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Analysis of $\delta^{15}N$ and $\delta^{18}O$ to identify nitrate sources and transformations in Songhua River, Northeast China

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SUMMARY

To understand the sources and transformations of nitrate in the Songhua River basin, which is one of seven largest river basins in China, the concentration of dissolved nitrogenous species, nitrogen and oxygen isotopes of NO_3^-, nitrogen isotopes of NH_4^+, and stable isotopes of water were determined in this study. Low NO_3^- concentrations and a high dissolved organic nitrogen/total dissolved nitrogen ratio (DON/TDN) were observed in the Nen River and other rivers originating from the mountains, which are covered by forest. NO_3^- and DON were the major nitrogenous compounds in aquatic systems, accounting for the TDN being about 90% during high flow season and about 85% during low flow season, respectively. The nitrogen efflux for the entire basin was estimated to be approximately 1.17×10^5 tons/yr, which represents an annual N output of 0.21 ton/(km^2 yr). The majority of the $\delta^{18}O$-NO_3^- values were between −4‰ and 4‰, reflecting nitrification. During the high flow season, the isotopic compositions of NO_3^- and the water chemistry suggest that NO_3^- in the Nen River was mainly derived from soil organic nitrogen (SON), whereas NO_3^- in the Songhua River originated from organic nitrogen, nitrogenous fertilizers and sewage waters. NO_3^- in the low flow season samples generally originates from SON and sewage waters. Moreover, the calculated loss of nitrate via the mass budget in rivers, together with isotopic values and water chemistry confirm that denitrification occurs during the high flow season, especially in the Songhua River. This study suggests that the mass calculation and isotopic proof provide a better understanding for riverine N budget and biogeochemical processes.

1. Introduction

The biogeochemical cycles of nitrogen plays an important role in the terrestrial and aquatic ecosystems. The N cycle is currently focused by numerous ecological and environmental researchers due to the influence of human activities, which has increased the formation rate of reactive N and greatly altered N cycling in terrestrial and aquatic ecosystems (Mayer et al., 2002; Galloway et al., 2004; Sebilo et al., 2006; Tobari et al., 2010). High concentrations of NO_3^- in drinking water are considered to be harmful to human health and may cause serious disease, such as methemoglobinemia in infants (Fewtrell, 2004). It is also a major reason for eutrophication in aquatic ecosystems (Galloway et al., 2004). NO_3^- has several sources including atmospheric deposition, nitrogenous fertilizers, animal manure, discharge of domestic sewage and soil organic nitrogen (SON). The effective management of NO_3^- to preserve water quality requires identification of actual N sources and an understanding of the processes affecting local NO_3^- concentrations. However, the determination of N concentrations cannot be solely used in order to detect the specific N sources and biogeochemical processes.

Stable nitrogen isotope techniques, enable the identification of sources based on the characteristic or distinctive nitrogen isotope compositions and are valuable tools for the detection of the origin of NO_3^- in water (Kellman and Hillaire-Marcel, 2003; Kendall et al., 2007; Koba et al., 1997; Li et al., 2010; Panno et al., 2008; Sebilo et al., 2006; Wexler et al., 2012). However, the chemical, biological and physical processes that accompany isotopic fractionation in the N cycle alter the original source characteristics and some sources had overlapping $\delta^{15}N$-NO_3^- values (Kendall et al., 2007). Accordingly, a dual isotope approach using both $\delta^{15}N$-NO_3^- and

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$\delta^{18}O\text{-}NO_3^-$ can provide more conclusive source information based on the wide range of $\delta^{18}O\text{-}NO_3^-$ in atmospheric deposition, NO_3^- fertilizers and soil organic nitrogen (SON) (Mayer et al., 2002; Deutsch et al., 2006). Therefore, dual isotopes of NO_3^- have been widely applied to identify the sources and understand the N cycle (Mayer et al., 2002; Tobari et al., 2010). Many scientists have successfully applied these techniques to case studies on aquatic ecosystems (Battaglin et al., 2001; Heaton et al., 2012; Li et al., 2010; Liu et al., 2006; Mayer et al., 2002; Spoelstra et al., 2004; Wassenaar, 1995; Widory et al., 2005).

The Songhua River Basin is one of seven primary river basins in China and the largest tributary of the Amur River. Two major agricultural regions of Northeast China, Sanjiang Plain and Songnen Plain, are also located within the basin. The study area hosts important grain farming areas (Yin et al., 2006), which have an environmental impact onto the aquatic systems in the study area. Studies of heavy metals and toxic organic pollutants in the Songhua River system have been conducted (Li et al., 2006; Lin et al., 2008; Wang et al., 2012). However, sources and transformation of NO_3^- in the river systems of Northeast China have not been thoroughly investigated based on the dual isotopes of NO_3^- and water chemistry. Therefore, in this study, we analyzed the chemical parameters, composition of dissolved nitrogen, dual isotopes of NO_3^- and water isotopes in the Songhua Rivers. The results were used to describe the seasonal and spatial variation of nitrogen species and dual isotopes of NO_3^-.

2. Material and methods

2.1. Study area

The Songhua River basin is located in NE China at 41°42′–51°38′N latitude and 119°52′–132°31′E longitude (Fig. 1). The entire study area of this river basin is about 55.7×10^4 km^2, with a length of 920 km and a width of 1070 km. The water system of Songhua River flows through Heilongjiang Province, Jilin Province and Inner Mongolia Autonomous Region, including the Upper Reach of Songhua River (URSHR), the Nen River (NR) and main stream of the Songhua River (SHR). The mainstream river length is over 939 km from the confluence of the Upper Reach of Songhua River and the Nen River. The south head water of the rivers is the Upper Reach of Songhua River, originating in the Changbai Mountains with an altitude of more than 2700 m. The north head water of the rivers is the Nen River, originating from the Daxingan and the Xiaoxingan Mountains with an altitude of more than 1000 m. After the confluence, the river is named as Songhua River, which is one of the biggest tributary of Amur River.

Mountains, hills, and plains account for 61%, 15% and 24% of the terrain in the study area, respectively. The mountains are mainly distributed on the boundaries of the basin, with the Changbai Mountains in the east, the Daxingan Mountains in the west, and the Xiaoxingan Mountains in the north. The forest and grassland are mainly distributed in the mountains and hills. The Songnen Plain and Sanjiang Plain are major agricultural areas in the basin, which show huge amounts of black soils. The crop-growing season in this area is generally from May to September and the majority of crops are paddy, corn and soybean. The major cities in the basin, such as Daqing, Harbin, Changchun, are located at the Songnen Plain. The Sanjiang Plain is located at the east of this basin, near the mouth of the Songhua River. The aquifer of two plains is mainly covered by the Quaternary alluvial deposits with a varying thickness from 30 m to 300 m. The aquifer consists principally of sandy gravels, medium and fine sands, sandy clays and silts (Zhang et al., 2004; Liu et al., 2012).

The Songhua River basin is located at the junction of the temperate and cold-temperate zones. In this basin, the atmospheric temperature is generally above 20 °C in summer and below 0 °C in winter. The study area is mainly influenced by the monsoonal climate, which has relative high frequent rain in summer. The precipitation in the mountains in the southeast part of the basin is about 800 mm, whereas it is about 400 mm in the plains in the southwest of the basin. The mean annual precipitation in the entire basin is about 500 mm, although the precipitation shows significant spatial and temporal differences. The precipitation from May to September accounted for nearly 74% of the total rainfall in 2010 (NBS of China, 2011).

2.2. Sampling

Samples were collected from the main stream and major tributaries of three major rivers (Songhua River, Upper Reach of Songhua River and Nen River) in July and November of 2010, corresponding to the high and low flow season, respectively. 54 water samples from rivers were collected during high flow season (Fig. 1). During the low flow season, some parts of the rivers (especially the Nen River) were frozen. Therefore, water samples were selectively obtained and mainly distributed in the main stream during the low flow season. In addition, 3 precipitation samples (rain and snow, Table 1) and 3 sewage samples were collected during the sampling periods. Sampling containers were washed with HCl acid and thoroughly rinsed prior to sampling. Water samples were filtered through 0.22 μm cellulose-acetate filter paper and then stored in the pre-washed polyethylene bottles in the refrigerator before analysis of nutrients and isotopes.

2.3. Analytical procedure

The water parameters (temperature, pH and electric conductivity (EC)) were measured at the sample site using portable sensors. Anions (Cl^-, SO_4^{2-}, NO_3^-) were determined by ionic chromatography using a Dionex ICS-90 (USA). The nutrient concentrations were measured using an automatic flow analyzer (SKALAR Sans Plus Systems) (EPA of China, 2002).The total dissolved nitrogen (TDN) was digested using alkaline potassium persulfate and analyzed spectrophotometrically after the reduction of NO_3^- to NO_2^-. The concentration of NH_4^+ was determined using sodium salicylate-sodium hypochlorite spectrophotometry. The concentration of dissolved organic N (DON) was calculated using the amounts of TDN and DIN (the sum of NO_3^-, NO_2^- and NH_4^+).

The samples used for the stable isotope analysis ($\delta^{18}O$, δD) were measured using a Finnigan MAT-253 isotope-ratio mass spectrometer. δD and $\delta^{18}O\text{-}H_2O$ have a precision of 1‰ and 0.2‰. The dual stable isotopic compositions in $NO_3^- + NO_2^-$ were analyzed by quantitative bacterial reduction of $NO_3^- + NO_2^-$ to nitrous oxide (N_2O) using a strain of denitrifier (*Pseudomonas chlororaphis* subsp. *aureofaciens* ATCC 13985), that lacks N_2O-reductase enzyme, followed by automated extraction, purification using Trace Gas Pre-concentrator unit and analysis of the N_2O product by an isotope ratio mass spectrometer. The process of culture and pretreatment followed the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002). The bacterium cells were concentrated 5-fold, and then split into 3-mL aliquots in 20-mL headspace vials. After purging with high-purity N_2 to ensure anaerobic conditions, a sample amount corresponding to 50 nmol of NO_3^- was then injected into sample vials and cultured overnight to allow complete conversion. Four international nitrate (USGS-32, USGS-34, USGS-35 and IAEA-N3) and experimental reference materials that were treated identically to the water samples were used to calibrate the measured sample data. For the $\delta^{18}O\text{-}NO_3^-$ correction, the Wankel et al.'s (2010) method for one sewage sample (S1) with

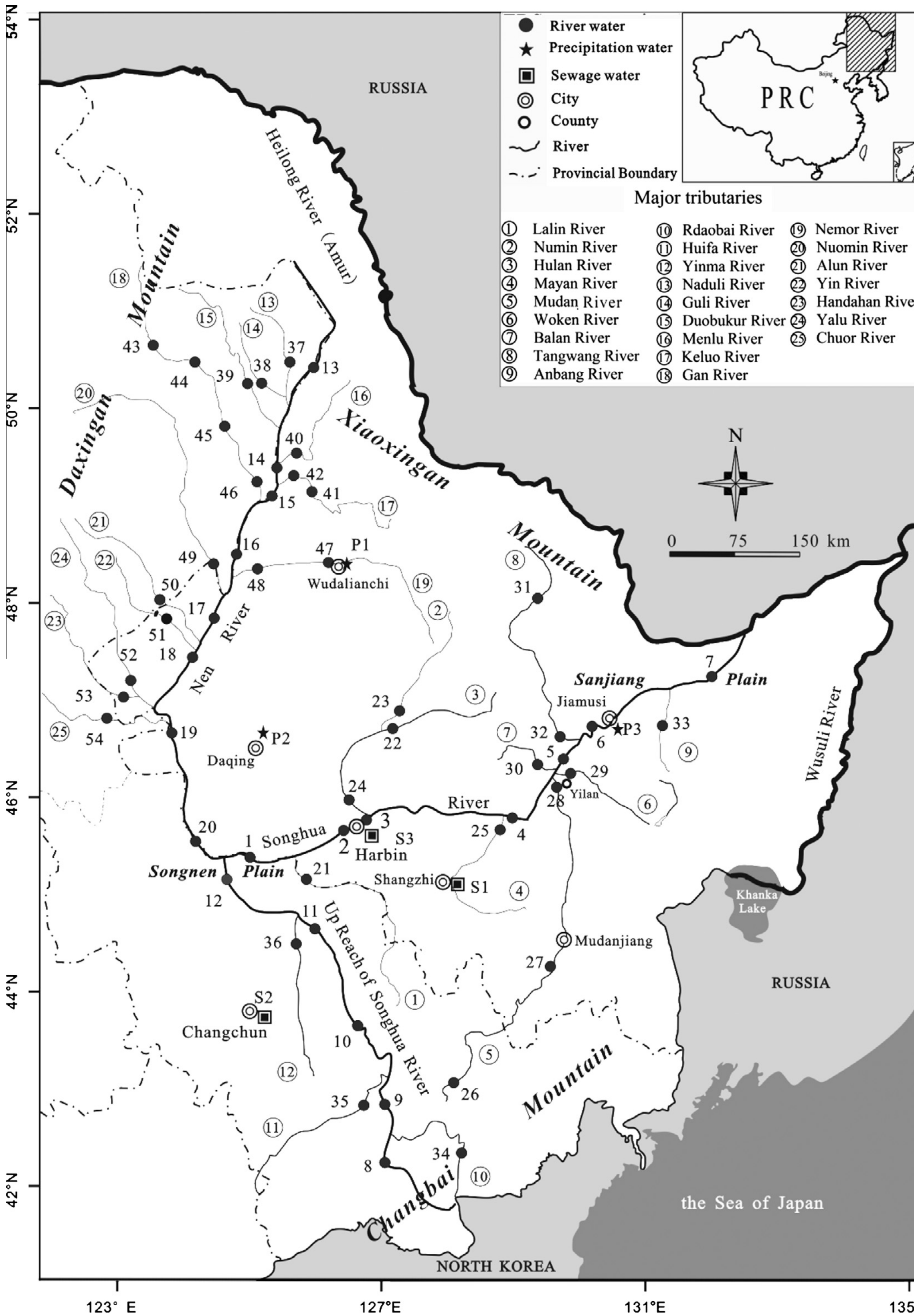

Fig. 1. Location of Songhua River basin and sampling sites modified from Liu et al. (2013). The Nos. 1–7 and 21–33 belongs to the Songhua River (SHR), Nos. 8–12 and 34–36 belongs to the Upper Reach of Songhua River (URSHR), Nos. 13–20 and 37–54 belongs to the Nen River (NR).

high concentration of NO_2^- was used. Each sample was measured in duplicate and the standard error was 0.3‰ for $\delta^{15}N\text{-}NO_3^-$ and 0.5‰ for $\delta^{18}O\text{-}NO_3^-$. For $\delta^{15}N\text{-}NH_4^+$ 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 a week, after which the trap was freeze dried and EA-MS was used to determine the isotopic compositions with a precision of 0.2‰ for $\delta^{15}N$ values. The isotopic characteristics of samples with low concentrations of NH_4^+ during the high flow season could not be determined.

Isotopic values are reported using delta (δ) expressing:

$$\delta(‰) = ((R_{sample}/R_{standard}) - 1) * 1000$$

Table 1
Concentration of chemical parameters, nitrogen species and the environmental isotopic ratios in water samples.

No.	Locations/type	[a]Cl^- mg/L	DON-N	NO_2^-	[a]NO_3^-	NH_4^+	$\delta^{15}N$-NO_3	$\delta^{18}O$-NO_3 ‰	$\delta^{15}N$-NH_4^+	δD-H_2O	$\delta^{18}O$-H_2O
The high flow season samples											
Mainstreams											
1	Songhua River	11.2	2.14	BDL	6.41	0.03	6.7	−1.3	–	−81.7	−11.3
2	Songhua River	11.6	3.21	0.13	7.96	0.10	6.3	0.1	–	−82.0	−11.0
3	Songhua River	11. 7	0.32	BDL	7.32	0.03	4.8	0.6	–	−82.2	−11.2
4	Songhua River	8.28	0.49	0.01	1.49	0.01	8.7	1.2	–	−82.2	−11.3
5	Songhua River	6.02	0.56	0.01	0.06	0.08	9.2	−0.1	–	−85.4	−11.8
6	Songhua River	7.59	0.25	BDL	3.54	0.03	4.3	0.4	–	−65.2	−9.2
7	Songhua River	8.97	0.51	0.01	6.18	0.04	6.9	−2.6	–	−84.9	−11.5
8	UPSHR	2.52	0.85	BDL	5.22	0.23	8.6	0.8	–	−91.3	−13.0
9	UPSHR	2.64	0.34	BDL	5.26	0.04	5.7	5.9	–	−89.0	−12.7
10	UPSHR	6.17	0.08	BDL	7.78	0.05	8.3	9.6	–	−85.1	−12.2
11	UPSHR	11.6	0.42	BDL	13.2	0.04	0.9	−0.7	–	−82.4	−11.6
12	UPSHR	16.2	3.50	BDL	13.0	0.04	8.1	2.9	–	−78.0	−11.1
13	Nen River	0.23	0.85	0.14	0.16	0.09	5.3	−1.6	–	−84.5	−12.0
14	Nen River	0.49	0.46	0.02	0.05	0.05	5.6	−0.5	–	−87.6	−11.9
15	Nen River	0.54	0.43	0.01	0.08	0.03	1.8	1.5	–	−87.8	−12.1
16	Nen River	1.37	0.52	0.01	0.14	0.04	3.9	−0.7	–	−90.5	−12.6
17	Nen River	1.31	0.41	BDL	0.12	0.05	2.3	4.0	–	−92.1	−12.7
18	Nen River	2.53	1.02	0.01	1.26	0.03	1.5	−0.6	–	−78.5	−10.0
19	Nen River	2.58	0.37	0.01	0.81	0.04	4.8	−3.6	–	−87.6	−11.9
20	Nen River	3.23	0.98	0.01	1.27	0.04	2.9	−3.7	–	−86.8	−11.8
Tributaries of Songhu River											
21	Lalin River	15.2	7.35	0.07	4.78	0.21	9.6	−6.0	–	−74.5	−9.7
22	Hulan River	7.95	0.65	0.01	0.25	0.05	7.1	−6.0	–	−80.0	−10.7
23	Numin River	8.14	0.38	0.01	0.01	0.03	–	–	–	−74.3	−9.3
24	Hulan River	14.3	1.53	0.02	11.5	0.13	3.2	−3.2	–	−75.3	−9.6
25	Mayi River	10.8	0.31	0.01	0.16	0.03	2.2	11.0	–	−80.7	−10.8
26	Mudan River	2.12	0.14	BDL	1.81	0.01	8.5	1.0	–	−85.4	−12.3
27	Mudan River	6.26	0.62	0.01	3.71	0.64	8.2	1.4	–	−80.6	−11.5
28	Mudan River	5.65	0.26	BDL	4.97	0.05	6.8	6.4	–	−92.4	−13.0
29	Woken River	11.8	1.26	0.01	0.8	0.04	7.3	−1.7	–	−80.9	−10.4
30	Balan River	1.58	–	BDL	4.43	BDL	4.4	1.6	–	−80.7	−10.4
31	Tangwang River	1.36	3.45	0.05	0.85	0.19	7.9	0.3	–	−94.8	−13.3
32	Tangwang River	2.49	0.16	BDL	2.11	0.03	6.2	0.2	–	−93.3	−13.0
33	Anbang River	28.3	0.05	BDL	20.9	0.01	0.3	−2.1	–	−82.2	−10.4
Tributaries of UPSHR											
34	Seconddao River	6.86	0.66	BDL	0.8	0.05	0.3	−5.9	–	−96.2	−13.8
35	Huifa River	8.99	0.90	BDL	17.6	0.04	5.4	1.7	–	−79.4	−11.3
36	Yinma River	19.1	1.57	0.01	4.82	0.04	4.3	0.6	–	−69.1	−9.3
Tributaries of Nen River											
37	Naduli River	0.40	5.20	0.07	0.1	0.55	0.8	−3.5	–	−85.8	−12.2
38	Guli River	0.12	0.72	0.01	0.02	0.04	–	–	–	−82.0	−10.6
39	Duobukuer River	0.52	0.33	BDL	BDL	0.04	–	–	–	−92.5	−14.0
40	Menlu River	0.88	0.18	BDL	0.58	0.01	3.7	−0.5	–	−85.5	−12.0
41	KeluoRiver	2.93	0.24	BDL	1.61	0.03	–	–	–	−49.5	−5.8
42	Keluo River	3.11	0.50	BDL	0.14	0.05	4.7	−4.4	–	−83.7	−11.1
43	Gan River	0.60	0.36	0.01	0.01	0.06	–	–	–	−102.4	−14.5
44	Gan River	0.62	0.46	0.01	0.34	0.06	11.7	−2.3	–	−98.1	−13.9
45	Gan River	1.05	0.36	0.01	0.05	0.06	–	–	–	−97.5	−13.7
46	Gan River	2.30	0.33	0.01	0.95	0.14	1.3	−2.0	–	−95.1	−13.3
47	NamoRiver	2.29	3.73	0.09	1.01	0.99	2.3	−1.2	–	−86.2	−12.1
48	NamoRiver	3.29	1.17	BDL	0.48	0.06	2.3	2.1	–	−79.2	−10.8
49	Nuomin River	1.16	2.22	0.03	1.73	0.72	11.2	0.0	–	−94.8	−13.2
50	Alun River	2.51	0.06	BDL	3.69	0.03	1.8	0.0	–	−91.5	−12.6
51	Yin River	5.83	0.23	BDL	BDL	0.06	–	–	–	−74.0	−9.4
52	Yalu River	3.78	0.22	BDL	1.05	0.05	7.4	2.5	–	−88.5	−12.5
53	Handahan River	2.46	0.99	0.03	7.07	0.26	8.5	3.1	–	−74.9	−10.5
54	Chuoer River	1.65	0.26	BDL	0.09	0.06	6.9	1.0	–	−90.1	−12.2
The low flow season samples											
1	Songhua River	14.0	2.82	0.09	6.97	0.66	8.7	0.2	25.1	−84.8	−11.4
2	Songhua River	11.8	3.11	0.07	8.54	0.54	7.6	−4.0	12.4	−82.3	−11.6
3	Songhua River	12.2	4.69	0.09	7.47	0.75	7.2	3.6	25.8	−81.7	−11.6
4	Songhua River	12.3	0.50	–	7.37	0.30	8.3	−1.3	11.8	−82.6	−11.5
5	Songhua River	16.4	5.63	0.08	5.88	1.07	8.7	−2.8	14.8	−82.7	−11.5
6	Songhua River	12.5	0.63	0.11	6.97	0.44	7.3	−2.9	10.3	−78.8	−9.9
28	Mudan River	69.9	1.73	0.08	105.8	4.10	18.5	6.7	7.3	−80.8	−11.4
10	UPSHR	12.1	4.61	0.09	16.6	0.77	9.6	6.9	8.1	−80.9	−11.6
11	UPSHR	8.83	0.79	0.07	13.9	0.04	8.1	−0.1	–	−76.7	−11.1
12	UPSHR	16.4	1.00	0.06	13.5	0.90	8.5	1.2	25.3	−78.6	−11.2
19	Nen River	4.58	0.42	–	2.24	0.59	7.8	11.9	8.5	−87.2	−12.0

Table 1 (*continued*)

No.	Locations/type	[a]Cl^- mg/L	DON-N	NO_2^-	[a]NO_3^-	NH_4^+	$\delta^{15}N$-NO_3	$\delta^{18}O$-NO_3 ‰	$\delta^{15}N$-NH_4^+	δD-H_2O	$\delta^{18}O$-H_2O
20	Nen River	3.77	0.28	–	1.85	0.72	6.4	0.5	3.1	−89.4	−12.2
Sewage and precipitation samples											
S1	Shangzhi, sewage	48.8	43.3	2.40	0.4	6.90	9.4	16.2	8.3	−83.0	−11.5
S2	Changchun, sweage	50.2	26.1	0.93	1.03	9.78	7.1	−3.5	6.4	−71.3	−10.0
S3	Harbin, sweage	110	62.9	0.52	2.26	28.7	−1.5	3.6	4.5	−82.4	−11.9
P1	Wudalianchi, rain	0.17	–	–	3.96	–	−6.1	61.5	–	−64.2	−9.8
P2	Daqing, rain	0.23	–	–	4.77	–	−2.2	47.3	–	−71.6	−10.0
P3	Jiamusi, snow	4.27	–	–	2.65	0.19	7.9	83.5	−14.6	−141.4	−20.6

[b]"URSHR" stands for the Upper Reach of Songhua River; "BDL" stands for below detection limit of 0.005 mg/L of NH_4^+-N, 0.02 mg/L of NO_3^- and 0.002 mg/L of NO_2^--N; "–" stands for not determined.

[a] Data during the high flow season from Liu et al. (2013).

where R = D/H, $^{15}N/^{14}N$ or $^{18}O/^{16}O$. The ratio of $^{15}N/^{14}N$ reference is N_2 in air, the D/H and $^{18}O/^{16}O$ reference is Vienna standard mean ocean water (VSMOW).

3. Results

3.1. The isotopic composition of water

The δD and $\delta^{18}O$ values of river water show isotopic values ranging from −102.4‰ to −49.5‰ (n = 54, mean −84.5‰) and from −14.5‰ to −5.8‰ (n = 54, mean −11.6‰), respectively, during the high flow season (Table 1). The δD-H_2O and $\delta^{18}O$-H_2O values are plotted in a diagram (Fig. 2). The local meteoric water line (LMWL) with $\delta D = 7.2\delta^{18}O - 2.39$ was generated by the published database in the Songhua River basin (Li et al., 2012). The LMWL of the Songhua plain differs clearly from the global meteoric water line (GMWL) with $\delta D = 8\delta^{18}O + 10$ (Craig, 1961). The Nen River showed relatively low average isotope values of H_2O, with −87.9‰ for δD and −12.2‰ for $\delta^{18}O$ (except sample No.41). The Upper Reach of Songhua River revealed mean isotopic values for δD and $\delta^{18}O$ of −83.8‰ and −11.9‰, respectively. The main stream and tributaries of the Songhua River had high average isotopic values of −81.9‰ for δD and −11.1‰ for $\delta^{18}O$. The relationship between δD and $\delta^{18}O$ were highly significant during the high flow season, with values of $\delta D = 5.8\delta^{18}O - 17.3$ (n = 26, R^2 = 0.97) for the Nen River, $\delta D = 6.2\delta^{18}O - 10.5$ (n = 8, R^2 = 0.99) for the Upper Reach of Songhua River and $\delta D = 5.4\delta^{18}O - 22.5$ (n = 20, R^2 = 0.88) for the Songhua River. The isotopic compositions of water in the low flow season ranged from −89.6‰ to −76.7‰ (n = 12, mean −82.2‰) for δD and from −12.2‰ to −9.9‰ (n = 12, mean −11.4‰) for $\delta^{18}O$.

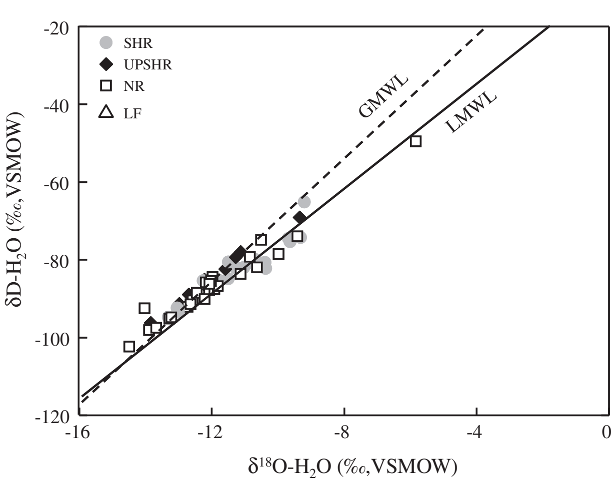

Fig. 2. Scatter plots for the correlation correlation of δD-H_2O verus $\delta^{18}O$-H_2O in the Songhua rivers during the two flow seasons. SHR stands for the Songhua River samples; URSHR stands for the Upper Reach of Songhua River samples; NR stands for the Nen River samples; LF stands for Low Flow samples.

3.2. The concentration of nitrogenous species

The concentrations of NO_3^- in the river samples ranged from below detection limit (BDL) to 20.9 mg/L during the high flow season. The NO_3^- levels showed significant spatial variation (Fig. 3 a). The NO_3^- contents in the samples from the Upper Reach of Songhua River were all higher than those in other rivers. The average NO_3^- contents in the Nen River, Upper Reach of Songhua River and Songhua River during the high flow season were 0.99 ± 3.14 mg/L, 8.47 ± 11.24 mg/L and 4.70 ± 10.01 mg/L, respectively. A similar variation among the river samples was observed during the low flow season, which displayed NO_3^- concentrations of from 1.03 mg/L to 105.8 mg/L (No.28). A correlation between the concentrations of the samples from the high flow season and the low flow season can be found as: $[NO_3]_{LF} = [NO_3]_{HF} * 0.79 + 3.78$ (not shown, R^2 = 0.64). The obtained NO_3^- contents for the samples collected during the low flow season were all higher than during the high flow season.

Two other important inorganic nitrogen compounds (NH_4^+ and NO_2^-) were present in low concentrations and showed no spatial variation during the high flow season. Specifically, NH_4^+ and NO_2^- ranged from BDL mg/L to 1.0 mg/L and from BDL to 0.14 mg/L during the high flow season, respectively. Unlike NO_3^-, the temporal variation of these compounds was significant, especially that of NH_4^+. The NH_4^+ and NO_2^- concentrations ranged from 0.03 mg/L to 4.1 mg/L and from BDL to 0.11 mg/L, respectively, during the low flow season. All samples that were collected during the low flow season had significantly higher NH_4^+ levels than those collected in the high flow season. The DON showed average concentrations of 1.03 ± 2.79 mg/L in the high flow season and 2.19 ± 3.85 mg/L in the low flow season, respectively. The average DON contents in the three major rivers during the high flow season were 1.24 ± 3.57 mg/L (Songhua River), 1.04 ± 2.18 mg/L (Upper Reach of Songhua River) and 0.87 ± 2.33 mg/L (Nen River) (Fig. 3a). No spatial and temporal variations for DON were observed. However, during the high flow season, the average DON/TDN values in the three major rivers were 52.4%, 35.4% and 73.0% for the Songhua River, the Upper Reach of Songhua River and the Nen River, respectively. DON was the dominant nitrogen species in both the main stream and tributaries of the Nen River. DON and NO_3^- were the two major N species in both the Songhua River and the Upper Reach of Songhua River. The average ratio of these two N compounds versus total dissolved nitrogen were about 90% in the high flow season and about 85% in the low flow season, which indicates that DON and NO_3^- were the major nitrogenous compounds in the collected river water samples.

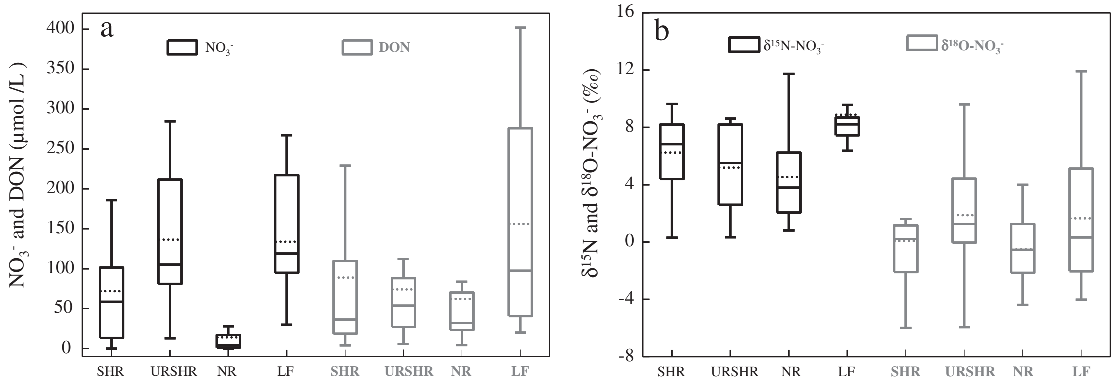

Fig. 3. (a) Boxplot for NO_3^- and DON concentration; (b) Boxplot for $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- the edges of the box represent the 75th and 25th percentiles respectively. The dashed line in the box represents the mean value. The solid line in the box represents the median value. The branch gives the range of the data except for the outliers. All the river samples were analyzed except No. 28 for the NO_3^- in the low flow season.

Additionally, three sewage samples revealed higher NH_4^+ contents than the river samples, but relative low NO_3^- concentrations (average = 1.23 ± 2.14 mg/L). NO_2^- showed low contents in the river samples, but high contents in sewage effluent. Moreover, the sewage samples with concentration values from 26.1 mg N/L to 62.9 mg N/L revealed higher DON contents than the river samples and represent the dominant nitrogen species in the sewage water.

3.3. The isotopic composition of nitrogenous species

During the high flow season, the $\delta^{15}N$-NO_3^- values ranged from 0.3‰ to 11.7‰ with a median value of 5.3‰ (n = 47), while the $\delta^{18}O$-NO_3^- values ranged from −6.0‰ to 11.0‰ with a median value of 0.0‰ (n = 47). The Songhua River showed higher average $\delta^{15}N$-NO_3^- values (6.3‰) than the Nen River, which displayed relatively low average $\delta^{15}N$-NO_3^- values (4.5‰) during the high flow season (Fig. 3b). During the low flow season, the $\delta^{15}N$-NO_3^- were generally higher and characterized by a smaller range from 6.4‰ to 9.6‰ with a median value of 8.2‰ (n = 11, except No. 28) compared to the high flow season, while the $\delta^{18}O$-NO_3^- ranged from −4.0‰ to 11.9‰ with a median value of 0.3‰ (n = 12) (Fig. 3b).

The $\delta^{15}N$-NH_4^+ displayed a wide range from 3.1‰ to 25.8‰ with a median value of 11.8‰ (n = 11). As indicated in Fig. 4, most samples had $\delta^{15}N$-NO_3^- values below 10‰. The isotopic values were primarily distributed in two ranges during the high flow season, 2–8‰ and greater than 8‰. During the low flow season, the river water samples showed higher $\delta^{15}N$-NO_3^- values compared to the high flow season and ranged primarily between 6‰ and 10‰. The $\delta^{18}O$-NO_3^- values scattered in the low flow season, while the $\delta^{18}O$-NO_3^- values in the high flow season were primarily between −4‰ and 4‰.

4. Discussion

4.1. The sources and pathways of water as determined by a dual isotopic approach

The stable isotopes of water are an ideal conservative geochemical tracer that can provide essential information about the origin of the water as well as hydrogeological processes (Gammons et al., 2006; Kendall and Coplen, 2001; Wassenaar et al., 2011). The air mass in the Songhua River basin is mainly from monsoon from the Pacific Ocean in summer and the westerly moisture in winter (Li et al., 2012). The hydrogen and oxygen isotopic composition of most river water samples collected in the study area was in agreement with the local meteoric water line indicating the dominance of local precipitation. The regression line for river water

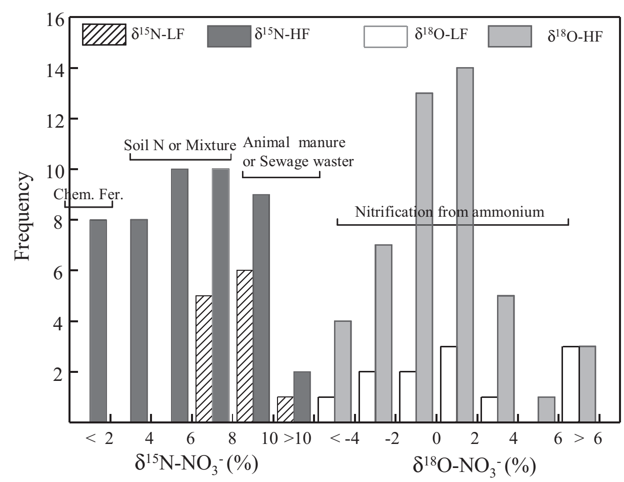

Fig. 4. Histograms of $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- in Songhua rivers (the isotopic compositions of various sources refer to Kendall et al., 2007). HF = the high flow season, LF = the low flow season.

from the Songhua River was generated using the results of the water isotopic analyses [$\delta D = 5.6\delta^{18}O - 19.0$ (R^2 = 0.92, not shown)]. The slope of the regression line for the Songhua River is lower than that of the Local Meteoric Water Line. It might be caused by evaporation of surface water increasing the oxygen and hydrogen isotopic values of the residual fraction and generating the systematic deviation from the Local Meteoric Water Line. Another possible reason might be part of local air mass contributing to precipitation (Li et al., 2012). The head waters from the west and south mountains showed generally relatively low isotopic values due to the altitude effect. The sample (No. 41) has the highest oxygen and hydrogen isotopic values showing large difference with adjacent samples, which reflect the evaporation effects. Moreover, the average δD-H_2O and $\delta^{18}O$-H_2O values of the mainstream of the Songhua River were slightly higher than those in the Nen River and the Upper Reach of Songhua River There is approximately 1‰ difference of $\delta^{18}O$-H_2O in mainstream waters among three rivers, which would indicate that no strong evaporation occur in the main channel.

4.2. The variation of contents and isotopes of nitrogenous species in the Songhua River Basin

The concentration of specific nitrogenous species that play important roles in the aquatic ecosystem can be used to indicate the level of health and potential environmental problems, especially for water quality (Fewtrell, 2004; Galloway et al., 2004). The Environmental Quality Standards for Drinking Water (GB5749-2006, China) suggest that the concentration of NO_3^- and NH_4^+ in drinking water should be lower than 10 mg N/L and 0.5 mg N/L, respectively. In this study, all river samples revealed concentrations of NO_3^- <10 mg N/L except one sample (No. 28). More than 10 water samples from rivers passing city areas showed NH_4^+ concentration higher than 0.5 mg N/L reflecting the effect of urban pollution.

In the studied area, waters in these areas have low contents of nitrate, especially in the Nen rivers (Fig. 5a and b). The rivers that form the Nen River originate in the Daxingan and Xiaoxingan Mountains, which are covered by native forest and not heavily affected by anthropogenic activities. The nitrogenous species in the Nen River watershed show low concentrations and high DON/TDN ratios. In rivers, the majority of DON is derived from terrestrial leaching and runoff, and consists mainly of humic substances (Perakis and Hedin, 2002). Up-stream of the Nen River (above sample location No. 15), the rivers flow through a mountain zone covered by vegetation, which prevents soil erosion and causes only a low nutrient input into the rivers. The middle part of the stream (between locations Nos. 15 and 16) represents the transition zone from the mountains to the plains and hosts only a few rivers merging with the main stream. The river passes then the Songnen Plain and widens out. Although the downstream portion of the Nen River watershed partly flows through an agricultural area (Songnen Plain), the low anthropogenic input causes low concentrations of DIN. Moreover, river samples show high concentrations of DON (Nos. 37, 48, 49, 53) or high DON/TDN ratios (except five samples Nos. 41, 46, 50, 51, 53) probably caused by humus-rich black soil and forest soil in the Nen River basin.

The Upper Reach of Songhua River can be divided into four zones, the headwater zone, upstream zone, hill zone and downstream zone. The headwater zone is characterized by a high amount of vegetation and low nitrogen contents in rivers. The other three zones are located in the main agricultural area of Jilin Province. The NO_3^- concentrations were high in the samples from the tributaries that flowed through agricultural land, suggesting agricultural inputs. Most of the tributaries of the Songhua Rivers originated from the Changbai or the Daxingan Mountain which are covered with native forest, and then pass the agricultural area. The low N levels in the water samples from these rivers were likely related to low agricultural activities. In contrast, the water samples have relative high contents of nitrate, when the rivers pass the two plains, which are characterized by industrial and agricultural activities.

In the low flow season, generally higher NH_4^+ and NO_3^- concentrations could be observed compared to the high flow season (Fig. 5b and c, Table 1). The phenomenon might be caused by dilution effects due to frequent rainfall in summer. The high fraction of sewage water in the low flow season could lead to the high NH_4^+ concentrations in rivers during the low flow season. Meanwhile, the low temperature in winter might limit the oxidation of ammonia. Sample No. 28 which showed the highest concentration of NH_4^+ and NO_3^- during the low flow season, was probably heavily polluted by episodic industrial and municipal wastewater, probably originating from the inputs at the Yilan County and the Mudanjiang City of the third largest city in the basin. The DON contents displayed a wide variation, but did not show great variation between the two flow seasons. This may be caused by recharge from local hydrological storage and municipal wastewater as well as the degradation of organic matter.

As indicated by Fig. 5, the $\delta^{15}N\text{-}NO_3^-$ values varied widely from upper reach to lower reach in the main channel during the high flow season. However, the isotopic values of nitrate in waters reveal a relatively narrow range in the low flow season. The agricultural activities and the frequent rainfall in summer could be responsible for the different patterns in the isotopic compositions of nitrate due to rapid water mixing and flow as well as biological processes in summer under higher temperature conditions compared to winter. The chemical composition and isotopic values of NO_3^- in the rivers indicate that the nitrogenous compounds might be affected by land use in the catchment. Similar to the results of a study conducted by Mayer et al. (2002) for sixteen watersheds in the north eastern U.S.A, riverine NO_3^- enriched in light isotopes (Fig. 5e) in nitrate from the Nen River suggest SON as the primary source. Samples collected in these regions typically have low concentration in rivers from forested catchments, except three samples with high isotopic compositions (Nos. 44, 49, 53). The isotopic composition of NO_3^- in the first water sample of the main stream of the Songhua River behind the confluence with the Nen River was likely affected by the mixing between other two rivers based on the contents and isotopic compositions of nitrate. The

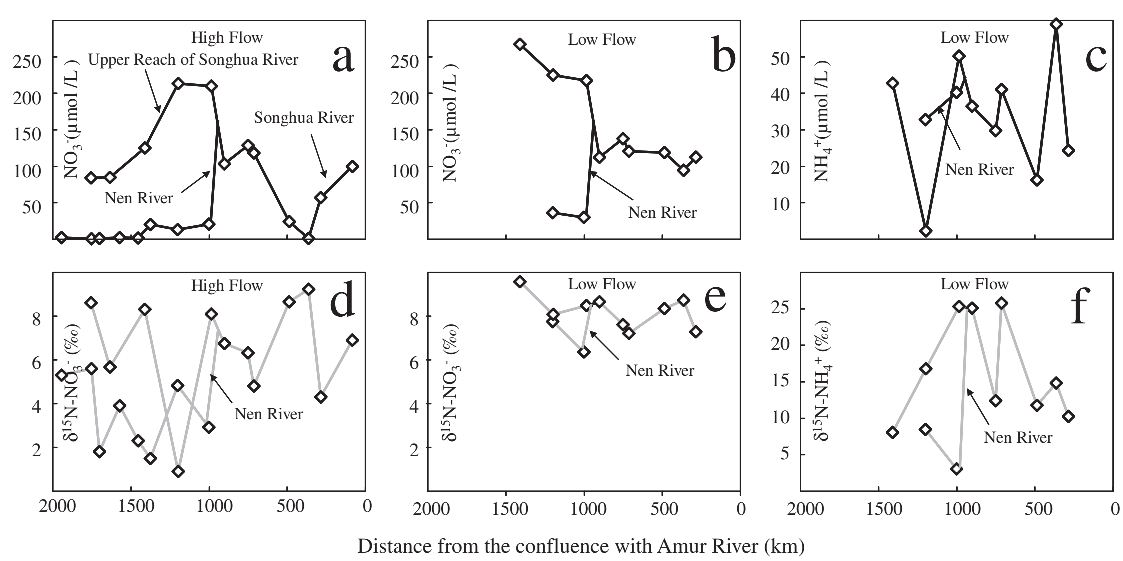

Fig. 5. Longitudinal plots of (a) NO_3^- in high flow season, (b) NO_3^- in low flow season, (c) NH_4^+ in low flow season, (d) $\delta^{15}N\text{-}NO_3^-$ in high flow season, (e) $\delta^{15}N\text{-}NO_3^-$ in low flow season and (f) $\delta^{15}N\text{-}NH_4^+$ in low flow season, referenced to kilometers from the confluence with the Amur River.

isotopic composition of NO_3^- in the main stream changed slightly downstream until sampling site No. 4, where a sample was taken showing a relatively low concentration and a high $\delta^{15}N-NO_3^-$ value.

Both the contents and isotopic values of NH_4^+ show a wide range without a distinct trend along the river flow (Fig. 5c and g). NH_4^+ was detected in waters, which passed cities and towns suggesting that the urban pollution was not managed well in this area. Ammonia volatilization and slow nitrification at low temperatures may have led to enrichments of heavy isotopes in the NH_4^+ (Kendall et al., 2007), which might be responsible for the high $\delta^{15}N-NH_4^+$ values in several water samples.

4.3. The N flux in three major rivers

The N flux from the Songhua River System was roughly estimated using the TDN and discharge values at the outlets of three major rivers and three tributaries located on the main reach of the Songhua River. Flux (F) is calculated by the concentration (C) and discharge (Q) through the equation: $F = C * Q$. F is the sum of the fluxes during the low flow (F_L) and high flow (F_H) periods. It is reported that the average proportion of precipitation during the high flow season (from May to September) in the studied area is about 74% of the entire year (NBS of China, 2011).

$$F = F_L + F_H = C_L * Q_L + C_H * Q_H = C_L * Q * 26\% + C_H * Q * 74\%$$

The TDN, which contains two fractions, DIN and DON, is described by $F_{DTN} = F_{DIN} + F_{DON}$. The downstream sample in the Songhua River (No. 7) was not collected during the low flow season, so sample No. 6 was used to estimate the N flux. We also roughly estimated the N export flux from the Nen River (No. 20) and the Upper Reach of Songhua River (No. 12) to the Songhua River.

The calculated results are listed in Table 2. The Upper Reach of Songhua River shows a relatively high contribution of nitrogen to the Songhua River compared with the Nen River. The sum of N flux in the Songhua River basin is about 1.17×10^5 ton/yr based on the discharge and concentration data, and the annual N output is 0.21 ton/(km^2 yr) for the entire basin. Statistical analyses revealed that the amount of synthetic fertilizer N used in the Songhua River basin is 1.85×10^6 ton/yr (NBS of China, 2011). Thus, the N flux via the Songhua River accounts for 6.3% of the synthetic fertilizer used in this basin. The management of water quality should be improved in order to reduce the nitrogen load in the Songhua rivers. The sewage effluent discharge into the Upper Reach of Songhua River and episodic pollution events at other rivers should be controlled and monitored.

It is calculated that the total fluxes of NO_3^- from the Upper Reach of Songhua River and Nen River is 1.33 times greater than the NO_3^- exported from the Songhua River during the high flow season. The N flux from the major tributaries of the Songhua River is 2.47 times higher than the export at the outlet of the Songhua River during the high flow season, suggesting the loss of a certain amount of nitrogen during transportation in the rivers. The unbalanced budget of the N flux suggests the existence of a nitrogen sink, such as denitrification and biological uptake during transportation in the river system.

4.4. The potential transformation of nitrogen species in the study basin

The isotopic composition of nitrate does not only depend on the different nitrate sources with their different isotopic compositions, but is also influenced by biological processes, such as nitrification and denitrification in aquatic systems. The oxygen isotopic composition of nitrate produced by nitrification is much lighter than nitrate from precipitation and chemical nitrate fertilizers (Kendall et al., 2007). The narrow range of $\delta^{18}O-NO_3^-$ values was found for samples from the mainstream of three rivers. Most water samples (>90%) from rivers showed $\delta^{18}O-NO_3^-$ values lower than 6‰ (Fig. 4). The $\delta^{18}O-NO_3^-$ values of precipitation in this study ranged from 47.3‰ to 83.5‰, which is in agreement with a previous study showing $\delta^{18}O-NO_3^-$ values generally above +60‰ (Kendall et al., 2007). The $\delta^{18}O$ of nitrate fertilizers is assigned as 23.5‰, which is close to that of atmospheric O_2 (Böttcher et al., 1990). In theory, NO_3^- formed by nitrification (microbial mediated oxidation) derives two of its oxygen atoms from water and one from dissolved O_2, which has a similar isotopic composition to atmospheric oxygen (Böttcher et al., 1990). As indicated by Fig. 6, most samples revealed $\delta^{18}O-NO_3^-$ values around this value, especially the Nen River samples, which suggests nitrification as the major source of nitrate in this area. The dilution effect during high flow season and nitrification under higher temperatures in summer compared to winter may be responsible for the low concentration of NH_4^+. However, several samples showed lower $\delta^{18}O-NO_3^-$ values compared to the theoretical values. Fractionation was found to depend greatly on the NH_4^+ concentration, with high concentrations of NH_4^+ increasing the nitrification rate (Zaman et al., 1999) and using more O atoms derived from H_2O. Meanwhile, it was found that oxygen exchange with water, which could alter the oxygen isotopic signature of nitrate in soil (Kool et al., 2011) and lead to low $\delta^{18}O-NO_3^-$ values in water (Wexler et al., 2012). Some samples tend to slightly higher $\delta^{18}O-NO_3^-$ values compared to the theoretical value, which may be caused by higher $\delta^{18}O-H_2O$ values due to evaporation of soil waters or because the O_2 was characterized by a high $\delta^{18}O$ value caused by bacterial respiration (Kendall et al., 2007). Moreover, other processes, such as denitrification, can also result in high $\delta^{18}O-NO_3^-$ values in some samples. In brief, the $\delta^{18}O-NO_3^-$ values for river water in this study are far below than those of precipitation and chemical fertilizers, suggesting that nitrification dominates the oxygen isotopic composition of nitrate.

Table 2
Estimated annual nitrogen flux from the Songhua River Basin.

Rivers	No.	DIN	DON	Normal mean flow[a]	%	DIN	DON	Flux
		mg/L		10^3 m^3/s		10^5 T/yr		
Songhua River	6-H	0.82	0.25	2.53	74	0.49	0.15	1.17
	6-L	1.95	0.63		26	0.40	0.13	
Upper Reach of Songhua River	12-H	2.97	3.50	0.54	74	0.37	0.44	1.02
	12-L	3.76	1.00		26	0.17	0.04	
Nen River	20-H	0.33	0.98	0.82	74	0.06	0.19	0.34
	20-L	0.98	0.28		26	0.07	0.02	
Lalin River	21-H	1.26	7.35	0.12	74	0.04	0.21	0.25
Hulan River	24-H	2.70	1.53	0.15	74	0.10	0.05	0.15
Mudan River	28-H	1.16	0.26	0.30	74	0.08	0.02	0.10

[a] Data from Zhu (2007); H = the high flow season; L = the low flow season.

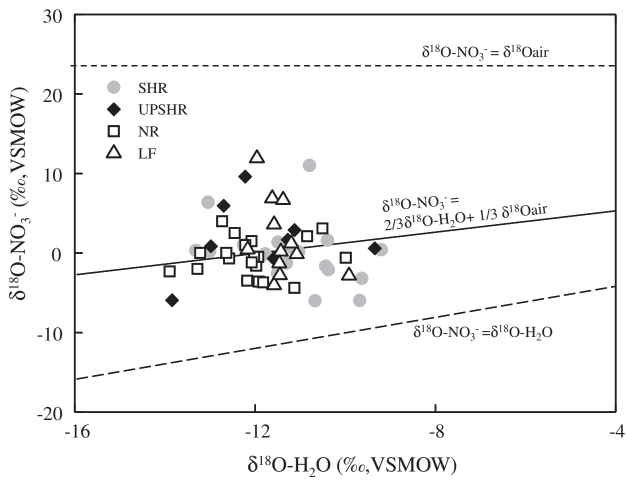

Fig. 6. $\delta^{18}O\text{-}H_2O$ versus $\delta^{18}O\text{-}NO_3^-$ in water samples from the Songhua rivers. Three lines represent the theory line in different condition.

Denitrification reduces NO_3^- to gaseous products, which are then lost to the atmosphere resulting in enrichment of $\delta^{15}N$ and $\delta^{18}O$ values in the residual NO_3^-, and this process leads to isotopic values for nitrate covering wide isotope ranges in $\delta^{15}N$ and $\delta^{18}O$ depending on the denitrification rate, temperature, and substrate concentrations (Böttcher et al., 1990; Kendall et al., 2007). The study suggests that denitrification causes a correlation between $\delta^{15}N$ and $\delta^{18}O$ in a ratio of 2:1 in the groundwater (Böttcher et al., 1990). In the present study, high isotopic values were not detected, (except sample No. 28), which indicates that denitrification did not have a great impact on the isotopic composition of NO_3^- of the investigated river water. However, a lack of isotopic evidence for denitrification does not mean that denitrification has not occurred. As indicated by Fig. 7a, there is a slightly negative correlation between the NO_3^- concentration (ln[NO_3]) and the $\delta^{15}N\text{-}NO_3^-$ values in the Songhua River expressed by a regression line of $y = -0.99x + 7.44$ ($R^2 = 0.36$) except for one tributary (sample No. 25). The negative relationship suggests that denitrification could have removed nitrate in the Songhua River. The denitrification might be responsible for the partial loss of nitrate flux due that the total flux of NO_3^- from major tributaries is 1.33 times of the flux at the mouth of the Songhua River. Several factors could have supported the process of denitrification in the Songhua River, such as flowing through two major plains with black soils and high thickness aquifers, river fall below 0.15 m/km and high temperatures in summer. Moreover, there is no significant isotope shift by denitrification apparent for two other rivers based on the relationship between the NO_3^- concentration and $\delta^{15}N\text{-}NO_3^-$ values (Fig. 7a). However, the samples (Nos. 44, 54) with low contents of nitrate in tributaries covering by forest in the Nen River have relative high $\delta^{15}N\text{-}NO_3^-$ value, which might suggest nitrate was impacted by natural denitrification in these rivers. In addition, the slightly negative relationship ($R^2 = 0.37$, except No. 28) between the NO_3^- concentration and $\delta^{15}N\text{-}NO_3^-$ values (Fig. 7b) indicates, that the mixing processes has influenced the nitrate content in the rivers during the low flow season. The wide variation of contents and isotopic values for nitrate during the high flow season compared to the low flow season indicates that several factors control the concentration and isotopic composition of nitrate in the rivers, such as intense biological processes and frequent rain in summer.

4.5. Identification of NO_3^- sources by dual isotopes and water chemistry

NO_3^- in the aquatic system has several major sources, including atmospheric deposition, leaching from chemical fertilizers, nitrification in soils and manure/sewage, and can be influenced by denitrification as well as biological uptake (Kendall et al., 2007). Cl^- is a good indicator for the impact of sewage on aquatic systems. Moreover, the NO_3^-/Cl^- method can provide more information about mixing processes or distinguish between dilution and denitrification (Koba et al., 1997; Liu et al., 2006; Widory et al., 2005). Plotting $\delta^{15}N$ 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 $\delta^{15}N\text{-}NO_3^-$ values in the water samples (Fig. 8). In the study area Cl^- is primarily derived from rain and anthropogenic inputs due to the lack of halite and low contents of Cl^- in the headwaters (Liu et al., 2013). High chloride contents were detected in some samples collected during low flow season and from municipal sewage. There is generally a positive correlation ($R^2 = 0.68$, $n = 20$) between Cl^- and NO_3^- in the main stream of the three major rivers, which indicates that the Cl^- is strongly influenced by anthropogenic input. The low Cl^- and NO_3^- concentrations in the Nen River indicate that this area is only slightly affected by anthropogenic activities. The sewage samples having high TDN/Cl^- molar ratios caused high NO_3^-/Cl^- molar ratios in the river water samples due to fast degradation of dissolved organic nitrogen and NH_4^+. Sample No. 28 had a similar chemical composition as sewage water with high Cl^- and NO_3^- concentrations in the low flow season, which indicated that this

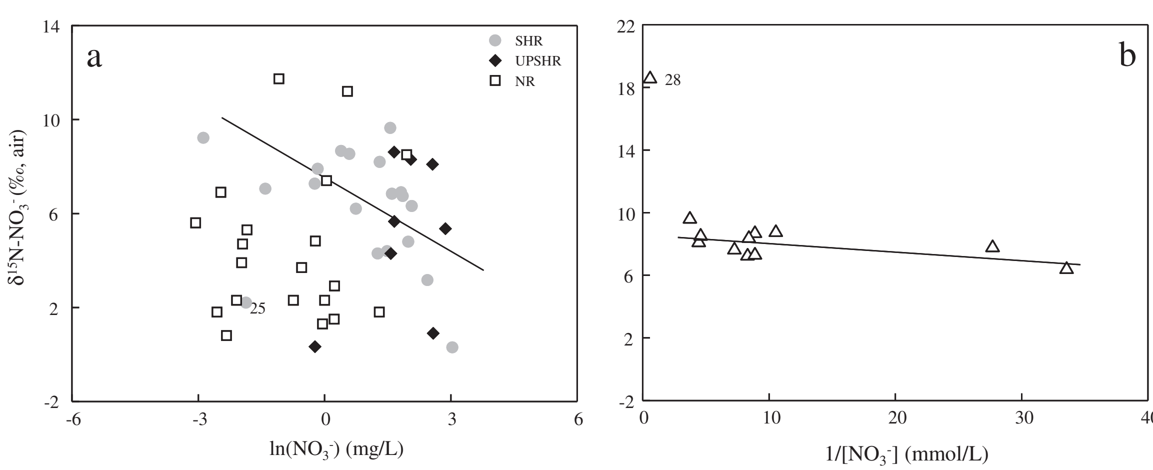

Fig. 7. The variation of nitrogen isotopic compositions of nitrate as a functions of ln[NO_3] (mg/L) during the high flow season (a) and 1/[NO_3](mol/L) during the low flow season (b) in the Songhua rivers.

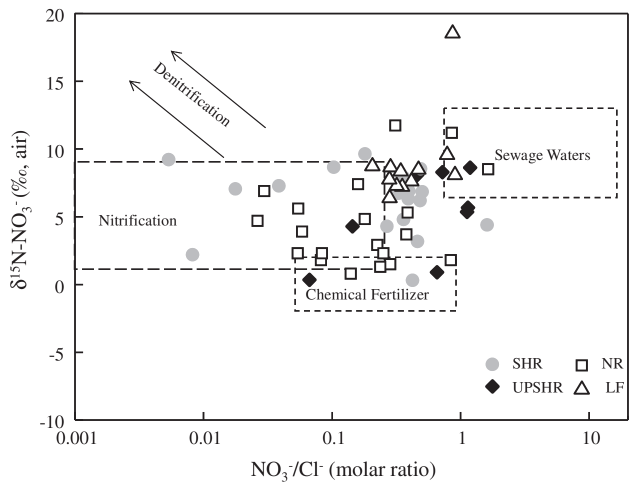

Fig. 8. The nitrogen isotope of NO_3^- versus the NO_3^-/Cl^- molar ratio in the Songhua rivers.

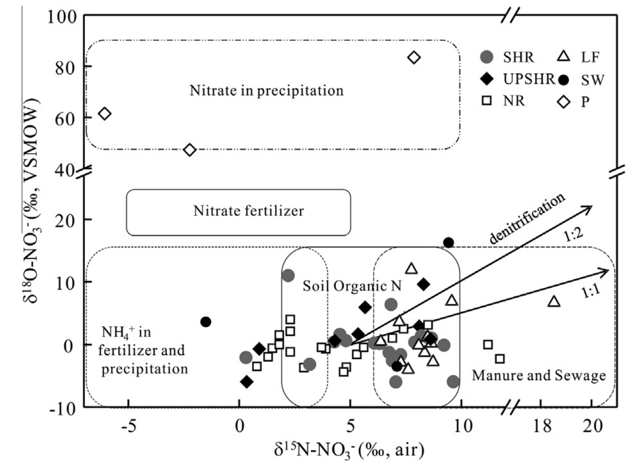

Fig. 9. Relationship between $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in the Songhua rivers, the isotopic composition of various sources in the diagram modified (Kendall et al., 2007; Widory et al., 2013).

sample had been contaminated with episodic wastewater at the sampling period when the river passed urban areas. It was reported that the NO_3^-/Cl^- molar ratio from suburb groundwater is below 0.1 and that from urban waste water >1 in Guiyang, Changjiang River basin (Liu et al., 2006). The high Cl^- concentrations and low NO_3^-/Cl^- molar ratios of the samples may be influenced by denitrification, which likely occurred in municipal sewage.

As indicated by Fig. 8, NO_3^- was derived from at least three different sources: one generating a low NO_3^-/Cl^- molar ratio and low $\delta^{15}N$-NO_3^- value, another with a variable but generally high NO_3^-/Cl^- molar ratio and high $\delta^{15}N$-NO_3^- value, the third source with a high NO_3^-/Cl^- molar ratio and low $\delta^{15}N$-NO_3^-. Microbial denitrification typically results in progressively increasing $\delta^{15}N$-NO_3^- values, whereas NO_3^-/Cl^- molar ratios decrease, and mixing of NO_3^- from two or more sources can result in patterns of increasing $\delta^{15}N$-NO_3^-. Most samples in the Nen River watershed showed $\delta^{15}N$-NO_3^- values below 5‰ and low contents of chemical compositions, which indicates that NO_3^- was mainly derived from SON. Some samples revealed high NO_3^-/Cl^- molar ratios with $\delta^{15}N$-NO_3^- values lower than 4‰, which indicates that NO_3^- may be derived from nitrogenous fertilizers. Moreover, some samples displayed high NO_3^-/Cl^- molar ratios with $\delta^{15}N$-NO_3^- values greater than 8.0‰, indicating that NO_3^- was influenced directly by sewage. Thus, NO_3^- in the URSHR and the Songhua River should mainly be affected by mixing of at least three sources.

NO_3^- isotopic values are plotted in Fig. 9. The NO_3^- derived from atmospheric deposition samples showed $\delta^{15}N$ values from −7.0‰ to +8.0‰ and relatively high $\delta^{18}O$ (>+45‰) in line with values reported in the literature (Kendall et al., 2007). NO_3^- from natural SON has isotopic compositions ranging from +2.0% to +8.0% (Wassenaar, 1995; Yue et al., 2013). Nitrogenous fertilizers generally have $\delta^{15}N$-NO_3^- values within a few permil around zero in China (Liu et al., 2006; Yue et al., 2013). In this study, the three sewage effluent samples had wide isotopic ranges. Actually, it is suggested that the isotope values of N in animal manure and sewage waters are usually characterized by heavy isotopes in a typical range of +8‰ to +25‰ (Kendall et al., 2007; Widory et al., 2013). The NO_3^- in samples collected during both flow seasons had low $\delta^{18}O$-NO_3^- values (Fig. 9), indicating that rain and nitrate fertilizer was not the major source of river NO_3^-. During the high flow season, the patterns of the dual isotopes of NO_3^- suggest that the NO_3^- in most samples likely originated from mixing by multi sources, especially for samples from in the Upper Reach of Songhua River and the Songhua River. The concentration and isotopic values of NO_3^- in the rivers might be affected by land use and urban inputs in the catchment (Douglas et al., 2002; Mayer et al., 2002; Ohte et al., 2012). The nitrate might derive from mixing of sewage and SON in the lower reach of the Nen River based on isotopes and contents of nitrate. The NO_3^- sources changed in the middle and downstream in the Upper Reach of Songhua River and the high nitrate concentrations and low $\delta^{15}N$-NO_3^- values show that nitrate in some water samples may be derived from chemical fertilizers (e.g. sample Nos. 11 and 35). The isotopic values of NO_3^- in major tributaries and downstream of the Songhua River indicate that sewage water and chemical fertilizers are the main sources during the high flow season, such as Lalin River (No. 21) and Mudan River (No. 28) as well as Anbang River (No. 33). The NO_3^- in rivers was mainly derived from sewage and SON during the low flow season based on the dual isotopic pattern and mixing diagram (Fig. 7b).

5. Conclusions

This study presents a detailed analysis of the water chemistry and multiple stable isotopes to identify the sources and fate of NO_3^- in a large river basin in Northeast China. The δD- and $\delta^{18}O$-H_2O values demonstrated that river water originated from local precipitation and was slightly affected by evaporation. The spatial variation of contents and isotopic compositions of nitrate in rivers reflected the impacting from vegetation, land use and anthropogenic input. The Nen River samples showed low concentrations of nitrogenous species and high DON/TDN values due to the influence of forest and grassland covering the area around the river. NO_3^- and DON were the dominant species of TDN during both flow seasons in the Songhua River Basin. The flux of total nitrogen was estimated to be approximately 1.17×10^5 ton/yr of nitrogen outflow at the mouth of the Songhua River, amounting to 6.3% of the synthetic fertilizer used in this basin. The rain and nitrate fertilizers were not the major source of river NO_3^- due that the $\delta^{18}O$-NO_3^- values mainly presented the nitrification characteristics in the Songhua rivers. SON was the major source of NO_3^- in in the Nen River based on the water chemistry and isotopic values. Moreover, nitrogenous fertilizers and sewage waters were the two major contributors to NO_3^- in most samples from the Upper Reach Songhua River and the Songhua River during the high flow season. Nitrate in the low flow season samples was mainly derived from mixing between SON and sewage water according to the isotopic compositions and water chemistry. The unbalanced budget of the N flux and isotopic proof in the Songhua River identified that denitrification would be partially responsible for the loss of river nitrate during transportation. The results suggest that the processes influencing nitrate in rivers should be considered for accurate riverine N budget. Furthermore, to improve the water quality and reduce the nitrogen load in the investigated rivers, point sources of sewage effluents need to be managed first.

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