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Application of chemical tracers to estimate benthic denitrification in the Okhotsk Sea

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Abstract

To estimate the benthic denitrification in a marginal sea, we assessed the usefulness of N_2^* using dissolved nitrogen (N_2) and argon (Ar) with N^* in the intermediate layer (26.6-27.4 σ_θ) of the Okhotsk Sea. The examined parameters capable of affecting N_2^* are denitrification, air injection and rapid cooling. We investigated the extents of these effects on N_2^* using multiple linear regression analysis. The best model included two examined parameters of denitrification and air injection based on the Akaike Information Criterion as a measure of the model fit to data. More than 80% of N_2^* was derived from the denitrification, followed in order by air injection. Denitrification over the Okhotsk Sea shelf region was estimated to be 5.6 ± 2.4 μmol kg^{-1}. The distribution of N_2^* was correlated with potential temperature (θ) between 26.6-27.4 σ_θ ($r = -0.55$). Therefore, we concluded that N_2^* and N^* as a complement can act as a quasi-conservative

tracer of benthic denitrification in the Okhotsk Sea. Our findings suggest that N_2^* in combination with N^* is a useful chemical tracer to estimate benthic denitrification in a marginal sea.

1. Introduction

The marine fixed nitrogen (N) cycle is highly dynamic due to large input-output rates and a shorter turnover time compared to the cycles of other biologically important nutrients. Despite recent developments in our understanding of the processes and magnitude of the pathways in the marine N cycle, a number of open questions remain (Brandes *et al.*, 2007). Denitrification in the water column and sediments, including canonical denitrification and anaerobic ammonium oxidation (anammox), is the primary sink of N from fixed forms to molecular nitrogen (N_2) (Brandes *et al.*, 2007; Eugster and Gruber, 2012). Conversely, N_2 fixation by diazotrophs is the major oceanic source of biologically available N in the open ocean (Galloway *et al.*, 2004). In particular, there is controversial disagreement as to how close the marine N cycle is balanced between N_2 fixation and denitrification (Codispoti, 2007; Codispoti *et al.*, 2001; Codispoti and Christensen, 1985; Gruber, 2004; Gruber and Sarmiento, 1997).

Denitrification occurs primarily in continental marginal sediments (Christensen *et al.*, 1987; Middelburg *et al.*, 1996) and in the three major oxygen deficient zones (ODZs) that experience oxygen concentrations less than 4 μmol/kg: the eastern tropical North and South Pacific and the Arabian Sea (Codispoti *et al.*, 2001; Codispoti and Christensen, 1985; Codispoti *et al.*, 2005). While reports related to nitrogen sources and sinks have increased over the past 20 years, estimation of benthic denitrification are the most poorly constrained (Codispoti, 2007; DeVries *et al.*, 2012; Liu and Kaplan, 1984; Middelburg *et al.*, 1996).

There are several ways to constrain the biologically available marine N budget in the global ocean (Codispoti, 2007; Gruber and Sarmiento, 1997). For example, Gruber and Sarmiento

(1997) proposed a quasi-conservative tracer N^* (= $([NO_3^-] - 16[PO_4^{3-}] + 2.9) \times 0.87$) as an index of N_2 fixation-denitrification using the observed nutrient data. In general, N^* increases with N_2 fixation because diazotrophs N_2 into the internal nitrogen cycle in the ocean, and decreases with denitrification because microbes consume nitrate. However, the distribution of N^* may reflect not only N_2 fixation and denitrification, but also other important processes. Such processes include variable phytoplankton stoichiometry (Weber and Deutsch, 2010), atmospheric deposition (Hansell *et al.*, 2007) and different remineralization rates for total organic phosphorus and nitrogen (Coles and Hood, 2007; Landolfi *et al.*, 2008; Monteiro and Follows, 2012; Yoshikawa *et al.*, 2013; Zamora *et al.*, 2009). These processes are capable of reducing the accuracy of N^*.

Another way to estimate the content of denitrification derived from excess N_2 has been developed based on comparison of the dissolved N_2/Ar ratio in the water mass between in and out of the denitrification regions (Codispoti, 2007; Devol *et al.*, 2006; Shigemitsu *et al.*, 2013a). The observations of N_2/Ar have already been used in the three ODZs to estimate water-column denitrification at depths above ~1000 m (Chang *et al.*, 2010; Devol *et al.*, 2006; DeVries *et al.*, 2012). However, the dissolved N_2/Ar is sensitive to not only denitrification process but also physical processes, e.g., lower atmospheric pressure, rapid cooling, and air injection by bubbles (Hamme and Severinghaus, 2007). Hamme and Emerson (2013) reported that both net denitrification and bubble injection are the most likely effects capable of causing large changes in the dissolved N_2/Ar ratio, because the N_2/Ar ratio in the air is almost double the ratio in seawater. Regarding constraint of excess N_2 derived from denitrification, Shigemitsu et al. (2013a) proposed a new tracer N_2^* based on N_2 and Ar in the denitrification region. However, since this method is difficult to distinguish excess N_2 produced during denitrification from that caused by physical processes, only this method could not estiamate the content of denitrification in the continental marginal sea.

The Okhotsk Sea (OS) is a subpolar marginal sea in the western North Pacific and the southernmost sea ice production area in the northern hemisphere. The densest water ventilating in the North Pacific region originates over the northwestern continental shelf of the OS (Kitani, 1973). This water is called the Dense Shelf Water (DSW, σ_θ = 26.7-27.0, where σ_θ □□□ the potential density, Nakatsuka *et al.*, 2004; Nakatsuka *et al.*, 2002), produced by brine rejection during sea ice formation (Kitani, 1973). The DSW penetrates into the intermediate depths to join with the Okhotsk Sea Intermediate Water (OSIW, σ_θ = 26.8-27.4, Yamamoto *et al.*, 2002; Yamamoto-Kawai *et al.*, 2004). The OSIW is transported into the North Pacific Intermediate Water (NPIW). The NPIW is widely distributed over the North Pacific, which influences the climate of the North Pacific. This water contains a large amount of anthropogenic CO_2 (e.g., Sabine *et al.*, 2004; Wakita *et al.*, 2005; Watanabe *et al.*, 2001). However, the density surface of 26.8 σ_θ does not outcrop to the atmosphere within the open North Pacific, even in winter (Yasuda, 1997). In addition, Yasuda (1997) found a pycnostad (referred to as the Okhotsk Sea Mode Water) in the 26.6-27.0 σ_θ density range.

Primary production in the northwestern shelf region in the OS is extremely high throughout the year, except the ice-covered season (Saitoh *et al.*, 1996; Sorokin and Sorokin, 1999). Because of the strong tidal mixing and extremely low temperature on this region, the particulate organic matter produced on the shelf is exported efficiently to the pelagic intermediate water (Nakatsuka *et al.*, 2002). Therefore, benthic flux of organic matter is possible to be high in this shelf region. Yoshikawa et al. (2006) found that there was possibility of large benthic denitrification in the continental region of the OS based on N^* (= $[NO_3^-] + [NO_2^-] + [NH_4^+] - 16[PO_4^{3-}] + 2.9) \times 0.87$) and the nitrogen isotopic ratio of nitrate. Furthermore, Shigemitsu et al. (2013b) indicated that Fe is also reduced in such sub-oxic sediments in addition to nitrate based on high Fe/Al ratio in the same region. However, the quantification of benthic denitrification has not been evaluated.

We here try to estimate the extent of benthic denitrification quantitatively in the continental shelf region. In this study, we also used the N^* ($([NO_3^-] + [NO_2^-] + [NH_4^+] - 16[PO_4^{3-}] + 2.9) \times 0.87$) defined in Yoshikawa et al. (2006). Because the N^* include nitrite and ammonium, the N^* would be affected by the anammox and would not be affected by dissimilatory nitrate reduction to ammonium (DNRA). To estimate the extent of benthic denitrification in the marginal sea shelf region, we focused on the density layer (σ_θ= 26.6-27.4) in the OS producing DSW and OSIW and attempted to assess the usefulness of N_2^* with N^* as the complement for detecting benthic denitrification. The DSW produced from sea-ice formation on the northwestern shelf in the OS flows out to intermediate depths along the Sakhalin coast to the southward and joins with the OSIW (Matsuda *et al.*, 2009). Yoshikawa et al. (2006) showed that N^* acts as a quasi-conservative tracer of denitrification during the OSIW formation process. No study that detects a sedimentary denitrification originating in the shelf region using N_2 and Ar exists. The OS is one of the best regions to evaluate the usefulness of this method.

2. Materials and Method

This investigation involved sampling and analyzing 28 sites to measure concentrations of dissolved gas using gas chromatography (Figure 1). The selected sites were located from the northwestern continental shelf in the OS to the Bussol Strait. Samples were collected in 24-liter X-Niskin bottles equipped with a CTD on the R/Vs Professor Khromov and Hokko-maru during August to early in September 2007 and May 2008, covering a formation area of the intermediate water in the OS (49-55°N, 142-148°E) and in the western North Pacific (40-42°N, 145-146°E), respectively.

Samples at each station were obtained at approximately 25 layers above 2000 m depth. The collected seawater for N_2 and Ar was directly transferred from the Niskin bottle to a 60 ml glass vial vessel. After opening the vent of the Niskin bottle, the vessel was washed twice and overflowed with three times the volume of the vessel to avoid air contamination during the transfer procedure. For the final filling, we added 50 μl of saturated $HgCl_2$ solution to prevent biological activity: the vial vessel was covered with a butyl rubber cap and aluminum seal, paying particular attention to assure that no air bubble contamination occurred. We preserved these vial vessels in the dark and in a cool seawater bath. The N_2 and Ar concentrations were determined by gas-chromatographic system with thermal conductivity detection (Tanaka and Watanabe, 2007) in our laboratory. Dissolved oxygen was also determined on board using the Winkler titration method (Carpenter, 1965). The analytical precisions for replicate measurements of gas concentrations were within 0.04% for N_2 and within 0.05% for Ar.

We have found systematic offsets in the data obtained below 1750m depth between the stations near the Bussol Strait (A1 and A2) and station KNOT (data from Hamme and Emerson (2002), http://web.uvic.ca/~rhamme/download.html). The average differences of temperature, salinity and density between the Bussol Strait and the station KNOT are 0.7 °C, 0.2, 0.2 σ_θ, respectively. The oxygen concentrations in both regions were more than 60 μmol kg^{-1}. Since the above results indicated that there were not any significant processes affecting Ar and N_2 concentrations, we have applied the following corrections to the N_2 and Ar concentrations in the OS and the western North Pacific. We thus made corrections to the N_2 and Ar data in the OS using the following relations: $[N_2]_{meas} = [N_2]_{meas0}$ + 16.97 μmol kg^{-1} and $[Ar]_{meas} = [Ar]_{meas0}$ + 0.87 μmol kg^{-1}, where meas and $meas_0$ represented the measured concentrations after the offset corrections and raw values of our observations. In this study, we used only dissolved gas concentrations near the center of DSW and OSIW layers between 26.6 and 27.4 σ_θ to estimate the extent of benthic denitrification that occurred in the northwestern shelf of the OS (Kitani, 1973; Wakita *et al.*, 2005; Wakita *et al.*, 2003; Yoshikawa *et al.*, 2006).

3. Approach: Concepts for estimating the benthic denitrification in the Okhotsk Sea

In this study, we used the chemical tracers N_2^{ex} and N_2^* to represent the excess N_2 with N^*. One approach to estimate denitrification involves the direct measurement of the denitrification end product, i.e., the excess N_2 above the background value. There is no significant sink of N_2 gas and the only source is denitrification in the interior of the ocean. Therefore, excess N_2 can be assumed to represent the amount of denitrification.

To estimate excess N_2, we employed a method analogous to Devol et al. (2006) that uses N_2/Ar ratios normalized to atmospheric equilibrium ratios:

$$N_2^{ex} = \left[\left(\mathrm{N_2/Ar}\right)_{\mathrm{norm}} - \left(\mathrm{N_2/Ar}\right)_{\mathrm{back}}\right] \times \left[\mathrm{N_2}\right]_{\mathrm{sat}} \times 2, \quad (1)$$

where $(\mathrm{N_2/Ar})_{\mathrm{norm}}$ ($\equiv \delta\mathrm{N_2}/\ \delta\mathrm{Ar} = ([\mathrm{N_2}]_{\mathrm{meas}}/[\mathrm{N_2}]_{\mathrm{sat}})/\ ([\mathrm{Ar}]_{\mathrm{meas}}/[\mathrm{Ar}]_{\mathrm{sat}})$) is the ratio normalized to the atmospheric equilibrium ratio within the denitrifying waters of the ODZs, $(\mathrm{N_2/Ar})_{\mathrm{back}}$ is the normalized ratio predicted for a parcel of water outside the denitrifying zone with the same density, $[\mathrm{N_2}]_{\mathrm{sat}}$ is the atmospheric equilibrium saturation of N_2 predicted from θ and S, and the factor of 2 converts to units of μM for monoatomic N.

Another way of estimating excess N_2 uses N_2^*, which has been developed recently (Shigemitsu *et al.*, 2013a) as follows:

$$N_2^* = \left[\mathrm{N_2}\right]_{\mathrm{meas}} - \left(\left[\mathrm{N_2}\right]_{\mathrm{sat}} / \left[\mathrm{Ar}\right]_{\mathrm{sat}}\right) \times \left[\mathrm{Ar}\right]_{\mathrm{meas}}, \quad (2)$$

As mentioned above, since N_2^{ex} largely depended on the $(\mathrm{N_2/Ar})_{\mathrm{back}}$ value in equation (1), we also used N_2^* with the assistance of multiple linear regression analysis (MLRA) to clarify the influence of denitrification and physical processes on N_2^* in this region. We considered the following factors to affect N_2^*: denitrification (J_{den}), bubble injection (J_{air}), change of solubility by rapid cooling (J_{cool}) and change of atmospheric air pressure (J_{pres}). In this study, because N_2 and Ar are similarly affected by J_{pres}, the influence of J_{pres} could be ignored.

We assumed that the observed N_2^* can be categorized into the fractions affected by J_{den}, J_{cool} and J_{air}, and used MLRA to fit the data to the following equation:

$$N_2^* = a_0 + a_1 \cdot J_{den} + a_2 \cdot J_{air} + a_3 \cdot J_{cool}, \quad (3)$$

where a_0, a_1, a_2 and a_3 are the partial regression coefficients. We here used the observed N^* values (μmol kg^{-1}) as the proxy for J_{den}, the combinations of the difference between observed and saturated Ar values (ΔAr, $[Ar]_{meas} - [Ar]_{sat}$) and the atmospheric mixing ratio of N_2 to Ar (χ_c = 78.084 / 0.934) for J_{air}, and the difference between the potential temperature (θ) and reference temperature (T_{ref}) defined as freezing temperature at the surface for J_{cool}, namely, we have

$$J_{den} = N^*, \quad (4)$$

$$J_{air} = \Delta\mathrm{Ar} \times \chi_c, \quad (5)$$

and

$$J_{cool} = \theta - T_{ref}. \quad (6)$$

In the MLRA analysis, we selected the candidate models using all possible combination of the three explanatory variables, i.e., 7 models. We selected the best model using the Akaike

Information Criterion (*AIC*, Akaike, 1973) as follows:

$$AIC = -2 \times L_M + 2 \times k, \quad (7)$$

where L_M is the maximized log-likelihood and k is the number of parameters for each model. More explanatory variables typically induce less deviation from the observed values. However, using explanatory variables in excess, we will have an instable model. The best model should include a combination of less explanatory variables and less deviation from the observed values. Using the *AIC* allows us to select the best model in this manner (Hilborn and Mangel, 1997; Shigemitsu *et al.*, 2013a; Shigemitsu *et al.*, 2010; Shimono and Kudo, 2003).

4. Results and Discussions

4.1. Comparison of the chemical tracers, N_2^{ex} and N_2^*

We found a significant positive correlation ($r^2 = 0.98$, $n = 45$, $p < 0.0001$; simple linear regression) between N_2^{ex} and N_2^* in the range 26.6-27.4 σ_θ□□Figure 2). In Eqn. (1), we determined $(N_2/Ar)_{back}$ using the curve fitted to $\delta N_2/\delta Ar$ observations in the western North Pacific outside of the OS (i.e., $(N_2/Ar)_{back}$ = 0.010$\sigma_\theta^{□}$□−□□□□□□$\sigma_\theta^{□}$□□□□□□□□□σ_θ□−□□□□□□□□□ in Figure 3). Although there were large and systematic variations in the hydrographic variables (e.g., O_2 and NO_2^-) at the three stations in the western North Pacific (KY08 st4, st7 and st11), the $\delta N_2/\delta Ar$ values for the these stations did not appreciably differ. Moreover, $\delta N_2/\delta Ar$ values were approximately 1.000 at the surface and increased to 1.020 ± 0.002 (standard error) with depth. However, since N_2^{ex} method can be a tracer of denitrification only when there is no change in Ar concentration from background and observed values (Codispoti, 2007), it is difficult to use as an appropriate tracer for denitrification in the OS. Because DSW is modified at the air-sea interface and thus gas exchange and cooling alters both Ar and N_2 concentrations. Therefore, we used N_2^* which is more useful to discuss the effects of denitrification, air injection and rapid cooling on excess N_2 because the estimate of N_2^{ex} largely depended on the $(N_2/Ar)_{back}$ value.

4.2. Model selection by AIC

In the case that a correlation among the explanatory variables in a multiple regression model is high, the multicollinearity problem may occur (Graham, 2003). Confounding in the regression is typically represented as a multicollinearity, of which deleterious effects on the interpretation of the regression coefficients are well known (e.g., Van Sickle, 2013). The explanatory variables were screened for multicollinearity as one of the assumptions in the MLRA. Multicollinearity in MLRA was studied by examining the tolerance, which is a statistic used to determine how much the independent variables are linearly related to one another. The tolerance is calculated as $1-R^2$ (R^2 is the multiple coefficient of determination) for an independent variable when it is predicted by the other independent variables already included in the analysis. The higher the intercorrelation of the independent variables, the closer the tolerance is to 0. Generally, the tolerance less than 0.2 indicates a multicollinearity problem and provides an increase in the standard error of the regression coefficients (Hart and Sailor, 2009; Rawlings *et al.*, 1998). To avoid this problem, we verified the combinations of the explanatory variables having a high correlation. The correlation of J_{air} and J_{cool} (0.66) was slightly high compared to the other combinations (Table 1). However, the tolerance values for all variables in our MLRA were greater than 0.2. Therefore, there were no multicollinearity problems between our explanatory variables. In our study, we used all possible explanatory variables, J_{den}, J_{air} and J_{cool} in the MLRA.

To clarify the amount of denitrification occurred in the northwestern shelf of the OS, we compared the results of 7 models (Table 2) and evaluated N_2^* between 26.6-27.4 σ_θ, using Eqns. (3) to (7). The best model was Model 1 due to having the smallest *AIC* for J_{den} and J_{air} (Table 2), ($R = 0.66$, $n = 45$, $p < 0.0001$; simple linear regression). Both denitrification and air injection primarily contributed to N_2^* in this region. Because our explanatory variables had different units, we evaluated which have larger effect on N_2^* by using each standardized dataset. We found that the largest effect was for J_{den}, followed by J_{air} based on the standard partial regression coefficient (a_i', i = 0 to 3) and that a_1' and a_2' were −0.51 and −0.27, respectively.

4.3. Evaluation of combination of N_2^* with N^* as a quasi-conservative tracer for benthic denitrification on the continental shelf

In order to evaluate the amount of denitrification by using N_2^* and N^*, it is necessary to validate the extent of N_2^* dynamics as a conservative property. In the relationship between N_2^* and potential temperature of the OSIW, we found a moderate linear relationship between the N_2^* and θ for 26.6-27.4 σ_θ in this region ($r = -0.55$, $n = 42$, $p < 0.0001$; simple linear regression), indicating that N_2^* may act as a quasi-conservative tracer for the OSIW formation process.

Plots for the Kuril Basin and Bussol Strait areas (sta. A1, A2, A4 and B1) were almost distributed below the regression line (open circles in Figure 4) due to the entrainment of low N_2^* waters from upper layer by active tidal mixing. The other data below the regression line were shallow layer (< 75m depth) except the deepest layer over the northwestern shelf. In this shallower layer, O_2 concentration was higher than oxygen deficient condition (< 4 µmol/kg) indicating that denitrification in the water column did not occur. Furthermore, the DSW may flow out of the shelf by the gravity current along the slope and/or a western boundary current (e.g., Nakatsuka *et al.*, 2002; Ohshima *et al.*, 2002). Therefore these plots may include only physical effects and consequently lower than the regression line.

On the other hand, plots above the regression line may be due to vertical transport of high N_2^* waters benthic denitrification occurred in the east Sakhalin sediment and northwestern shelf, because the intermediate water in this study includes the layer below the DSW (> 27.0 σ_θ). This relationship between N_2^* vs. θ suggests that N_2^* at intermediate depths was not essentially changed, i.e., N_2^* levels were not affected by regeneration and the high N_2^* signature in DSW is not modified by in situ denitrification or regeneration during the formation process of OSIW and its pathways. Moreover, our finding demonstrates that N_2^* acts as a quasi-conservative tracer for the benthic denitrification.

Therefore, since both N_2^* along with N^* act as quasi-conservative tracer in the DSW, $a_1 \times J_{den}$ in the MLRA act conservatively in this layer. Thus, we concluded that the combination of N_2^* with N^* is useful to estimate a benthic denitrification in the OS.

4.4. Estimate of denitrification, air injection and rapid cooling using MLRA and AIC

Using N_2^* along with N^*, ΔAr *and* θ in the MLRA (Eqns. (3)-(6)), we estimated contributions of denitrification, air injection and rapid cooling to excess N_2 for the first time. By multiplying partial regression coefficients (a_i (i = 0 to 3) in Table 2) by each explanatory variable value, we can estimate the contribution of each explanatory variables as follows.

In the best Model 1, the obtained partial regression coefficient, a_1 for J_{den} was −1.09 (Table 2). We estimated the benthic denitrification in this intermediate water to be approximately 5.6 ± 2.4 μmol kg^{-1} (SD) from $a_1 \times J_{\mathrm{den}}$ values (Figure 5 a-1). In the same water, observed N^* value was −5.1 ± 2.2 μmol kg^{-1}. The N^* value was approximately 2.6 ± 1.1 μmol kg^{-1} converted to N_2 yield during denitrification. These results demonstrate that excess N_2 calculated by using N_2^* along with N^* was found to be at least twice N^* alone, which agrees with the difference between N_2^{ex} and N^* in the Arabian Sea (Devol *et al.*, 2006). What cause the difference of denitrification between excess N_2 and N^* in this region?

In the Arabian Sea, Devol et al. (2006) deduced from previous studies that there are several possible mechanisms to increase the N_2 yield during denitrification: (1) oxidation of the ammonium regenerated during water-column denitrification to N_2, (2) contributions of N_2 resulting from processes taking place within the sediments in contact with the ODZ waters, (3) high N:P ratio material produced during N_2 fixation or (4) denitrification fueled by non-Redfield organic matter such as preferential degradation of proteins, and (5) other reactions between metals, iodine and various N species that lead to N_2 production,.

First, N^* in this study would be affected by anammox and would not be affected by DNRA in common with N_2^*, because the N^* includes nitrite and ammonium. The rate of DNRA is three orders of magnitude lower than denitrification and anammox and is therefore insignificant to nitrogen cycle (Crowe *et al.*, 2012). Second, in the most intermediate water of the OS, oxygen concentration was larger than the oxygen deficient condition (< 4 μmol/kg, Codispoti *et al.*, 2005). Third, N_2 fixation does not occur in the high-latitude OS, which is outside the habitat range of nitrogen fixers (Capone *et al.*, 1997). Therefore, since the cases of (1) to (3) can be ignored, although we have no data about the case of (5), (4) could possibly result in most of the increases of the N_2 yield during denitrification in this study.

The N^* is a useful tracer for denitrification and N_2 fixation, inputs from terrestrial and atmosphere, but in the OS, quantification of benthic denitrification by N^* alone is possible to underestimate/overestimate. That is, there are possible mechanisms to increase/decrease the N^* in this region: dissolved organic nitrogen (DON) input from the Amur River and phosphate elution from sediments.

During early sedimentary diagenesis, particulate organic N is hydrolyzed to DON by bacterial hydrolytic enzymes, which can increase the N^*. A large fraction of DON is ultimately remineralized to ammonium (NH_4^+). In the presence of O_2, a portion of the regenerated NH_4^+ is oxidized to NO_3^- (nitrification) before it can escape from the sediments. This NO_3^- may, in turn, be used as a terminal electron acceptor by denitrifying bacteria producing gaseous forms of N (coupled nitrification-denitrification) (Lehmann *et al.*, 2007; Thibodeau *et al.*, 2010).

Under anaerobic sediment conditions, phosphate may also be eluted from the sediment (e.g.., Sundby *et al.*, 1992), which can decrease the N^*. Yoshikawa et al. (2006) showed that the DSW reflects not only sedimentary denitrification but also phosphate elution from sediment from relationships between apparent oxygen utilization and phosphate concentration and nitrate concentration.

Therefore, the best way to estimate a benthic denitrification in this region is to make up for each other complementarily N_2^* and N^*. The physical processes are disadvantage for N_2^*. The phosphate elution, DON input from the river and remineralization with non-Redfield ratio are disadvantage for N^*. On the other hand, the advantage for N_2^* is independent from Redfield ratio and it can directly estimate end product of denitrification as excess N_2, if there are unknown biological processes. The advantage for N^* is to be used as index of denitrification including anammox and N_2 fixation.

On the other hand, the obtained partial regression coefficient, a_2 for J_{air} was −2.38 (Table 2) and the average air injection value in the intermediate water was 0.8 ± 1.3 μmol kg^{-1} (Figure 5 a-2). The maximum air injection (c.a. 2.4 μmol kg^{-1}) was found at approximately 26.7 σ_θ in this region. Note that the air injection values should not be represented strictly only air injection, but also other physical effects: rapid cooling, heating, slow gas exchange and mixing of water masses, because ΔAr used in J_{air} includes effects of all processes resulting in disequilibrium in Ar concentration. However, it is difficult to estimate ΔAr due only to air injection. Therefore, as we mentioned above, we confirmed how much the independent variables are linearly related to one another by examining the tolerance. Since the tolerance values for all variables in our MLRA were greater than 0.2, our analysis may be insensitive to this error. Craig and Weiss (1971) found that the mean N_2 saturation anomaly due to air injection was 1.9% in the Pacific Deep Water. However, compared with the maximum N_2 concentration due to air injection predicted by Model 1, approximately < 1%, only half the air injection is predicted with the model. The unexpected low value of air injection may be caused by sea ice formation when DSW originates. When forming the dense shelf water in the wintertime, sea surface water of the OS is covered by ice due to the strong cooling from the air mass of Siberia. Therefore, the air-sea gas exchange may be weakened by the sea ice coverage, and consequently we could observe the low value of the air injection (Tanaka and Watanabe, 2007). The effect of air injection is propagated into the abyssal zone over the northwestern shelf and the surface layer of OS. Its effect disappears below 100 m in open waters (Figure 5 a-2). In the best Model 1, we found that N_2^* was very sensitive to denitrification and air injection and insensitive to rapid cooling (< 0.5 μmol kg^{-1}, Figure 5 b-4) over the OS.

In the continental shelf region, more than 80% of N_2^* was derived from the denitrification, followed in order by air injection. The N_2^* in the intermediate water in the continental shelf (except A and B stations) was 8.3 ± 2.4 μmol kg^{-1}. The benthic denitrification ($a_1 \times J_{\mathrm{den}}$) was 6.8 ± 2.4 μmol kg^{-1}. The air injection ($a_2 \times J_{\mathrm{air}}$) was 1.9 ± 0.4 μmol kg^{-1}. Therefore the effect of benthic denitrification was about 80% or more than.

5. Conclusions

This study indicates the N_2^* along with N^* may be useful tracers to estimate the benthic denitrification in the OS. Most notably, this is the first study to estimate the contributions of denitrification and other factors on N_2^* separately. Our results provide compelling evidence for evaluate the benthic denitrification in the continental marginal sediments where denitrification occurs primarily (Christensen *et al.*, 1987; Middelburg *et al.*, 1996). However, some limitations are worth noting. Although our hypotheses were supported statistically, J_{air} cannot represent only air injection, but also other physical effects and MLRA in this study can be used only when properties in the water mass behave conservatively. Future work should therefore include follow-up work designed to evaluate whether the air injection was represented strictly and also whether N_2^* along with N^* continue to be used to improve understanding of the benthic denitrification.

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Figure Legends

Figure 1. Sampling points for dissolved gas and nutrients (black filled circles) in the Okhotsk Sea (49—55°N, 142—148°E) and the western North Pacific (40-42°N, 145-146°E). The dissolved gas samples from station KNOT (44°N, 155°E) and the western North Pacific (KY08 st04, st07 and st11) were used as background values. The solid line represents vertical sections from the stations in Figure 5. We used dissolved gas data from station KNOT from R. C. Hamme's data (http://web.uvic.ca/~rhamme/download.html).

Figure 2. The relationship between N_2^{ex} (μmol kg^{-1}) and N_2^* (μmol kg^{-1}) in the intermediate water (26.6-27.4 σ_θ□ in the Okhotsk Sea. The dashed line represents 1:1 line: the solid line shows a simple linear regression based on all our data (open symbols), (y = 5.68 + 0.47x; r = 0.99).

Figure 3. Profiles of $\delta N_2/\delta Ar$ in the Okhotsk Sea and throughout the western North Pacific versus σ_θ. Open circles denote samples near the Bussol Strait (A1, A2, A4 and KC2-5), triangles denote samples from the east of Sakhalin (B1, B4 and C5), pluses denote samples from Line D, and crosses denote samples from the northwestern shelf region. Closed circles denote average values outside the denitrifying zone of the OS (KY08 st4, st7, st11 and KNOT). Also shown is the best fit line for $\delta N_2/\delta Ar$ in waters outside the denitrifying zone $(N_2/Ar)_{back}$. Error bars represent standard errors in the range 0.001-0.004.

Figure 4. Relationship between calculated N_2^* (μmol kg^{-1}) and □ between 26.6-27.4 σ_θ. The solid line denotes the regression line (y = 7.20 −1.43x□; r = −0.55).

Figure 5. Vertical cross sections of N_2^*, denitrification, air injection and rapid cooling from

the northwestern shelf (140˚E) to the Bussol Strait, east of Sakhalin (153˚E) (the solid line in Figure 1) in (a) Model 1 and (b) Model 2; (a-1) and (b-1): N_2^*; (a-2) and (b-2): denitrification; (a-3) and (b-3): air injection; (b-4): rapid cooling. All units were μmol kg^{-1}. White contour lines indicated density surfaces with thin lines (contour interval 0.2 σ_θ□. The shading color indicated the calculated values of each property. Ocean Data View was used to draw these figures (Schlitzer, 2001).