

Nitrate sources and dynamics in the salinized rivers and estuaries – a $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}\text{-NO}_3^-$ isotope approach

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Abstract

To trace NO_3^- sources and assess NO_3^- dynamics in the salinized rivers and estuaries, three rivers (HH River, CB River and JY River) and two estuaries (HH Estuary and CJ Estuary) along the Bohai Bay (China) have been selected to determine DIN and $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}$ - NO_3^- . Upstream of the HH River NO_3^- was removed $30.9 \pm 22.1\%$ by aerobic denitrification, resulting from effects of the floodgate: limiting water exchange with downstream and prolonging water residence time to remove NO_3^- . Downstream of the HH River NO_3^- was removed $2.5 \pm 13.3\%$ by NO_3^- turnover processes. Conversely, NO_3^- was increased $36.6 \pm 25.2\%$ by external N source addition in the CB River and $34.6 \pm 35.1\%$ by in-stream nitrification in the JY River, respectively. The HH and CY Estuaries behaved mostly conservative excluding the sewage input in the CJ Estuary. Hydrodynamics in estuaries have been changed by the ongoing reclamation projects, aggravating the estuaries losing the attenuation function of NO_3^- .

1 Introduction

Increasing population, extensive agricultural activities and rapid development of urbanization in coastal areas have dramatically increased N loading to rivers and coastal waters (Seitzinger and Kroeze, 1998; Jennerjahn et al., 2004; Umezawa et al., 2008). Estuaries play a prominent role for delivery of terrestrially derived N to coastal water through physical, chemical, and biological processes (Mulholland, 1992; Bernhardt et al., 2003; Sebilo et al., 2006; Hartzell and Jordan, 2012).

Many estuarine studies have focused on tracing N sources and assessing N dynamics in large estuarine systems, such as the Elbe Estuary (Dähnke et al., 2008) and the Atlantic coast (Middelburg and Herman, 2007) in Europe, the San Francisco Bay Estuary (Wankel et al., 2006), the Mississippi River Estuary (Rabalais et al., 1996), and the Mid-Atlantic coast (Dafner et al., 2007) in the United States, and the Yangtze River Estuary (Chai et al., 2009) and Pearl River Estuary (Dai et al., 2008) in China. Sel-

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dom 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). How do these salinized estuaries respond to increased N loadings? How do physico-chemical and biological processes control DIN (NH_4^+ , NO_2^- and NO_3^-) concentration variations?

To answer these questions, an intensive study was conducted in three rivers and the corresponding estuaries characterized by different levels of salinization in a coastal municipality (Tianjin) along the Bohai Bay (China). Two investigated rivers with mean salinities around 0.5 and 0.7 flow through a rural area and are converged before entering into the estuary. The third one with mean salinity around 2.2 flows through Tianjin municipality and is separated into three parts by two floodgates cross the river, for providing water supply for the residents living along the river bank. Since the rapid urbanization and population growth in Tianjin municipality, NO_3^- loading progressively increased in rivers and estuaries associated with human activities, such as agricultural runoff, untreated domestic and industrial wastewater (Gao et al., 2011). Furthermore, port constructions and reclamation projects along the coastline of the municipality even aggravate NO_3^- pollution (Zhang et al., 2004). Thus, tracing NO_3^- sources and assessing NO_3^- dynamics in the salinized rivers and estuaries represent fundamental goals in this study.

More than concentration data alone, the combined use of N ($\delta^{15}\text{N}$) and O ($\delta^{18}\text{O}$) isotopes of NO_3^- has provided a powerful tool to investigate NO_3^- dynamics and identify NO_3^- sources in estuaries (Middelburg and Nieuwenhuize, 2001; Sebilo et al., 2006; Wankel et al., 2006; Dähnke et al., 2008; Miyajima et al., 2009). Therefore, in the present study, a combined approach based on the mixing curves of DIN concentration vs. salinity and $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}$ - NO_3^- is applied to (1) identify potential dominant NO_3^- sources responsible for NO_3^- contamination; and (2) elucidate possible NO_3^- dynamics in the different salinized rivers and the estuaries.

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2 Material and method

2.1 Study area

The investigated three rivers are located in a coastal municipality, Tianjin, China (Fig. 1). The survey took place in three rivers along a salinity gradient, the Haihe River (HH River) on 7 November 2012, the Chaobaixin River (CB River) on 9 November 2012 and the Jiyun River (JY River) on 10 November 2012 (Fig. 1). Water samples were also taken along the estuary of the HH River (HH Estuary) and the mixing estuary of the CB River and the JY River (CJ Estuary) on 16 November 2012 to study reactive N transformation processes from the river to the estuary (Fig. 1). The river-path length from the upstream sampling location to the river mouth sampling location of the HH River was 72 km with a watershed area of 2066 km² (Liu et al., 2001). Since the separation by the floodgate, 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 is located at the end of the HH River serves as flood discharging, tidal blocking and ship traffic. The CB River flows through the rural area with a total length of 81 km and the watershed area is about 1387 km² (Gburek et al., 1998). Animal manure could be a potential dominant NO₃⁻ 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 total length of the Jiyun River is 144 km with a watershed area of about 2146 km² (Chen et al., 2000).

2.2 Sampling and analysis

Water samples were taken on transects spanning the freshwater portion of the rivers to coastal water of the Bohai Bay. Water samples were stored frozen in 1 L HDPE (High Density Polyethylene) bottles for determination of physico-chemical properties and $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}$ -NO₃⁻. Salinity, temperature (T), pH and dissolved oxygen (DO)

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were measured by a portable water quality probe (Thermo Orion, USA). Laboratory analyses included NO_3^- , NO_2^- and NH_4^+ . All samples were filtered through 0.45 μm membrane filters and stored at 4 °C until analysis. Nitrate (NO_3^-), NO_2^- and NH_4^+ concentrations were analyzed on a continuous flow analyzer (Auto Analyzer 3, Seal, Germany).

The $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}$ - NO_3^- values were determined by the “Bacterial denitrification method” (Sigman et al., 2001; Casciotti et al., 2002; Xue et al., 2010) in the UC Davis Stable Isotope Facility of California University, which allows for the simultaneous determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N_2O produced from the conversion of NO_3^- by denitrifying bacteria, which naturally lack N_2O – reductase activity. Isotope ratios of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ are measured using a Thermo Finnigan GasBench + PreCon trace gas concentration system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). The N_2O sample is purged from vials through a double-needle sampler into a helium carrier stream (25 mL min^{-1}) and CO_2 is removed using scrubber (Ascarite). By cryogenic trapping and focusing, the N_2O is compressed onto an Agilent GS-Q capillary column (30 m \times 0.32 mm, 40 °C, 1.0 mL min^{-1}) and subsequently analyzed by IRMS.

Stable isotope data were expressed in delta (δ) units in per mil (‰) relative to the respective international standards:

$$\delta_{\text{sample}}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where R_{sample} and R_{standard} are the $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$ ratio of the sample and standard for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively. Values of $\delta^{15}\text{N}$ are reported relative to atmospheric air (AIR) and $\delta^{18}\text{O}$ values are reported relative to Vienna Standard Mean Ocean Water 2 (VSMOW 2). The calibration standards are the nitrates USGS 32, USGS 34, and USGS 35, and are supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD).

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3 Results

3.1 Physicochemical properties

Table 1 summarizes the data of physicochemical properties collected in this study in the rivers and estuaries. Obviously, the salinities of the HH River (ranging from 0.7 to 4.9 with a mean value of 2.2) and its estuary (ranging from 18.6 to 24.1 with a mean value of 21.2) is higher than the rivers of CB (ranging from 0.5 to 0.6 with a mean value of 0.5) and JY (ranging from 0.6 to 0.8 with a mean value of 0.7) and the corresponding estuary (ranging from 2.0 to 20.0 with a mean value of 7.7), respectively. The municipality had been suffering multiple seawater intrusion and regression, which results in the salinization of the rivers and soil (Wang, 2004), while the greater salinization level of the HH River is also related to seawater intrusion over the floodgate until upstream of the HH River in a relatively long distance. The rivers and the estuaries showed similar pH values between 7.5 and 8.6. The temperature of the HH River varied around 12.3 °C slightly higher than the CB River (mean is 10.9 °C) and the JY River (11.5 °C). The mean temperature of the HH Estuary (9.7) is also higher than that of the CJ Estuary (6.7). Dissolved oxygen (DO) concentrations were relatively enriched in this study (higher than 7.2 mgL⁻¹), excluding the DO depleted area in the upstream of the HH River (lower than 5.0 mgL⁻¹).

3.2 DIN species

Wide concentration variations were noticeable for DIN (NH₄⁺, NO₂⁻ and NO₃⁻) species in Table 1. In the HH River, the NH₄⁺ concentrations varied from 124.1 to 332.6 μmolL⁻¹, the NO₃⁻ concentrations varied from 62.5 to 219.0 μmolL⁻¹ and the NO₂⁻ concentrations varied from 7.2 to 20.8 μmolL⁻¹. The DIN concentrations of the HH Estuary varied smoothly (5.6–6.7 μmolL⁻¹ for NO₂⁻, 7.1–25.7 μmolL⁻¹ for NO₃⁻, and 65.7–88.1 μmolL⁻¹ for NH₄⁺) and were quite low compared to the HH River. Nitrate concentra-

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tions in the CB river were relatively elevated ($120.0\text{--}171.5\ \mu\text{mol L}^{-1}$) with a continuous accumulation along the entire salinity gradient, while NO_2^- concentrations decreased from 12.0 to $6.0\ \mu\text{mol L}^{-1}$. Ammonium concentrations in the CB River varied from 143.9 to $380.0\ \mu\text{mol L}^{-1}$. The JY River also showed NO_3^- accumulation (increased from 40.0 to $83.3\ \mu\text{mol L}^{-1}$) along the entire salinity gradient, while a decreasing trend was observed for both NO_2^- (decreased from 7.0 to $2.1\ \mu\text{mol L}^{-1}$) and NH_4^+ (decreased from 72.8 to $11.1\ \mu\text{mol L}^{-1}$) concentrations. The CJ Estuary displayed a sea-ward decreasing trend with relatively elevated concentrations in NH_4^+ ($328.4\text{--}43.2\ \mu\text{mol L}^{-1}$), NO_2^- ($7.8\text{--}3.4\ \mu\text{mol L}^{-1}$) and NO_3^- ($153.4\text{--}6.1\ \mu\text{mol L}^{-1}$). Compared to the other river and estuaries, DIN results of this study are similar to that in the Pearl River Estuary (Dai et al., 2008) in South China Sea, but higher than that in the Elbe Estuary (Dähnke et al., 2008) in Europe and the San Francisco Bay Estuary (Wankel et al., 2006) in the United States. The specific reasons to cause such variations could be potentially linking to internal/external NO_3^- source contributions and different NO_3^- dynamics in the rivers and the estuaries.

3.3 Isotopic composition of NO_3^-

The isotopic composition of NO_3^- varied spatially among the rivers and the estuaries (Table 1). The $\delta^{15}\text{N}\text{-NO}_3^-$ values in the HH River varied from -0.7 to 8.4‰ and the $\delta^{18}\text{O}\text{-NO}_3^-$ values varied from -1.7 to 1.5‰ . The isotopic composition of NO_3^- in the HH Estuary remained stable around 8.1‰ for $\delta^{15}\text{N}\text{-NO}_3^-$ and 5.6‰ for the $\delta^{18}\text{O}\text{-NO}_3^-$. In the CB River, the $\delta^{15}\text{N}\text{-NO}_3^-$ values were enriched with a mean of 13.6‰ , and the $\delta^{18}\text{O}\text{-NO}_3^-$ values were in a range between 3.9 and 5.6‰ . A decrease in $\delta^{15}\text{N}\text{-NO}_3^-$ (from 6.5 to 4.4‰) and an increase in $\delta^{18}\text{O}\text{-NO}_3^-$ (from 0.9 to 5.3‰) values along the salinity were observed in the JY River. The CY Estuary demonstrated a wide range of $\delta^{15}\text{N}\text{-NO}_3^-$ (from 7.1 to 15.0‰), while a narrow range of $\delta^{18}\text{O}\text{-NO}_3^-$ (from 5.9 to 6.9‰).

4 Discussion

4.1 Potential dominant NO_3^- sources

To derive qualitative information on the predominant NO_3^- sources in the rivers and the corresponding estuaries, a classical dual isotope approach ($\delta^{15}\text{N}\text{-NO}_3^-$ vs. $\delta^{18}\text{O}\text{-NO}_3^-$) has been applied (Fig. 2). It is clear that the isotope signatures of all the sampling locations showed in a scattered distribution, indicating different NO_3^- source contamination in the rivers and the estuaries. Upstream of the HH River at a salinity of 1.0, a floodgate separates the river into two parts; and at the end of the river at the salinity of 4.9, the other floodgate controls the connection of the river to the HH Estuary. Hence, the $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}\text{-NO}_3^-$ values of the HH River behaved quite differently, which moved from the overlapping area of the “ NH_4^+ fertilizer” and “soil N” source boxes for the majority of the upstream sampling locations, to the overlapping area of the “soil N” and “manure and sewage” source boxes at the end of the river. In this study, the majority of the sampling locations were potentially influenced by the source of “soil N” or “sewage” not the “mineral fertilizer”, as the HH River flows through the municipality without agricultural activities. In addition, it can no be excluded the influence from salt water intrusion from the estuary, which showed similar isotopic values to that at the end of the HH River. The distribution of the HH Estuary does not show a landward trend due to the floodgate at the end of the HH River, but falls into the range of marine NO_3^- reported by Kendall et al. (2007).

Animal manure could be a potential dominant NO_3^- source in the CB River as this watershed plays the role of important livestock breeding base for the municipality (Shao et al., 2010). Furthermore, the $\delta^{15}\text{N}\text{-NO}_3^-$ values were enriched and varied around 14‰, indicating anthropogenic NO_3^- derived from manure (Kendall et al., 2007; Xue et al., 2009). The isotope signatures of the JY River were mainly concentrated in the “soil N” source box. The $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ values of the CJ Estuary suggested an influence of the CB River. In addition, quite high DIN concentrations (Table 1) ap-

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peared in this estuary, due to sewage discharge of mooring ships in the vicinity of the sampling area. Thus, the influence of sewage and the CB River was considered as the dominant NO_3^- source.

4.2 Nitrate dynamics in the salinized rivers and the corresponding estuaries

4.2.1 Nitrate dynamics in the HH River and its estuary

A mixing line (HH1-E) was setup between the initial sampling location in the HH River and the last sampling location in the HH Estuary (Fig. 3). After the separation of the floodgate, the upstream of the HH River serves as a river-type reservoir. Thus, a new mixing line (HH2-E mixing line) was re-calculated between the sampling location after the floodgate and estuarine water (Fig. 3). The salinity gradient sampled in the HH River and its estuary showed a seaward decreasing trend in DIN (NH_4^+ , NO_2^- and NO_3^-) concentrations and an increasing trend in $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ values throughout the entire salinity gradient (Fig. 3). However, the DIN and isotopic trends did not behave conservatively, as most of the measured data deviated from the calculated mixing lines, especially from HH1-E between the riverine concentration and the coastal water.

It is clear that in the upstream part of the HH River before the floodgate 1, NO_2^- and NH_4^+ were above (a source) while NO_3^- was below (a sink) the HH1-E mixing line. Hence, NO_3^- removal processes are expected to occur in the river. Denitrification has been considered as a dominant NO_3^- reduction pathway, which reduces NO_3^- to gaseous N compounds (NO , N_2O and N_2) under anaerobic conditions. However, a large number of laboratory studies have reported that denitrification occurs under aerobic conditions in pure cultures of bacteria (Robertson and Kuenen, 1984; Ronner and Sorensson, 1985; Trevors and Starodub, 1987; Robertson et al., 1995). Various heterotrophic nitrifiers have been tested and found to be capable of simultaneous heterotrophic nitrification and aerobic denitrification (Castignetti and Hollocher, 1984; Robertson et al., 1988; Robertson et al., 1989). Some researchers have found aerobic

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Furthermore, a regular source-sink pattern was observed for NH_4^+ concentrations while a decrease for NO_2^- . The sharp increase in NH_4^+ concentrations was probably linked to manure discharge in the rural area. The added NH_4^+ was then rapidly oxidized to NO_2^- and NO_3^- during nitrification. Hence, $\delta^{15}\text{N-NO}_3^-$ values were enriched and varied around 13.6‰, indicating NO_3^- derived from manure (Kendall et al., 2007; Xue et al., 2009). As NO_3^- from these origins is produced via nitrification, its $\delta^{18}\text{O}$ values would not be very different from ambient water. Thus, the gradual increase in $\delta^{18}\text{O-NO}_3^-$ values along the salinity gradient above the respected mixing line confirmed the in situ nitrification (see the discussion above). Thus, in the CB River, the NO_3^- turnover is mainly regulated by nitrification from external livestock N loadings.

The JY River became a significant source for NO_3^- in concert with a sink for NO_2^- and NH_4^+ species. The accumulation of NO_3^- was linked to the in-stream nitrification, resulting from the consumption of NO_2^- and NH_4^+ . Evidence for this may be indicated by decreasing $\delta^{15}\text{N-NO}_3^-$ and increasing $\delta^{18}\text{O-NO}_3^-$ values along the river.

The salinity gradient sampled in the corresponding estuary showed a sea-ward decreasing trend in NH_4^+ , NO_2^- and NO_3^- concentrations. The measured data in the CJ Estuary were expected to fall between the two calculated mixing lines generated from the rivers of CB and JY, because they both discharge into the same estuary. A major DIN source (above the two calculated mixing lines) appeared in the salinity zone between 2.0 and 4.2. This was probably from sewage discharge of mooring ships in the vicinity of the sampling area. The typically high $\delta^{15}\text{N-NO}_3^-$ (13.6 to 15.0‰) values confirmed 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. The $\delta^{18}\text{O-NO}_3^-$ values of the estuarine water were quite close to the $\delta^{18}\text{O-NO}_3^-$ derived from the nitrification of sewage, thus $\delta^{18}\text{O-NO}_3^-$ values were expected to retain stable.

4.4 Nitrate removal efficiency in the rivers and the estuaries

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 NO_3^- removal efficiency for the rivers and estuaries (Fig. 5). Interestingly, in the upstream part of the HH River before the floodgate 1, NO_3^- was removed $30.9 \pm 22.1\%$ compared to the calculated mixing line. Denitrification could be the dominant NO_3^- removal process. This potentially results from the separation of the floodgate which limited water exchange with downstream water enriched in DO. Furthermore, the floodgate 1 might prolong water residence time in the upstream part to remove a significant part of riverine N loadings. The downstream part of the HH River between floodgate 1 and floodgate 2 showed an extremely weak NO_3^- removal tendency (remove $2.5 \pm 13.3\%$ of NO_3^-) from active NO_3^- turnover processes and the HH Estuary demonstrated a conservative behavior with respect to NO_3^- . In contrast, a significant source of NO_3^- is present in the CB ($36.6 \pm 25.2\%$) and JY ($34.6 \pm 35.1\%$) rivers compared to the calculated mixing line, explained by external N source addition and in-stream nitrification, respectively. Moreover, the CJ Estuary demonstrated higher NO_3^- accumulation efficiency ($82.1 \pm 78.8\%$) as a result of an external N source input. Great variation percentages were observed between the sampling points from the same river or estuary, possibly resulting from different N dynamics and/or external source input.

Estuaries of rivers are considered as active sites of massive NO_3^- losses (Brion et al., 2004; Seitzinger et al., 2006), removing up to 50% of NO_3^- (OsparCom, 2000). However, our data do not support this view as in the HH and the CJ estuaries. First, DO concentrations were higher than 10 mg L^{-1} not favorable for water column NO_3^- removal processes. Second, dredging and diking work to deepen the ship channel decreased the sediment area (where denitrification mainly occurred) that is in contact with the overlying water column in the rivers (Dähnke et al., 2008), thus the NO_3^- removal ability was reduced. Third, water residence time is not long enough to remove N loadings in

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the estuaries by NO_3^- removing processes as reclamation projects for the regional and national economy leading to the hydrodynamics of circulation in Tianjin section disappearance (Qin et al., 2012). This phenomenon could reduce water residence time and force NO_3^- pollutants moving to the northern part of Bohai Bay, aggravating NO_3^- contamination.

Furthermore, this wintertime situation, with water temperature around 10°C , ruled out most biological activity, and conservative mixing behavior in the HH River Estuary was not overly surprising. However, the CJ Estuary became a NO_3^- source, linking to sewage discharge of mooring ships.

5 Conclusions

The combined use of salinity, DIN concentrations and NO_3^- isotopic composition revealed NO_3^- sources and dynamics in the salinized rivers of HH, CB and JY and elucidated mixing patterns of NO_3^- in the corresponding estuarine system. The HH River demonstrated a significant NO_3^- sink appeared in the upstream part of the HH River by aerobic denitrification process. This potentially results from the separation of the floodgate 1 which limited water exchange with downstream water enriched in DO and prolong water residence time in the upstream to remove a significant part of riverine N loadings. The downstream of the HH River showed an extremely weak NO_3^- removal tendency from active NO_3^- turnover processes. In contrast, a significant source of NO_3^- is present in the rivers of CB and JY, linking to external N source addition and in-stream nitrification, respectively. We found that the estuarine mixing behavior is mostly conservative excluding the point source input appearing in the CJ Estuary. Data indicate that the rivers and their corresponding estuaries have lost their natural capacity of NO_3^- removal but turned into a significant source of NO_3^- for the adjacent Bohai Bay.

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Table 1. Physicochemical properties and isotopic composition of NO_3^- for the three investigated rivers and the corresponding estuaries.

Location	Salinity	pH	T ($^{\circ}\text{C}$)	DO (mgL^{-1})	NO_2^- μmolL^{-1}	NO_3^-	NH_4^+	$\delta^{15}\text{N-NO}_3^-$ ‰	$\delta^{18}\text{O-NO}_3^-$
HH ^a	0.7	7.5	11.4	2.7	16.6	219.0	221.4	-0.2	-0.5
	0.7	7.7	12.1	4.0	18.0	145.6	332.6	0.5	0.2
	0.7	7.7	12.2	4.8	18.6	134.4	311.3	0.6	0.2
	0.8	7.9	13.2	5.0	20.8	105.0	326.9	1.1	0.5
	1.0	8.1	13.1	8.2	10.0	94.7	157.9	4.5	0.6
	2.3	8.4	12.1	10.4	7.2	90.2	124.1	4.6	1.1
	2.4	8.5	11.9	10.5	8.6	94.0	127.1	4.3	1.3
	3.7	8.3	12.7	10.4	8.8	89.0	127.3	3.9	1.2
	4.6	8.3	12.1	9.9	15.5	62.5	156.8	8.4	1.5
	4.9	8.2	11.7	9.4	14.5	70.0	149.5	7.4	1.4
Average	2.2 ± 1.7	8.1 ± 0.3	12.3 ± 0.6	7.5 ± 3.1	13.9 ± 4.8	110.4 ± 45.9	203.5 ± 87.5	3.5 ± 3.0	0.8 ± 0.7
HH ^b	18.6	8.2	10.1	10.7	6.7	25.7	88.1	8.0	5.4
	20.6	8.2	10.2	10.7	6.3	17.8	79.9	7.9	5.6
	21.3	8.1	9.6	10.7	6.2	15.1	76.9	8.1	5.7
	24.1	8.1	9.0	10.7	5.6	7.1	65.7	8.3	5.8
Average	21.2 ± 2.3	8.2 ± 0.1	9.7 ± 0.6	10.7 ± 0.0	6.2 ± 0.5	16.4 ± 7.7	77.7 ± 9.3	8.1 ± 0.2	5.6 ± 0.2
CB ^a	0.5	7.9	11.3	8.9	12.0	120.0	333.4	13.7	4.0
	0.5	8.6	10.8	10.5	7.0	134.1	167.1	14.0	4.8
	0.5	8.5	10.7	9.1	10.6	157.8	380.0	13.9	3.9
	0.5	8.5	10.5	9.9	9.9	171.5	143.9	12.2	4.3
	0.6	8.6	11.0	10.4	6.0	171.1	367.0	13.7	4.8
	0.6	8.2	10.8	10.0	8.5	152.4	210.1	14.1	5.6
Average	0.5 ± 0.1	8.4 ± 0.3	10.9 ± 0.3	9.8 ± 0.7	9.0 ± 2.3	151.2 ± 20.6	266.9 ± 105.4	13.6 ± 0.7	4.6 ± 0.6
JY ^a	0.6	8.1	9.9	7.2	7.0	40.0	72.8	6.5	0.9
	0.7	8.2	11.0	8.7	6.9	42.0	64.4	6.3	2.0
	0.7	8.2	11.3	7.5	4.4	44.0	57.6	6.4	1.4
	0.7	8.4	11.7	9.3	4.9	46.0	48.1	5.8	0.8
	0.8	8.4	12.4	9.3	4.0	76.6	12.7	5.3	1.3
	0.8	8.4	11.8	9.7	3.8	78.0	25.9	5.3	1.1
	0.8	8.5	11.8	9.9	2.2	83.3	11.1	4.4	2.8
	0.8	8.5	11.8	9.9	2.1	81.5	35.1	4.4	5.3
Average	0.7 ± 0.1	8.3 ± 0.2	11.5 ± 0.8	8.9 ± 1.1	4.4 ± 1.8	61.4 ± 19.9	41.0 ± 23.4	5.6 ± 0.8	2.0 ± 1.5
CJ ^b	2.0	8.2	7.8	10.6	7.7	153.4	328.4	13.6	5.9
	2.5	8.2	6.9	11.4	7.8	120.0	304.7	15.0	6.1
	2.7	8.3	5.7	11.5	7.6	110.0	283.3	14.7	6.4
	4.2	8.3	5.7	11.1	7.0	130.0	286.3	13.6	6.4
	9.0	8.3	5.7	11.4	5.8	37.0	180.7	11.9	6.2
	13.7	8.3	6.2	11.3	4.9	24.0	120.4	9.3	6.7
	20.0	8.2	8.6	11.2	3.4	6.1	43.2	7.1	6.9
	Average	7.7 ± 6.9	8.3 ± 0.1	6.7 ± 1.2	11.2 ± 0.3	6.3 ± 1.7	82.9 ± 58.8	221.0 ± 108.0	12.2 ± 3.0

^a represents river;

^b represents estuary.

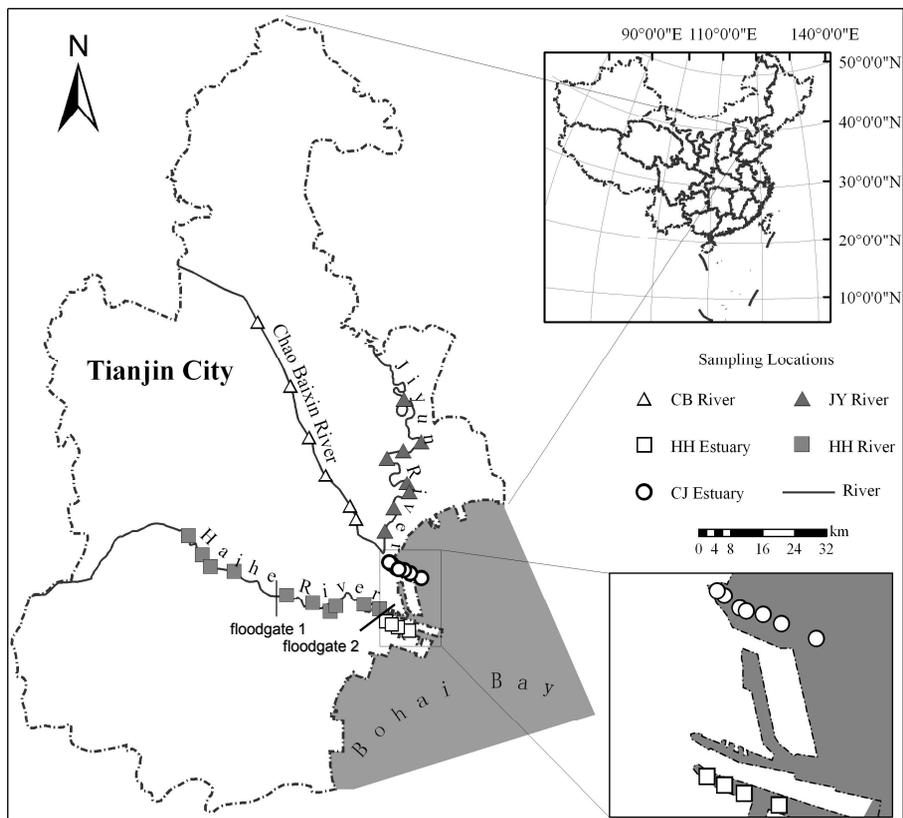


Fig. 1. Sampling location for the three investigated rivers.

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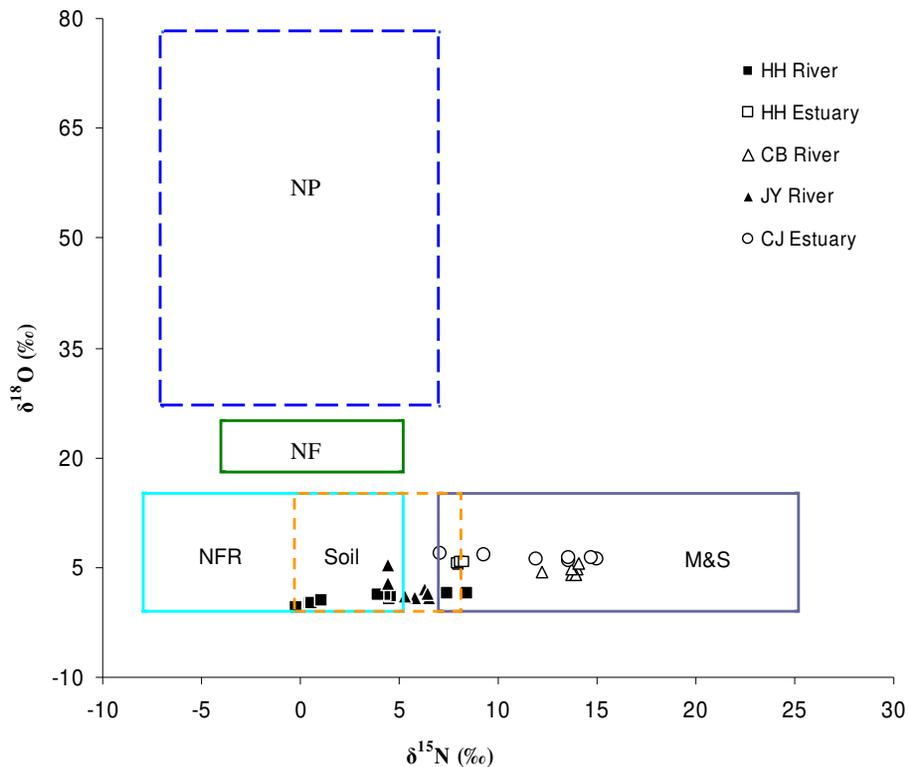


Fig. 2. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}\text{-NO}_3^-$ of the salinized rivers and estuaries. Ranges of isotopic composition for five potential NO_3^- sources are adapted from Xue et al. (2009) and indicated by boxes: NO_3^- in precipitation (NP), NO_3^- fertilizer (NF), NH_4^+ in fertilizer and rain (NFR), soil N (Soil) and manure and sewage (M and S).

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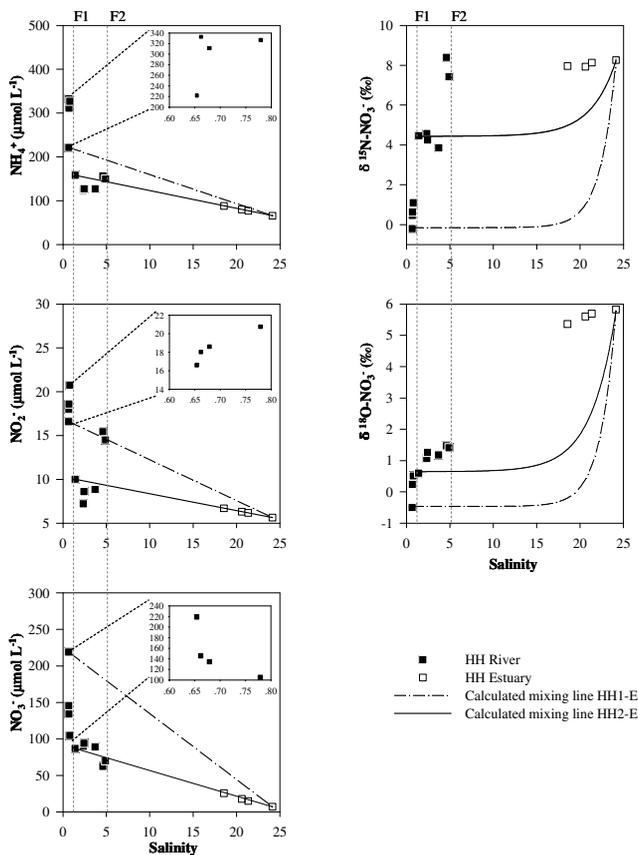


Fig. 3. DIN (NH_4^+ , NO_2^- , NO_3^-) concentrations and isotopic composition of NO_3^- vs. salinity in the HH River and the HH Estuary. HH1-E represented the calculated mixing line between the initial upstream and the estuary; HH2-E represented the calculated mixing line between the floodgate 1 and the estuary; F represents floodgate.

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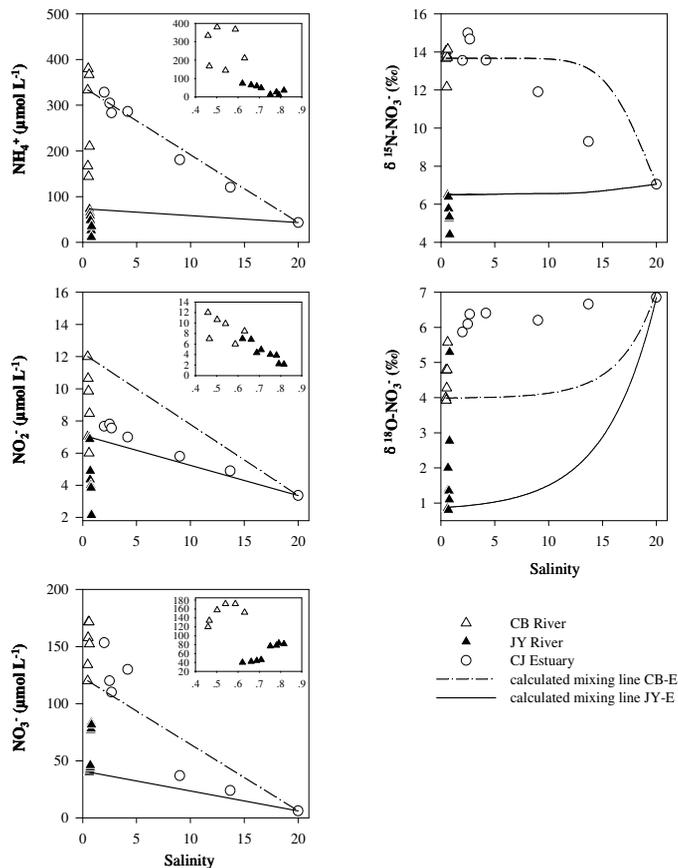


Fig. 4. DIN (NH₄⁺, NO₂⁻, NO₃⁻) concentrations and isotopic composition of NO₃⁻ vs. salinity in the CB River and the JY River and the CJ Estuary. CB-E represented the calculated mixing line between the CB River and the CJ Estuary; JY-E represented the calculated mixing line between the JY River and the CJ Estuary.

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