



Identifying the sources of nitrate in a small watershed using $\delta^{15}\text{N}$ - $\delta^{18}\text{O}$ isotopes of nitrate in the Kelan Reservoir, Guangxi, China

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ABSTRACT

China has one of the largest areas of agriculture and one of the highest application rates of chemical fertilizer in the world. Agricultural non-point source pollution has become important source of nitrogen (N) pollution of surface water. In this study, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes of nitrate as a tool were used to reveal the sources of nitrate in water bodies in the Nala watershed, at a sugarcane planted area in Guangxi, China. According to the analyses based on a stable isotope analysis in R (SIAR), the mean contributions of different nitrate sources in the Nala watershed could be ranked as follows: manure (39.90 %) > soil N (SN) (34.94 %) > chemical fertilizer (CF) (17.77 %) > rainfall (7.39 %) in July; manure (42.30 %) > SN (33.54 %) > CF (24.16 %) in December. The CF and SN collectively mean contributing > 50 % of nitrate both in July and in December in the watershed, therefore, reducing the use of CF is the key to control nitrate pollution in the Nala watershed. Manure also composed a substantial contribution (mean 39.68 %, in July and December) to nitrate in the small watershed, it is necessary to provide recycle treatment for manure for the purpose of alleviating the pollution. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes of nitrate can accurately identify various sources of nitrate and SIAR offers a useful method to quantify the contribution rates of different N sources to the N pollution of watersheds.

1. Introduction

China has a large area of agricultural land, and is one of the largest consumers of nitrogen (N) fertilizer in the world. At present, the rate of N fertilizer application in China accounts for 32 % of its total amount of application in the world (Yan et al., 2014; Heffer, 2009; Ata-Ul-Karim et al., 2017; Cui et al., 2010). The original intention of extensively applying N fertilizer is to increase crops yield and meet the demands for grain by China's ever-increasing population; however, when used unreasonably, N fertilizer not only has a low utilization rate, but also posed serious threats to aquatic ecosystems. At crops planted areas, especially areas with high rainfall, large amounts of N fertilizer are carried by rain into water bodies before being absorbed by crops, resulting in the eutrophication of water bodies (Ying et al., 2017; Tayefeh et al., 2018). In fact, eutrophication and water quality deterioration, caused by the continuously increased nitrate (NO_3^-) concentration in water bodies, have become worldwide concerns (Lau and Lane, 2002; Zhao et al., 2010; Li et al., 2014). To protect the water and solve the

urgent global problem of water pollution, the key technical challenge faced by the world is how to identify the sources of N pollution in water bodies and quantify their rates of contribution.

To identify the sources of nitrate in water bodies, the traditional approach is to investigate the land use types around polluted areas and analyze the spatio-temporal variations of the water chemistry composition of runoff and receiving water bodies. This approach requires an extensive workload, and its conclusions are largely uncertain (Hall and Risser, 1993). In the 1970s, Kohl et al. (1971) used the concentration and $\delta^{15}\text{N}$ isotope composition of nitrate to identify the sources of nitrate in surface water, the authors found that 55–60 % of NO_3^- in surface water bodies derived from chemical fertilizer (CF). Since then, the $\delta^{15}\text{N}$ isotope of nitrate has been gradually used to quantify identify the sources of nitrate in water bodies (Kellman and Hillaire-Marcel, 1998; Dignazio et al., 1998; Williams et al., 1998; Sigman et al., 2000). However, relying on $\delta^{15}\text{N}$ isotope alone for source identification still faces challenges because different sources of nitrate frequently resulted in overlapping the values of $\delta^{15}\text{N}$ (Kellman and Hillaire-Marcel, 1998).

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Fortunately, the $\delta^{18}\text{O}$ of nitrate can also provide some useful information about the sources of nitrate (Amberger and Schmidt, 1987; Li et al., 2010). Therefore, using of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes of nitrate makes it possible to accurately indicate sources of nitrate in water bodies (Xue et al., 2009; Granger et al., 2010). Recently, with the progress of isotope testing techniques, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ double isotopes of nitrate have been used to trace the sources of nitrate in water bodies (Li et al., 2013, 2019; Wang et al., 2018; Zhang et al., 2014). The Bayesian isotope mixing model was developed to evaluate the contributions of different sources of nitrate in the watershed (Xue et al., 2012).

South China was located in the center of South Asia, which is one of the largest karst landforms in the world. In the surface water of agricultural catchments in these karst areas, nitrate pollution is serious, which threatens safety of regional drinking water. However, quantifying the contribution of different pollution sources to nitrate is rarely reported in these small watersheds. In this study, Nala small watershed was selected as the research object for the following reasons: first, this small watershed was located in the karst area of south China, and the research on it is of practical significance to guide the prevention and control of pollution in the karst area. Second, this small watershed is a typical agricultural catchment, where agricultural non-point source pollution dominates the input of N pollutants. Third, this small watershed is the major inflow of drinking water source reservoir (Kelán Reservoir), and the study will help to protect the water quality of this water source reservoir. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ double isotopes of nitrate were used to trace the sources of nitrate, and the Bayesian isotope mixing model was employed to calculate the rates of contribution of different nitrate sources, providing a data basic to control N pollution of nitrate in this karst classic agricultural area, south China. This study aimed to: 1) quantify the rates of contribution of different sources of nitrate; and 2) highlight the key to control agricultural non-point source pollution in small watersheds.

2. Methods

2.1. Sample sites

The Kelán Reservoir (107°61'–107°67' E, 22°31'–22°39' N) was located in Fusui County, Guangxi, China. This watershed has a subtropical monsoon climate, an annual mean temperature of 20.8 °C–22.4 °C, and an annual rainfall of approximately 1200 mm, was mainly concentrated in May to September every year. The rainfall accumulation area and storage capacity of the reservoir amount are 35 km² and 323 million m³, respectively. The Kelán Reservoir serves as the source of drinking water for nearby towns and villages, and as an alternate source of drinking water for Chongzuo City.

The Kelán Reservoir is currently one of the largest reservoirs in Guangxi, China. Completed for impoundment in 1957, it is mainly used for irrigation, flood control, power generation, and drinking water. Distributed around the reservoir are the major agricultural areas of this region; crops, such as eucalyptus, sugarcane, rice, and orange are mainly planted around the banks. The planted area is mainly covered by low-fertility red soil, so a large amount of fertilizer need to be applied during crops growth.

2.2. Sample collection

Samples were collected from the sampling sites distributed in the Nala watershed of the Kelán Reservoir in July and December 2019 (Fig. 1). The sampling sites were distributed in villages and crops planted areas (sugarcane and rice). The watershed was primarily planted with sugarcane and a little rice. Four ponds (sampling sites 1–4) were located in the village were used to collect domestic sewage, aquaculture wastewater discharged by village, sampling sites 5–13 and 16 distributed in sugarcane planted area; sampling sites 14 and 15 distributed in sugarcane and rice mixed planted areas. Approximately

30 cattles and 400 ducks/chickens were feed by villager.

River water and rain water samples were collected with plastic buckets and stored in polyethylene bottles. Before chemical analysis, each water sample was divided into two portions, one portion was used to determine the anions and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes in nitrates, the other portion was used to test cations after adding 0.1 M HNO₃ solution to make pH less than 2. Surface soil (0–20 cm) and manure samples were collected from the vicinity of sampling sites, and contained in sealed bags. To analyze the influence of agricultural activities on nitrate in the watershed, chemical fertilizer samples were collected from town/township outlets selling CF in the watershed. All samples were put in ice boxes, immediately sent to the laboratory and stored in refrigerators (4 °C) until chemical analysis.

2.3. Sample analysis

After being filtered with a 0.45 μm filter membrane, water samples were cryopreserved in polyethylene bottles and stored in the dark. A total of 60 g soil, 40 g CF and manure samples were weighed, then were put into 250 mL high-density polyethylene bottles, 200 mL ultrapure water was added (Rock and Ellert, 2007) and samples were shaken for 60 min. After being filtered with a 0.45 μm filter membrane, the leaching liquors obtained were cryopreserved in polyethylene bottles and stored in the dark. The concentrations of cations in water samples were determined with an inductively coupled plasma Optical emission spectrometer (ICP-OES-Vista-Mpx, Varian Co, USA), and the concentrations of anions in water samples were determined with a Dionex ion chromatography (IC) system 90 (Dionex Co., Sunnyvale, CA, USA). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate in the above samples were determined by denitrifier bacteria method at the Environmental Stable Isotope Lab, Chinese Academy of Agricultural Science (CAAS) (Casciotti et al., 2002; Sigman et al., 2001). The specific test flow is as follows: The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO₃⁻ were determined by the “denitrifier bacteria method”, which allows for the simultaneous determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N₂O produced from the conversion of NO₃⁻ by denitrifying bacteria (*Pseudomonas aureofaciens*, ATCC13985, USA), which naturally lack N₂O-reductase activity. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ analysis of the produced N₂O was carried out using a trace gas preparation unit (Precon, Finnigan, Germany) coupled to an isotope ratio mass spectrometer (IRMS) (Delta V plus, Finnigan, Germany). The N₂O sample is injected via an autosampler and H₂O/CO₂ is removed using scrubbers (Magnesium Perchlorate and Carbosorb, Merck KGaA). By cryogenic trapping and focusing, the N₂O is compressed onto a capillary column (PoraPlot Q, 25 m, 0.32 mm id, 10 mm df, Agilent Technologies, US) at 35 °C and subsequently analyzed by isotope ratio mass spectrometer (IRMS). The $\delta^{15}\text{N}$ were calibrated with USGS32 (180.0 ± 1.0‰ for $\delta^{15}\text{N}$), USGS34 (-1.8 ± 0.2‰ for $\delta^{15}\text{N}$) and IAEA N3 (4.7 ± 0.2‰ for $\delta^{15}\text{N}$), $\delta^{18}\text{O}$ were calibrated with USGS34 (-27.8 ± 0.4‰ for $\delta^{18}\text{O}$), IAEA N3 (25.6 ± 0.4‰ for $\delta^{18}\text{O}$) and USGS35 (56.8 ± 0.3‰ for $\delta^{18}\text{O}$).

During water sample collection, a multi-parameter water quality monitor (YSI6600-V2, YSI Co, USA) was used to determine the temperature (T), dissolved oxygen (DO), pH, and electrical conductivity (EC) of water bodies.

2.4. Calculation of the rates of contribution of different sources of nitrate

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes of nitrate were expressed with δ and calculated according to the following formula (Kohl et al., 1971):

$$\delta(\%) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

R_{sample} and R_{standard} denote the $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$ ratios of samples/standard samples, that is, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively; N isotope adopts atmospheric N (N₂) as reference standard; O isotope adopts Vienna Standard Mean Ocean Water as reference standard (Vienna standard mean ocean water, V-SMOW).

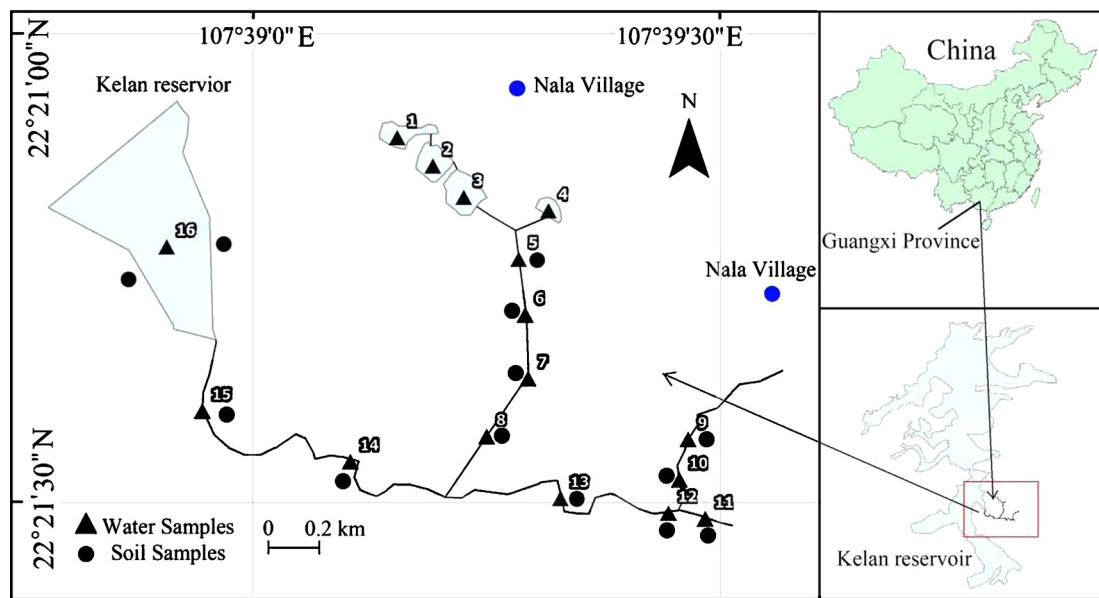


Fig. 1. Sampling points in the Nala watershed of the Kelan Reservoir, Guangxi Province, China.

The rates of contribution of different sources of nitrate were calculated using the stable isotope analysis in R (SIAR, Parnell et al., 2010):

$$X_{ij} = \frac{\sum_{K=1}^K P_k q_{jk} (S_{jk} + C_{jk})}{\sum_{K=1}^K P_k q_{jk}} + \varepsilon_{ij} \quad (2)$$

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

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

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

where X_{ij} denotes the j^{th} isotope of sample i ; S_{jk} denotes the j^{th} isotope of the K^{th} source; μ_{jk} denotes the mean value; ω_{jk}^2 denotes variance; C_{jk} denotes the fractionation factor of the j^{th} isotope of the K^{th} source; λ_{jk} denotes the mean value; τ_{jk}^2 denotes variance; p_k denotes the contribution rate of the K^{th} source calculated from the model; q_{jk} denotes the concentration of the j^{th} isotope of the K^{th} source; ε_{ij} denotes residual error with a mean value of 0; σ_j^2 denotes variance.

Previous studies showed that different sources of nitrate have obviously different ranges of isotope values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (rainfall: $\delta^{15}\text{N}_{\text{nitrate}}$ (-5‰ to 10‰) and $\delta^{18}\text{O}$ (60‰ to 80‰); SON: $\delta^{15}\text{N}_{\text{nitrate}}$ (0‰ to 8‰) and $\delta^{18}\text{O}$ (-10‰ to 14‰); Manure & Sewage: $\delta^{15}\text{N}_{\text{nitrate}}$ (8‰ to 20‰) and $\delta^{18}\text{O}$ (-10‰ to 14‰); NH_4^+ fertilizer: $\delta^{15}\text{N}_{\text{nitrate}}$ (-5‰ to 0‰) and $\delta^{18}\text{O}$ (-10‰ to 14‰); NO_3^- fertilizer: $\delta^{15}\text{N}_{\text{nitrate}}$ (-5‰ to 5‰) and $\delta^{18}\text{O}$ (16‰ to 25‰)) (Yue et al., 2014; Wang et al., 2016; Fenech et al., 2012; Yang and Toor, 2016). In this study, the values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate of different sources are listed in Table S2. The isotopic values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in different sources (including rainfall, manure, CF and SN in July; manure, SN and CF in December) of nitrate was used to calculate the rates of contribution in nitrate for river water in the Nala watershed, due to there are not obvious nitrification and denitrification in the watershed, so this study set C_{jk} as 0 in Formula (2). Detailed calculation steps refer to Parnell et al. (2010).

3. Results

3.1. Water chemistry

T, DO, pH, EC, anions and cations concentration of water samples in the Nala watershed were showed in Table 1. In July, the values of the

concentration of NO_3^- in sample sites 1–4 in the Nala watershed ranged from 0.22 mg L^{-1} to 0.55 mg L^{-1} and mean 0.33 mg L^{-1} , the values of NO_3^- in surface water (sites 5–16) ranged from 0.49 mg L^{-1} to 22.04 mg L^{-1} and mean 9.80 mg L^{-1} , respectively (Table 1). In December, the values of the concentration of NO_3^- in sample sites 1–4 in the Nala watershed ranged from 0.02 mg L^{-1} to 0.06 mg L^{-1} and mean 0.04 mg L^{-1} , the values of NO_3^- in surface water (sites 5–16) ranged from 0.09 mg L^{-1} to 38.21 mg L^{-1} and mean 14.92 mg L^{-1} , respectively (Table 1). The concentration of NO_3^- exceeded the environmental quality standard for surface water class II (0.5 mg L^{-1} , GB3838-2002, 2002) specified by the Ministry of Ecological Environment of China in 75 % (July) and 57 % (December) of river water sampling points. The concentration of NO_3^- in the leaching liquors of soil (rice soil, sugarcane soil) ranged from 0.25 mg L^{-1} to 261.70 mg L^{-1} and mean 44.88 mg L^{-1} in July. The concentration of NO_3^- in the leaching liquors of soil (rice soil, sugarcane soil) ranged from 0.25 mg L^{-1} to 74.87 mg L^{-1} and mean 27.45 mg L^{-1} in December, significantly lower than July (Table S1).

The concentration of NO_3^- in leaching liquor of soil was significantly higher than surface water in the small watershed, possibly because of the application of CF on the soil system (Table 1 and Table S1). It could be inferred that both SN and CF were two important sources of NO_3^- in the watershed.

In total, the concentration of NO_3^- in water bodies in the Nala watershed firstly increased and then decreased from upstream to downstream (Fig. 2). The NO_3^- concentration peaked (22.04 mg L^{-1}) at site 11 of water samples; the concentration of NO_3^- in soil leaching liquors around the watershed showed irregular variations and peaked (261.70 mg L^{-1}) at site soil 15 in July (Fig. 2). In December, the NO_3^- concentration peaked (38.21 mg L^{-1}) at site 9 of water samples; the concentration of NO_3^- in soil leaching liquors around the watershed also showed irregular variations and peaked (74.87 mg L^{-1}) at site soil 14, possibly because of the application of CF at the crops planted areas around the watershed (Fig. 2). The spatial variation characteristics of the concentration of Cl^- in water bodies in the small watershed were similar to NO_3^- (Fig. 2).

3.2. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotope compositions of nitrate in the Nala watershed

In July, the $\delta^{15}\text{N}$ values of nitrate in sample sites 1–4 ranged from 1.06‰ to 17.88‰ (mean 9.71‰). The $\delta^{15}\text{N}$ values in surface water (sites 5–16), in soil leaching liquors, in rainwater, in compound fertilizer and in manure leaching liquors ranged from 6.62‰ to 15.92‰

Table 1

Water quality characteristics of water samples in the Nala watershed of the Kelan Reservoir, Guangxi, China (In July and December 2019).

Sites	Date	T (°C)	DO (mg·L ⁻¹)	pH	EC (μscm ⁻¹)	K ⁺ (mg·L ⁻¹)	Ca ²⁺ (mg·L ⁻¹)	Na ⁺ (mg·L ⁻¹)	Mg ²⁺ (mg·L ⁻¹)	SiO ₂ (mg·L ⁻¹)	F ⁻ (mg·L ⁻¹)	Cl ⁻ (mg·L ⁻¹)	NO ₃ ⁻ (mg·L ⁻¹)	SO ₄ ²⁻ (mg·L ⁻¹)
1	July	30.8	1.69	7.52	423	28.51	30.02	8.02	4.87	13.35	0.37	23.24	0.31	15.66
	Dec.	14.9	7.16	8.18	318	30.38	45.84	10.05	8.30	6.63	0.44	31.74	0.06	19.32
2	July	30.7	3.79	7.64	389	23.98	38.60	9.26	6.11	9.40	0.35	33.18	0.55	20.29
	Dec.	14.9	7.16	8.18	318	30.38	45.84	10.05	8.30	6.63	0.44	31.74	0.06	19.32
3	July	28.0	1.40	7.54	500	33.88	47.00	14.04	8.84	21.67	0.26	37.76	0.22	24.22
	Dec.	10.1	5.38	7.15	250	3.09	—	11.56	9.33	20.87	0.29	0.18	0.05	4.09
4	July	30.3	2.15	7.67	375	10.88	49.54	6.47	5.43	11.69	0.46	37.86	0.25	19.21
	Dec.	15.1	6.28	8.01	329	11.68	57.95	7.92	6.63	0.04	0.47	38.88	0.02	22.74
5	July	26.4	2.80	7.64	364	24.81	44.96	7.91	6.38	12.41	0.29	40.36	0.49	17.93
	Dec.	16.3	8.35	7.68	372	13.70	62.74	9.37	8.43	8.12	0.20	42.23	0.79	24.41
6	July	26.7	3.17	7.62	393	26.27	39.99	7.55	6.06	11.94	0.27	39.88	1.94	18.65
	Dec.	10.6	8.40	7.90	333	13.12	63.97	9.44	9.26	7.81	0.22	41.97	0.74	22.97
7	July	27.6	3.52	7.72	375	24.68	39.92	7.24	5.86	11.17	0.27	38.93	1.86	17.65
	Dec.	14.3	9.20	8.05	353	13.42	63.02	9.44	8.99	6.16	0.28	44.01	0.23	22.68
8	July	28.4	3.59	7.78	368	20.94	38.52	6.89	5.38	9.98	0.27	37.20	2.67	18.28
	Dec.	11.2	9.11	8.40	312	15.48	53.91	10.78	9.80	4.40	0.37	44.93	0.09	23.55
9	July	29.3	3.55	7.92	372	1.05	51.96	2.59	1.98	8.37	0.30	72.62	20.05	9.17
	Dec.	11.6	6.62	7.63	439	0.35	122.06	2.25	3.27	8.77	0.24	27.42	38.21	8.02
10	July	29.0	3.79	7.90	437	2.35	72.28	1.94	2.24	7.39	0.33	41.71	17.40	10.62
	Dec.	16.4	10.63	8.09	460	4.22	101.69	2.32	3.51	6.76	0.30	35.44	30.83	10.09
11	July	29.5	4.61	7.99	442	1.46	69.12	2.70	2.25	6.82	0.27	49.23	22.04	11.22
	Dec.	29.2	3.77	7.89	438	2.17	69.81	2.21	2.30	7.32	0.31	44.01	17.69	10.22
12	July	29.2	3.77	7.89	438	2.17	69.81	2.21	2.30	7.32	0.31	44.01	17.69	10.22
	Dec.	15.7	9.96	8.12	532	4.43	104.40	2.29	3.77	6.18	0.29	35.01	30.01	10.22
13	July	27.9	4.56	7.85	391	3.28	59.03	2.28	2.30	6.83	0.31	43.73	14.42	9.35
	Dec.	10.5	10.43	8.18	271	2.94	63.73	3.99	4.36	1.00	0.47	40.44	—	11.90
14	July	28.1	2.00	7.56	467	2.50	78.01	2.83	3.10	10.85	0.26	22.22	8.31	9.72
	Dec.	13.3	7.88	7.69	400	0.53	106.42	2.42	2.95	9.55	0.19	12.88	21.42	10.18
15	July	27.4	3.56	7.57	389	2.47	80.87	2.99	3.35	10.79	0.27	22.80	8.68	10.43
	Dec.	14.6	8.51	7.84	425	0.72	103.79	2.80	3.30	9.13	0.25	15.54	21.94	12.47
16	July	28.3	3.96	7.75	345	2.09	41.16	2.31	2.97	7.97	0.15	15.59	2.08	14.77
	Dec.	15.4	6.90	8.05	386	1.97	68.36	2.37	3.77	7.79	0.17	11.97	4.92	17.06

— Not determined.

(mean 11.01‰), from -17.87‰ to 15.59‰ (mean 0.64‰), from -1.97‰ to -1.55‰ (mean -1.77‰), from -0.22‰ to 0.87‰ (mean 0.36‰) and from 16.28‰ to 16.89‰ (mean 16.66‰) (Fig. 3 and Table S2). The $\delta^{18}\text{O}$ values of nitrate in sample sites 1-4 ranged from 11.27‰ to 27.24‰ (mean 20.39‰). The $\delta^{18}\text{O}$ values of nitrate in surface water (sites 5-16), in soil leaching liquors, in rainwater, in compound fertilizer and in manure leaching liquors ranged from 6.42‰ to 22.84‰ (mean 11.04‰), from 0.99‰ to 16.41‰ (mean 6.52‰), from 64.59‰ to 72.16‰ (mean 67.69‰), from 14.21‰ to 15.11‰ (mean 14.73‰) and from 8.6‰ to 15.1‰ (mean 10.8‰) (Fig. 3 and Table S2).

In December, the $\delta^{15}\text{N}$ values of nitrate in surface water (sites 5-16, except 1-4, 8, 11 and 13; not determined) ranged from 6.47‰ to 10.26‰ (mean 8.23‰) (Fig. 3). The $\delta^{15}\text{N}$ values of nitrate in soil leaching liquors and in manure leaching liquors ranged from -2.10‰ to 8.44‰ (mean 4.40‰) and from 9.12‰ to 10.74‰ (mean 10.17‰) (Fig. 3 and Table S2). Values of the $\delta^{18}\text{O}$ isotope of nitrate in surface water (sites 5-16, except 1-4, 8, 11 and 13 not determined) ranged from 5.82‰ to 15.50‰ (mean 10.99‰). The $\delta^{18}\text{O}$ values of nitrate in soil leaching liquors and in manure leaching liquors ranged from 5.66‰ to 15.12‰ (mean 9.67‰) and from 8.62‰ to 13.96‰ (mean 11.79‰) (Fig. 3 and Table S2).

In general, the isotopic composition of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate in surface water samples had no obvious difference between in summer and winter in the Nala watershed. The values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate in water samples 1 and 3 were significantly higher than other samples in July. It is likely mixed other nitrate sources, such as rainfall and manure (Fig. 3). Compared with in December, some values of $\delta^{15}\text{N}$ of nitrate in soil samples were less than 0‰ in July, due to the plenty of application of ammonia fertilizer in soil in summer that led negative values of $\delta^{15}\text{N}$.

4. Discussion

4.1. Identification of the sources of NO₃⁻ in the Nala watershed based on water chemistry composition

Cl⁻ can be used to trace mixing in nature water, because it is conservative ion, almost unaffected by physical, chemical, or biological processes (Yue et al., 2013; Chen et al., 2014). In addition, the relationship between Cl⁻ and NO₃⁻ concentration, between the NO₃⁻/Cl⁻ molar ratio and Cl⁻ concentration are widely used to judge mixing process or biological process of nitrate in the watershed (Liu et al., 2006; Yue et al., 2013, 2014; Jin et al., 2018). The sources of Cl⁻ include domestic sewage, CF, dissolved minerals, and industrial wastewater (Liu et al., 2006; Jin et al., 2018). The presence of a high-concentration of Cl⁻ in water bodies suggests that pollution might have come from domestic sewage, manure, or industrial wastewater (Yao et al., 2007; Jin et al., 2018). In the case of a high NO₃⁻/Cl⁻ ratio but low Cl⁻ concentration, NO₃⁻ was mainly contributed by agricultural activities; a low NO₃⁻/Cl⁻ ratio but high Cl⁻ concentration, NO₃⁻ mainly derived from domestic sewage or manure. When the NO₃⁻ and Cl⁻ concentrations are both low, NO₃⁻ mainly derived from SN. In the case of a constant NO₃⁻/Cl⁻ ratio and low Cl⁻ concentration, the water bodies had gone through mixing processes. Furthermore, chemical fertilizer contain high NO₃⁻ concentration and high NO₃⁻/Cl⁻ (Chen et al., 2014; Liu et al., 2006; Yue et al., 2017).

There was no significant positive or negative correlation between Cl⁻ and NO₃⁻ in July in surface water in the Nala watershed (Fig. 4). The presence of low Cl⁻ concentration (< 0.1 mmol·L⁻¹) and high NO₃⁻/Cl⁻ ratio (> 1) in soil leaching liquors (sites s6, s7, s11, and s13; Fig. 4 (July)) suggested that NO₃⁻ mainly derived from agriculture activities. In the case of high Cl⁻ concentration (> 3 mmol) but low NO₃⁻/Cl⁻ ratio (< 0.1), the NO₃⁻ in water samples derived from domestic sewage or manure (site s14(paddy), Fig. 4 (July)). At other

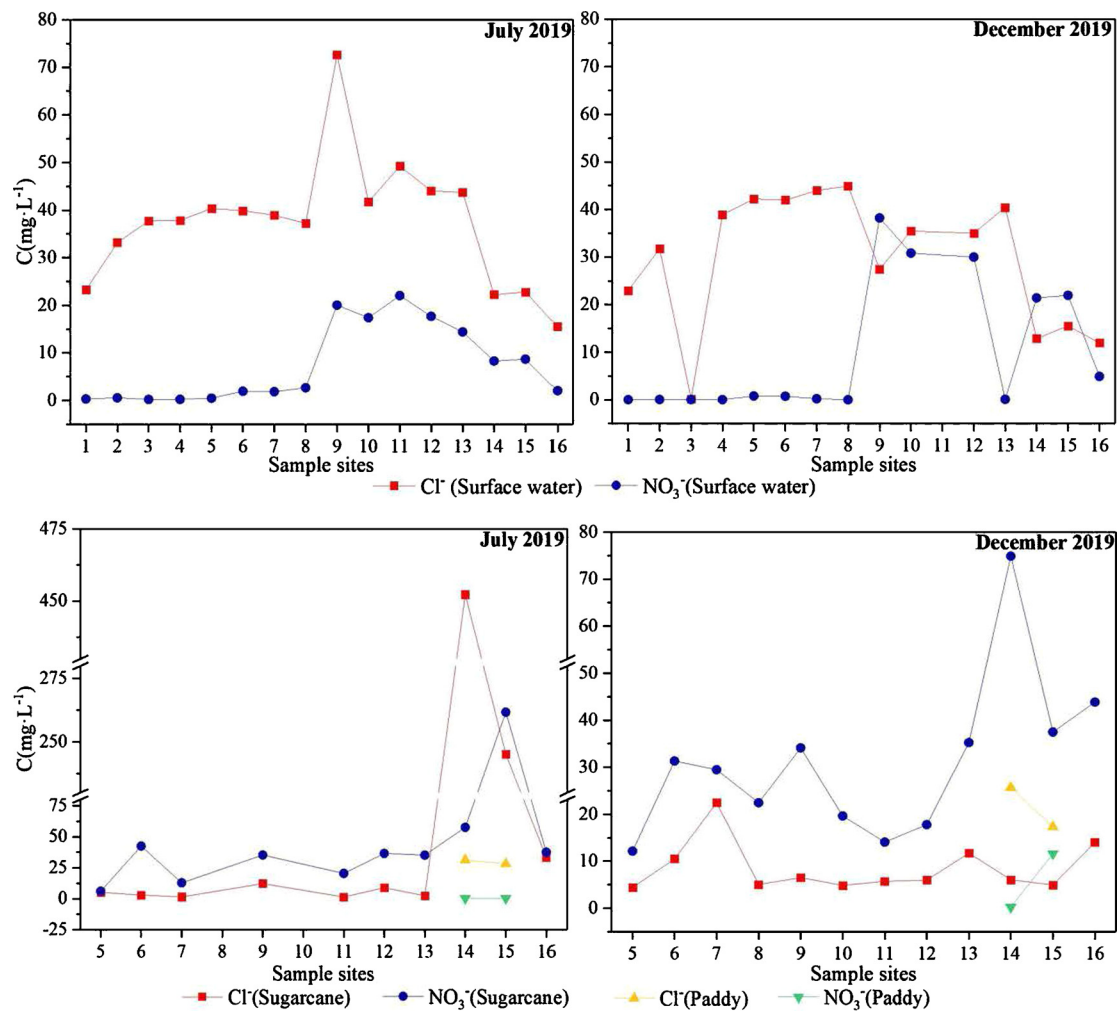


Fig. 2. Variations of the NO_3^- and Cl^- concentrations of water bodies and soil leaching liquors.

sampling points, the $\text{NO}_3^-/\text{Cl}^-$ molar ratio ranged from 0.2 to 1 and Cl^- concentration ranged from $0.2 \text{ mmol}\cdot\text{L}^{-1}$ to $0.5 \text{ mmol}\cdot\text{L}^{-1}$, suggesting that nitrate mixed multiple sources (including manure, CF and SN) at these points in the watershed (Fig. 4) (Amberger and Schmidt, 1987; Desimone and Howes, 1998). Overall, the NO_3^- in the Nala watershed mainly derived from CF, manure, and SN in July, but differences were observed in different sampling points.

In December, there was also no relation between Cl^- and NO_3^- . Sampling sites (5, 6, 7, 8, 13 and s14 (paddy)) exhibited high Cl^- concentration and low $\text{NO}_3^-/\text{Cl}^-$, suggesting a constant manure nitrogen sources (Fig. 4). At other sampling points, the $\text{NO}_3^-/\text{Cl}^-$ molar ratio ranged from 0.2 to 1 and Cl^- concentration ranged from $0.2 \text{ mmol}\cdot\text{L}^{-1}$ to $0.5 \text{ mmol}\cdot\text{L}^{-1}$, suggesting that nitrate derived from multiple sources (including manure, CF and SN) at these points in the watershed (Fig. 4) (Amberger and Schmidt, 1987; Desimone and Howes, 1998). Furthermore, sampling sites (s8-s12, s14-s15; Fig. 4) showed high $\text{NO}_3^-/\text{Cl}^-$ and low Cl^- concentration, suggesting a agricultural activities.

Above all, mixing processes were the main factor influencing the migration of N in the Nala watershed.

4.2. Quantification of the sources of NO_3^- in the Nala watershed using the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ double isotopes composition of nitrate and SIAR model

In July, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotope composition of NO_3^- in four ponds (Sites 1–4) was ranged from 1.06‰ to 17.88‰ (mean 9.71‰), and from 11.27‰ to 27.24‰ (mean 20.39‰). At the crops planted

areas (Sites 5–16), the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes composition were ranged from 6.62‰ to 15.92‰ (mean 10.72‰), and from 6.42‰ to 15.28‰ (mean 9.97‰). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopic values of nitrate in domestic sewage were not fallen into the range of manure & sewage, that's because these ponds are also collected by rain water, SN, and fertilizer besides domestic sewage (Fig. 5, 6 and Table S2). At the crops planted areas, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopes composition in most water samples had fallen into the ranges of manure, sewage and SN except Sites 11. At site 11, the $\delta^{18}\text{O}$ in nitrate of 22.84‰ was heavier than others, likely attributed to the less contribution of rain water with lighter $\delta^{18}\text{O}$ value (Fig. 5 and Table S2). Thus, the manure, CF, SN, and rainfall were main nitrate sources in Nala watershed in July, consistent with the results of the sources of nitrate revealed by water chemistry (4.1) and the results obtained by Xing and Liu (2016). In addition, there were high $\text{NO}_3\text{-N}$ in soil leaching liquors (soil 5, 7, and 11; Table S1) and low $\delta^{15}\text{N}$ (-3.77‰ , -2.31‰ , and -2.57‰) and $\delta^{18}\text{O}$ (1.94‰ , 4.17‰ , and 1.35‰) values (Fig. 3 or Table S2), indicating ammoniafertilizer was used in soil system and as a major formed sources of SN.

In December, the NO_3^- concentration was low ($0.02 \text{ mg}\cdot\text{L}^{-1}$ to $0.06 \text{ mg}\cdot\text{L}^{-1}$, Table 1) at sites 1–4, and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopic composition of nitrate was not obtained. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in nitrate in water samples at Sites 5–16 ranged from 6.47‰ to 10.26‰ (mean 8.23‰), and from 5.82‰ to 15.50‰ (10.99‰), respectively. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotope values in most surface water samples fallen into the isotopic range of SN, manure and sewage, suggests that the NO_3^- sources mainly derived from SN and manure (Fig. 5 and Fig. 6). In addition, the application amount of chemical fertilizer in winter is less

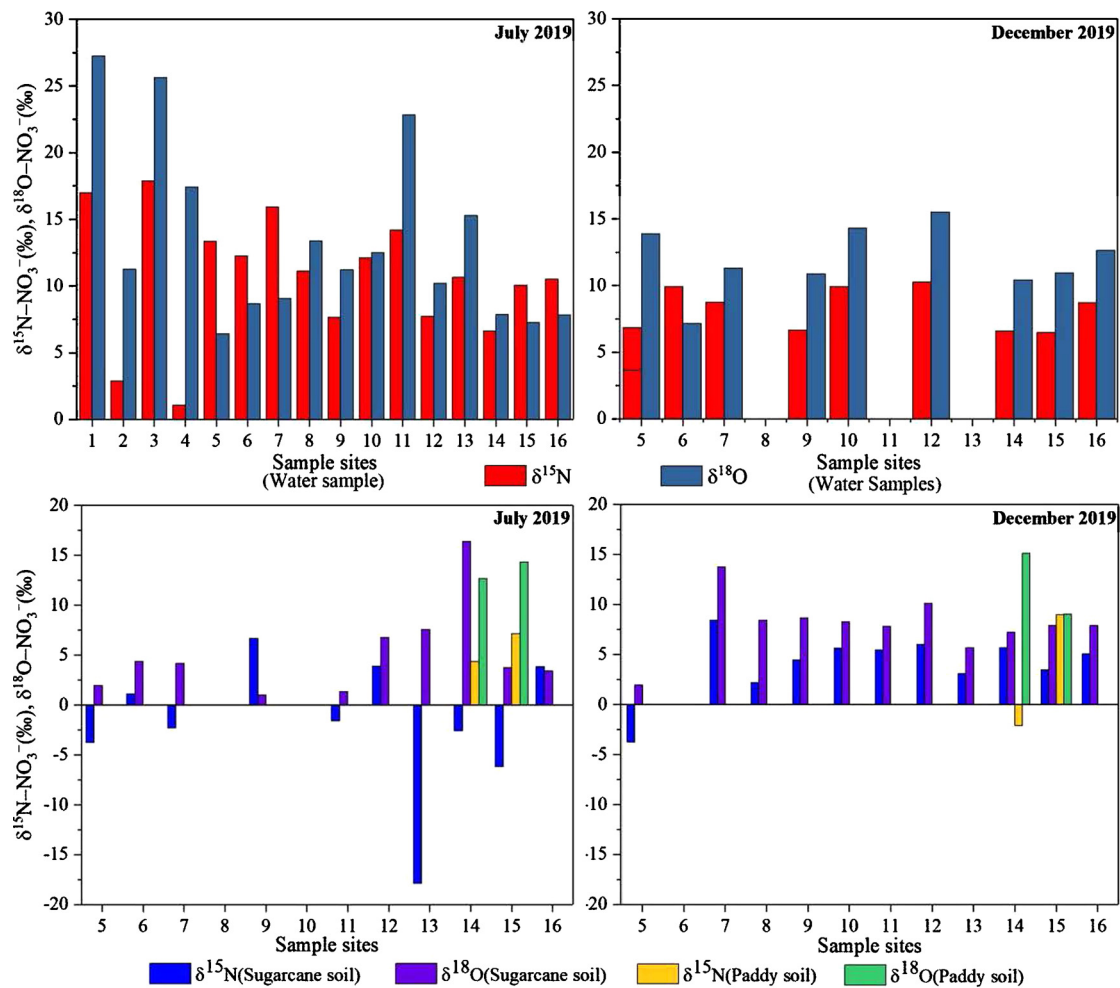


Fig. 3. Variation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate for samples in the Nala watershed.

than summer, thus the runoff formed livestock manure make a great contribution to the nitrate pollution of surface water in the watershed. Overall, at crops planted areas (Sites 5–16), the nitrate sources mainly derived from manure and SN in December.

In total, nitrate sources mainly derived from manure, SN, CF, and rainfall in July; the manure and SN are the main nitrate sources in December in the Nala watershed. The mixing process was an important factor affecting nitrate pollution in Nala watershed.

Although the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotope compositions of NO_3^- are related to their sources, but affected by the activities of aquatic organisms, such as denitrification and nitrification (Pastén-Zapata et al., 2014; Yue et al., 2017; Puig et al., 2017). Influenced by T, DO, NO_3^- concentration, and other factors in denitrification (Chen et al., 2014). Previous studies showed that when denitrification occurs in the water body, the nitrogen and oxygen isotope of nitrate are positively correlated with 1.3–2.1 ratio (Yue et al., 2014; Xia et al., 2016; Liu et al., 2018). In this study, relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in surface water in the Nala watershed were showed in Fig. S1. No significant positive or negative relationships between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in all water samples ($r^2 = 0.15$, $p > 0.05$ in July; $r^2 = 0.20$, $p > 0.05$ in December) were observed for surface water samples in the Nala watershed. This suggests that denitrification was weak in the Nala watershed. Moreover, the DO level in surface water in the Nala watershed ranged from 2.0 $\text{mg}\cdot\text{L}^{-1}$ to 4.6 $\text{mg}\cdot\text{L}^{-1}$ (mean: 3.6 $\text{mg}\cdot\text{L}^{-1}$) in July and from 5.4 $\text{mg}\cdot\text{L}^{-1}$ to 10.8 $\text{mg}\cdot\text{L}^{-1}$ (mean 8.2 $\text{mg}\cdot\text{L}^{-1}$) in December (Table 2), denitrification is considered to be not a significant process due to the presence of dissolved oxygen $> 1\text{ mg}\cdot\text{L}^{-1}$ in all water samples (Desimone and Howes, 1998; Yang and Toor, 2016;

Pastén-Zapata et al., 2014). Some high dual nitrate isotopes were found in water samples in July (Fig. 3), these high values were likely caused by the mixing of rainwater and fertilizer. For example, sampling sites 5–8 showed low molar ratio of $\text{NO}_3^-/\text{Cl}^-$ (< 0.05), high Cl^- concentration and $\delta^{15}\text{N}_{\text{nitrate}}$ values greater than 8.0‰, suggesting mixed manure or sewage (Fig. 3 and Fig. 4); the Cl^- concentration was hardly changeable, and molar ratio of $\text{NO}_3^-/\text{Cl}^-$ increased, which suggested the contribution of manure decreased, especially the high flow season (Sampling sites 5–8, Fig. 4); furthermore, NO_3^- mixed with more than two sources (including two) can cause an increasing in $\delta^{15}\text{N}_{\text{nitrate}}$ values (Jin et al., 2018; Yue et al., 2017). Moreover, nitrate derived from the rainfall can make $\delta^{18}\text{O}_{\text{nitrate}}$ enrichment and thus has higher $\delta^{18}\text{O}_{\text{nitrate}}$ values than other nitrate sources (Yue et al., 2017). The same reason to other high dual nitrate isotopes, such as sampling sites 1 and 3 in July. The above evidences demonstrated that denitrification was not a major factor influencing the isotopic composition of nitrate in the Nala watershed. Besides, the $\delta^{18}\text{O}$ values of nitrate ranged from 0.7‰ to 2.8‰ (Yue et al., 2017), from 0‰ to 3‰ (Sebilo et al., 2006) and from 1.5‰ to 5.4‰ (Yue et al., 2013) due to microbial nitrification. In theory, about 1/3 of the O in the NO_3^- produced by nitrification is derived from atmospheric O_2 , while the remaining 2/3 is derived from water ($\delta^{18}\text{O}_{\text{nitrate}} = 1/3 \delta^{18}\text{O}_{\text{water}} + 2/3 \delta^{18}\text{O}_{\text{air}}$) (Andersson and Hooper, 1983). The range of $\delta^{18}\text{O}_{\text{water}}$ according to Han et al. (2017) studied sites where closed to Kelan reservoir ranged from -8.9‰ to -6.8‰ during the high and low flow seasons. the $\delta^{18}\text{O}_{\text{nitrate}}$ would be 1.9‰ to 3.3‰ according to the calculation based on $\delta^{18}\text{O}_{\text{O}_2}$ of 23.5‰, the results are similar to Yue et al. (2013, 2017) and Sebilo et al. (2006). But in this study, all of water samples had $\delta^{18}\text{O}_{\text{nitrate}}$ values higher than

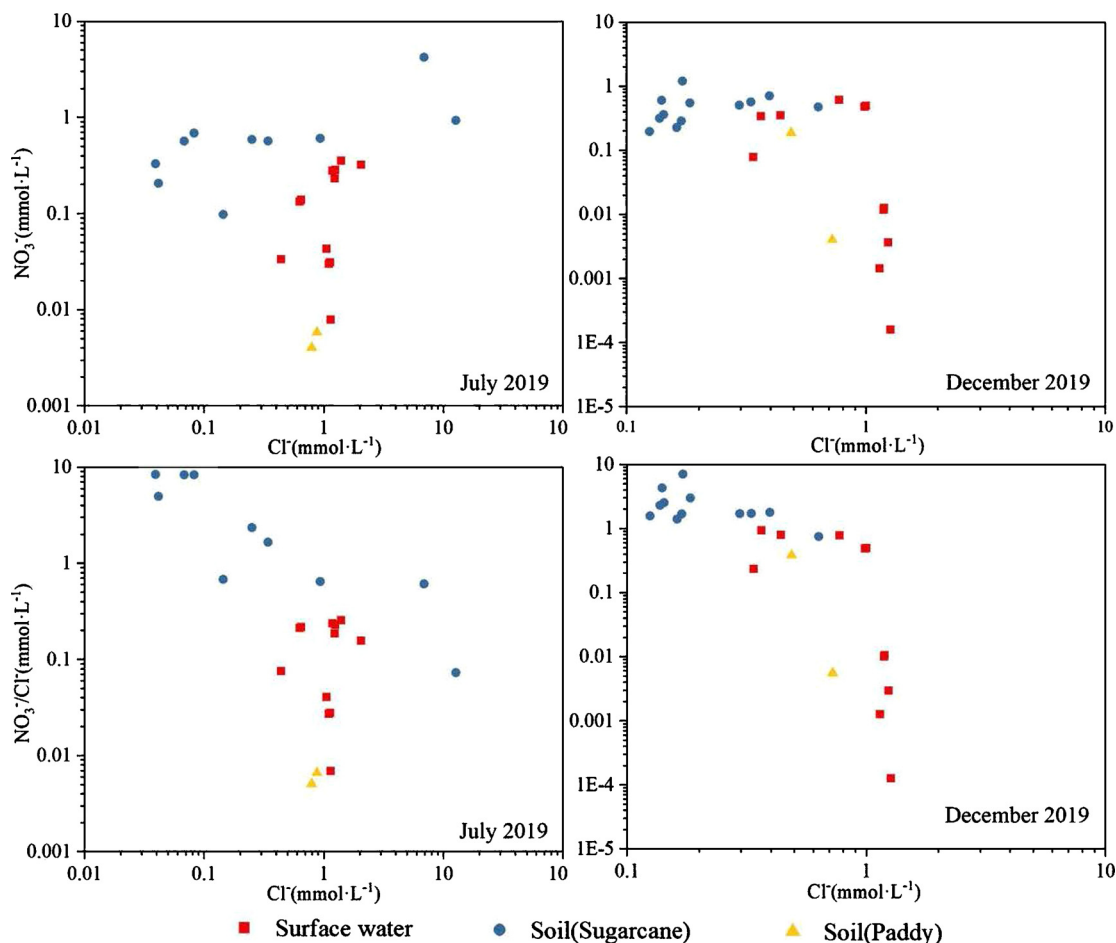


Fig. 4. Relationships among NO_3^- and Cl^- concentrations, $\text{NO}_3^-/\text{Cl}^-$ and Cl^- concentration.

3.3‰ (Table S2), suggesting nitrification process is not significant in river water, thus nitrification process is considered to be weak and mixing process constitute the main factor influencing the migration of nitrate in surface water in Nala small watershed.

The soil samples $\delta^{15}\text{N}_{\text{SN}}$ showed irregular variation from upstream to downstream and ranged from -6.18‰ to 7.15‰ in all soil samples expect one sample (soil 13) with lower $\delta^{15}\text{N}_{\text{SN}}$ (-17.87‰) in July and the values $\delta^{15}\text{N}_{\text{SN}}$ ranged from 1.49‰ to 9.00‰ in all soil samples expect one sample (soil 14 (paddy)) with lower $\delta^{15}\text{N}_{\text{SN}}$ (-2.10‰) in

December; low isotope values (-6.18‰ to 4.34‰ (n = 10) in July and -2.10‰ to 5.70‰ (n = 8) in December) major forms of N in manure and hydrolysis fertilizers are NH_4^+ (NH_3) and organic N, which are subsequently converted into NO_3^- or N_2 by denitrification, volatilization and nitrification (Yue et al., 2017).

Although the $\delta^{15}\text{N}$ - $\delta^{18}\text{O}$ isotopes values of nitrate in some soil samples had fallen into the isotopic range of nitrification in July (Fig. 5), that's because ammonia fertilizer was applied to the soil, and nitrification is widespread in the soil system. However, this nitrification

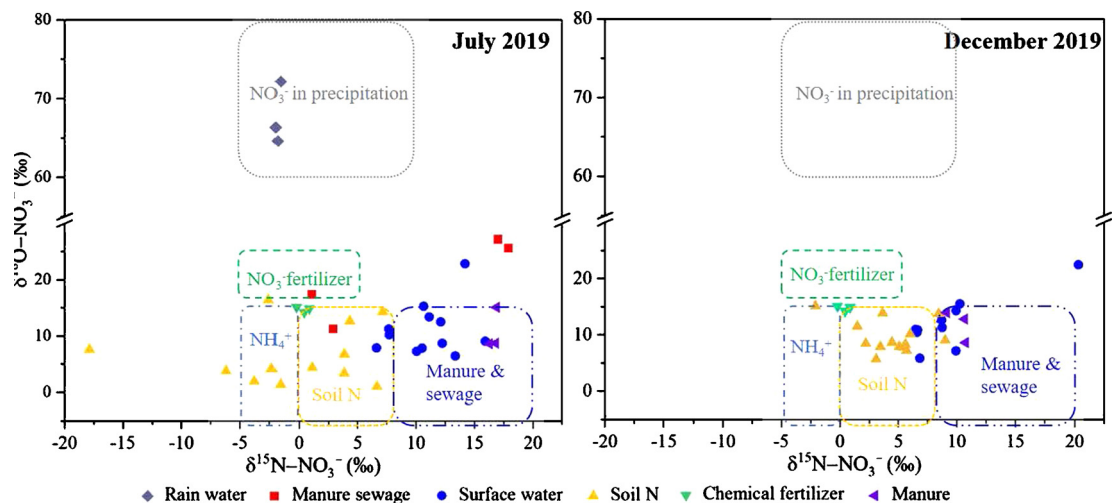


Fig. 5. The relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate at different sampling points.

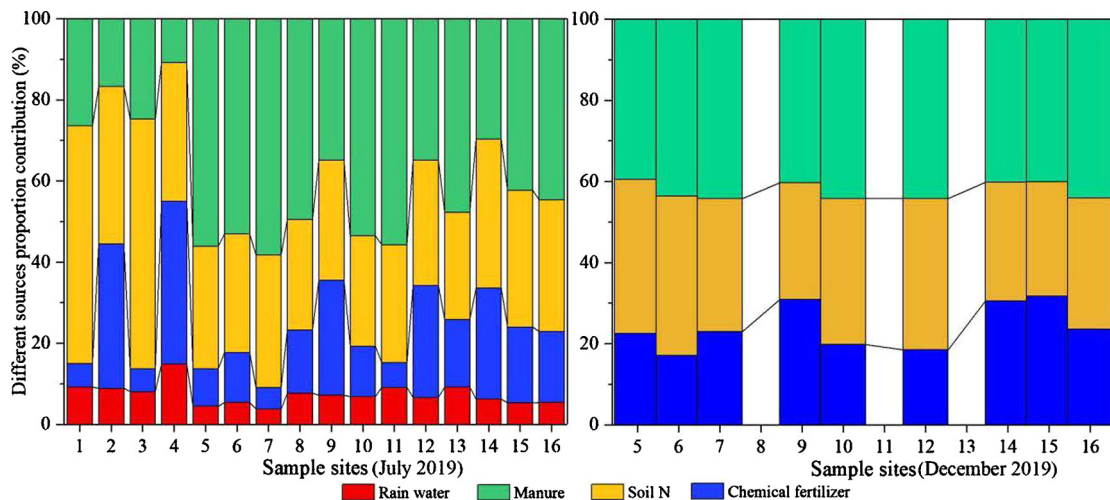


Fig. 6. The contribution rates of sources of NO_3^- in different surface water sampling points.

process only affects the $\delta^{18}\text{O}$ of nitrate in soil system. In order to deduct the influence of the nitrification process, the isotopic values of these soils which are obviously affected by nitrification are eliminated when calculating the source contribution of nitrate. Obvious nitrification was not found in surface water, and rainwater samples.

According to the analyses based on the SIAR, the mean contribution rates of NO_3^- sources at different sampling points in the Nala watershed could be ranked as follows: manure (39.90 %) > SN (34.94 %) > CF (17.77 %) > rainfall (7.39 %) in July, and manure (42.30 %) > SN (33.54 %) > CF (24.16 %) in December (Fig. 6). CF and SN collectively mean contributed 56.63 % of NO_3^- in July and December in the small watershed, roughly accounting for 3/5 of total input. This could be explained by the massive application of CF at crops planted areas. Clearly, manure source pollution around the Kelan Reservoir has made a huge contribution to the N pollution of the water. Therefore reducing fertilizer application (improved utilization rate of fertilizer) and controlling manure source pollution are the key to protect this reservoir in the future.

5. Conclusions

According to the results of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ double isotopes and water chemistry analysis, nitrate pollutant derived from rainfall, CF, SN, and manure in July, and from manure, SN, and CF in December 2019 in the Nala watershed. Calculations based on SIAR showed that SN and CF collectively contributed 52.71 % in July and 57.70 % in December of nitrate to the water sources within the watershed, respectively; thus reducing the use of CF in agricultural planted areas provides the key to control N pollution. Manure also made a substantial contribution (mean 41.10 % in July and December) to nitrate in the small watershed, so it is suggested that manure need to be treated. The results of this study can provide an important basis to control N pollution in small watersheds; however, in larger watersheds, the migration of nitrate results from the fractionation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ due to nitrification and denitrification by microorganisms. For this reason, future studies should take the isotope fractionation into account caused by biological processes. In addition, possible errors caused by the adoption of the isotope technique and a Bayesian isotope mixing model should be considered when distinguishing sources of pollution.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2020.106936>.

References

- Amberger, A., Schmidt, H.L., 1987. Natürliche isotopengehalte von nitratsindikatoren für dessen herkunft. *Geochim. Cosmochim. Ac.* 175 (4016), 1331–1334.
- Andersson, K.K., Hooper, A.B., 1983. O_2 and H_2O are each the source of one O in NO_2^- produced from NH_3 by Nitrosomonas: ^{15}N -NMR evidence. *FEBS Lett.* 164, 236–240.
- Ata-Ul-Karim, S.T., Liu, X., Lu, Z., Zheng, H., Cao, W., Zhu, Y., 2017. Estimation of nitrogen fertilizer requirement for rice crop using critical nitrogen dilution curve. *Field Crop Res.* 201, 32–40.
- Casciotti, K.L., Sigman, D.M., Hastings, M.G., Böhlke, J.K., Hilkert, A., 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Anal. Chem.* 74 (19), 4905–4912.
- Chen, Z.X., Yu, L., Liu, W.G., Lam, M.H.W., Liu, G.J., Yin, X.B., 2014. Nitrogen and oxygen isotopic compositions of water-soluble nitrate in Taihu Lake water system, China: Implication for nitrate sources and biogeochemical process. *Environ. Earth Sci.* 71 (1), 217–223.
- Cui, Z.L., Chen, X.P., Zhang, F.S., 2010. Current nitrogen management status and measures to improve the intensive wheat-maize system in China. *Ambio* 39, 376–384.
- Desimone, L.A., Howes, B.L., 1998. Nitrogen transport and transformations in a shallow aquifer receiving wastewater discharge: a mass balance approach. *Water Resour. Res.* 34 (2), 271–285.
- Dignazio, F.J., Krothe, N.C., Baedke, S.J., Spalding, R.F., 1998. $\delta^{15}\text{N}$ of nitrate derived from explosive sources in a karst aquifer beneath the ammunition burning ground, crane naval surface warfare center, Indiana, USA. *J. Hydrol. (Amst)* 206 (3–4), 164–175.
- Fenech, C., Rock, L., Nolan, K., Tobin, J., Morrissey, A., 2012. The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination: a review. *Water Res.* 46 (7), 2023–2041.
- GB3838-2002, 2002. China's National Standard "Environmental Quality Standards for Surface Water", Ministry of Environmental Protection of the People's Republic of China and General Administration of Quality Supervision. Inspection and Quarantine of the People's Republic of China.
- Granger, J., Sigman, D.M., Rohde, M.M., Maldonado, M.T., Tortell, P.D., 2010. N and O isotope effects during nitrate assimilation by unicellular prokaryotic and eukaryotic plankton cultures. *Geochim. Cosmochim. Ac.* 74 (3), 1030–1040.
- Hall, D.W., Risser, D.W., 1993. Effects of agricultural nutrient management on nitrogen fate and transport in Lancaster county pennsylvania. *J. Am. Water Resour. As.* 29

- (1), 55–76.
- Han, G.L., Lv, P., Tang, Y., Song, Z.L., 2017. Spatial and temporal variation of H and O isotopic compositions of the Xijiang River system, Southwest China. *Isotopes Environ. Health Stud.* 54 (1–3), 137–146.
- Heffer, P., 2009. Assessment of Fertilizer Use by Crop at the Global Level. International Fertilizer Industry Association, Paris. www.fertilizer.org/ifa/Home-Page/LIBRARY/Publication-database.html/Assessment-of-FertilizerUse-by-Crop-at-the-Global-Level-2006-07-2007-08.html2.
- Jin, Z.F., Qi, Z., Zhu, C.Y., Wang, Y., Cen, J.R., Li, F.L., 2018. Contribution of nitrate sources in surface water in multiple land use areas by combining isotopes and a Bayesian isotope mixing model. *Appl. Geochem.* 93, 10–19.
- Kellman, L., Hillaire-Marcel, C., 1998. Nitrate cycling in streams: Using natural abundances of $\text{NO}_3^- \delta^{15}\text{N}$ to measure *in-situ* denitrification. *Biogeochemistry.* 43 (3), 273–292.
- Kohl, D.H., Shearer, G.B., Compton, B., 1971. Fertilizer nitrogen: contribution to nitrate in surface water in a corn belt watershed. *Science* 174 (4016), 1331–1334.
- Lau, S.S., Lane, S.N., 2002. Biological and chemical factors influencing shallow lake eutrophication: a long-term study. *Sci. Total Environ.* 288 (3), 167–181.
- Li, S.L., Liu, C.Q., Li, J., Liu, X., Chetelat, B., Wang, B.L., Wang, F.S., 2010. Assessment of the sources of nitrate in the Changjiang River, China using a nitrogen and oxygen isotopic approach. *Environ. Sci. Technol.* 44 (5), 1573–1578.
- Li, S.L., Liu, C.Q., Li, J., Xue, Z.C., Guan, J., Lang, Y.C., Ding, H., Li, L.B., 2013. Evaluation of nitrate source in surface water of southwestern China based on stable isotopes. *Environ. Earth Sci.* 68 (1), 219–228.
- Li, Y.P., Tang, C.Y., Yu, Z.B., Acharya, K., 2014. Correlations between algae and water quality: factors driving eutrophication in Lake Taihu. *China. Int. J. Environ. Sci. Technol.* 11, 169–182.
- Li, C., Li, S.L., Yue, F.J., Liu, J., Zhong, J., Yan, Z.F., Zhang, R.C., Wang, Z.J., Xu, S., 2019. Identification of sources and transformations of nitrate in the Xijiang River using nitrate isotopes and Bayesian model. *Sci. Total Environ.* 646, 801–810.
- Liu, C.Q., Li, S.L., Lang, Y.C., Xiao, H.Y., 2006. Using $\delta^{15}\text{N}$ - and $\delta^{18}\text{O}$ -values to identify nitrate sources in karst ground water, Guiyang, Southwest China. *Environ. Sci. Technol.* 40 (22), 6928–6933.
- Liu, S.S., Wu, F.C., Feng, W.Y., Guo, W.J., Song, F.H., Wang, H., Wang, Y., He, Z.Q., Giesy, J.P., Zhu, P., Tang, Z., 2018. Using dual isotopes and a Bayesian isotope mixing model to evaluate sources of nitrate of Tai Lake. *China. Environ. Sci. Pollut. Res.* 25, 32631–32639.
- Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS One* 5 (3), e9672.
- Pastén-Zapata, E., Ledesma-Ruiz, R., Harter, T., Ramírez, A.I., Mahlknecht, J., 2014. Assessment of sources and fate of nitrate in shallow groundwater of an agricultural area by using a multi-tracer approach. *Sci. Total Environ.* 470–471, 855–864.
- Puig, R., Soler, A., Widory, D., Mas-Pla, J., Domènech, C., Otero, N., 2017. Characterizing sources and natural attenuation of nitrate contamination in the Baix Ter aquifer system (NE Spain) using a multi-isotope approach. *Sci. Total Environ.* 580, 518–532.
- Rock, L., Ellert, B.H., 2007. Nitrogen-15 and oxygen-18 natural abundance of potassium chloride extractable soil nitrate using the denitrifier method. *Soil Sci. Soc. Am. J.* 71 (2), 355–361.
- Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, M., Mariotti, A., 2006. Assessing nitrification and denitrification in the Seine river and Estuary using chemical and isotopic techniques. *Ecosystems* 9 (4), 564–577.
- Sigman, D.M., Altabet, M.A., Mccorkle, D.C., Francois, R., Fischer, G., 2000. The $\delta^{15}\text{N}$ of nitrate in the southern ocean: Nitrogen cycling and circulation in the ocean interior. *J. Geophys. Res.* 105 (C8), 19599–19614.
- Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., Böhlke, J.K., 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* 73 (17), 4145–4153.
- Tayefeh, M., Sadeghi, S.M., Noorhosseini, S.A., Bacenetti, J., Damalas, C.A., 2018. Environmental impact of rice production based on nitrogen fertilizer use. *Environ. Sci. Pollut. Res.* 25, 15885–15895.
- Wang, W., Song, X., Ying, M., 2016. Identification of nitrate source using isotopic and geochemical data in the lower reaches of the Yellow River irrigation district (China). *Environ. Earth Sci.* 75 (11), 1–13.
- Wang, A., Fang, Y.T., Chen, D.X., Phillips, O., Koba, K., Zhu, W.X., 2018. High nitrogen isotope fractionation of nitrate during denitrification in four forest soils and its implications for denitrification rate estimates. *Sci. Total Environ.* 633, 1078–1088.
- Williams, A.E., Lund, L.J., Johnson, J.A., Kabala, Z.J., 1998. Natural and anthropogenic nitrate contamination of groundwater in a rural community. *California. Environ. Sci. Technol.* 32 (1), 32–39.
- Xia, Y., Weller, D.E., Williams, M.N., Jordan, T.E., Yan, X., 2016. Using Bayesian hierarchical models to better understand nitrate sources and sinks in agricultural watersheds. *Water Res.* 105, 527–539.
- Xing, M., Liu, W., 2016. Using dual isotopes to identify sources and transformations of nitrogen in water catchments with different land uses, Loess Plateau of China. *Environ. Sci. Pollut. Res.* 23 (1), 388–401.
- Xue, D.M., Botte, J., Baets, B.D., Accoe, F., Nestler, A., Taylor, P., Cleemput, O.V., Berglund, M., Boeckx, P., 2009. Present limitations and future prospects of stable isotope methods for nitrate source identification in surface and groundwater. *Water Res.* 43 (5), 1159–1170.
- Xue, D.M., Baets, B.D., Cleemput, O.V., Hennessy, C., Berglund, M., Boeckx, P., 2012. Use of a Bayesian isotope mixing model to estimate proportional contributions of multiple nitrate sources in surface water. *Environ. Pollut.* 161, 43–49.
- Yan, X.Y., Ti, C.P., Vitousek, P., Chen, D., Leip, A., Cai, Z.C., Zhu, Z.L., 2014. Fertilizer nitrogen recovery efficiencies in crop production systems of china with and without consideration of the residual effect of nitrogen. *Environ. Res. Lett.* 9 (9), 095002. <https://doi.org/10.1088/1748-9326/9/9/095002>.
- Yang, Y.Y., Toor, G.S., 2016. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ reveal the sources of nitrate-nitrogen in urban residential storm water runoff. *Environ. Sci. Technol.* 50 (6), 2881–2889.
- Yao, L.X., Li, G.L., Tu, S.H., Sulewski, G., He, Z.H., 2007. Salinity of animal manure and potential risk of secondary soil salinization through successive manure application. *Sci. Total Environ.* 383 (1–3), 106–114.
- Ying, H., Ye, Y., Cui, Z., Chen, X., 2017. Managing nitrogen for sustainable wheat production. *J. Clean. Prod.* 162, 1308–1316.
- Yue, F.J., Li, S.L., Liu, C.Q., Zhao, Z.Q., Hu, J., 2013. Using dual isotopes to evaluate sources and transformation of nitrogen in the Liao River, Northeast China. *Appl. Geochem.* 36, 1–9.
- Yue, F.J., Liu, C.Q., Li, S.L., Zhao, Z.Q., Liu, X.L., Ding, H., Liu, B.J., 2014. Analysis of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to identify nitrate sources and transformations in Songhua River, Northeast China. *J. Hydrol. (Amst)* 519, 329–339.
- Yue, F.J., Li, S.L., Liu, C.Q., Zhao, Z.Q., Ding, H., 2017. Tracing nitrate sources with dual isotopes and long term monitoring of nitrogen species in the Yellow River. *China. Sci. Rep.* 7 (1), 1–11.
- Zhang, Y., Li, F.D., Zhang, Q.Y., Li, J., Liu, Q., 2014. Tracing nitrate pollution sources and transformation in surface- and ground-waters using environmental isotopes. *Sci. Total Environ.* 490, 213–222.
- Zhao, Y.H., Deng, X.Z., Zhan, J.Y., Xi, B.D., Qi, L., 2010. Progress on preventing and controlling strategies of lake eutrophication in China. *Environ. Sci. Technol.* 33 (3), 92–98.