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ORIGINAL PAPER

Sources and Processes Affecting Nitrate in a Dam-Controlled Subtropical River, Southwest China

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Abstract Excess nutrient (N and P) loads are recognized as the major cause of serious water quality problems in China. River systems play a very important role in nitrate (NO_3^-) transportation and transformation in the aquatic environment. To understand and clarify the sources and processes affecting NO_3^- in river basins, we have examined spatial and temporal variations of concentration and dual-isotopic composition of NO_3^- in the dam-controlled Jialing River, a major tributary of the Yangtze River where land use is dominated by agriculture. Water samples were collected in July 2008 and February 2009 from the main channel of the Jialing River and its major tributaries. The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- range from 1.5 to 11.0 ‰ (average 6.2 ‰) and −5.0 to 11.1 ‰ (average, 1.6 ‰), respectively. NO_3^- isotope data and $\delta^{18}O$ of water interpreted in combination with hydrological and chemical data suggest that most of the NO_3^- input is from nitrification during the rainy season, and discharge of sewage and manure in the upper course and from cities accounts for much of the NO_3^- load during the dry season. The construction of cascade dams has led to retention of Si and a decrease in the Si/N ratio, implying that assimilation and/or denitrification may significantly affect NO_3^- in the dam area, as demonstrated by NO_3^- and dissolved Si concentrations, and $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ values. This study indicates that dual-isotopic data can be used to identify NO_3^- pollution sources

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and the processes NO_3^- has undergone during its retention and transport in the watershed of the dam-controlled Jialing River.

Keywords Nitrate isotope · Jialing River · Nitrification · Cascade dams · Assimilation

1 Introduction

In recent years, increasing population, industrial and agricultural development, and urbanization in many developing countries have resulted in excess concentrations of nitrate (NO_3^-) in rivers, lakes, and coastal areas (Carpenter et al. 1998; Canfield et al. 2010). As nitrogen (N) travels down a river, it usually undergoes numerous transformations which play a key role in the transport of N and related N export, so the identification of the underlying processes is a major topic in river research.

Generally, each biogeochemical transformation process of N (i.e., assimilation, nitrification, and denitrification) is characterized by significant isotopic fractionation, which commonly result in increases in $\delta^{15}N$ of the substrate and decreases in $\delta^{15}N$ of the product, unless the reactions go to completion (Kendall et al. 2007). Processes that consume NO_3^- (primarily denitrification and assimilation by phytoplankton and/or prokaryotes) generally cause the $\delta^{15}N$ and $\delta^{18}O$ in the remaining pool of NO_3^- to increase in a relatively predictable pattern (Kendall et al. 2007; Deutsch et al. 2009). In combination with land-use type, the isotopic ratios of N and oxygen (O) in NO_3^- can provide insights into the sources, sinks, and transport processes of the N cycle within a river basin that are difficult to be achieved through traditional measurements of solute concentration and flux (Lee et al. 2008; Panno et al. 2008; Burns et al. 2009; Miyajima et al. 2009; Li et al. 2010; Ohte et al. 2010; Nestler et al. 2011; Cohen et al. 2012).

River systems can be perturbed by human activities through pollution and river regulation processes that generally occur simultaneously (Milliman 1997; Li et al. 2010). Numerous artificial dams constructed on rivers around the world may exert significant impacts on river biogeochemistry (Ligon et al. 1995; Friedl et al. 2004). The resulting man-made stagnant systems must be considered as a part of a river basin that may influence the biogeochemical transformation and circulation of biogenic elements, such as N, phosphorus (P), and silicon (Si) (Jossette et al. 1999; Humborg et al. 2002; Friedl et al. 2004). Reservoirs increase water residence times, thus allowing greater assimilation of nutrients (N, P, and Si) by inducing algal proliferation that, in excess, may lead to anoxic bottom water conditions that promote denitrification, which further reduces exports of N to downstream waters (Ligon et al. 1995; Birgand et al. 2007). Loss of N and P might be overcompensated by anthropogenic inputs downstream of the reservoirs, but no such compensation has been observed for Si. The construction of dams in rivers will cause an imbalance of these nutrients and enhance cultural eutrophication (Liu et al. 2006; Yan et al. 2010).

Concentrations of NO_3^- (average 48.7 μmol/L) in the Jialing River are high among the observed large tributaries feeding the upper Yangtze River (Chen et al. 2000; Liu et al. 2003). Because the Jialing River watershed accounts for 35 % of the total N transported to the upper Yangtze River—and ultimately the Three Gorges Reservoir (TGR)—the sources and fate of NO_3^- within the Jialing River basin raise great concern (Liu et al. 2003).

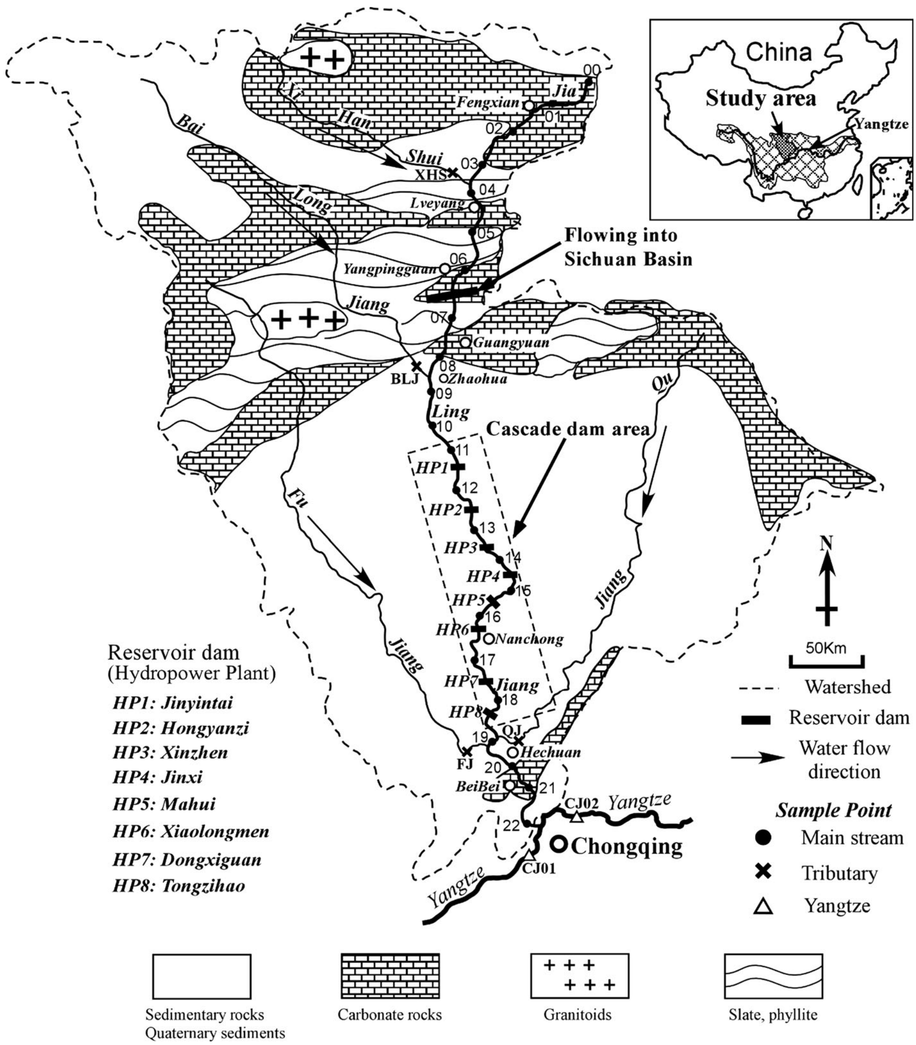

Fig. 1 *Sketch map* showing the drainage basin of the Jialing River, major tributaries, and main provenance rock types, along with sampling locations and *sample numbers*. The lithology of the drainage basin of the Jialing River is modified from the Geological Map of China (1:2,500,000, China Geological Survey 2004)

Moreover, due to the drastic increase in industrial and agricultural activities along with the great demand for electricity, dam construction in the Jialing River has become particularly significant over the past two decades. Of the sixteen cascade dams proposed for construction on the Jialing River, eight were completed in 2008 (Fig. 1). In the present study, spatial and temporal (the samples were taken in July 2008 and February 2009) variations of concentration and isotopic composition of NO_3^- in the dam-controlled Jialing River were examined. By using dual-isotopic values of NO_3^-, this study aims to identify the major sources of NO_3^- and trace the potential N transformation processes in the dam-controlled Jialing River.

2 Study Site

2.1 Topography

The mainstream of the Jialing River, a major tributary of the upper Yangtze River, is 1,120 km long and has a drainage area of 160,000 km^2, which constitutes the largest drainage area of the Yangtze basin. The Jialing River itself has four major tributaries, Xihanshui, Bailongjiang, Qujiang, and Fujiang (Fig. 1). In the upper course of the Jialing River, the lithology consists of carbonate rocks intercalated with carbonaceous slate and phyllite (Fig. 1; Bureau of Geology and Mineral Resources of Sichuan Province 1991). In its middle and lower courses, the Sichuan Basin comprises a relatively undeformed part of the Yangtze Platform. The study area is covered with fluvial deposits and reddish sandstone and mudstone (Zhang et al. 2008).

2.2 Climate and Land Use

The Jialing River is located in a zone of subtropical humid monsoon climate. The mean annual precipitation is 1,010–1,250 mm in the middle and lower Jialing River regions (Sichuan Basin). The climate is highly seasonal with a rainy period (May to September) accounting for about 80 % of the total annual precipitation (Changjiang Water Resources Commission 2003). The percentage of land involved with agriculture is higher in the middle and lower courses where large residential areas are present (Table 1; Bureau of Geology and Mineral Resources of Sichuan Province 1991; Wang et al. 2007). The Sichuan Basin is a key center for rice and wheat farming, and has undergone rapid development since the end of 1970s. In this region, synthetic fertilizer [mainly urea—$CO(NH_2)_2$ and ammonium bicarbonate (ABC)—NH_4HCO_3] input has increased greatly (about 50–70 %) over time (Tang 2010).

3 Methodology

3.1 Water Samples

A total of 58 samples were collected from the source area to Chongqing, where the Jialing River joins the Yangtze. Samples were taken from the same locations during the rainy (July 2008) and dry (February 2009) seasons (Fig. 1). These samples included 46 samples from the mainstream, eight samples from major tributaries (Xihanshui, Bailongjiang,

Table 1 Geographical characteristics of the Jialing River

Reaches	Length (km)	Drainage area (× 10^4 km^2)	Average annual discharge (× 10^8 m^3/a)	Population density (person/km^2)	Agricultural land (%)	Forest (%)
Upper	379	5.28	140	15.2	<20	65.87
Middle and lower						
Main stream	741	2.98	281	697	67.19	27.96
Branch–Qujiang	723	4.10	227	320	17.71	–
Branch–Fujiang	670	3.64	152	330	23.81	–

–: represents no data

Qujiang, and Fujiang), and four samples from the Yangtze (before and after joining with the Jialing River; Fig. 1). Specifically, samples JL00 (headwater) were collected from pristine forest at the source area of the Jialing River (Fig. 1).

Water samples were collected midstream at ~0.5 m from the surface using a polyethylene (PE) bucket dropped from a bridge or ferryboat. The samples were filtered using 0.45-μm pore-sized membrane filters (Millipore) at each site to remove suspended particles. The filtered water samples were then stored in pre-rinsed, air-tight, high-density polyethylene (HDPE) bottles, one of which was amended with saturated $HgCl_2$ to inhibit biological activity for NO_3^- and SiO_2 analyses, while another was left untreated for the analyses of Cl^-, and $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ isotopes.

3.2 Analytical Methods

NO_3^- and Cl^- were analyzed by ion chromatography (ICS-90, DIONEX). Dissolved silicon (DSi, denoted as SiO_2) was analyzed by colorimetry using a silicon–molybdenum blue complex. The precision was better than 0.5 % for NO_3^- and Cl^-, and 2.5 % for SiO_2.

Frozen aliquots were stored, thawed, and analyzed for $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ using the microbial denitrifier method (Sigman et al. 2001; Casciotti et al. 2002) at the Tokyo University of Agriculture and Technology, Japan. The denitrifying bacterium, *Pseudomonas chlororaphis* f. sp. *aureofaciens* (ATCC 13985), was used to convert 25 nmoles of NO_3^- into gaseous N_2O in 20-mL vials prior to isotope analysis. Isotopic data were analyzed with an isotope-ratio mass spectrometer (Delta XP; Thermo Fisher Scientific K.K., Yokohama, Japan) coupled with a gas chromatograph (HP6890; Hewlett Packard Co., Palo Alto, CA, USA) equipped with a PoraPLOT column (25 m × 0.32 mm) and GC interface III (Thermo Fisher Scientific K.K., Yokohama, Japan). We ran several standards (USGS32, 34, and 35, and IAEA NO_3^-) to obtain the calibration curve to correct for drift and oxygen isotope exchange. The average standard deviations for replicate analysis of an individual sample were ±0.2 ‰ for $\delta^{15}N$ of NO_3^- and ±0.5 ‰ for $\delta^{18}O$. To evaluate the influence of the $\delta^{18}O$ of water on that of NO_3^-, the $\delta^{18}O$ of water (hereafter, $\delta^{18}O_{H_2O}$) in each sample was measured by CO_2–H_2O equilibrium method on an IsoPrime Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS, GV Instruments, Manchester, UK) with Multiflow preparation lines, at the Institute of Geochemistry, Chinese Academy of Sciences. The precision was typically better than 0.2 ‰ for $\delta^{18}O_{H_2O}$.

The N and O stable isotope ratios are expressed in the following generally accepted delta notation as δ values in parts per thousand (‰):

$$\delta_{\text{sample}}(‰) = \left(\left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}}\right) \times 1{,}000,$$

where R is the $^{15}N/^{14}N$ ratio of NO_3^- or $^{18}O/^{16}O$ ratio of NO_3^- and H_2O of a sample and the standard. The $\delta^{15}N_{NO_3}$ values are reported relative to air, and the $\delta^{18}O_{NO_3}$ and $\delta^{18}O_{H_2O}$ values are reported relative to the Vienna Standard Mean Ocean Water (VSMOW).

4 Results

4.1 NO_3^- and SiO_2

A summary of the chemical and isotopic compositions of waters in the Jialing River collected during two sampling seasons is presented in Table 2. The concentrations of

Table 2 Summary of the chemical and isotopic compositions of waters in the Jialing and Yangtze Rivers during two sampling seasons

River	Sampling time	NO_3 (μmol/L)		Cl (μmol/L)		SiO_2 (μmol/L)		$\delta^{15}N_{NO_3}$ (‰)		$\delta^{18}O_{NO_3}$ (‰)		$\delta^{18}O_{H_2O}$ (‰)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Jialing (23 sites)	Jul. 2008 (rainy)	43.1 ~ 147	100 ± 48	25 ~ 181	117 ± 105	108 ~ 181	131 ± 16	+1.5 ~ +10.0	+5.6 ± 4.3	+0.8 ~ +11.1	+3.2 ± 1.5	−10.0 ~ −7.6	−8.5 ± 1.3
	Feb.2009 (dry)	37.7 ~ 108	69 ± 35	32 ~ 300	175 ± 189	3.93 ~ 133	63 ± 79	+1.5 ~ +11.0	+6.9 ± 4.0	−5.0 ~ +7.3	−0.1 ± 2.0	−10.9 ~ −7.3	−8.6 ± 1.4
Xihanshui	Jul. 2008 (rainy)	46.6		173		118		4.6		2.7		−8	
(XHS)	Feb.2009 (dry)	53.2		230		110		9.8		4.2		−8.4	
Bailongjiang	Jul. 2008 (rainy)	69.3		74.3		109		3.1		2		−9.5	
(BLJ)	Feb.2009 (dry)	53.2		103		68.2		7		−3.9		−9.7	
Qujiang	Jul. 2008 (rainy)	82.5		183		126		5.2		1.8		−7.1	
(QJ)	Feb.2009 (dry)	76.7		274		50.7		8.9		0.4		−7.6	
Fujiang	Jul. 2008 (rainy)	198		246		114		4.5		3.1		−8.5	
(FJ)	Feb.2009 (dry)	130		459		43.2		7.6		−1.2		−8.8	
Yangtze[a]	Jul. 2008 (rainy)	79.3		268		121		6.2		1.9		−12.5	
(CJ01)	Feb.2009 (dry)	73.8		292		99.3		5.9		−2.5		−12.3	

Table 2 continued

River	Sampling time	NO_3 (μmol/L)		Cl (μmol/L)		SiO_2 (μmol/L)		$\delta^{15}N_{NO_3}$ (‰)		$\delta^{18}O_{NO_3}$ (‰)		$\delta^{18}O_{H_2O}$ (‰)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Yangtze[b]	Jul. 2008 (rainy)	88.4		250		120		7.5		7		−11.7	
(CJ02)	Feb.2009 (dry)	77.3		297		78.2		7.1		−2.1		−11.7	

The detailed data were shown in Li et al. (2011), which have examined sulfate dual-isotopic evidence to identify dissolved sulfate sources, while the novel nitrate dual-isotopic data are not discussed

[a, b] Samples from the Yangtze before and after joining with the Jialing River

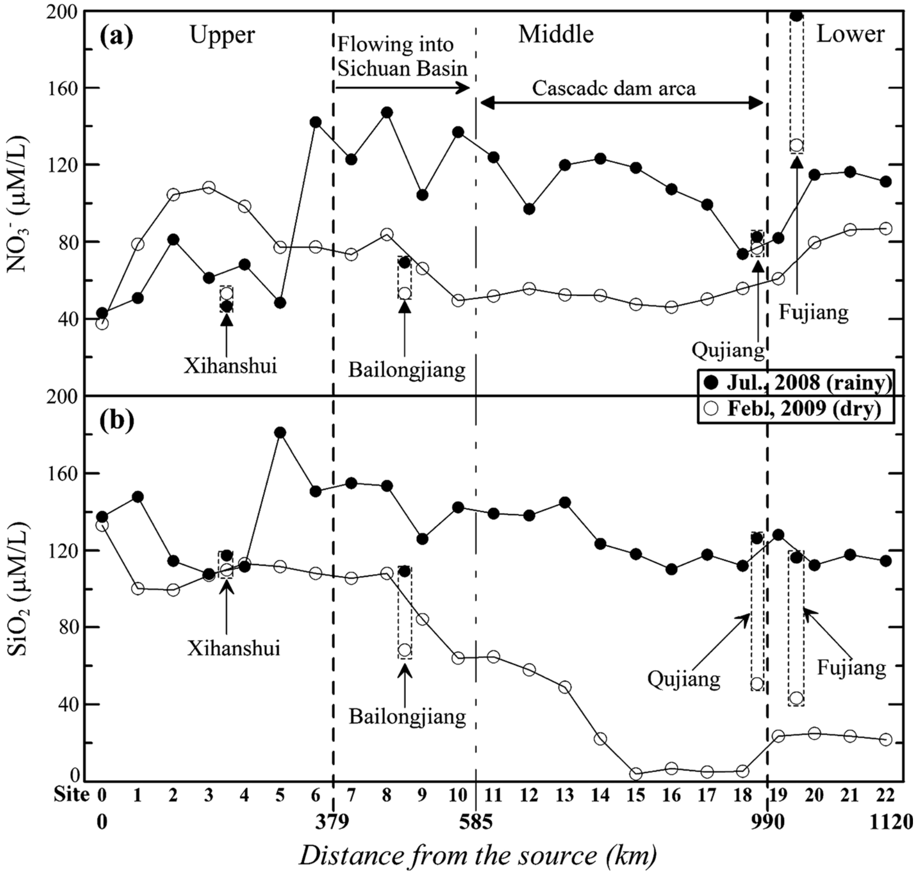

Fig. 2 Concentration of NO_3^- (**a**) and SiO_2 (**b**) in the waters sampled along the Jialing River during two seasons. The points of samples from the tributaries are in the *boxes*

NO_3^- in the river as it flowed into the Sichuan Basin were higher in the rainy season (Fig. 2a), and the decrease in NO_3^- concentrations was especially conspicuous during summer in the middle course. Spatially, during the dry season, the concentrations of NO_3^- were lower in the middle Jialing River compared with other sampling locations except the headwater JL00. During the rainy season, the concentrations decreased from 124 μmol/L at site 11 to 74 μmol/L at site 18 across the dam area.

SiO_2 concentration was also higher in the rainy season, and it gradually decreased downstream from site 05 in both seasons, especially within the dam area (Fig. 2b). During the dry season, the SiO_2 concentration decreased from 64 μmol/L at site 11 to the lowest level, 4 μmol/L, at site 15 locating at the reservoir-Mahui (HP5) constructed in 1992, the earliest of the cascade dams (Figs. 1, 2b).

4.2 $\delta^{15}N$ and $\delta^{18}O$ in Nitrate

Seasonally, the $\delta^{15}N_{NO_3}$ values were heavier in the dry season, whereas the $\delta^{18}O_{NO_3}$ values were heavier in the rainy season in the middle and lower courses (Fig. 3). Additionally, the

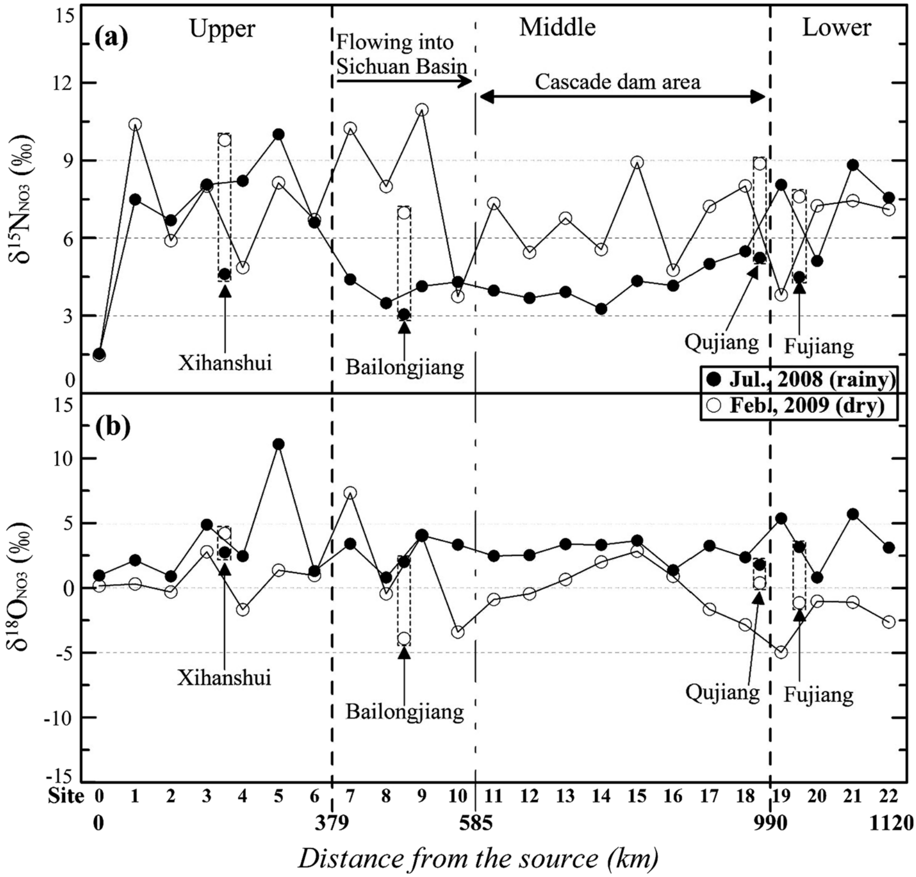

Fig. 3 $\delta^{15}N_{NO_3}$ values (**a**) and $\delta^{18}O_{NO_3}$ values (**b**) in the waters sampled along the Jialing River during two seasons. The points of samples from the tributaries are in the *boxes*

spatial variations of these dual-isotopic values showed complicated patterns due to the comprehensive effects of many factors. At the headwater site 0 on the Jialing River, NO_3^- concentrations (40 μmol/L) were low and $\delta^{15}N_{NO_3}$ values (1.5 ‰) were light (Figs. 2a, 3a). However, NO_3^- concentrations and $\delta^{15}N_{NO_3}$ values increased markedly at the next sample point, site 1, which is located 50 km downstream from the headwater below the first town. Light $\delta^{18}O_{NO_3}$ values were found in the headwaters (1.0 ‰ for summer and 0.2 ‰ for winter), and they were slightly elevated in the upper course of the Jialing River (Fig. 3b).

During the rainy season, between sites 5 and 7 (where the river flowed into the Sichuan Basin), NO_3^- concentrations steeply increased and $\delta^{15}N_{NO_3}$ values gradually decreased. In contrast, within the area of the cascade dams, the NO_3^- concentrations gradually decreased, but the $\delta^{15}N_{NO_3}$ values slightly increased and kept within 3–6 ‰ (Figs. 2a, 3a). Downstream from the cascade dams (in the lower course of the Jialing River), even though the river was affected by input from Qujiang and Fujiang tributaries, the $\delta^{15}N_{NO_3}$ of the mainstream almost increased to 6–9 ‰—values that were similar to those observed in the upper course of the Jialing River. In contrast, the $\delta^{18}O_{NO_3}$ values of waters in the Jialing River were mainly between 1 and 4 ‰ and remained relatively stable during the rainy season (Fig. 3b).

During the dry season, in the middle course of Jialing River, even though NO_3^- concentrations decreased slightly and then kept stable within the area of cascade dams, the spatial changes of the $\delta^{15}N_{NO_3}$ values were rather complex and had no obvious regularity (Figs. 2a, 3a). Nonetheless, the $\delta^{18}O_{NO_3}$ values increased slightly and then decreased sharply within the area of the cascade dams (Fig. 3b).

5 Discussion

5.1 Covariations of NO_3^- and SiO_2

The average concentration of NO_3^- (84.5 μmol/L) in the present study was higher than the data (48.7 μmol/L) from 1997 obtained by Liu et al. (2003) and the data (64.7 μmol/L) from 2006 reported by Li et al. (2010). Here, compared with the NO_3^- concentration (40 μmol/L) of sample JL00 collected from pristine forest, high NO_3^- concentrations examined in the middle and lower courses should be resulted from anthropogenic activities, especially in the rainy season (Fig. 2a). Excess fertilization and manure production create an N surplus on agricultural lands (Long et al. 2008). Surplus N is mobile in many soils and much of it leaches into surface waters or percolates into groundwater. Currently, cropland dominates land use in the Jialing River basin and accounts for about 25 % of the total area (Wang et al. 2007). Heavy use of N fertilizer (urea and ABC, 180–190 kgN/ha) with low fertilization efficiency (22–33 %) is often responsible for N loss from the soil in the Sichuan Basin (Zhou et al. 2006; Tang 2010). Compared with point sources, agrichemical N accounted for 60–80 % of the total N load (Xing and Zhu 2000; Zheng et al. 2009). Based on the geographical information technology, the yearly load of the nitrogen pollution due to soil erosion in the Jialing River basin was estimated at 34,423 t/a in 2005 (Long et al. 2008). High efflux of N from soil and widely distributed steep slope cultivation are the key factors that cause substantial NO_3^- loss in the Jialing River watershed, which would be responsible for the high NO_3^- concentrations in the middle course comparing with the upper course particularly during the rainy season (Fig. 2a).

Historical measurements of nutrient SiO_2 concentrations in Jialing River waters during the period 1958–1990 revealed that the average was approximately 103 μmol/L (Chen et al. 2002), the same as the average value of the Yangtze River, and lower than the world average of 150 μmol/L (Chen et al. 2002). In this study, the annual average concentration of SiO_2 was 96.7 μmol/L, which is lower than all the above values. It is well known that Si is the second most abundant element in the Earth's crust, and DSi is an important nutrient for the growth of terrestrial and aquatic organisms. DSi is essentially of a diffuse origin that depends on basin lithology and other environmental factors such as vegetation, hydrology, temperature, and water residence times in soil and riverbed (Sferratore et al. 2006). In addition, biological processes, especially the biogeochemical cycling of Si through terrestrial ecosystems, will exert a strong control on chemical weathering rates and significantly affect SiO_2 content in rivers (Derry et al. 2005). During the rainy season, the high efflux of nutrients from croplands and soil erosion along with the surface runoff, as with NO_3^-, may introduce much Si into the Jialing River watershed which might correspond with higher SiO_2 concentrations in the rainy season (Fig. 2b).

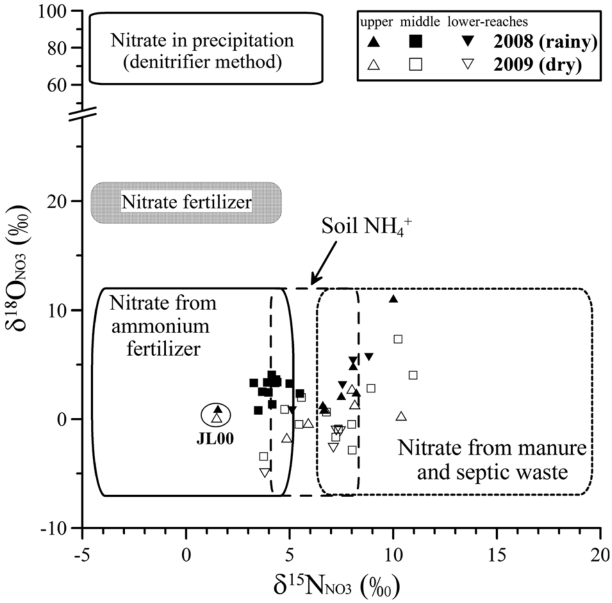

Fig. 4 $\delta^{15}N_{NO_3}$ versus $\delta^{18}O_{NO_3}$ values for river waters sampled in the mainstream of the Jialing River during two seasons. The $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ values predicted for nitrate in rain, nitrate fertilizer, nitrate from ammonium fertilizer, nitrate from soil NH_4^+, and nitrate from manure and septic waste end-members, which were cited from Kendall et al. (2007). Please see the text for details

Generally, construction of cascade dams leads to the conditions of low flow and long water residence time in the dry season, and naturally high temperature and more terrestrial nutrients input along with surface runoff during the rainy season, both of which are favorable for phytoplankton proliferations (Conley 2002; Humborg et al. 2002; Triplett et al. 2008). In the present study, the growth of algae or diatom species consumes SiO_2 and NO_3^- simultaneously, which can account for the decreasing trend of SiO_2 and NO_3^- concentrations within the area of cascade dams, especially for the rainy season (Fig. 2).

5.2 Dual-Isotopic Approach and Major Sources of Nitrate

A $\delta^{15}N_{NO_3}$ versus $\delta^{18}O_{NO_3}$ plot shows that the dual-isotopic composition of NO_3^- in water samples from the Jialing River might be mixtures of any pairing of the three end-members or a mixture of all three: (1) ammonium fertilizer, (2) soil NH_4^+, and (3) manure and domestic wastewater (Fig. 4). In particular, the $\delta^{15}N_{NO_3}$ data of sample JL00 were 1.5 ‰ in both seasons and just fall within nitrate from ammonium fertilizer, not the soil NH_4^+ region. Generally, the $\delta^{15}N$ of soil nitrate ranges from about −10 to +15 ‰, with most soils having $\delta^{15}N_{NO_3}$ values in the range from +1 to +5 ‰ (Kendall et al. 2007). The $\delta^{15}N$ of soil nitrate is strongly affected by drainage, topographic position, vegetation, plant litter, land use, temperature, and rain amount. Therefore, depending on the land use and possible sources of anthropogenic contaminants from pristine forest at the source area of the Jialing River, the nitrate $\delta^{15}N$ (1.5 ‰) of sample JL00 may reflect natural source and not the effects of anthropogenic activities. Downstream JL00, due to the land use in the upper course of the Jialing River (Table 1), sewage, and manure from both residential and agricultural areas were probably the largest anthropogenic sources of N affecting NO_3^- concentrations and $\delta^{15}N_{NO_3}$ of these river waters (Figs. 1, 2a, 3a).

The temporal variability in the dual-isotopic composition is obvious. During the rainy season, river water samples from the middle course plotted closest to those of nitrate from ammonium fertilizer (Fig. 4) and a subset of summer samples from the upper and lower courses had isotopic values plotting near and within the domain that characterizes manure and sewage. During the dry season, a greater influence of manure and domestic wastewater was observed in the isotopic composition (Fig. 4). Generally, from the $\delta^{15}N_{NO_3}$ versus $\delta^{18}O_{NO_3}$ plot, the upper and lower Jialing River samples clustered together but had heavier $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ value relative to the middle Jialing River samples, probably due to more discharge of domestic wastewater with heavy $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ values (Fig. 4).

The measured values of $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ in the middle Jialing River agree with the previously reported range of microbially nitrified NO_3^- from soil NH_4^+ and/or fertilizer NH_4^+. The simultaneous increase in both population density and agricultural lands along the middle course of the Jialing River has resulted in the input of synthetic NH_4^+ fertilizer (mainly as urea and ABC) nitrified NO_3^- with a lighter $\delta^{15}N_{NO_3}$ value which might have weakened the increase of $\delta^{15}N_{NO_3}$ in river water caused by the input of NO_3^- from sewage efflux (Figs. 2a, 3a, 4). Combining plots of $\delta^{15}N_{NO_3}$ versus $\delta^{18}O_{NO_3}$ and $\delta^{18}O_{NO_3}$ versus $\delta^{18}O_{H_2O}$ indicates that NO_3^- in river water did not come from NO_3^- in atmospheric deposition. Such deposits would have substantially heavy $\delta^{18}O$ values (Kendall et al. 2007), instead of NO_3^- formed from nitrification of the applied urea and ABC fertilizers (Figs. 4, 5).

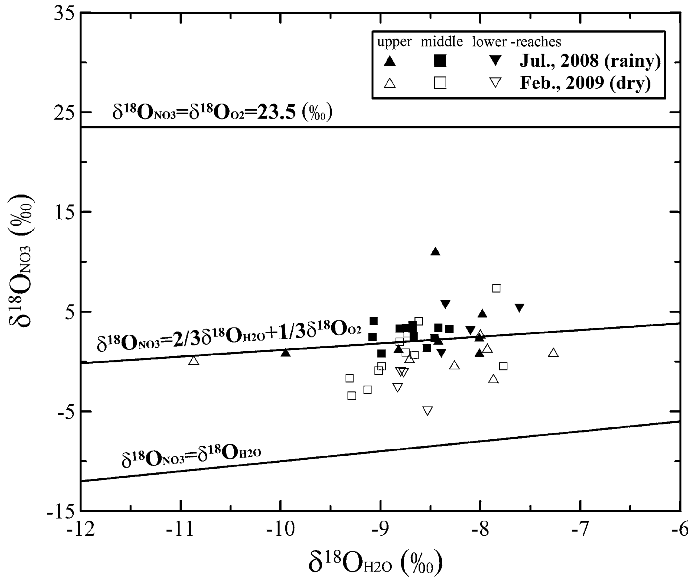

Fig. 5 $\delta^{18}O$ of water versus $\delta^{18}O$ of nitrate. The *three lines* indicate the oxygen atoms of NO_3^- formed during the process of nitrification originate from water and/or dissolved oxygen (Anderson and Hooper 1983; Hollocher 1984; McMahon and Böhlke 2006)

5.3 Major Processes Affecting Concentration and Isotopic Values of Nitrate

In this study, the longitudinal changes of NO_3^- concentrations and stable isotope values demonstrate strong evidence of in-stream N processing within the dam area in the middle course of the Jialing River (Figs. 2a, 3).

5.3.1 Nitrification

The middle and lower courses of Jialing River flow through a typical agricultural area, with about 25 % of land being used for cultivation. Rice, planted in late April, is the dominant crop in the river basin. The heavy application of fertilizers (urea and ABC) takes place around May, when rice and fruit trees grow rapidly. The application of urea and ABC could induce NH_3 volatilization, and the volatilized NH_3 would be ^{15}N depleted. The remaining N would therefore be enriched in ^{15}N. Additionally, products of nitrification like NO_3^- were also ^{15}N depleted (Kendall et al. 2007). Along with the nitrification process, the remaining NH_4^+ would become ^{15}N enriched, which in turn would increase ^{15}N values for NO_3^-. As the NH_4^+ applied as fertilizer is consumed, the nitrification rate decreases, and ultimately, the $\delta^{15}N$ of the total resulting NO_3^- may increase toward a few permil heavier than the original NH_4^+ (Feigin et al. 1974). In a survey of fertilized soils in Texas, Kreitler (1975) attributed a 2–3 ‰ increase in $\delta^{15}N_{NO_3}$ in underlying groundwater relative to the applied fertilizer. Li and Wang (2008) determined concentrations and N isotopic signatures of NH_4^+ and NO_3^- from cropland soils fertilized with urea and ABC. They found NO_3^- pools increased with the decline of NH_4^+ pools in both urea- and ABC-treated plots, and the final $\delta^{15}N$ values of NO_3^- were a little heavier than those of applied fertilizer. This variation was ascribed to the existence of nitrification. In the present study, during the rainy season in July and August, the NO_3^- from the nitrification of NH_4^+ fertilizer widely applied in May was transported into the river along with surface runoff, leading to a high concentration of NO_3^-. Simultaneously, the $\delta^{15}N$ values of NO_3^- in river water were 3–4.5 ‰ in the middle course of the Jialing River (Fig. 3a), which is 2–3.5 ‰ heavier than those of the applied NH_4^+ fertilizers (+1±2.6 ‰, Li et al. 2007).

During the process of nitrification, ammonium–nitrogen is transformed into NO_3^-, simultaneously acquiring three oxygen atoms. Two of these are assumed to be derived from water, whereas the third comes from dissolved oxygen (Anderson and Hooper 1983; Hollocher 1984; DiSpirito and Hooper 1986; Mayer et al. 2001). If isotopic fractionation is neglected during the incorporation of oxygen, the final NO_3^- product should have an isotopic signature equal to 2/3 $\delta^{18}O$ of water and 1/3 $\delta^{18}O$ of dissolved oxygen, as shown by the following equation:

$$\delta^{18}O_{NO_3} = 2/3 \times \delta^{18}O_{H_2O} + 1/3 \times \delta^{18}O_{O_2}$$

Atmospheric oxygen has a $\delta^{18}O$ value of 23.5 ‰, and the $\delta^{18}O_{NO_3}$ values of the generated NO_3^- would cover a wide range between −5 and 15 ‰ (Mayer et al. 2001; Kendall et al. 2007). In one of the few studies that dealt with riverine nitrification, Sebilo et al. (2006) calculated a $\delta^{18}O$ value of 3 ‰ for newly produced NO_3^- in the Seine River catchment. In this study, the Jialing River watershed has $\delta^{18}O_{H_2O}$ values ranging from −10.9 to −7.3 ‰. Thus, the expected $\delta^{18}O_{NO_3}$ values for NO_3^- produced by nitrification should be in the range of 1.0–4.0 ‰, which matched well with the $\delta^{18}O_{NO_3}$ values of waters in the middle Jialing River during the rainy season (Fig. 3b).

In addition, plotting $\delta^{18}O_{NO_3}$ versus $\delta^{18}O_{H_2O}$ could also be useful to determine nitrification, because nitrification in contact with the ambient water would result in $\delta^{18}O_{NO_3}$ values that show a positive correlation with $\delta^{18}O_{H_2O}$ (Wankel et al. 2006; McMahon and Böhlke 2006). As shown in Fig. 5, values of $\delta^{18}O_{NO_3}$ for samples taken in the middle course of the Jialing River during the rainy season mostly aligned along line 2 were defined as $\delta^{18}O_{NO_3} = 2/3 \times \delta^{18}O_{H_2O} + 1/3 \times \delta^{18}O_{O_2}$. Along this line 2, the expected $\delta^{18}O_{NO_3}$ values for NO_3^- of the studied samples are caused by microbial nitrification, which further suggests that the input of NO_3^- originating from the nitrification of the applied fertilizers in the Jialing River watershed, consistent with the published estimates of N inputs to the Yangtze River (Li et al. 2010).

5.3.2 Assimilation and Denitrification

Losses of Si are attributed to the growth of epilithic diatoms, whereas NO_3^- losses are consistent with a number of processes including the growth of aquatic plants, the development of epilithic biofilms (assimilation), and denitrification (House et al. 2001; Cook et al. 2010). Fractionation due to assimilation of NO_3^- by phytoplankton as well as denitrification leaves the residual NO_3^- enriched in ^{15}N and ^{18}O because of a preferential uptake of "isotopically light" NO_3^- (^{14}N and ^{16}O) by phytoplankton and bacteria (Kendall et al. 2007). Although the degree of isotopic fractionation changes, depending on many factors such as environmental conditions and reaction rates, the ratios of the increase in $\delta^{15}N$ and $\delta^{18}O$ values of the remaining NO_3^- are assumed to be close to 1:1 during assimilation (Granger et al. 2004), and 1.5:1 or even 2:1 during denitrification in freshwater (Panno et al. 2006, David et al. 2006). Therefore, the processes of assimilation and denitrification can possibly be evaluated by means of the ratios of these increases (Kendall et al. 2007; Granger et al. 2008; Deutsch et al. 2009).

During the rainy season, the NO_3^- concentrations typically decreased within the dam area in the middle course of the Jialing River, which was caused not only by dilution (rain) but also by uptake by aquatic biota (e.g., algae) and/or in-stream denitrification (Figs. 2a, 3). Based on productivity indirectly calculated from Si removal rates, House et al. (2001) estimated the proportion of NO_3^- assimilated by diatoms to reach 30 % of the measured NO_3^- removal in a stream reach during summer. Generally, while N assimilation by phytoplankton is a major process in eutrophic lakes (Cook et al. 2010), it has not been given much attention in rivers—probably because the role of primary producers for nutrient transformations in rivers has been underestimated (Deutsch et al. 2009). Additionally, in rivers, denitrification mostly takes place at the river—groundwater interface, in the riparian zone, and in the sediments just below the water/sediment interface (Birgand et al. 2007). In the present study, concomitantly with a decrease in NO_3^- concentration and an increase in $\delta^{15}N_{NO_3}$ values during the rainy season, we measured a decrease in the SiO_2 content across the dam area, indicating that assimilation occurs and that N uptake by phytoplankton is at least partly responsible for the loss of NO_3^- (Figs. 2, 3) besides dilution by precipitation. In addition, to control flooding and generate electricity, the water level in these reservoirs was increased, and near-stream rice paddies were submerged from mid-June to October and long stretches of low-gradient streams are common, which would cause riparian denitrification in these areas. For denitrification occurring in the sediments, because the limiting step is the diffusion of water column NO_3^- into the anoxic sediment layer where NO_3^- is denitrified, this process might be inconspicuous and not result in significant isotopic fractionation (approximately −1.5 ‰) compared with riparian denitrification (Sebilo et al. 2006; Kendall et al. 2007).

During the dry season, although the NO_3^- concentrations remained stable within the dam area and then increased slightly in the lower course of the Jialing River, the SiO_2 concentrations decreased sharply to the lowest silica concentration at site 15 and then became stable downstream (Fig. 2). In fact, the contribution of domestic sewage and manure should be greater in the dry season, which is indicated by the higher concentrations of Cl^-, and lower NO_3^-/Cl^- ratios in February 2009 (Table 2; Li et al. 2011), because domestic sewage and manure have been found to be high in Cl^- and SO_4^{2-} concentrations (Krapac et al. 2002; Li et al. 2011) and low in NO_3^-/Cl^- ratios (Liu et al. 2006; Chen et al. 2009). Therefore, constant concentrations of NO_3^- during the dry season are likely due to the following: (a) the minimal input from nitrification of fertilizer and (b) mass-balance between sewage and manure inputs and low biological activity with cold temperatures, as described previously. In view of the nonpoint sources of NO_3^- with greatly variable dual-isotopic values, the assimilation and denitrification and mixing processes should be considered to explain the mismatch in the $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- during the dry season (Figs. 2a, 3).

Even though the $\delta^{18}O_{NO_3}$ values and the SiO_2 concentrations had a negative correlation between sites 11 and 15 during the dry season (Figs. 2b, 3b), there was no obvious isotopic evidence of the $\delta^{18}O_{NO_3}$ values for assimilation and denitrification within the dam area of the Jialing River. Most values of $\delta^{18}O_{NO_3}$ in the catchment were found to be less than 5 ‰, less than what has been reported in previous studies in the large rivers of the world (Panno et al. 2008; Lee et al. 2008; Li et al. 2010). This can be explained mainly by the following: (a) the nitrification of fertilizer and wastewater NH_4^+ produced NO_3^- with a light $\delta^{18}O_{NO_3}$ values in the middle and lower courses of the Jialing River and (b) the effect of in-stream nutrient cycling as mentioned above. This suggests that the NO_3^- in the middle to lower course was affected by a higher degree of NO_3^- recycling and mixture input, similar to that already reported in the Oldman River in western Canada (Rock and Mayer 2004).

6 Conclusion

This study of the Jialing River has shown that NO_3^- concentrations are much higher in the rainy season than in the dry season in the middle and lower courses and are closely related to land-use and seasonal precipitation patterns. The increase in the use of synthetic fertilizers is considered to be the major driving factor resulting in the large amount of riverine N transport, and discharge of sewage and manure effluent in the upper course and from large cities accounts for a considerable amount of the NO_3^- load to the river, as demonstrated by NO_3^- concentrations and dual (N and O) isotopic compositions. Dams along the Jialing River significantly affect the water chemistry and N transformation, and change the SiO_2 and Si/N ratio in river waters. As a major source of runoff and sediment into the Three Gorges Reservoir, the increasing N loads and decreasing SiO_2 concentration and Si/N ratio in the waters of the Jialing River might have profound impacts on a variety of biogeochemical cycles on a basin scale and in the upper Yangtze River. It is deduced that the intense reservoir development coupled with rapid economic growth in the Jialing River basin should trigger more significant anthropogenic impacts on the water chemistry in the near future.

The data collected in this study represent a comprehensive evaluation of the dual-isotopic compositions of riverine NO_3^- in a dam-controlled subtropical river in Asia. The impact of fertilizer NH_4^+ on the NO_3^- concentration and $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ values of river water along with the influence of human-derived sewage may be common to many agricultural regions in Asia.

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