frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 26 July 2022
DOI 10.3389/fmars.2022.956474

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SPECIALTY SECTION
This article was submitted to Marine Pollution, a section of the journal Frontiers in Marine Science

RECEIVED 30 May 2022
ACCEPTED 28 June 2022
PUBLISHED 26 July 2022

CITATION
Cai S, Lao Q, Jin G, Chen C, Zhou X, Zhu Q and Lu X (2022) Sources of nitrate in a heavily nitrogen pollution bay in Beibu Gulf, as identified using stable isotopes.
Front. Mar. Sci. 9:956474.
doi: 10.3389/fmars.2022.956474

Sources of nitrate in a heavily nitrogen pollution bay in Beibu Gulf, as identified using stable isotopes

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Eutrophication, mainly caused by the oversupply of inorganic nitrogen and phosphate, has increased and become a serious environmental problem in the coastal bays of Beibu Gulf, a newly developing industry and port in South China. However, the sources of nitrate are poorly understood in the gulf. In this study, nitrate dual isotopes ($\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) and ammonium isotopes ($\delta^{15}N\text{-}NH_4^+$) were measured during the rainy season to identify the nitrate sources and elucidate their biogeochemical processes in Xi Bay, a semi-enclosed bay that is strongly affected by human activities in the Beibu Gulf. The results showed that a high dissolved inorganic nitrogen (DIN, 10.24-99.09 μmol L^{-1}) was observed in Xi Bay, particularly in the bay mouth. The concentrations of DIN in the bay were 1.5 times higher than that in Qinzhou Bay and 1.7 times than that in Tieshangang Bay, which mainly influenced by the intensive human activities (i.e., industrial and port activities). In addition, lower values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ and higher values of $\delta^{15}N\text{-}NH_4^+$ were observed in the upper bay, suggesting that microbial nitrification occurs in the upper bay, which was the dominant nitrate source in the upper bay (39%). In addition to nitrification, external sources, including sewage and manure (33%), soil N (15%) and fertilizer (11%), contributed to the higher nutrients in the upper bay. In the lower bay, severe nitrogen pollution led to a weaker impact of biological processes on isotopic fractionation, although a high Chl *a* level (average of 7.47 μg L^{-1}) was found in this region. The heavy nitrate pollution in the lower bay mainly originated from sewage and manure (54%), followed by soil N (26%) and fertilizer (17%). The contribution of the nitrate source from atmospheric deposition was relatively low in the bay (<3%). This study suggests that biogeochemical processes have little impact on nitrate dual isotopes under heavy nitrogen pollution, and isotopes are an ideal proxy for tracing nitrogen sources.

KEYWORDS

nitrate dual isotopes, ammonium isotope, nitrogen pollution, source, Beibu Gulf

Introduction

Coastal bays receive terrestrial and riverine nutrient inputs, which have increased considerably in recent decades (Jickells, 1998; Gruber and Galloway, 2008; Savage et al., 2010; Yan et al., 2017). The increase in anthropogenic inputs has resulted in a host of environmental problems, such as eutrophication (Malone and Newton, 2020; Zhou et al., 2020), seasonal hypoxia (Rabalais et al., 2002; Howarth et al., 2011; Yu et al., 2021) and harmful algal blooms (Li et al., 2014; Glibert et al., 2014), in coastal bays, which greatly affect biogeochemical cycles and coastal ecosystems. Nitrogen (N) plays a key role in regulating marine primary productivity (Falkowski et al., 1998; Moore et al., 2013), and thus, its availability influences the sequestration of anthropogenic CO_2 in coastal seas and climate changes. However, increasing N loads to coastal bays, associated with industrial, agricultural, urban and domestic waste, have aroused widespread public concern (Yang et al., 2018). Therefore, a better understanding of N sources and their recycling processes is beneficial for designing effective management practices to protect and improve aquatic ecosystems.

The stable N isotopes ($\delta^{15}N$) of various pools of N, combined with the oxygen isotope of nitrate ($\delta^{18}O$-NO_3^-), have been proven to be useful in identifying nitrogen sources and their biogeochemical processes (Kendall, 1998; Ye et al., 2016; Yan et al., 2017; Yang et al., 2018; Lao et al., 2019a; Chen et al., 2020). For example, sewage and manure have higher $\delta^{15}N$ values (4-25‰) than soil N (-0.1-8.3‰), fertilizer (-1.9-3.0‰) and atmospheric deposition (-1.8-4.1‰) (Chen et al., 2019a; Kendall, 1998; Xue et al., 2009; Zhang et al., 2018) because the volatilization of $\delta^{15}N$-depleted ammonia is produced from human and animal waste and thus results in the enrichment of $\delta^{15}N$ in the residual N pools (Xue et al., 2009; Yang et al., 2018). In addition, the applicability of this technique can also reflect biogeochemical transformations associated with N cycles. Nitrification first oxidizes ammonia to nitrite and then further oxidizes it to nitrate (Casciotti et al., 2011). A nitrogen isotope effect of 14-42‰ has been found for the former step (Casciotti et al., 2003 and Casciotti et al., 2010), whereas an inverse kinetic isotope effect occurs for the latter step (Casciotti, 2009); thus, $\delta^{15}N$-NO_3^- values are close to 0‰ during nitrification (Sigman et al., 2005 and Sigman et al., 2009; Smart et al., 2015). In addition, oxygen (O) is sourced from water (H_2O) and dioxygen (O_2) during nitrification. Typically, one-third of O in the newly formed nitrate is considered to originate from O_2, with two-thirds originating from H_2O, and $\delta^{18}O$-NO_3^- values are 7.8‰ during nitrification (Kendall, 1998; Wankel et al., 2006; Chen et al., 2009). Moreover, assimilation and denitrification cause an increase in $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values in the residual nitrate pool, as lighter isotopes (^{14}N and ^{16}O) are preferentially utilized by phytoplankton and microorganisms, with an increase in the $\delta^{15}N$-NO_3^-:$\delta^{18}O$-NO_3^- ratio of 1 (Granger et al., 2004; Sigman et al., 2005; Granger et al., 2008). Thus, stable isotopes can serve as a useful tool in advancing our understanding of nitrogen dynamics in coastal bays.

Beibu Gulf is a semi-enclosed gulf and a newly developing industry and port area in the northwestern South China Sea (SCS). There are several rivers around the northern Beibu Gulf, and the influence of runoff input by the rivers and tidal influx from the outer gulf constitutes a well estuary-bay multi-ecosystem and has become an important mariculture area in China (Lao et al., 2020 and Liu et al., 2020; Xu et al., 2020: Xu et al., 2021; Lao et al., 2021a; Lao et al., 2022a and 2022b). However, with the rapid development of local urbanization and industrialization, the coastal bays in Beibu Gulf have been subjected to a high loading of contaminants from anthropogenic inputs, such as domestic, industrial, port and agricultural waste (Lao et al., 2019b; Xu et al., 2020: Lao et al., 2021a; Lao et al., 2021b; Lao et al., 2021c; Lao et al., 2021d: Xu et al., 2021 and Lao et al., 2022a). The nutrients discharged into coastal bays from runoff input have increased over the past 20 years, and the increase in phosphorus input has decreased the N/P ratios in several coastal bays in the Beibu Gulf (Lao et al., 2020 and Lao et al., 2021c). Moreover, the eutrophication of some coastal bays in the Beibu Gulf has increased in recent decades due to the increase in terrestrial nutrient input, particularly the nitrogen input (Lai et al., 2014; Guo et al., 2020; Lao et al., 2021a and Lao et al., 2021c; Pan et al., 2021). In addition, sewage is the dominant nitrate source in most bays/areas in the Beibu Gulf, which is identified by the dual nitrate isotopes (Pan et al., 2021; Lao et al., 2021b; Chen et al., 2022). This has resulted in an increase in the frequency of harmful algal blooms in the Beibu Gulf over the past decades, particularly in the past ten years (Xu et al., 2019).

Xi Bay is a semi-enclosed bay located on the coast of Fangchenggang city, which is an important industrial and port area in the northwestern Beibu Gulf. In addition to being affected by industrial activities, Xi Bay is also affected by a large amount of terrestrial contaminant input from the Fangcheng River in the upper bay (Lao et al., 2020). It is reported that a higher contaminant level was found in the coastal bay, including high nutrient loads, particularly in the rainy seasons (Lao et al., 2019b; Lao et al., 2021a). This is mainly affected by the heavy land-source discharge during the rainy seasons (Lao et al., 2021a). However, in such bays that are seriously affected by intensive human activities (i.e., industrial and port activities), the sources of nitrate are poorly understood. In this study, nitrate dual isotopes ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) and ammonium isotopes ($\delta^{15}N$-NH_4^+) were measured during the rainy season (July 2021) to determine the nitrate sources and their biogeochemical processes in Xi Bay.

Materials and methods

Study area and sampling

Xi Bay is a semi-enclosed bay in the northern Beibu Gulf, located on the coast of Fangchenggang city, South China. The climate of the bay is influenced by the East Asian monsoon, and the southwest monsoon prevails during summer and the northeast monsoon prevails during winter. The annual rainfall in the bay is 2441 mm; most rainfall occurs in the rainy season (April to October, accounting for 91% of the annual rainfall), and the highest rainfall occurs in July (accounting for 23% of the annual rainfall) (China Meteorological Data Sharing Service System, http://data.cma.cn/site/index.html). The monthly mean air temperature ranges from 14.3 to 28.6 °C, with an annual average of 22.7 °C. There is the Fangcheng River in the upper bay (**Figure 1**). The Fangcheng River is approximately 98 km long with a drainage area of 843 km^2. The annual runoff of the Fangcheng River is 542.25 × 10^8 m^3, with the highest runoff in July (127.41 × 10^8 m^3), and flows into the northern end of Xi Bay (Lao et al., 2020; Lao et al., 2022b). The eastern part of Xi Bay is the urban center of Fangchenggang city. The eastern bay mouth of Xi Bay is the industrial and port area. Thus, Xi Bay is a coastal bay greatly affected by human activities in the Beibu Gulf.

In this study, a cruise was conducted in Xi Bay in July (rainy season) 2021. A total of 16 seawater stations were collected in the bay, including a station (X1) in the Fangcheng River estuary and a station (X16) in the outer bay, using a rosette sampler fitted with 5-L Niskin bottles. The pH was measured in-suit with a pH meter (PHS-3C, Shanghai, China), and salinity was measured with a salinometer (SYA2-2). Dissolved oxygen (DO) was immediately determined using the Winkler titration method, with a precision of 0.07mg L^{-1}. COD samples were determined by the potassium permanganate oxidation method with a precision of 0.15 mg L^{-1}. The seawater samples for the dual nitrate isotopes ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) and ammonium isotope ($\delta^{15}N$-NH_4^+) were filtered by precombustion (450°C, 4 h) GF/F (glass fiber filters, 47-mm diameter, Whatman). The filtrate was transferred into a 250-ml precleaned (acid clean) polyethylene bottle and stored at -20°C until further analysis. Seawater was filtered by GF/F for the chlorophyll *a* (Chl *a*) sample, and the filtered GF/F was stored at -20°C until further analysis.

Chemical analysis

DO samples were measured on site by using the Winkler titration method. The Chl *a* samples were extracted by acetone (9:1) and measured by a spectrophotometer (Lorenzen, 1967). Nutrients, including PO_4^{3-}, NO_2^-, NO_3^- and NH_4^+, were determined using a San++ continuous flow analyzer (Skalar, Netherlands). The detection limit of PO_4^{3-} was 0.02 μg L^{-1}, and the detection limits of NO_2^-, NO_3^- and NH_4^+ were 0.1 μg L^{-1}. The $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-values were measured by the cadmium-azide method (McIlvin and Altabet, 2005) using a GasBench II-MAT 253 (253 Plus, Thermo Scientific, United States). Briefly, the NO_2^- in the seawater was removed by sulfamic acid (Granger et al., 2008). NO_3^- was reduced to NO_2^-

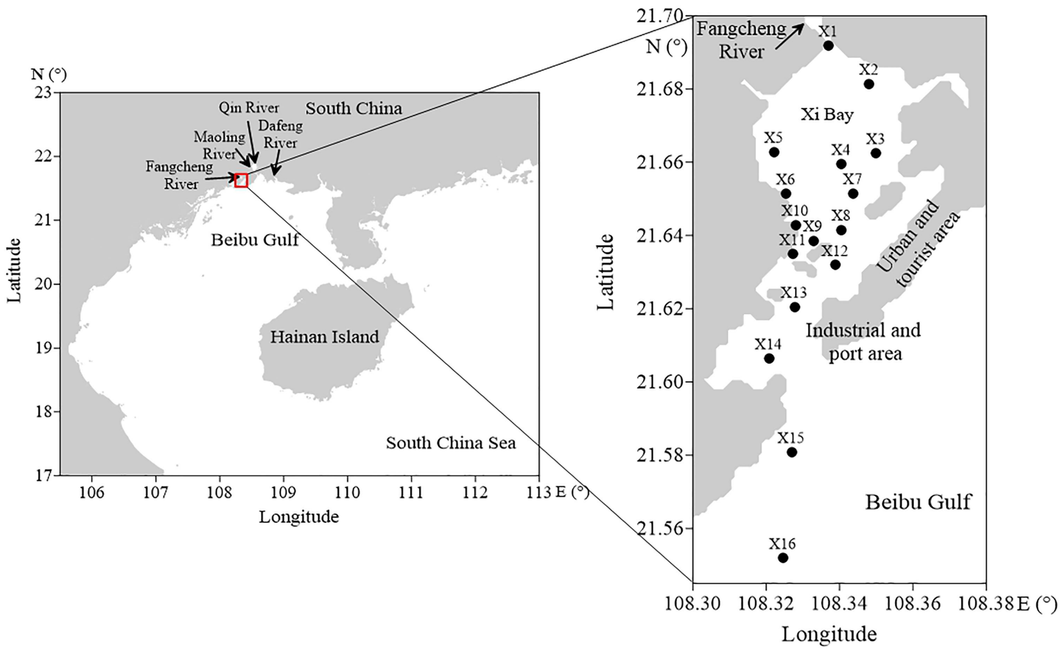

FIGURE 1
Study area and the sampling stations in Xi Bay, Beibu Gulf.

by adding Cd and then further reduced to N_2O by adding sodium azide buffer. For the analysis of $\delta^{15}N\text{-}NH_4^+$, BrO^- (pH 12) was added to the seawater to oxidize NH_4^+ to NO_2^-, and then NaAsO2 was added to remove excess $NaAsO_2$. The yield was verified *via* colorimetric nitrite determination. Then, sodium azide buffered with acetic acid was added to reduce NO_2^- to N_2O (Zhang et al., 2007). The N_2O was purified and separated by TraceGas (Isoprime), and the compositions of $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ were determined by GasBench II-MAT 253. The IAEA-N3 international standard was used to calibrate the values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, and the international standards of USGS 25, USGS26 and IAEA-N1 were used to calibrate the values of $\delta^{15}N\text{-}NH_4^+$. The reproducibility of duplicate analysis of $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ was<0.3‰ (mean ± 0.1‰),<0.6‰ (mean ± 0.3‰) and<0.3‰ (mean ± 0.1‰), respectively.

Salinity-based conservative mixing model

Isotopic values and nutrient concentrations from simple physical mixing can be calculated by the salinity-based conservative mixing model (Fry, 2002; Ye et al., 2016; Lao et al., 2019a). The equation is as follows:

$$q_1 + q_2 = 1 \quad (1)$$

$$q_1 S_1 + q_2 S_2 = S_{mix} \quad (2)$$

$$q_1 N_1 + q_2 N_2 = N_{mix} \quad (3)$$

$$q_1 N_1 \delta_1 + q_2 N_2 \delta_2 = N_{mix} \delta_{mix} \quad (4)$$

where q is the proportional contributions of the two endmembers. The terms S, N and δ are the salinity, nutrient and isotopic values of the two endmembers, and S_{mix}, N_{mix} and δ_{mix} are the theoretical values of mixing of the two endmembers. According to equations (1) to (4):

$$q_1 = (S_{mix} - S_2)/(S_1 - S_2) \quad (5)$$

$$N_{mix} = N2 + (N1 - N2) q_1 \quad (6)$$

$$\delta_{mix} = [q_1(\delta_1 N_1 - \delta_2 N_2) + \delta_2 N_2]/N_{mix} \quad (7)$$

Thus, salinity-based nutrient mixing exhibits linear conservative mixing (6), whereas isotopic mixing exhibits curvilinear mixing. The distributions of isotopic values and nutrient levels are expected to fall on the mixing line between two endmembers. The deviation from the mixing line suggests external sources or the presence of transformation processes (Wankel et al., 2006; Yang et al., 2018; Lao et al., 2019a; Lao et al., 2021b; Chen et al., 2022).

The Bayesian mixing model

The proportional contributions of potential nitrate sources can be quantified by a Bayesian mixing model, which was created and run in the SIAR (stable isotope analysis in R 4.1.2) package. This model has been widely used to quantify the potential nitrate sources in marine systems (Xue et al., 2009; Korth et al., 2014; Davis et al., 2015; Zhang et al., 2018: Lao et al., 2019a; Lao et al., 2021b and Chen et al., 2022). The framework can be found in Xue et al. (2009) and Moore and Semmens (2008) as follows:

$$X_{ij} = \sum_{k=1}^{k} P_k (S_{jk} + \quad c_{jk}) + \epsilon_{ij} \quad (8)$$

$$S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2) \quad (9)$$

$$c_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2) \quad (10)$$

$$\epsilon_{jk} \sim N(0, \sigma_j^2) \quad (11)$$

where X_{ij} represents the isotopic value j of the mixed water i (i =1, 2, 3,…, N and j =1, 2, 3,…, j), S_{jk} represents the source value k (k =1, 2, 3,…, k) on isotope j and represents the normal distribution with an average of μ and a standard deviation of ω, P_k represents the proportional contribution of source k, c_{jk} represents the fractionation factor and represents a normal distribution with an average of λ and a standard deviation of τ, and ϵ_{jk} represents the residual error of the additional unquantified variation between individual mixed samples and represents a normal distribution with an average of 0 and a standard deviation of σ.

Statistical analyses

Statistical analyses were conducted by the software SPSS 19.0 for Windows. The linear regression in this study was conducted by SigmaPlot 11.0.

Results

Physicochemical characteristics

The distributions of physicochemical parameters are presented in **Figure 2**. The surface seawater temperature ranged from 31.90 to 32.40 °C (an average of 32.13 °C). The salinity ranged from 8.97 to 24.22 (an average of 18.60), with a decreasing trend from the upper bay to the outer bay and the lowest salinity in the Fangcheng River estuary and the highest salinity in the outer bay. This suggests that the seawater in Xi Bay

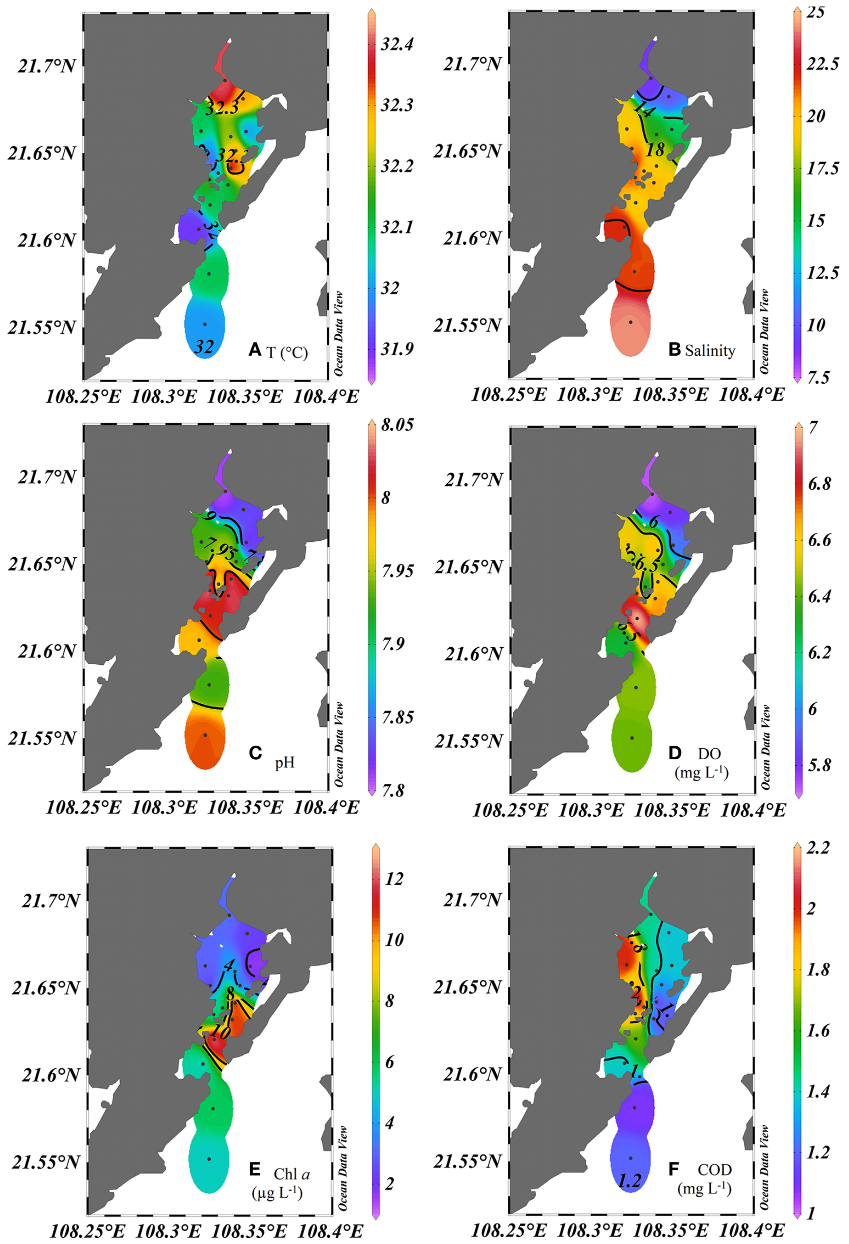

FIGURE 2
Spatial distributions of physicochemical parameters, including temperature **(A)**, salinity **(B)**, pH **(C)**, DO **(D)**, Chl *a* **(E)** and COD **(F)**, in Xi Bay during the rainy season.

is greatly affected by freshwater input from the Fangcheng River. The pH values ranged from 7.81 to 8.04, with an average of 7.95. The DO level ranged from 5.73 mg L^{-1} to 7.00 mg L^{-1} (an average of 6.41 mg L^{-1}), with a lower DO level in the upper bay (< 6 mg L^{-1}). The Chl *a* level ranged from 1.60 to 12.30 μg L^{-1} (average of 5.41 μg L^{-1}), and a higher Chl *a* level was found in the bay mouth. The COD concentrations ranged from 1.09 to 2.18 mg L^{-1}, with an average of 1.52 mg L^{-1}.

Characteristics and distributions of nutrients

The distributions of nutrients are presented in **Figure 3**. The concentrations of PO_4^{3-} ranged from 0.36 to 1.17 μmol L^{-1} (an average of 0.67 μmol L^{-1}). A higher PO_4^{3-} concentration was found in the estuary and the upper bay and exhibited a decreasing trend from the upper bay to the outer bay

(**Figure 3A**). This result suggests that the PO_4^{3-} in Xi Bay is mainly influenced by the runoff input. High DIN (dissolved inorganic nitrogen, the sum of NO_3^-, NO_2^- and NH_4^+) concentrations were found in Xi Bay. The concentration of NO_3^- ranged from 8.21 to 89.93 μmol L^{-1}, with an average of 31.05 μmol L^{-1}. Although the NO_3^- concentration exhibited a decreasing trend from the upper bay to near the bay mouth, a significantly high NO_3^- concentration was found in the bay mouth (stations X13, X14 and X15, over 3 times than that in the Fangcheng River estuary), which is located in the industrial and port areas (**Figures 3B, C**). Similar distribution patterns were also found in the concentrations of NO_2^- and NH_4^+ (**Figure 3**). The concentrations of NO_2^- and NH_4^+ ranged from 0.61 to 2.54 μmol L^{-1} (an average of 1.11 μmol L^{-1}) and 1.26 to 7.36 μmol L^{-1} (an average of 3.01 μmol L^{-1}), and high concentrations were all found in the bay mouth. Notably, the concentrations of DIN in the bay mouth (an average of 98.28 μmol L^{-1}) were significantly higher than those in the Fangcheng River (an average of 58.64 μmol L^{-1}) during a similar rainy month (Lao et al., 2020). This result suggests that the discharge of DIN caused by intensive human activities (i.e., industrial and port activities) in the bay mouth is much higher than the runoff input in Xi Bay. In addition, the concentrations of DIN in Xi Bay (an average of 35.17 μmol L^{-1}) during a similar rainy month (August 2018) were 1.5 times those in Qinzhou Bay (22.99 μmol L^{-1}) and 1.7 times those in Tieshangang Bay (20.51 μmol L^{-1}) (Lao et al., 2021a), which are semi-enclosed bays that are greatly affected by intensive human activities in the northern Beibu Gulf (Chen et al., 2022). Thus, it can be concluded that Xi Bay is a bay seriously affected by artificial inorganic nitrogen input in the Beibu Gulf.

Characteristics and distributions of isotopic compositions

The distribution characteristics of $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ in Xi Bay during the rainy season are presented in **Figure 4**. The values of $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ ranged from 4.6 to 13.7‰, 3.1 to 15.3‰ and -3.0 to 26.3‰, with averages of 7.5‰, 7.1‰ and 13.5‰, respectively. Generally, the values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were lower in the upper bay and exhibited an increasing trend to the bay mouth (**Figure 4**). Corresponding to the DIN, the highest values

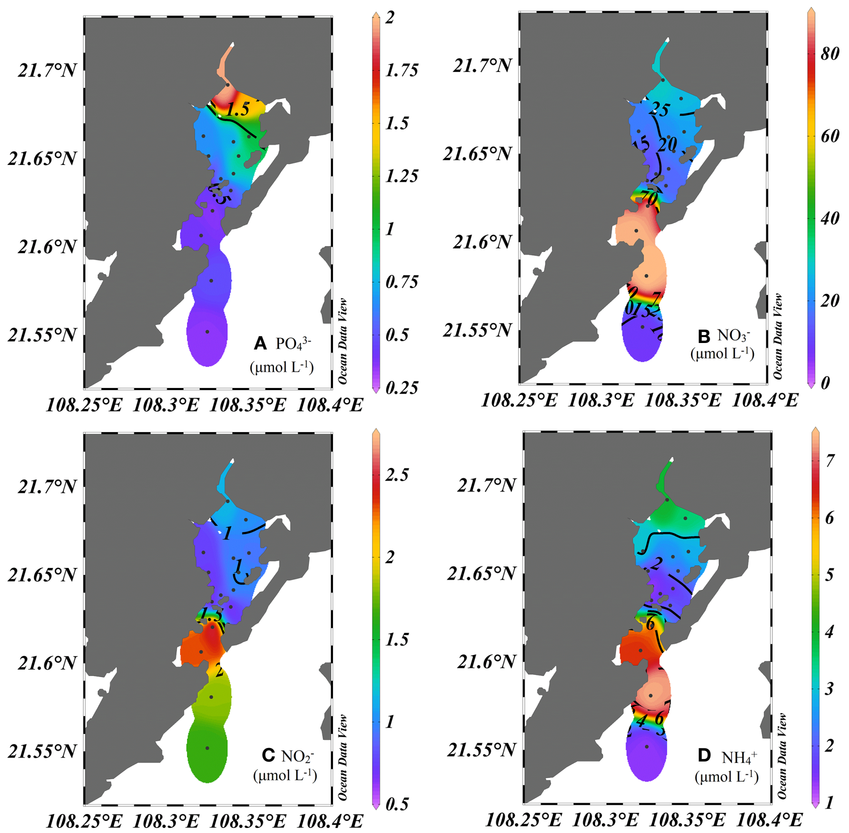

FIGURE 3
Spatial distributions of nutrients in Xi Bay during the rainy season. **(A)**, PO_4^{3-} **(B)**, NO_3^- **(C)**, NO_2^- **(D)**, NH_4^+.

of $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ were all observed in the bay mouth. In contrast, higher values of $\delta^{15}N-NH_4^+$ were observed in the upper bay (**Figure 4A**). The difference in isotopic values in the upper bay and bay mouth suggests that different biogeochemical processes or sources of nitrogen could have occurred in the two regions.

Riverine and marine endmember mixing in the bay

In this study, the lowest salinity at station X1 was selected as the freshwater endmember, and the highest salinity at the outer bay (station X16) was selected as the marine endmember. The hydrological characteristics, nutrient concentrations and isotopic values of freshwater and marine endmembers are presented in **Table 1**.

Discussion

Characteristics of nutrients in Xi Bay

Higher nutrient concentrations were also observed in the other coastal bays of Beibu Gulf during the rainy seasons (Lai et al., 2014; Lao et al., 2021a and Lao et al., 2021c), which were

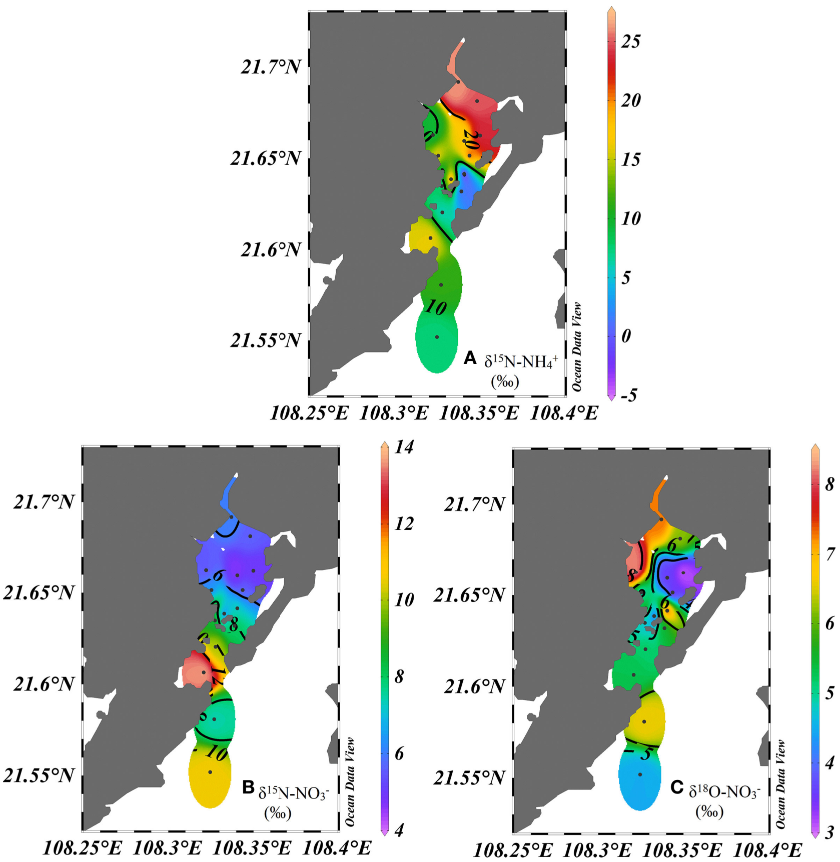

FIGURE 4
Spatial distributions of nutrients in Xi Bay during the rainy season. **(A)**, δ15N-NO_3^- **(B)**, δ18O-NO_3-**(C)**, δ15N-NH4$^+$

TABLE 1 The hydrological characteristics, nutrient concentrations and isotopic values of freshwater and marine endmembers of Xi Bay.

Station	Salinity	PO_4^{3-}	NH_4^+[μmol L^{-1}]	NO_2^-	NO_3^-	$\delta^{15}N-NO_3^-$ [‰]	$\delta^{18}O-NO_3^-$ [‰]	$\delta^{15}N-NH_4^+$ [‰]
X1	8.97	1.17	3.15	1.15	29.22	6.1	7.3	26.3
X16	24.22	0.36	1.38	0.65	8.21	10.6	4.5	7.6

mainly related to the heavy land-source discharge induced by the increasing runoff around the coastal Beibu Gulf (Lao et al., 2020; Lao et al., 2021a). Particularly in Qinzhou Bay, because there are several rivers, such as the Qin River, Maoling River and Dafeng River, that directly input a large amount of terrestrial contaminants into the bay (Chen et al., 2019a; Zhang et al., 2019; Lao et al., 2020; Lao et al., 2021c; Lao et al., 2022b; Lyu et al., 2022). However, heavier DIN pollution was found in Xi Bay. Similarly, there is a Fangcheng River that directly inputs into Xi Bay, and 626,669 t of nitrogen and 4,987 t of phosphorus are discharged directly into the bay every year, including 69% of nitrogen and 60% of phosphorus in the rainy season (Lao et al., 2020). In addition, a significant correlation between PO_4^{3-} concentrations and salinity was found in Xi Bay (**Figure 5A**), suggesting that the PO_4^{3-} in the bay was mainly affected by terrestrial input. There was no relationship between DIN concentration and salinity (**Figure 5B**). This was mainly because the high DIN concentration occurred in the bay mouth. However, if the stations in the bay mouth (X13, X14 and X15) were excluded, a significant correlation between DIN concentration and salinity was found in Xi Bay (**Figure 5C**). This result suggests that the DIN in the bay is also affected by terrestrial input. An extremely high DIN level was observed in the bay mouth, suggesting that the DIN in Xi Bay was also greatly influenced by anthropogenic discharge. Fangchenggang is a major industrial and port city in the Beibu Gulf, and industrial and port transportation activities may cause a great load of environmental pollution in the coastal area (Lai et al., 2014; Chen et al., 2019a; Lao et al., 2021a and Xu et al., 2019; Lao et al., 2021d). Thus, except for the runoff input, industrial and port transportation also contributed greatly to the DIN load in Xi Bay, which could be responsible for the high DIN pollution compared to the other coastal bays in the Beibu Gulf. In addition, the N/P ratios were high in Xi Bay (ranging from 21 to 272, with an average of 69), particularly in the bay mouth (an average of 241), which was significantly higher than the Redfield ratio of 16 (the ratio at which nutrients are utilized by phytoplankton). This result is similar to previous studies in coastal Fangchenggang city (Lai et al., 2014; Lao et al., 2021a). The high DIN discharge could be responsible for the high N/P ratios in the coastal bay. Moreover, Fangchenggang city is an important production area of phosphate fertilizer around the Beibu Gulf urban agglomeration, and the PO_4^{3-} concentration in the coastal area is relatively higher than that in other coastal bays in the Beibu Gulf, which is mainly influenced by the waste produced from the phosphate fertilizer plant in the city (Lai

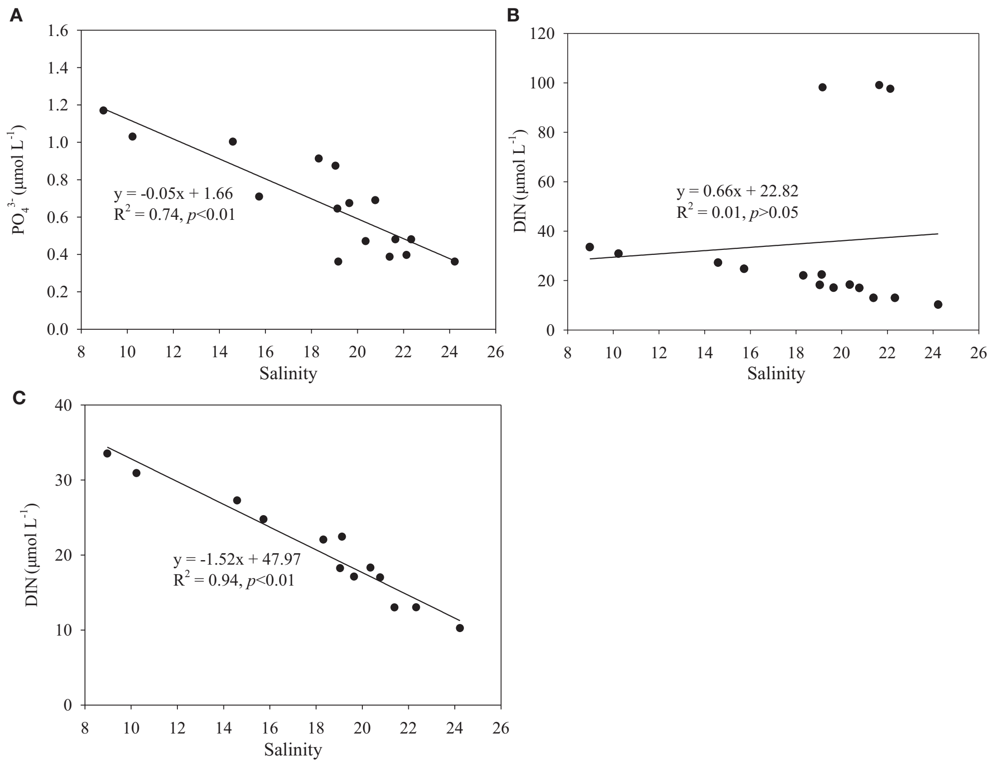

FIGURE 5
The linear relationship between PO_4^{3-} and salinity all stations of Xi Bay **(A)**; the linear relationship between DIN and salinity all stations of Xi Bay **(B)**; the linear relationship between DIN and salinity in Xi bay that exclude the stations in the bay mouth **(C)**.

et al., 2014; Lao et al., 2021a). In this study, higher PO_4^{3-} concentrations (with a minimum $[PO_4^{3-}]$ of >0.4 μmol L^{-1}) suggest that neither P nor N acted as a limiting nutrient in this ecosystem despite higher N/P ratios found in the bay and that environmental conditions were favorable for phytoplankton blooms. In addition, a decreasing N/P ratio was found in the coastal bays of Beibu Gulf due to increasing P input (Lao et al., 2021a and Lao et al., 2021c; Chen et al., 2022), which has resulted in harmful algal blooms increasing in frequency in recent years, especially in the past ten years (Xu et al., 2019; Kang et al., 2020; Guan et al., 2022). This could be responsible for the high Chl *a* level in the bay, particularly in the bay mouth (**Figure 2**). A similar situation has been documented in Xiangshan Bay of the East China Sea (Yang et al., 2018).

Sources and biogeochemical processes of nitrate in Xi Bay

Lower values of dual nitrate isotopes ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) were observed, whereas higher $\delta^{15}N$-NH_4^+ values were observed in the upper bay (stations X1 to X7). In addition, the nutrients in the bay are slightly above the mixing lines, particularly in the bay mouth (except for PO_4^{3-}) (**Figure 6**). These results suggest that decomposition/nitrification and/or external sources may occur in the bay. During the nitrification process, light N ($\delta^{14}N$) in the NH_4^+ pool is preferentially utilized by microorganisms, resulting in the enrichment of $\delta^{15}N$-NH_4^+ while adding depleted $\delta^{14}N$ to the residual nitrate pool (Sigman et al., 2005; Chen et al., 2009; Ye et al., 2016; Lao et al., 2021b). If nitrification is the dominant process in the bay, isotopic fractionation during the process can be estimated by open-system Rayleigh fractionation (Altabet, 2006; Ye et al., 2016; Chen et al., 2022) as follows:

$$\delta_m = \delta_i + \epsilon \times f_{NH4} \quad (12)$$

where δ_m is the measured $\delta^{15}N$-NH_4^+ value; δ_i is the initial $\delta^{15}N$-NH_4^+ value; ϵ is the fractionation factor; and f_{NH4} is the fraction of measured NH_4^+ concentration relative to the initial NH_4^+ concentration ($[NH_4^+]_m/[NH_4^+]_i$). The slope of the $\delta^{15}N$-NH_4^+-f_{NH4} linear regression line corresponds to the isotopic effects of NH_4^+ oxidation in seawater (Ye et al., 2016). In our results, a weak but still significant correlation was found between $\delta^{15}N$-NH_4^+ values and f_{NH4} in the upper bay (**Figure 7**). In addition, the overall estimated ϵ (-25.21‰) falls well within the reported values for N fractionation factors during nitrification in field studies (-14 to -38‰) (Casciotti et al., 2003; Ye et al., 2016). This further suggests that microbial nitrification occurs in the upper bay. Microbial decomposition/nitrification could consume oxygen and release carbon dioxide in the water column, which may be responsible for the lower DO level (an average of 6.20) and pH value (an average of 7.88) in the upper bay (**Figure 2**).

However, if nitrification was the dominant process in the upper bay, the $\delta^{15}N$-NO_3^- values should be close to 0‰ (Sigman et al., 2005 and Sigman et al., 2009; Smart et al., 2015). Higher $\delta^{15}N$-NO_3^- (ranging from 4.9 to 6.1‰) and $\delta^{18}O$-NO_3^- (ranging from 3.1 to 8.4‰) values were found in the upper bay. This indicates that other processes or external sources may also occur in the bay. Both the processes of assimilation and denitrification can enrich the dual nitrate isotopes in the residual water column, with coupled $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- fractionation effects of ~1 (Granger et al., 2004; Sigman et al., 2005; Granger et al., 2011). However, both assimilation and denitrification consume nutrients, which is consistent with the distribution of nutrients above the mixing lines (**Figure 6**). In addition, there is no relationship between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values (R^2 = 0.13, p>0.05). This further confirms that the processes of assimilation and denitrification are not responsible for the slight enrichment of dual nitrate isotopes in the upper bay, which can be related to external sources. Sewage and manure are usually characterized by relatively high $\delta^{15}N$-NO_3^- values (4‰ to 25‰) because of the volatilization of $\delta^{15}N$-depleted ammonia produced from human and animal waste (Xue et al., 2009). Soil N is another source that is characterized by relatively higher $\delta^{15}N$-NO_3^- values (0‰ to 8‰) (Xue et al., 2009). Previous studies suggested that a higher proportional contribution of soil N (30%) was found in strong hydrodynamic conditions with several river inputs in Qinzhou Bay, whereas a lower contribution of soil N (20%) was found in weak hydrodynamic conditions with river inputs in Tieshanggang Bay (Chen et al., 2022). Particularly in the rainy seasons, heavy rainfall could carry more land-source nutrients into the coastal Beibu Gulf (Lao et al., 2021a). In addition, manure and sewage were the dominant nitrate sources in the coastal bays of Beibu Gulf, which were mainly influenced by intensive human activities, such as mariculture, industrial and shipping activities (Lao et al., 2021b; Chen et al., 2022).

In the lower bay (stations X8 to X12) and the bay mouth (stations X13 to X15), the increased $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values may be influenced by the characteristics of high isotopic nitrate sources (i.e., sewage and manure) and/or the processes of denitrification and assimilation. However, denitrification in water column can be first ruled out due to the high DO levels (> 6 mg L^{-1}) in the lower bay and the bay mouth. The high Chl *a* level indicates that assimilation may occur in the lower bay and the bay mouth. In addition, the PO_4^{3-} concentrations were below the mixing line (**Figure 6**), which may be related to consumption by phytoplankton. However, there is no correlation between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values (R^2 = 0.07, p>0.05). In addition, according to the open-system Rayleigh fractionation (equation 12), the fractionation during the process of assimilation can be calculated. There is no relationship between $\delta^{15}N$-NO_3^- and f_{NO3} (R^2 = 0.25, p>0.05) in the lower bay and the bay mouth, and the overall estimated ϵ (0.7‰) significantly deviates from the range of reported values (-3 to

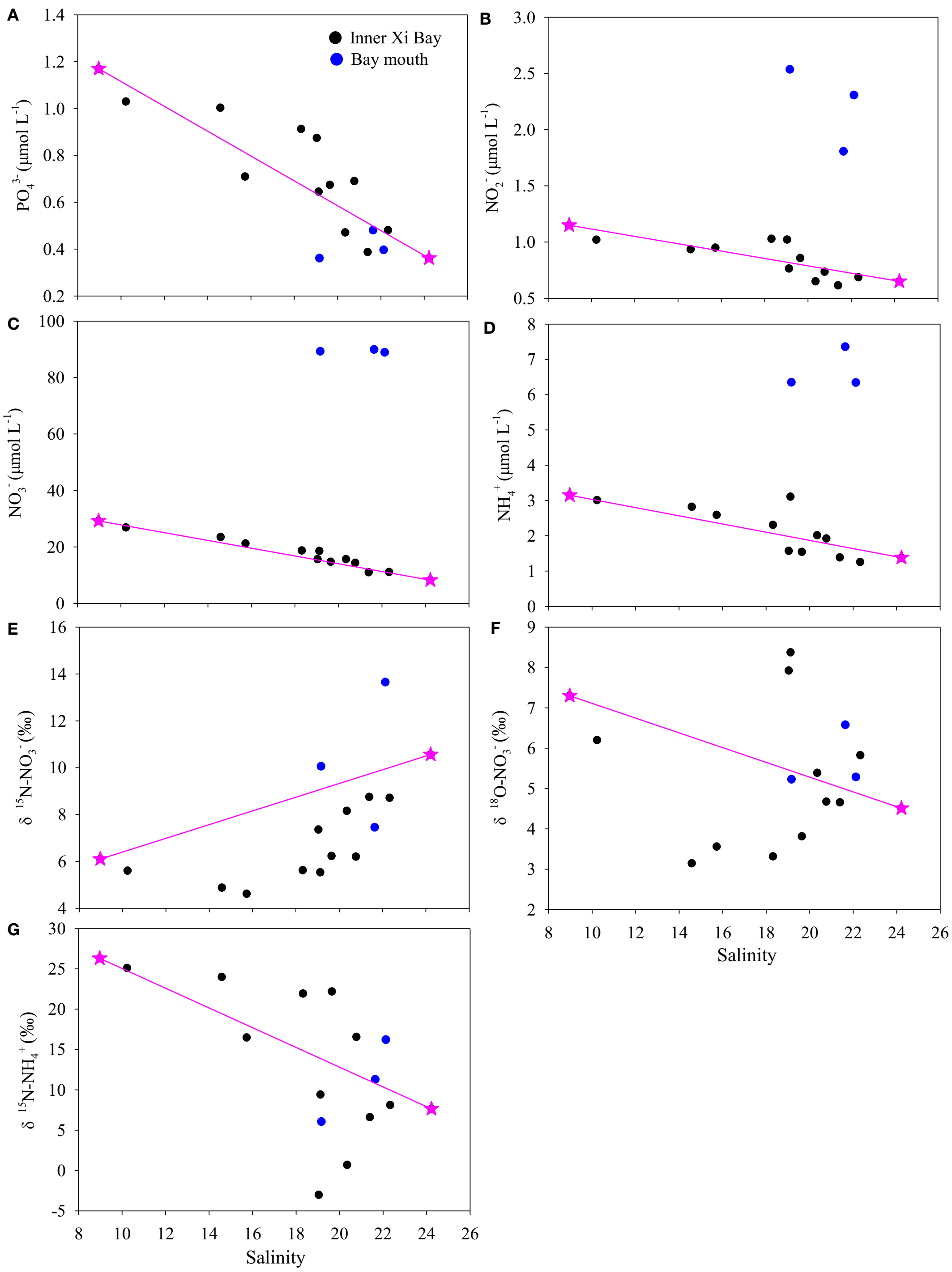

FIGURE 6
Distribution of nutrients and isotopic values against salinity in Xi Bay. The black dots are the samples excluding the stations in the bay mouth of Xi Bay; the blue dots are the samples in the bay mouth. The pink lines indicate the mixing line. The inner bay includes the upper bay and lower bay (stations X1 to X12); the bay mouth includes stations X13 to X15. **(A)**, PO_{43}^{-} **(B)**, NO_2^- **(C)** NO_3^- **(D)** NH_4^+ **(E)**, δ15N-NO_3^- **(F)**, δ18O-NO_3^- **(G)**, δ15N-NH_4^+

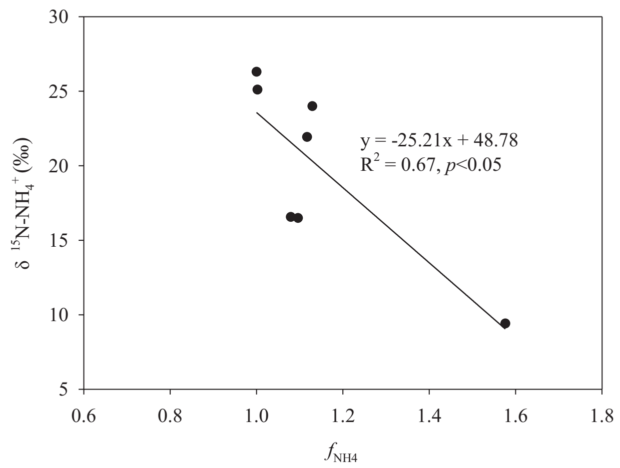

FIGURE 7
Linear relationship between $\delta^{15}N\text{-}NH_4^+$ and f_{NH4} in the upper bay.

-9‰) during assimilation (York et al., 2007; Ye et al., 2016; Chen et al., 2022). This suggests that assimilation is unlikely to be the dominant process causing the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values in the lower bay and the bay mouth. Moreover, a high NH_4^+ concentration (>6.30 μmol L^{-1}) was found in the bay mouth, indicating that NH_4^+ is sufficient, and phytoplankton preferentially assimilate NH_4^+ (Glibert et al., 2016). However, if the assimilated NH_4^+ by phytoplankton was the dominant process in the lower bay and the bay mouth, increasing $\delta^{15}N\text{-}NH_4^+$ values can be expected. In contrast, lower $\delta^{15}N\text{-}NH_4^+$ values were found in the lower bay and the bay mouth (**Figure 4**). Thus, the external DIN sources could be responsible for the high DIN level in the bay mouth. The coast of the bay mouth is an important port and industrial area in Fangchenggang city. Intensive human activities and industrial emissions may be responsible for the high DIN concentration in the bay mouth (Lao et al., 2021a). In addition, heavy rainfall during the rainy seasons could also scour masses of pollutants into the bay, particularly in heavily polluted wharves and industrial areas (Lao et al., 2021a).

Quantification of nitrate sources in Xi Bay

Because of the lower influence of isotopic fractionation caused by biological processes, such as denitrification and assimilation, isotopic characteristics may provide a signal on the various nitrate sources in Xi Bay, and the Bayesian mixing model can be used to quantify the potential nitrate sources (Lao et al., 2019a). In previous studies, potential nitrate sources, including sewage and manure, soil N, fertilizer and atmospheric deposition, have been reported in the coastal Beibu Gulf (Lao et al., 2021b; Chen et al., 2022). In Beibu Gulf, sewage and manure are considered as the most important nitrate sources in the coastal areas (Pan et al., 2021; Lao et al., 2021b; Chen et al., 2022). In addition, due to the input of many rivers into the Beibu Gulf, the rivers can input a large amount of agricultural nitrogen fertilizer and soil N into the coastal waters (Pan et al., 2021; Chen et al., 2022). Therefore, based on previous literature, we carried out a Bayesian mixed model calculation for four potential sources that may affect nitrate content in Xi Bay. In this study, the potential nitrate source from sewage and manure was selected from the measured values in the Beibu Gulf (Pan et al., 2021), and nitrate sources from soil N and fertilizer were selected from the coastal areas in the gulf (Jin et al., 2020), and the source from precipitation was selected near the Beibu Gulf in Zhanjiang city (Chen et al., 2019c). The $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values of these four potential sources are presented in **Table 2**. As discussed above, nitrification is another important nitrate source in the upper bay, and thus, the isotopic values of $\delta^{15}N\text{-}NO_3^-$ (0‰) and $\delta^{18}O\text{-}NO_3^-$ (7.8‰) (Chen et al., 2009 and Chen et al., 2019b) are considered a nitrate source endmember in the upper bay. The results of the quantification of potential nitrate sources in Xi Bay are presented in **Figure 8**. In the upper bay, the nitrate is mainly formed by nitrification (accounted for 39%). Except for the nitrate formed by nitrification, the nitrate from sewage and manure was another dominant external source in the upper bay (ranging from 28% to 38%, average of 33%), followed by soil N (ranging from 0% to 31%, average of 15%) and fertilizer (ranging from 0% to 29%, average of 11%), while the source from atmospheric deposition was the lowest (2%). In the lower bay and the bay mouth, due to the influence of intensive human activities, heavy nitrate pollution mainly originated from sewage and manure (ranging from 44% to 64%, average of 54%),

TABLE 2 The values (‰) of dual isotopes for the four potential nitrate sources at the river mouth.

Source	$\delta^{15}N\text{-}NO_3^-$ Range	$\delta^{15}N\text{-}NO_3^-$ Mean ± SD	$\delta^{15}N\text{-}NO_3^-$ Reference	$\delta^{18}O\text{-}NO_3^-$ Range	$\delta^{18}O\text{-}NO_3^-$ Mean ± SD	$\delta^{18}O\text{-}NO_3^-$ Reference
Manure and sewage	–	15.5 ± 0.5	(Pan et al., 2021)	–	-1.2 ± 0.2	(Pan et al., 2021)
Soil N	-6.2~9.0	2.9 ± 4.0	(Jin et al., 2020)	1.0~16.4	8.2 ± 4.4	(Jin et al., 2020)
Fertilizer	-0.2~0.9	0.4 ± 0.5	(Jin et al., 2020)	14.2~15.1	14.7 ± 0.4	(Jin et al., 2020)
Atmospheric deposition	-1.8~4.1	0.8 ± 1.5	(Chen et al., 2019c)	42.7~61.6	52.4 ± 5.1	(Chen et al., 2019c)

followed by soil N (ranging from 4% to 45%, average of 26%) and fertilizer (ranging from 1% to 33%, average of 17%), and atmospheric deposition accounted for only 3%. According to the calculated results, the high proportion of sewage and manure was responsible for the high DIN pollution in Xi Bay, particularly in the bay moth. Similar results were also reported in the other coastal bays in the Beibu Gulf, which suggests that sewage and manure were the dominant nitrate sources (Lao et al., 2021b; Pan et al., 2021; Chen et al., 2022). With the rapid development of urbanization and industrialization in the Beibu Gulf, the population density of coastal cities has gradually increased, and land-based wastewater discharge has increased (Lao et al., 2019a; Lao et al., 2020; Liu et al., 2020; Lao et al., 2021d), resulting in an increase in eutrophication in the coastal seawater of Beibu Gulf in recent decades (Lao et al., 2021a and Lao et al., 2021c). In addition, soil N was another important source that contributed to the heavy nitrogen pollution in the bay. With the increase in rainfall in the rainy season, a large amount of soil N on the land can be washed away and enter the coastal bay with the river (Lao et al., 2022b). Previous studies suggested that the nitrate source from the soil N in a coastal bay with riverine input was significantly higher than that in a bay without riverine input during the same period (Chen et al., 2022; Lao et al., 2022b). Moreover, nitrogen fertilizer (almost in the reduced form) is heavily applied in catchment agriculture in the Beibu Gulf during the rainy seasons (Xia et al., 2011; Kaiser et al., 2014; Lao et al., 2021a: Lao et al., 2021b), which could leak and contribute to the bay in this rainy season.

Conclusion

Nitrate and aammonium isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$) were measured to determine the nitrate sources and the biogeochemical processes associated with nitrogen cycling in Xi Bay in Beibu Gulf, a semi-enclosed bay strongly affected by human activities. A high DIN concentration was observed in Xi Bay, particularly in the bay mouth. In addition, the DIN concentration was significantly higher than that in the other coastal bays in Beibu Gulf (the concentration in Xi Bay is 1.5 times that in Qinzhou Bay and 1.7 times that in Tieshangang Bay), suggesting high nitrogen pollution in the bay due to intensive human activities (i.e., industrial and port activities). Lower values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ and higher $\delta^{15}N\text{-}NH_4^+$ values were observed in the upper bay, suggesting that microbial nitrification occurs in the upper bay, and the nitrate

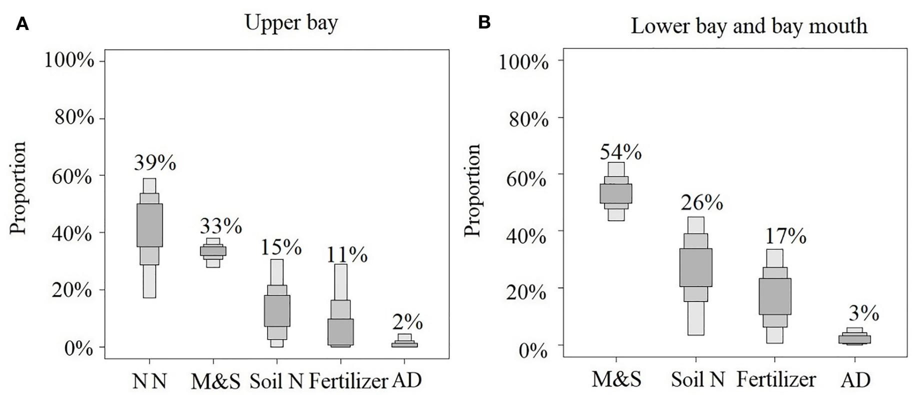

FIGURE 8
Proportion of potential nitrate sources in bay A during rainy season. **(A)** Proportion of potential nitrate sources in Upper bay, **(B)** Proportion of potential nitrate sources in Low bay and bay mouth. NN: nitrate from nitrification.

formed by nitrification accounted for 39%, which is the dominant nitrate source in the upper bay. Except for nitrification, external sources, including sewage and manure (accounting for 33%), soil N (accounting for 15%) and fertilizer (accounting for 11%), contributed to the higher nutrients in the upper bay. In the lower bay and the bay mouth, severe nitrogen pollution resulted in less impact of biological processes on isotopic fractionation. The heavy nitrate pollution in the lower bay and the bay mouth mainly originated from sewage and manure (accounting for 54%), followed by soil N (accounting for 26%) and fertilizer (accounting for 17%), and atmospheric deposition accounted for only 3%. Overall, biogeochemical processes have little impact on nitrate dual isotopes under heavy nitrogen pollution, and isotopes are an ideal proxy for tracing nitrogen sources.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

GJ was responsible for the conceptualization. SC and QL prepared and wrote the original draft. SC, QL and GJ wrote, reviewed, and edited the manuscript. QL were responsible for the data curation. CC, QZ and XL were responsible for the experimental operation. SC, QL and XL were responsible for field sampling. QL and GJ funded the acquisition. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank Marine Environmental Monitoring Centre of Beihai, State Oceanic Administration, for their support in collecting water samples. This study was supported by the Guangdong Provincial College Innovation Team Project (2019KCXTF021), the Guangxi Natural Science Foundation of China (2020GXNSFBA297065) and First-class Discipline Plan of Guangdong Province (080503032101, 231420003).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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