RESEARCH ARTICLE

Sources and transformations of anthropogenic nitrogen in the highly disturbed Huai River Basin, Eastern China

Pei Ma[1] • Shuaixia Liu[1] • Qibiao Yu[2] • Xinyan Li[3] • Xinqing Han[4]

Abstract

Due to serious nitrogen pollution in the Huai River, Eastern China, nitrogenous concentrations and dual stable isotopes (δ^{15}N and δ^{18}O) were measured to ascertain the sources and transformation of nitrogen in the Shaying River, the largest and most polluted tributary of the Huai River during the summer and winter seasons. Total nitrogen (TN), NO_3^-, and NH_4^+ were significantly higher in winter, with values of 7.84 ± 3.44 mg L^{-1}, 2.31 ± 0.81 mg L^{-1}, and 3.00 ± 2.24 mg L^{-1}, respectively, while the highest nitrogen compounds occurred in the Jialu River, one of the tributaries of the Shaying River, in both summer and winter. Isotope characteristics of nitrate reveal that manure and sewage were the principal nitrate sources in both summer ($62.44 \pm 19.66\%$) and winter ($67.33 \pm 15.45\%$), followed by soil organic nitrogen, with $24.94 \pm 15.52\%$ in summer and $26.33 \pm 9.45\%$ in winter. Values of δ^{15}N-suspended particulate nitrogen (SPN) ranged from 0.78 to 13.51‰, revealing that point source from industrial and domestic sewage accounted for the largest input to SPN at most sites, whereas soil organic nitrogen and agricultural fertilizers were found in the Jialu River in both sampling periods. Point sources from septic/manure and household waste were the main contributors to ammonium in most river water samples in both summer and winter; most wastewater discharged into the river was untreated, which was one of the main reasons for the high level of ammonium in winter. Nitrogen pollution and the dams had an effect on N transformation in the river. Significant assimilation of NH_4^+ and aerobic denitrification competed for NH_4^+, resulting in the weakness of nitrification in the summer. Denitrification was also an important process of nitrate removal during the summer, whereas nitrification was a key N transformation process in the river in the winter time. To reduce nitrogen pollution and improve water quality, greater effort should be focused on the management of sources from urban input as well as on the improvement in sewage treatment.

Keywords Isotopic composition · Pollution source identification · Nitrate · Ammonium · Huai River

✉ Pei Ma
map55912@yahoo.com

1 Henan University of Engineering, No. 1 Xianghe Road, Zhengzhou 451191, Henan, China

2 Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

3 Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

4 Zhoukou hydrology and Water Resources Survey Bureau, Zhoukou 466000, Henan, China

Introduction

Rapid urbanization has caused a wide range of environmental problems, including increase in riverine nitrogen (N) pollution (Pernet-Oudrier et al. 2012; Zhang et al. 2015a). To develop effective strategies for the mitigation of nitrogen pollution and to achieve the sustainability of water resources, it is vitally important to determine the nitrogen sources and their transformation processes in the rivers. In the past several decades, strong efforts have been made to identify nitrogen pollution sources entering into rivers (Mayer et al. 2001; Anisfeld et al. 2007; Kaushal et al. 2011). Indeed, there are many methods for identifying nitrogen sources from a watershed to the receiving waters. In general, point source pollution, including domestic sewage, industry discharge, and livestock manure, can be determined through detailed statistics, whereas the nonpoint source, including

agricultural fertilizer, soil erosion, and atmospheric precipitation, can be estimated through the use of watershed models (Strokal et al. 2015; Álvarez-Cabria et al. 2016). Despite great attempts to identify nitrogen sources, N source identification remains challenging due to its complexity in many river basins worldwide (Lai et al. 2006; Zhang et al. 2010).

Isotope composition of nitrogen compounds is to be a promising important tool for identifying nitrogen sources and the nitrogen transformation process in aquatic systems(Ding et al. 2014; Yue et al. 2014; Xing and Liu 2016; Yuan et al. 2017; Du et al. 2017; Yi et al. 2017; Bu et al. 2017a). In general, riverine nitrogen can be classified into dissolved and particulate species (Yan 2006). Dissolved inorganic nitrogen (DIN) includes NO_3^-, NH_4^+, and NO_2^-. The contents of NO_2^- are often minimal in the surface water, and NO_3^- is reported to be the dominant form of DIN in many aquatic environments (Bu et al. 2011). NH_4^+ is another important DIN, in fact, it is the dominant form of DIN in some surface waters, such as the Huai River (Zhang et al. 2017). Particulate N is also an important form of N in rivers and can provide significant information about the N cycle (Middelburg and Nieuwenhuize 1998). Dual isotopes in nitrate provide unique information about NO_3^- sources and their transformation from atmospheric precipitation, soils, chemical fertilizers, as well as from sewage and manure in aquatic systems (Panno et al. 2001; Amundson et al. 2003; Widory et al. 2005; Sebilo et al. 2006; Oelmann et al. 2007; Xue et al. 2012). Similar to nitrates, $\delta^{15}N-NH_4^+$ and suspended particulate matter also varies in isotopic signatures depending on its different sources (Du et al. 2017; Nikolenko et al. 2018; Cifuentes et al. 1988; Goering et al. 1990; Kreitler and Browning 1983; Wassenaar 1995). Several studies have been conducted to assess the riverine N source in NH_4^+ and the particulate matter in rivers by focusing on $\delta^{15}N$ in NH_4^+ and SPN (Norrman et al. 2015; Nikolenko et al. 2018; Kendall et al. 2001; Gao et al. 2014). Of course, there are still many uncertainties when using isotope to partition the sources. For instance, the isotope signature is not clear for every sources, or the isotope signature is overlapped for some of sources. Besides, isotope fractionation for some N cycling processes is not clear yet.

The Huai River, the third longest river in China, is one of the most polluted rivers in China, especially in terms of nitrogen pollution (Yang et al. 2016; Zhang et al. 2017). Since the 1990s, the Chinese government has invested greatly in effort to reverse the quality of deteriorated water in the basin. However, despite the enormous financial investment, the water pollution remains a serious problem. Besides being one of the world's most polluted watersheds (Bai and Shi 2006), a large number of dams have been built on the Huai River, causing a low flow rate in many parts of the river (Shi et al. 2016). The long residence time and high nutrient concentrations could facilitate bacterial uptake in the river system (Balls et al. 1996; Brion et al. 2000), which might cause changes in the N transformation process in the river systems. Previous studies on the Huai River focus mainly on

nutrient concentrations, the effect of dams on water quality, and metal contamination and anthropogenic contributions to N loads in the river watershed (Zhai et al. 2014, 2017; Zuo et al. 2015; Shi et al. 2016; Xia et al. 2018; Ni et al. 2011; Zhang et al. 2015b, 2017; Yang et al. 2016, 2018). However, there is little study about source identification of N and the effects of dams and pollution on N transformation in the Huai River. Therefore, it is of critical importance to identify N sources and N transformation, and then provide theoretical study in order to develop effective N pollution control strategies.

The Shaying River is the largest and the most polluted tributary of the Huai River Basin, and the amounts of its COD and NH_3^+ discharges are more than one-third of the amounts in the main stream of Huai River (Duo et al. 2015). More than 60 dams over the Shaying River, together with rapid urbanization and economic growth, have caused the river basin to become highly disturbed (Duo et al. 2015; Zhang et al. 2017). By combining analysis of water quality and N isotopic composition, the main objectives of this study are to (1) identify the sources of NO_3^-, NH_4^+, and SPN, and (2) to reveal the N transformation processes under the influence of dams and nitrogen pollution during different seasons in the Shaying River watershed.

Materials and methods

Study area

The Shaying River, originated in Xinmi County, Henan Province, flows through Zhengzhou city, Zhoukou city, and Fuyang city, then down into the Huai River mainstream near Mohekou in Anhui Province (Fig. 1). The main river is composed of the Jialu River, the Ying River, and the Sha River, which converge at Zhoukou city. The length of the river is 620 km and its basin area is 40,000 km^2. The upperstream is from the source to the Zhoukou hydrological station (M-a), while the midstream is from M-a to Fuyang hydrological station (M-c). The river basin is mostly agricultural land, which accounts for 76.24% of its land coverage (Zuo et al. 2016). It is located in the typical East Asian monsoonal climate with four distinct seasons. Annual mean temperature is from 14 to 16 °C and the average annual rainfall is 770 mm in the basin (Zhang et al. 2013). Under the significant influence of monsoon, its precipitation is usually concentrated in the summer from June to August. Figure 2a demonstrates monthly variations of temperature, and precipitation at M-a in the Shaying River during 1960–2015.

The Shaying River has experienced rapid urbanization and industrialization in the last decades, resulting in a massive discharge of wastewater and declining surface water quality. The concentration of NH_4^+ was greater than 2.0 mg L^{-1} in most months of the winter in Fig. 2b, failing to meet the Chinese Surface Water Quality Standards (GB3838-2002), and sometimes even three times greater than the standard value.
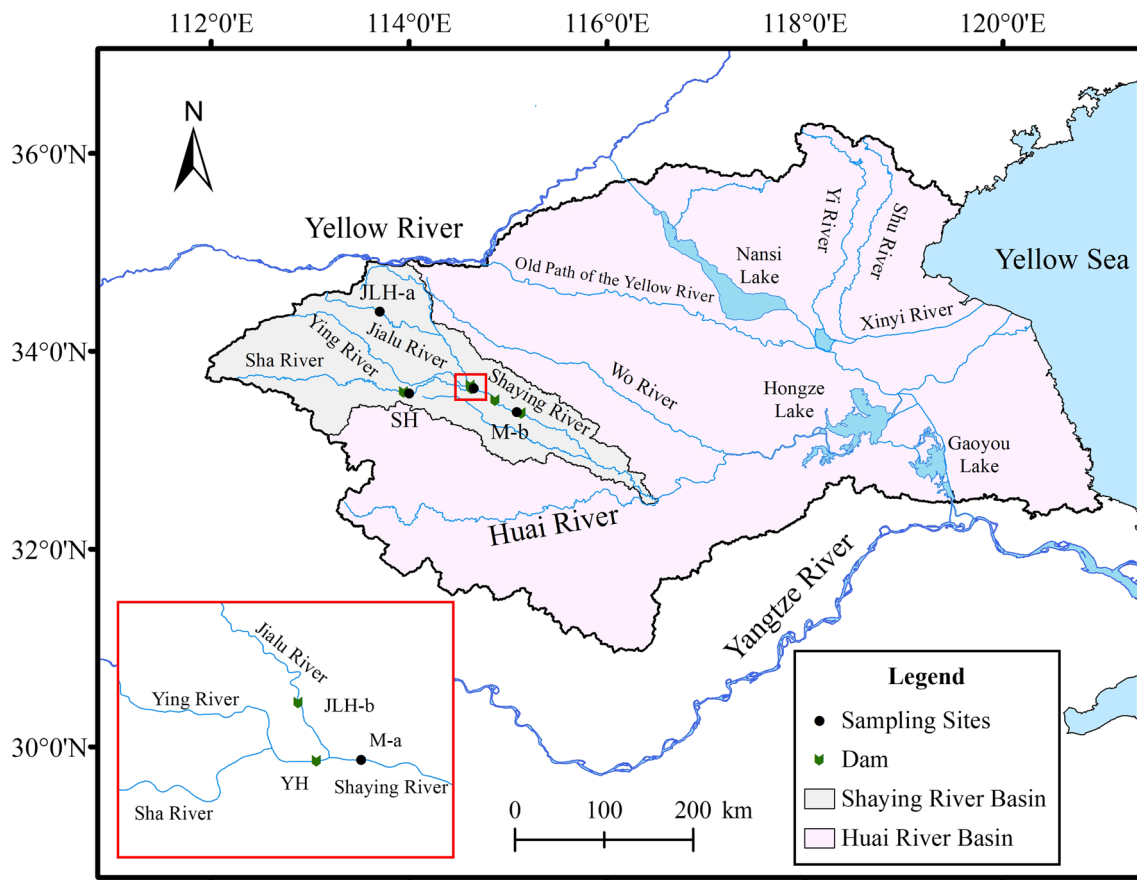
Fig. 1 The Shaying River Basin and locations of sampling sites, YH and JLH-b were located at the front of dams

Field sampling

Water samples at a depth of 20–30 cm were collected from the main stream of the Shaying River and its major tributaries (the Yinghe, the Shahe, and the Jialuhe rivers), as shown in Fig. 1. JLH-b and YH were at the front of dams, and all the dams located before and after the sampling sites are marked in Fig. 1. Samplings were conducted once a month in the summer and the winter. Table 1 presents the research contents and sampling time at each of the sampling sites.

The water samples were filtered with 0.45-μm membrane filters (MF-Millipore, USA) in the field. Part of the filtered water was kept refrigerated and frozen for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ analysis at the laboratory, while the remainder of the filtered water was treated with hydrochloric acid to pH 5–6 and then kept refrigerated and frozen for $\delta^{15}N\text{-}NH_4^+$ analysis. Large water samples (5 L) were filtered through precombusted and tared GF/F filters for $\delta^{15}N$ of suspended particulate nitrogen ($\delta^{15}N\text{-}SPN$). Filters were frozen in the field, and then freeze-dried in the laboratory.

One-liter water samples were placed in polyethylene plastic bottles and acidified to pH < 2 by sulfate in situ and then stored below 4 °C for laboratory analysis of NH_4^+, NO_3^-, NO_2^-, and total nitrogen (TN). Water samples for dissolved

N_2 and Ar analysis were given a small volume of saturated $HgCl_2$ solution (the final concentration was approximately 3%, v/v) to stop biological activity, sealed in 60 ml digests, then stored to avoid light.

Water samples at the depth of 20–30 cm and surface sediment samples of 1 cm thickness were collected at sites JLH-a, JLH-b, SH, YH, M-a, and M-b in June and November 2015 for the analysis of microbial flora involved in metabolism of inorganic nitrogen compounds. The samples were refrigerated until analysis. Water samples for Chl-a analysis were filtered with 0.45-μm pore cellulose filters and then stored frozen in the dark until analysis.

Sample analysis

The pH values were measured in situ using a portable meter (pHB-4). Dissolved oxygen (DO) was determined according to the iodometric method (GB/T 7489-87) in the field. In the laboratory, NH_4^+ was measured by Nessler's reagent spectrophotometry method (HJ535-2009); NO_3^- was quantified according to UV spectrophotometry standard (HJ/346-2007); and NO_2^- was measured according to naphthyl ethylenediamine spectrophotometry standard (GB17378.4-1988). Detection limits were 0.025 mg L^{-1}, 0.02 mg L^{-1},

Fig. 2 The mean monthly variations of temperature, and precipitation at Zhoukou hydrological station (M-a) during 1960–2015 (**a**); Monthly variations of NH$_4^+$ concentrations at M-a during 2010–2015 (**b**)
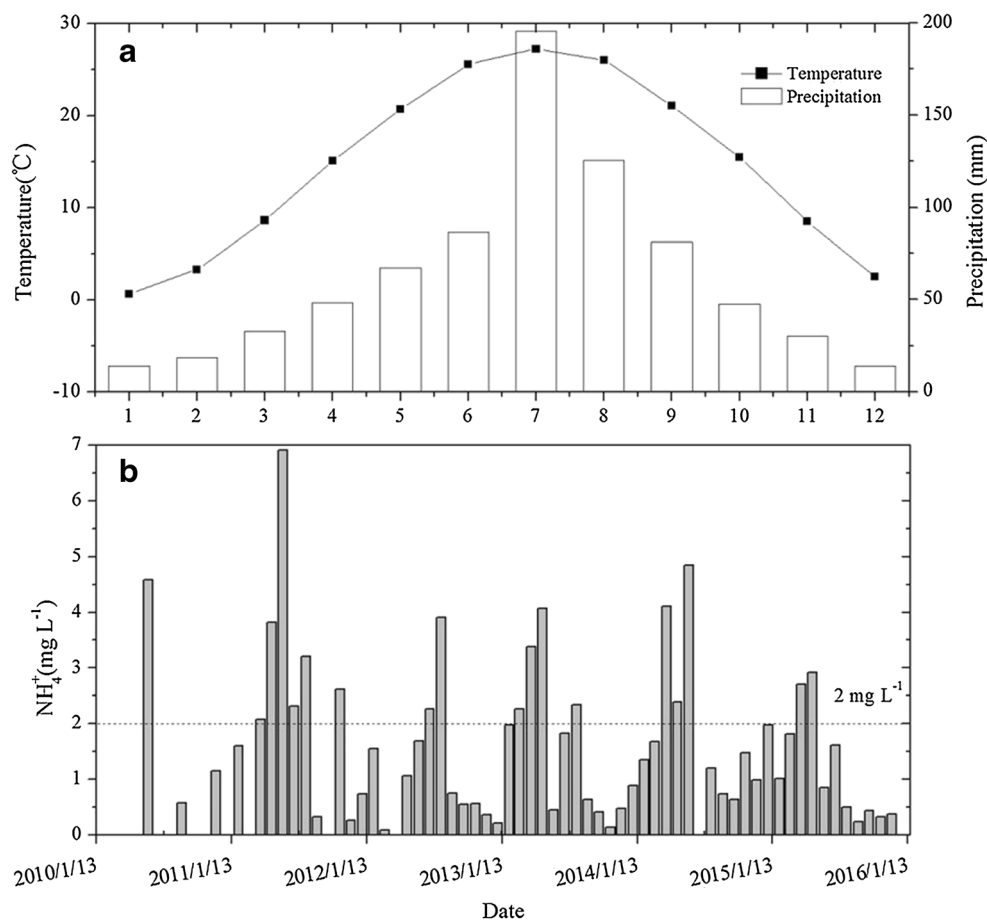
and 0.003 mg L^{-1} for NH$_4^+$, NO$_3^-$, and NO$_2^-$, in respectively. The concentrations of Cl$^-$ in water samples were determined with ionic chromatography method using a Dionex ICS-2100 (USA). Microbial flora involved in metabolism of inorganic nitrogen compounds in waters and sediment were estimated by most probable number (MPN) procedures (Rowe et al. 1977; Papen and Berg 1998; Jiao et al. 2009) after arrival at the laboratory.

To determine the N and O isotopic compositions of NO$_3^-$ in water samples, a denitrifier method was used (Casciotti et al. 2002). First, nitrate was quantitatively converted to N$_2$O by *pseudomonas aureofaciens*, which lacks the active N$_2$O-reductase enzyme. Then, N$_2$O gas was purified and analyzed by isotope ratio mass spectrometry (IRMS, Isoprime 100, Isoprime, Cheadle, UK), with precision of 0.25%. For δ^{15}N-NH$_4^+$ pre-treatment, the NH$_4^+$ diffusion method described by Holmes et al. (1998) was used. An acid trap (KHSO$_4$) was used to absorb the NH$_3$ in a closed system for 1 week, after which the trap was freeze-dried and EA-MS (an Elemental Analyzer coupled to a Mass Spectrometer; Fry et al. 1996) was used to determine the isotopic compositions with a precision of 0.25% for δ^{15}N values.

The δ^{15}N in SPN was determined by a Finnigan MAT 252 gas isotope mass spectrometer coupled to an elemental analyzer. Pure tank N$_2$ calibrated against the reference standards for the International Atomic Energy Agency (IAEA)-N-1 and IAEA-N-2 was used as a working standard. δ^{15}N-SPN has a precision of 0.25%. δ^{18}O-H$_2$O was analyzed in a LGR isotopic Water Analyzer (IWA-45EP, Los Gatos Research, Inc., CA, USA). δ^{18}O-H$_2$O has a precision of 0.25%.

The isotopic compositions are expressed as:

$$\partial(\%) = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) \times 100 \tag{1}$$

Water R = ^{15}N/^{14}N or ^{18}O/^{16}O. The ratios of ^{15}N/^{14}N and ^{18}O/^{16}O are expressed relative to air for N and to Vienna standard mean ocean water (V-SMOW) for O.

Dissolved N$_2$ in river water was determined by the use of a Membrane Inlet Mass Spectrometry (MIMS) system (Bay Instruments, Easton, MD, USA). For details on the analysis and calculation of dissolved Ar and N$_2$ concentrations, see the description of the open-channel method in McCutchan et al. (2003) and Baulch et al. (2010). The net production of N$_2$ (△N$_2$) was then calculated as: △N$_2$ = [N$_2$]$_{water}$-[N$_2$]$_{equilibrium}$.

Table 1 Experimental manipulations of the study in 2015 and 2016

Sampling location	Sampling time	Analyzed items[a]
JLH-a (113° 46′48″E, 34° 20′20″ N) JLH-b (114° 38′24″E, 33° 37′48″ N) SH (114° 01′12″E, 33° 34′48″ N) YH (114° 36′36″E, 33° 37′45″ N) M-a (114° 39′36″E, 33° 37′33″ N) M-b (115° 5′24″E, 33° 23′9″ N)	Summer: July 23 and 24, August 25 and 26 in 2015; August 29 and 30 in 2016; Winter: November 26 and 27, December 29 and 30 in 2015, December 28 and 29 in 2016	NO_3^-, NO_2^-, NH_4^+, TN, Cl^-, $[N_2]$, Chl.a, pH, microbial flora involved in metabolism of inorganic nitrogen compounds, $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N\text{-}NH_4^+$, $\delta^{18}O\text{-}H_2O$, $\delta^{15}N\text{-}SPN$
M-c (116° 16′48″E, 32° 39′00″ N)	Summer: August 24, 2015 Winter: December 28 in 2015	NO_3^-, NH_4^+, TN, $[N_2]$, Chl.a, $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N\text{-}NH_4^+$, $\delta^{18}O\text{-}H_2O$
HR' (117° 23′24″E, 32° 57′36″ N)	Summer: August 24, 2015 Winter: December 28, 2015	

a. $[N_2]$, dissolved N_2 concentration (mg/L); NO_2^-, $\delta^{15}N\text{-}SPN$ and $\delta^{15}N\text{-}NH_4^+$ were only analyzed in 2016; The analysis of microbial flora involved in metabolism of inorganic nitrogen compounds was only carried out in June and November in 2015

Data for NH_4^+ content during 2010–2015 at site M-a in Fig. 2 were obtained from the Zhoukou hydrological station, whereas data for discharges of pollution and ammonium from the upperstream watershed to the Shaying River were acquired from the Huai River Water Resources Commission. Data for temperature, and precipitation at M-a were obtained from http://www.cma.gov.cn/2011qxfw/2011qsjcx/.

Estimation of nitrate source contributions

The contributions of NO_3^- sources to a mixture were quantified through the Stable Isotope Analysis in R (SIAR) model (Parnell et al. 2010) as follows:

$$X_{ij} = \sum_{k=1}^{k} p_k \left(S_{jk} + c_{jk} + \varepsilon_{ij} \right) \tag{2}$$

$$S_{jk} \sim N\left(\mu_{jk}, \ \omega_{jk}^2 \right) \tag{3}$$

$$C_{jk} \sim N\left(\lambda_{jk}, \ \tau_{jk}^2 \right) \tag{4}$$

$$\varepsilon_{ij} \sim N\left(0, \sigma_j^2 \right) \tag{5}$$

where X_{ij} is the isotope value j of the mixture i; S_{jk} is the source value k on isotope j and is normally distributed with mean μ_{jk} and standard deviation (SD) ω_{jk}; pk is the proportion of source k; C_{jk} is the fractionation factor for isotope j on source k, normally distributed with mean λ_{jk} and SD τ_{jk}; and ε_{ij} is the residual error, representing additional unquantified variation between individual mixtures and is normally distributed with mean 0 and SD σ_j (Parnell et al. 2010).

Nitrate source endmember values for $\delta^{15}N$ and $\delta^{18}O$ were obtained from the literature, except for atmospheric precipitation and wastewater. The actual values of $\delta^{15}N$ and $\delta^{18}O$ in atmospheric precipitation and wastewater were analyzed from typical samples collected within the study area (Table 2).

Data analysis

Correlations between variables were analyzed using Pearson correlations, and statistical analyses were carried out to determine the differences in water quality parameters at a significance level of $P \leq 0.05$ in the Origin8 software package.

Results

Spatial-temporal variations of the nitrogen species and water chemistry

Concentrations of TN, NO_3^-, NO_2^-, and NH_4^+ in the Shaying River ranged from 0.43 to 13.19 mg L^{-1}, 0.04 to 4.72 mg L^{-1}, 0.01 to 0.32 mg L^{-1}, and 0.01 to 7.59 mg L^{-1}, respectively. Nitrogen pollution in the Jialu River (JLH-a, JLH-b) was most serious in both summer and winter (Table 3). The nitrogen pollution in the Sha River was the lightest, where the NH_4^+

Table 2 Specific $\delta^{15}N$ and $\delta^{18}O$ values of potential NO_3^- sources

Source	n	$\delta^{15}N(\%)$		$\delta^{18}O(\%)$	
		Mean	SD	Mean	SD
Precipitation	4	− 2.71	2.11	61.68	13.69
Fertilizer N	–	− 0.20[a]	2.28	− 2.00[a]	8.00
Soil N	–	7.50[b]	5.23	− 2.00[b]	8.00
Manure and Sewage	4	15.71	4.72	3.5	2.89

a. Data obtained from (Li et al. 2007; Black and Waring 1977; Kim et al. 2015; Widory et al. 2004; Curt et al. 2004; Choi et al. 2002, 2003, 2007). In China, urea and ammonium salt were the majority of synthetic fertilizer, so we use the values for NH_4^+ fertilizers (Li et al. 2007)

b. Data obtained from (Divers et al. 2014; Black and Waring 1977; Widory et al. 2004; Curt et al. 2004; Choi et al. 2007; Kendall et al. 2007; Sturm et al. 2011; Spoelstra et al. 2007; Kellman 2005; Kaushal et al. 2011; Bedard-Haughn et al. 2003)

Table 3 Concentrations of nitrogen and ΔN_2, dual isotopes ($\delta^{15}N$, $\delta^{18}O$), and chemical indicators in water samples from the Shaying River basin in 2015 and 2016

	Sampling periods	Sampling sites							
		JLH-a (n = 9)	JLH-b (n = 9)	SH (n = 9)	YH (n = 9)	M-a (n = 9)	M-b (n = 9)	M-c (n = 3)	HR (n = 3)
NH_4^+-N (mg L^{-1})	Summer	1.72 ± 1.19	1.22 ± 0.63	0.84 ± 0.10	0.22 ± 0.15	0.73 ± 0.60	1.18 ± 0.88	0.3	0.42
	Winter	0.69 ± 0.41	6.79 ± 0.18	0.98 ± 0.13	0.83 ± 0.55	4.48 ± 0.21	3.58 ± 2.42	2.94	1.54
NO_3^--N (mg L^{-1})	Summer	0.35 ± 0.56	2.20 ± 1.40	0.06 ± 0.03	0.31 ± 0.35	1.44 ± 1.27	0.97 ± 0.42	0.85	0.64
	Winter	1.39 ± 0.22	3.50 ± 0.07	1.26 ± 0.42	2.44 ± 0.73	2.86 ± 0.15	2.59 ± 1.01	2.1	1.4
NO_2^--N (mg L^{-1})	Summer	0.01	0.15	0.01	0.03	0.05	0.32	–	–
	Winter	0.02	0.19	0.02	0.13	0.16	0.14	–	–
TN (mg L^{-1})	Summer	4.10 ± 0.70	8.79 ± 3.32	0.98 ± 0.04	4.51 ± 3.85	6.30 ± 3.07	7.35 ± 2.52	3.08	1.68
	Winter	5.18 ± 2.18	10.22 ± 0.01	2.24 ± 0.42	6.44 ± 2.86	9.65 ± 0.28	8.08 ± 1.89	6.72	4.62
DO (mg L^{-1})	Summer	1.46 ± 1.16	5.94 ± 1.34	9.60 ± 0.88	7.86 ± 2.68	7.80 ± 2.41	5.02 ± 1.86	6.32	4.8
	Winter	4.20 ± 0.28	11.00 ± 1.41	13.20 ± 0.72	11.89 ± 3.32	10.28 ± 1.75	8.00 ± 0.968	10.88	10.64
Chl-a (μg L^{-1})	Summer	6.07 ± 3.84	13.97 ± 3.00	16.26 ± 1.78	26.17 ± 13.02	34.47 ± 7.54	24.15 ± 5.53	13.23	31.98
	Winter	5.51 ± 3.59	16.25 ± 16.51	3.77 ± 1.05	8.58 ± 7.88	12.85 ± 8.75	5.33 ± 2.36	9.23	10.53
Cl$^-$ (mg L^{-1})	Summer	87.15 ± 4.62	101.35 ± 7.10	31.84 ± 4.62	42.67 ± 3.91	64.75 ± 6.04	78.95 ± 8.17	74.44	34.26
	Winter	110.41 ± 10.65	115.55 ± 3.91	47.14 ± 8.17	59.57 ± 4.62	94.39 ± 3.91	93.15 ± 7.10	95.89	36.81
ΔN_2 (mg L^{-1})	Summer	4.277 ± 2.84	3.50 ± 0.37	3.42 ± 0.47	3.48 ± 0.91	3.79 ± 0.93	3.66 ± 0.60	3.72	3.59
	Winter	−2.71 ± 1.13	−2.72 ± 0.76	0.09 ± 1.00	−3.12 ± 0.89	−2.58 ± 0.42	0.11 ± 1.66	−2.8196	−2.688
$\delta^{15}N$-NO_3^- (‰)	Summer	11.57 ± 4.48	12.41 ± 1.14	10.28 ± 0.64	16.62 ± 1.98	12.71 ± 1.50	11.19 ± 0.15	13.3	5.25
	Winter	11.39 ± 0.54	13.03 ± 0.80	20.96 ± 0.30	15.00 ± 0.89	11.91 ± 2.87	13.29 ± 1.30	13.9	7.12
$\delta^{18}O$-NO_3^- (‰)	Summer	13.21 ± 1.71	0.87 ± 1.79	10.44 ± 0.78	5.11 ± 1.90	3.59 ± 2.12	0.26 ± 3.81	4.2	6.95
	Winter	4.85 ± 3.35	−0.40 ± 0.59	4.02 ± 0.78	0.17 ± 0.96	3.28 ± 3.07	−1.64 ± 0.12	2.38	3.12
$\delta^{15}N$-NH_4^+ (‰)	Summer	17.685	16.44	4.38	10.07	20.92	8.56	9.27	13.07
	Winter	7.67	5.18	−7.01	15.63	11.2	5.99	7.19	9.98
$\delta^{15}N$-SPN (‰)	Summer	1.56	5.69	13.51	12.73	11.07	11.48	–	–
	Winter	1.46	0.78	4.55	11.26	10.79	13.41	–	–
$\delta^{18}O$-H_2O (‰)	Summer	−8.18 ± 0.51	−6.75 ± 0.09	−3.79 ± 0.28	−5.75 ± 1.31	−6.29 ± 0.88	−6.68 ± 0.08	−7.35	−6.77
	Winter	−8.38 ± 0.30	−7.96 ± 0.11	−5.30 ± 0.29	−6.81 ± 0.98	−7.1 ± 0.38	−7.39 ± 0.77	−7.75	−7.03

*n is the sample number

and NO_3^- level was less than 2 mg L^{-1} (Chinese Surface Water Quality Standard GB3838-2002). NO_2^- ranged from 0.01 to 0.15 mg L^{-1} in summer, and from 0.01 mg L^{-1} to 0.32 mg L^{-1} in winter. The concentrations of TN, NO_3^-, and NH_4^+ in the Shaying River were 5.26 ± 3.09 mg L^{-1}, 1.05 ± 1.11 mg L^{-1}, and 1.22 ± 0.92 mg L^{-1} in summer, respectively, whereas they were 7.84 ± 3.44 mg L^{-1}, 2.31 ± 0.81 mg L^{-1}, and 3.00 ± 2.24 mg L^{-1}, respectively, in winter. Nitrogen concentrations (TN, NO_3^-, NH_4^+) in most samples were significantly higher in winter than those of in summer, which might be due to the low precipitation and microbial activity. The Jialu River, with the highest level of ammonium in winter, should be the main ammonium contributor to the mainstream of the Shaying River due to the low level of ammonium in other tributaries (the Sha River, the Ying River). Finally, unlike in the other rivers, NH_4^+ in the Shaying River was always the leading proportion (range, 9.22 to 95.17%; mean, 49.66%) of DIN.

The DO concentrations ranged from 0.64 to 12.40 mg L^{-1} in summer, and from 3.36 to 14.24 mg L^{-1} in winter. Compared to other rivers, Chl-a concentrations in the Shaying River were high (20.17 ± 11.12 μg L^{-1}), even though the precipitation was great during the summer, which could be attributed to the low flow rate caused by dams (Balls et al. 1996; Brion et al. 2000). The Chl-a dropped to 9.21 ± 6.13 μg L^{-1} in the winter because of low water temperature, as seen in Table 3. Cl^- ranged from 23.79 to 112.18 mg L^{-1} in summer, and from 79.52 to 129.58 mg L^{-1} in winter, and no obvious seasonal variations were observed.

Isotope compositions of nitrogenous species

$\delta^{15}N$-NO_3^- in the Shaying River ranged from 5.25 to 18.02% in summer, and 8.82 to 20.96% in winter, respectively, while $\delta^{18}O$-NO_3^- ranged from -2.43 to 14.76% in summer and from -1.64 to 7.22% in winter. $\delta^{18}O$-NO_3^- in the three tributaries (Jialu, Ying, and Sha) experienced an obvious decrease in winter, whereas no obvious $\delta^{18}O$-NO_3^- seasonal variations were observed in the main stream of the Shaying River (M-a, M-b, M-c) and the Huai River (HR). The higher values of $\delta^{18}O$-NO_3^- in tributaries during the summer may be attributed to intensive denitrification or assimilation. There were no seasonal variations for most values of $\delta^{15}N$-NO_3^-, whereas $\delta^{15}N$-NO_3^- at SH in the Sha River experienced an obvious increase in winter (Table 3). If the increase of $\delta^{15}N$ was attributed to intensive denitrification or uptake of nitrate during winter in the Sha River, both values of $\delta^{15}N$ and $\delta^{18}O$ should have increased at the same time (Kendall et al. 2001). Nevertheless, our data show that the values of $\delta^{18}O$ decreased in the Sha River (SH) during winter. Therefore, this phenomenon may be explained as a result of mixing of different pollution sources.

$\delta^{15}N$-NH_4^+ ranged from 4.38 to 20.92% in summer, and from -7.01 to 15.63% in winter. Values of ^{15}N-NH_4^+ at JLH-a, JLH-b, SH, and M-a during the summer were significantly higher than those of in winter. No significant seasonal variations were found in $\delta^{15}N$-NH_4^+ at YH, M-b, and M-c. $\delta^{18}O$-H_2O ranged from -8.64 to -3.32% in summer, significantly higher than those of in winter (-8.77% to -5.09%), which may be due to the intensive evaporation during summer. $\delta^{15}N$-SPN ranged from 1.56 to 13.51% in summer, and 0.78 to 13.41% in winter. The values of $\delta^{15}N$-SPN in the Sha River demonstrate an apparent seasonal variation, whereas no apparent seasonal variations were observed in the other values of $\delta^{15}N$-SPN (Table 3).

Relationships between nitrogen isotopic composition and chemical variables in different seasons

During summer, DO was negatively correlated with NH_4^+ and positively correlated with NO_3^- (both $p < 0.05$), as seen in Table 4. The NH_4^+ was more easily oxidized, with the increase of DO; because of nitrification, the NO_3^- could increase and NH_4^+ might decrease, even though no significant negative correlation was found between NO_3^- and NH_4^+. A negative correlation between NH_4^+ and $\delta^{15}N$-SPN was observed, indicating the occurrence of NH_4^+ uptake during summer (Bardhan et al. 2017), which could be partly responsible for the low level of NH_4^+ in summer. NO_3^- was positively correlated with TN ($p < 0.01$), suggesting that they share a same/similar source. $\delta^{18}O$-NO_3^- was negatively correlated with NO_3^-, which could be attributed to the denitrification in the river during summer (Kellman and Hillaire-Marcel 2003; Kendall et al. 2001). The values of Chl-a concentrations show a positive correlation with $\delta^{15}N$-SPN, which could suggest that the assimilation process was intensive in summer. During winter, NO_2^- was negatively correlated with $\delta^{15}N$-NO_3^- ($p < 0.05$), and NO_3^- was positively correlated with NH_4^+ ($p < 0.01$). The process of nitrification first turned NH_4^+ into NO_2^-, then turned NO_2^- into NO_3^-, causing the increase of NO_3^- concentration and the decrease of $\delta^{15}N$-NO_3^-. Therefore, it could be that the nitrification process that caused the negative correlation between NO_2^- and $\delta^{15}N$-NO_3^-. The positive correlation between NO_3^- and NH_4^+ in winter suggests that they share a same/similar source.

Denitrification variations and the distribution of microbial flora involved in metabolism of inorganic nitrogen compounds in the Shaying River

During summer, N_2 in water samples was oversaturated, with ΔN_2 ranging from 2.15 to 4.92 mg L^{-1}, with the mean of 3.50 ± 0.69 mg L^{-1}; the highest ΔN_2 was at JLH-a, and lowest at SH (Table 3), whereas N_2 in most water samples during winter was unsaturated, with ΔN_2 ranging from -4.28 to

Table 4 Pearson's correlation coefficients among nitrogen, dual isotopes (δ^{15}N, δ^{18}O), and chemical indicators during the sampling periods in the Shaying River, Eastern China

|	NH_4^+	NO_2^-	NO_3^-	TN	DO	Cl^-	Chl-a	δ^{15}N-SPN	δ^{15}N-NH_4^+	δ^{18}O-H_2O	δ^{15}N-NO_3^-	δ^{18}O-NO_3^-
Summer												
NH_4^+	1											
NO_2^-	0.0011	1										
NO_3^-	−0.1175	0.5994	1									
TN	−0.0591	0.5991	0.5188[**]	1								
DO	−0.6569[**]	0.1805	0.2810[*]	−0.3665	1							
Cl^-	−0.1106	0.0132	0.4735	−0.6913[*]	−0.1309	1						
Chl-a	−0.3131	0.4887	0.0676	−0.0972	0.6489[*]	−0.0654	1					
δ^{15}N-SPN	−0.9045[*]	0.0939	−0.1412	−0.9439[*]	0.9398[**]	0.0123	0.7676[*]	1				
δ^{15}N-NH_4^+	0.0507	0.6666	−0.1086	−0.2436	0.3596	−0.1669	0.1962	0.4769	1			
δ^{18}O-H_2O	−0.1415	−0.2885	−0.0452	−0.0325	0.4674	−0.6153[*]	0.0111	0.8959[*]	0.2775	1		
δ^{15}N-NO_3^-	−0.2719	−0.2214	−0.0293	−0.0055	−0.4157	0.4047	−0.3951	0.1323	−0.1259	−0.0492	1	
δ^{18}O-NO_3^-	0.1817	−0.7833	−0.6300[**]	−0.2285	−0.3617	0.1073	−0.1358	−0.3531	−0.2203	−0.3954	0.0746	1
Winter												
NH_4^+	1											
NO_2^-	0.6275	1										
NO_3^-	0.7262[*]	0.8447	1									
TN	0.4605	0.8166	0.4564	1								
DO	0.5593	0.0011	0.4130	0.4526	1							
Cl^-	−0.3213	0.0032	0.4356	0.5843	0.0333	1						
Chl-a	0.3162	0.7091	0.1170	0.1117	0.9567[*]	0.2286	1					
δ^{15}N-SPN	−0.0637	0.0599	0.4851	0.0973	0.0077	0.0032	−0.0749	1				
δ^{15}N-NH_4^+	−0.0794	0.5941	0.8041	0.3522	0.0011	0.0001	0.6273	0.6566	1			
δ^{18}O-H_2O	0.0026	−0.9884[**]	0.2015	−0.3518	0.3444	−0.3981	−0.1618	−0.0748	−0.5250	1		
δ^{15}N-NO_3^-	−0.5420	−0.9547[*]	−0.3700	−0.3011	0.3979	0.8306	−0.0899	−0.2526	−0.6370	0.6702[*]	1	
δ^{18}O-NO_3^-	0.1951	−0.7562	−0.2510	−0.4450	−0.4054	0.3489	−0.0237	−0.6397	−0.7283	0.1091	−0.2364	1

1.29 mg L^{-1}. The mean of ΔN_2 was −2.40 ± 1.05 mg L^{-1}, with the highest value at M-b and the lowest at M-a.

The distribution of microbial flora involved in metabolism of inorganic nitrogen compounds in water and sediment in the

Table 5 Distribution of microbial flora involved in metabolism of inorganic nitrogen compounds in water and in sediment in June and November

Sampling sites		Aerobic denitrifying bacteria (MPN L^{-1})		Anaerobic denitrifying bacteria (MPN L^{-1})		Heterotrophic nitrifying bacterial (MPN L^{-1})		Autotrophic nitrifying bacteria (MPN L^{-1})	
		June	November	June	November	June	November	June	November
JLH-a	Water	1.25×10^4	70	2.0×10^2	60	60	0	0	30
JLH-b		2.25×10^4	70	4.5×10^4	2.5×10^4	70	0	2.0×10^2	200
SH		2.25×10^3	70	4.5×10^3	60	1.5×10^2	0	90	30
YH		2.25×10^3	110	2.5×10^2	900	0	0	70	0
M-a		4.75×10^3	150	1.5×10^2	60	2.0×10^2	0	7.5×10^2	30
JLH-a	Sediment	7.00×10^7	2.0×10^5	7.5×10^4	6.0×10^3	2.5×10^2	0	7.5×10^3	9.5×10^4
JLH-b		2.25×10^6	2.0×10^5	1.5×10^5	6.0×10^3	4.5×10^3	0	1.5×10^4	2.5×10^4
SH		4.75×10^6	4.5×10^4	2.5×10^4	9.5×10^4	2.5×10^2	0	4.5×10^3	250
YH		1.50×10^6	2.0×10^4	9.5×10^4	6.0×10^3	1.1×10^2	0	7.5×10^3	4.5×10^3
M-a		1.50×10^5	3.0×10^3	9.5×10^4	3.0×10^3	2.0×10^2	0	7.5×10^3	2.5×10^5
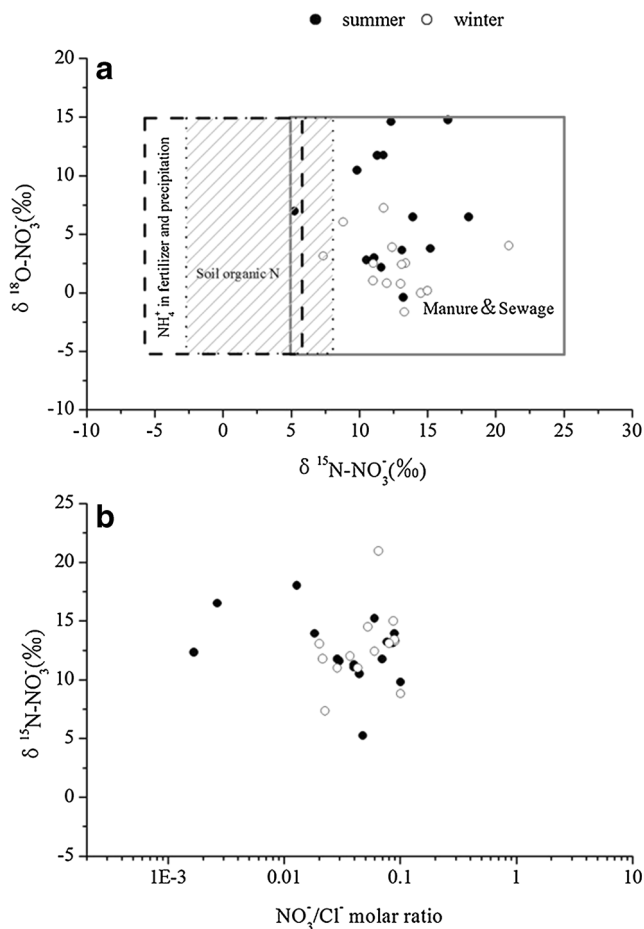
Fig. 3 Dual $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- source plots for water samples collected during August and December 2015 and 2016 (**a**); Plots of $\delta^{15}N$-NO_3^- vs NO_3^-/Cl^- molar ratio in the Shaying River water during August and December 2015 and 2016 (**b**)

Shaying River system was also studied (Table 5). During June, aerobic denitrifying bacteria were the dominant microorganism in both water and sediment samples. The MPN of aerobic denitrifying bacteria ranged from 2.25×10^3 MPN L^{-1} to 2.25×10^4 MPN L^{-1} in the water samples, and from 1.50×10^5 MPN L^{-1} to 7.00×10^7 MPN L^{-1} in the sediments, whereas the anaerobic denitrifying bacteria was second only to the aerobic denitrifying bacteria. MPN of both the heterotrophic and autotrophic nitrifying bacteria were low in the water samples.

During November, the amount of bacteria dropped dramatically. The MPN of aerobic denitrifying bacteria was on average 150 MPN L^{-1} in water and 2.0×10^5 MPN L^{-1} in the sediment. The MPN of anaerobic denitrifying bacteria in water was significantly higher than that of aerobic denitrifying bacteria, whereas the MPN of aerobic denitrifying bacteria in sediment was still bigger than the MPN of anaerobic denitrifying bacteria. The MPN of autotrophic nitrifying bacteria in the sediment was at the same level as the aerobic denitrifying bacteria, whereas the MPN of autotrophic

nitrifying bacteria in water was small, from 0 to 200 MPN L^{-1}. The MPN of heterotrophic nitrifying bacteria in both water and sediment was negligible.

Discussion

Quantitative identification of NO_3^- pollution sources

NO_3^- in the aquatic system has several major sources, including atmospheric precipitation, leaching from chemical fertilizers, nitrification in soils, and manure and sewage (Kendall et al. 2001). NO_3^- derived from atmospheric precipitation has low values of $\delta^{15}N$ in the range of -13.0 to $+13.0\%$ and relatively high values of $\delta^{18}O$ ($>+20\%$, Kendall et al. 2001; Widory et al. 2005). Nitrogenous fertilizers generally have $\delta^{15}N$-NO_3^- values within a few permil around zero in China (Yue et al. 2013). $\delta^{15}N$-NO_3^- in the Shaying River ranged from 5.25 to 20.96%, and $\delta^{18}O$-NO_3^- from -2.43 to 14.76%. The low values of $\delta^{18}O$ and high level of $\delta^{15}N$ indicate that precipitation and nitrogenous fertilizers were not the major sources of riverine NO_3^- during our sampling periods. Figure 3 shows that most data are distributed in the manure and sewage source window, suggesting that manure and sewage were the main source of NO_3^- in the research area during both sampling periods. Nevertheless, values at HR in summer and M-a in winter indicate that nitrate was originated from the mixing of soil-induced N with manure and sewage.

Cl^- is biologically and chemically inert, thus a good indicator for the impact of sewage on aquatic systems (Liu et al. 2006). High Cl^- concentrations have been detected in sewage and livestock effluent (Yao et al. 2007), but no significant correlation has been found between Cl^- and NO_3^- in summer and winter, separately (Table 4). However, if we combine all data, a significant positive correlation between Cl^- and NO_3^- ($R^2 = 0.7972$, $n = 28$) can be seen, which indicates that Cl^- is strongly influenced by anthropogenic input. Furthermore, plots of $\delta^{15}N$ values versus the NO_3^-/Cl^- molar ratio can reveal whether denitrification or mixing of NO_3^- from various sources is responsible for the increasing of $\delta^{15}N$-NO_3^- values in the water body (Koba et al. 1997; Liu et al. 2006; Widory et al. 2005). Most samples in the Shaying River show high ^{15}N-NO_3^- values ($>9.00\%$) and Cl^- concentrations (>34 mg L^{-1}), as well as low in NO_3^-/Cl^- molar ratios (<0.11), which suggests that NO_3^- in the Shaying River derived mainly from manure and sewage influenced by denitrification (Yue et al. 2013, 2014; Vystavna et al. 2017).

In order to further estimate the proportional contributions of different potential NO_3^- sources quantitively, a SIAR mixing model was employed. Figure 4 shows that manure and sewage contributed to the maximal NO_3^-, at $62.44 \pm 19.66\%$ in summer and $67.33 \pm 15.45\%$ in winter,

Fig. 4 Proportional contributions of four potential NO_3^- sources estimated by the SIAR mixing model in the Shaying River watershed (box plot denotes 5th, 25th, 50th, 75th, and 95th percentiles; white square represents the mean values)
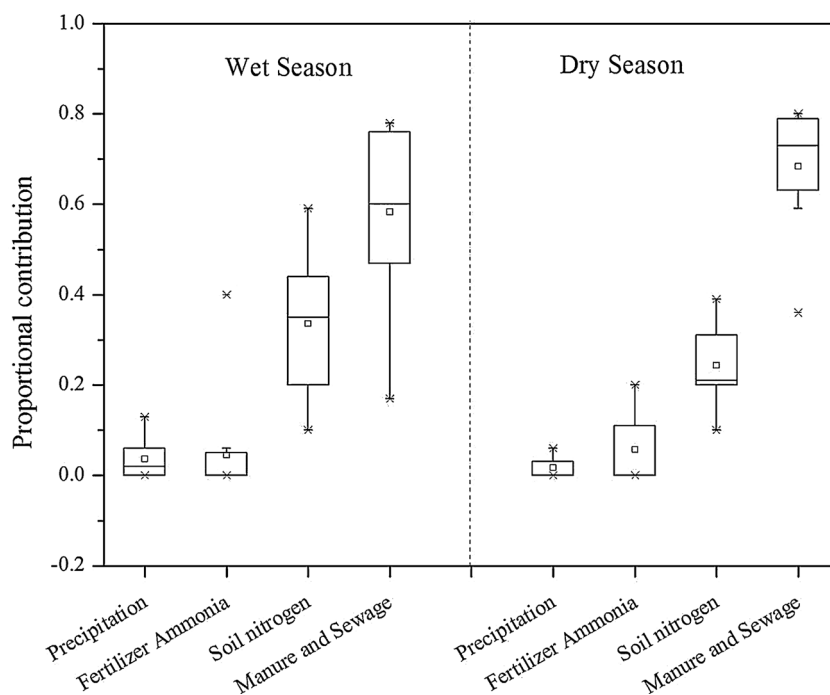

respectively, followed by soil organic N ($24.94 \pm 15.52\%$ and $26.33 \pm 9.45\%$ for summer and winter, respectively), chemical fertilizer nitrification ($7.81 \pm 6.77\%$ and $3.96 \pm 5.57\%$ for summer and winter, respectively) and atmospheric precipitation ($4.81 \pm 5.65\%$ and $2.38 \pm 3.55\%$ for summer and winter, respectively). The contribution of chemical fertilizer nitrification was significantly higher in summer than in winter ($P < 0.05$). The Shaying River watershed is an agricultural drainage stream network, and 76.24% of the research area is cultivated land (Zuo et al. 2016). Intensive farming, as well as high water precipitation during summer, has increased the chemical fertilizer nitrification contribution to riverine nitrate. Although manure and sewage was the foremost source for the riverine nitrate in this river, soil organic nitrate was also important. The contributions of manure and sewage, soil organic N, and atmospheric precipitation demonstrate no significant seasonal variations in most samples, whereas atmospheric precipitation contributed more than 10% of the nitrate at SH, JLH-a, and HR in the summer, and it only contributed less than 4% in winter.

Nitrogen source in NH_4^+ and suspended matter

Ammonium, unlike in other river systems, was the dominant form of DIN in the Shaying River system, especially in winter (Table 3). Every year, there was a large amount of ammonium discharged to the Shaying River (Fig. 5a). The ammonia wastewater from the upperstream watershed discharged to the Shaying River decreased dramatically during 2010–2015, from 1.29×10^7 Kg N year^{-1} in 2010 to 0.89×10^7 Kg N year^{-1} in 2015. Normally, as input of ammonia

decreases, the concentrations of riverine ammonia decrease accordingly. Figure 5b shows a comparison of the level of ammonium between 2010–2012 and 2013–2015, and the results indicate that the NH_4^+ concentrations in summer significantly decreased from 1.40 ± 1.54 mg L^{-1} in 2010–2012 to 0.84 ± 0.56 mg L^{-1} in 2013–2015, whereas no such difference in the concentrations of NH_4^+ was found between the two periods in winter (Fig. 5b). Even though ammonium discharged decreased dramatically, the accelerated urban development due to rapid population migration from rural to urban lands may have led to high level of untreated sewage entering directly into aquatic ecosystems.

It is known that both concentration and isotopic characteristics of N species in treated and untreated wastewater are different. The untreated sewage water has a high δ^{15}N-NO_3^- and low δ^{15}N-NH4 value, with a low level of NO_3^- and a high level of NH_4^+. However, the treated sewage water, because of nitrification, has a low level of NH_4^+ and high level of NO_3^-, with a high δ^{15}N-NH4 and low δ^{15}N-NO_3^-. Figure 5c suggests that about 50% of the δ^{15}N-NO_3^- values were higher than the values of δ^{15}N-NH_4^+ during summer, whereas the contents of nitrate were similar to the level of ammonium in water samples in summer (Table 3). Thus, we can infer that about half of the wastewater was treated before entering the river during summer. On the other hand, most δ^{15}N values in nitrate were higher than δ^{15}N-NH_4^+ during winter and the contents of nitrate were lower than the level of ammonium (Fig. 5c, Table 3), revealing that most of the wastewater discharged into the Shaying River was untreated, which could be one of the main reasons for the high concentrations of NH_4^+ in winter.
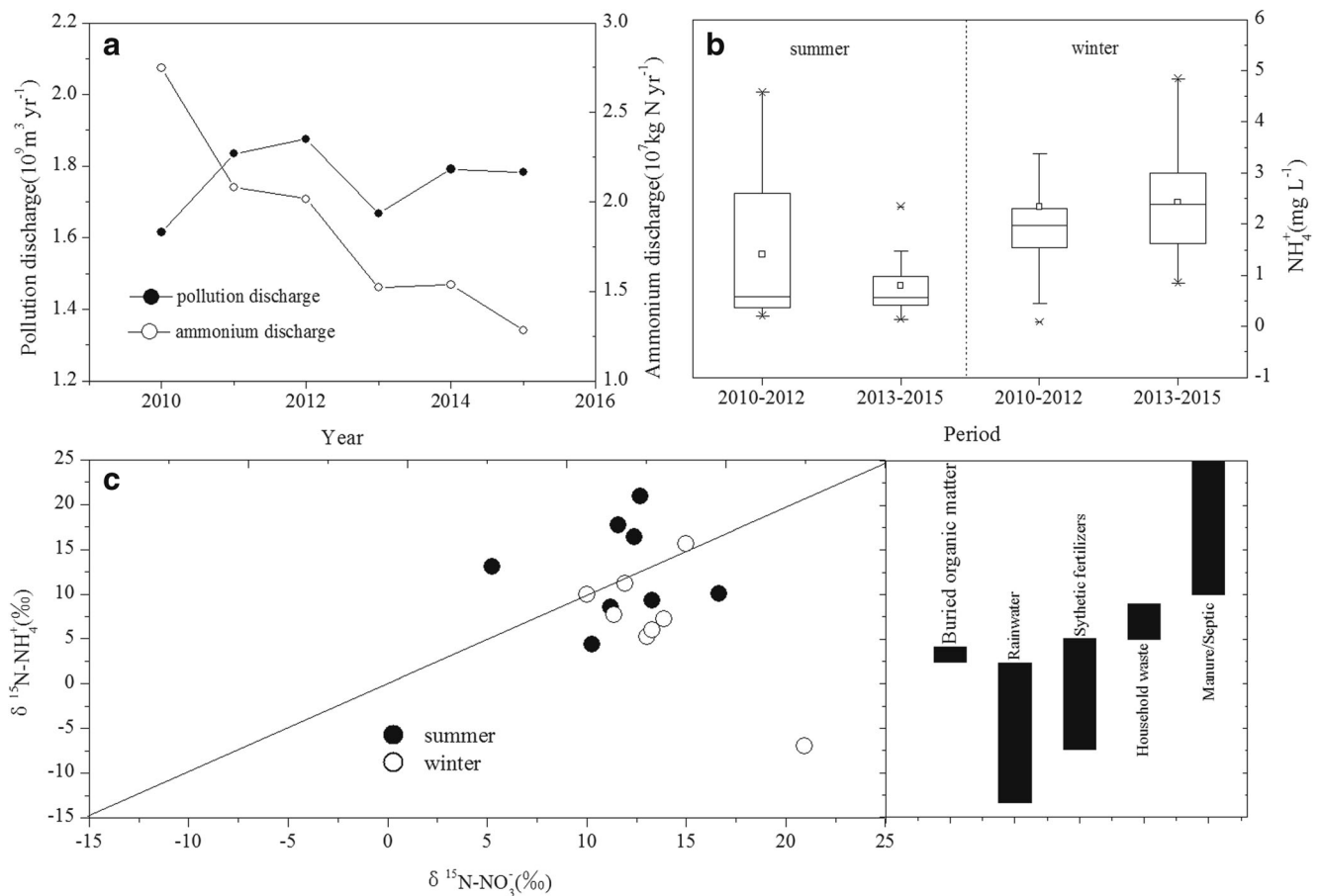
Fig. 5 Discharge of pollution and ammonium to the Shaying River from the upperstream of the Shaying River watershed (**a**); The comparison of ammonium concentrations at M-a in the river water between periods of 2010 to 2012 and 2013 to 2015 (**b**; Data for pollution discharge an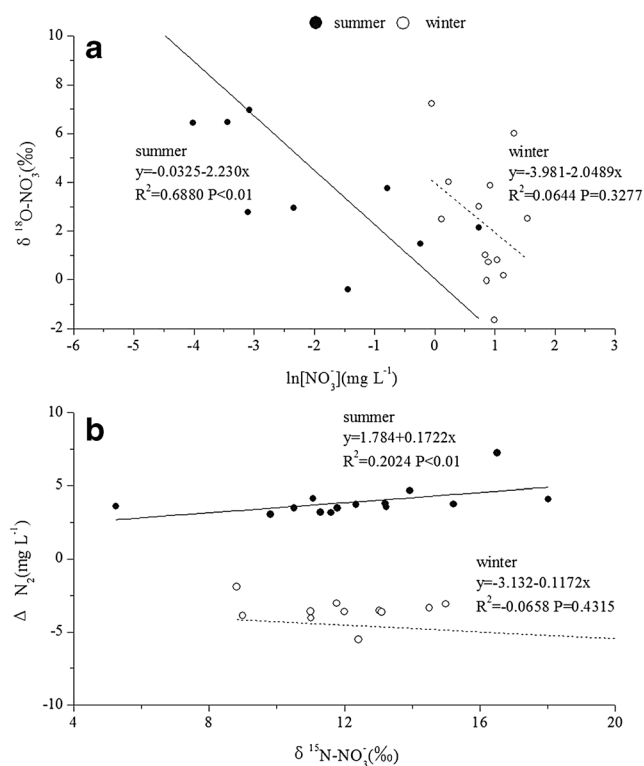d ammonium discharge were obtained from the Huaihe River water Resources Commission, while data for NH_4^+-N content at M-a during 2010–2015 were obtained from the Zhoukou hydrological station); Plots of $\delta^{15}N$-NH_4^+ vs $\delta^{15}N$-NO_3^- in the river water in August and December 2016 with reported ranges of $\delta^{15}N$-NH_4^+ values for different NH_4^+ sources on the right side (**c**)

Similar to nitrate, $\delta^{15}N$-NH_4^+ varies according to its source. Values of $\delta^{15}N$-NH_4^+ in the Shaying River suggest that ammonium at most sites was mainly from septic/manure, except at SH (4.38‰) in summer (Hao et al. 2018; Du et al. 2017; Nikolenko et al. 2018). In winter, values of $\delta^{15}N$-NH_4^+ at JLH-a, JLH-b, M-b, and M-c (5.18‰ to 7.67‰) suggest that household waste was the main source of ammonium, whereas values of $\delta^{15}N$-NH_4^+ at YH and M-a (11.20‰ to 15.63‰) reveal that ammonium was mainly from septic/manure (Fig. 8c). Thus, point source pollution, i.e., septic/manure and domestic sewage, was an important source of ammonium in the Shaying River system. Zeng (2015), by applying the SWAT model, estimated that nonpoint source contributed just 5.1% of the total ammonium loading, whereas point source was the main source of ammonium in the upper stream of the Shaying River basin.

Nitrogen pollution was slight in the Sha River, where the concentrations of ammonium were less than 2 mg L^{-1} (Chinese Surface Water Quality Standard (GB3838-2002)) during the two sampling periods. The low values of $\delta^{15}N$-NH_4^+(4.38‰) in the summer indicate that ammonium in the river derived from buried organic matter mineralization, similar to that of the Red River delta of Southeast Asia (Norrman et al. 2015). The low value of $\delta^{15}N$-NH_4^+ (− 7.01‰) at SH in winter may suggest that ammonium derived from rainwater and synthetic fertilizers. Agricultural and microbial activities were low in winter; however, NH_4^+ can be concentrated in the rain if vaporization of NH_3 occurs from the low flow surface of soil particles that adsorb NH_4^+ (Russell et al. 1998). This could be the reason for the significant influence of rainwater and synthetic fertilizers on $\delta^{15}N$-NH_4^+ in the Sha River.

SPN was an important form of nitrogen in river water (Middelburg and Nieuwenhuize 1998). The assimilation of sewage-derived NH_4^+ could contribute to enriching the SPN of ^{15}N in the Shaying River during summer, consistent with what has been observed in other systems (Owens 1985; Cifuentes et al. 1988; Cole et al. 2004). Microbial activity was usually low in December. The effects of

Fig. 6 $\ln[NO_3^-]$ versus $\delta^{15}N\text{-}NO_3^-$ (**a**); and $\delta^{15}N\text{-}NO_3^-$ versus ΔN_2 (**b**) in water samples in the Shaying River

Roles of denitrification, nitrification, and assimilation in the river system

A negative linear relationship was found between $\delta^{18}O\text{-}NO_3$ and $\ln[NO_3^-]$ during summer ($R^2 = 0.7282$, $p < 0.01$), seen in Fig. 6a, revealing that denitrification occurred in river water during the summer (Yue et al. 2014). However, no notable negative relationship between $\ln[NO_3^-]$ and $\delta^{15}N\text{-}NO_3$ was observed, perhaps because nitrate consumption processes could be overprinted by stronger signals due to external N loading. N_2 was the main product of the denitrification process, i.e., $NO_3^- \rightarrow NO_2^- \rightarrow N_2$; thus, the linear positive correlation between $\delta^{15}N\text{-}NO_3$ and ΔN_2 during summer further verifies the important influence of denitrification on nitrate isotopes in the river system during summer (Fig. 6b). Long residence time caused by dam would enhance the denitrification at YH and JLH-b, but no spatial differences were observed in ΔN_2. There were a lot of dams in our research area, and the discharge was mostly controlled by dams, except that at JLH-a (Fig. 1). The denitrification process was enhanced, as indicated by high values of ΔN_2 in summer (Table 3). Even though there was no dam at or near the JLH-a, but the low level of DO (< 5.00 mg/L) was benefit for denitrification, thus ΔN_2 at JLH-a was high in summer.

Table 5 demonstrates that the aerobic denitrifying bacteria, not the anaerobic denitrifying bacteria, were the dominant population of microorganisms both in the water and the sediment in summer (June). The aerobic denitrification process, i.e., coupled nitrification-denitrification, can remove NO_3^- provided by nitrification from NH_4^+ (Joo et al. 2006). This would have had little effect on nitrate isotopic composition, but could increase the residual $\delta^{15}N\text{-}NH_4^+$ (Wells et al. 2016), which might be the reason for the high level of $^{15}N\text{-}NH_4^+$ in most water samples during summer. Therefore, we can infer that the aerobic denitrification process is important to the removal of NH_4^+, which could attribute for the low level of ammonium in summer. Although most DO concentrations in the surface water were above 2 mg L^{-1}, they were not suitable for the anaerobic denitrification process (Baron et al. 2013), but instead suitable for the aerobic denitrification process (Robertson and Kuenen 1984, Robertson et al., 1985). The notable correlation between $\ln[NO_3^-]$ and $\delta^{15}N\text{-}NO_3$, as well as the significant correlation between $\delta^{15}N\text{-}NO_3$ and ΔN_2 during summer, should prove the existence of anaerobic denitrification. Table 4 indicates that the MPN of anaerobic denitrifying bacteria was also significant in summer (June), just next to the MPN of aerobic denitrifying bacteria in both water and sediment.

In winter, there was no significant relationship between $\delta^{15}N\text{-}NO_3$ and ΔN_2, or between $\ln[NO_3^-]$ and $\delta^{15}N\text{-}NO_3$, which could be attributed to the stop/weakness of denitrification in the river system. Low water temperature (1.0 °C to 11.2 °C) suppressed the denitrification progress, despite high nitrate concentrations in winter (Silvennoinen et al. 2008;

isotopic fractionation by microbial processes, therefore negligible (Lehmann et al. 2004). However, the values of $\delta^{15}N\text{-SPN}$ were still high in the winter, ranging from 0.78 to 13.41%, which may be attributed to its source. Generally, evaporation caused $\delta^{15}N$ values enrichment, whereas no such difference was observed between $\delta^{15}N$ values in the two seasons. Likewise, there were no significant correlations between $\delta^{15}N\text{-SPN}$ and NO_3^- in August and December (Table 4), which suggests that terrestrial N contributed to much of the change in $\delta^{15}N\text{-SPN}$ during the two sampling periods. The inputs of terrestrial sources of particulate organic N via rain runoff may have contributed to the increase in $\delta^{15}N\text{-SPN}$; nevertheless, values of $\delta^{15}N\text{-}$SPN originated from industrial and domestic sewage were high (Li and Zhang 2010), which may be one reason for the high level of $\delta^{15}N\text{-SPN}$ in both seasons. Most values of $\delta^{15}N\text{-SPN}$ (YH, M-a, and M-b in both sampling periods, and SH in summer) distributed into the industrial and domestic sewage ranges (Kreitler and Browning 1983; Wassenaar 1995), which suggests that industrial and domestic sewage was the main source for the riverine SPN. Values of $\delta^{15}N$ in the Jialu River (JLH-a, JLH-b) during both seasons and $\delta^{15}N$ in the Sha River (SH) in winter demonstrate that the N in suspended matter came from mixed sources, i.e., soil organic nitrogen and agricultural fertilizers (Kreitler and Browning 1983; Wassenaar 1995).
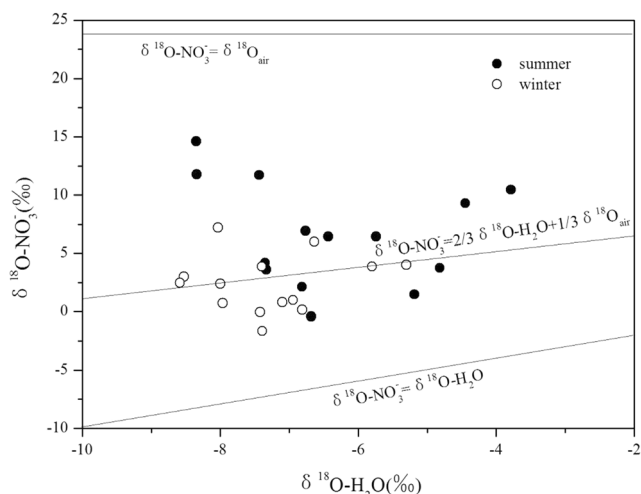
Fig. 7 $\delta^{18}O-H_2O$ versus $\delta^{18}O-NO_3^-$ in water samples in the Shaying River. Three lines represent the theory line in different conditions

Nowicki 1994). Furthermore, both aerobic and anaerobic denitrifying bacteria decreased dramatically in November (Table 5). The MPN of aerobic denitrifying bacteria in sediment decreased from 1.57×10^7 MPN L^{-1} (mean) in June to 9.36×10^4 MPN L^{-1} (mean) in November, whereas that of in water decreased from 8.85×10^3 MPN L^{-1} in June to 94 MPN L^{-1} in November. Moreover, pH can directly affect the activity of reductase of the denitrifying bacteria (Zhang et al. 2012). Pan et al. (2012) found up to 20% of the nitrate-nitrogen accumulated as N$_2$O when the pH dropped to 6.0–6.5 (Pan et al. 2012; Hanaki et al. 1992). The pH values in the Shaying River ranged from 6.0 to 6.6 during winter, which could have further reduced the production of N$_2$. Finally, along with increase of ln[NO$_3^-$], there was a slight decrease in $\delta^{15}N-NO_3^-$, though not to a significant degree (Fig. 6a). This further indicates that the denitrification in the Shaying River was weak, but not stopped during this period.

Theoretically, if denitrification, i.e., anaerobic denitrification, was significant in summer, the $\delta^{18}O$ and $\delta^{15}N$ in nitrate should have been elevated. However, these elevations were not reflected in the changes of $^{15}N-NO_3^-$, indicating that it was mainly precipitation, not denitrification, in the summer that lead to the low NO$_3^-$ concentrations. The effect of denitrification on nitrate isotopes was overprinted by anthropogenic nitrate source, i.e., mainly manure and sewage, so no significant correlation was observed between $\delta^{18}O$ and $\delta^{15}N$ in nitrate (Fig. 3). An increase of $\delta^{15}N-NO_3^-$ and decrease of $\delta^{18}O-NO_3^-$ in winter at SH may be mainly due to the previously mentioned multiple nitrate sources. More specifically, the contribution of precipitation was up to 10% at SH in summer, according to the SIAR mixing model estimation, while it dropped to 0.01% in winter. As can be seen (Table 2), rainwater was low in $\delta^{15}N$ and high in $\delta^{18}O$. Therefore, the drop in rainwater contribution to the NO$_3^-$ may be the main reason for the seasonal variations of $\delta^{15}N$ and $\delta^{18}O$ in NO$_3^-$ at SH.

Ammonium and DO were abundant in the Shaying River, so the nitrification process was considered to be significant, but the MPN data illustrate that MPN of the nitrifying bacteria was not great; in fact, it was even absent in some water samples in both summer and winter (Table 5). In theory, about one third of the O in the NO$_3^-$ produced by nitrification is derived from DO, whereas the remaining two-thirds is derived from water O during oxidation of NH$_4^+$ to NO$_3^-$ (Böttcher et al. 1990). If the O from these two sources is incorporated without isotopic fractionation, the values of $\delta^{18}O-NO_3^-$ would be + 3.47% (mean) during summer and 2.98% (mean) during winter, according to calculations based on the $\delta^{18}O-O_2$ of + 23.5%. The $\delta^{15}N$ values of the samples (mean, + 6.10%) during summer were significantly higher than the theoretical values, while $^{18}O-NO_3^-$ in samples (mean, 3.10%) was similar to the theoretical values during winter (Fig. 7). Thus, nitrification during summer may not have been important, whereas it was dominant in winter, which is also supported by the significant negative correlation between $\delta^{15}N-NO_3^-$ and NO$_2^-$ (Table 4). This was similar to the upper Han River, where nitrification was dominant in January (Yuan 2017). Finally, although nitrification during summer was not significant, it should not be neglected, because the content of heterotrophic nitrifiers and autotrophic nitrifiers in the sediment was not small (Table 5).

The high level of Chl-a and the significant positive correlation between Chl-a and $\delta^{15}N-SPN$ during summer (Table 3) verify that the assimilation process was important during summer. In freshwater systems, N uptake rates often follow patterns expected from N assimilation energies (i.e., NH$_4^+$ > NO$_3^-$ > N$_2$) (Présing et al. 2001). The significant negative correlation between NH$_4^+$ and $\delta^{15}N-SPN$ (Table 3) indicates that the uptake of NH$_4^+$ in the Shaying River was dominant during summer (Bardhan et al. 2017; Owens 1985; Cifuentes et al. 1988; Cole et al. 2004). No significant correlation was found either between NO$_3^-$ and $\delta^{15}N-SPN$ or between NH$_4^+$ and $\delta^{15}N-SPN$, suggesting that assimilation process was weak during winter. However, Chl.a concentrations at most sites, except SH, in winter were still high, suggesting assimilation was an important N transformation process in winter. Because of the dam, the flow speed in this river was low, which was of beneficial for the occurrence of assimilation even in winter. However, high anthropogenic NH$_4^+$ loading along with the mild assimilation process resulted in assimilation that had little effect on NH$_4^+$ isotopic composition in winter.

Assimilation of NH$_4^+$, intensified by low flow speed, along with aerobic denitrification competed for NH$_4^+$, bringing about the weakness of nitrification in the Shaying River system in summer. Thus, N transformation in summer changed from traditional pattern A into pattern B (Fig. 8). Instead of turning into NO$_3^-$ through nitrification, NH$_4^+$ turned into N$_2$ through the aerobic denitrification process, or turned into organic N through assimilation. Nitrate in the river system, originated from sewage and manure or soil-N, turned into N$_2$

Fig. 8 The scheme of traditional N transformation pattern (pattern A) and the N transformation progress in summer in the Shaying River system (pattern B)
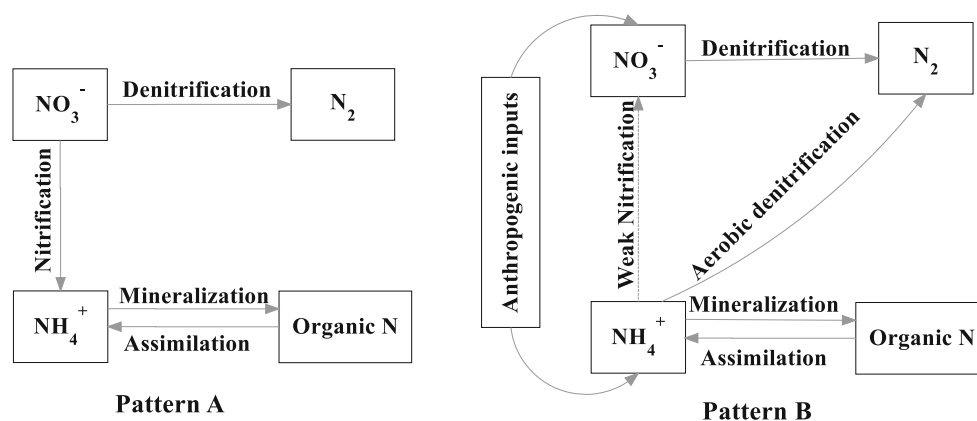
through denitrification. Consequently, heavy nitrogen pollution and the presence of dams caused a difference in the N transformation in the Shaying River.

Besides denitrification, nitrification, and assimilation, anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA) are also two important N transformation processes in rivers (Hou et al. 2013; Yin et al. 2017). However, processes of anammox and DNRA cannot be quantified by simply analyzing the isotopic composition of nitrate, ammonium, and SPN. Isotopic composition of NO_2^-, together with $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- were used to identify the process of DNRA (Hu et al. 2016), which was lacking in our study. Mostly, isotope-tracing method was applied to quantify the relevance of anammox and DNRA to nitrogen retention in soils and rivers (Hoagland et al. 2019; Bu et al. 2017b). Of course, the nitrogen isotopic characteristics can be altered by anammox and DNRA, thus affecting the identification of nitrogen sources. For instance, DNRA produce a strong enrichment in both $\delta^{15}N$ and $\delta^{18}O$ in the residual NO_3^- (Hu et al. 2016).

Uncertainty analysis

In this study, the sample sizes for each site were small, which might result in insufficient data to support the conclusion. Also, the nitrogen isotopic composition fraction caused by bio-chemical processes was ignored in the SIAR mixing model, which might lead to uncertainty of the quantification of NO_3^- sources.

Conclusions

Our results demonstrate that point source (household and industrial sewage, and manure), not the non-point source, was the dominant source for nitrate, ammonium, and SPN in the Shaying River. Isotope characteristics of nitrate and the SIAR mixing model reveal manure and sewage were

the dominant nitrate source in both summer ($62.44 \pm 19.66\%$) and winter ($67.33 \pm 15.45\%$), and soil organic nitrogen generated substantial amounts of nitrate in summer ($24.94 \pm 15.52\%$) and winter ($26.33 \pm 9.45\%$). Data of $\delta^{15}N\text{-}NH_4^+$ (-7.54% to 20.92%) suggest that septic/manure and household waste was the main source to ammonium in most sampling sites in both summer and winter. Most wastewater discharged into the river was untreated, which was one of the main reasons for the high level of ammonium in winter. $\delta^{15}N$-SPN, ranging from 0.78 to 13.51‰, suggest that SPN was mainly from industrial and domestic sewage at most sites in both seasons. Nitrogen pollution, along with the dams, caused the difference in the N transformation in the river. Significant assimilation of NH_4^+ and aerobic denitrification competed for NH_4^+, resulting in the weakness of nitrification in summer. Denitrification was an important anthropogenic nitrate removal process in summer, while nitrification was a key N transformation process in the river in the winter. Accelerated urban development resulting from rapid population migration from rural to urban lands has led to high level of untreated sewage directly entering the Shaying River, which has caused the high level of nitrogen in the Shaying River. We recommend that more efforts should be directed toward the reduction of domestic and industrial waste discharges and manure.

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