Using δ 15 N- and δ 18 O-Values To Identify Nitrate Sources in Karst Ground Water, Guiyang, Southwest China

4 AUTHORS, INCLUDING:

Si-Liang Li
Tianjin University

39 PUBLICATIONS   **474** CITATIONS

Using δ^{15}N- and δ^{18}O-Values To Identify Nitrate Sources in Karst Ground Water, Guiyang, Southwest China

CONG-QIANG LIU,* SI-LIANG LI,
YUN-CHAO LANG, AND HUA-YUN XIAO

*The State Key Laboratory of Environmental Geochemistry,
Institute of Geochemistry, Chinese Academy of Sciences,
Guiyang 550002, China*

Nitrate pollution of the karstic groundwater is an increasingly serious problem with the development of Guiyang, the capital city of Guizhou Province, southwest China. The higher content of NO_3^- in groundwater compared to surface water during both summer and winter seasons indicates that the karstic groundwater system cannot easily recover once contaminated with nitrate. In order to assess the sources and conversion of nitrate in the groundwater of Guiyang, we analyzed the major ions, δ^{15}N-NH_4^+, δ^{15}N-NO_3^-, and δ^{18}O-NO_3^- in surface and groundwater samples collected during both summer and winter seasons. The results show that nitrate is the major dominant species of nitrogen in most water samples and there is a big variation of nitrate sources in groundwater between winter and summer season, due to fast response of groundwater to rain or surface water in the karst area. Combined with information on NO_3^-/Cl^-, the variations of the isotope values of nitrate in the groundwater show a mixing process of multiple sources of nitrate, especially in the summer season. Chemical fertilizer and nitrification of nitrogen-containing organic materials contribute nitrate to suburban groundwater, while the sewage effluents and denitrification mainly control the nitrate distribution in urban groundwater.

Introduction

Nitrate is possibly the most widespread groundwater contaminant in the world caused by anthropogenic activities, imposing a serious threat to drinking water supplies and promoting eutrophication (*1−4*). In drinking water, high nitrate concentrations are believed to be a health hazard because it may cause methemeoglobinemia in infants and be responsible for increases in stomach cancer in others (*5*). Groundwater is a major source of drinking water for a part of the residents of Guiyang, a city with a typical karstic landform in southwestern China. Nitrate, along with SO_4^{2-} and Cl^-, pollution of groundwater is an increasingly serious problem with the development of the city (*6, 7*). According to Lang et al. (*7*), the higher content of NO_3^- in groundwater as compared to surface water during the summer and winter seasons indicates that the karstic groundwater system is not capable of denitrification and therefore does not easily recover once contaminated with nitrates. Therefore, it is important to assess the source and transformation of nitrate in the groundwater.

Stable isotope composition of nitrogen has been used extensively to provide information on the origins and transformations of inorganic N in a hydrosphere (*1*). Nitrate is the dominant nitrogen species in groundwater, which may be derived from soil organic nitrogen, synthetic fertilizer, livestock waste, sewage effluent, and atmosphere precipitation. In many cases, these sources produce nitrate with distinguishable ^{15}N/^{14}N ratios: δ^{15}N values are from −1‰ to +2‰ for synthetic fertilizer, from +2‰ to +8‰ for soil organic nitrogen, and from +8‰ to +20‰ for livestock waste and sewage (*1, 2, 8, 9*). However, a disadvantage of the single isotope approach is its inability to identify sources and transformation of NO_3^- in groundwater due to the nonconservative behavior of nitrogen in the earth's surface (*2*). The δ^{18}O of NO_3^- has been developed to provide more information about the nitrate cycling in groundwater (*2, 10, 11*). According to Andersson and Hooper (*12*), theoretically, two of the oxygens in nitrate derive from H_2O and one derives from air during microbial nitrification. The isotopic composition of oxygen in chemical nitrate fertilizers is similar to air oxygen, which is about +23.5‰ (*10, 13, 14*). The δ^{18}O of NO_3^- ranges from −2‰ to +6‰ when only microbial nitrification occurs (*15*). Denitrification can cause enrichment in heavy isotope of nitrogen and oxygen in the residual NO_3^- (*10, 11, 16−19*). However, the δ^{18}O has some limitations to trace cycling of nitrate due to the complication of soil microbial processes (*2, 20*).

Recently, combined use of isotope ratios of boron (B), strontium (Sr), and N and O of nitrate has been proved to be successful as an isotopic multitracer approach for tracing contaminants in groundwater (*4, 21, 22*). The newly measured nitrogen and oxygen isotope data, together with chemical composition (e.g., NO_3^-, NH_4^+, NO_2^-, Cl^-) previously discussed in our papers (*7, 23*) for the karstic ground and surface water of the city of Guiyang are used to identify the sources of nitrate, to determine the species of nitrogen, to understand their transformation, and to understand the relationship between the nitrate and other anthropogenic components in the ground and surface water of the karstic area.

Study Area

The city of Guiyang, in the center of Guizhou Province, has a population of more than 1.5 million and is located in a karstic basin, close to the coteau that separates the Yangtze River and Zhu River basins. The city has diverse karstic landforms and abundant karst groundwater sources. The majority of aquifers are pre-Jurassic carbonate (limestone and dolomite) and clastic sedimentary rocks (shale, sandstone, and shale), with sulfate evaporite (gypsum) and coal seams occurring locally. The area studied is located between 26°28′ and 26°42′N latitude and 106°38′ to 106°50′E longitude (Figure 1). The elevation of Guiyang is between 1100 and 1300 m above mean sea level. The climate is subtropical with an average temperature of 16 °C and annual precipitation of about 1200 mm. A monsoonal climate often results in high precipitation during summer and much less during winter, although the humidity is often high during most of the year (*24*). Because there was rapid influx of livestock waste and sewage effluent directly into the shallow karst aquifer in the urban area, water quality has seriously deteriorated, with high nitrogen contents in many domestic and municipal wells for years (*6, 24*). Agriculture is a major land use in order to produce the vegetables and foods in the suburb of Guiyang.

* Corresponding author phone: +8613608554829; fax: +86 851 5891609; E-mail: liucongqiang@vip.skleg.cn.
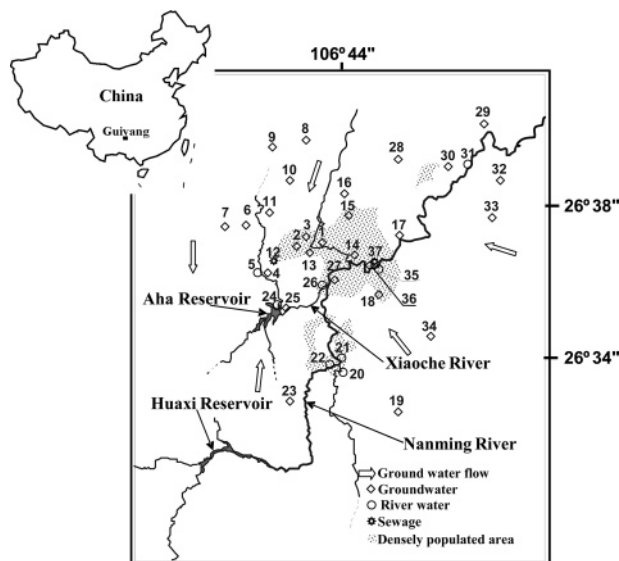
FIGURE 1. Map showing the location of Guiyang city and the sampling sites.

Sampling and Analytical Methods

Water samples were collected in January and August of 2002, corresponding to the low-flow and high-flow seasons, respectively. The sampling sites for groundwater and surface water are shown in Figure 1. The groundwater samples were collected from wells (bore holes) and various types of springs (*7*).

Temperature, electrical conductivity, pH values, and dissolved oxygen contents of the samples were measured on site. Alkalinity was measured by titration with HCl within 12 h. Major cations (Mg^{2+}, Ca^{2+}, K^+, and Na^+) were analyzed by atomic absorption spectrometry (AAS) and the anions (SO_4^{2-}, Cl^-, and NO_3^-) by high performance liquid chromatography (HPLC).

A portion of the water sample (200−2000 mL) was filtered through 0.45-μm cellulose-acetate filter paper and collected in polyethylene bottles with airtight caps and preserved with HCl to prevent biological activity. Ammonium and nitrite concentrations were determined by spectrophotometry. The precisions of major ions, NH_4^+ and NO_2^-, were about ±5%.

For nitrogen isotope analysis of water samples, nitrate and ammonium were first collected on the ion-exchange columns with anion resin (Dowex 1-X8, 200-mesh) and cation resin (Dowex 50-X8, 100-mesh), respectively (*25−27*), and then excess 2 M KCl was used to elute nitrate or ammonium from the resins. N was trapped with an acid trap (H_2SO_4) from the eluant according to the diffusion method described by Xiao and Liu (*28*). The acid traps were frozen to dry for isotopic analysis by the combustion tube method (*29*). For nitrogen isotope determination of soil organic nitrogen, synthetic fertilizer, livestock waste, and particulate organic nitrogen of sewage effluent collected on a Whatman GF/F filter (precombusted), the samples were dried at 40 °C and ground into a fine powder. Approximately 20−100 mg of dried samples was combusted by the combustion tube method (*29*).

The eluant of KNO3/KCl was converted into $AgNO_3$ for δ ^{18}O analyses by using the modified procedure descried by Silva et al. (*26*). The SO_4^{2-} and PO_4^{3-} were removed by precipitation with excess $BaCl_2$ according to SO_4^{2-} contents. To remove the DOC, 20 mg of activated carbon was added, and the mixture was shaken in an orbital shaker and then filtered through a nylon filter. Next, the sample was passed through the cation exchange resin and collected in a 50-mL glass beaker with a stirrer. Using an excess of Ag_2O removed

Cl and neutralized the solution to achieve a pH value of about 6, and then the AgCl precipitate was removed by filtration and rinsed with distilled water. After freeze-drying of the solution, the $AgNO_3$ precipitate was converted to CO_2 gas by combustion of the $AgNO_3$ with 4-5 mg of finely ground spectrographic graphite at a shut-off temperature of 850 °C.

N and O isotope ratios were determined on a Finnigan MAT 252 mass spectrometer at the Institute of Geochemistry, Chinese Academy of Sciences, and reported in the δ notation relative to N_2(air) and Standard Mean Ocean Water (SMOW) (in parts per thousand). The isotope results are given in δ units defined as

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

$\delta^{15}N$ and $\delta^{18}O$ values have a precision of 0.2‰ and 0.6‰, respectively. $\delta^{15}N$ standards N-1(IAEA), N-3(IAEA), and KNO_3 were provided by Shoko Co., Ltd., Tokyo, Japan (+1.9‰), and working standards were converted to N_2 as a check on reproducibility. The value of $\delta^{18}O$-N3(IAEA) was measured to be around +22.3‰, similar to other results of from +22.7‰ to +25.3‰ measured in other laboratories (*26, 30−32*). Révész and Böhlke (*33*) measured $\delta^{18}O$ values of nitrate in different KNO_3 salts with a wide range of $\delta^{18}O$ values relative to VSMOW by using off-line, sealed glass tube combustion methods and an on-line carbon combustion technique, and they found that all methods yielded roughly similar results for the samples with $\delta^{18}O$ values near the midpoint but different results for the samples with $\delta^{18}O$ values far from the midpoint (around +20‰) due to O isotope exchange between the sample and the glass combustion tubes. Although our samples were measured by using the high-temperature graphite off-line combustion method of Silva et al. (*26*), most of the $\delta^{18}O$ values of our samples range from +5‰ to +20‰ and are close to the midpoint, near which the off-line and on-line techniques may yield roughly similar results (*33*). In addition, a number of duplicate samples and standards (IAEA-NO-3) were measured during our experiments, and the results showed that the differences were less than the range of measurement accuracy.

Rock Weathering and Anthropogenic Inputs of Major Ions into Groundwater

Concentrations of Main Cations and Anions. In the groundwater, Ca^{2+} and Mg^{2+} were the dominant cations and HCO_3^- and SO_4^{2-} the dominant anions (*7*). Groundwater, according to stoichiometric analyses, can be mainly characterized as a SO$_4$·HCO$_3$−Ca·Mg type. The groundwater concentrations of Ca^{2+}, Mg^{2+}, and HCO_3^- were generally higher than those of the surface water, indicating extensive dissolution of carbonate rocks and greater solubility of the dissolved solutes, probably because of lower pH of the groundwater. In general, the average concentrations of Ca^{2+}, Mg^{2+}, HCO_3^-, and SO_4^{2-} in summer were lower than those in winter (*23*), indicating a dilution process during the wet season by the mixing of infiltrated meteoric water of low solutes with the aquifer groundwater.

The outcrops in the study area are mainly carbonate rocks (limestone and dolomite) of Triassic and Paleogene age, with a minor amount of sandstone. This geological feature of the study area is responsible for the high concentrations of Ca^{2+}, Mg^{2+}, and HCO_3^- in the ground and surface water. Some of the aquifers contain gypsum. Since there are some coal-bearing clastic rocks in the study area, which are enriched in sulfide minerals, oxidation of sulfide minerals such as FeS_2 will contribute sulfate to the groundwater, in addition to dissolution of gypsum. These chemical characteristics, together with Sr isotopic compositions, show the important contributions principally from carbonate weathering and

TABLE 1. Chemical and Isotopic Analysis of Groundwater Samples in Guiyang, China

sample	Cl^a(mg/L)* winter	summer	N-NO$_3^-$ ^{a}(mg/L) winter	summer	δ^{15}N-NO$_3^-$ (‰) winter	summer	δ^{18}O-NO$_3^-$ (‰) winter	summer	N-NH$_4^+$(mg/L) winter	summer	δ^{15}N-NH$_4^+$ (‰) winter	summer	N-NO$_2^-$ (mg/L) winter	summer
Groundwater in the City of Guiyang														
03	115.5	5.8	2.09	3.51	−^{b}	3.9	−	4.1	6.04	1.03	3.93	1.02	0.02	0.44
14	38.1	33.4	7.71	8.36	6.2	4.3	15.7	11.1	bd^c	bd			bd	bd
15	138.9	28.0	3.75	10.7	14.7	12.3	17.0	5.1	1.44	bd	1.26		0.08	0.10
18	25.5	18.6	3.65	4.59	15.0	2.5	15.6	2.8	0.45	bd	−0.76		0.01	bd
27	42.2	42.0	1.71	11.2	15.4	12.5	18.7	7.5	1.22	1.09	−0.61	0.04	0.04	0.36
36	33.3	31.6	8.89	8.61	9.6	14.9	12.7	8.7	bd	bd			bd	bd
Groundwater in the Suburb of Guiyang														
04	36.0	20.9	4.53	11.7	2.8	0.5	7.4	5.7	1.52	bd	−1.65		0.09	bd
06	6.9	−	1.89	−	3.8	−	12.1	−	bd	−		−	bd	−
07	14.4	−	bd	−		−		−	9.06	−	1.41	−	bd	−
08	6.15	2.57	6.28	2.33	7.0	1.6	5.8	15.5	bd	0.26		0.49	bd	bd
09	31.6	9.24	0.05	2.21	−	−0.4	−	9.9	2.40	bd	0.45		0.10	0.01
10	7.10	4.61	5.53	6.03	4.3	2.0	15.7	8.9	bd	0.10			bd	0.01
11	14.3	9.27	1.95	2.77	11.8	4.0	6.2	11.3	bd	0.06			bd	0.11
16	13.2	7.67	4.86	3.18	−0.1	3.9	16.3	9.6	bd	bd			bd	bd
17	38.3	25.8	6.68	11.7	9.7	6.2	7.2	15.5	0.58	bd			0.29	bd
19	8.81	5.22	1.99	1.89	−0.1	0.5	4.9	16.3	bd	bd			bd	bd
23	5.19	3.85	1.17	1.76	1.9	0.2	17.6	16.8	bd	bd			bd	bd
25	2.50	1.17	1.33	0.29	2.9	1.9	23.5	6.1	bd	bd			bd	bd
28	18.0	5.79	1.76	0.56	6.6	7.1	4.3	18.2	0.04	0.04			bd	0.01
29	25.7	0.95	0.88	1.12	7.3	−1.4	11.6	4.5	bd	bd			bd	bd
30	40.3	10.0	bd	2.51		1.6		13.6	17.98	3.64	2.30	0.99	bd	0.41
32	25.3	4.40	2.12	7.47	−	4.0	−	10.7	bd	0.05			bd	bd
33	4.79	2.28	1.24	1.87	7.6	0.9	12.7	18.1	bd	bd			bd	bd
34	10.2	3.51	3.83	4.54	7.4	7.7	−	16.7	bd	bd			bd	bd
Surface Water														
05	32.7	8.16	2.66	3.43	8.8	7.8	4.5	10.6	0.06	0.42		6.19	0.02	0.05
20	19.9	4.75	0.59	0.91	−2.4	−2.7	18.4	27.0	6.27	0.02	2.35		0.10	bd
21	13.2	6.89	2.17	2.08	2.8	−0.2	17.2	23.2	1.02	0.03	−1.46		0.14	0.11
22	12.0	5.25	2.20	3.11	3.7	2.2	18.5	21.7	0.67	0.03	−0.51		0.15	0.15
24	11.0	7.00	0.28	1.38	−2.4	−1.4	−	26.8	0.11	bd			0.02	0.03
26	20.1	7.28	7.34	2.10	1.7	2.9	15.5	11.2	0.06	bd			0.01	0.04
31	63.5	12.3	0.28	2.19	−	1.7	−	4.7	13.70	2.10	3.98	4.55	0.09	0.45
35	46.5	10.5	1.50	1.98	1.2	2.5	−	5.8	7.73	1.08	−1.62	1.01	0.17	0.15
Sewage Effluent														
12	45.4	36.6	bd	1.85		−2.4		−	5.11	3.92	5.47	5.63	0.16	0.57
37	64.3	37.9	bd	bd					18.30	10.76	−	4.67	0.26	0.31

^{a} Data from Lang et al.(2006). ^{b} − stands for not detected. ^{c} bd stands for below detection limit of 0.05 mg NO$_3^-$/L, 0.02 mg NH$_4^+$/L, and 0.003 mg NO$_2^-$/L.

oxidation of FeS$_2$ and/or dissolution of gypsum strata to the solutes of groundwater (7).

Nitrogen-Containing Species. On the basis of the variations in chemical and isotopic (^{87}Sr/^{86}Sr) composition of surface and groundwater together with municipal sewage samples, Lang et al. (7) conclude that the major anthropogenic components in the groundwater include Cl$^-$ and NO$_3^-$, as well as SO$_4^{2-}$ and probably some Na$^+$ and K$^+$, and that they were mainly derived from industrial and agricultural activities. Concentrations of NO$_3^-$ (Table 1) in groundwater in summer were generally higher than in the winter, in the range of 0.29−11.7 and 0−8.9 mg of N-NO$_3^-$/L, with mean values of 5.0 and 3.1 mg of N-NO$_3^-$/L, respectively. There were higher nitrate contents in some urban groundwater samples than those in suburban groundwater, but the nitrate concentration was much lower in several deep groundwater samples, which were also characterized by lower contents of anthropogenic ions (Cl$^-$, K$^+$, and Na$^+$), showing the lack of contamination (7).

Nitrate was the major dominant specie of nitrogen in most water samples (Figure 2), but high concentrations of ammonium were detected, and NH$_4^+$ became by far the dominant specie especially in sewage effluent and contaminated waters and in winter. The higher detection of ammonium in winter was found because biological activities (nitrification) were limited by lower temperature as compared
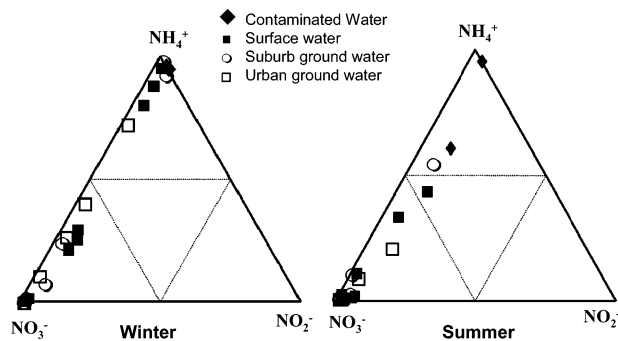

FIGURE 2. Ternary diagram showing relative concentrations of different nitrogen species in surface and groundwater samples collected in both winter and summer seasons.

with that of summer. Nitrite concentration was low due to unstable chemical characteristics but was higher in sewage effluent and surface water than in groundwater. The inorganic nitrogen species distribution might indicate that septic effluents including high contents of organic nitrogen and ammonium have an important side effect on water quality.

NO$_3^-$/Cl$^-$ Concentration Ratio Variations. Concentrations of Cl$^-$ in groundwater were in the range of 2.5−139 mg/L in winter and 1.0−42.0 mg/L in summer, with mean
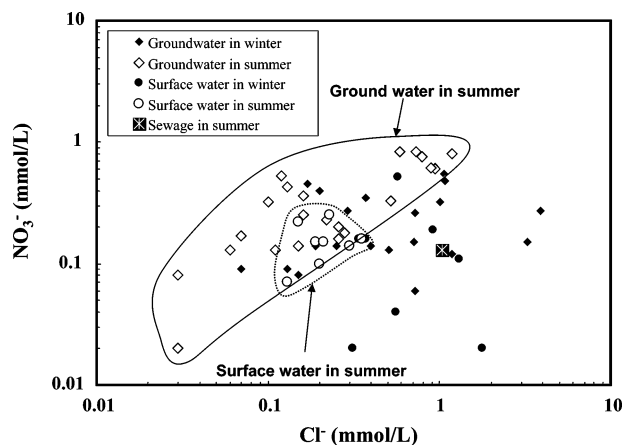
FIGURE 3. Relationship between Cl^- and NO_3^- concentration of the surface and groundwater.
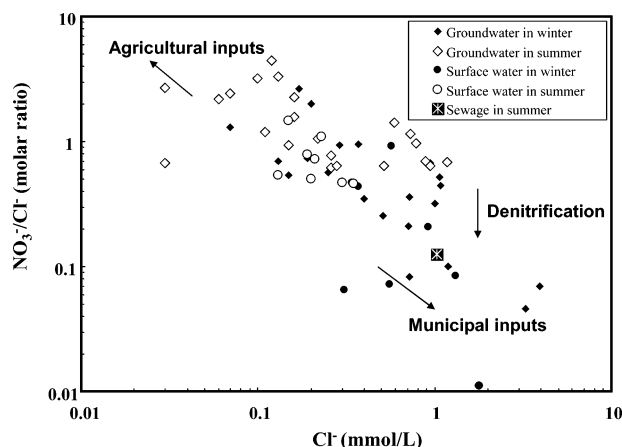

FIGURE 4. Variations of NO_3^-/Cl^- molar ratios with Cl^- molar concentrations of groundwater, surface water, and sewage in both winter and summer seasons.

values of 29.3 and 12.6 mg/L, respectively (Table 1). The distribution of natural solutes in surface and groundwater was generally different from that of anthropogenic sources. Potential sources for Cl^- include natural sources (dissolution of minerals), agricultural chemicals (potash or KCl), animal waste, septic effluent, and road salt. There were high chloride contents in the municipal sewage samples and some contaminated groundwaters, which maybe indicate that most of the Cl^- was mainly of anthropogenic origin.

Chloride is a good indicator of sewage impacts because it is not subject to physical, chemical, and microbiological processes occurring in groundwater. NO_3^-/Cl^- method can provide more information to distinguish the effect of N removal processes by dilution from denitrification (18, 21, 22, 34, 35). In the water samples studied here, there was no correlation between Cl^- and NO_3^- in winter but a generally positive correlation ($r^2 = 0.66$) in summer (Figure 3), which might indicate that the mixing process has a major effect on nitrate transportation in summer. Figure 4 shows the variation of the NO_3^-/Cl^- molar ratios with $Cl-$ concentrations in the surface and groundwater samples as well as a sewage sample in both summer and winter seasons. There were higher NO_3^-/Cl^- ratios of both surface and groundwater samples in summer than in winter. The surface water samples in summer showed similar Cl^- concentrations and NO_3^-/Cl^- ratios as groundwater, which can be useful to understand the fast response of groundwater to rain and surface water in the southwestern karstic area of China. Most of ground and surface water as well as the municipal sewage had significantly higher Cl^- concentrations but much lower NO_3^-/Cl^- ratios,
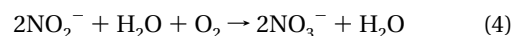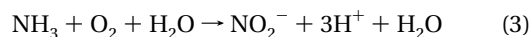
TABLE 2. Isotopic Signature of Some Nitrogen Source Materials in Guiyang

nitrogen source	$\delta^{15}N$ signature (‰)
chemical fertilizers	0 ± 1.4 ($n = 3$)
soil organic matter	5.7 ± 2.0 ($n = 6$)
PON from sewage effluent	3.7 ± 0.8 ($n = 3$)
livestock waste	7.0 ± 3.2 ($n = 5$)

suggesting that nitrate contents increased in most case in groundwater with Cl^- decreasing and that some potential nitrate input into groundwater might have been ascribed to rain, in addition to agricultural activities in summer season, since the rainwater in Guiyang showed obvious enrichment of NO_3^- and SO_4^{2-} (36).

Origin and Isotopic Characteristics of Nitrogen-Containing Compounds

Potential Nitrate Source Materials. Potential sources of NO_3^- in the study area include chemical fertilizer (urea, ammonium sulfate, and N/P/K fertilizer mix) used in the suburb, soil organic matter, effluent derived from the septic system, livestock waste, and wet deposition. The nitrogen isotope compositions of different sources are listed in Table 2. The isotopic signatures of the potential sources are similar to published data (1, 2, 9, 22, 37). The production of nitrate by organic nitrogen can be considered in terms of the following equations:

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

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

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

In general, the values of $\delta^{15}N$ of the organic nitrogen and ammonium are similar to that of nitrate by mineralization of the nitrogen if the mineralization process is limited by the eq 2. A large amount of ammonium may strongly deplete the ^{15}N of nitrate by mineralization if high ammonium concentrations are kept in the environment for a long time. In this study, there might be a little fractionation during mineralization because of the low ammonium concentrations in most water samples. Rain is probably not significant as a major cause of nitrate pollution because NH_4^+ is the dominant nitrogen species with very negative $\delta^{15}N$, and low nitrate contents were detected with lower $\delta^{15}N$ values (27).

$\delta^{15}N$**-Value of Nitrate and Ammonium.** The N isotopic compositions of nitrate and ammonium are listed in Table 1. The $\delta^{15}N$ of NO_3^- in groundwater ranged from -0.1‰ to $+15.4$‰ ($n = 19$, mean $+7.0$‰) in winter, and from -1.4‰ to $+14.9$‰ ($n = 22$, mean $+4.1$‰) in summer, which showed that nitrate in winter was enriched in ^{15}N relative to that in summer. The mean values of $\delta^{15}N$-NO_3^- in surface water were $+1.9$‰ ($n = 7$) in summer and $+1.6$‰ ($n = 8$) in winter, showing a little difference. The N isotopic compositions of ammonium in water samples had lower values than $\delta^{15}N$-NO_3^-, in the range of -1.7‰ and $+6.2$‰, with a mean value of $+1.2$‰ ($n = 20$). However, the values of $\delta^{15}N$-NH_4^+ in sewage effluent were a mean value of $+5.3$‰ ($n = 3$), suggesting that the residual ammonium was enriched in ^{15}N due to nitrification and volatilization. Nitrate in groundwater was enriched in ^{15}N as compared to that of surface water and ammonium. It might indicate that denitrification occurs in the deep soil and groundwater.

There was no correlation between $\delta^{15}N$ and N-NO_3^- in winter, which is similar to other results (9, 38), but there was a positive correlation ($r^2 = 0.46$, except for sample 25) in
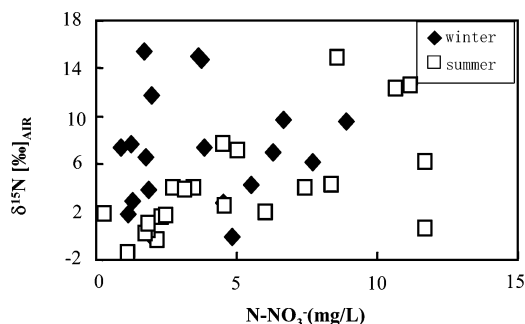
FIGURE 5. Cross-plot of the $\delta^{15}N_{nitrate}$ versus the N-NO$_3^-$ concentrations in the groundwaters.

FIGURE 6. Cross-plot of the $\delta^{18}O_{nitrate}$ versus the $\delta^{15}N_{nitrate}$ in the groundwaters.

most cases in summer (Figure 5). It might indicate that mixing was the major cause controlling the transportation of nitrate in groundwater in summer, and the mixing might have occurred between old groundwater and meteoric water with chemical fertilizers and soil organic nitrogen. The low $\delta^{15}N$ values of the groundwater in summer were indicative of NO$_3^-$ derived from chemical fertilizers and mineralization of soil organic nitrogen (39). The high $\delta^{15}N$ values of the groundwater in winter might be relative to the typical denitrification trend (40). The anomalously heavy $\delta^{15}N$-NO$_3^-$ value of groundwater and high nitrate concentrations with high Cl$^-$ contents observed in the urban area suggest that these wells had a significant contribution of NO$_3^-$ from an isotopically heavy $\delta^{15}N$ source, denitrification probably occurred, and there was a serious anthropogenic pollution input into the groundwater systems in urban Guiyang.

$\delta^{18}O$-Value of Nitrate. The $\delta^{18}O$ of NO$_3^-$ provided a further indication of sources and transformation of nitrate in groundwater. The $\delta^{18}O$ values of NO$_3^-$ in groundwater ranged from +4.3‰ to +23.5‰ ($n = 18$, mean +12.5‰) in winter and from +2.8‰ to +18.2‰ ($n = 22$, mean +10.7‰) in summer, showing that nitrate in winter was slightly enriched in ^{18}O relative to that in summer. The range of $\delta^{18}O$ values of NO$_3^-$ is similar to other results (11, 16−19). There were higher $\delta^{18}O$ values of NO$_3^-$ in surface water than those in groundwater, suggesting that rain has more effect on surface water nitrate due to the high $\delta^{18}O$ of NO$_3^-$ in rain. The $\delta^{18}O$ of groundwater in Guiyang was between about −9‰ and −6‰ (Lang et al., unpublished data). The expected $\delta^{18}O$ values of NO$_3^-$ in the groundwater from nitrification would be below +4‰ if one-third of the oxygen in the NO$_3^-$ had been derived from air (+23.5‰) and two-thirds derived from groundwater oxygen (12). There is only the $\delta^{18}O$ of NO$_3^-$ in one groundwater sample below +4‰, as if the nitrification was not the dominant process to contribute nitrate to the groundwater. However, the $\delta^{18}O$ value of NO$_3^-$ might be higher than the calculated result because the residual oxygen became heavy due to evaporation of water and diffusion of oxygen, so it is difficult to ascertain the contribution of nitrification to the nitrate sources only using $\delta^{18}O$ of NO$_3^-$ due to complicated microbial process occurring in the soil zone (2, 20). The $\delta^{18}O$ value of NO$_3^-$ fertilizer would be similar to the $\delta^{18}O$ value of air, and nitrate in groundwater might be more affected by chemical NO$_3^-$ fertilizer according to high $\delta^{18}O$ values of NO$_3^-$ in the groundwater. So the range of $\delta^{18}O$ values of NO$_3^-$ in the groundwater might suggest that nitrification and nitrate fertilizer affect the oxygen isotope composition.

Using the Isotopic Data To Trace the Nitrate Sources and Their Transformation. The mean $\delta^{15}N$ and $\delta^{18}O$ values of NO$_3^-$ in rainwater were +1.5‰ ($n = 21$) and +34.2‰ ($n = 14$) during 1 year, respectively (Li et al., unpublished data). There were lower $\delta^{15}N$ and higher $\delta^{18}O$ values and also lower contents (mean content, 0.51 mg of N/L) of nitrate in the rainwater as compared to the groundwater, suggesting that
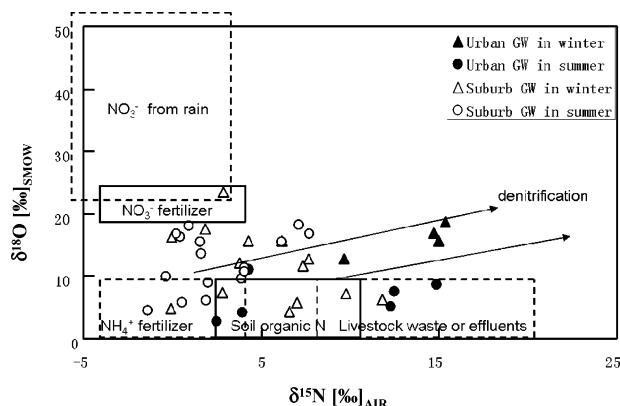
nitrate from rain was not the major source input into the groundwater.

Denitrification can remove nitrate from groundwater and cause nitrogen and oxygen isotope enrichment in the residual NO$_3^-$. The enrichment factors for ^{15}N and ^{18}O can be estimated using a simplified Rayleigh equation (11):

$$\delta_s = \delta_{s,0} + \epsilon \ln(f) \qquad (5)$$

where δ_s = delta values in the substrate, $\delta_{s,0}$ = initial delta values in the substrate, ϵ = enrichment factor, and f = substrate concentration divided by the initial substrate concentration.

It is reported that the ratios of $\delta^{15}N/\delta^{18}O$ range from 1.3 to 2.1 (11, 16−19). In this study, there was no negative correlation between $\delta^{15}N$-NO$_3^-$ and N-NO$_3^-$ (Figure 5) and no distinct positive correlation between $\delta^{15}N$ and $\delta^{18}O$ (Figure 6), which indicated that denitrification had not predominantly occurred in the groundwater. As indicated by Figure 6, there was some distinct NO$_3^-$ fertilizer input into the suburban groundwater due to low $\delta^{15}N$ values and high $\delta^{18}O$ values, especially in summer. And the denitrification might affect the nitrate in some urban groundwater, as suggested by the high $\delta^{15}N$ values and high $\delta^{18}O$ values.

The average values of $\delta^{15}N$ and $\delta^{18}O$ of nitrate were higher in winter than those in summer, showing that seasonal variation of nitrate source affected the isotopic composition and that denitrification might affect the isotopes of nitrate. Panno et al. (40) have shown that the trend in isotopic composition of nitrate from lower to higher values generally follows the seasons from spring to summer and fall to winter, which is also the case of this study. However, it is difficult to ascertain the time scale of isotope fractionation according to the difference in isotope values between two seasons by the Rayleigh equation because of seasonal variations of nitrate sources and water sources.

Furthermore, there were multiple potential sources of nitrate affecting groundwater quality in the study area, and mixing between point and nonpoint sources along shallow flow paths made determination of sources and the extent of nitrate transformation very difficult. But it might be sure that chemical fertilizer and nitrification mainly contributed nitrate to suburban groundwater due to the ranges of $\delta^{15}N$ values and $\delta^{18}O$ values of nitrate, and the sewage effluents and denitrification might mainly affect the nitrate in urban groundwater due to high $\delta^{15}N$ values and high $\delta^{18}O$ values of nitrate.

Literature Cited

(1) Heaton, T. H. E. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. *Chem. Geol.* **1986**, *59*, 87−102.

(2) Kendall, C.; Tracing sources and cycling of nitrate in catchments. In: *Isotope Tracers in Catchment Hydrology*; Kendall, C., McDonnell, J. J., Eds.; Elsevier: Amsterdam, 1998.

(3) Silva, S. R.; Ging, P. B.; Lee, R. W.; Ebbert, J. C.; Tesoriero, A. J.; Inkpen, E. L. Forensic applications of nitrogen and oxygen isotopes in tracing nitrate sources in urban environments. *Environ. Forensics* **2002**, *3*, 125−130.

(4) Seiler, R. L. Combined use of ^{15}N and ^{18}O of nitrate and ^{11}B to evaluate nitrate contamination in ground water. *Appl. Geochem.* **2005**, *20*, 1626−1636.

(5) Comely, H. H. Cyanosis in infants caused by nitrate in well water. *J. Am. Med. Assoc.* **1945**, *129*, 112−116.

(6) Cao, W.F. A prediction for ground water supplies and environmental geological issues of 2000−2030 in Guiyang city. *Guizhou Geol.* **1998**, *15*, 368−376 (in Chinese).

(7) Lang, Y. C.; Liu, C.-Q.; Zhao, Z. Q.; Li, S. L.; Han, G. L. Geochemistry of surface and ground water in Guiyang city, China: Water/rock interaction and pollution in a karst hydrological system. *Appl. Geochem.* **2006**, *21*, 887−903.

(8) Mariotti, A.; Landreau, A.; Simon, B. 15N isotope biogeochemistry and natural denitrification process in ground water: Application to the chalk aquifer of northern France. *Geochim. Cosmochim. Acta* **1988**, *52*, 1869−1878.

(9) Wassenaar, L. I. Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. *Appl. Geochem.* **1995**, *10*, 391−405.

(10) Amberger, A.; Schmit, H. L. Naturliche isotopengehalte von nitrat als indikatoren fur dessen Herkunft. *Geochim. Cosmochim. Acta* **1987**, *51*, 2699−2705.

(11) Böttcher, J.; Strebel, O.; Voerkelius, S.; Schmidt, H. L. Using isotope fractionation of nitrate−nitrogen and nitrate−oxygen for evaluation of microbial denitrification in a sandy aquifer. *J. Hydrol.* **1990**, *114*, 413−424.

(12) Andersson, K. K.; Hooper, A. B. 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.* **1983**, *64*, 236−240.

(13) Kroopnick, P. M.; Craig, H. Atmospheric oxygen: isotopic composition and solubility fractionation. *Science* **1972**, *175*, 54−55.

(14) Horibe, Y.; Shigehara, K.; Takakuwa, Y. Isotopic separation factors of carbon-dioxide−water system and isotopic composition of atmospheric oxygen. *J. Geophys. Res.* **1973**, *78*, 2625−2629.

(15) Durka, W.; Schulze, E. D.; Gebauer, G.; Voerkelius, S. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. *Nature* **1994**, *372*, 765−767.

(16) Aravena, R.; Robertson, W. D. Use of multiple isotope tracers to evaluate denitrification in ground water: Study of nitrate from a large-flux septic system plume. *Ground Water* **1998**, *36*, 975−982.

(17) Cey, E. E.; Rudolph, D. L.; Aravena, R.; Parkin, G. Role of the riparian zone in controlling the distribution and fate of agricultural nitrogen near a small stream in southern Ontario. *J. Contam. Hydrol.* **1999**, *37*, 45−67.

(18) Mengis, M.; Schiff, S. L.; Harris, M.; English, M. C.; Aravena, R.; Elgood, R. J.; Maclean, A. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. *Ground Water* **1999**, *37*, 448−457.

(19) Fukada, T.; Kevin, M.; Dennis, P. F.; Grischek, T. A dual isotope approach to identify denitrification in ground water at a river-bank infiltration site. *Water Res.* **2003**, *37*, 3070−3078.

(20) Mengis, M.; Walther, U.; Bernasconi, S. M.; Wehrli, B. Limitations of using $\delta^{18}O$ for the source identification of nitrate in agricultural soils. *Environ. Sci. Technol.* **2001**, *35*, 1840−1844.

(21) Widory, D.; Kloppmann, W.; Chery, L.; Bonnin, J.; Rochdi, H.; Huinamant, J.-L. Nitrate in groundwater: An isotopic multi-trace approach. *J. Contam. Hydrol.* **2004**, *72*, 165−188.

(22) Widory, D.; Petelet-Giraud, E.; Négrel, P.; Ladouche, B. Tracking the sources of nitrate in groundwater using coupled nitrogen and boron isotopes: A synthesis. *Environ. Sci. Technol.* **2005**, *39*, 539−548.

(23) Li, S. L.; Liu, C.-Q.; Tao, F. X.; Lang, Y. C.; Han, G. L. Carbon biogeochemistry of ground water, Guiyang, Southwest China. *Ground Water* **2005**, *43*, 494−499.

(24) Han, Z. J.; Jin, Z. S. *Hydrogeology of Guizhou Province, China*; Seismic Publication: Beijing, 1996 (in Chinese).

(25) Garten, C. T., Jr. Nitrogen isotope composition of ammonium and nitrate in bulk precipitation and forest throughfall. *Int. J. Environ. Anal. Chem.* **1992**, *47*, 33−45.

(26) Silva, S. R.; Kendall, C.; Wilkison, D. H.; Zergler, A. C.; Chang, C. C. Y.; Avanzino, R. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen ratios. *J. Hydrol.* **2000**, *228*, 22−36.

(27) Xiao, H. Y.; Liu, C.-Q. Sources of nitrogen and sulfur in wet deposition at Guiyang, southwest China. *Atmos. Environ.* **2002**, *36*, 5121−5130.

(28) Xiao, H. Y.; Liu, C.-Q. Discussion on a method for preparation nitrate in water samples for $\delta^{15}N$ analysis. *Rock Miner. Anal.* **2002**, *21*, 105−108 (in Chinese).

(29) Kendall, C.; Grim, E. Combustion tube method for measurement of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water. *Anal. Chem.* **1990**, *62*, 526−529.

(30) Revesz, K.; Böhlke, J. K.; Yoshinari, Determination of $\delta^{18}O$ and $\delta^{15}N$ in nitrate. *Anal. Chem.* **1997**, 75−4380.

(31) Kornexl, B. E.; Gehre, M.; Höfling, R.; Werner, R. A. On-line $\delta^{18}O$ measurement of organic and inorganic substances. *Rapid Commun. Mass Spectrom* **1999**, *13*, 1685−1693.

(32) Bräuer, K.; Strauch, G. An alternative procedure for the ^{18}O measurement of nitrate oxygen. *Chem. Geol.* **2000**, *168*, 283−290.

(33) Révész, K.; Böhlke, J.-K. Comparison of $\delta^{18}O$ measurements in nitrate by different combustion techniques. *Anal. Chem.* **2002**, *74*, 5410−5413.

(34) Koba, L.; Tokuchi, N.; Wada, E.; Nakajiama, T.; Iwatsubo, G. Intermittent denitrification: the application of a ^{15}N natural abundance method to a forested ecosystem. *Geochim. Cosmochim. Acta* **1997**, *61*, 5043−5050.

(35) Kellman, L. M.; Hillaire-Marcel, C. Nitrate cycling in streams: Using natural abundances of NO_3^--$\delta^{15}N$ to measure in-situ denitrification. *Biogeochemistry* **1998**, *43*, 273−292.

(36) Han, G.; Liu, C.-Q. Strontium isotope and major ion chemistry of the rainwaters from Guiyang, Guizhou Province, China. *Sci. Total Environ.* **2006**, *364*, 165−174.

(37) Victòria, L.; Otero, N.; Soler, A.; Canals, À. Fertilizer characterization: Isotopic data (N, S, O, and Sr). *Environ. Sci. Technol.* **2004**, *38*, 3254−3262.

(38) Feast, N. A.; Hiscock, K. M.; Dennis, P. F.; Andrews, J. N. Nitrogen isotope hydrochemistry and denitrification within the Chalk aquifer system of north Norfolk, UK. *J. Hydrol.* **1998**, *211*, 233−252.

(39) Ostrom, N. E.; Knoke, K. E.; Hedin, L. O.; Robertson, G. P.; Smucker, A. J. M. Temporal trends in nitrogen isotope values of nitrate leaching from an agricultural soil. *Chem. Geol.* **1998**, *146*, 219−227.

(40) Panno, S. V.; Hackley, K. C.; Hwang, H. H.; Kelly, W. R. Determination of the sources of nitrate contamination in karst springs using isotopic and chemical indicators. *Chem. Geol.* **2001**, *179*, 113−128.