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Accepted Manuscript

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Excessively high concentrations of nitrate in surface water have become one of the commonly concerned problems in the world. In this paper, multiple isotopes (^{15}N, ^{18}O and ^{13}C) have been successfully used to distinguish between nitrate sources of chemical fertilizer, soil N and livestock waste in the upper stream of Chaobai River Beijing, China. It has proved that the $\delta^{13}C_{DIC}$ values could provide additional information for the identification of soil N and mineralization process of nitrate in wet season in the watersheds with dominantly forested land. This is useful to exactly determine the source and fate of nitrate in surface water.

Environmental Science:
Processes & Impacts

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Multi-isotope (^{15}N, ^{18}O and ^{13}C) indicators of sources and fate of nitrate in the upper stream of Chaobai River, Beijing, China

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Abstract Dual isotopes of nitrate (^{15}N and ^{18}O) and carbon isotopes of dissolved inorganic carbon (^{13}C) together with water chemistry were used to identify the sources and fate of nitrate in the upper stream of Chaobai River, north China. The results show that NO$_3^-$ concentrations ranges from 0.03 mmol L^{-1} to 0.80 mmol L^{-1}. Sampling sites from watershed with dominant forest land had higher NO$_3^-$ concentrations and lower δ^{15}N-NO$_3^-$ (<10‰) in wet season than in dry season, while those from watershed with more anthropogenic activities had lower NO$_3^-$ concentrations and higher δ^{15}N-NO$_3^-$ (>10‰) in wet season. Compositions of isotopes and chemistry indicated that NO$_3^-$ originated mainly from soil N, sewage and livestock wastes and atmospheric nitrogen. Furthermore, the mixing model suggested that soil N was the major NO$_3^-$ source in wet season, while the sewage and livestock wastes contributed the most in dry season. Compared to rivers, Miyun Reservoir had a higher contribution of atmospheric N and the N input from the upper rivers exerted significant influence over the reservoir. Mineralization and nitrification played an important role in N biogeochemical based on the isotopes (^{15}N and ^{18}O and ^{13}C) and chemical data. There appeared to be no significant denitrification in the watershed according to the three isotopes and chemical ions. The combined use of ^{15}N, ^{18}O and ^{13}C approved to be useful for further identification of sources and fate of nitrate in watersheds with dominant forest land in wet season.

Keywords： NO$_3^-$ source · NO$_3^-$ fate ·Dual isotopes · Carbon isotope · Surface water

1 Introduction

Nitrate contamination in waters has been increasingly concerned in the world.[1-3] High concentration of nitrate is related to anthropogenic activities involving nitrogen compounds such as inorganic fertilizer and by-products of organic matter from agriculture, septic systems, and animal manure.[4,5] Excessively high levels of nitrate in waters not only increase human health risks but also cause environmental and ecological problems, such as water acidification and eutrophication.[5] It is important to identify the sources and fate of nitrate for improving the water quality.

Nitrogen and oxygen isotopes of nitrate have been widely used to identify sources and fate of nitrate in surface water and ground water based on distinctive isotope compositions from different nitrate sources.[6-9] However, physicochemical processes that can alter the original isotope compositions of sources during the nitrate transport must be considered. For example, denitrification (NO$_3^-$→N$_2$) and dilution process produce similar decrease of nitrate concentrations, while denitrification process can increase δ^{15}N and δ^{18}O values of the remaining nitrate in waters.[10] In addition to the nitrogen and oxygen isotopes, other isotopes have been used to further study in the investigation of nitrate sources and fate. For example, Cravotta distinguished the animal manure, human waste and forest-leaf litter on the basis of δ^{13}C, even though different nitrogen sources had δ^{15}N and δ^{34}S values with wide ranges and overlap of compositions.[11] Use of δ^{13}C$_{DIC}$ and δ^{34}S-SO$_4^{2-}$ has proved to be helpful to evaluate which electron donors are related to the denitrification.[12-15] Two characteristics make the isotope tracers favorable to elucidate the sources and fate of nitrate. First, these elements co-exist with nitrogen among the nitrate sources and differences in isotopic composition of nitrogen sources can be measured. Next, these elements co-migrate with nitrate and retain the original isotopic composition of sources during the nitrate transport.[4,11] Numerous studies have focused on ground waters to constrain the sources and fate of nitrate by multiple isotopes while multi-isotope is less applied to river waters. Moreover, δ^{13}C have been extensively used to identify the denitrification processes in which heterotrophic denitrifiers utilize organic compounds as electron donors for reduction of NO$_3^-$ to N$_2$,[13,14] while δ^{13}C is less used from view of source identification and other NO$_3^-$ transformations.

The upper stream of Chaobai River watershed is located in Beijing, China, with the largest reservoir of North China in this watershed. Forested land use dominates the Chaobai River watershed, while the influence of anthropogenic activities has increased with the development of tourism and breeding industry in recent years.[16,19] TN concentrations in the watershed have been elevated significantly from 1990 to 2010.[16] Traditional hydrochemical analyses only show the levels of nitrate contamination not the source of nitrate. Previous studies have identified organic matter source by analyzing δ^{13}C and δ^{15}N of soil, plants, surface sediments of river, and suspended particulate matter of this watershed.[17,18] However, sources and fate of nitrate is not clearly identified by previous carbon and nitrogen isotopes of organic matter in this watershed. Therefore, the objective of this study is to identify nitrate sources and to elucidate transport and chemical transformations of nitrate by using multiple isotopes (^{15}N, ^{18}O, ^{13}C) and chemical water compositions, combined with previous carbon and nitrogen isotopes of organic matter in the Chaobai River watershed.

2 Material and Methods

2.1 Study site description

The upper stream of Chaobai River is located in the northern part of Beijing. The study area includes Miyun Reservoir and its two major tributaries, Chaohe River and Baihe River, with 400 km^2 of the Chaohe River and 3114 km^2 of the Baihe River. Miyun Reservoir was built in 1960, which has been used as the major surface drinking water storage of Beijing. The terrain is high in the northwest and low in the southeast and more than 60% of the areas have a slope greater than 25°. Brown soil and cinnamon soil are the dominant soil categories, accounting for 80% or more of the total area. Land use pattern in this region is dominated by forest land (91.4%), water bodies (4.8%), and cultivated land (3.3%).[19] The annual average soil loss in 2010 is 16.80 t ha^{-1} and 14.8% of total area suffers from severe water soil erosion (>50 t ha^{-1}).[20] The watershed is subject to a semi-humid monsoon climate with an average annual precipitation of 585 mm from 1951 to 2010.[21] The high rainfall season is from June to September, accounting for 83% of the annual rainfall.[22] More precipitations appear as rainstorm, which increase the risk of soil and water erosion. The long-term average annual potential evaporation is about 1800 mm, higher than the annual rainfall.[21] As reported by previous studies, influence of non-point source pollution such as domestic sewage, chemical fertilizer and livestock raising has increased.[16,17] According to the statistical yearbook of Beijing and local counties,[23] chemical fertilizer application in 2012 in Beijing was approximately 1.37×10^5 t. Take Miyun County, where the Miyun Reservoir located, as an example, 18,264 t chemical fertilizers were applied in 2012, dominated by nitrogen fertilizer (55%) and compound fertilizer (34%).[24] In recent years, the application amount of chemical fertilizer has decreased along with the increase in manure application in the cultivated land.[16]

2.2 Sampling and Analyses

Sampling sites are located in Miyun Reservoir and its upper reaches including Chaohe River, Baihe River, Baimaguan River (Fig. 1). River water samples were collected three times from the 29 sites in September 2012, December 2012 and June 2013. Meanwhile, two snow water samples and two rain water samples were taken in December 2012 and June 2013, respectively. In situ analyses included electrical conductivity, pH and alkalinity. Laboratory analyses included cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), anions (Cl^-, NO_3^-, SO_4^{2-}) and dissolved organic carbon (DOC). All samples were filtered through 0.45 μm membrane filters and stored at 4□ until analysis. Electrical conductivity and pH values were measured by portable meter and alkalinity was determined by titration with HCl acid. Cations were analyzed by ion chromatography Dionex 1500 and anions were analyzed by ion chromatography Dionex 90. DOC was determined by Liqui□TOC (Germany). The δ^{15}N- and ^{18}O-NO_3^- values were analyzed using the Denitrifier Method, in which δ^{15}N and δ^{18}O values were determined by measuring N_2O (produced by the denitrifying bacteria which was lack of N_2O-reductase activity).[25] The produced N_2O was then carried out using a trace gas coupled to an isotope ratio mass spectrometer (Isoprime 100, UK). The method of Atekwana and Krishnamurthy was modified for the $\delta^{13}C_{DIC}$ measurement.[26] First, the filtered water samples were injected into vacuumed glass bottles with pure phosphate and heated in a water bath. The produced CO_2 in the bottle was extracted and cryogenically purified into a tube using a high vacuum line. Finally, $\delta^{13}C_{DIC}$ values were determined on a isotope ratio mass spectrometer (Isoprime 100, UK). The nitrate dual isotopes and carbon isotope were measured in Dry land Farming and Water-Saving Laboratory of the Ministry of Agriculture, Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agriculture Sciences. The stable isotope ratios are expressed in delta (δ) units and a permil (‰) notation relative to an international standard:

$$\delta X(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (1)$$

Where, R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$, $^{18}O/^{16}O$ or $^{13}C/^{12}C$ ratio of the sample and sample standard for δ^{15}N, δ^{18}O and $\delta^{13}C_{DIC}$, respectively. Values of δ^{15}N are reported relative to N_2 in atmospheric air (AIR), δ^{18}O values are reported relative to VSMOW and $\delta^{13}C_{DIC}$ values are reported relative to PDB. Precision averaged ±0.3‰ for δ^{15}N, ±0.5‰ for δ^{18}O and ±0.3‰ for $\delta^{13}C_{DIC}$ values.

3 Results

3.1 Chemical compositions of the collected water

The spatial and seasonal distribution of TDS, NO_3^- and HCO_3^- are presented in Fig. 2a-c. The average TDS values for September, June and December were 384±91 mg L^{-1}, 362±73 mg L^{-1} and 427±106 mg L^{-1}, respectively (Table 1 in supplementary information). The TDS concentrations were lower in wet season than in dry season, which partly reflected the dilution effect of rainwater on chemical ions during high flow period.[32,34] Spatially, the TDS concentrations were higher in draining areas with more population and mineral activities. Assuming that one or more chemical ions level is significantly elevated by human activities, then the TDS concentration will become higher than that of the natural environment. Thus, TDS value might reveal the impact of anthropogenic activities. For example, the TDS values in Chaohe River in September and December exceeded the average value of TDS (500 mg L^{-1}) from the most polluted rivers among the world's 60 largest rivers, indicating anthropogenic influences to some extent in the studied basin.[27] Ca^{2+} and Mg^{2+} were the dominant cation for these waters, accounting for more than 80% of the total cation concentrations in the river waters. On average, HCO_3^- was the most abundant anion (64%), followed by SO_4^{2-} (22%) and Cl^-+NO_3^- (14%). The water composition reflected the effect of carbonate rock dissolution.[21]

Concentrations of NO_3^- ranged from 0.03 mmol L^{-1} to 0.80 mmol L^{-1}, with an average value of 0.26 mmol L^{-1}. Forty-four percent of the obtained samples had NO_3^- concentrations above the human affected values (0.21 mmol L^{-1}),[28] with water samples located in sampling site DJH close to the limits for drinking water (50 mg L^{-1}) by the World Health Organization. Seasonal variations of NO_3^- concentrations were not significant, while the spatial difference was significant in the watershed. Varying characteristics such as drained area and vegetation cover rate could result in the differences between distinct watersheds and streams.[7,29] In the present study, the NO_3^- concentrations were lower in the reservoir than in river waters, reflecting the differences in source and fate of nitrate between reservoir and river water. This is similar to the results found in the Three Gorges Dam Reservoir, Dongting Lake and Poyang Lake in the Changjiang River.[8] The NO_3^- concentrations in waters draining areas with more forest coverage ranged from 0.07 to 0.25 mmol L^{-1} and the concentrations were higher in June. The NO_3^- concentrations in tributaries (from T1 to T5) were high on average, which indicated that these tributaries contributed a large amount of nitrate to the main channel of the Baihe River.

Chloride concentrations ranged from 0.17 mmol L^{-1} to 0.83 mmol L^{-1}, showing little seasonal variations (Table 1 in supplementary information). However, Cl^- concentrations varied spatially, with high levels of Cl^- found in waters where there were intense human activities, which was similar to the above NO_3^- distribution. Because Cl^- is biologically and chemically conservative, the NO_3^-/Cl^- ratios have been used to identify NO_3^- sources and transformations in many studies.[14,29,34,35]

DOC concentrations ranged from 0.07 mmol L^{-1} to 0.39 mmol L^{-1}, typical for the unpolluted rivers.[30] Generally, DOC concentrations in reservoir were higher compared with the upper rivers (Fig. 2c), which agreed with the distribution of particulate organic matter in this watershed reported by Li et al.[18] According to Ogrinc et al.,[31] lentic conditions in natural lakes or man-made reservoirs facilitate the in situ growth of phytoplanktonic algae, which may contribute more DOC and POC to the waters. In addition, elevated DOC concentrations may be related to anthropogenic influence such as agricultural activities and/or septic systems, and donate electrons during denitrification.[1,13,14]

3.2 The nitrate isotopes and dissolved inorganic carbon isotopes

$\delta^{15}N\text{-}NO_3^-$ varied between -1.12 and +13.61‰ with the average value of +8.86‰; $\delta^{18}O\text{-}NO_3^-$ varied between -3.75 and +21.26‰, and averaged +7.4‰. Recent studies based on the denitrifier method have reported that $\delta^{18}O$ values of microbial NO_3^- are much lower than previous studies using the $AgNO_3$ method to determine the $\delta^{18}O\text{-}NO_3^-$.[32,33] Seasonally, $\delta^{15}N\text{-}NO_3^-$ values were low in June and high in September (Fig. 2d). As shown in the histogram of $\delta^{15}N\text{-}NO_3^-$ (Fig. 3), approximately 80% of the sites had $\delta^{15}N\text{-}NO_3^-$ values <10‰ in June and December, while 62% of those sites had $\delta^{15}N\text{-}NO_3^-$ values >10‰ in September. Unlike the $\delta^{15}N\text{-}NO_3^-$ variations, $\delta^{18}O\text{-}NO_3^-$ values were higher in June and September than in December (Fig. 2e), suggesting that rain water had more effect on nitrate in wet season due to the high $\delta^{18}O\text{-}NO_3^-$ values in rain.[34] Intra-seasonal correlationship was not observed for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values. However, the nitrate for the three months can be clearly distinguished by the $\delta^{15}N$ values, showing an increasing trend from June to December to September. A different increasing trend was displayed for $\delta^{18}O\text{-}NO_3^-$ values (from December to September and to June). The above relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ was different from rivers where $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ River were lower than Chaohe River, in agreement with the distribution of nitrate concentrations. The average values of $\delta^{15}N$ and $\delta^{18}O$ in rain water were +1.07‰ and +59.84‰ and in snowmelt water were +7.20‰ and +80.94‰, respectively, falling in the typical range of atmospheric nitrate.[33]

Measured $\delta^{13}C_{DIC}$ in river waters ranged from -12.02 to -3.63‰, with mean values of -9.07‰ in June and -6.29‰ in December, with 76% of the samples had $\delta^{13}C_{DIC}$ values between -14 and -8‰ in June (Table 1 in supplementary information; Fig. 3). This is similar to other studies.[38,43] The low $\delta^{13}C_{DIC}$ values in summer (Fig. 2f) suggested that the soil CO_2 (mainly biogenic, hence enriched in ^{12}C) made a larger contribution to the DIC of river water in high flow season than in low flow season.[51] The DIC was enriched in $\delta^{13}C_{DIC}$ in reservoir comparing to the rivers, which might result from enhancement of CO_2 evasion from the river system due to increased water residence time.[38]

4 Discussion

4.1 Nitrate origins based on the isotopes (^{15}N, ^{18}O and ^{13}C) and chemical compositions

The potential NO_3^- sources in the study area include soil organic materials, livestock wastes and effluents from septic systems, chemical fertilizer and precipitation. Given that different nitrate sources have typical isotopic compositions, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values provide forceful evidence for identifying nitrate sources. $\delta^{13}C_{DIC}$ and chemical compositions show different variation trend in the natural environment and anthropogenic areas, which make it possible to distinguish nitrate sources.

The measured isotopic values of NO_3^- in river water are plotted in Fig. 4 together with typical isotopic composition from different nitrate sources. As shown in Fig. 2b and Fig. 4, spatial variations of $\delta^{15}N\text{-}NO_3^-$ values and NO_3^- concentrations indicated different nitrate origins and impacting factors in different rivers. The highest NO_3^- concentrations were observed in areas where commercial crops such as walnut and ansu apricot were widely planted on the hills with slop greater than 25°. Leaching of soil containing chemical fertilizer from slops to rivers was more intensive during high flow season. The corresponding $\delta^{15}N\text{-}NO_3^-$ values (-0.24±0.64‰) in two tributaries DJH and T5 revealed the nitrate source from chemical fertilizer. It has been reported that inorganic fertilizers usually have $\delta^{15}N$ values of -4 to +4‰ and nitrate fertilizers often have slightly higher $\delta^{15}N$ values than NH_4^+fertilizer. [33] Unlike ammonium fertilizers, nitrate fertilizers have higher $\delta^{18}O\text{-}NO_3^-$ values from +17 to +25‰ because the O originates from atmospheric O_2 (+23.5‰).[39] The $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values in DJH and T5 fell in the range of NH_4^+ fertilizer and NO_3^- fertilizer suggesting that high concentrations of nitrate was derived from chemical fertilizer. Additionally, NO_3^-/Cl^- ratios could provide further information regarding the effect of chemical fertilizer. Chemical fertilizer applied in this watershed is mainly nitrogen fertilizer and compound fertilizer, which would produce a high level of NO_3^- concentration and a low ratio NO_3^-/Cl^- if heavy application of this fertilizer takes place. As shown in Fig. 5a, the highest NO_3^-/Cl^- ratios and the lowest Cl^- concentrations were found in DJH and T5, which confirmed the chemical fertilizer inputs as identified by nitrate dual isotopes. The ratio of NO_3^-/Cl^- as an indicator of nitrate sources was also used in other studies.[29,34,35] The nitrogen isotopic values of sewage and livestock waste are generally in the range of +10 to +20‰.[5] Two of the sampling rivers (Chaohe River and Baimaguan River) with $\delta^{15}N$ values >10‰ had high population density and livestock and poultry raising and may therefore be affected by sewage and livestock effluent. According to the statistical data in the studied watershed,[24,36,37] the amount of animals increased rapidly due to the development of stockbreeding. The annual nitrogen yield of animal waste in eleven villages or towns was calculated based on the statistical data of the local counties and previous study.[24,36,37,38] Sites of B1 and B2, outside of the Beijing city (Fig. 1), are not included. The computed $\delta^{15}N$ vs. nitrogen yield is plotted in Fig. 5b, and specific data in each village or town can be found in supplementary Table 2. Nitrogen isotopes of nitrate and N yield of animal waste showed a positive relation (R^2=0.45, P=0.01), indicating that the elevated $\delta^{15}N$ values were related to animal waste, which was similar to the study by Mayer et al.[9] However, this is just a simple relation analysis. The N load of animal waste, which is flushed to rivers by soil erosion, should be further studied. The high concentrations of Cl^- and SO_4^{2-} and the relatively low NO_3^-/Cl^- ratios could confirm this nitrate source (Fig. 5). Previous studies found that livestock effluent had high concentrations of Cl^- and SO_4^{2-} and low NO_3^-/Cl^- ratios.[29,34,35] The low NO_3^-/Cl^- ratios in livestock effluent were related to two factors: (1) weak nitrification process. As a major N species, ammonium in livestock effluent had not been nitrified to nitrate and consequently nitrate contents was low;[35] (2) denitrification process. NO_3^-/Cl^- would decrease if NO_3^- was transformed to N_2 during denitrification.[34]

Forty-one percent of the sites had $\delta^{15}N$-NO_3^- values falling in the typical range of soil organic nitrogen (+2 and +8‰)[5] in June. These drain watersheds are predominately forested with the coverage of forested land around 90%, reflecting the potential soil N sources for the N input. Such low $\delta^{15}N$-NO_3^- values were observed in the main stream of Baihe River with NO_3^- concentrations less than 0.2 mmol L^{-1}. However, as shown in the histogram of $\delta^{15}N$-NO_3^- (Fig. 3), 55% of the samples in winter were within the overlapping range (+8~+10‰) of the nitrogen isotopic composition of soil nitrogen and livestock effluents, which made it difficult to differentiate among these nitrogen sources based only on $\delta^{15}N$-NO_3^- values. Cravotta[11] used stable isotopes of carbon and nitrogen to identify nitrogen sources in the Lower Susquehanna River Basin given the different carbon compositions of natural leaf litter and farm-animal manure. C3 plants are expected to have more negative $\delta^{13}C_{DIC}$ values than C4 plants due to differences in fractionation during photosynthesis.[41] While $\delta^{13}C_{DIC}$ of livestock waste would inherit the carbon compositions of C4 plants if farm animals are feed on corn and maize[11] and these variations of $\delta^{13}C_{DIC}$ would eventually be reflected on riverine $\delta^{13}C_{DIC}$ values. Therefore, $\delta^{13}C_{DIC}$ is likely to be useful to differentiate manure and natural soil nitrogen. The related equations of nitrate production by organic matter can be obtained from Liu et al.[34] :

$$RCHNH_2COOH+O_2 \rightarrow RCOOH+CO_2+NH_3 \qquad (2)$$

$$NH_3+O_2+H_2O \rightarrow NO_2^-+3H^++H_2O \qquad (3)$$

$$2NO_2^-+H_2O+O_2 \rightarrow 2NO_3^-+H_2O \qquad (4)$$

The studied areas belong to a warm-temperature zone and thus C3 plants are more dominant. Carbon compositions of C3 plants and C4 plants in the studied basin were in a range of -29~-22‰ and -17~-11‰, respectively.[17] After accounting for the diffusional fractionation +4.4‰ between soil CO_2 and soil organic matter[42] and +9‰ between soil CO_2 and HCO_3^-,[43] the resulting $\delta^{13}C_{DIC}$ from C3 plants and C4 plants should be in the range of -15.6~-8.6‰ and -3.6~+2.4‰, respectively. The theoretic calculation process of $\delta^{13}C_{DIC}$ and carbon variations in aqueous systems were shown in Fig. 6a. Values of $\delta^{13}C_{DIC}$ <-8.6‰ represented the watersheds with a higher percentage of forested land. For example, more negative $\delta^{13}C_{DIC}$ values were distributed in Baihe River, where approximately 90% of the land was covered by forested land. This indicated that nitrate in sites with $\delta^{15}N$-NO_3^- values between +8 and +10‰ and $\delta^{13}C_{DIC}$ <-8.6‰ could be originated from soil organic matter. For sites with elevated $\delta^{13}C_{DIC}$ values and higher $\delta^{15}N$-NO_3^- values such as Chaohe River, sewage and livestock waste was the major nitrate source. The trend of elevated $\delta^{13}C_{DIC}$ values in areas with more anthropogenic activities was similar to other studies.[39,44] However, $\delta^{13}C_{DIC}$ values in December were not indicative of nitrogen sources because these higher $\delta^{13}C_{DIC}$ values exceeded the theoretical range. Seasonal variations of $\delta^{13}C_{DIC}$ might be a result of mixture of endmembers and/or in-stream process.[42,45]

In contrast with the upper river, the reservoir had lower NO_3^- concentrations and mediate $\delta^{15}N$-NO_3^- values, suggesting the input from rivers and multi end member mixing for NO_3^-. As identified in Fig. 2, NO_3^- concentrations and $\delta^{15}N$-NO_3^- values in sites of reservoir (M2 and M4) were lower than the corresponding river mouth (C4 and B15). Whereas, variations of $\delta^{18}O$-NO_3^- were opposite to the trend of $\delta^{15}N$-NO_3^-. $\delta^{18}O$-NO_3^- can be useful to distinguish atmospheric nitrate from nitrification-derived NO_3^- when sources and fate of NO_3^- cannot be fully elucidated using only N isotopes. $\delta^{18}O$-NO_3^- values in precipitation were higher than those derived from nitrification.[33] The elevated $\delta^{18}O$-NO_3^- values in reservoir suggested that nitrogen sources with heavy $\delta^{18}O$ compositions contributed to riverine NO_3^-. Ammonium-nitrate fertilizer might contribute NO_3^- to these samples for the heavier $\delta^{18}O$ values. However, the more positive $\delta^{18}O$-NO_3^- values in September and December were not consistent with the applied time of nitrogen fertilizer and the low nitrate concentration of these samples. Another source for the higher $\delta^{18}O$-NO_3^- values was atmospheric nitrate. The collected samples of rain water and snow water had $\delta^{18}O$-NO_3^- values ranging from +56.37‰ to +81.10‰. Influence of atmospheric nitrate would probably be pronounced on samples when nitrate concentration in precipitation was high and nitrate concentration in rivers was relatively low.[1] The average concentrations of NO_3^- in Miyun Reservoir were 0.05 mmol L^{-1}, according to meteorological data,[46] which was close to the mean NO_3^- concentrations in the present study (0.07 mmol L^{-1}). The combined $\delta^{18}O$-NO_3^- values and nitrate concentrations indicated that atmospheric nitrate had more contribution to reservoir than river. Besides, evidence using NO_3^-/Cl^- ratios supported the influence of atmospheric nitrate in reservoir. As reported by the meteorological data,[46] NH_4^+ was the main N form in rain water in the studied watershed. Thus, NO_3^-/Cl^- ratios should be low if atmospheric nitrate has significant effect on river water. As shown in the plot of NO_3^-/Cl^- ratios vs. Cl^- concentrations (Fig. 5), the lowest NO_3^-/Cl^- ratio was found in the Miyun Reservoir, which confirmed the above conclusion that atmospheric nitrate had more influence on reservoir than river. Another possible explanation for the low NO_3^-/Cl^- ratios in reservoir is biological uptake of NO_3^-. However, this process could be negligible as discussed in the next section. It was reported that δ^{18}O-NO$_3^-$ values from atmospheric deposition was modified by nitrification before entering the river and less than 3% of the unmodified atmospheric nitrate directly entered the stream.[47,48] Thus, nitrification production of precipitation should be one major source of nitrate for the reservoir.

In general, nitrate was mainly derived from soil nitrogen, livestock waste or sewage and atmospheric nitrogen. Also, several sites were affected by chemical fertilizer. Differences of nitrate source in seasons and sites suggested that there were varying impacting factors such as forest coverage, climate and human activities. δ^{13}C$_{DIC}$ can provide evidence for the identification of nitrogen source in wet season. However, δ^{13}C$_{DIC}$ in dry season appeared to reflect the mixture of endmembers and/or transformation process, not the composition of the N sources.

4.2 Transformations of nitrate

Processes influencing dual isotopic compositions of NO$_3^-$ include fixation, assimilation, mineralization, volatilization, nitrification, denitrification and other dissimilatory N transformations. These processes can alter the original NO$_3^-$ isotopic compositions, potentially biasing identification of NO$_3^-$ sources.[3,33,48] Biochemical N cycle is closely linked to organic matter, which makes it possible to identify NO$_3^-$ transformations by δ^{15}N- and δ^{18}O-NO$_3^-$ together with δ^{13}C$_{DIC}$.

Denitrification is an important process to reduce NO$_3^-$ load in the contaminated basin. Denitrification requires anoxic condition, where NO$_3^-$ is reduced to N$_2$ or N$_2$O by organic matter or sulfide. Apart from the dual isotopes of nitrate used to identify denitrification process, studying the isotopic composition of DIC and chemical composition can further reveal whether or not organic matter and/or sulfides play a significant role as electron donors if denitrification process occurs. Variations of isotopes and corresponding ions during denitrification are characterized as follows: (1) nitrate concentrations tend to decrease while values of δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ simultaneously increase with enrichment ratios ranging from 2.1 to 1.3.[10,33] (2) carbonic isotope of DIC would decrease due to the preferential utilization of ^{12}C when organic matters are oxidized as electron donors during the denitrification process.[13,14] Correspondingly, a decrease of DOC concentrations and an increase of DIC concentrations would be observed as a result of the oxidation of organic matters. Therefore, plots of δ^{15}N-NO$_3^-$ vs. NO$_3^-$/Cl$^-$ (Fig. 7a-b), δ^{13}C$_{DIC}$ vs. NO$_3^-$/Cl$^-$ (Fig. 7c) and δ^{13}C$_{DIC}$ vs. HCO$_3^-$ (Fig. 8) are made to identify the denitrification process. In the present study, the intra-seasonal and inter-seasonal increasing trend for δ^{15}N and δ^{18}O was not obtained (Fig. 4). Thus, denitrification had no significant influence on removing nitrate, although there was a significant negative correlationship between δ^{15}N-NO$_3^-$ and NO$_3^-$/Cl$^-$ (Fig. 7a-b). Indeed, the observed relationship of δ^{13}C$_{DIC}$ values vs. HCO$_3^-$ concentrations (Fig. 8) and δ^{13}C$_{DIC}$ values vs. DOC concentrations (not shown) support this conclusion. Previous studies argued that some anthropogenic sources might mask any isotopic denitrification signal[13,32] and denitrificaion could occur in situ and groundwater flow systems.[1,35] It should be noted that sampling location B3 (Baihepu Reservoir) showed a simultaneous increase in the δ^{15}N and δ^{18}O-NO$_3^-$ values (ratio of 1.63:1) during the sampling period associated with a strong decrease in NO$_3^-$ concentrations. This might be an indicator for denitrification in the Baihepu Reservoir. Since denitrification process was excluded for the negative correlationship between δ^{15}N-NO$_3^-$ and NO$_3^-$/Cl$^-$, assimilation could be one mechanism for the trend of δ^{15}N-NO$_3^-$ values and NO$_3^-$/Cl$^-$ ratios. In general, isotopes with the lower mass were preferentially transformed during assimilation. δ^{15}N-NO$_3^-$ values of non-fixing plants and particulate organic matter (POM) would be lower than or equal that of the dissolved inorganic nitrogen during assimilation.[45,49] In the studied watershed, the enriched δ^{15}N-NO$_3^-$ relative to the δ^{15}N-POM with a range of -0.66 to +4.79‰[18] suggested the assimilation process. Similar δ^{15}N-NO$_3^-$ enrichments relative to POM have been found in Mississippi River and Sava River.[31,45] Needoba et al.[50] demonstrated that influence of assimilation fractionation on external nitrate pool is through efflux of nitrate with enriched δ^{15}N-NO$_3^-$ values from phytoplankton cells. The slightly higher riverine δ^{15}N-NO$_3^-$ in December relative to June maybe provided evidence for assimilation fractionation effect. However, negative relationship of δ^{15}N-NO$_3^-$ and NO$_3^-$/Cl$^-$ was not obtained in the Miyun Reservoir (Fig. 7a-b). One mechanism might be related to uptake discrimination among nitrogen species in waters. Phytoplankton uptake of other forms of DIC was strongly suppressed by NH$_4^+$ concentrations above 1.5 μmol L^{-1}.[51] NH$_4^+$ in the Miyun Reservoir is higher than 1.5 μmol L^{-1} and consequently NO$_3^-$ uptake is limited, which could be one of reasons for the scattered trend of δ^{15}N-NO$_3^-$ values and NO$_3^-$/Cl$^-$ ratios.

Mineralization is connected to organic nitrogen and inorganic nitrogen, which plays an important role in N cycle. Although nitrate isotopes could better identify nitrification and denitrification and other N transformations, these two isotopes could not further identify mineralization. Combined use of δ^{13}C$_{DIC}$ and NO$_3^-$/Cl$^-$ was attempted to identify mineralization given the variations of carbon and nitrogen during the mineralization. Assuming that nitrogen-containing organic compounds of mineralization was a major NO$_3^-$ source, δ^{13}C$_{DIC}$ would tend to be more negative with increasing production of NO$_3^-$ because organic matter with lower carbon isotopes was preferentially used. The expected negative relationship between δ^{13}C$_{DIC}$ values and NO$_3^-$/Cl$^-$ ratios was found in this study (Fig. 7c), confirming the above assumption. Similar trend of δ^{13}C$_{DIC}$ vs. NO$_3^-$/Cl$^-$ was found in Lee et al.[52] As indicated from equations (2) ~ (4), the partial pressure of CO$_2$ (pCO$_2$) could provide additional information if mineralization had significant influence on N cycle. The pCO$_2$ was calculated using the WATSPEC software.[53] The computed pCO$_2$ were several times higher than atmospheric equilibrium values as reported in other studies.[31,52] Two major processes increasing the riverine pCO$_2$ level include in situ respiration of organic carbon and influx of soil CO$_2$ through baseflow and interflow.[54] In the studied watershed, the former process could be ignored due to the weak influence of plankton and macrophytes on the riverine organic matter.[17] Hence, influx of soil CO$_2$ could be the major control on pCO$_2$ level. Therefore, the positive relationship of pCO$_2$ vs. NO$_3^-$ concentrations in June (Fig. 7d) suggested that NO$_3^-$ as products of mineralization and nitrification was flushing into rivers via soil erosion in wet season. Note that some values in site B1, B2 and B3 were not included in the regression model because they deviated significantly from the regression lines. The specific values excluded were marked with the corresponding sampling location in the Fig. 7. Previous studies have suggested that the sites B1 and B2, outside of the Beijing City, have different N sources and impacting factors.[17,18] Therefore, the observed scattered values might be related to the different N transformations. In addition to the above regression model applied to identify the nitrate transformation process, recent work using multiple statistical analyses of chemical data revealed that cluster analysis and principal component analysis have potential for distinguishing natural processes and anthropogenic inputs.[55,56]

$\delta^{18}O$-NO_3^- has been successfully used to identify nitrification, since nitrification process usually has $\delta^{18}O$-NO_3^- values in the range of -10 to +10‰.[33] In theory, NH_4^+ is firstly oxidized to NO_2 by using one oxygen atom from ambient O_2 and one oxygen atom from ambient H_2O as electron acceptor, and NO_2 is then oxidized to NO_3^- by using one oxygen atom from ambient H_2O.[57] Accordingly, an estimated $\delta^{18}O$-NO_3^- range of +1.2 to +5.7‰ can be obtained based on the $\delta^{18}O$-O_2 of +23.5‰[39] and local $\delta^{18}O$-H_2O range of surface water of -9.9 to -3.2‰ from Beijing.[21] As shown in Fig. 4, most samples had $\delta^{18}O$-NO_3^- values above the calculated range. Researchers have offered a variety of explanations for these high $\delta^{18}O$-NO_3^- values, such as $\delta^{18}O$-H_2O and O_2 incorporation and $\delta^{18}O$-H_2O fractionation caused by respiration and evaporation.[5,33] As for Beijing, the long-term average annual potential evaporation is higher than the annual rainfall, and evidence has been found to show that enrichment of heavy isotopes of ground water and surface water results from evaporation in Beijing.[21] Hence, evaporation could be the main reason for higher $\delta^{18}O$-NO_3^- values. Similar result was found in Changjiang River, China.[8] For samples with $\delta^{18}O$-NO_3^- values below the expected range, the ratio of oxygen from H_2O and O_2 might be higher than the expected 0.67 during nitrification.[32,58]

In order to further estimate the isotopic fractionation during the nitrate transformations, the Rayleigh distillation equation and typical values of fractionation factors were used. Fig. 6b showed the fractionation of nitrogen isotopes by a series of transport and transformation of nitrogen, modified from Cravotta.[11] $\delta^{15}N$ of soil N was assumed to be +1.8‰, which was the average value of soil organic nitrogen in the studied watershed.[17] Approximately 10% of the soil organic N was mineralized based on the mineralization proportion 7~10% in forested land.[59] The nitrification proportion was calculated from the ratio of NO_3^-/NH_4^+ in river water and fractionation factors were taken from Cravotta.[11] The calculated $\delta^{15}N$-NO_3^- was +7.4‰, close to the average $\delta^{15}N$-NO_3^- value (+7.65‰) in Baihe River in June, suggesting that nitrate in Baihe River could be derived from transport and transformation of soil organic nitrogen. This was in line with the nitrate source deduced from $\delta^{13}C_{DIC}$ and $\delta^{15}N$-NO_3^- values discussed above. However, $\delta^{15}N$-NO_3^- values in September was much higher than the computed $\delta^{15}N$-NO_3^- value, which could be related to high microbial activity and transformation proportion in late summer.

4.3 Contributions of nitrate sources

Contribution of three nitrate sources can be calculated by dual isotope mixing models based on mass balance, which assumed that no major isotopic change occurred during NO_3^- transformations.[7,28]

$$\delta^{15}N_m = f_1 \times \delta^{15}N_1 + f_2 \times \delta^{15}N_2 + f_3 \times \delta^{15}N_3 \qquad (5)$$

$$\delta^{18}O_m = f_1 \times \delta^{18}O_1 + f_2 \times \delta^{18}O_2 + f_3 \times \delta^{18}O_3 \qquad (6)$$

$$1 = f_1 + f_2 + f_3 \qquad (7)$$

where $\delta^{15}N_m$ and $\delta^{18}O_m$ are the NO_3^- isotopes values measured in the study, and f_1, f_2 and f_3 represent the proportional contribution of the three NO_3^- sources, which include soil N, precipitation and sewage and livestock wastes. Determination of the different end-members is as follows. (1) soil N: the $\delta^{15}N$ of soil organic matter was assumed to be +7.4‰, which was the calculated value of soil N based on nitrogen-isotopic fractionation during mineralization and nitrification (Fig. 6b). (2) Precipitation: the $\delta^{15}N$ for precipitation was determined to be +1.39‰ which was the average $\delta^{15}N$ values of precipitation in the suburb of Beijing, China.[60] (3) sewage and livestock waste: the highest $\delta^{15}N$ value of +13.61‰ in the study area was used. The $\delta^{18}O$ of precipitation was determined to be +70.4‰, which was the average $\delta^{18}O$ value of rain water and snow water sampled in the studied basin. The $\delta^{18}O$ for soil N and manure was the theoretical value of nitrification process (+1.2‰) in this study. Given the high microbial activity and isotopic fractionation of nitrogen in late summer, nitrate isotopes in June and December were used in this model. For sites with $\delta^{18}O$-NO_3^- values below the lower limit of the calculated range of nitrification process, these sites was not included in the mixing model.

The ranges of contributions of each NO_3^- source are shown in boxplots (Fig. 9). In summer, the contribution of soil N was the highest, followed by sewage and livestock effluents and atmospheric nitrogen. In winter, livestock effluents contributed the most, atmospheric nitrogen contributed the least and soil N was intermediate. The contributions of NO_3^- source in wet season exhibited more scatter than winter, reflecting more mixing effects and biological activities impacted NO_3^- due to the high rainfall and temperatures. Compared to rivers, an increase of atmospheric NO_3^- contribution occurred in reservoir, which resulted in dilute effect of varying NO_3^- sources. The atmospheric contribution proportion agreed with the reported range of riverine NO_3^- from storm events (<33%) and snowmelt water.[6,61] The contributions of livestock waste and soil N in reservoir were between those of the upstream rivers such as Chaohe River and Baihe River, which verified the significant N input from the upper rivers to the reservoir.

5 Conclusions

The multi-isotopic method (^{15}N, ^{18}O and ^{13}C) together with chemical composition of water is used to elucidate sources and fate of nitrate in the up-stream Chaobai River for the first time. The results of the present study showed that the concentrations of NO_3^- varied significantly between reservoir and river, with much lower levels of NO_3^- concentrations in reservoir than in river. The isotopic data indicated that NO_3^- was derived mainly from soil N, sewage and livestock waste and atmospheric deposition, except for two tributaries which had NO_3^- derived from chemical fertilizer. Carbonic isotope of DIC can be used to identify nitrate sources in wet season when using $\delta^{15}N$-NO_3^- was difficult to distinguish soil N and animal waste. That said, for sites with $\delta^{15}N$-NO_3^- ranging from +8 to +10‰, lower $\delta^{13}C_{DIC}$ values (<-

8.6‰) indicated the nitrate source from soil N while the sites with elevated $\delta^{13}C_{DIC}$ values (>-8.6‰) was mainly affected by animal waste. However, $\delta^{13}C_{DIC}$ in dry season tended to reflect the mixture of endmember or transformation processes, not the composition of nitrogen sources. The results of mixing-model indicated that NO_3^- was mainly related to soil N and nitrification in wet season, while sewage and livestock waste contributed the most in dry season. On the other hand, there was an increase in atmospheric N contribution in the reservoir relative to the upper river and nitrate sources from the rivers exerted important influence over the reservoir. The dual isotopes of NO_3^- and carbon isotope of DIC revealed that mineralization and nitrification played an important role in N biogeochemical process in wet season. Specifically, the negative relationship of $\delta^{13}C_{DIC}$ values vs. NO_3^-/Cl^- and positive relationship of NO_3^- concentrations vs. pCO_2 indicated that mineralization was a major control on nitrate transformation in wet season and nitrate as products of the mineralization and nitrification was flushing into river through soil and water erosion. In addition, assimilation process and water evaporation affected the nitrate isotopes. However, the nitrate isotopes and carbon isotope as well as water chemistry suggested that denitrification was absent or weak in the watershed.

This study shows that the combination of nitrate isotopes and $\delta^{13}C_{DIC}$ could provide additional information for the identification of sources and transformations of nitrate in watersheds with dominantly forested land in wet season. It is useful to improve the approach of nitrate identification and further access nitrate pollution. Additionally, the source and fate of nitrate indicate that much attention should be paid in the upper rivers with expanding livestock breeding, and suitable soil conservation measures should be adopted in this watershed.

Acknowledgements

Sincere thanks to Huang Xingxing and Liang Xiatian, who helped to collect water samples in the studied basin. This work was financially supported by the National Science Foundation of China Funded Project (41173113) and the program of "One Hundred Talented People" of Chinese Academy of Science.

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Figure captions

Fig. 1 Location of sampling sites in the upper stream of Chaobai River, Beijing. T1, T2 and T3 are sampling sites in tributaries of Baihe River.

Fig. 2 Temporal and spatial variations of TDS, NO$_3^-$, DOC, δ^{15}N-NO$_3^-$, δ^{18}O-NO$_3^-$ and δ^{13}C$_{DIC}$ in river water and reservoir water sampled in September, December and June on the Miyun Reservoir watershed. The dash line in Fig. 2a is the average value of TDS (500 mg L^{-1}) from the most polluted rivers among the world's 60 largest rivers.[27] The dash line in Fig. 2b is the human affected values of NO$_3^-$ (0.21 mmol L-1).[28]

Fig. 3 The histogram of δ^{15}N-NO$_3^-$ and δ^{13}C$_{DIC}$ in sampling waters.

Fig. 4 Composition of dual isotopes of nitrate from different sources and values measured in the studied watershed. Typical ranges of isotopic composition for nitrate sources are adopted from Kendall et al.[33]and Xue et al.[3].

Fig. 5 (a) Variations of NO$_3^-$/Cl$^-$ molar ratios with Cl$^-$ molar concentrations in the upper stream Chaobai River; (b) δ^{15}N vs. N yield of animal waste in 2012 in the watershed.

Fig. 6 (a) DIC sources and potential variations of δ^{13}C$_{DIC}$ in aqueous system modified from Jiang.42 (b) Isotopic fractionation of nitrogen during mineralization, volatilization and nitrification modified from Cravotta.[11]

Fig. 7 (a-b) Relationship between NO$_3^-$/Cl$^-$ molar ratios and δ^{15}N-NO$_3^-$. (c) Relationship between NO$_3^-$/Cl$^-$ molar ratios and δ^{13}C$_{DIC}$. (d) Variations of NO$_3^-$ concentrations with pCO$_2$ in June.

Fig. 8 Variations of δ^{13}C$_{DIC}$ relative to HCO$_3^-$ concentrations. The dashed line was the upper limit of resulting δ^{13}C$_{DIC}$ values (-15.6~-8.6‰) from C3 plants

Fig. 9 Seasonal and spatial proportional contribution of three major NO$_3^-$ sources for the studied watershed estimated by mixing model. Boxplots illustrate the 25th, 50th, and 75th percentiles; the whiskers indicate the 5th and 95th percentiles; the squares indicate the mean values.

Table 1 Mean and standard deviation (SD) of chemical compositions and isotopes (^{15}N, ^{18}O and ^{13}C) in the upper stream of Chaobai River[a]

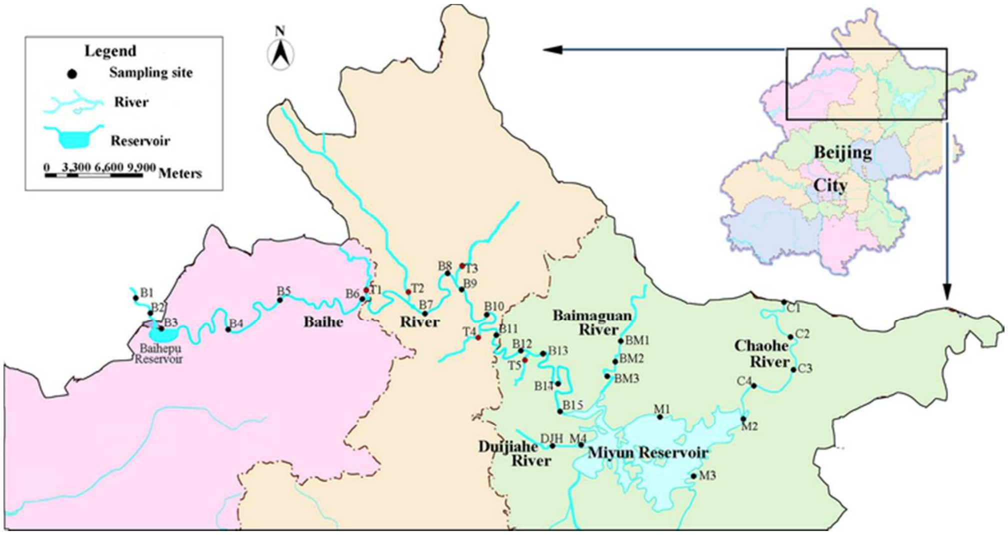
Sample	Mean/ SD	pH	HCO_3^-	Cl^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}	$\delta^{15}N$	$\delta^{18}O$	$\delta^{13}C$	TDS
June	mean	8.11	3.10	0.45	0.25	0.55	0.59	0.08	0.63	1.44	7.86	8.89	-9.07	362
	SD	0.22	0.85	0.16	0.17	0.20	0.16	0.03	0.17	0.32	2.99	5.42	2.20	73
September	mean	7.88	3.45	0.47	0.24	0.58	0.60	0.08	0.63	1.37	9.88	6.98	-	384
	SD	0.20	1.02	0.17	0.17	0.26	0.14	0.04	0.15	0.31	3.45	4.05	-	91
November	mean	7.83	3.94	0.47	0.30	0.58	0.59	0.07	0.76	1.52	8.83	6.32	-6.29	427
	SD	0.39	1.17	0.18	0.20	0.28	0.18	0.05	0.21	0.38	3.03	7.17	1.23	106
Main stream of Baihe River	mean	7.96	3.88	0.40	0.19	0.44	0.59	0.06	0.66	1.47	8.85	4.20	-5.35	396
	SD	0.25	0.77	0.07	0.09	0.11	0.08	0.02	0.14	0.16	1.91	4.57	4.44	50
Chaohe River	mean	7.98	3.90	0.77	0.42	0.85	0.84	0.08	0.74	1.85	12.04	6.98	-4.66	486
	SD	0.20	0.52	0.05	0.09	0.06	0.04	0.01	0.06	0.15	0.98	1.66	3.74	35
Baimaguan River	mean	7.97	4.08	0.64	0.49	1.10	0.60	0.19	1.00	1.88	9.93	11.09	-4.97	529
	SD	0.22	1.06	0.08	0.08	0.31	0.11	0.06	0.17	0.15	2.72	2.59	5.04	93
Reservoirs	mean	8.05	3.03	0.41	0.10	0.46	0.57	0.07	0.66	1.06	9.22	12.74	-3.60	323
	SD	0.34	0.49	0.07	0.07	0.13	0.11	0.02	0.12	0.17	2.16	5.68	2.50	44
Tributaries	mean	7.85	2.87	0.40	0.28	0.54	0.49	0.07	0.59	1.32	7.11	7.38	-6.38	338
	SD	0.34	1.21	0.17	0.15	0.14	0.19	0.02	0.18	0.32	4.29	6.01	4.04	89

[a] ions and DOC (mmol L^{-1}), TDS (mg L^{-1}), isotopes (‰). –, not determined.

Table 2 The N yield of animal waste in each village or town[a] and average nitrogen isotopes of nitrate and the related nitrogen isotope of nitrate

Name of village/ town	Sampling sites	Area /Km^2	Average $\delta^{15}N$ values	N yield (liquid waste and solid waste)/t					N yield /t km^{-2}
				Pig	Beef	Sheep	Broiler	Total/	
Gubeikou	C1, C2	83.1	11.99	7.96	1.01	0	0.50	9.48	1.01
Gaoling	C3, C4	105	12.09	6.86	0.87	0	78.21	85.94	2.82
Fengjiayu	BM1-BM3	214.25	9.93	48.55	26.49	5.03	0	80.07	0.64
Bulaotun	M1	193.2	7.81	6.93	1.56	7.73	8.66	24.88	0.51
Taishitun	M2, M3	202	11.11	22.93	9.17	0	153.40	185.50	1.57
Shicheng	B12-B15, T5, M4	252.8	6.07	14.87	0	0	0	14.87	0.10
Liulimiao	B11, T4	226.5	9.35	5.62	4.20	9.94	118.19	137.96	0.04
Tanghekou	B8-B10, T3	224.8	9.76	22.32	17.43	25.38	32.89	98.02	0.38
Baoshan	B7, T2	248.6	9.89	67.28	44.68	52.17	153.75	317.88	0.75
Xiangying	B3	117.00	9.69	109.63	73.63	56.06	0.01	239.33	0.68
Qianjiadian	B4-B6, T1	363.49	9.23	56.36	9.57	16.75	0	82.67	0.04

[a] Related statistical data can be found in local statistical yearbook,[24,36,37] and the nitrogen concentration of liquid waste and solid waste in Beijing suburb is taken from the study by Jia et al.[38]



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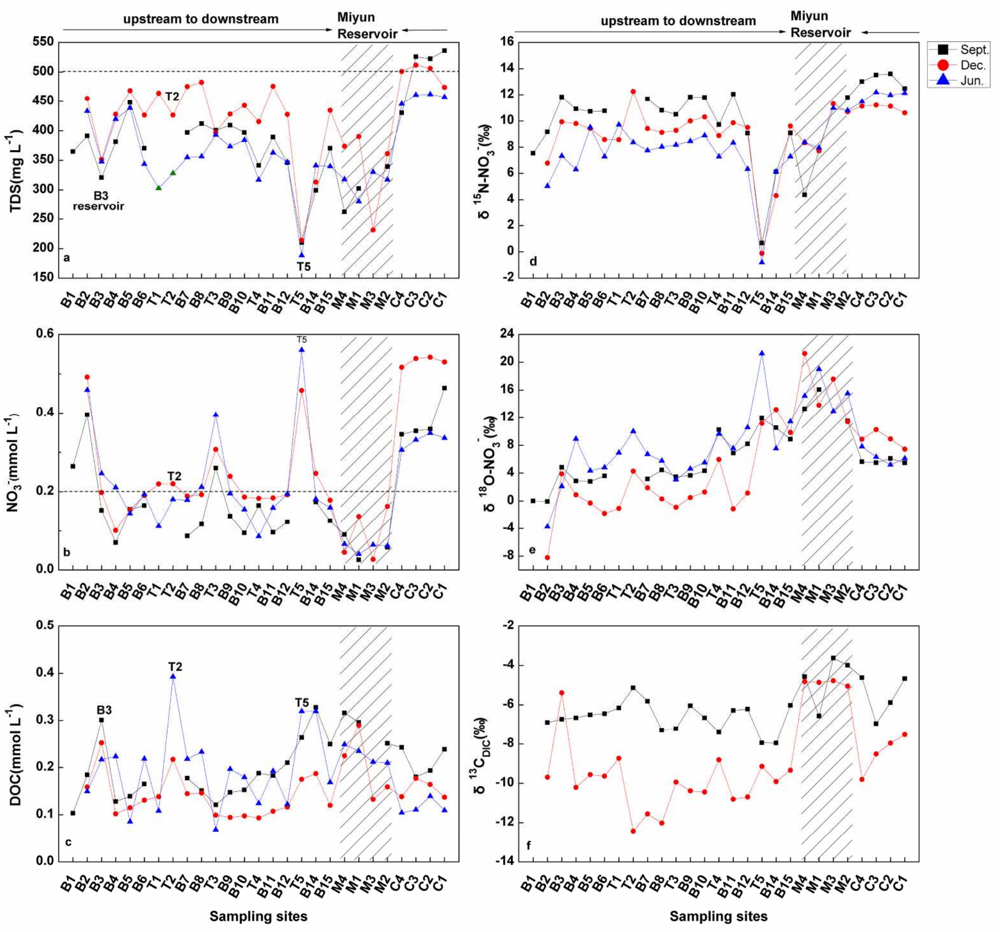

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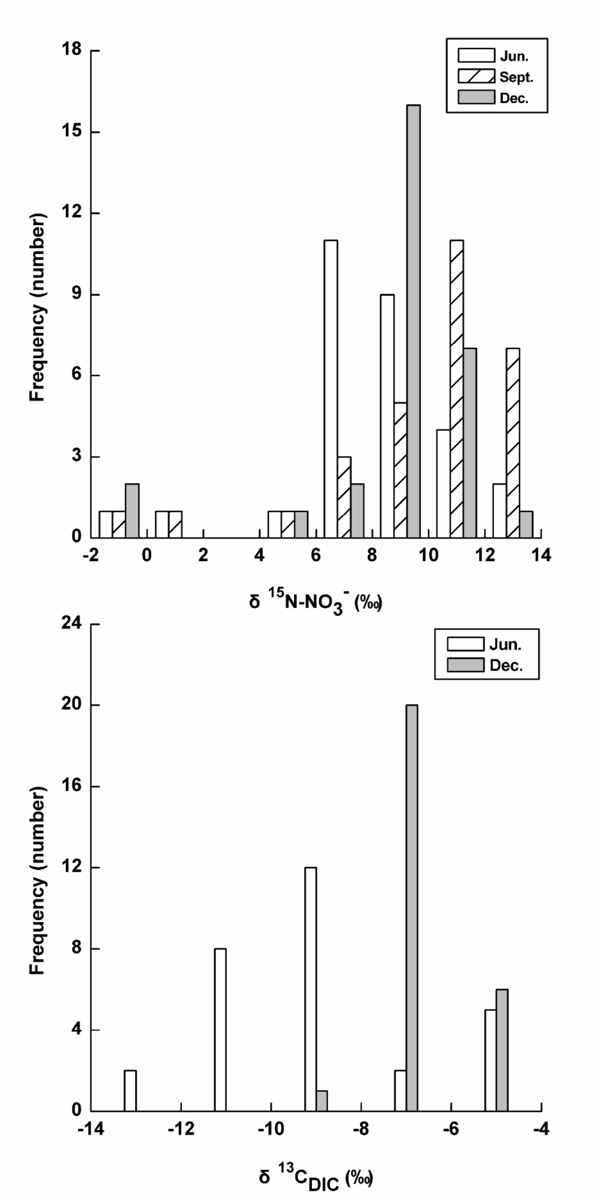

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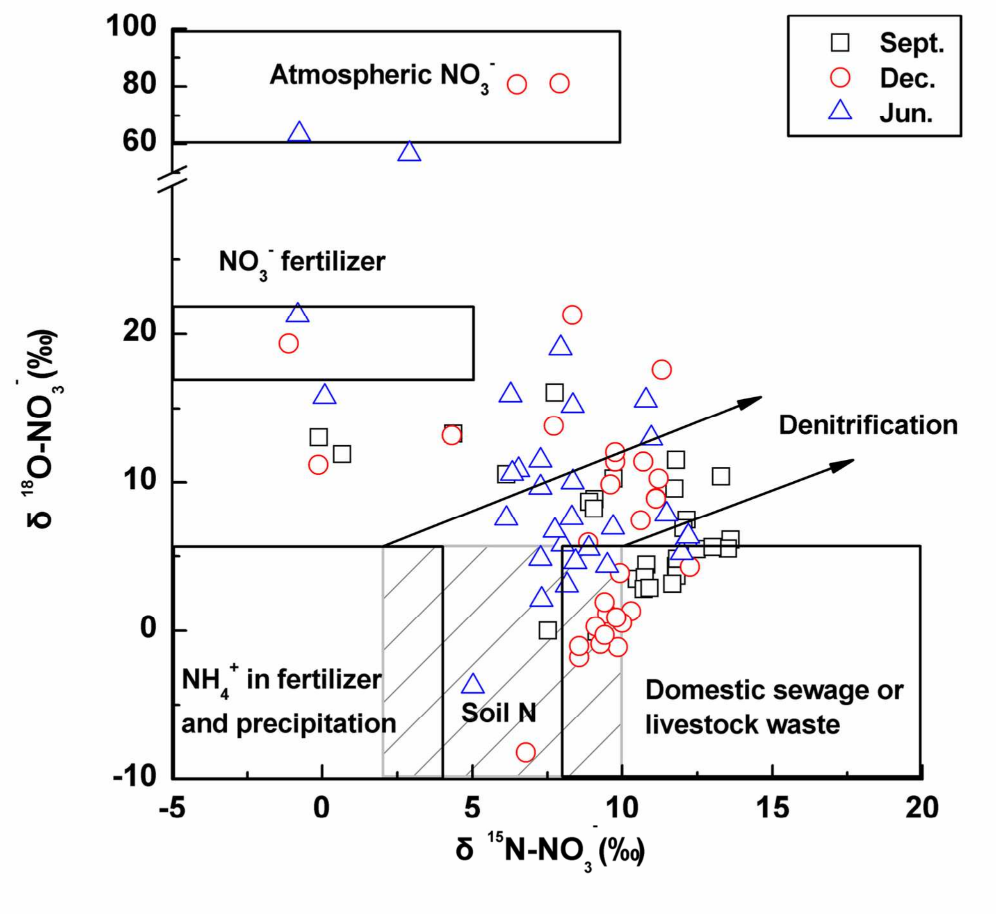

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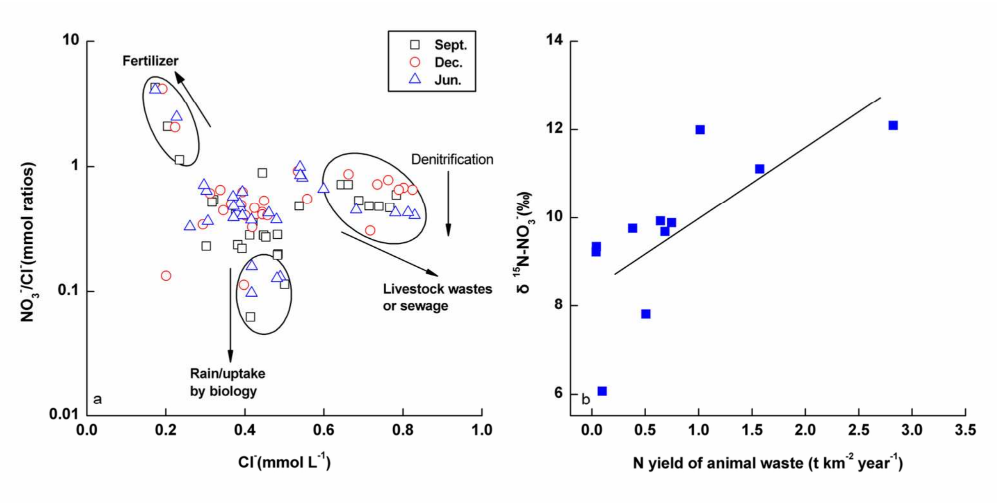

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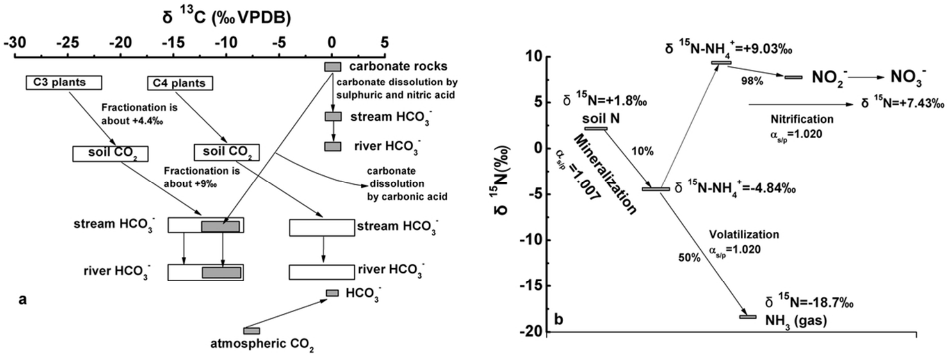

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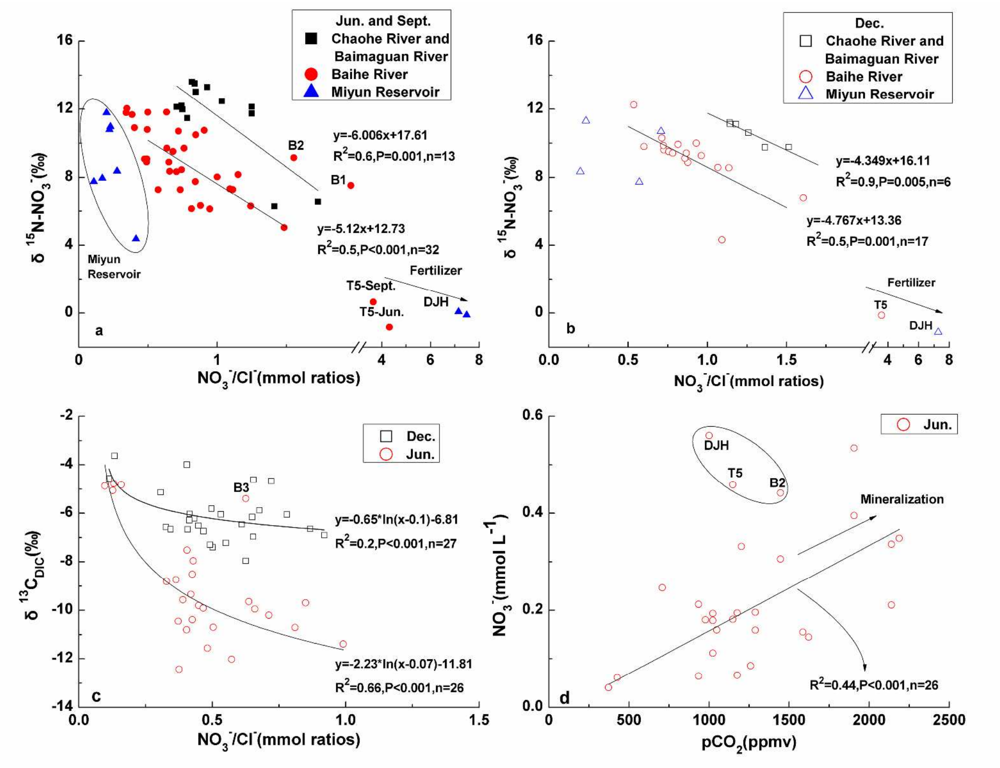

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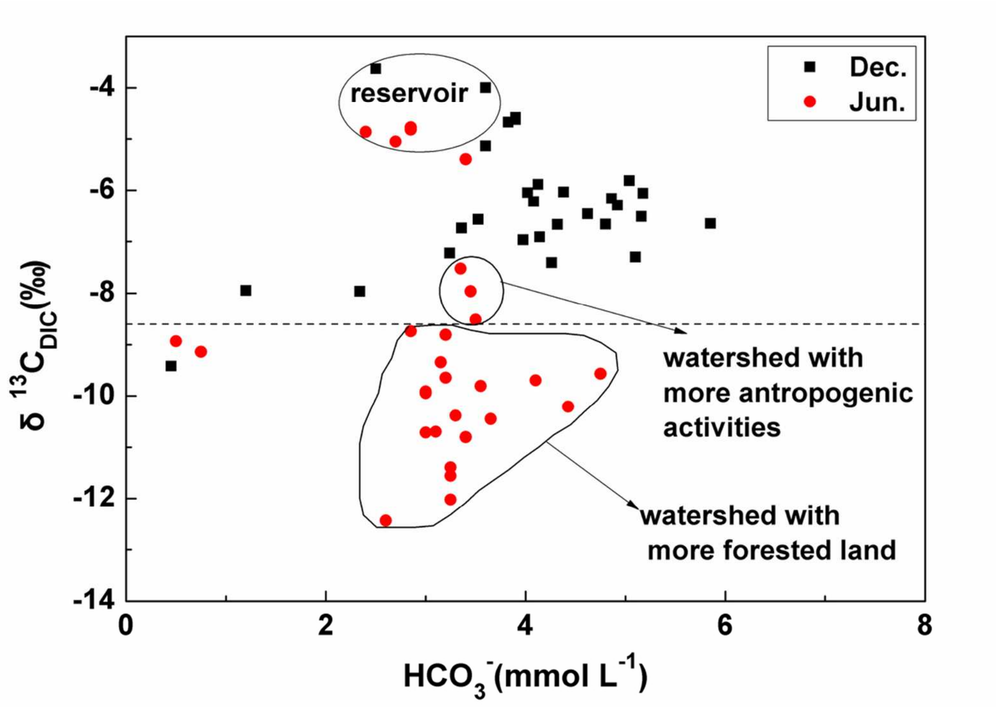

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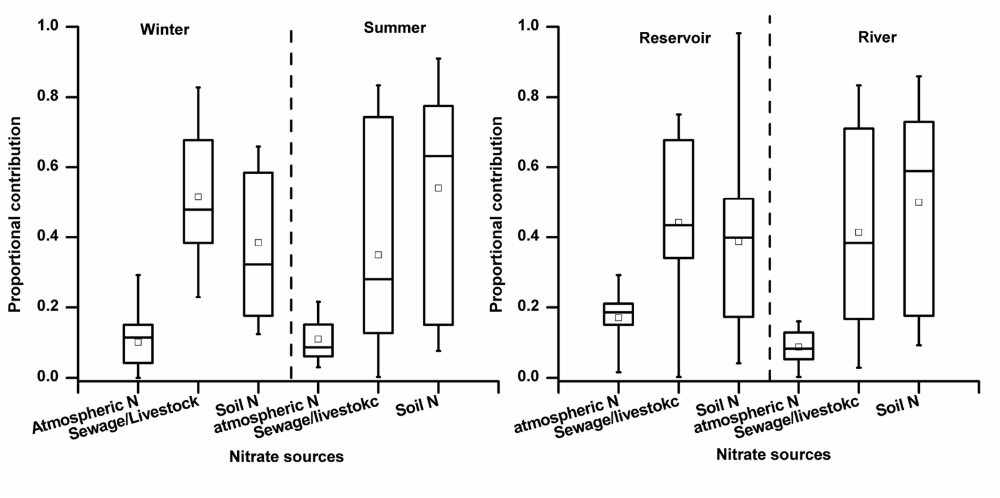

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