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Author(s)	Sugimoto, Ryo; Kasai, Akihide
Citation	Biogeochemistry, 128(1-2), 1-17 https://doi.org/10.1007/s10533-016-0190-z
Issue Date	2016-03
Doc URL	http://hdl.handle.net/2115/64622
Rights	The final publication is available at link.springer.com
Rights(URL)	http://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	Kasai Revision 3-1.pdf



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1 Key biogeochemical processes evaluated by the stable nitrogen isotopes of dissolved inorganic
2 nitrogen in the Yodo River estuary, Japan: Significance of estuarine nutrient recycling as a
3 possible source for coastal production

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5 Ryo Sugimoto^{1*}, Akihide Kasai²

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8 (1) Research Center for Marine Bioresources, Fukui Prefectural University, 49-8-2 Katsumi,
9 Obama, Fukui 917-0116, Japan

10 (2) Field Science Education and Research Center, Kyoto University, Oiwake, Kitashirakawa,
11 Sakyo, Kyoto, 606-8502, Japan

12

13 Present address: A. Kasai

14 Faculty of Fisheries Sciences, Hokkaido University, 3-1-1, Minato-cho, Hakodate, Hokkaido 041-
15 8611, Japan

16

17 * Corresponding author: R. Sugimoto

18 Tel: +81-770-52-7305

19 Fax: +81-770-52-7306

20 E-mail: sugiryo@fpu.ac.jp

21

1 **Abstract**

2 Isotope analysis of dissolved inorganic nitrogen (DIN) and a mixing model along the salinity
3 gradient were used to clarify the dominant biogeochemical processes controlling nutrient
4 dynamics within a shallow eutrophic estuary in Japan. Although delivery of riverine DIN into the
5 estuary is largely dominated by mixing with seawater during most months, internal
6 biogeochemical processes of DIN assimilation by phytoplankton and DIN production by
7 remineralization and subsequent nitrification within the estuary offer the most reasonable
8 explanation for observed deviations in the isotopic compositions of NO_3^- from mixing behavior.
9 However, the balance of each process changed over time. During phytoplankton blooming in
10 summer, co-occurrence of NO_3^- assimilation and NO_3^- regeneration inhibited the accumulation of
11 NO_3^- within the estuary. Moreover, assimilation of NH_4^+ as well as NO_3^- by phytoplankton
12 complicates the nutrient dynamics within the estuary. However, mostly conservative or productive
13 behavior of DIN as well as PO_4^{3-} showed that recycled nutrients are significant net source within
14 the estuary. These results suggest recycled nutrients within the estuary could have a non-
15 negligible impact on eutrophication in Osaka Bay.

16

17 **Keywords**

18 Nutrient, Stable isotope, Eutrophication, Regeneration, Estuary

19

1 **1. Introduction**

2 Estuaries have an important role in regulating nutrient fluxes from land to sea. The
3 estuaries of major rivers are considered as active sites for reducing riverine nitrogen loads to
4 coastal seas (Brion et al. 2004; Seitzinger et al. 2006). However, several studies suggest that
5 estuarine removal of reactive nitrogen may be significantly overrated (Jickells et al. 2000; Tobias
6 et al. 2003). Several recent studies showed that estuaries may be a significant source of nitrogen
7 to coastal systems (Dänke et al. 2008; Xue et al. 2014). Therefore, global concerns about the
8 nutrient enrichment of estuaries have directed considerable focus toward understanding how
9 nutrients are transported and transformed at the land–sea interface.

10 A variety of biogeochemical processes distributes nutrients between inorganic and
11 organic forms, and between the pelagic and benthic compartments of estuaries. To clarify the roles
12 of biogeochemical processes such as denitrification, nitrification, and primary production, rate
13 measurements of each process at a variety of spatial and temporal scales are effective (Thompson
14 1998; Ragueneau et al. 2002; Giblin et al. 2010; Roberts et al. 2012). However, a large number
15 of rate measurements are needed to describe the system thoroughly, which requires considerable
16 technical expertise and intense research efforts.

17 A mixing diagram of concentration data against salinity provides a convenient method
18 to display the net result of nutrient transformations in an estuary and gives an indication of likely
19 occurring biogeochemical processes (Eyre 2000). However, it is not possible to determine
20 whether conservative behavior is due to a lack of activity or to an approximate balance between
21 production and consumption. Additional measurements of stable nitrogen isotopes ($\delta^{15}\text{N}$) of
22 dissolved inorganic nitrogen (DIN) provide an effective method to assess the internal turnover
23 processes of nitrogen and sources in estuaries (Middelburg and Nieuwenhuize 2001). The ability
24 to detect small differences in the $^{15}\text{N}:^{14}\text{N}$ ratio of various pools of nitrogen, combined with

1 knowledge of kinetic isotope fractionation in chemical and biological reactions, potentially
2 provides ways to monitor nitrogen transformations on various temporal and spatial scales
3 (Sugimoto et al. 2010; 2014). For instance, denitrification leaves behind NO_3^- enriched in ^{15}N ,
4 whereas nitrification results in the formation of ^{15}N depleted NO_3^- relative to the original NH_4^+
5 pool. The combined use of nutrient concentrations and $\delta^{15}\text{N}$ values of DIN makes it possible to
6 determine whether observed concentrations represent conservative or balance between sources
7 and sinks.

8 This study was conducted in the estuarine zone of the Yodo River, which empties into
9 Osaka Bay (Fig. 1). Osaka Bay is one of the most eutrophic coastal areas in Japan (Tsujimoto et
10 al. 2006; Yashuhara et al. 2007). Whereas efforts have been made to reduce nutrient loads from
11 the rivers in the last few decades (Nakatani et al. 2011), riverine nutrient discharge is thought to
12 have significant effects on the formation of hypoxic or anoxic conditions in the bottom water at
13 the head of the bay during summer (Nakajima and Fujiwara 2007), and red tides often occur
14 throughout the year in the bay (Hayashi and Yanagi 2008). In recent years, a harmful algal bloom
15 (HAB) of *Alexandrium tamarense* has been observed at the head of the bay (Yamamoto et al.
16 2009). Although the intrusion of saltwater into the estuary is well documented (Yamamoto et al.
17 2013), there is little knowledge about estuarine nutrient dynamics.

18 The goal of this study was to account for DIN behavior within the Yodo River estuary
19 and to assess DIN losses in the estuary to better constrain the effect of riverine nutrients on
20 eutrophication in Osaka Bay. Therefore, we examined the concentrations and $\delta^{15}\text{N}$ values of NO_3^-
21 and NH_4^+ , as well as other parameters such as phosphorus and chlorophyll-*a* (Chl-*a*)
22 concentrations to clarify how the dominant biogeochemical processes affect nutrient dynamics
23 within the eutrophic Yodo River estuary.

24

1 **2. Materials and methods**

2 **2.1. Study site**

3 The Yodo River is the largest river flowing into Osaka Bay (Fig. 1). It drains a catchment
4 area of 8240 km², which contains a population of approximately 11 million. Large amounts of
5 nutrients are released into the inner part of the bay through the Yodo River, causing significant
6 eutrophication in the region. The estuarine zone in the Yodo River is shallow and shortened by a
7 weir located 15 km upstream from the river mouth (Fig. 1). The weir is used for flood control and
8 ensures that enough water is available for various upstream uses. During low precipitation and/or
9 low freshwater discharge periods, seawater extends up to the weir (Yamamoto et al. 2013).

10 Nutrient concentrations in the river are monitored by the Ministry of Land,
11 Infrastructure and Transport (MLIT), Japan (<http://www1.river.go.jp/>) (a triangle in Fig. 2).
12 Riverine nitrogen is composed mainly of DIN (76% on average), although the proportion changes
13 seasonally, becoming lower in summer (June to July) and higher in winter (December to January).
14 NO₃⁻ is the dominant form of DIN. Proportions of NH₄⁺ in DIN are less than 10%. Although
15 dissolved inorganic phosphorus (DIP) concentrations change from 0.4 to 2.9 μM, the DIN/DIP
16 (N/P) ratios are always higher than the Redfield ratio of 16 (Redfield et al. 1963).

17

18 **2.2. Field sampling**

19 Transect surveys from the bay head of Osaka Bay to the lower part of the Yodo River
20 (circles in Fig. 1) were conducted during the spring tide in each season: spring (30 May 2010 and
21 17 May 2011), summer (15 July 2011), autumn (8 November 2011) and winter (21 February 2012).
22 The observations were started from the offshore station (Stn. Y10) to the upper estuarine station
23 (Stn. Y5) during the morning ebb tide. At each station, water temperature, salinity, and dissolved
24 oxygen (DO) concentration were measured vertically by a CTD (JFE Advantech, ASTD102).

1 Water samples (5L) for chemical analysis were collected vertically from the surface to bottom
2 layers (three to five layers; Tables 1 to 5) at each station using a 6-L Van Dorn Water Sampler
3 (RIGO, Horizontal type).

4 In the laboratory, 100 mL water was filtered through precombusted (450°C, 3 h) glass
5 fiber filters (25 mm diameter, Whatman GF/F). The filters were soaked separately in 5 mL *N,N*-
6 dimethylformamide (DMF) in polypropylene tubes and stored in the dark at 4°C for subsequent
7 Chl-*a* analysis. Other water samples were filtered through precombusted glass fiber filters (25 or
8 47 mm diameter and 0.7 µm pore size, Whatman GF/F), and the filters were stored individually
9 in glass vials at -30°C until later analysis for nitrogen and carbon isotopes of particulate organic
10 matter (POM). Filtrate was stored separately at -30°C for later analyses of concentration and $\delta^{15}\text{N}$
11 of NO_3^- and NH_4^+ .

12

13 **2.2. Chemical analyses**

14 The Chl-*a* concentration in the bulk POM on GF/F was quantified using a calibrated
15 fluorometer (Trilogy, Turner Design). The NO_3^- , NO_2^- , and PO_4^{3-} concentrations were measured
16 using an autoanalyzer (Bran-Luebbe, TRAACS-800). The NH_4^+ concentration was measured
17 fluorometrically by application of the ortho-phthaldialdehyde (OPA) method (Holmes et al. 1999)
18 using a Turner Design Trilogy fluorometer.

19 The measurement of $\delta^{15}\text{N}$ of NH_4^+ was based on the method by Holmes et al. (1998). In
20 this study, we analyzed the samples that contained over 2 µM NH_4^+ to minimize isotope
21 fractionation due to recovery rate. The filtrate was transferred to incubation bottles, and NaCl,
22 MgO, and an NH_3 trap were added. The NH_3 trap consisted of an acidified 10-mm diameter GF/D
23 filter sandwiched between two 25-mm diameter, 10-µm pore size Teflon membrane filters that
24 floated on the saline samples. Di-ammonium hydrogen citrate was used as the reference for

1 sample correction of isotope deviations due to recovery rate. The samples and references were
2 incubated for 10 to 14 days depending on the incubation bottle sizes such that all NH_4^+ could
3 diffuse out of the solution and be trapped by the filters. During incubation, samples were mixed
4 gently and maintained at 40°C . After incubation, filter packages were removed from the
5 incubation bottles, dried, and stored prior to isotope analysis in a desiccator with sulfuric acid.
6 The $\delta^{15}\text{N}$ values were measured using a mass spectrometer (delta S, Finnigan MAT) coupled
7 online via a ConFlo-2 interface (Finnigan MAT) with an elemental analyzer (EA1108, Carlo Erba).
8 The recovery of the NH_4^+ retrieval procedure was $>80\%$ with a tendency toward better precision
9 at higher concentrations. Measured $\delta^{15}\text{N}$ values of samples were corrected by the fractionation of
10 each reference. The reproducibility of the running standard (DL-alanine) was better than $\pm 0.2\%$.

11 The measurement of $\delta^{15}\text{N}$ of $\text{NO}_3^- + \text{NO}_2^-$ was conducted using the denitrifier method
12 (Sigman et al. 2001) with a Thermo Finnigan GasBench and PreCon trace gas concentration
13 system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer at the
14 University of California- Davis Stable Isotope Facility (SIF). Full procedure was done by SIF
15 staff. Except in May 2011, the value of $\delta^{18}\text{O}$ of $\text{NO}_3^- + \text{NO}_2^-$, were measured simultaneously with
16 $\delta^{15}\text{N}$ (Casciotti et al. 2002). Because the majority samples included a non-negligible amount of
17 NO_2^- ($> 20\%$ of $\text{NO}_2^- + \text{NO}_3^-$) at offshore sites in July 2011, we show $\delta^{18}\text{O}$ data for $< 20\%$ of the
18 samples. Hereafter, $\text{NO}_3^- + \text{NO}_2^-$ is described simply as NO_3^- unless specified otherwise.

19 POM samples were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. GF/F filters for this analysis
20 were stored overnight in a desiccator with HCl fumes to remove inorganic carbon. Each dried
21 filter was folded and packed into a tin capsule (Thermo Scientific, 240 06400). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
22 values of the POM were measured on a mass spectrometer (Finnigan Mat, delta S) and an
23 elemental analyzer (Carlo Erba, EA1108). Center of Ecological Research-certified reference
24 materials (Tayasu et al. 2011) were used for calibration and verification processes. The

1 reproducibilities of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were better than ± 0.3 and $\pm 0.2\%$, respectively. All isotope data
2 are reported in conventional delta notation and are relative to atmospheric N_2 and VPDB standard.

4 **2.3. Mixing model**

5 As a conservative parameter, salinity is commonly used in estuarine studies to derive
6 sources and sinks of nutrients deviating from conservative mixing (Middelburg and
7 Nieuwenhuize 2001; Kasai et al. 2010). When the estuarine nutrient concentration is determined
8 simply by the conservative mixing of river water and seawater nutrients, the concentration of
9 nutrients in estuarine waters (N_{MIX}) depends linearly on salinity:

$$11 \quad N_{\text{MIX}} = \frac{(S_s \times N_m) + ((S_m - S_s) \times N_r)}{S_m}, \quad (1)$$

12
13 in which s denotes the sampling point, m the marine endmember, r the river endmember, S the
14 salinity and N the concentrations of the nutrients (NO_3^- , NH_4^+ and PO_4^{3-}). The salinity of the river
15 endmember is zero. Similarly, $\delta^{15}\text{N}$ of DIN is expressed as follows:

$$17 \quad \delta N_{\text{MIX}} = \frac{S_s \times N_m \times \delta N_m + (S_m - S_s) \times N_r \times \delta N_r}{S_s N_m + (S_m - S_s) \times N_r}, \quad (2)$$

18
19 in which δN denotes the $\delta^{15}\text{N}$, and δN_{MIX} is the expected $\delta^{15}\text{N}$ from the model as a function of S .
20 In this study, we calculated only δN_{MIX} of $\delta^{15}\text{N}_{\text{NO}_3}$, because it was impossible to characterize the
21 $\delta^{15}\text{N}_{\text{NH}_4}$ of the riverine endmember due to analytical limitations. Values of marine endmembers
22 were from the bottom water (highest salinity water) at Stn. Y10, whereas those of riverine
23 endmembers were obtained from the monitoring data in each month (Figs. 1 and 2) by MLITT

1 and the literature ($\delta^{15}\text{N}_{\text{NO}_3} = 10\%$; Wada 2009).

2 Deviations of each constituent from conservative behavior were calculated on the basis
3 of the expected change in concentration or $\delta^{15}\text{N}$ due to the conservative mixing model for
4 concentrations (Eq. 1) or $\delta^{15}\text{N}$ (Eq. 2) versus the actual change in concentrations, that is, $\Delta N = N_s$
5 $- N_{\text{MIX}}$ or $\Delta\delta^{15}\text{N} = \delta N_s - \delta N_{\text{MIX}}$. A positive ΔN corresponds to an increase in analyte concentration
6 (production) compared with the conservative estimate, whereas a negative ΔN corresponds to a
7 decrease in the analyte (consumption) (Roberts et al. 2012). A positive $\Delta\delta^{15}\text{N}$ of NO_3^- ($\Delta\delta^{15}\text{N}_{\text{NO}_3}$)
8 suggests an isotope effect due to assimilation and/or denitrification, whereas a negative $\Delta\delta^{15}\text{N}_{\text{NO}_3}$
9 suggests an isotope effect from, for example, partial nitrification (Dähnke et al. 2008; Wankel et
10 al. 2009; Miyajima et al. 2009; Sugimoto et al. 2014).

11

12 **2.4. Rayleigh equations**

13 The quantitative relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and NO_3^- concentrations can be
14 theoretically described by the first-order Rayleigh fractionation model. If phytoplankton take up
15 NO_3^- supplied from the river, $\delta^{15}\text{N}_{\text{NO}_3}$ in the estuary can follow the theoretical relationship
16 between $\delta^{15}\text{N}_{\text{NO}_3}$ and NO_3^- concentrations with some isotopic discrimination as expressed by the
17 following equation (Altabet and Francois 2001; Sugimoto et al. 2009b):

18

$$19 \quad \delta^{15}\text{N}_{\text{NO}_3_{\text{ass}}} = \delta^{15}\text{N}_{\text{NO}_3_{\text{ini}}} + \varepsilon_{\text{NO}_3} \times \ln f_{\text{NO}_3} \quad , \quad (3)$$

20

21 in which $\delta^{15}\text{N}_{\text{NO}_3_{\text{ass}}}$ is the $\delta^{15}\text{N}_{\text{NO}_3}$ expected by NO_3^- assimilation, $\delta^{15}\text{N}_{\text{NO}_3_{\text{ini}}}$ is the $\delta^{15}\text{N}$ of the
22 supplied NO_3^- , $\varepsilon_{\text{NO}_3}$ is the isotope discrimination of NO_3^- assimilation by phytoplankton and f_{NO_3}
23 ($[\text{NO}_3^-]_s/[\text{NO}_3^-]_{\text{ini}}$) is the residual fraction of NO_3^- utilized by phytoplankton. The values of $[\text{NO}_3^-]_s$
24 and $[\text{NO}_3^-]_{\text{ini}}$ denote the measured and the initial NO_3^- concentrations of the source, respectively.

1 On the other hand, $\delta^{15}\text{N}_{\text{NO}_3}$ regenerated by nitrification ($\delta^{15}\text{N}_{\text{NO}_3_{\text{nit}}}$) can be simulated as follows
2 (Southwell et al. 2008; Sugimoto et al. 2008):

$$3 \quad \delta^{15}\text{N}_{\text{NO}_3_{\text{nit}}} = \delta^{15}\text{N}_{\text{NH}_4_{\text{ini}}} - \varepsilon_{\text{nit}} \times f_{\text{NH}_4} \times \ln f_{\text{NH}_4} / (1 - f_{\text{NH}_4}) \quad , \quad (4)$$

5
6 in which ε_{nit} is the apparent isotopic discrimination for nitrification in ‰ units and f_{NH_4}
7 ($[\text{NH}_4^+]_s / [\text{DIN}]_s$) is the proportion of NH_4^+ remaining in the water. $[\text{NH}_4^+]_s$ and $[\text{DIN}]_s$ denote the
8 measured NH_4^+ and DIN concentrations.

9 Eqs. (3) and (4) assume a closed system (i.e., neither new NO_3^- nor regenerated NH_4^+ is
10 supplied continuously). However, the substrate NO_3^- for assimilation or NH_4^+ for nitrification may
11 be supplied continuously from the upper site or from sufficient organic materials in the estuary,
12 respectively, which conflict with the assumption of the closed system model. Therefore, it is
13 important to assume that the theoretical changes in an open system at a steady state are as follows
14 (Southwell et al. 2008; Sugimoto et al. 2009a):

$$15 \quad \delta^{15}\text{N}_{\text{NO}_3_{\text{ass}}} = \delta^{15}\text{N}_{\text{NO}_3_{\text{ini}}} - \varepsilon_{\text{NO}_3} \times \ln(1 - f_{\text{NO}_3}) \quad , \quad (5)$$

$$16 \quad \delta^{15}\text{N}_{\text{NO}_3_{\text{nit}}} = \delta^{15}\text{N}_{\text{NH}_4_{\text{ini}}} + \varepsilon_{\text{nit}} \times f_{\text{NH}_4} \quad . \quad (6)$$

17
18
19
20 In reality, the reaction probably represents and an intermediate between these two extreme
21 situations, but we can constrain the results by considering both models.

22 3. Results

23 3.1. Physical and biogeochemical properties on each sampling date

1 Our snapshot sampling for physical and biogeochemical parameters within the estuaries
2 found marked differences among sampling dates, especially in spring 2010 and 2011 (Fig. 3). In
3 May 2010, saltwater showed extensive intrusion into the estuary compared to May 2011 (Fig. 3B).
4 DO concentrations were significantly lower in 2010, with a minimum of 49% (4.1 mg L^{-1}), than
5 those in 2011 (Fig. 3C). Although NO_3^- concentrations were not significantly different between
6 the two years, $\delta^{15}\text{N}_{\text{NO}_3}$ values in 2010 were significantly lower than those in 2011 (Fig. 3E, I,
7 Tables 1, 2). $\delta^{15}\text{N}_{\text{NO}_3}$ in the bottom water at Stn. Y9 showed minimum value (0.1‰, Table 1). The
8 concentrations of NH_4^+ and PO_4^{3-} in 2010 were significantly higher than those in 2011 (Fig. 3F,
9 G), while the ratios of DIN to PO_4^{3-} (N/P) were significantly low in 2010 (Fig. 3H).

10 In July 2011, saltwater did not show extensive intrusion (Fig. 3B). Water temperature,
11 Chl-a concentrations and $\delta^{15}\text{N}_{\text{PN}}$ values showed highest values between sampling dates (Fig. 3A,
12 D, L, Table 3). The DO values showed widest range from 15% (1.2 mg L^{-1}) in the bottom layer to
13 129% (9.1 mg L^{-1}) in the surface layer (Fig. 3C). PO_4^{3-} concentrations significantly increased from
14 spring to summer, while N/P ratio decreased to around Redfield ratio of 16 (Fig. 3G, H).

15 Saltwater occupied the majority of the estuary in November 2011 (Fig. 3B), since the
16 proportion of freshwater were lowest between sampling dates. The values of DO, Chl-a, $\delta^{15}\text{N}_{\text{NO}_3}$
17 and $\delta^{15}\text{N}_{\text{PN}}$ significantly decreased from summer to fall (Fig. 3C, D, I, K), although PO_4^{3-}
18 concentrations and N/P ratios showed higher and lower values as well as in summer, respectively
19 (Fig. 3G, H).

20 In the coldest water ($< 10^\circ\text{C}$) period in February 2012, DO concentrations showed near
21 saturated values (Fig. 3A, C). Saltwater intruded extensively into the estuary, but freshwater
22 occupied upper layer (Fig. 3B). From fall to winter, PO_4^{3-} concentrations and N/P ratio
23 significantly decreased and increased, respectively (Fig. 3G, H).

24 There was no significant difference in $\delta^{15}\text{N}_{\text{NH}_4}$ values on each sampling date, but $\delta^{15}\text{N}_{\text{NH}_4}$

1 were always higher than $\delta^{15}\text{N}_{\text{NO}_3}$. The C/N ratios of bulk POM during all months showed similar
2 values from 5 to 10, which are similar to those of phytoplankton (6.6; Redfield et al. 1963).

3 4 **3.2. Concentrations and isotope ratios along the salinity gradient**

5 The concentrations of NO_3^- and NH_4^+ in May 2010 showed conservative or slightly
6 higher values than the mixing line, whereas $\delta^{15}\text{N}_{\text{NO}_3}$ showed lower values (Fig. 4). The mean
7 values of ΔNO_3^- , ΔNH_4^+ and ΔPO_4^{3-} in May 2010 were averaged to near zero, although the
8 $\Delta\delta^{15}\text{N}_{\text{NO}_3}$ in May 2010 showed a mean negative value (Table 6), indicating an importance of
9 productive processes on average across the transect. Contrastingly, net consumptive processes
10 dominated in May 2011 (Table 6), producing lower NO_3^- , NH_4^+ and PO_4^{3-} values than predicted
11 by the mixing line. However, this was not uniform across the salinity gradient. NO_3^-
12 concentrations were lower than the mixing line at low salinity water (<10), but shifted to higher
13 concentrations than predicted by the mixing at the high salinity water.

14 In July 2011, the concentrations of NO_3^- and NH_4^+ in low-salinity waters (< 10) were
15 lower than and similar to the mixing line, respectively, whereas both concentrations in brackish
16 waters (10–25) were higher than the mixing line (Fig. 4). Positive average values of ΔNO_3^- ,
17 ΔNH_4^+ and ΔPO_4^{3-} across the transect (Table 6) indicated that net productive process dominated
18 in summer, although non-negligible consumptive process occurred within the estuary. Moving
19 toward the higher salinity area, there was a shift from $\delta^{15}\text{N}_{\text{NO}_3}$ higher than the mixing line to
20 $\delta^{15}\text{N}_{\text{NO}_3}$ lower than the mixing line (Fig. 4). This means that the consumptive process occurred in
21 the lower salinity area, but productive process occurred in the higher salinity area.

22 In November 2011, NO_3^- concentrations considerably increased from mixing line. High
23 average values of ΔNO_3^- , ΔNH_4^+ and ΔPO_4^{3-} (Table 6) indicated that net productive process
24 dominated within the estuary, although the deviations of $\delta^{15}\text{N}_{\text{NO}_3}$ from the mixing line were

1 slightly positive (Fig. 4). In February 2012, in comparison with the mixing line, mean ΔNO_3^-
2 showed highest values with higher values, whereas mean ΔPO_4^{3-} was zero (Table 6). The $\delta^{15}\text{N}_{\text{NO}_3}$
3 values showed conservative mixing, and the ranges of $\Delta\delta^{15}\text{N}_{\text{NO}_3}$ were the smallest during all
4 months (Fig. 4 and Table 6).

5

6 **3.3 Relationships among isotope ratios, nitrogen concentrations and DO**

7 When the NO_3^- concentrations were higher than $\sim 40 \mu\text{M}$, $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ showed
8 relatively constant values around 8–12‰ and 0–4‰, respectively (Fig. 5), indicating isotope
9 ratios of riverine NO_3^- have almost constant values regardless of the season. With the lower NO_3^-
10 concentrations ($< \sim 40 \mu\text{M}$), however, $\delta^{15}\text{N}_{\text{NO}_3}$ values showed two contrasting trends. In May 2011
11 and February 2012, $\delta^{15}\text{N}_{\text{NO}_3}$ values slightly increased with a decrease in NO_3^- concentrations,
12 whereas $\delta^{15}\text{N}_{\text{NO}_3}$ values considerably decreased with the NO_3^- concentration in May 2010 and
13 November 2011. The values of $\delta^{15}\text{N}_{\text{NO}_3}$ in July 2011 showed both trends: an increase until ~ 20
14 μM but a decrease from $\sim 20 \mu\text{M}$ with a decrease in NO_3^- concentrations. $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$
15 showed higher values in near-saturated DO conditions ($> 80\%$), and the lowest $\delta^{15}\text{N}_{\text{NO}_3}$ and
16 $\delta^{18}\text{O}_{\text{NO}_3}$ values were observed when the DO concentration was 40–80% and $< 80\%$, respectively.
17 On the other hand, $\delta^{15}\text{N}_{\text{NH}_4}$ values converged to around 12 to 18‰ which corresponded to waters
18 with low DO ($< 80\%$), where they showed low values with $< 5 \mu\text{M}$ of NH_4^+ and $> 80\%$ of DO.

19

20 **4. Discussion**

21 **4.1. Significance of internal biogeochemical processes within the estuary**

22 We found that, regardless of the season of sampling, the mean ΔDIN and ΔPO_4^{3-} values
23 were mostly positive and/or conservative, except in May 2011 (Table 6). This indicates that on
24 average across the transect net productive process dominated within the estuary. Nutrient supply

1 from remineralization would play a prominent role in sources in most months, particularly in
2 summer and autumn, since direct nutrient supply from external sources such as groundwater is
3 minor component in this region (Yasumoto et al. 2010). However, this was not uniform across the
4 salinity gradient. For example, in July 2011, nutrient concentrations tended to be lower than
5 predicted by the mixing in the lower salinity water, but shifted to concentrations higher than
6 predicted by the mixing in the higher salinity water (Fig. 4). Similarly, $\delta^{15}\text{N}_{\text{NO}_3}$ showed higher
7 values than predicted by the mixing line in the lower salinity water, but decreased to $\delta^{15}\text{N}_{\text{NO}_3}$
8 lower than predicted by the mixing line in the higher salinity water. These results indicate a change
9 in dominant process from consumption to production moving toward the higher salinity area.

10 Positive and negative deviations in the $\delta^{15}\text{N}$ of NO_3^- , which is the major form of DIN,
11 showed the importance of internal biogeochemical processes (Fig. 4 and Table 6). The negative
12 relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and NO_3^- concentrations manifests as both spatial and temporal
13 phenomena of NO_3^- assimilation by phytoplankton (Altabet and Francois 2001; Sugimoto et al.
14 2009b). In contrast, a positive exponential relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and NO_3^- concentrations
15 was obtained when nitrification was dominant in nitrogen cycle (Sugimoto et al., 2009a). Both
16 relationships were clearly found below $40 \mu\text{M NO}_3^-$ (Fig. 5), and thus the isotope effects of NO_3^-
17 assimilation and/or nitrification likely causes spatial and temporal variations in $\delta^{15}\text{N}_{\text{NO}_3}$.

18

19 **4.2. Evaluation of NO_3^- assimilation and nitrification within the estuary**

20 Quantitative influence of NO_3^- assimilation and nitrification on DIN dynamics can be
21 evaluated using Rayleigh equations. To calculate Eqs. (3) and (5), we assumed $\delta^{15}\text{N}_{\text{NO}_3\text{-ini}} = 10\text{‰}$,
22 because $\delta^{15}\text{N}_{\text{NO}_3}$ values converged to approximately 10‰ above $40 \mu\text{M NO}_3^-$ (Fig. 5), and this
23 value is similar to the $\delta^{15}\text{N}_{\text{NO}_3}$ value of the freshwater source in the Yodo River (9.6 to 10.5‰;
24 Wada 2009). The values of $[\text{NO}_3^-]_{\text{ini}}$ were from the monitoring data in each month (Fig. 2). As a

1 result, in the case of NO_3^- assimilation (Fig. 6A), most $\delta^{15}\text{N}_{\text{NO}_3}$ values in May 2011, July 2011,
2 and February 2012 plotted within the range of -1 to -7‰ of ϵ_{ass} , which is similar to previously
3 reported values from field studies (Horrigan et al. 1990; Lehmann et al. 2004).

4 On the other hand, to calculate Eqs. (4) and (6), we have used 12‰ as $\delta^{15}\text{N}_{\text{NH}_4\text{-ini}}$. This
5 value was estimated from the minimum case of $\delta^{15}\text{N}_{\text{NH}_4}$ convergence value in low DO waters (12
6 to 18‰, Fig. 5). In Ise Bay, negative $\delta^{15}\text{N}_{\text{NO}_3}$ values caused by nitrification were observed in
7 slightly depleted DO waters (approximately 2.6–6.4 mg L⁻¹) in spring (Sugimoto et al. 2009a).
8 Similar DO values (40–80% = 2.5–7 mg L⁻¹, Fig. 5) suggest the dominant influence of DIN
9 remineralization and subsequent nitrification. In May 2010, most $\delta^{15}\text{N}_{\text{NO}_3}$ values were plotted
10 within the ϵ_{nit} range of -15 to -25‰ of the both models (Fig. 6B), but $\delta^{15}\text{N}_{\text{NO}_3}$ values in November
11 2011 could be explained by the steady-state model of Eq. (6) with -15 to -25‰ of ϵ_{nit} . These
12 results may be supported by $\delta^{18}\text{O}_{\text{NO}_3}$ reflecting the $\delta^{18}\text{O}$ of ambient water ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$), because it is
13 directly sensitive to that degree of nitrification (Casciotti et al. 2002). If we assume that the
14 $\delta^{18}\text{O}_{\text{NO}_3}$ produced by nitrification is slightly higher than that of ambient estuarine water ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$
15 = -7 to 0‰, Nishida et al. 2011), the $\delta^{18}\text{O}_{\text{NO}_3}$ would tend to have relatively negative values near
16 0‰. The $\delta^{18}\text{O}_{\text{NO}_3}$ could also be affected by the $\delta^{18}\text{O}$ of dissolved O₂ in low DO water, in which
17 O₂ has high $\delta^{18}\text{O}$ due to respiration (Quay et al. 1995). However, no increasing trends of $\delta^{18}\text{O}_{\text{NO}_3}$
18 below 80% DO (Fig. 5) indicate the non-negligible effect of dissolved O₂ incorporation.

20 **4.3. Co-occurrence of DIN assimilation and nitrification**

21 The mean ΔNO_3^- in July 2011 was significantly lower than that in November 2011,
22 although the mean values of ΔNH_4^+ and ΔPO_4^{3-} in both months showed similar positive values
23 (Table 6). The $\Delta\delta^{15}\text{N}_{\text{NO}_3}$ values showed positive linear relationships with DO% ($r^2 = 0.88$, $p <$
24 0.01) and Chl-*a* concentrations ($r^2 = 0.63$, $p < 0.01$) in July, indicating co-occurrence of NO_3^-

1 regeneration (nitrification) and NO_3^- assimilation within the estuary. These findings indicate that
 2 NO_3^- assimilation by phytoplankton blooming is likely to inhibit the accumulation of NO_3^- . These
 3 results were supported by a slope of 1.1 of $\delta^{15}\text{N}_{\text{NO}_3}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$ in July 2011 ($r^2 = 0.49$, $p < 0.01$),
 4 because NO_3^- assimilation leads to an enrichment of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ on a slope of 1 to 1
 5 (Granger et al. 2004). The contribution rates of NO_3^- assimilation on $\delta^{15}\text{N}_{\text{NO}_3}$ signatures (f_{ass}) can
 6 be expressed simply as:

7

$$8 \quad f_{\text{ass}} = 100 \times \frac{\delta^{15}\text{N}_{\text{NO}_3\text{S}} - \delta^{15}\text{N}_{\text{NO}_3\text{nit}}}{\delta^{15}\text{N}_{\text{NO}_3\text{ass}} - \delta^{15}\text{N}_{\text{NO}_3\text{nit}}} , \quad (7)$$

9

10 in which $\delta^{15}\text{N}_{\text{NO}_3\text{S}}$ is the observed isotopic value. If f_{ass} shows unreasonable estimates (i.e., $>100\%$
 11 or negative values), we assumed it to be 100% or 0%. Using equations (3), (4), (5), and (6),
 12 contribution rates were estimated as $72 \pm 26\%$ (mean \pm SD) with changing values of ϵ_{nit} (-15 to -
 13 25‰) and ϵ_{ass} (-4 to -7‰), although f_{ass} decreased moving toward the ocean (Fig. 7). These results
 14 suggest that NO_3^- assimilation by phytoplankton contributed to drawdown of NO_3^- and limited its
 15 accumulation in the estuary.

16 However, if NO_3^- assimilation is the major regulator of the $\delta^{15}\text{N}$ values in phytoplankton,
 17 its $\delta^{15}\text{N}$ should be equal to or less than that of NO_3^- . The observed $\delta^{15}\text{N}$ differences between PN
 18 and NO_3^- in July 2011 ranged from -1.0 to 14.5‰, with a mean \pm SD value of $6.6 \pm 4.4\%$, which
 19 was significantly higher than those during the other months (Fig. 8). The positive differences in
 20 July 2011, when Chl-a concentrations were significantly high, are explained only by the
 21 assimilation of ^{15}N -enriched NH_4^+ (Fig. 3 and Table 3). Because an assimilation of NH_4^+ by
 22 phytoplankton disturbs the oxidation of NH_4^+ to NO_3^- via NO_2^- , competition for NH_4^+ uptake by
 23 phytoplankton and NH_4^+ oxidizing bacteria would explain the limited increase in NO_3^-
 24 concentrations.

1 Sufficient concentrations of NH_4^+ ($> 1 \mu\text{M}$, Figs. 3 and 4) for phytoplankton demand
 2 would inhibit the assimilation of NO_3^- , since phytoplankton uptake NH_4^+ preferentially than NO_3^-
 3 (Dortch 1990; Pennock et al. 1987; York et al. 2007). The % NH_4^+ utilization (u_{NH_4}) during
 4 phytoplankton blooming in July 2011 could be determined by applying the following equation
 5 (York et al. 2007),

$$6 \quad u_{\text{NH}_4} = 100 \times \frac{[(\delta^{15}\text{N}_{\text{NO}_3} - \varepsilon_{\text{NO}_3}) - \delta^{15}\text{N}_{\text{PHY}}]}{(\delta^{15}\text{N}_{\text{NO}_3} - \varepsilon_{\text{NO}_3}) - (\delta^{15}\text{N}_{\text{NH}_4} - \varepsilon_{\text{NH}_4})} \quad , \quad (8)$$

8
 9 In which the $\delta^{15}\text{N}_{\text{PHY}}$ is the $\delta^{15}\text{N}$ value of phytoplankton. In this study, we assumed $\delta^{15}\text{N}_{\text{PN}}$ as
 10 $\delta^{15}\text{N}_{\text{PHY}}$, because low C/N ratios showed the dominance of phytoplankton (Fig. 3). The
 11 fractionation factors for NH_4^+ assimilation and NO_3^- assimilation are $\varepsilon_{\text{NH}_4}$ and $\varepsilon_{\text{NO}_3}$, respectively.
 12 The factor $\varepsilon_{\text{NO}_3}$ had values of -4‰ and -7‰, which were obtained from the Rayleigh fractionation
 13 model in Fig. 6. The value of $\varepsilon_{\text{NH}_4}$ may be determined by applying the following empirical
 14 equation (Liu et al. 2013):

$$15 \quad \varepsilon_{\text{NH}_4} = -39 \exp \frac{-0.0025C}{1 + 55/C} - 2[1 - \exp(-0.0025C)] \quad , \quad (9)$$

16
 17
 18 in which C is the NH_4^+ concentration (μM). In this case, $\varepsilon_{\text{NH}_4}$ varied from -9.7 to -1.4‰. If u_{NH_4}
 19 shows unreasonable estimates (i.e., $>100\%$ or negative values), we assume it to be 100% or 0%.
 20 As a result, we found that phytoplankton assimilated from 43.8 to 44.7% on average, of their N
 21 as NH_4^+ . These results imply that co-assimilation of NH_4^+ and NO_3^- by phytoplankton inhibited
 22 NO_3^- accumulation in July 2011.

1 **5. Conclusions**

2 Although delivery of riverine DIN, which is mainly composed of NO_3^- , into the Yodo
3 River estuary is usually dominated largely by seawater mixing, internal biogeochemical processes
4 of DIN assimilation by phytoplankton and DIN production by remineralization and subsequent
5 nitrification within the estuary offer the most reasonable explanations for the observed deviations
6 in NO_3^- isotopic compositions from mixing behavior. Co-assimilation of regenerated NH_4^+ within
7 the estuary and riverine NO_3^- as well as co-occurrence of DIN assimilation and regeneration
8 complicate nutrient dynamics. However, we found that, regardless of the season, the estuarine
9 mixing behavior is mostly conservative or productive. A significant net source of DIN as well as
10 DIP is present within the Yodo River estuary. These results suggest that recycled nutrients within
11 the estuary could have a non-negligible impact on eutrophication in Osaka Bay, despite the
12 general belief that riverine nitrogen loadings from the land are major trigger of eutrophication in
13 the bay (Yasuhara et al. 2007; Nakatani et al. 2011).

14

15 **Acknowledgments**

16 We are grateful to three anonymous reviewers for their helpful comments and suggestions. Isotope
17 analysis was conducted using Cooperative Research Facilities (Isotope Ratio Mass Spectrometer)
18 of Center for Ecological Research, Kyoto University. This work was supported by JSPS
19 KAKENHI Grant Number 24658175.

20

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1 **Figure captions**

2

3 Figure 1. Map of the study site. Circles are sampling stations and a triangle indicate the monitoring
4 site of water quality by the Ministry of Land, Infrastructure and Transport, Japan.

5

6 Figure 2. Monthly variation in nitrogen concentrations and dissolved inorganic nitrogen to
7 phosphorous (DIN/DIP) ratios in the Yodo River. Data were obtained from the monitoring site
8 of water information system of Ministry of Land, Infrastructure and Transport, Japan. Vertical
9 gray lines show the sampling dates of the estuary. Horizontal dashed line shows the Redfield
10 ratio (DIN/DIP = 16).

11

12 Figure 3. Box plots of temperature, salinity, DO concentrations, Chl-*a* concentrations, NO₃⁻
13 concentrations, NH₄⁺ concentrations, PO₄³⁻ concentrations, δ¹⁵N-NO₃⁻ and δ¹⁵N-NH₄⁺, DIN to
14 PO₄³⁻ ratio, δ¹⁵N-PN, C/N ratio from Stn. Y5 to Stn. Y9 on each observation date (medians;
15 box = 25-75% CI; error bar 5-95% CI; and plots maximum and minimum). ND means no data.
16 Dates with different letters are significantly different (Kruskal-Wallis ANOVA on ranks, $p <$
17 0.05).

18

19 Figure 4. Salinity versus NO₃⁻ concentrations, NH₄⁺ concentrations, δ¹⁵N-NO₃⁻ and δ¹⁵N-NH₄⁺ in
20 the Yodo River estuary. Solid and dashed lines are the conservative mixing lines for NH₄⁺ and
21 NO₃⁻, respectively.

22

23 Figure 5. δ¹⁵N-NO₃⁻, δ¹⁸O-NO₃⁻ and δ¹⁵N-NH₄⁺ plotted in relation to each concentration (left
24 panels) and DO saturation (right panels) for all water samples in May 2010, May 2011, July

1 2011, November 2011 and February 2012.

2
3 Figure 6. (A) Relationships between the residual fraction of NO_3^- ($= f_{\text{NO}_3}$) and the $\delta^{15}\text{N-NO}_3^-$.
4 Solid curve (closed system model of Eq. (3)) and dashed lines (open system model of Eq. (5))
5 indicate the theoretical changes in $\delta^{15}\text{N-NO}_3^-$ by NO_3^- assimilation with ϵ_{NO_3} of -1, -4 and -7‰.
6 (B) Relationships between the residual fraction of NH_4^+ ($= f_{\text{NH}_4}$) and the $\delta^{15}\text{N-NO}_3^-$. Solid curve
7 (closed system model of Eq. (4)) and dashed lines (open system model of Eq. (5)) indicate the
8 theoretical changes in $\delta^{15}\text{N-NO}_3^-$ by nitrification with ϵ_{nit} of -15, -20 and -25‰. Different plots
9 indicate different observations; May 2010 (\blacktriangle), May 2011 (\blacksquare), July 2011 (\circ), November 2011
10 (\times) and February 2012 (\bullet).

11
12 Figure 7. Salinity versus the contribution rates of NO_3^- assimilation on $\delta^{15}\text{N}_{\text{NO}_3}$ (f_{ass}) in July 2011.
13 Plots and error bars are mean value and SD changing values of ϵ_{nit} (-15‰ and -25‰) and ϵ_{ass}
14 (-4‰ and -7‰) for equations (3), (4), (5), and (6).

15
16 Figure 8. Relationships between Chl-a concentrations and $\delta^{15}\text{N}$ differences between PN and NO_3^-
17 in May 2011 (\blacksquare), July 2011 (\circ), November 2011 (\times) and February 2012 (\bullet).

Table 1. Results of elemental and isotopic analyses on 30 May 2010. The depth in parentheses indicates the water depth.

Station	Layer (m)	NO ₃ ⁻ + NO ₂ ⁻ (μM)	NH ₄ ⁺ (μM)	PO ₄ ³⁻ (μM)	δ ¹⁵ N _{NO3} (‰)	δ ¹⁸ O _{NO3} (‰)	δ ¹⁵ N _{NH4} (‰)	δ ¹⁵ N _{P_N} (‰)	δ ¹³ C _{POC} (‰)	C/N (mol/mol)	Chl-a (μg/L)
Y5	0	58.8	4.7	1.3	8.6	-	-	-	-	-	2.0
	1.5	19.8	12.3	1.6	6.2	-	-	-	-	-	8.0
	2.5 (2.8)	15.2	13.1	1.7	4.7	-	-	-	-	-	18.9
Y6	0	60.2	6.2	1.5	3.6	-	-	-	-	-	1.8
	1.5	16.4	6.7	1.1	5.3	-	-	-	-	-	16.2
	2.7 (3.0)	10.8	6.3	1.5	3.1	-	-	-	-	-	14.1
Y7	0	58.1	6.7	1.6	8.0	-	-	-	-	-	2.5
	1.5	8.3	5.7	1.1	3.0	-	-	-	-	-	12.4
	2.7 (3.0)	6.1	7.2	1.2	1.7	-	-	-	-	-	10.0
Y8	0	44.3	5.5	1.3	8.1	-	-	-	-	-	2.4
	2.0	5.5	2.2	0.7	3.0	-	-	-	-	-	11.4
	3.0	5.3	3.3	0.7	4.2	-	-	-	-	-	7.9
	3.2 (3.5)	6.3	3.1	0.7	3.6	-	-	-	-	-	13.7
Y9	0	56.9	7.1	1.6	6.8	-	-	-	-	-	3.6
	3.0	6.7	2.9	0.7	2.2	-	-	-	-	-	7.3
	6.0	5.3	6.9	1.0	2.8	-	-	-	-	-	9.4
	9.0	4.6	10.1	1.2	2.0	-	-	-	-	-	17.2
	10.5 (11.0)	4.3	12.5	1.5	0.1	-	-	-	-	-	17.4
Y10	0	46.6	8.1	1.5	7.3	-	-	-	-	-	3.5
	3.0	7.4	1.3	0.4	3.5	-	-	-	-	-	2.7
	6.0	7.0	4.7	0.7	0.8	-	-	-	-	-	1.8
	10.0	5.1	4.3	0.6	3.4	-	-	-	-	-	1.4
	11.5 (12.0)	4.6	7.1	0.9	0.5	-	-	-	-	-	8.1

1

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Table 2. Results of elemental and isotopic analyses on 17 May 2011. The depth in parentheses indicates the water depth.

Station	Layer (m)	NO ₃ ⁻ + NO ₂ ⁻ (μM)	NH ₄ ⁺ (μM)	PO ₄ ³⁻ (μM)	δ ¹⁵ N _{NO3} (‰)	δ ¹⁸ O _{NO3} (‰)	δ ¹⁵ N _{NH4} (‰)	δ ¹⁵ N _{PN} (‰)	δ ¹³ C _{POC} (‰)	C/N (mol/mol)	Chl-a (μg/L)
Y5	0	28.4	0.9	0.4	9.6	4.1	-	7.0	-26.2	7.5	19.1
	1.0	28.1	0.8	0.4	9.5	3.9	-	6.2	-26.5	6.3	19.3
	1.7 (2.0)	27.4	0.8	0.4	9.3	5.0	-	7.3	-26.3	7.7	18.7
Y6	0	27.5	0.9	0.4	9.2	4.3	-	6.5	-26.3	7.5	18.1
	1.0	27.4	1.0	0.4	9.3	4.2	-	7.4	-26.2	7.8	24.5
	2.6 (2.9)	27.5	1.0	0.4	9.3	4.2	-	7.4	-26.4	8.6	19.2
Y7	0	21.8	1.1	0.4	9.4	3.7	-	7.0	-26.4	7.0	16.1
	1.0	27.7	1.2	0.5	9.1	4.3	-	6.6	-26.2	8.0	18.6
	2.4 (2.7)	27.5	4.8	0.6	9.0	4.8	16.1	6.9	-26.2	8.5	16.2
Y8	0	27.9	1.0	0.4	9.2	4.8	-	7.3	-26.8	6.2	15.9
	2.0	27.8	1.3	0.4	9.1	4.7	-	6.3	-27.3	6.1	15.1
	2.7 (3.0)	22.0	9.3	1.0	9.3	6.1	11.7	6.5	-	6.6	7.6
Y9	0	14.5	1.2	0.2	9.3	2.6	-	6.6	-26.9	5.8	13.1
	3.0	2.9	6.7	0.8	11.6	12.5	20.2	9.9	-24.3	4.8	22.5
	6.0	4.7	6.6	0.7	11.8	24.7	14.6	9.3	-22.8	6.0	1.3
	8.5 (9.0)	2.7	10.7	0.8	10.6	25.8	12.3	8.8	-23.0	6.3	2.2
Y10	0	27.9	4.7	0.3	8.8	4.1	16.4	7.8	-26.4	5.9	11.3
	3.0	11.2	3.9	0.1	11.2	14.3	4.3	10.6	-20.9	5.1	9.8
	6.0	4.6	3.8	0.1	12.4	14.1	12.2	9.7	-21.2	5.8	3.1
	10.0	2.4	4.1	0.3	11.0	7.5	10.5	8.4	-21.7	5.7	1.6
	12.0 (13.5)	1.8	10.7	1.1	6.0	14.7	14.6	7.6	-22.6	7.5	5.9

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Table 3. Results of elemental and isotopic analyses on 15 July 2011. The depth in parentheses indicates the water depth, and the $\text{NO}_3^- + \text{NO}_2^-$ with asterisks indicates the higher NO_2^- fractions ($\geq 20\%$).

Station	Layer (m)	$\text{NO}_3^- + \text{NO}_2^-$ (μM)	NH_4^+ (μM)	PO_4^{3-} (μM)	$\delta^{15}\text{N}_{\text{NO}_3}$ (‰)	$\delta^{18}\text{O}_{\text{NO}_3}$ (‰)	$\delta^{15}\text{N}_{\text{NH}_4}$ (‰)	$\delta^{15}\text{N}_{\text{PN}}$ (‰)	$\delta^{13}\text{C}_{\text{POC}}$ (‰)	C/N (mol/mol)	Chl-a ($\mu\text{g/L}$)
Y5	0	33.1	0.8	1.2	12.7	5.0	-	18.3	-28.1	6.1	41.7
	0.5	33.9	1.3	1.4	12.4	4.7	-	16.1	-27.8	8.0	40.8
	1.4 (1.7)	25.2	1.1	1.6	12.7	4.1	-	23.1	-26.8	4.4	41.3
Y6	0	31.9	1.1	1.6	12.3	4.3	-	17.6	-26.2	5.9	55.8
	1	44.2	6.8	3.0	11.2	2.8	20.6	22.9	-24.3	4.5	33.7
	2.8 (3.1)	45.2	15.5	4.2	11.1	1.4	18.0	12.8	-23.3	6.0	36.2
Y7	0	31.5	2.5	1.5	12.6	4.2	3.8	23.1	-24.9	4.7	53.7
	1.5	38.0	13.5	3.6	11.0	2.8	17.3	23.7	-23.0	4.9	47.6
	2.4 (2.7)	26.4	11.8	3.4	10.8	2.8	17.8	18.7	-22.6	6.4	45.7
Y8	0	52.1	5.8	3.4	10.8	2.8	18.2	25.3	-23.8	4.9	35.8
	1	26.2	7.9	1.9	11.6	2.7	14.4	21.0	-22.7	5.5	68.0
	2	32.7	7.8	3.8	11.4	3.4	20.5	12.3	-20.8	6.5	40.1
	3.1 (3.4)	48.1	19.3	3.6	12.8	7.3	15.0	11.7	-24.8	9.2	51.3
Y9	0	83.6	9.5	3.9	12.5	2.3	27.0	19.3	-23.5	5.7	39.1
	3	17.7	10.0	1.9	9.9	2.3	18.3	20.1	-19.5	4.7	29.8
	6	11.9*	10.3	1.8	8.0	-	17.6	14.5	-19.8	6.8	17.0
	9.5 (10.0)	11.1*	9.2	2.1	7.3	-	17.4	11.1	-22.9	8.6	14.8
Y10	0	15.1*	5.1	0.8	16.0	-	13.09	19.1	-19.5	4.9	82.7
	3	3.6*	2.2	0.1	12.0	-	5.89	11.0	-18.1	6.4	11.5
	6	2.1*	2.4	0.3	7.6	-	14.45	18.8	-19.1	5.6	21.3
	10	4.5*	3.7	0.8	3.9	-	12.91	12.9	-18.1	6.4	12.9
	12.0 (12.7)	6.0*	2.0	0.2	11.2	-	14.85	13.2	-17.9	6.4	38.4

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Table 4. Results of elemental and isotopic analyses on 8 November 2011. The depth in parentheses indicates the water depth.

Station	Depth (m)	NO ₃ ⁻ + NO ₂ ⁻ (μM)	NH ₄ ⁺ (μM)	PO ₄ ³⁻ (μM)	δ ¹⁵ N _{NO3} (‰)	δ ¹⁸ O _{NO3} (‰)	δ ¹⁵ N _{NH4} (‰)	δ ¹⁵ N _{PN} (‰)	δ ¹³ C _{POC} (‰)	C/N (mol/mol)	Chl-a (μg/L)
Y5	0	37.2	10.6	2.8	10.6	-0.3	20.1	5.5	-25.4	6.2	7.6
	1.0	25.5	12.5	2.9	9.0	-0.4	18.2	5.6	-24.8	6.4	11.8
	1.7 (2.0)	28.3	12.8	3.5	8.8	-0.2	-	5.8	-24.9	7.1	12.8
Y6	0	39.4	10.7	3.0	10.0	-0.5	19.6	5.5	-24.7	5.5	8.0
	1.5	28.2	11.1	3.4	8.4	-0.4	17.8	6.5	-23.9	6.5	10.1
	2.3 (2.6)	21.4	11.0	3.2	7.0	-0.8	17.7	6.0	-24.9	8.4	10.2
Y7	0	42.3	10.0	3.1	8.4	-0.7	17.2	5.3	-25.4	5.6	11.6
	1.5	43.5	10.0	3.3	8.9	-0.7	19.5	5.4	-25.5	5.8	11.9
	2.7 (3.0)	29.8	10.9	3.3	7.5	-1.5	17.5	6.2	-24.5	6.6	9.1
Y8	0	52.1	9.7	3.0	10.9	1.6	19.1	6.2	-24.7	5.6	9.6
	1.0	31.4	6.4	1.9	9.2	-0.8	14.2	7.7	-23.8	6.1	8.4
	2.0	23.3	5.4	2.3	7.7	-0.9	17.6	8.4	-23.6	6.3	7.6
	2.7 (3.0)	41.1	10.0	2.2	9.6	-0.5	14.0	7.2	-24.5	8.6	7.4
Y9	0	54.5	7.6	4.4	10.0	-0.4	17.1	5.2	-24.6	4.9	8.3
	3.0	18.8	1.3	1.1	9.2	-1.5	-	12.8	-20.2	5.7	8.2
	6.0	22.4	1.3	1.4	9.2	-1.5	-	12.8	-20.7	5.3	6.4
	8.5 (9.3)	14.9	3.6	1.3	7.2	-2.9	19.0	10.1	-22.6	7.5	3.3
Y10	0	26.4	5.2	1.1	12.0	1.2	17.7	9.3	-20.8	5.1	4.0
	3.0	16.7	1.6	0.8	9.6	-1.0	-	11.8	-20.7	5.8	4.7
	6.0	16.5	1.1	0.8	9.7	-1.7	-	12.0	-20.5	5.6	4.3
	10.0	12.9	1.9	0.8	7.6	-2.4	-	11.5	-21.0	6.5	2.6
	12.0 (13.1)	8.9	4.1	0.7	6.5	-2.0	15.7	9.9	-21.4	7.4	2.4

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Table 5. Results of elemental and isotopic analyses on 21 February 2012. The depth in parentheses indicates the water depth.

Station	Depth (m)	NO ₃ ⁻ + NO ₂ ⁻ (μM)	NH ₄ ⁺ (μM)	PO ₄ ³⁻ (μM)	δ ¹⁵ N _{NO3} (‰)	δ ¹⁸ O _{NO3} (‰)	δ ¹⁵ N _{NH4} (‰)	δ ¹⁵ N _{PN} (‰)	δ ¹³ C _{POC} (‰)	C/N (mol/mol)	Chl-a (μg/L)
Y5	0	58.9	3.8	0.6	10.1	0.0	15.3	7.5	-26.0	6.5	11.1
	1.0	55.8	5.1	0.8	9.8	1.3	21.5	7.4	-25.9	6.7	9.8
	2.2 (2.5)	46.4	12.1	1.5	9.6	1.3	16.7	5.8	-23.5	7.4	12.1
Y6	0	59.7	3.7	0.6	9.9	1.1	20.1	6.9	-26.6	6.1	9.9
	1.5	47.6	7.9	1.1	10.0	2.4	17.8	8.4	-24.2	6.8	9.3
	2.7 (3.0)	42.6	8.8	1.2	10.1	2.2	18.2	5.8	-24.9	8.1	12.0
Y7	0	58.3	26.1	0.6	9.9	0.0	18.4	8.7	-26.8	6.4	8.9
	1.5	38.4	5.4	0.7	9.9	1.3	12.1	6.1	-21.9	6.8	16.1
	2.5 (2.8)	18.6	2.6	0.5	11.7	4.5	15.1	7.7	-23.0	7.1	12.2
Y8	0	57.9	5.2	0.8	10.2	0.5	14.3	7.2	-25.8	6.3	7.4
	1.0	37.9	10.3	1.0	10.1	1.4	20.3	7.7	-22.1	6.4	22.6
	2.0	31.6	4.8	0.6	10.0	1.3	18.3	7.9	-22.0	6.5	13.5
	2.8 (3.1)	19.8	3.1	0.4	10.7	2.8	11.1	4.4	-21.1	6.0	10.7
Y9	0	65.2	14.1	1.6	9.8	1.1	20.3	6.6	-25.8	7.2	4.1
	3.0	29.9	5.4	0.6	10.5	2.0	16.4	8.5	-20.5	5.4	22.5
	6.0	15.9	3.0	0.3	12.0	5.4	9.8	10.2	-20.7	5.7	28.0
	9.0 (9.6)	7.3	4.8	0.6	11.2	7.4	14.9	9.8	-21.9	5.8	29.5
Y10	0	84.2	24.4	2.8	10.5	-0.2	15.2	4.2	-25.3	5.9	3.9
	3.0	26.7	6.1	0.5	11.5	5.9	15.7	9.4	-20.5	5.9	27.0
	6.0	7.3	2.4	0.3	14.1	12.5	11.2	10.1	-20.8	5.9	22.4
	10.0	8.4	7.1	0.5	11.4	4.3	12.8	10.2	-20.8	5.7	16.0
	12.0 (13.1)	8.5	5.1	0.5	11.5	9.9	12.3	9.0	-21.3	5.6	20.7

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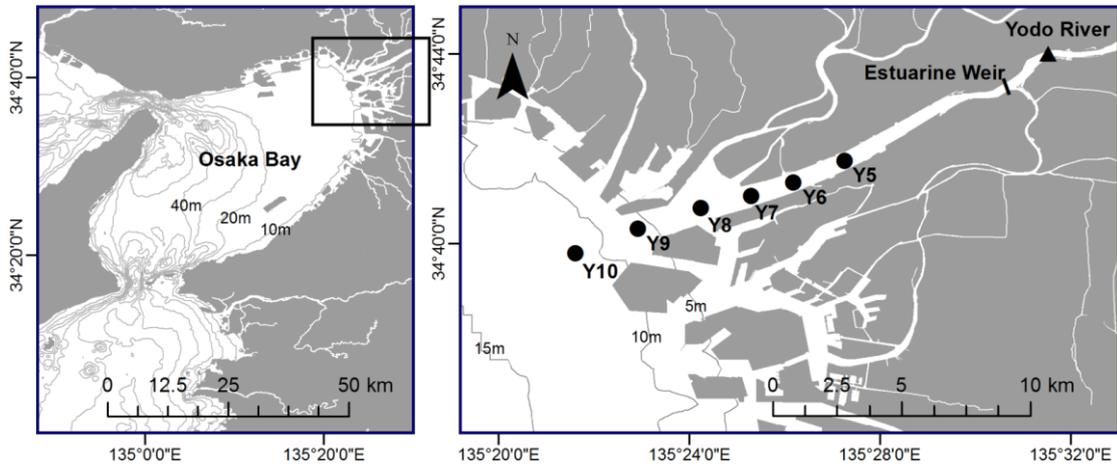
Table 6. Deviations (mean \pm SD, range) of NO_3^- , NH_4^+ , DIN, PO_4^{3-} and $\delta^{15}\text{N}_{\text{NO}_3}$ from the conservative mixing values.

Date	ΔNO_3^- (μM)	ΔNH_4^+ (μM)	ΔDIN (μM)	ΔPO_4^{3-} (μM)	$\Delta\delta^{15}\text{N-NO}_3^-$ (‰)
30 May 2010	0.9 ± 4.9 (-11.4 to 14.3, $n = 19$)	-0.2 ± 3.2 (-5.7 to 5.4, $n = 19$)	0.8 ± 6.0 (-9.3 to 17.3, $n = 19$)	0.0 ± 0.3 (-0.5 to 0.6, $n = 19$)	-0.8 ± 1.8 (-6.3 to 2.1, $n = 19$)
17 May 2011	-10.4 ± 8.1 (-27.7 to 4.5, $n = 19$)	-3.0 ± 2.2 (-6.7 to 2.8, $n = 19$)	-13.3 ± 7.9 (-30.8 to -0.2, $n = 19$)	-0.5 ± 0.2 (-1.0 to 0.0, $n = 19$)	0.7 ± 2.0 (-1.2 to 4.6, $n = 16$)
15 Jul 2011	1.3 ± 15.0 (-13.5 to 49.5, $n = 20$)	4.2 ± 4.7 (-1.3 to 13.4, $n = 20$)	5.5 ± 18.5 (-14.5 to 57.0, $n = 20$)	1.3 ± 1.2 (-0.3 to 3.5, $n = 19$)	0.5 ± 3.0 (-7.1 to 5.9, $n = 19$)
8 Nov 2011	13.1 ± 8.9 (0.1 to 31.8, $n = 21$)	3.3 ± 4.1 (-2.9 to 8.9, $n = 21$)	16.3 ± 11.0 (1.0 to 36.9, $n = 21$)	1.5 ± 1.0 (0.1 to 3.4, $n = 19$)	1.1 ± 1.2 (-0.9 to 3.7, $n = 19$)
21 Feb 2012	15.7 ± 13.0 (-2.2 to 55.9, $n = 20$)	1.2 ± 5.9 (-6.2 to 17.2, $n = 20$)	16.9 ± 16.9 (-5.0 to 73.1, $n = 20$)	0.0 ± 0.6 (-0.8 to 1.9, $n = 19$)	-0.1 ± 0.8 (-1.0 to 2.8, $n = 19$)

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1 Figure 1 (Sugimoto and Kasai)

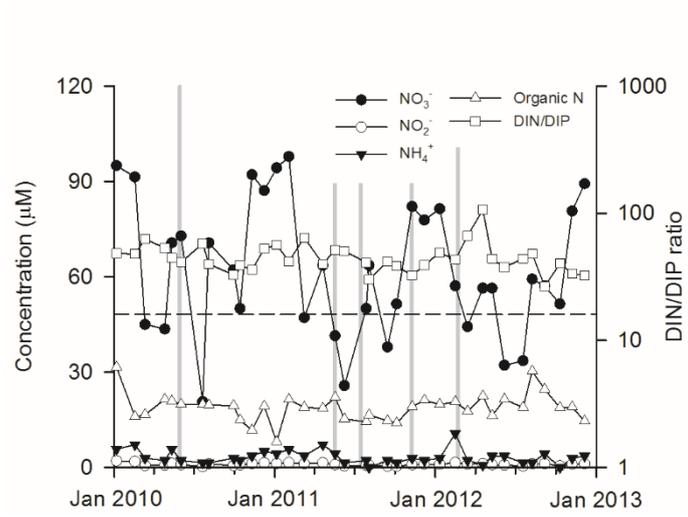
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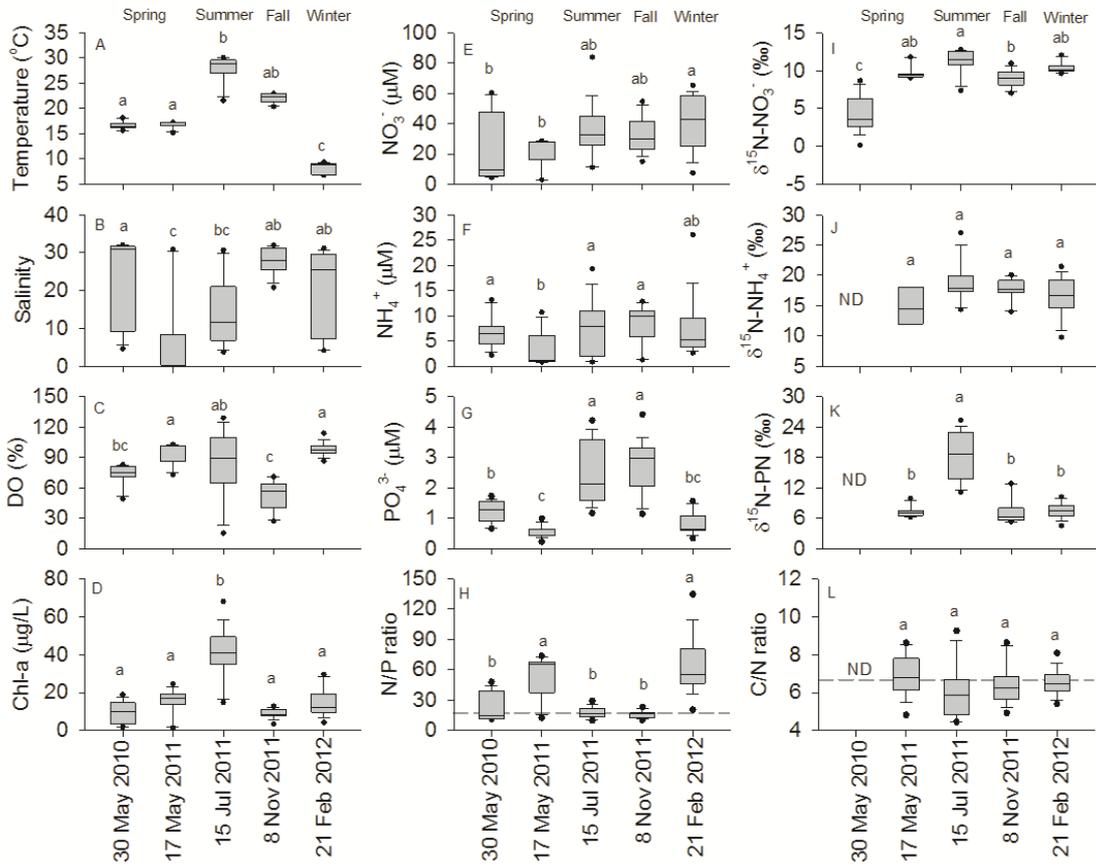
1 Figure 2 (Sugimoto and Kasai)



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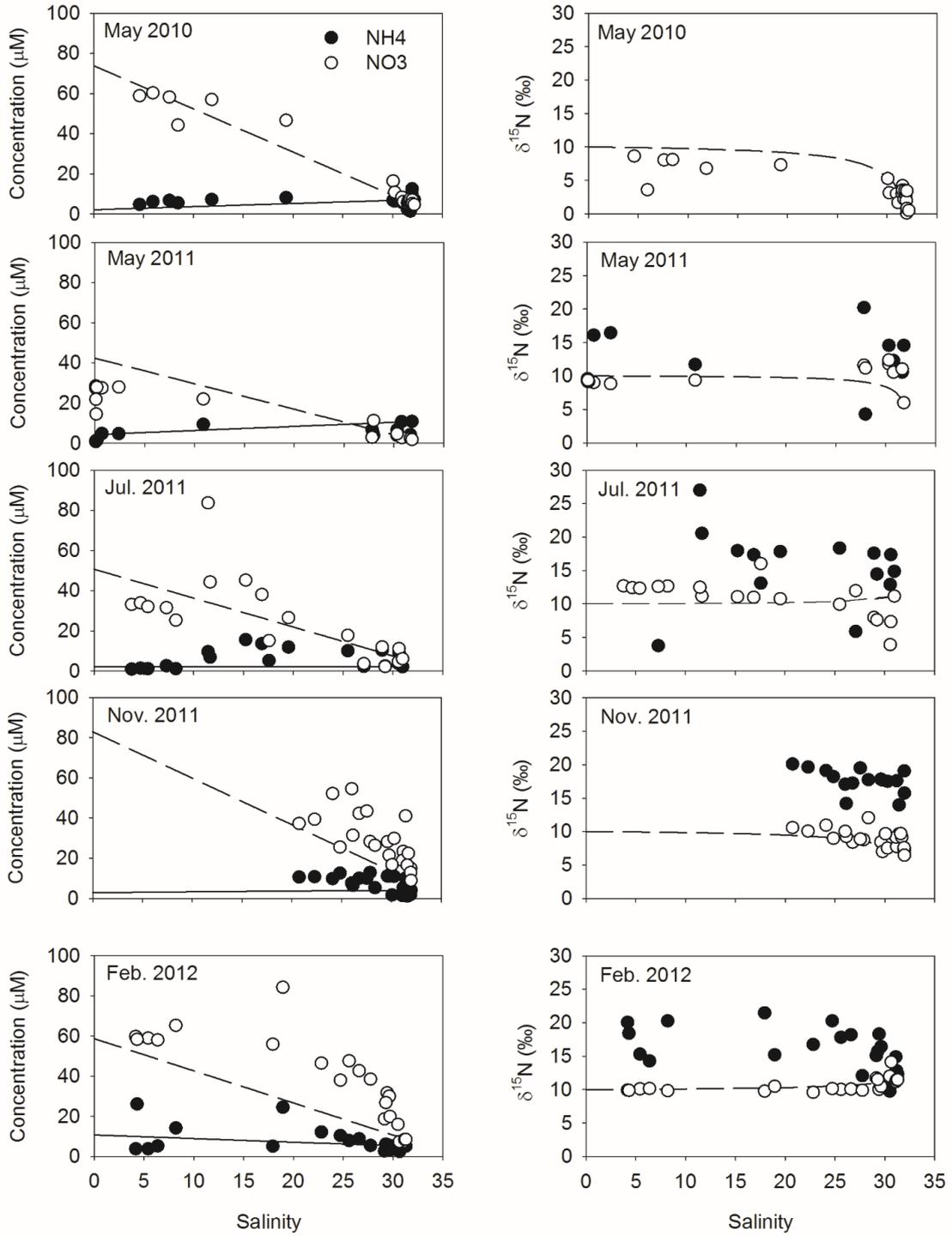
1 Figure 3 (Sugimoto and Kasai)



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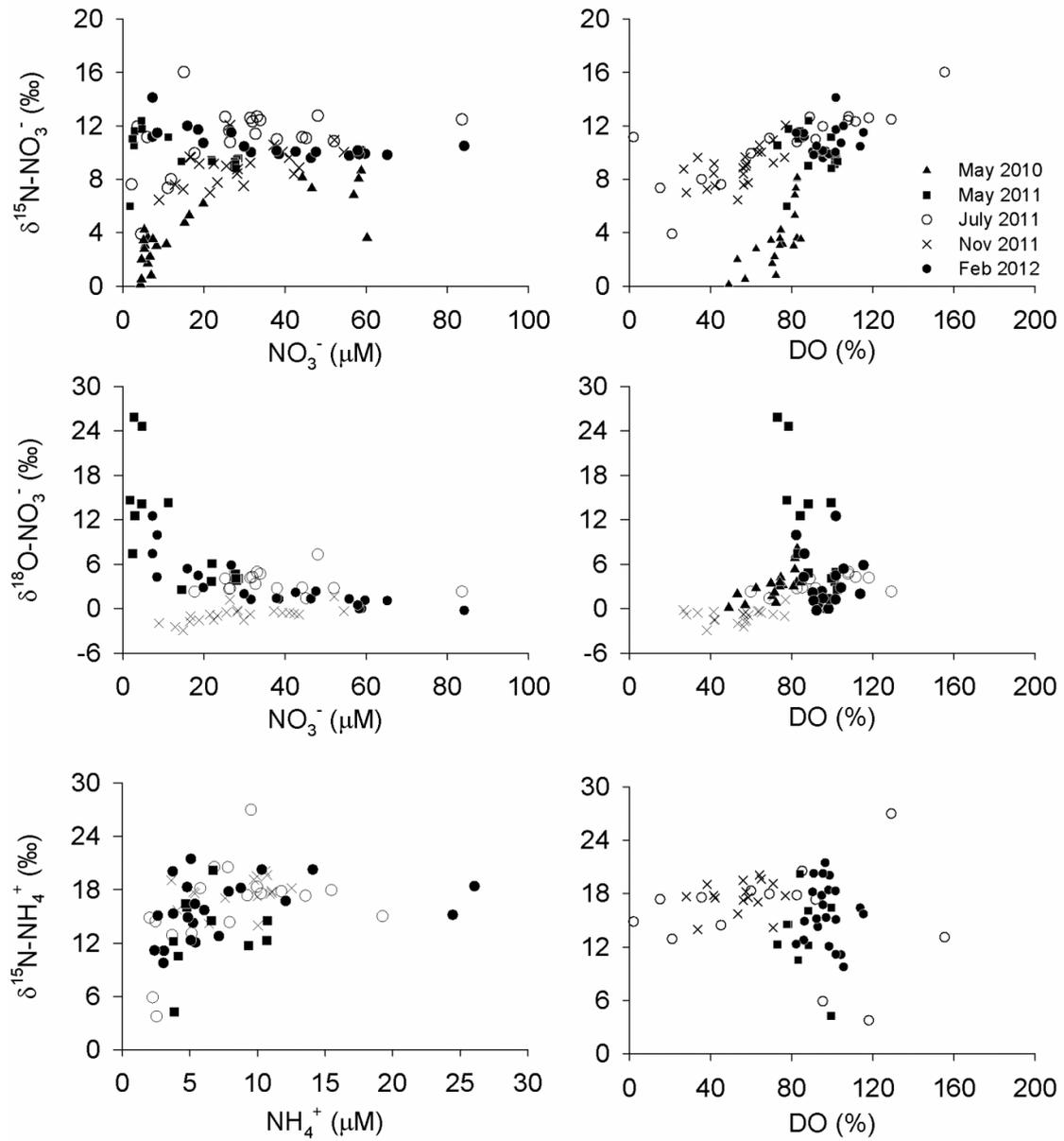
1 Figure 4 (Sugimoto and Kasai)



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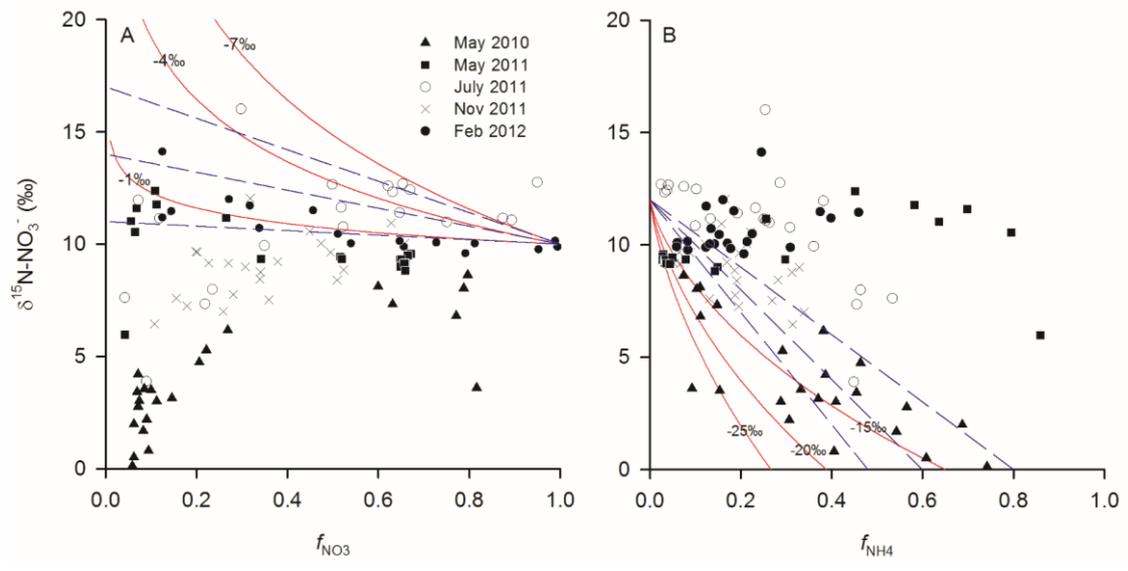
1 Figure 5 (Sugimoto and Kasai)



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1 Figure 7 (Sugimoto and Kasai)

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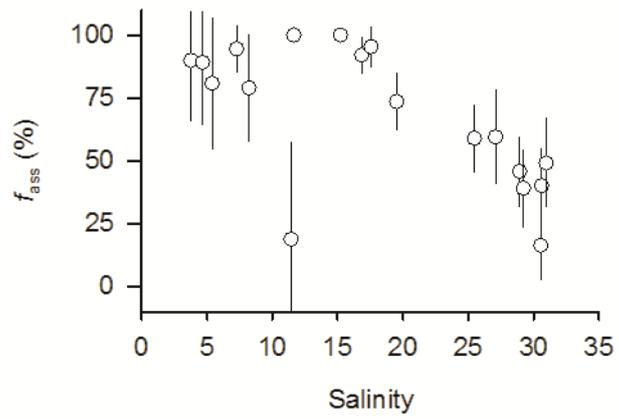


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1 Figure 7 (Sugimoto and Kasai)

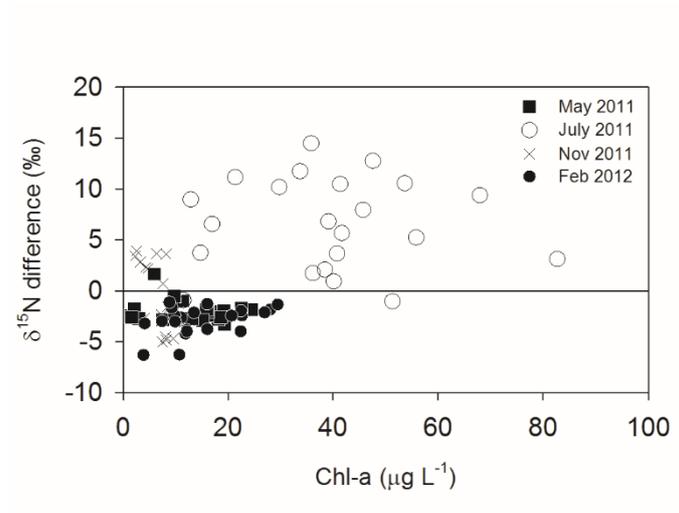
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1 Figure 8 (Sugimoto and Kasai)



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