as (N-a) with the expanded scale on the left y-axis, and (N-d) the same panel as (N-b) with the expanded scale on the left y-axis. Right panels: simulated results for (A-a) the contribution of each process to the flux budget of ammonium, (A-b) $T_N$ for ammonium, the tendencies of ammonium $\delta^{15}N$ from each process (see Section 4.1), (A-c) the same panel as (A-a) with the expanded scale on the left y-axis, and (A-d) the same panel as (A-b) with the expanded scale on the left y-axis. In N-a to N-d, Ex (brown dashed line), Nit (green dashed line), PPSHOTO (blue dash–dotted line), PLPHOTO (black dotted line), and Total (pink solid line) indicate the effects from excetration by ZF, ZL, and ZS, remineralization of DON and PON, nitrification, photosynthesis by PS and PL, and their total, respectively. In A-a to A-d, ZPEX (light blue dashed line), ZLEX (pink dashed line), DONRE (gray dash–dotted line), PONRE (yellow dotted line), Nit (green dashed line), PPSHOTO (blue dash–dotted line), PLPHOTO (black dotted line), and Total (pink solid line) indicate the effects from excretion by ZP, ZL, and ZS, remineralization of DON and PON, nitrification, photosynthesis by PS and PL, and their total, respectively. For Total, the expanded scales on the right y-axis are used to readily see the changes.
excretion by ZS, ZL and ZP (Fig. 6A-a to A-d). In contrast to nitrate, however, the δ\textsuperscript{15}N of ammonium continued to increase from fall to maximum values in winter, leading to the increased δ\textsuperscript{15}N of PON (Fig. 4c). This occurred mainly because the flux from nitrification was much larger than other fluxes in winter (Fig. 6A-c and A-d). In other words, the ratio of the phytoplankton ammonium assimilation rate to the nitrification rate is important for determining the δ\textsuperscript{15}N of ammonium, and ultimately the δ\textsuperscript{15}N of PON. This mechanism is the same as the interpretation by Yoshikawa et al. (2005) for the nearby Okhotsk Sea, and might be common in the WSP, including the proximal marginal seas.

The spring minimum PON δ\textsuperscript{15}N can be explained by three factors: spring had the highest f-ratio, the highest relative proportion of dead diatom (PL) in PON, and the lowest δ\textsuperscript{15}N of nitrate over the course of a year. The summer minimum of PON δ\textsuperscript{15}N was mainly due to the high relative proportion of dead non-diatom small phytoplankton (PS) and microzooplankton (ZS) fecal pellet which have the lowest δ\textsuperscript{15}N values among all the PON components in our model. The progressive increase in PON δ\textsuperscript{15}N from summer to fall was mainly due to the progressive increase in δ\textsuperscript{15}N of nitrate and ammonium resulting from isotope fractionations during assimilation by non-diatom small phytoplankton (PS) and diatom (PL). The winter maximum in PON δ\textsuperscript{15}N can be explained mainly by two factors: the highest relative proportion of predatory zooplankton (ZP) with the highest δ\textsuperscript{15}N values among our model PON components in a year, and the increase of δ\textsuperscript{15}N of ammonium because of nitrification and the subsequent assimilation of this ammonium by non-diatom small phytoplankton (PS) and diatom (PL).

4.2. Sensitivities of modeled δ\textsuperscript{15}N of PON to isotope fractionation effects in each process, and the isotope fractionation effect values

To investigate the sensitivity of the seasonal PON δ\textsuperscript{15}N variations in our model to each isotopic fractionation effect, we carried out a sensitivity analysis by using a Monte Carlo analysis. In the Monte Carlo experiment, 1000 model simulations were performed with the optimized isotope effects to which Gaussian noises with means equal to respective optimized values and coefficients of variation of 10% (i.e., standard deviation/mean = 0.1) are added for each simulation. As the correlation between varied parameter values and prognostic variable value is a good indicator of sensitivity for the Monte Carlo approach (Rose et al. 1991), we computed correlations between the respective isotope effects and PON δ\textsuperscript{15}N values. For PON δ\textsuperscript{15}N values, we used seasonally averaged values (spring (March to May), summer (June to August), fall (September to November) and winter (December to February)), and the annual flux-weighted mean of PON δ\textsuperscript{15}N. We divided the Monte Carlo results into three groups based on the magnitude of their correlation as in Yoshie et al. (2007): (1) insignificant effect: |r|< 0.25, (2) small effect: 0.25 ≤ |r| < 0.4, and (3) large effect: |r| ≥ 0.4. The results are shown in Table 2.

Alteration of the isotope fractionation effect of diatom (PL) nitrate assimilation had a large effect on δ\textsuperscript{15}N variations of PON in fall to winter, and the largest effect on those in spring. Larger values for the isotope fractionation effect tend to yield lower values for δ\textsuperscript{15}N of PON in the seasons. The effect of altering the isotope fractionation effect for nitrate assimilation by non-diatom small phytoplankton (PS) was large, especially in summer to fall. These two isotope effects also have large effects on the annual flux-weighted mean of δ\textsuperscript{15}N of PON. Note that the larger isotope effect of non-diatom small phytoplankton (PS) compared to that of diatom (PL) is appropriate for reproducing the observed PON δ\textsuperscript{15}N (Table 1). This larger isotope effect of non-diatom small phytoplankton (PS) than that of diatom (PL) is consistent with field observations (e.g., Wu et al., 1999; Karsh et al., 2003) but not with the results of culture experiments (Montoya and McCarthy, 1995). In addition, the value of the isotope effects of nitrate assimilation by diatom (PL) is close to the lower limit within the previously reported values (4 to 12) in the fields, and much smaller than the values (8 to 15) from an iron enrichment experiment of the northeastern subarctic Pacific (Needoba et al., 2006). Granger et al. (2004) reported that the lower isotope effect of nitrate assimilation by phytoplankton seems to be associated with iron-limited cultures. If this is the case, the difference between the optimized isotope effect of nitrate assimilation by diatom (PL) in this study and the values obtained in an iron enrichment experiment by Needoba et al. (2006) may be related to the ambient iron concentrations. For ammonium assimilation by non-diatom small phytoplankton (PS) and diatom (PL), the isotope fractionation effect of diatom (PL) had a small impact on δ\textsuperscript{15}N of PON only in winter, and that of non-diatom small phytoplankton (PS) was insignificant to δ\textsuperscript{15}N of PON. The estimated large error of the isotope effect of ammonium assimilation by non-diatom small phytoplankton (PS) must be related to this insignificant sensitivity to δ\textsuperscript{15}N of PON. Nevertheless, the fact that the isotope effects for ammonium assimilation are much smaller than the previously reported values (Table 1) is interesting. Pennock et al. (1996) estimated the isotope effect in culture experiments, and that was dependent on the ambient ammonium concentrations. From the result, they expected that as ammonium

<p>| Table 2 |</p>
<table>
<thead>
<tr>
<th>Isotope effect</th>
<th>Annual flux-weighted mean of PON δ\textsuperscript{15}N</th>
<th>Seasonally averaged δ\textsuperscript{15}N of PON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring (3–5)</td>
<td>Summer (6–8)</td>
</tr>
<tr>
<td>NO\textsubscript{2}\textsuperscript{+} assimilation by PL</td>
<td>-0.58</td>
<td>-0.84</td>
</tr>
<tr>
<td>NO\textsubscript{2}\textsuperscript{+} assimilation by PS</td>
<td>-0.72</td>
<td>-0.20</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+} assimilation by PL</td>
<td>-0.05</td>
<td>-0.18</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+} assimilation by PS</td>
<td>-0.07</td>
<td>-0.01</td>
</tr>
<tr>
<td>Excretion by ZS</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Excretion by ZL</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>Excretion by ZP</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Egestion by ZS</td>
<td>-0.05</td>
<td>-0.04</td>
</tr>
<tr>
<td>Egestion by ZL</td>
<td>-0.04</td>
<td>-0.08</td>
</tr>
<tr>
<td>Egestion by ZP</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Excretion by PL</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Excretion by ZS</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Excretion by ZL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Excretion by ZP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Decomposition (PON to DON)</td>
<td>-0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Characters in black: |r|<0.25; bold black: 0.25 ≤ |r|<0.4; bold black with gray hatch: |r|≥0.4.
concentrations approach zero, the isotope effect of ammonium assimilation by phytoplankton will approach zero. Our optimized values may support their expectation. It is natural of us to think that at very low ambient ammonium concentrations typical of the open ocean, the value could be lower as diffusion of ammonium into the cells becomes the rate-limiting process. The isotope effects of excretion and egestion by zooplankton, and of decomposition from PON to DON, had little impact on δ15N of PON with the exception of small effect in winter by excretion of predatory zooplankton (ZP). In Section 4.1, we mentioned that the highest relative proportion of ZP in winter is responsible for the highest values of δ15N in this season. The isotope effect of excretion by predatory zooplankton (ZP) was also found to be important for keeping the δ15N values high in winter. The isotope effect of nitrification exerted a large influence on δ15N of PON, especially in winter, with larger isotope effects resulting in higher δ15N of PON. This effect is opposite to the effect of ammonium assimilation by diatom (PL) in this season, which means that the δ15N of PON increases with increases in the difference between the isotope fractionation effects of ammonium assimilation by diatom (PL) and nitrification. Interestingly, this isotope effect also has a small impact on the annual flux-weighted mean of δ15N, and the value is close to the lower limit within the previously reported values (Table 1). The latter may indicate that similarly to ammonium assimilation, diffusion of ammonium into nitrifiers' cells becomes the rate-limiting process at very low ambient ammonium concentrations.

From this sensitivity analysis, we found that isotope fractionation effects of two processes—nitrate assimilation by non-diatom small phytoplankton (PS) and diatom (PL), and nitrification—have large and small influences over the δ15N of PON, respectively. By classifying phytoplankton into two size categories in our model, we were able to investigate the possibility of different isotope effects in diatom (PL) and non-diatom small phytoplankton (PS) for nitrate assimilation. On the basis of a field study, Karsh et al. (2003) suggested that the isotope fractionation effect for nitrate assimilation by diatoms may be smaller than that for assimilation by small phytoplankton. Wu et al. (1999) also found that the δ15N of diatoms is higher than that of nanoplankton, indicating that the isotope fractionation effect during nitrate uptake by diatoms might be smaller than that in nanoplankton. Combining model and field studies would enhance our understanding of the relative importance of isotope fractionation effects during various processes.

4.3. Paleoceanographic use of δ15N in sediments: implications from model results

From the paleoceanographic point of view, the factors affecting the annual flux-weighted mean of δ15N of PON are important. The results of our optimization of each isotope effect and sensitivity analysis showed two possible factors with large and small effects on δ15N of PON: (1) the difference between the isotope fractionation effects during nitrate assimilation by the non-diatom small phytoplankton (PS) and diatom (PL); and (2) the variations in the isotope fractionation effect as a function of nitrification. The first factor is very important for the paleoceanographic interpretation of δ15N in sediments because there is evidence that during the glacial periods in the WSAP, diatoms in the phytoplankton assemblage shifted toward cold-water, bloom-related larger species (e.g., Katsuki et al., 2003; Katsuki and Takahashi, 2005). Brunelle et al. (2007) found higher δ15N in Bering Sea sediments from glacial periods than from interglacials. In contrast, Shigemitsu et al. (2008) showed that δ15N in sediments of the open WSAP was apparently lower during glacial periods than during interglacials. They interpreted the difference between the two locations as possibly reflecting differences in the amount of iron supplied to the surface waters during glacial times. Galbraith et al. (2008) also found the similar δ15N results to that of Shigemitsu et al. (2008) in the WSAP, and suggested that a large part of the glacial–interglacial δ15N signal in the WSAP arose from changes in the basin-wide δ15N signature, caused by changes in denitrification rates. However, if the larger diatom species present during glacial times had smaller isotope fractionation effects during nitrate assimilation as shown in this study, then the difference in the glacial to interglacial patterns of δ15N variations in sediments between the Bering Sea and the open WSAP might reflect differences in the relative proportion of large diatom species at the two locations. Our present study has shown that the isotope fractionation effect of nitrate assimilation by diatom (PL) is smaller than that by non-diatom small phytoplankton (PS), and these results are consistent with recent field observations (e.g., Wu et al., 1999; Karsh et al., 2003). This, however, contradicts the results of Montoya and McCarthy (1995), who found that the isotope effect of nitrate is larger in diatoms than in flagellates in culture experiments. Further study is needed to explain this difference.

For the second factor, nitrification has not been considered to affect δ15N in marine sediment. However, our sensitivity analysis showed that nitrification isotope effect could affect the annual flux-weighted mean of δ15N of PON. Combining with the sensitivity analysis result of ammonium assimilation isotope effect by diatom (PL), if the isotope effect of ammonium assimilation by phytoplankton is smaller than that of nitrification as in this study, then nitrification brings nitrate with lower δ15N values into the pool and makes δ15N values in the ammonium pool higher, leading to higher δ15N values of PON. This is consistent with the interpretation obtained by Winkel et al. (2007). Because the PON flux is small in winter when the nitrification has the largest impact on the δ15N of PON, this effect has only a small influence on the annual flux-weighted mean of δ15N of PON as seen in the sensitivity analysis (Table 2). However, some researches (Yoshikawa et al., 2005; Winkel et al., 2007) including this study show the importance of nitrification for δ15N variations of PON. Hence, the nitrification effect should be interpreted carefully when using δ15N in sediments to reconstruct past nitrate utilization in the ocean. We notice that our zero-dimensional model does not consider the mixing of ammonium and isotopic mass balance between the mixed and below layers, and this may affect the result that nitrification isotope effect has an influence on the annual flux-weighted mean of δ15N of PON. Further researches to investigate the effect are needed.

5. Conclusions

We developed an ecosystem model that included nitrogen isotopes and validated this model by comparing the simulated results with observations. Results of our model showed that the factors controlling the seasonal variations in δ15N of PON are: (1) In spring, the highest f-ratio, the highest relative proportion of dead diatoms (PL) in PON, and the lowest δ15N of nitrate of the year induce the lowest values for δ15N of PON in a year. (2) In summer, the highest relative proportion of non-diatom small phytoplankton (PS) and microzooplankton (ZS) fecal pellet with the lowest δ15N values among all the PON components also leads to the lowest values for PON δ15N in a year. (3) In summer to fall, a progressive increase in δ15N of nitrate and ammonium resulting from the process associated with their uptake by phytoplankton causes δ15N of PON to gradually increase. (4) In winter, the nitrification-induced increase in δ15N of ammonium, and the higher relative proportion of predatory zooplankton (ZP) in PON result in the highest δ15N of PON during the year. The nitrification process in winter is the same as previous finding in the proximal marginal sea, the Okhotsk Sea, suggesting that this process can occur in both the marginal seas and the open ocean of the WSAP. Future studies could provide direct evidence for this by observing increases in δ15N of ammonium in winter. In our model, the process previously proposed to explain the maximum δ15N of PON in winter, (1), stated in Section 1, are not explicitly represented. Thus,
some influences of these processes on $\delta^{15}N$ of PON should be examined in the future.

The following results are important for the paleoceanographic application of $\delta^{15}N$ in sediments.

(1) We found that the isotope effects of nutrient assimilation appear to be different for non-diatom small phytoplankton (PS) and the larger diatoms (PL). A usual assumption when applying $\delta^{15}N$ in sediments to paleoceanographic studies is a constant isotope effect for nutrient assimilation by phytoplankton, but our results suggest the need for caution and to consider how the relative proportions of phytoplankton species have varied on the geological timescale of interest.

(2) We also found that the variations in the isotope effect of nitrification has a small impact on the annual flux-weighted mean of PON $\delta^{15}N$, which indicates that the nitrification might play a role in determining $\delta^{15}N$ of sea-floor sediments. However, this might be an artifact due to our using zero-dimensional model without considering the mixing of ammonium and isotopic mass balance between the mixed and below layers. Further researches to investigate the effect are needed.

Acknowledgements

We thank Prof. Masao Minagawa for his help in measuring the nitrogen isotopes of settling particles, and wish to thank Mrs. Maki N. Aita and Dr. Naoki Yoshiie for providing the data necessary for this study and help in carrying out sensitivity analysis, respectively. We also thank three anonymous reviewers and an editor for constructive comments. This work was partially supported by CREST/JST and the Grant-in-Aid for Scientific Research in Priority Areas "Western Pacific Air-Sea Interaction Study (W-PASS)" under Grant No.18067004.

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