

Reply to the referee 1#

In general, the manuscript is well written and contains interesting information regarding the nitrate sources and transformations in the salinized rivers and estuaries in China based on DIN and dual isotopic compositions. However, the paper needs some clarification and additional support for many of its interpretations.

1. Page 4568, line 19 and 25. When an enriched external source or biological transformation contributes into the river, DIN distribution is expected to fall above the mixing line. In turn, when a depleted external source or the internal removal processes appears in the river, DIN distribution is expected to fall below the mixing line. As the base of the base, the authors need to briefly interpret the mechanisms regarding the judgment of the sink/source of DIN by the mixing curves or lines.

*Reply: Thanks for the comments of the referee. In this manuscript, we used a combined approach based on the mixing curves of DIN concentration versus salinity and determined  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  to illustrate possible transformation processes in the estuarine cycling of reactive nitrogen. The mixing curves of DIN concentrations versus salinity between riverine and estuarine end-members follow a linear mixing line. When an enriched external source or biological transformation (e.g. mineralization, nitrification, etc.) contributes into the river, DIN distribution is expected to fall above the mixing line. In turn, when a depleted external source or the internal removal processes (e.g. denitrification, assimilation, etc.) appears in the river, DIN distribution is expected to fall below the mixing line. The curvilinear mixing curves of determined  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  provide better information for transformation processes: an isotopic enriched  $\text{NO}_3^-$  source or internal removal processes (e.g. denitrification, assimilation, etc.) will result in a distribution of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  falling above the mixing line, while an isotopic depleted  $\text{NO}_3^-$  source or internal nitrification will result in a distribution of  $\delta^{15}\text{N}$  and/or  $\delta^{18}\text{O}$  falling below the mixing line. This expanded part can be found in the revised manuscript page 8 lines 179-191.*

2. About the nitrogen transformations, such as denitrification and nitrification, the authors must need to examine and probe those data carefully before drawing some conclusions.

*Reply: Thanks for the referee reminder. We indeed carefully check the mixing lines of DIN and isotopic values and found different rivers and estuaries were regulated by different transformation processes: 1) in the upstream of the HH River,  $\text{NO}_3^-$  was reduced (below the mixing line) by denitrification, indicated by an enrichment of  $^{15}\text{N}$  relative to  $^{18}\text{O}$  by a factor of 0.8 falling in the typical literature range of 0.8-2.0 for denitrification in the revised manuscript of page 13 lines 303-322; 2) in the downstream of the HH River,  $\text{NO}_3^-$  was accumulated (above the mixing line) by an in-stream nitrification process ( $\text{NO}_2^-$  and  $\text{NH}_4^+$  were consumed to produce  $\text{NO}_3^-$ ), resulting in  $^{15}\text{N}$  depleted  $\text{NO}_3^-$  and  $^{18}\text{O}$  enriched  $\text{NO}_3^-$  (taking similar  $\delta^{18}\text{O}$  values to the ambient water from freshwater with relatively low  $\delta^{18}\text{O}$  to estuarine water with relatively high  $\delta^{18}\text{O}$ ), in the revised manuscript of page 14 lines 333-346; 3) in the CB River,  $\text{NO}_3^-$  was continuously accumulated linked to external livestock N loading (an*

intensive livestock production along the river), as  $\delta^{15}\text{N-NO}_3^-$  values were in the range of manure and  $\delta^{18}\text{O-NO}_3^-$  values increased along the salinity gradient in the revised manuscript of page 16 lines 378-391; 4) the JY River became a source for  $\text{NO}_3^-$  linked to the in-stream nitrification, as  $\delta^{15}\text{N-NO}_3^-$  values were decreasing while  $\delta^{18}\text{O-NO}_3^-$  values were increasing along the river in the revised manuscript of page 16 lines 392-395; and 5) the estuarine mixing behavior is mostly conservative (on the mixing line for the HH Estuary and between the two mixing lines for the CJ Estuary) excluding the point source input appeared in the CJ Estuary in the revised manuscript of page 15 lines 362-369 and page 16-17 lines 396-407. Thus, we try our best to assess the  $\text{NO}_3^-$  sources and cycling in a scientific and reasonable way.

3. As the river having floodgates, the authors need to review and check the rationality of the mixing curves or lines being adopted.

*Reply: The mixing lines were developed between the riverine and marine end-members. The HH River has two floodgates crossing the river and limit water exchange. Therefore we developed two mixing lines to illustrate  $\text{NO}_3^-$  source/sink and cycling. Furthermore, the corresponding  $\delta^{15}\text{N-}$  and  $\delta^{18}\text{O-NO}_3^-$  values support the source/sink results obtained from DIN mixing lines: 1) the first mixing line (HH1-E mixing line) indicated a reduced  $\text{NO}_3^-$  which was attributed to denitrification where an enrichment of  $^{15}\text{N}$  relative to  $^{18}\text{O}$  by a factor of 0.8 has been observed in the revised manuscript of page 13 lines 303-322; and 2) the second mixing line (HH2-E mixing line) indicated an accumulated  $\text{NO}_3^-$  which was attributed to in-stream nitrification where a decrease in  $^{15}\text{N}$  and an increase in  $^{18}\text{O}$  have been observed in the HH River in the revised manuscript of page 14 lines 333-346. In conclusion, we carefully check and assess the rationality of the adopted mixing lines.*

Page 4571, line 7-9: The  $\text{NH}_4^+$  species was accumulated as a source, potentially originating from organic matter decomposition not sewage discharge, as the  $\delta^{15}\text{N-NO}_3^-$  values ( $-0.7$   $-1.1$  ‰) were out of the sewage range. ( $-0.7$   $-1.1$  ‰) is ( $-0.2$   $-1.1$  ‰)? So, please give the nitrate source of first point ( $\delta^{15}\text{N}$  as  $-0.2$ ‰).

*Reply: The first point of  $\text{NO}_3^-$  source originated from organic matter decomposition as well. Normally, when a river passed through a municipality, the relatively high  $\text{NH}_4^+$  concentrations were attributed to sewage discharge. Sewage is enriched in  $^{15}\text{N}$  relative to other N sources, as ammonia volatilization causes a large enrichment of  $^{15}\text{N}$  in the residual  $\text{NH}_4^+$ . This  $\text{NH}_4^+$  is subsequently converted into  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$ . Thus,  $\delta^{15}\text{N}$  values of  $\text{NO}_3^-$  originating from sewage are higher than  $+7$ ‰. However, in this study,  $\delta^{15}\text{N-NO}_3^-$  values ( $-0.2$ - $1.1$ ‰) were out of sewage range. There is no agricultural activity in the upstream of the HH River, thus  $\text{NH}_4^+$  accumulation is linked to organic matter decomposition. We feel sorry for the incorrect typing ( $-0.7$ - $1.1$ ‰) and have corrected in the revised manuscript on page of lines.*

Page 4572 line 7-10. After the separation of the floodgate F1, the upstream of the HH River serves as a river-type reservoir. Thus, a new mixing line (HH2-E mixing line) was recalculated between the sampling location after the floodgate F1 and estuarine

water (Fig. 3). But it also includes the floodgate F2, which is different from F1? How to consider the sampling points between F1 and F2 when new mixing line was re-calculated? So, if the river has floodgates, is it appropriate that some results and conclusions were obtained based on those mixing curves or lines?

*Reply: We refer to the previous reply 3.*

Page 4572 line16–page 4573 line 3. Denitrification usually occurs only where  $O_2$  concentrations are less than 20  $\mu M$ . If denitrification really occurred in the HH river before F1, it will cause the  $\delta^{15}N$  of the residual nitrate to increase exponentially as nitrate concentrations decrease, and also cause the  $\delta^{18}O$  values to increase. Please interpret the increase of  $\delta^{15}N$  and  $\delta^{18}O$  of  $NO_3^-$  in these four points.

*Reply: It is clear that in the upstream part of the HH River before the floodgate F1 (the first four sampling locations),  $NO_3^-$  was below (a sink) the HH1-E mixing line. Normally, the reductive removal of  $NO_3^-$  due to denitrification is accompanied with N and O isotope fractionations. Some studies reported that a linear relationship indicating an enrichment of  $^{15}N$  relative to  $^{18}O$  by a factor between 0.8 and 2.0 gives evidence for denitrification. In our study, the ratio of N and O isotopic enrichment is 0.8, apparently implying that the removal process of  $NO_3^-$  in this river was predominated by denitrification. The enrichment factor  $\epsilon$  for  $^{15}N$  is -1.8 and  $^{18}O$  is -1.4‰. The information can be found in the revised manuscript of page 13 lines 303-322.*

Page 4573 line 13 - page 4574 line 9. In fact, between F1 and F2, these six sampling points can be divided into two groups, as front four (salinity 1.0, 2.3, 2.4, 3.7) and last two (salinity 4.6, 4.9). Except for  $NO_2^-$  and  $NH_4^+$  content of that point-salinity 1.0, the DIN and isotopic compositions of front four samples were almost same, and there is no nitrate accumulation (94.7, 90.2, 94.0, 89.0) and no obvious ammonium decreasing (124.1, 127.1, 127.3). Therefore, whether concerned the analytical precision or not, the variations in  $\delta^{15}N$ - (4.5, 4.6, 4.3, 3.9‰) and in  $\delta^{18}O$  (0.6, 1.1, 1.3, 1.2 ‰) will never draw a conclusion that nitrification occurred. As for last two points between F1 and F2, The  $NH_4^+$  concentrations ..., probably from the release of particle-bound  $NH_4^+$ ... could explain the sharp increase of the  $\delta^{15}N$  from 3.9 to 8.4 ‰ while the  $\delta^{18}O$  only increased slightly from 1.2 to 1.5 ‰. That is to say, particle-bound  $NH_4^+$  leads to the sharp increase of the  $\delta^{15}N$ ? Why? Where are the particle-bound  $NH_4^+$  originated from? The  $\delta^{15}N$ -N values of particle-bound  $NH_4^+$  are high?

*Reply: In this study, when a conservative mixing appeared between the riverine (high DIN concentrations) and estuarine (low DIN concentrations) end-members, DIN distribution is expected to show a sea-ward decreasing trend and fall on the linear mixing line. When an enriched external source or biological transformation (e.g. mineralization, nitrification, etc.) contributes into the river, DIN distribution is expected to fall above the mixing line. As illustrated in the HH2-E mixing line, the front four (salinity 1.0, 2.3, 2.4, 3.7) were above the mixing line indicating  $NO_3^-$  in this section as a source. Nitrate accumulation may be linked to an in-stream nitrification process, in which  $NO_2^-$  and  $NH_4^+$  were consumed to produce  $NO_3^-$ . The*

*nitrification results in a decrease in  $\delta^{15}\text{N}$ - (4.6-3.9‰) and an increase in  $\delta^{18}\text{O}-\text{NO}_3^-$  (0.6-1.2‰) occurred downstream of the HH River and confirmed the in-stream nitrification process as a  $\text{NO}_3^-$  source. The information can be found in the revised manuscript on page 14 lines 333-346.*

*The  $\text{NH}_4^+$  concentrations suddenly increased at a salinity of ~5 (a maximum turbidity zone), probably from the release of particle-bound  $\text{NH}_4^+$  (Seitzinger et al., 1991; Schlarbaum et al., 2010). Results (Kranck, 1984; Eisma, 1986; Schlarbaum et al., 2010) have been reported that this  $\text{NH}_4^+$  could originate from the mineralization of  $^{15}\text{N}$ -enriched DON adsorbed onto the particles and was released with the estuarine turbidity maximum. The  $^{15}\text{N}$ -enriched  $\text{NH}_4^+$  was further converted to  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$ . Thus, the  $\delta^{15}\text{N}-\text{NO}_3^-$  increased sharply from 3.9 to 8.4‰ while the  $\delta^{18}\text{O}-\text{NO}_3^-$  only increased slightly from 1.2 to 1.5‰, resulting from taking similar  $\delta^{18}\text{O}$  values to the ambient water. Another candidate reason to cause a sharp increase in  $\text{NH}_4^+$  concentration could be sewage discharge. Sewage is enriched in  $^{15}\text{N}$  relative to other N sources, as ammonia volatilization causes a large enrichment of  $^{15}\text{N}$  in the residual  $\text{NH}_4^+$ . This  $\text{NH}_4^+$  is subsequently converted into  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$ . This information can be found in the revised manuscript of page 14-15 lines 346-358.*

Page 4576 line 11-14. The downstream part of the HH River between floodgate 1 and floodgate 2 showed an extremely weak  $\text{NO}_3^-$  removal tendency (remove  $2.5 \pm 13.3\%$  of  $\text{NO}_3^-$ ) from active  $\text{NO}_3^-$  turnover processes and the HH Estuary demonstrated a conservative behavior with respect to  $\text{NO}_3^-$ . What are the active  $\text{NO}_3^-$  turnover processes in the HH River between floodgate 1 and floodgate 2? The concentrations of  $\text{NO}_3^-$  in the HH Estuary decreased from 25.7 to 17.8, 15.1, 7.1, why is it a conservative behavior?

*Reply: Concerning the  $\text{NO}_3^-$  turnover of the HH River between floodgate 1 and floodgate 2 refer to reply for the previous comment of Page 4573 line 13 - page 4574 line 9. Since the concentrations of  $\text{NO}_3^-$  in the HH Estuary were located on the HH2-E mixing line, it is a conservative mixing.*

Page 4576 line 21-23. Estuaries of rivers are considered as active sites of massive  $\text{NO}_3^-$  losses (Brion et al., 2004; Seitzinger et al., 2006), removing up to 50 % of  $\text{NO}_3^-$  (OsparCom, 2000). However, our data do not support this view as in the HH and the CJ estuaries. From table 1, along with the increase of salinity, the  $\text{NO}_3^-$  decreased from 25.7 to 7.1 in HH estuary, from 153.4 to 6.1 in CJ estuary, respectively. Please interpret those decreases.

*Reply: Refer to the reply for the previous comments of Page 4573 line 13 - page 4574 line 9 regarding mixing issue between riverine and marine end-members.*

Specific Comments:

Page 4567, line 24-26, please give the analytical precision or the average standard deviations for replicate analysis of an individual sample for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ .

*Reply: The  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}-\text{NO}_3^-$  values of the samples were determined in the UC Davis Stable Isotope Facility of California University. The average standard*

deviation for  $\delta^{15}\text{N}$  is 0.17‰ and  $\delta^{18}\text{O}$  is 0.22‰, respectively.

Page 4568, line 12 and 17, Dähnke et al., 2006 and Dähnke, 2006 should be Dähnke et al., 2008? Check all the other references again.

*Reply: We feel sorry for the incorrect typing, we have modified as Dähnke et al., 2008 in the revised manuscript on page lines. The references have been carefully checked through the entire manuscript.*

Page 4571, line 6, ...NO<sub>3</sub><sup>-</sup> source contamination... why contamination detected here? how to define and judge it?

*Reply: The word “contamination” has been modified as “influence” in the revised manuscript of page 11 line 263.*

Page 4571, line 7, ... a salinity of 1.0, a floodgate ..., should be “the floodgate F1” . And make clearly all the floodgates as F1 or F2 throughout the entire article.

*Reply: Thanks for the comment of the referee. The floodgates have been marked as floodgate F1 or floodgate F2 throughout the entire revised manuscript.*

Table 1 please give the unit of Salinity, ‰ or g/kg? and for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>.

*Reply: The unit of salinity is ‰, and NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> unit is  $\mu\text{mol L}^{-1}$  in Table 1 in the revised manuscript.*

Figure 2, please break Y axis, and make the range of  $\delta^{18}\text{O}$  more wide and clear. “Ranges of isotopic composition for five potential NO<sub>3</sub><sup>-</sup> sources are adapted from Xue et al. (2009)...” is better referred from “Kendall et al., 2007” .

*Reply: Thanks for the comment of the referee. We modified the figure as the referee suggested. We add “Kendall et al., 2007” as a reference in the revised manuscript in Figure 2.*

Figure 5, “HH\*downm” is “HH\*down”?

*Reply: The “HH\*downm” has been corrected as “HH\*down” in the revised manuscript in Figure 5.*

## Reply to the referee 2#

General: This paper presents some interesting results about the use of nitrate isotopes to study DIN dynamics in human influenced river- estuary. Generally, the text is clearly written but some important shortcomings were identified. First, there is a lack of information about the study area – especially hydrology of the systems is poorly described- and about the sampling (see below). However most important, the interpretation of the data mainly based on comparisons towards mixing lines, present important weaknesses. This is mainly linked to the authors choice of considering a continuous water body from the most upstream station to the most downstream station (2 extreme end-members) while the system is characterized by non-continuous hydrological characteristics such as the presence of gates or river confluences. When performing Nitrogen budgets, this clearly needs to be taken into consideration in order to avoid misinterpretation. More details and suggestions here bellow.

*Reply: The referee provides significant comments for this manuscript and we have carefully read and improved our manuscript based on the comments of the referee. Regarding the lack of hydrologic information about the study area, we added more information in the revised manuscript and the reply is linked to the relative comment on the part of Material and methods.*

*More important, the referee showed his concern about data interpretation based on the mixing curves. In this study, a combination of mixing curves of DIN concentration versus salinity and determined  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  has been applied to assess the potential source/sink and the corresponding possible transformation processes in the estuarine cycling of reactive nitrogen. When a conservative mixing appeared between the riverine (high DIN concentrations) and estuarine (low DIN concentrations) end-members, DIN distribution is expected to show a sea-ward decreasing trend and fall on the linear mixing line. A contribution from an enriched external source or biological transformation (e.g. mineralization, nitrification, etc.) in the river, DIN distribution is expected to fall above the mixing line. In turn, a contribution from a depleted external source or the internal removal processes (e.g. denitrification, assimilation, etc.) in the river, DIN distribution is expected to fall below the mixing line. In our study, the HH River has two floodgates which limit and prolong but unblock water exchange. Therefore we developed two mixing lines to illustrate  $\text{NO}_3^-$  source/sink and cycling. Furthermore, the corresponding  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  values provide strong evidence for DIN mixing results: 1) the first mixing line (HH1-E mixing line) indicated a  $\text{NO}_3^-$  sink (below the mixing line) which was attributed to denitrification where an enrichment of  $^{15}\text{N}$  relative to  $^{18}\text{O}$  by a factor of 0.8 has been observed for denitrification; and 2) the second mixing line (HH2-E mixing line) indicated a  $\text{NO}_3^-$  source (above the mixing line) which was attributed to in-stream nitrification where a decrease in  $^{15}\text{N}$  and an increase in  $^{18}\text{O}$  have been observed in the HH River. Since the CB and JY rivers converge before entering the CJ Estuary, two mixing lines were calculated between the CB and JY rivers and the estuarine water, respectively. Furthermore, the corresponding  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  values provide strong evidence for  $\text{NO}_3^-$  mixing results. The CB River became a  $\text{NO}_3^-$  source by*

*external N source addition, and the  $\delta^{15}\text{N-NO}_3^-$  values were enriched and varied around 13.6‰, indicating  $\text{NO}_3^-$  derived from manure. The gradual increase in  $\delta^{18}\text{O-NO}_3^-$  values along the salinity gradient confirmed the in situ nitrification. In the JY River,  $\text{NO}_3^-$  is accumulated by in-stream nitrification, as a decreasing  $\delta^{15}\text{N-NO}_3^-$  and an increasing  $\delta^{18}\text{O-NO}_3^-$  values were observed along the river. In conclusion, we carefully check and assess the rationality of the adopted mixing lines.*

Important comments:

#### Introduction

P4564, Line 26 - “Seldom researchers carried out research in small estuarine systems (Caffrey et al., 2007; Teixeira et al., 2010; Kaiser et al., 2013). Even more, less is known about small estuarine systems with salinization from sea-water intrusion upstream the estuarine channel (Graas and Savenije, 2008).” These statements should be checked carefully. To my knowledge, studies on N dynamics in small estuaries are not so seldom (many studies on small tropical estuaries for example), and most (all) of small studied estuaries have salinity gradients inside the estuarine-river bed because of low freshwater discharge and tidal mixing. I do not know any freshwater small estuary:

*Reply: The basic idea we addressed here is to mention that compared to the large estuarine systems with large discharge of freshwater, the levels of freshwater discharge are relatively low in the small estuaries, which are characterized by salinization from sea-water intrusion for rather long distances upstream. Thus, question will be highlighted as: how do these salinized estuaries respond to increased N loading? How do physico-chemical processes control DIN concentration variation? The information has been corrected in the revised manuscript on page 3 lines 64-69.*

#### Material and methods

Study area P4566. Missing useful information: What are the average freshwater river discharge of the 3 rivers? Climate characteristics of the study area (seasons)? Tidal amplitude at river mouth? How are the gates functioning? Info about geometry (depth?, width?, residence times?) Population in Tianjin (density)? Presence of Wastewater treatment facilities?

*Reply: The missing information regarding the sampling area has been added and improved as: “The study region is located in the warm temperate semi-humid monsoon climate with an average annual temperature of 11.4–12.9°C. The annual precipitation is 520–660 mm, with 75% of the total precipitation occurring in June, July and August. The population of Tianjin municipality is ca.16 million and the density is 1100 inhabitants km<sup>-2</sup>. The HH River is characterized by 72km in length, ca. 100m in width, 3-5m in depth, and a watershed area of 2066 km<sup>2</sup> (Liu et al., 2001). Since the separation by the floodgate F1, the upstream part of the HH River serves as a river-type reservoir for the purpose of supplying water to the residents living along the river bank. The other floodgate F2 is located at the end of the HH River serves as flood discharging, tidal blocking and ship traffic. Although there were eight sewage outlets along the HH River, they were all forbidden to discharge. The average runoff*

of the HH River is  $12.36 \times 10^8 \text{ m}^3/\text{a}$ ; the average tidal amplitude is 2.43m; and the average flow velocity is 0.3-0.4 m/S (Wen and Xing, 2004). The CB River flows through a rural area and is characterized by 81km in length, ca. 700 m in width, 5-7m in depth, and a watershed area of 1387 km<sup>2</sup> (Gburek et al., 1998). Animal manure could be a potential dominant NO<sub>3</sub><sup>-</sup> source in the CB River as this watershed has important livestock breeding base for the municipality (Shao et al., 2010). The JY River flows through agricultural area and is considered as a significant water source for agricultural and domestic use. The JY River is characterized by 144km in length, ca. 300 m in width, less than 7 m in depth, and a watershed area of 2146 km<sup>2</sup> (Chen et al., 2000). The average runoff of the converged river mouth of the CB River and the JY River is  $16.03 \times 10^8 \text{ m}^3/\text{a}$ ; the average tidal amplitude is 2.45m; and the average flow velocity is 0.5-0.7 m/S (Liang and Xing, 1999)". The information can be found in the revised manuscript on page 4-5 lines 96-125.

### Sampling

P4566. How do you take the samples? From a ship? From bridges? With a bucket (surface water)? With a Niskin bottle? Is the water column well mixed? At which moment of the tide? What was the river discharge (dry weather condition or not) and seasonal condition? Please give more details... P4566, Line 25: what physico-chemical property do you analyze on the frozen sample?

*Reply: Surface river was sampled using a bucket from bridge serially from upstream downwards. We put the bucket into the river until it reached ~0.5m below the river surface to sample the water. We sampled in a dry season. Unfortunately, we have no hydrological data for these rivers during the study period. The frozen water was kept until the laboratory analyses of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and δ<sup>15</sup>N- and δ<sup>18</sup>O-NO<sub>3</sub><sup>-</sup>. The information can be found in the revised manuscript on page 6 lines 128-130, page 6 lines 134-136.*

### Results

P4570, Line 13 "The specific reasons to cause such variations could be potentially linking to internal/external NO<sub>3</sub><sup>-</sup> source contributions and different NO<sub>3</sub><sup>-</sup> dynamics in the rivers and the estuaries". Not only nitrate – also NH<sub>4</sub>, organic N, etc. . .

*Reply: We modified "NO<sub>3</sub><sup>-</sup>" as "N" in the revised manuscript on page 10 lines 242-243.*

### Discussion

P4571 – Potential dominant nitrate sources: what is the influence of internal processes in changing the original signal of the nitrate source? The use of the dual approach to identify major sources may be biased in a system with active Nitrate transformation pathways as here . . .

*Reply: The five potential NO<sub>3</sub><sup>-</sup> sources (NO<sub>3</sub><sup>-</sup> in precipitation, NO<sub>3</sub><sup>-</sup> fertilizer, NH<sub>4</sub><sup>+</sup> in fertilizer and rain, soil N and manure and sewage) in Fig. 2 are adapted from Kendall et al., (2007) and Xue et al. (2009). Each source box has a wide range of isotopic composition. Although the HH River was regulated by denitrification and in-stream*



*nitrification processes, most of the  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  measurements fell in the “soil N” source box. We exclude the influence of “ $\text{NH}_4^+$  in fertilizer and rain”, as the HH River flows through the municipality without agricultural activities. The CB River is influenced by nitrification from manure input. The JY River is influenced by in-stream nitrification falling in the “soil N” source box. The isotopic measurements of estuaries fell into the range of marine  $\text{NO}_3^-$  reported by Kendall et al. (2007), excluding sewage discharge of mooring ships in the vicinity of the CJ Estuary. Thus, a plot of  $\delta^{15}\text{N-NO}_3^-$  vs.  $\delta^{18}\text{O-NO}_3^-$  provides qualitative information on the predominant  $\text{NO}_3^-$  sources. Although the active transformation processes may derivate the original isotopic values, the altered values were still in the same source box.*

P4572, L6: What is the “initial sampling location”? The most upstream station? Similar: “last sampling location” should be “most downstream sampling location”. There is a general problem with the mixing lines in river HH as presented. Actually, there is no continuity between the water masses located in the different river stretches separated by gates. So you cannot consider a mixing line crossing the gates because you do not have a continuous mixing pattern between your chosen end-members. . . If you want to compare observed concentration with conservative mixing lines, you should do this separately in each of your stretches: (1) Freshwater HH = before gate 1, (2) region between gate 1 and 2, and (3) estuary below gate 2

*Reply: Thanks for the comments of the referee. We corrected “initial sampling location” as “the most upstream sampling location” and “last sampling location” as “the most downstream sampling location” in the revised manuscript of page 12 lines 291-292. Regarding the uncontinuity of the mixing in the HH River, we refer to the reply to the general comment at the beginning. Even we set up three mixing lines for the HH River, we got the same results as the two mixing lines.*

P4572- Line 16. In the upstream HH river you have freshwater and you should look at the evolution of concentration as a function of the distances as plotted in you figure 3. Line 18 and on: discussion about anaerobic-aerobic denitrification. You can have anaerobic denitrification in the sediments of your river. This is probably more likely than aerobic denitrification in the water column, which although may occur. Do not neglect sediment-water interaction in your system – especially as it is a small river.

*Reply: The salinity increasing trend in the upstream of the HH River is following the sampling location from the top down distribution. In the upstream part of the HH River before the floodgate F1 (the first four sampling locations),  $\text{NO}_3^-$  was reduced due to denitrification, which is accompanied with N and O isotope fractionations. In our study, the ratio of N and O isotopic enrichment is 0.8, falling in the range of 0.8-2.0 reported by literature for denitrification. Plotting  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  vs. logarithm of residual  $\text{NO}_3^-$ , the enrichment factors were calculated as -1.8‰ for  $\epsilon^{15}\text{N}$  and -1.4‰ for  $\epsilon^{18}\text{O}$ . The relatively small enrichment factors were potentially linked to sedimentary denitrification, as diffusion limits the effects of fractionations in the sediments on the  $\delta^{15}\text{N-}$  and  $\delta^{18}\text{O NO}_3^-$  in the overlying water column (Sebilo et al., 2003, Lehmann et al., 2004; Sigman et al., 2005). Denitrification in the upstream of*

*the HH River was re-write in the revised manuscript on page 12-13 lines 301-322.*

P4574 L 4:  $\text{NH}_4$  is not only the preferred nutrient, it also inhibits  $\text{NO}_3^-$  assimilation by phytoplankton. Hence, assimilation cannot be ignored but may be considered as unlikely to be significant.

*Reply: We think the referee is referring to P4573 L4. We addressed that assimilation is not the predominant process to remove  $\text{NO}_3^-$  in the upstream of the HH River, as  $\text{NH}_4^+$  is the preferred nutrient and elevated  $\text{NH}_4^+$  will inhibit  $\text{NO}_3^-$  assimilation for Phytoplankton. The information was modified in the revised manuscript one page 13 lines 313-315.*

P4574 L 9: replace “aerobic denit” by “denit” (see comment above). You cannot exclude nitrification, especially as the  $\text{O}_2$  levels are relatively low which favors the unbalance between the 2 groups of nitrifiers (ammonium oxidizers more sensitive to low  $\text{O}_2$  than nitrite oxidizers –see old paper by Helder and de Vries 1983 - Netherlands Journal of Sea Research, 17(1) 1–18). For example in the Scheldt estuary in the 1970’s,  $\text{NH}_4$  levels where up to 750  $\mu\text{M}$  and still nitrification was intense (Somville, 1984 – APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1984, p. 424-426)

*Reply: We think the referee is referring to P4573 L9. The “aerobic denitrification” has been replaced by “denitrification” in the revised manuscript on page 13 lines 303-322. The referee provided valuable information regarding  $\text{NO}_2^-$  accumulation in the upstream of the HH River. Yes, we agree that nitrification especially oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  could contribute as well. The significant information has been added in the revised manuscript on page 13-14 lines 326-328.*

P4574 L18: for CB and JY rivers and estuaries: here also you cannot consider continuity between most upstream river station and most downstream marine station as you have another important end-member coming into your system. Thus, you should separate the 2 freshwater rivers from the mixed estuary. Evaluate the 2 freshwater zones by looking at evolution of C as a function of distance (there is almost no salinity gradient there), and then the estuary by plotting a mixing line between most upstream station after the confluence with the marine end-member. This will make evaluation much more clear—and change the story... Hence freshwater rivers: sink of  $\text{NO}_2$ , source of  $\text{NO}_3$ , sink of  $\text{NH}_4$  (JY) or scattered distribution of  $\text{NH}_4$  (CB-difficult to say if there is a source or sink). Estuary: sink of  $\text{NH}_4$ , conservative  $\text{NO}_2$  and sink of  $\text{NO}_3$  (and not a source as you conclude...) Please rewrite your discussion ad hoc and reconsider your conclusion.

*Reply: Since the CB and JY rivers converge before entering the CJ Estuary, two mixing lines were calculated between the CB and JY rivers and the estuarine water, respectively. Furthermore, the corresponding  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}-\text{NO}_3^-$  values provide strong evidence for  $\text{NO}_3^-$  mixing results. The CB River became a  $\text{NO}_3^-$  source by external N source addition, and the  $\delta^{15}\text{N}-\text{NO}_3^-$  values were enriched and varied around 13.6‰, indicating  $\text{NO}_3^-$  derived from manure. The gradual increase in*

$\delta^{18}\text{O-NO}_3^-$  values along the salinity gradient confirmed the *in situ* nitrification. In the JY River,  $\text{NO}_3^-$  is accumulated by *in-stream* nitrification, as a decreasing  $\delta^{15}\text{N-NO}_3^-$  and an increasing  $\delta^{18}\text{O-NO}_3^-$  values were observed along the river. Based on the referee comments, we obtained the similar results for the two rivers. However, plotting a mixing line between the conjunct and the estuarine end-member, we obtained different results.

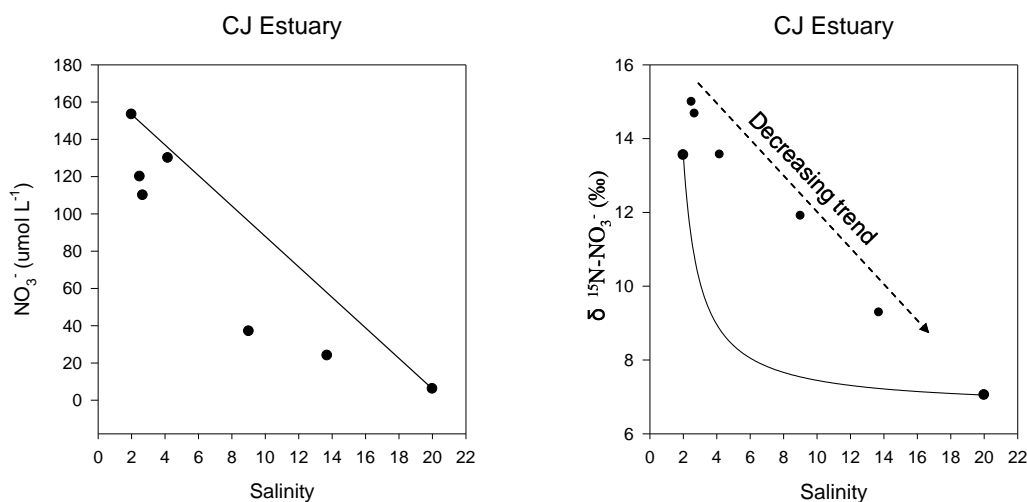


Fig.1 Mixing line calculated between conjunct of the CB and JY rivers and estuarine end-member.

As shown in Fig.1, the  $\text{NO}_3^-$  became a sink in the CJ Estuary suggested by the referee. A reduced  $\text{NO}_3^-$  can be linked to denitrification or assimilation processes, which will result in an increase in  $\delta^{15}\text{N-NO}_3^-$ . However, the  $\delta^{15}\text{N-NO}_3^-$  values demonstrated a decreasing trend (from 15.0‰ to 9.3‰) in the CJ Estuary, indicating a dilution. Thus, the isotopic values did not support the mixing line as suggested by the referee. Actually, as our analysis, a major DIN source from sewage discharge of mooring ships appeared in the salinity zone between 2.0 and 4.2. The typically high  $\delta^{15}\text{N-NO}_3^-$  (13.6 to 15.0‰) values confirmed  $\text{NO}_3^-$  derivation from sewage. This point-source contamination was diluted by the estuarine water when salinity higher than 4.2, where the DIN concentrations and  $\delta^{15}\text{N-NO}_3^-$  values fall between the two mixing lines. This information can be found in the revised manuscript on page 16-17 lines 399-405

P4576, line 3 – how do you practically calculate the removal efficiency? This is not clear at all.

Reply: In this study, most of the measured data deviated from the calculated mixing lines, indicating rivers and estuaries becoming either a source or a sink. Thus, variation percentages of the measured data compared to the calculated mixing lines were computed to assess the  $\text{NO}_3^-$  removal efficiency for the rivers and estuaries as follows:

$$\text{Variation (\%)} = \frac{C_{\text{measured}} - C_{\text{theoretical}}}{C_{\text{theoretical}}} \times 100\% \quad (1)$$

where  $C_{\text{measured}}$  represents the measured  $\text{NO}_3^-$  concentration; and  $C_{\text{theoretical}}$  represents the theoretical  $\text{NO}_3^-$  concentration on the mixing line. A variation percentage  $> 0$

*represents a source; a variation percentage < 0 represents a sink; and a variation percentage equal to 0 represents a mixing. This information has been added in the revised manuscript on page 8 lines 194-201.*

P4576 line 28: what is the water residence time?

*Reply: Sorry, we do not have relevant data to assess water residence time.*

Small remarks

P4548–“Normally, a mixture (MIX) can be calculated via a basic mixing model”–should be: “The concentration of a mixture can be calculated . . .”

*Reply: We think the referee is referring to page 4568. The sentence is corrected as the referee suggested in the revised manuscript on page 7 line 161.*

P4565, Line 10: replace “are converged” by “converge” P4565, Line 12 “and is separated into three parts by two floodgates cross the river” replace by “. . . crossing the river” or “...that cross the river” Fig3 and 4: no info in the legend about the small graphs inside the NH<sub>4</sub>, NO<sub>2</sub> and NO<sub>3</sub> figures (I guess a plot across distance in the freshwater zones).

*Reply: The word “converged” has been replaced by “converge” in the revised manuscript on page 3 line 73. The sentence has been corrected as “...crossing the river” in the revised manuscript on page 3 line 75. As the small graphs were extracted from the large one in Fig. 3 and 4, thus they should have the same legend.*