

Nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ evidence for active biological transformation in the Changjiang Estuary and the adjacent East China Sea

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Abstract

The Changjiang Estuary has been considered as one of the most polluted estuaries in the world due to high nitrate (NO_3^-) input, especially in spring and summer. In this study, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- , along with other chemical parameters in this area, were measured in spring to evaluate NO_3^- biogeochemical processes. A simple two end-members mixing model was used to examine the relative contribution of the Changjiang River Diluted Water and marine water to NO_3^- sources in the Changjiang Estuary and the adjacent East China Sea. The isotopic signals show that NO_3^- behaved relatively and conservatively in Transect F and Transect P where assimilation was weak possibly due to vertical mixing, while active assimilation and weak nitrification occurred in Transect D. Spatial difference in assimilation was indicated by the ~1:1 enrichment of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in the three transects, while spatial difference in nitrification was reflected by deviations of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ from assimilation line. Our results suggest that the input of the Changjiang River Diluted Water promoted NO_3^- assimilation possibly by stratifying the water column which favored the phytoplankton growth.

Key words: Changjiang Estuary, nitrate, nitrogen isotope, oxygen isotope

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1 Introduction

Nitrate is the most important form of bioavailable nitrogen in the ocean. Anthropogenic activities, such as increased urban development in coastal areas and intensified agriculture, have greatly increased the input of nutrients to estuaries (Galloway et al., 2004; Gruber and Galloway, 2008; Liu et al., 2009). Eutrophication has been a major concern in estuaries and coastal areas worldwide (Galloway et al., 2004; Dumont et al., 2005; Gruber and Galloway, 2008).

The isotope method, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- , as a powerful “tracer” and/or “integrator” of N cycle processes (Brandes et al., 1998; Robinson, 2001), provides an insight into the fate of dissolved NO_3^- in marine environments. Isotopic signals suggest the biogeochemical processes of nitrogen in various marine environments (Horrihan et al., 1990; Sigman et al., 1999, 2000; Ahad et al., 2006; Granger et al., 2010), such as algal assimilation, mineralization, nitrification and denitrification (Cline

and Kaplan, 1975; Sugimoto et al., 2008). Biological transformation of NO_3^- often results in isotopic fractionation because ^{14}N is preferentially transformed or assimilated over ^{15}N (Waser et al., 1998; Ahad et al., 2006). For instance, nitrification results in the formation of ^{15}N -depleted NO_3^- , while denitrification leaves behind NO_3^- enriched in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ by 1:1 ratio (Sigman et al., 2003). An increase in $\delta^{15}\text{N}$ of the residual NO_3^- is also found when microbial and algal assimilation of NO_3^- occurs (Waser et al., 1998; Ahad et al., 2006). In contrast, remineralization of sinking organic N leads to a rise in the percentage of light N in seawater. To date, this dual isotopic approach has been used in a wide variety of systems other than marine environments, including agriculturally impacted areas (Böhlke and Denver, 1995; Böttcher et al., 1990; Cey et al., 1999), groundwater (Aravena et al., 1993; Böhlke and Denver, 1995; Cole et al., 2006; McMahan and Böhlke, 2006), precipitation (Jia and Chen, 2010), and freshwater aquatic environments, such as lakes and

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rivers (Ostrom et al., 2002; Sebilo et al., 2003; Chen et al., 2009). However, the dual isotopic composition of NO_3^- has not been applied to marine environments until recently (Brandes et al., 1998; Casciotti et al., 2002; Sigman et al., 1999, 2009; Wankel et al., 2006, 2007, 2009). Few studies were conducted on the dual isotopic composition of NO_3^- in estuaries (Wankel et al., 2006, 2009; Liu et al., 2009).

The Changjiang River ranks third in length (6 300 km), and fifth in freshwater discharge in the world (Tian et al., 1993). Many famous large fisheries are located in the Changjiang Estuary, such as Zhoushan fishery. NO_3^- concentration has increased dramatically over the last decades due to increased anthropogenic activities, resulting in severe eutrophication impacts on the East China Sea during spring (Edmond et al., 1985; Zhang et al., 1999; Bao et al., 2006). Therefore, it is important to examine nitrogen biogeochemical cycling in the Changjiang Estuary and the adjacent East China Sea in spring. Although $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ in Changjiang River Basin have been examined (Li et al., 2010), the previous studies focused on the distribution of NO_3^- in the Changjiang Estuary and the adjacent East China Sea. So far, only one study has been conducted to study surface NO_3^- biogeochemistry in the Changjiang Estuary using single $\delta^{15}\text{N}-\text{NO}_3^-$, implying that active NO_3^- biological transformation occurred in the Changjiang Estuary (Liu et al., 2009). However, it may be biased to estimate the contribution of various NO_3^- sources and the degree of NO_3^- biological transformation using $\delta^{15}\text{N}-\text{NO}_3^-$ alone due to its limitation. In this study, nitrogen and oxygen isotopes of NO_3^- and other chemical parameters in water column were determined to distinguish various sources of NO_3^- , and provide an insight into NO_3^- biological transformation in the water column in spring.

2 Materials and methods

2.1 Field sampling

A cruise was carried out in spring (March to April) of 2011. The twenty-three sampling stations were visited in three representative transects (D, F, P) (Fig.1). Vertical profiles of salinity, and temperature were measured with a CTD. For nitrate (NO_3^- -N), and the isotopic composition of NO_3^- ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$), water samples were filtered through cellulose acetate membrane (0.45 μm in pore size). For samples of NO_3^- concentration, and NO_3^- isotopic composition, mercuric chloride (HgCl_2) was added to kill bacteria and then samples were stored at -20°C until analyzed.

2.2 Chemical analysis

NO_3^- was determined by the cadmium-copper reduction method (Grasshoff, 1976) with a SKALAR Flow autoanalyzer. The data quality was monitored by intercalibrations and the detection limit was 0.2 $\mu\text{mol/L}$ for nitrate. Dissolved oxygen concentration was measured by the Winkler titration method (Strickland and Parsons, 1972) and the precision was 0.07 mg/L.

2.3 Stable isotopic analysis

After nitrite was removed by adding Sulfamic acid (Granger and Sigman, 2009), isotopic analysis of dissolved NO_3^- followed a chemical conversion method modified from Melvin and Altabet (2005). According to this method, NO_3^- is reduced to nitrite with spongy cadmium and further reduced to nitrous oxide with sodium azide in an acetic acid buffer. Subsequently, nitrous oxide is separated and purified in TraceGas (Isoprime), and used to analyze $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ with Isoprime 1000. The

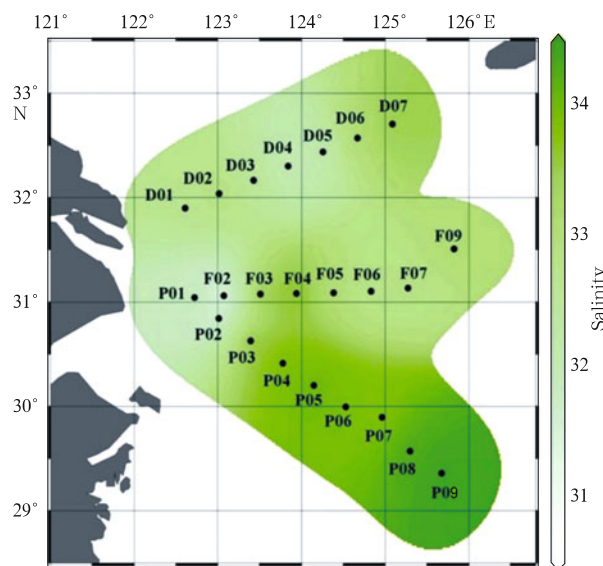


Fig.1. Sampling sites and salinity in the Changjiang Estuary and adjacent East China Sea.

standard deviation was less than 0.2×10^{-3} for nitrogen isotope and less than 0.5×10^{-3} for oxygen isotope. International standard IAEA-N3 was used to calibrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. Reproducibility of duplicate analyzes was less than 0.6×10^{-3} for $\delta^{18}\text{O}$ (average $\pm 0.3 \times 10^{-3}$), and less than 0.3×10^{-3} for $\delta^{15}\text{N}$ (average $\pm 0.1 \times 10^{-3}$).

3 Results

3.1 Chemical parameters

Salinity, temperature, nitrate concentration, DO concentration were relatively uniform in the water column, except Stas P01, F02 and P02, which were near the Changjiang Estuary (Figs 2–4). There appeared to be a spatial variation in salinity, temperature, nitrate concentration, DO concentration among stations, with lower salinity and higher nitrate and DO concentration at Stas P01, F02, P02 and D3–6. NO_3^- concentrations ranged from 2.80 to 20.07 $\mu\text{mol/L}$, with mean value of 9.25 $\mu\text{mol/L}$. In Figs 2–4, NO_3^- concentration decreased offshore, with high in the Changjiang Estuary and low in the outer continental shelf of the East China Sea. Mean NO_3^- concentrations were 10.68, 9.83, 7.91 $\mu\text{mol/L}$ for Transects D, F and P, respectively, with high in the north (Transect D) and low in the south (Transects F and P). NO_3^- concentrations at the surface and 10 m layer were higher than that at 30 m and the bottom, in the Changjiang Estuary (mainly P01, P02, F02), while it is vertically uniform in the outer continental shelf of the East China Sea (Figs 2–4).

3.2 Isotopic composition

As is showed in Figs 2–4, the average $\delta^{15}\text{N}-\text{NO}_3^-$ value in the Changjiang Estuary and the adjacent East China Sea was 6.9×10^{-3} , ranging from 0.5×10^{-3} to 22.3×10^{-3} . $\delta^{18}\text{O}-\text{NO}_3^-$ values ranged from 0.10×10^{-3} to 27.0×10^{-3} , with an average of 7.4×10^{-3} . Figures 2–4 showed large variations in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- in the water columns. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- at the surface showed the similar pattern and they were higher in the subsurface water (10 m and 30 m), especially in Transect D. There also appeared to be large variations in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- between transects, with high in the north (Transect D), and low in the south (Transects F and P). This was similar to the distribution pattern of NO_3^- concentration.

4 Discussion

4.1 Can end-members mixing explain nitrate isotopes?

Generally, the Changjiang River Diluted Water near the

mouth of the Changjiang exhibited a bimodal distribution, with one water mass extending in a band to the south along the coast, and the other water mass extending offshore on average towards the northeast (Beardsley et al., 1985; Wang et al., 2002).

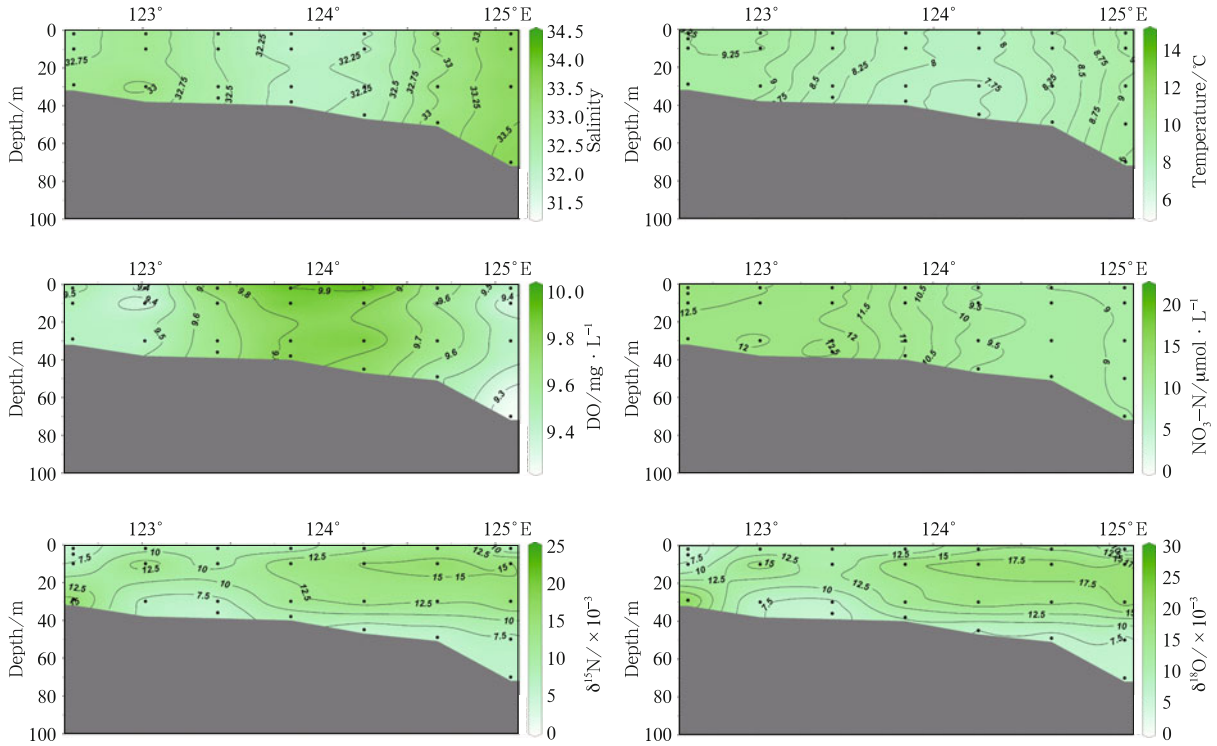


Fig.2. Salinity, temperature, nitrate concentration, DO concentration, and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate along Transect D in Changjiang Estuary and adjacent East China Sea.

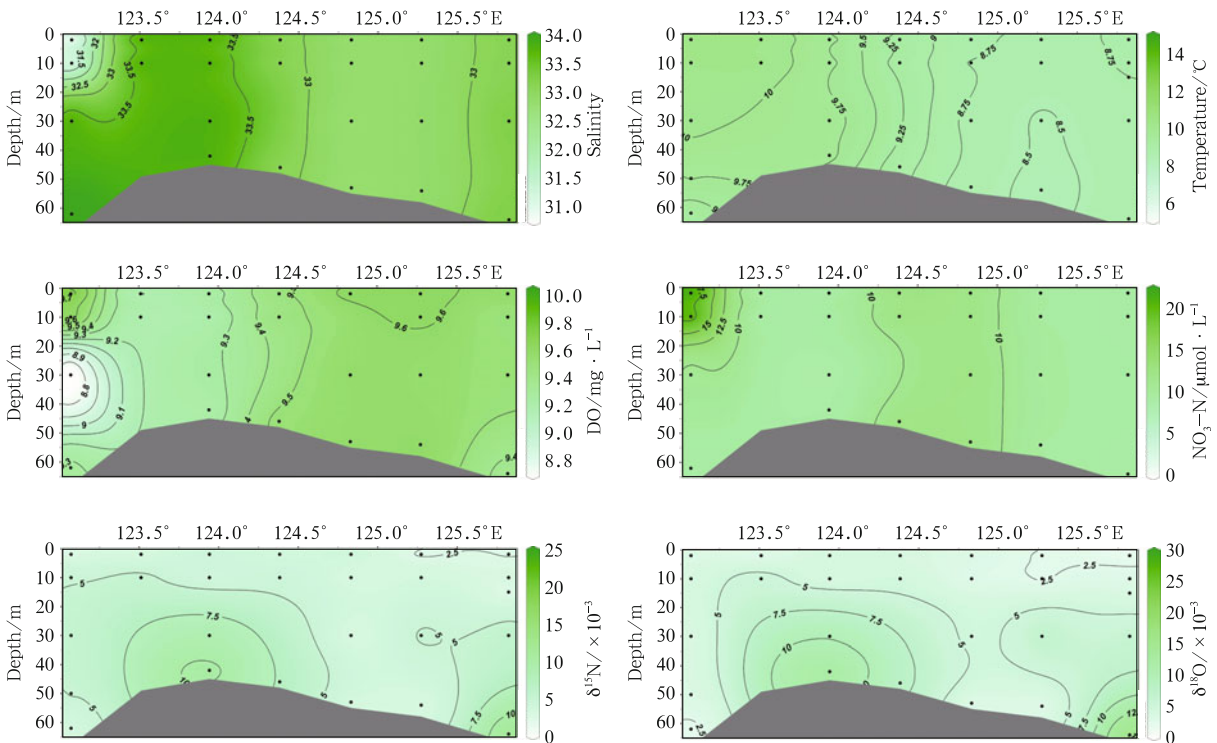


Fig.3. Salinity, temperature, nitrate concentration, DO concentration and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate along Transect F in Changjiang Estuary and adjacent East China Sea.

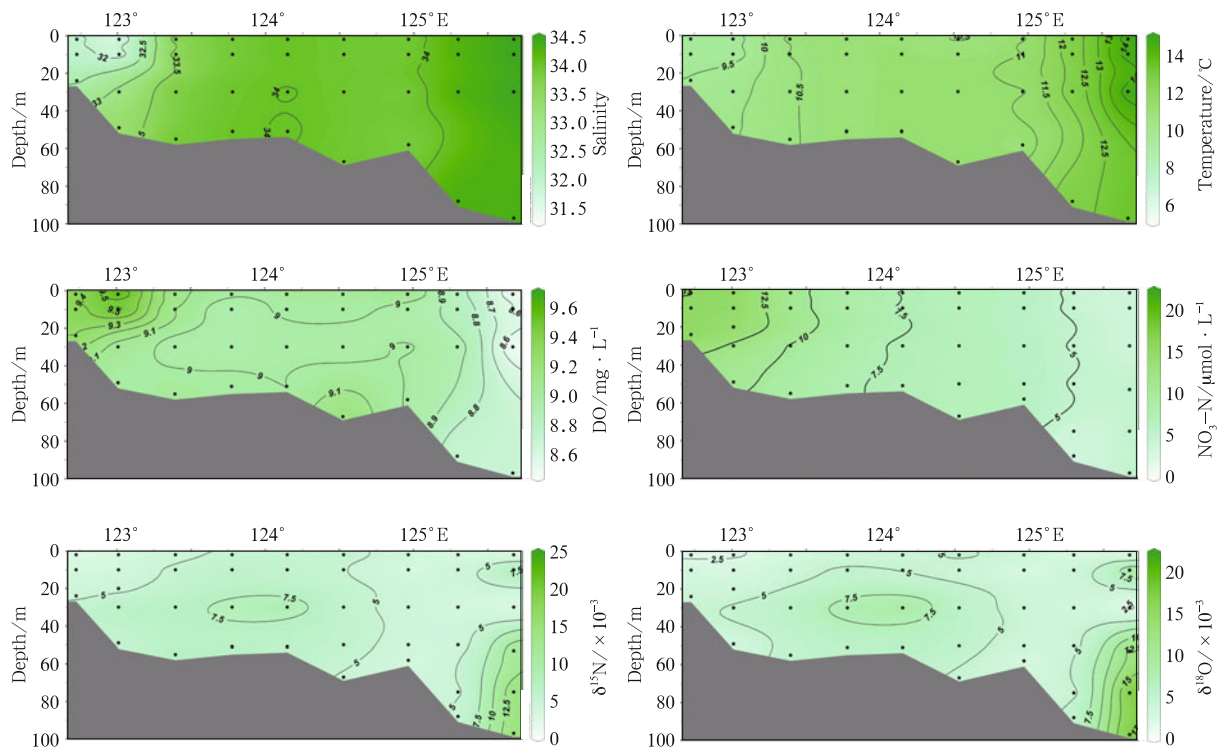


Fig.4. Salinity, temperature, nitrate concentration, DO concentration and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate along Transect P in Changjiang Estuary and adjacent East China Sea.

Spring is a transitional period when the Changjiang River Diluted Water extends offshore towards the northeast near the mouth of the Changjiang, driven by south wind (Beardsley et al., 1985; Wang et al., 2002). Thus, the transport path of the Changjiang River Diluted Water was toward the northeast near the mouth of the Changjiang. As is shown in Figs 2–4, relatively low salinity, relatively high temperatures and weak stratification at Stas P01, F02 and P02, and D 3–6 were observed, accompanied by higher NO_3^- concentration than the adjacent stations, suggesting that these stations were influenced by the Changjiang River Diluted Water. From Figs 2–4, in the transport path of the Changjiang River Diluted Water, $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ increased as they moved offshore, while $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ did not show apparent variable pattern at other stations that were not influenced by river discharge. This might be due to the different contribution of mixing between the Changjiang River Diluted Water and marine water or biological transformation in three transects.

To examine whether the mixing was primarily responsible for the spatial pattern of $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$, it is necessary to distinguish $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ of marine and the Changjiang River Diluted Water end-members. At river-influenced stations (P01, F02 and P02), $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ data showed that NO_3^- mainly resulted from the Changjiang River Diluted Water delivery (Figs 2–4). In addition, the Chl a level in this area (Stas P01, F02 and P02) was always as low as that in the Changjiang River, due to the turbid water there (Zhu, 2004), suggesting that NO_3^- biological uptake was less important in Stas P01, F02 and P02 during the study period (early spring with relatively low temperature). Hence, it might be appropriate to use the average value of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- at the surface at these three stations as nitrate $\delta^{15}\text{N}$ and

$\delta^{18}\text{O}$ of the Changjiang River Diluted Water end-member. Nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for the Changjiang Diluted Water were estimated to be 2.0×10^{-3} and 1.9×10^{-3} , respectively. The average $\delta^{15}\text{N}$ value was in agreement with the previous observations (2.1×10^{-3} in February) in the Changjiang River Diluted Water (Liu et al., 2009). This further confirmed that the average $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ value of NO_3^- in these three stations could be used as nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the Changjiang River Diluted Water end-member.

The isotopic feature of marine end-member can be estimated by the isotopes of NO_3^- in the Kuroshio Current, because the $\delta^{15}\text{N}$ of NO_3^- for the Kuroshio Current, which has significant influence on the East China Sea, is about 5.6×10^{-3} (Liu et al., 1996), consistent with the average value of nitrate $\delta^{15}\text{N}$ (5.0×10^{-3}) in the oceanic water over the global ocean (Sigman et al., 2003). Thus, the $\delta^{15}\text{N}$ of NO_3^- in marine end-member was 5.6×10^{-3} . There were currently some arguments about the ratio of oxygen atoms in NO_3^- deriving from O_2 and water during nitrification. Although recent study suggests that nitrification in deep-ocean incorporates five-sixth of the oxygen in NO_3^- from water (Casciotti et al., 2002), the $\delta^{18}\text{O}$ of NO_3^- was commonly calculated by the nitrification rule (one-third of the oxygen in NO_3^- from O_2 and two-thirds from the surrounding water) in estuaries (Böttcher et al., 1990; Kendall, 1998; Sebilo et al., 2006; Wankel et al., 2006; Casciotti et al., 2010). Since atmospheric oxygen isotope value is 23.5×10^{-3} (Mayer et al., 2001), nitrate $\delta^{18}\text{O}$ value was estimated to be 7.8×10^{-3} for the reduced N sources of NO_3^- . The estimated value seems to be realistic, because nitrate $\delta^{18}\text{O}$ value in shallow inshore water was generally slightly higher than that of mean North Pacific deep water nitrate $\delta^{18}\text{O}$ ($+3 \times 10^{-3}$) (Sigman, 2000, 2008; Wankel et al., 2007). Therefore, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- was 5.6×10^{-3} and

7.8×10^{-3} in marine, respectively.

Based on the above discussion, a mixing line was obtained by plotting the isotope values of the Changjiang River Diluted Water versus marine end-member (Fig. 5). As is shown in Fig. 5, relatively low nitrate $\delta^{15}\text{N}$ ($<10 \times 10^{-3}$) and $\delta^{18}\text{O}$ (mostly distributed in Transects F and P) in this study well fit to the mixing line, although mixing line did not pass through the center of these data. This indicates that mixing was a major factor controlling the isotopic pattern in Transects F and P, but other minor factors were also involved since the relationship between nitrate $\delta^{15}\text{N}$ ($<10 \times 10^{-3}$) and $\delta^{18}\text{O}$ deviated from the mixing line. In contrast, high nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (mostly distributed in Transect D) did not fit to the linear line. This suggests the conservative mixing was not a major process regulating the pattern of nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in Transects D. These were supported by the relationship between salinity and NO_3^- concentrations in three transects. In Fig. 6, NO_3^- concentrations were significantly correlated with salinity in Transects F and P, while NO_3^- concentrations were not significantly correlated with salinity in Transect D, suggesting that NO_3^- behaved relatively conservatively in Transect F and P, but unconservatively in Transect D. It was biased to judge whether NO_3^- biogeochemical processes occurred based on the linear relationship between NO_3^- concentration and salinity alone. The dual isotopic composition of nitrate ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) is robust to address nitrogen sources and cycling in aquatic environments (Kendall, 1998).

4.2 Evidence of nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for NO_3^- biological transformation

NO_3^- assimilation by phytoplankton or denitrification makes the remaining NO_3^- pools simultaneously enriching in ^{15}N and ^{18}O at a ratio of 1:1, while nitrification results in the decoupling of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ as a decrease in $\delta^{18}\text{O}$ smaller than that for $\delta^{15}\text{N}$ (Granger et al., 2004, Wankel et al., 2009). In this study, $\delta^{15}\text{N}$ in Transects F and P were mostly lower than 10×10^{-3} , except at Sta. P09 (Figs 3–5), while $\delta^{15}\text{N}$ in Transect D were mostly higher than 10×10^{-3} (most of the data points within the ellipse in Fig. 5), except at the bottom (Figs 2–4). DO concentrations were high (8.43–10.03 mg/L), suggesting that denitrification was not the process causing the enrichment of ^{15}N and ^{18}O in the study area since denitrification generally occurs under the low DO concentration (<2 mg/L) condition (Kendall, 1998). Hence, assimilation primarily contributed to the enrichment of ^{15}N and ^{18}O in the Changjiang Estuary and the adjacent East China Sea. Spatial variations in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- indicated that NO_3^- assimilation was relatively more active in the river-impacted Transect D than that in Transects F and P where vertical mixing probably restricted NO_3^- assimilation by phytoplankton (Figs 3 and 4). The weak assimilation in Transects F and P resulted in the deviation from the mixing line (Fig. 5).

The plots of $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}$ from two groups (all data pools vs $\delta^{15}\text{N}$ with lower than 10×10^{-3}) revealed that the slope of the linear regression was 0.998 4 for $\delta^{15}\text{N}$ lower than 10×10^{-3} , closer to 1:1 ratio than that (1.286 7) of all the data pools (Fig. 5). The slope (0.998 4:1) of $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}$ in Transects F and P was surprisingly consistent with the theoretical value (1:1), suggesting that the processes other than assimilation might be negligible in Transects F and P. Hence, the linear line from data points of $\delta^{15}\text{N}$ lower than 10×10^{-3} could be considered as “assimilation line”. Contrarily, $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- at Sta. P09 were higher than 10×10^{-3} (Figs 4 and 5), demonstrating stronger NO_3^- as-

simulation occurring, which was likely related to the Kuroshio Current. As shown in Fig. 4, high salinity (>34), and relatively high temperature at Sta. P09 suggested that the front of the Kuroshio Current occurred there. In an earlier study, active biological processes were observed in the front of the Kuroshio Current, near Sta. P09 in this study (Lu, 1998).

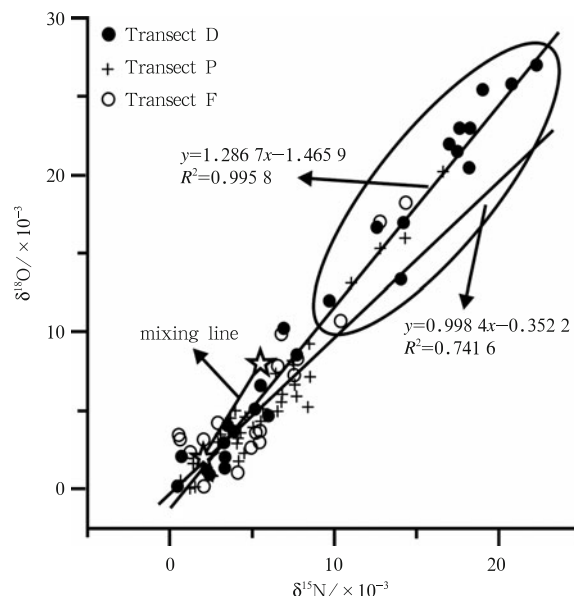


Fig. 5. Ranges of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values measured in the Changjiang River Estuary and the adjacent East China Sea. The two horizontal dashed lines delineate the local $\delta^{18}\text{O}$ of NO_3^- from these reduced N sources as described in the discussion. One regression line is calculated from all the data ($y=1.286\ 7x-1.465\ 9$, $R^2=0.995\ 8$). The other regression line is calculated from data points with $\delta^{15}\text{N}$ lower than 10×10^{-3} ($y=0.998\ 4x-0.352\ 2$, $R^2=0.741\ 6$). Data points within the ellipse are data of $\delta^{15}\text{N}$ higher than 10×10^{-3} . The dashed “mixing line” shows the compositions of marine and freshwater end-members (marked by two hollow star).

However, the regression line did not pass through the center of the isotopic data for $\delta^{15}\text{N}$ with higher than 10×10^{-3} , and most of them were above the line (Fig. 5). This slight deviation from the assimilation line indicated that nitrification possibly occurred in Transect D. This was supported by the relatively high slope (1.286 7) of the plot of $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ higher than 15×10^{-3} . The assimilation should be more important than nitrification in Transect D as $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ of nitrate were high. The input of high nutrient from the Changjiang River Diluted Water delivered nutrient with high concentration triggered the active assimilation, as indicated by low nitrate concentrations at relatively low salinity (<32) stations in Transect D. In the water column, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- were higher in subsurface water (10 m or 30 m) than at the surface, indicating that biological assimilation might be active at 10 m or 30 m depth where the maximum Chl a concentration occurs (Lu, 1998) likely since light level is more appropriate for phytoplankton there. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- increased from Stas D3 to D6 in Figs 2–4, indicating that assimilation occurred when the Changjiang River Diluted water transported offshore beyond the Changjiang mouth. In contrast, at the bottom in Transect D, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- were lower (Figs 2 and 5), suggesting that relatively weak biological transformation took place since low light availability limited nitrate uptake by phytoplankton.

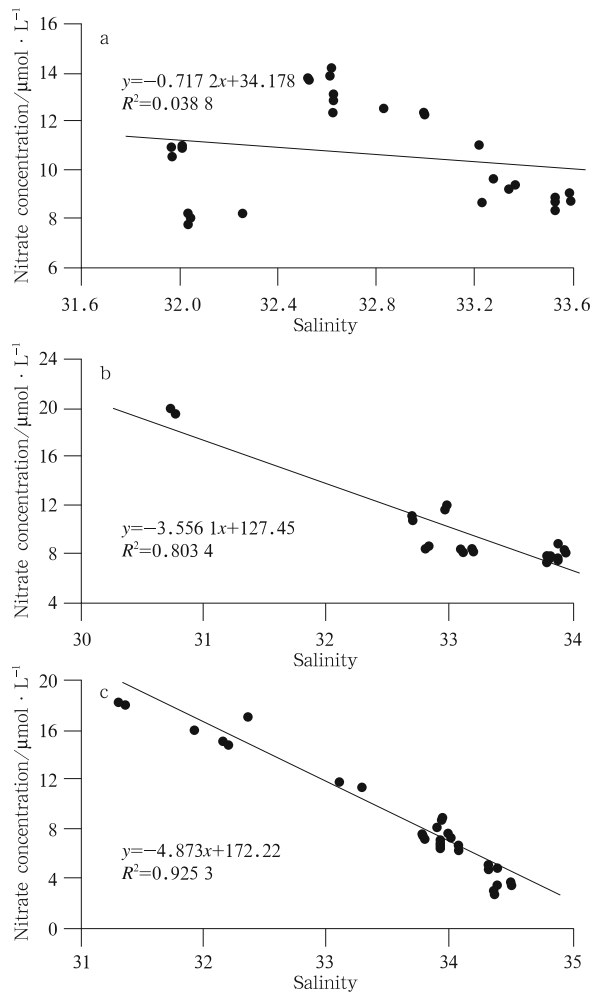


Fig. 6. Linear relationship between nitrate concentration and salinity. a. Transect D, b. Transect F and c. Transect P.

5 Conclusions

A simple two end-members mixing model was used to examine the relative contribution of the Changjiang River Diluted Water and marine water to NO_3^- sources in the Changjiang Estuary and the adjacent East China Sea. The isotopic signals showed that NO_3^- behaved relatively conservatively in Transect F and P likely due to vertical mixing and unconservatively in the river-impacted Transect D. Biological processes (i. e., assimilation and nitrification) were less significant in Transects F and P with low nutrient. High assimilation and weak nitrification took place in Transect D with high nutrient (the transport path of the Changjiang River Diluted Water in the estuary and adjacent sea). Consequently, our results demonstrate that the NO_3^- biological transformation is very active in areas influenced by the Changjiang River Diluted Water.

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