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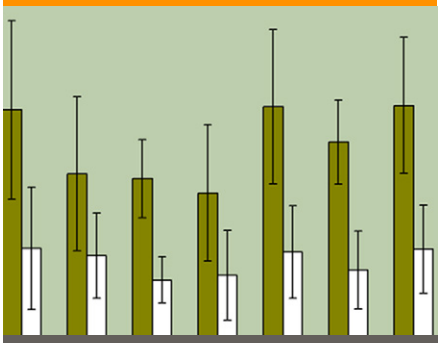
Frontiers in Hydropedology: Interdisciplinary Research from Soil Architecture to the Critical Zone



Core Ideas

- High NO_3^- concentrations were detected in an area with developed conduit networks and sinkholes.
- Stable isotopes of water and NO_3^- were used to identify their transport.
- Isotopic composition of NO_3^- was affected by denitrification, particularly in the wet season.
- Water management should consider the linkage between N flux and rainfall.

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Evaluation of Factors Driving Seasonal Nitrate Variations in Surface and Underground Systems of a Karst Catchment

Fu-Jun Yue, Si-Liang Li*, Jun Zhong, and Jing Liu

A typical mixed-land-use karst catchment in Guizhou Province, southwestern China, with well-developed conduit networks was studied to understand the spatiotemporal variability of nitrate sources and transformation processes. High nitrate concentrations were detected in the karstic study area, which is particularly vulnerable to anthropogenic pollution and is considered a representative catchment to understand the surface–underground system in southwestern China. The stable isotopes of water and nitrate (δD_{water}, $\delta^{18}O_{water}$, $\delta^{15}N_{nitrate}$, and $\delta^{18}O_{nitrate}$) were used to identify nitrate sources and to assess seasonal variations in hydrological processes affecting nitrate levels. Monthly sampling indicated that nitrate flux is strongly related to agricultural activities and the intensity of rainfall, which can accelerate transport between the surface water (SFW) and underground water (UGW) systems. Nitrate in water samples showed nitrification characteristics and was mainly derived from the oxidation of reductive fertilizers including organic N fertilizer. The isotopic composition of nitrate was affected by denitrification, particularly in the wet season. Isotopic signatures indicate that moderate evaporation occurs during the dry season. Denitrification in underground conduits appears to be relatively minor compared with that in SFW. The results of this study suggest that rainfall is an important factor influencing N flux, which has implications for water management.

Abbreviations: HRB, Houzhai River Basin; SFW, surface water; UGW, underground water.

Underground water in karstic regions is particularly vulnerable to anthropogenic pollution because of the direct connectivity between SFW and UGW systems, high transmissivity, and often poor waste disposal habits (Hiscock, 2005; Ford and Williams, 2007). Nitrate is possibly the most widespread contaminant in UGW and SFW systems in intensive agricultural karstic areas as a result of high fertilizer application and water management methods (Katz et al., 2001; Perrin et al., 2008; El Gaoizo et al., 2013). Previous studies have found high nitrate concentrations in karst regions, particularly in agricultural areas (Perrin et al., 2008; El Gaoizo et al., 2013; McMullen et al., 2014; Yue et al., 2015). Understanding the sources and fate of nitrate in SFW and UGW is critical to improve management practices in this infertile environment, to reduce nutrient loss, and to protect water quality.

The karst area of southwestern China is particularly vulnerable; the vadose zone consists of thin soils underlain by fractured rock, which provides minimal buffering of the UGW aquifer (Liu et al., 2009; Chen et al., 2008). There is rapid transfer between the UGW and SFW systems by conduit networks and sinkholes. Surface water, for example, overland flow on hillslopes and surface river streamflow, mostly occurs during heavy rainfall periods. Therefore, both UGW and SFW are highly sensitive to pollution by anthropogenic activities such as fertilization. Underground water and SFW are the primary water resources for agriculture, industry, and domestic use in the karst area of southwestern China. Hence, prevention of water pollution is of paramount concern for local government and residents (Wang et al., 2004; Chen et al., 2008; Liu, 2009). Also, as a soil macronutrient, leaching of N from the thin soil layer will decrease the productivity of cultivated land in the karst area of southwestern China (Wang et al., 2004; Liu, 2009).

Nitrate in aquatic environments is generally derived from chemical fertilizer, soil nitrification, atmospheric deposition, and sewage effluent (Kendall et al., 2007). Stable isotopic techniques have been successfully applied to track the sources and fate of nitrate in numerous watersheds (Heaton, 1986; Wassenaar, 1995; Katz et al., 2001; Liu et al., 2006; Panno et al., 2006; Kendall et al., 2007; Jiang et al., 2009; Li et al., 2010b, 2014; El Gaouzi et al., 2013; Jin et al., 2015; Peters et al., 2015). Nitrate that originates from atmospheric precipitation and chemical fertilizer (nitrate and reductive fertilizers) generally has a lighter N isotopic value and greater concentrations in the chemical fertilizer than that from urban sewage and livestock manure (Kendall et al., 2007). However, the use of the single-isotope approach to evaluate the sources and fate of nitrate in water is restricted by the overlap of $\delta^{15}N$ values for nitrate from different sources and the nonconservative behavior of nitrate. The dual isotopes of NO_3^- are more useful than $\delta^{15}N$ for separating nitrate from wet deposition, microbial nitrification, and chemical fertilizer because there is large variability in $\delta^{18}O_{nitrate}$ among sources (Wassenaar, 1995; Kendall et al., 2007). Nitrate from atmospheric precipitation is generally enriched in ^{18}O (Kendall et al., 2007). In general, the $\delta^{18}O$ value of nitrate produced by nitrification ranges from 0 to 15‰ because of the range of conditions and complexity involved in biological processes (Mengis et al., 2001). Nitrate fertilizers typically have $\delta^{18}O$ values of 23.5‰, which is close to atmospheric O_2 (Amberger and Schmidt, 1987).

The isotopic composition of water is a valuable additional dataset when tracing hydrological processes relevant to contaminant dynamics. Variation in stable isotope ratios of water bodies, expressed using standard $\delta^{18}O_{water}$ and δD notation, has been shown to reflect evaporation (Katz et al., 1997; Winter et al., 1998). The deuterium excess (*d*-excess) is a second-order isotope parameter that was first shown to reflect evaporation conditions at the source of precipitation but has also been correlated with the land surface processes of evaporation and plant transpiration (Rozanski et al., 1993; Clark and Fritz, 1997). It is a measure of deviation from the global meteoric water line and defined by the equation $d = \delta D - 8\delta^{18}O_{water}$ (Dansgaard, 1964; Rozanski et al., 1993; Clark and Fritz, 1997). The parameters of $\delta^{18}O_{water}$, δD, and *d*-excess are useful to infer processes relevant to nitrate levels.

In nonkarstic areas of southwestern China, UGW normally has better quality than SFW as a result of filtering in the vadose zone (Li et al., 2013). However, in karst areas, the nitrate concentration has been found to be higher in UGW than in SFW, for example, in Guiyang City, southwestern China (Liu et al., 2006). In the Houzhai River Basin (HRB) (Fig. 1), our previous studies also indicated that UGW conduit flow exhibits higher nitrate concentrations than corresponding SFW flow (Li et al., 2010a; Yue et al., 2015). Biannual sampling indicated significant spatial and seasonal variation in nitrate concentrations, denitrification rate, and nitrate flux. Because marked seasonal variability in streamflow exists in the whole karst area of southwestern China, biannual sampling is not sufficient to understand the transport of nitrate in the SFW–UGW system and its relationship with streamflow variability.

This study aimed to identify spatial and temporal characteristics of nitrate concentration variability and potential influencing sources in the HRB, a typical karst catchment in the karst area of southwestern China. The stable isotopes ($\delta^{18}O_{water}$, δD, $\delta^{15}N_{nitrate}$, and $\delta^{18}O_{nitrate}$) and water chemistry of the samples were used to identify UGW and SFW nitrate sources in wet and dry seasons, elucidate the mechanism of nitrate transport in the UGW and SFW systems, and understand biogeochemical processes potentially affecting nitrate fate in the catchment.

Materials and Methods

Site Description

The HRB, with an area of 73.5 km^2, was selected for this study and is located at the center of the Southeast Asian karst region

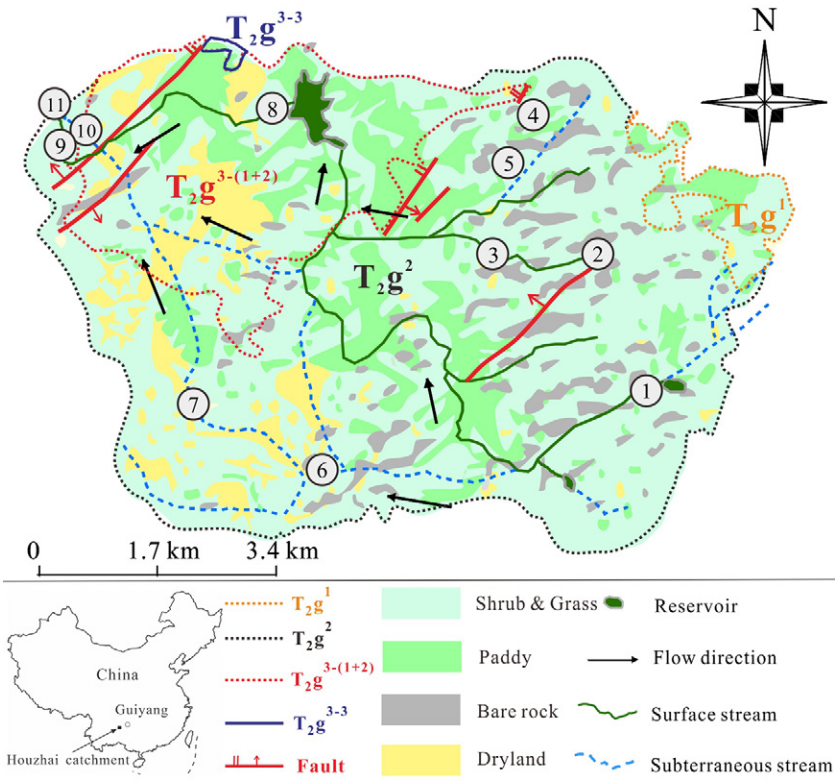

Fig. 1. The monthly sampling sites in the Houzhai catchment during 2013: T_2g^1, mudstone and argillaceous dolomite of Middle Triassic; T_2g^2, limestone and dolomite of Middle Triassic; $T_2g^{3-(1+2)}$, dolomite of Middle Triassic; T_2g^{3-3}, breccia of Middle Triassic.

(Fig. 1). The HRB lies within Guizhou Province and is characterized by a subtropical, monsoonal climate, where ~80% of the annual precipitation occurs during the wet season (May–October) (Supplemental Fig. S1) (Li et al., 2010a; Yue et al., 2015). It is a typical karst catchment comprising mid-Triassic period limestone and dolomite across the basin (Chen et al., 2008). The topography of the basin can be split into the eastern mountainous region, characterized by high relief and steep hillslopes, and the low-relief plain of the western region with an elevation range of 1218 to 1565 m asl. The geomorphology changes from peak clusters with many depressions in the upper reaches to peak forests with valleys in the middle reaches, to vast plains in the lower reaches of the catchment (Fig. 1). Surface streams are formed in the relatively thick clay soil in the north and are usually dry as a result of high percolation through cracks and fractures into underlying carbonate rock. The Qingshan Reservoir (No. 8) receives SFW from upper reaches during flood periods. The reservoir outflow discharges into the surface outlet of the HRB (e.g., No. 9 in Fig. 1). The primary underground conduit in the south of the catchment originates from the eastern mountainous area, where most surface and subsurface flow recharges into the underground conduit through sinkholes. The conduit is continuous through the relatively flat plain of the middle and lower catchments and discharges at the catchment underground outlet (No. 10 in Fig. 1). The underground conduit is well developed and connects numerous larger fractures.

The soils are thin (average <50 cm) with a low water retention capacity. The soil profile is thinnest and discontinuous in the upper reaches of the study area and relatively thick in the depression areas, which are mostly distributed in the middle and lower reaches (Liu et al., 2009). The land cover and land use can be divided into four main types in the HRB: bare rock, forest, cultivated agriculture, and grassland (Fig. 1). The cultivated area covers 20.4% of the HRB, approximately two-thirds of which is paddies of rice (*Oryza sativa* L.) grown in the wet season (Hu et al., 2001). Other crops in the wet season are corn (*Zea mays* L.) and various vegetables. The main crops in the dry season are canola [*Brassica juncea* (L.) Czern. ssp. *juncea*] and a few vegetables. Farmers apply fertilizer including urea, diammonium phosphate, and organic fertilizers. During the period April through July, these fertilizers are typically applied three times at proportions of 40 to 60, 20 to 30, and 20 to 30% (Feng et al., 2009). Organic fertilizers are generally used for rice seedlings in April and May.

Sampling and Analyses

Water samples were collected monthly throughout 2013 at 11 selected sites and one rainfall event in June (Fig. 1). The annual rainfall in 2013 (732.5 mm) was significantly lower than the average annual rainfall between 2006 and 2012 (1246.1 mm) (Supplemental Fig. S1). The rainfall event captured on 9 June was typical of the heavy rainfall events that characterize the wet season (55.8 mm) and at sampling sites similar to our previous sites (Li et al., 2010a; Yue et al., 2015). After 12 d, the second sampling was carried out on 21 June. The 11 sampling sites can be separated into two SFW sites, the outlet of the Qingshan reservoir (No. 8) and a surface river (No. 9), and nine UGW sites (upper reaches, No. 1–5; middle reaches, No. 6–7; outlet of lower reaches, No. 10). All UGW samples were collected at spring outlets. Among the UGW sites, No. 4 was excluded from the nitrate concentration and isotope statistical analyses because the NO_3^-–N concentration was below the detection limit (Supplemental Table S1). This site sampled a deep aquifer with relatively stable groundwater table and outflow (Fig. 1).

The major anions (Cl^- and NO_3^-) were analyzed by ion chromatography using a Dionex ICS-90. Deuterium and O isotopes of water were measured using a liquid-water isotope analyzer (IWA-35EP, LGR Company). Three standards (ranging from −96.4 to −9.5‰ for δD and from −13.1 to −2.8‰ for $\delta^{18}O_{water}$) were used for isotopic calibration. The standard error was 0.2‰ for $\delta^{18}O_{water}$ and 0.5‰ for δD. The denitrifier method was used to measure nitrate isotopes (McIlvin and Casciotti, 2011). A denitrifier lacking the N_2O-reductase enzyme [*Pseudomonas chlororaphis* ssp. *aureofaciens* (Kluyver) ATCC 13985] was used to convert NO_3^- into N_2O. Then simultaneous $\delta^{15}N$ and $\delta^{18}O$ analysis of the N_2O was performed using an isotope ratio mass spectrometer (IsoPrime, GV). The $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ values were normalized and calibrated relative to the international reference materials USGS-32, USGS-34, USGS-35, and IAEA-N3 after blank correction (McIlvin and Casciotti, 2011). In general, $\delta^{15}N$ and $\delta^{18}O$ for nitrate have a precision of 0.3 and 0.5‰, respectively.

Results

Spatial and Temporal Variations of the Stable Water Isotopes

The $\delta^{18}O_{water}$ and δD values in the water samples from the study area ranged from −9.1 to −5.8‰ (mean = −7.9 ± 0.6‰, n = 110) and −63.2 to −46.9‰ (mean = −55.6 ± 3.2‰, n = 110), respectively (Supplemental Table S1). The lowest $\delta^{18}O_{water}$ and δD values for each month were observed at the deep groundwater site (No. 4, n = 8), −9.0 ± 0.4 and −63.2 ± 0.8‰, respectively (Fig. 2). As indicated in Fig. 2a, water samples in the wet months between June and September had more depleted $\delta^{18}O_{water}$ values than those in dry months (February, April, and November). This seasonal shift in the isotopic composition reflects the effect of evaporation because of lower rainfall and longer sunshine hours during the dry season. Additionally, the longer residence times encountered in the dry season may increase this effect, causing an enrichment of heavy isotopes relative to the wet season. The water samples at Sites 9, 10, and 11 represent the outlets of SFW, UGW, and the entire basin (a mixture of SFW and UGW),

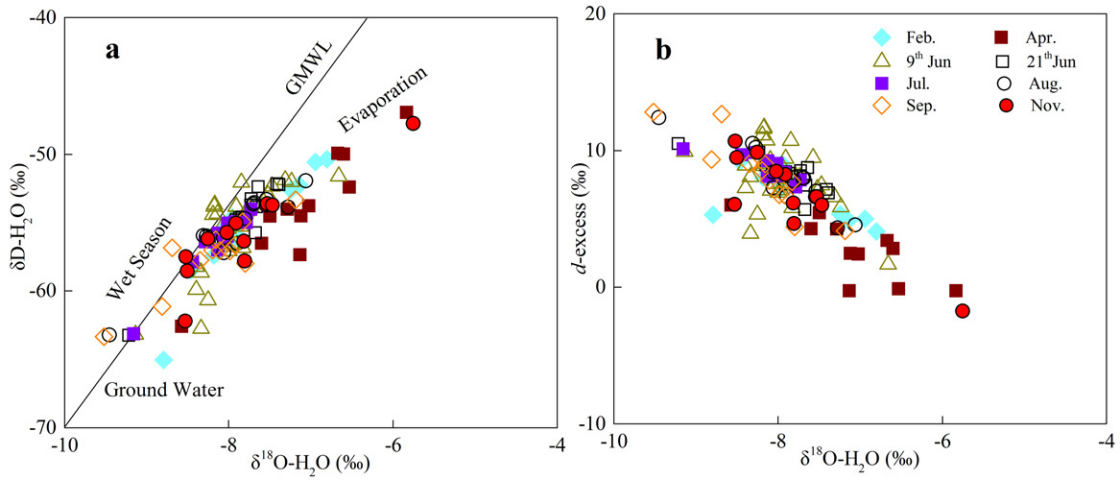

Fig. 2. Scatterplots for the correlation (a) between $\delta^{18}O_{water}$ and δD and (b) of $\delta^{18}O_{water}$ and deuterium excess (*d*-excess) values in water samples.

respectively. During the dry season, the $\delta^{18}O_{water}$ values at the SFW outlet (average −6.6 ± 0.9‰, *n* = 3) were heavier (more positive) than those at the UGW outlet (average −7.1 ± 0.4‰, *n* = 3), suggesting that evaporation had affected samples from the SFW outlet more than those from the UGW outlet. The isotopic composition of water at the basin outlet (average −7.1 ± 0.4‰, *n* = 3) was similar to that at the UGW outlet, indicating a relatively stable UGW flow regime and that the underground system contributed a major proportion of water to the catchment outlet. In the wet season, there was little difference in the water isotopic values between the basin outlet (−7.9 ± 0.3‰, *n* = 5), UGW outlet (−7.9 ± 0.1‰, *n* = 5), and SFW outlet (−7.6 ± 0.4‰, *n* = 5), which illustrates high levels of exchange between the UGW and SFW systems in the HRB under high-flow conditions.

Spatial and Temporal Variations of Nitrate Concentrations

Nitrate was the major dissolved N species, accounting for 85% of the total dissolved N in most water samples. The NO_3^-–N concentration ranged from 0.2 to 14.5 mg L^{-1} across the catchment (Supplemental Table S1). The temporal variation in average monthly nitrate and Cl^- concentrations for HRB is shown in Fig. 3a. The nitrate concentration in water samples was higher in the wet season (particularly in June and July) and lower in the dry season (particularly in February through April), especially at the surface stream site (Fig. 4). Higher nitrate concentrations generally correspond to the time period between May and July when farmers use fertilizers (Feng et al., 2009). The nitrate concentrations measured during the heavy rainfall event (55.8 mm

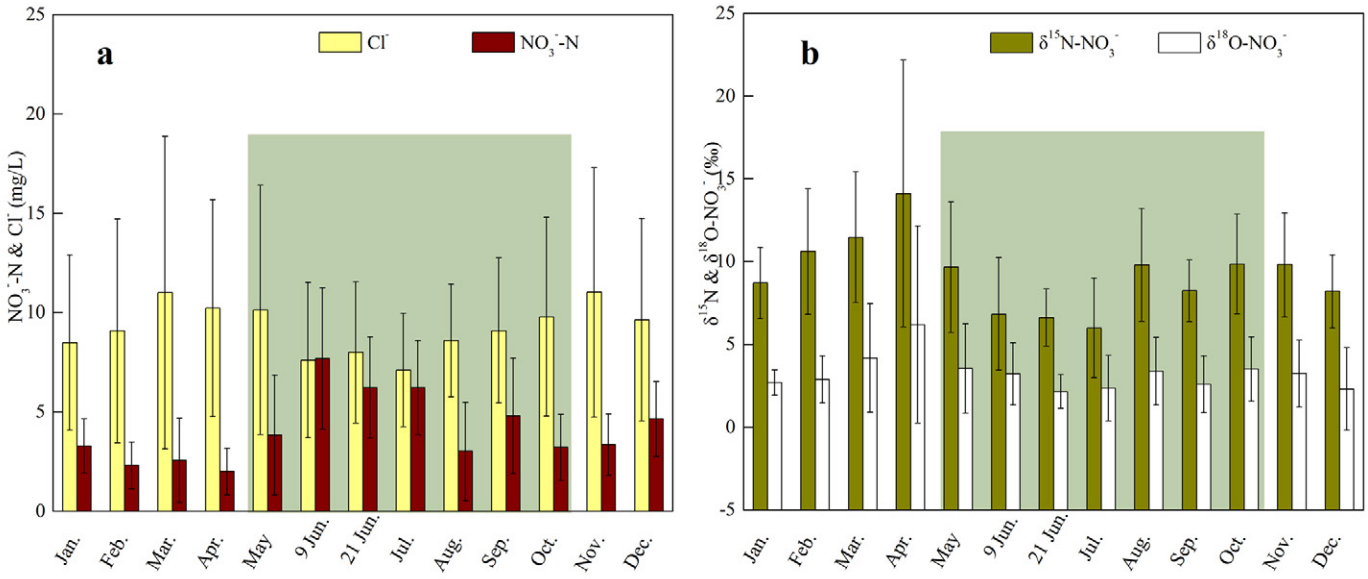

Fig. 3. Monthly variations for the concentrations of (a) NO_3^-–N and Cl^- and (b) nitrate isotopes.

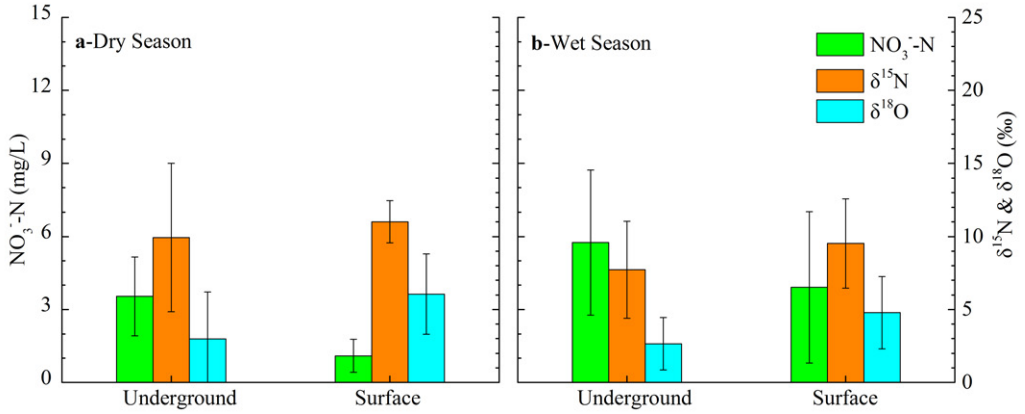

Fig. 4. The seasonal variation between underground stream and surface stream for NO_3^-–N and ^{15}N and ^{18}O isotopes.

on 9 June) was higher than that measured during the subsequent period of no rainfall (12 d later on 21 June) for each sample site, which shows that storm rainfall accelerates the N loss in agricultural areas.

Spatially, during the dry season, the highest nitrate concentration occurred in the middle reaches of the underground conduit in the flat plains of the western part of the catchment (e.g., Laoheitan Site 6 in Fig. 5), where the land use is dominated by agriculture and villages. During the wet season, the headwater samples (e.g., Muzhu and Changchong, Sites 1 and 2 in Fig. 5) had higher nitrate concentrations than the samples in the middle and lower reaches, possibly a result of increased mixing of streamflow and exchange between UGW and SFW in the HRB. There is no distinct spatial pattern in the variation of nitrate concentrations based on the analysis of 32 water samples in June after heavy rainfall, which is similar to the results of our previous study (Yue et al., 2015).

Results indicate that the export of N from the catchment mainly occurs in June and July from surface streams, while the UGW system becomes a dominant contributor to export in lower rainfall months. For example, considering samples from the outlets of the UGW, SFW, and the total catchment (No. 9, 10, and 11), the ratio of SFW nitrate to the total catchment outlet nitrate ($nitrate_{SFW}/nitrate_{total}$) is 0.40 ± 0.19 ($n$ = 7), much lower than the ratio of UGW nitrate to the total catchment outlet ($nitrate_{UGW}/nitrate_{total}$) (1.13 ± 0.12) for the samples during the dry season, but the difference between these ratios is reduced during the wet season (0.65 ± 0.37, n = 6 for SFW; 1.03 ± 0.05, n = 6 for UGW). This indicates mixing of SFW and UGW at various sites

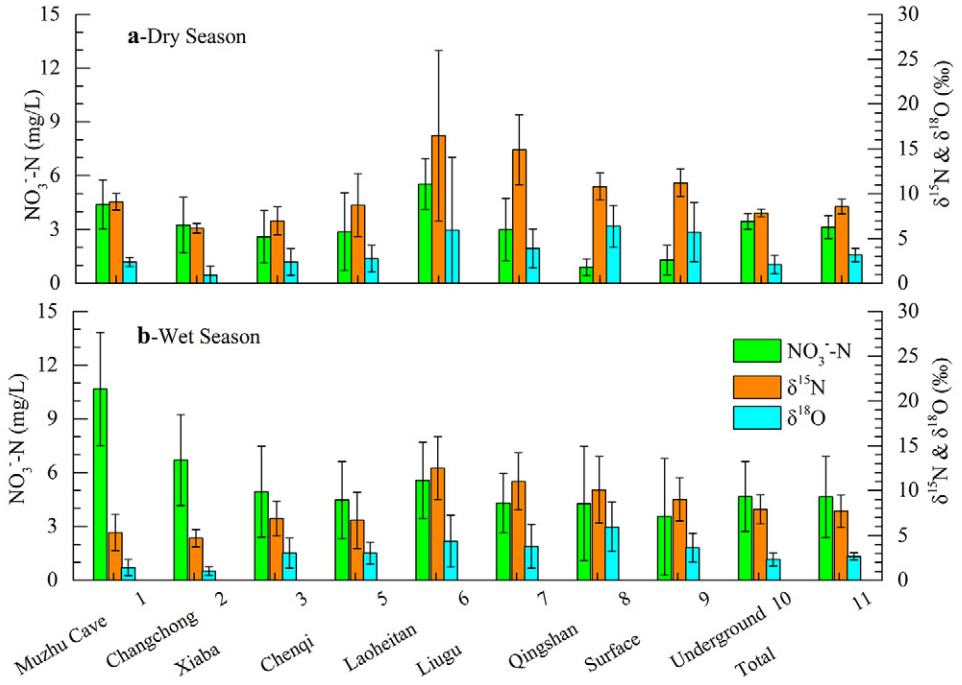

Fig. 5. The average values for (a) NO_3^-–N and (b) ^{15}N and ^{18}O isotopes for the sampling sites during the dry season.

in the wet season. Compared with our previous study results in 2007 (Yue et al., 2015), the variation of the nitrate concentration during April and July in 2013 was opposite at the same sample sites. In April, the NO_3^-–N concentration of 2013 (2.1 ± 1.2 mg L^{-1}, n = 9) was lower than that in 2007 (3.3 ± 1.9 mg L^{-1}, n = 9), but in July, the NO_3^-–N concentration in 2013 (6.9 ± 1.6 mg L^{-1}, n = 10) was higher than that in 2007 (5.0 ± 2.5 mg L^{-1}, n = 10), possibly a result of the larger rainfall in 2007 having a dilution effect. The N flux in July 2013 may be significantly lower than in July 2007, as the rainfall in July 2013 (86 mm) was 22% of that in July 2007 (393 mm), and the timing of agricultural activities (e.g., fertilization) remains similar from year to year.

Spatial and Temporal Variations of Nitrate Isotopes

The $\delta^{15}N_{nitrate}$ ranged from 2.8 to 35.5‰, with a median value of 3.9‰ (n = 152) in the HRB. Meanwhile, the $\delta^{18}O_{nitrate}$ was found to range from −0.2 to 22.4‰ in the catchment, with a median value of 2.7‰ (n = 152). The isotopic data showed that >90% of water samples have $\delta^{15}N_{nitrate}$ values from 2 to 15‰ and $\delta^{18}O_{nitrate}$ values from 0 to 6‰ (Fig. 6), similar to the previous study where they ranged from 1.7 to 14 ‰ for $\delta^{15}N_{nitrate}$ and 0.7 to 9.8‰ for $\delta^{18}O_{nitrate}$ (Yue et al., 2015). The isotopic values of nitrate were found to be lower in the wet-season months (June and July had an average $\delta^{15}N$ <6.8‰) than in the dry-season months (average $\delta^{15}N$ >7.9‰) (Fig. 3b and 4; Supplemental Table S1). The seasonal variation of isotopic values of nitrate is inverse to the seasonal variation in nitrate concentrations, with nitrate being generally enriched in heavy isotopes during the dry season relative to the wet season (Fig. 4). Lower $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values and high $[NO_3^-$–N] were observed in the wet season, while higher $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values and lower $[NO_3^-$–N] were observed in the dry season. The spatial variation in $\delta^{15}N_{nitrate}$, $\delta^{18}O_{nitrate}$, and $[NO_3^-$–N] in Fig. 5 indicates that the general trend is decreasing $[NO_3^-$–N] and increasing isotopic values moving from the upper to the lower reaches of the catchment during the wet season. The high $\delta^{15}N$ of the outlet SFW is similar to the $\delta^{15}N$ observed at the Qingshan outlet (No. 7), indicating that the SFW outlet is probably strongly regulated by the Qingshan Reservoir outflow.

Discussion

Seasonal Variation of Water Isotopes

In this study, the d-excess values ranged from −1.7 to 12.8‰, and >90% of the water samples had a d-excess value >4‰. Figure 2b shows a significant negative relationship between d-excess and $\delta^{18}O$ values in the water samples from the HRB ($d = -3.4\delta^{18}O - 19.7$, $R^2 = 0.62$, $p < 0.001$). More negative d-excess values may suggest that evapotranspiration has significantly shifted the water isotopic composition. In this study, water samples with d-excess values <4‰ generally had high $\delta^{18}O$ values, and most samples with these values were obtained only in April when water was brought to the surface by pumping for paddy field irrigation. The relatively low rainfall during this period (Supplemental Fig. S1) coupled with high evaporation rates resulted in very low stream discharge and a low water table for UGW during the sampling period. Thus, evapotranspiration significantly shifted the isotopic composition in these samples.

Nitrate Sources Constrained by Dual Isotopes

Dissolved NO_3^- in SFW and UGW originates from rain, chemical fertilizer, soil nitrification, and sewage effluent, which have different characteristic signatures of combined $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ isotopes. For example, nitrate from rain samples in the HRB has $\delta^{15}N_{nitrate}$ values between 1.0 and 4.7‰ and $\delta^{18}O_{nitrate}$

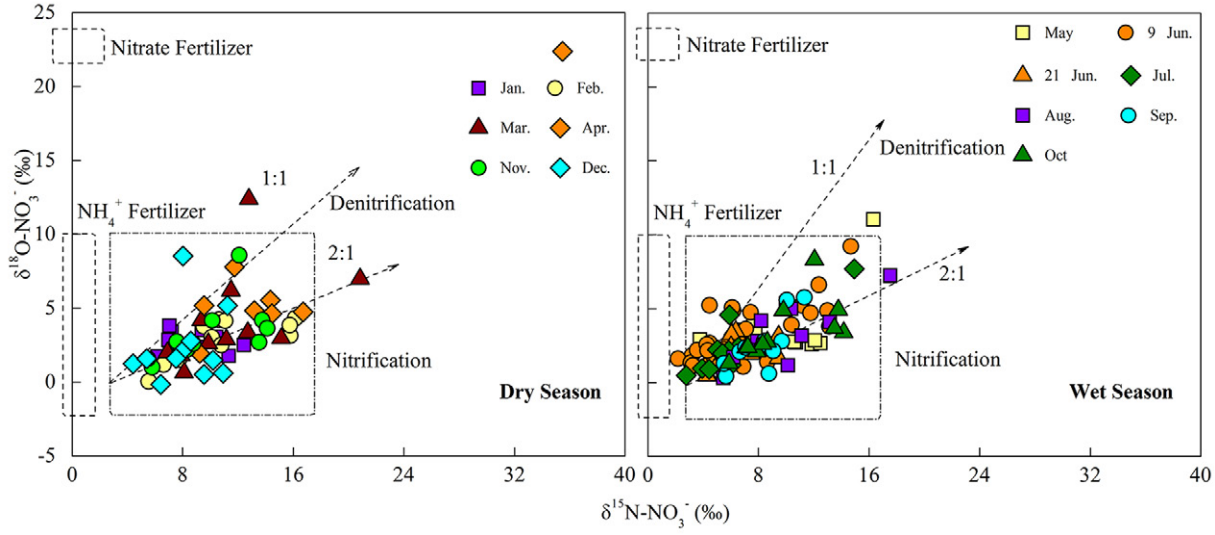

Fig. 6. Scatterplots between $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ in different seasons of 2013 at the Houzhai catchment, southwestern China.

values generally >30‰ (30.4–75.8‰) (Yue et al., 2015). Chemical fertilizer and manure samples taken from a nearby region of Guizhou Province with similar farming, environmental, and climatic conditions exhibited $\delta^{15}N$ ratios of $\sim 0 \pm 1.4$‰ and 7.0 ± 3.2‰, respectively (Liu et al., 2006). The applied chemical fertilizers in the HRB are mainly reductive N fertilizers such as urea and ammonium. Because the $\delta^{18}O_{nitrate}$ values of rain and nitrate fertilizer are much higher than those from the HRB water samples, the dual isotope patterns observed in this study indicate that they were not the dominant sources of nitrate in the water samples. In addition, soil organic N may not be a major source of nitrate in the water samples considering the thin soil profile and rapid water movement in the karst system (Chen et al., 2008; Liu et al., 2009), particularly for the water samples from the mountainous upper reaches where flow rates are much faster than in the lower reaches of the catchment (Fig. 1).

Chloride is a useful indicator in understanding mixing and biological processes because of its stable physical and chemical characteristics in water, high concentrations in domestic and agricultural runoff, and relatively low natural levels in many environments (Koba et al., 1997; Li et al., 2010b, 2014). Low [Cl^-] in the water samples indicates minimal or no influence of contamination sources on the water (Liu et al., 2006). For example, Cl^- and nitrate concentrations measured at Site no. 4 (UGW from a deep aquifer) were 1.0 ± 0.1 mg L^{-1} and below the detection limit, respectively ($n = 13$). These values are much lower than those from samples from the shallow aquifer directly above. Figure 3 illustrates that relatively low Cl^- concentrations were measured in all samples during June and July and high concentrations were measured in the dry season, while high nitrate concentrations occurred in the wet season and lower levels in the dry season. This trend in Cl^- concentration probably reflects a dilution effect in the wet season (Fig. 3a). The high NO_3^- concentration observed in the wet season (e.g., June) despite dilution effects illustrates the very high loading from crops and consequently large flux into rivers.

The relationship between NO_3^-/Cl^- and $\delta^{15}N_{nitrate}$ values illustrates the major sources of nitrate in waters. No halite is observed in the HRB, so the high Cl^- levels are probably a result of agricultural inputs including chemical fertilizer and manure. The water samples containing reductive N fertilizers may have a high NO_3^-/Cl^- ratio and low $\delta^{15}N_{nitrate}$ values. Manure contains a mixture of animal excrements and bedding straw and may have higher [Cl^-] and $\delta^{15}N_{nitrate}$ values of 6 to 25‰ than chemical fertilizer (Kendall et al., 2007; Widory et al., 2013). As indicated in Fig. 7, the $\delta^{15}N_{nitrate}$ values are logarithmically correlated with NO_3^-/Cl^-, which indicates that reductive N fertilizer and manure may be the major sources for N loading in the Houzhai catchment. The high nitrate concentration and dual isotopic composition at Site no. 6 suggest that manure may be a more significant contributor of nitrate in the middle reaches, particularly in the dry season (Fig. 5).

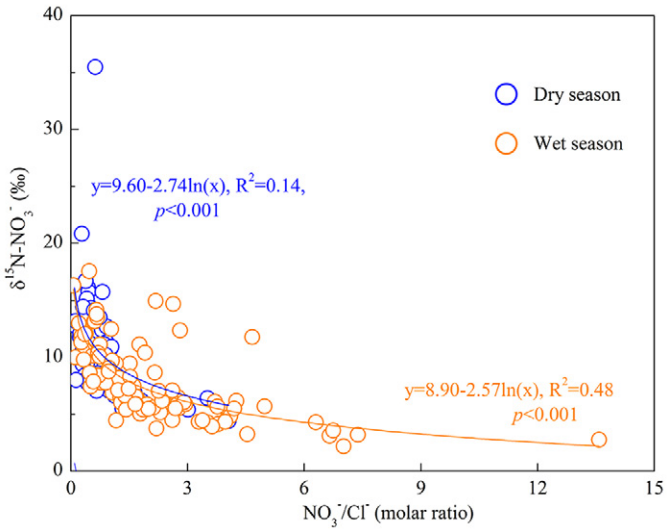

Fig. 7. The relationship between $\delta^{15}N_{nitrate}$ and NO_3^-/Cl^-.

The seasonal variation in the dual isotopes indicates that generally, nitrate in the water samples was enriched in light isotopes during the wet season relative to the dry season (Fig. 3b and 4). The opposite trends in $\delta^{15}N_{nitrate}$, $\delta^{18}O_{nitrate}$, and [NO_3^-–N] (Fig. 4a and 6b) indicates that nitrate in most water samples is mainly derived from a mix of reductive N fertilizer and manure in the wet season. In the dry season, few chemical fertilizers and only a small quantity of manure are used in dryland cropping in southwestern China. In addition, only a small proportion of annual precipitation occurs during the dry season and typically in low-intensity events. This is likely to result in the majority remaining as SFW and low infiltration to the UGW system. Thus, the majority of nitrate loss from soil to water is likely to occur during the wet season. The SFW samples with low nitrate concentrations have high isotopic values (Fig. 4), which may derive from manure and stored nitrate in the low-permeability, thicker soil region. For the UGW outlet, however, low [NO_3^-–N] combined with high $\delta^{15}N$ was observed during the dry season and high [NO_3^-–N] with low $\delta^{15}N$ during the wet season. This indicates that several complex biochemical processes control nitrate levels in water. For example, the nitrate in the UGW during the first period of heavy rainfall had a higher $\delta^{15}N$ value than that during the second period after rainfall in this study (Supplemental Table S1), which suggests that nitrate derived from denitrification may contribute more to the nitrate flux during rainfall.

Identifying Denitrification of Nitrate in Water

The biogeochemical controls on NO_3^-–N levels in water can be identified by differences in the $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ values in water and identification of possible contamination sources. Because the ranges of $\delta^{15}N_{nitrate}$ values in water are greater than those from rainfall (1.0–4.7‰) and chemical fertilizer ($\sim 0 \pm 1.4$‰), denitrification is clearly significantly changing the isotopic composition. Figure 8 shows a highly negative relationship between $\delta^{15}N$ and

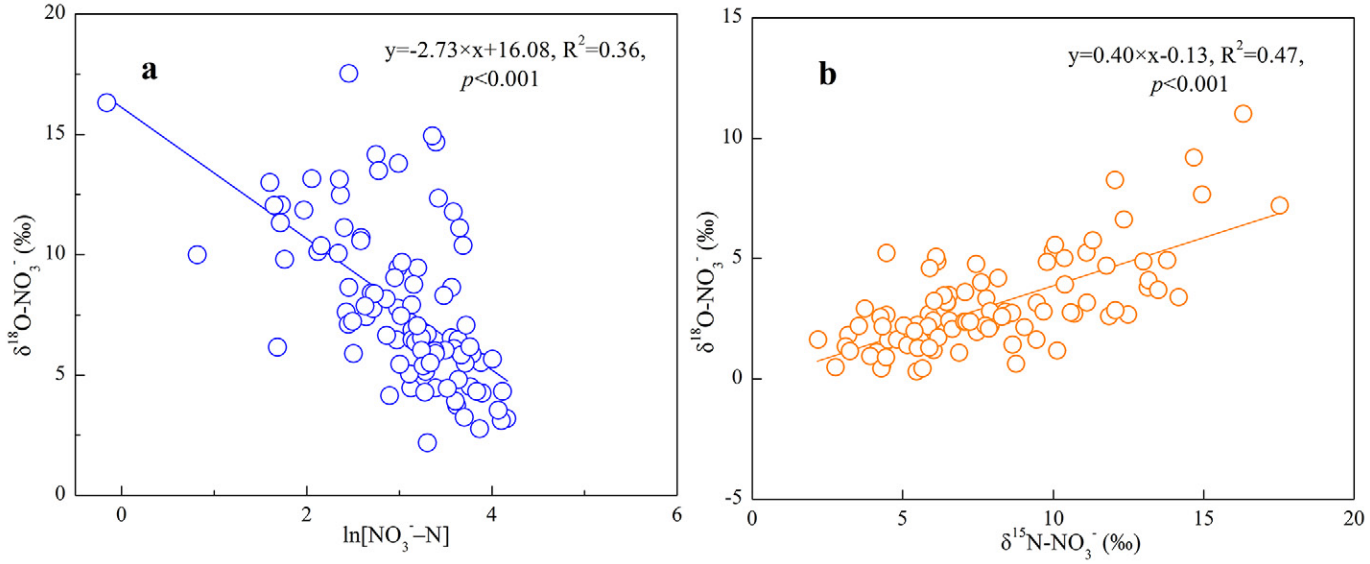

Fig. 8. The relationship between (a) $\delta^{15}N$ and $\ln[NO_3^- - N]$ and (b) $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ during the wet season.

$\ln[NO_3^- - N]$ and a positive relationship between $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ values from water samples collected during the wet season (Fig. 8). Linear regression analysis produced a reasonable relationship between $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ ($\delta^{18}O_{nitrate} = 0.40\delta^{15}N - 0.13$, $R^2 = 0.47$, $p < 0.001$). The slope of the linear regression close to 0.5 is consistent with denitrification characteristics found in previous studies (Wassenaar, 1995; Panno et al., 2006).

A positive relationship between $\delta^{15}N$ and $\Delta^{18}O$ was found ($R^2 = 0.62$, $p < 0.001$). The $\Delta^{18}O$ denotes the difference between measured and expected $\delta^{18}O_{nitrate}$ values after the process shown in Fig. 9. The value of $\Delta^{18}O$ is calculated as

$$\Delta^{18}O = \delta^{18}O_{nitrate} - \left(\frac{2}{3}\delta^{18}O_{water} + \frac{1}{3}\delta^{18}O_{O_2}\right)$$

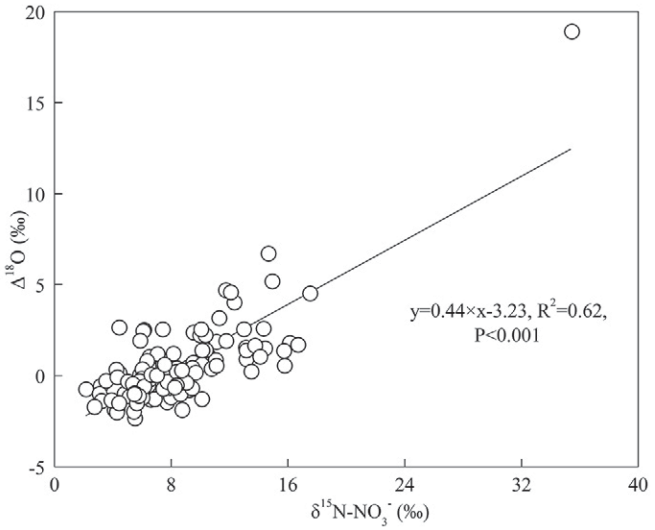

Fig. 9. The relationship between $\delta^{15}N$ values and the difference of $\Delta^{18}O$ values [$= \delta^{18}O_{nitrate} - (2\delta^{18}O_{water} + \delta^{18}O_{O2})/3$] between measured $\delta^{18}O_{nitrate}$ values and expected values via nitrification.

The observed $\delta^{18}O_{nitrate}$ values were lower than those calculated for some water samples. This is a result of O exchange between water and nitrite during nitrification (Casciotti et al., 2010; Kool et al., 2011) or different ratios of water O and atmospheric O_2 during nitrification than those assumed in the theoretical calculation (Kendall et al., 2007).

Denitrification in the HRB occurs predominantly in the wet season under conditions of high temperature and rainfall, particularly in the depression areas, which are characterized by a relatively thick soil zone (Yue et al., 2015). This increases the dual isotopic values of nitrate and the difference between measured and expected $\delta^{18}O_{nitrate}$ values. It is difficult to distinguish between biological uptake processes and denitrification using the dual isotopes of nitrate technique because algae and plants may preferentially uptake light isotopes (Granger et al., 2010). Amundson et al. (2003) found that plant $\delta^{15}N$ values are more negative than the values in soils worldwide. However, Evans et al. (1996) reported little N isotope fractionation between the plant and the source in a tomato (*Solanum lycopersicum* L.) cultivar experiment. An experiment on rice cultivation found that the difference between the $\delta^{15}N_{nitrate}$ signature of initial NO_3^- sources and NO_3^- remaining in the solution is generally lower than 1‰ for most cultivation methods (Yoneyama et al., 2001). In this study, no significant algal biomass was observed across the HRB during the sampling period, and NO_3^- may easily leach, which supports the hypothesis that denitrification is the major cause of heavy isotope enrichment in nitrate from the HRB.

Nitrate concentrations in the water samples decreased with increasing dual isotope values of nitrate along the water path for most

months during the wet season (Fig. 5b). For example, the highest observed nitrate concentrations in the headwaters correspond to $\delta^{15}N_{nitrate}$ values close to 3‰ in June and July, which are slightly higher than the $\delta^{15}N$ values of fertilizer if volatilization is ignored (Liu et al., 2006). This implies that little denitrification is occurring in the fast flow of sections of the upper reaches of the catchment, where bare rock and thin soil are widely distributed. Denitrification generally occurs in the middle and lower reaches, where low permeability and thick soils are widely distributed in the depressions.

A negative relationship between $\delta^{15}N$ and $\ln[NO_3^-–N]$ was observed ($\delta^{15}N = -1.10 \times \ln[NO_3^-–N] + 12.71$; $R^2 = 0.004$; $p < 0.05$), with the exception of the highest observed $\delta^{15}N$ in April. A weak but insignificant positive relationship between $\delta^{15}N$ and $\delta^{18}O_{nitrate}$ values ($\delta^{18}O_{nitrate} = 0.34\delta^{15}N - 0.20$; $R^2 = 0.25$; $p < 0.05$) was observed for the water samples collected during the dry season. Meanwhile, the difference in $\delta^{15}N_{nitrate}$ values of water samples taken along the primary underground conduit (e.g., a downstream transect from Muzhu through Laoheitan, Liugu, and the basin outlet in Fig. 5) during the dry season was much less than those during the wet season. This suggests that denitrification had a smaller effect on the isotopic signature of nitrate during the dry season.

Nitrate generally had higher isotopic values in surface streams than in underground conduits at the outlets of this catchment (Fig. 4b), which may indicate that denitrification is a more significant process in surface streams. This could be explained by the rapid passage of rainfall through the soil profile to the underground system and the continual contribution of stored water and nutrients from the rock matrix to UGW. Rock is a much less suitable environment for denitrification than soil. The nitrate analyzed from UGW during the first period (9 June) had a higher $\delta^{15}N$ value than that during the succeeding dry period (21 June), which suggests that nitrate derived from denitrification may contribute more to the nitrate flux during rainfall. Rainfall events appear to be a critical control of nutrient transport in this karstic area, but the precise mechanisms by which nitrate is transformed and transported are still not properly understood need further work.

Conclusion

This study aimed to identify the sources and fate of NO_3^- in a typical mixed-land-use catchment at the center of the Southeast Asian karst region through detailed analysis of the water chemistry and stable isotope signature of ground and SFWs. The δD and $\delta^{18}O_{water}$ values demonstrate that water is affected by evaporation during the dry season. The $\delta^{18}O_{nitrate}$ values indicate that nitrate fertilizers and rain are not the major sources of NO_3^- in the water samples and that nitrification is a significant process controlling nitrate levels in the HRB. High nitrate concentrations were generally observed in UGW samples, particularly in the upper reaches with a thin soil profile, suggesting direct nutrient transport following rainfall in the karst system. The outlets of the surface watershed and underground system within the HRB are in close proximity and combine to form the total outlet. A stronger denitrification signal was found in the dual isotopes of nitrate in the SFW outlet than the UGW conduit outlet. Denitrification removes part of the nitrate in the HRB, shifting the nitrate isotope values, particularly in the depression areas with relatively thick soils.

Acknowledgments

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