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Combined use of stable nitrogen and oxygen isotopes to constrain the nitrate sources in a karst lake

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A R T I C L E I N F O

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A B S T R A C T

Nitrate is a highly concerned pollutant in global aquatic ecosystem, resulting in eutrophication and water quality deterioration. As a result of the dissolved inorganic carbon fertilization effect, lake ecosystem is expected to respond more sensitively to nitrogen (N) addition in karst region than in non-karst region. Identifying accurately the sources of nitrate in lake system is an important prerequisite for formulating effective strategies on reducing nitrate and restoring water ecosystem. Quantitative identification of nitrate sources to lakes in karst region is limited until now. In this study, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were jointly used to identify the nitrate sources in Caohai Lake, a typical karst lake. The $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ of lake water displayed significant seasonal variations. The average $\delta^{15}N\text{-}NO_3^-$ values during normal, dry and wet seasons were 6.6‰, 12.7‰ and 0.9‰, respectively. Accordingly, the average $\delta^{18}O\text{-}NO_3^-$ values were 11‰, 13‰ and 16‰, respectively. The average contribution percentages of nitrate from agricultural activities, precipitation and sewage were 42 %, 41 % and 17 %, respectively. Strict measures should be taken to prohibit unreasonable agricultural activities and to improve nutrient use efficiency through optimized fertilization and irrigation management. In view of the sensitivity of karst lake ecosystem to N/P addition, higher discharge standard requirement is necessary for restoring water ecosystem and maintaining good water quality. This study proved that the combined use of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ is a promising method for identifying quantitatively the nitrate sources in karst lake system.

1. Introduction

Biogeochemical cycle of nitrogen (N) in global ecosystems has been significantly affected by the agricultural and industrial activities (Jin et al., 2020; Paredes et al., 2020; Xue et al., 2012). Agricultural activities (AA) have discharged large amounts of nitrate into surface water and ground water, accelerating the eutrophication all over the world (Hess et al., 2020; Romanelli et al., 2020; Wang et al., 2019). It is an important prerequisite to identify the sources of nitrate in lake system for formulating effective strategies on reducing nitrate and restoring water ecosystem. The NO_3^-/Cl^- ratio and $\delta^{15}N$ can provide valuable information about the NO_3^- sources (Jin et al., 2015). However, the $\delta^{15}N$ signals of different nitrate sources may overlap. This problem may be solved by the combined use of $\delta^{15}N$ and $\delta^{18}O$ of nitrate in aquatic systems (Archana et al., 2016; Hu et al., 2019). Generally, the potential sources of NO_3^- include 1) atmospheric precipitation (AP) ($\delta^{15}N$: -13 to 13‰, $\delta^{18}O$: 25 to 75‰) (Kendall, 1998; Xue et al., 2009), 2) sewage (SW) and manure (M) ($\delta^{15}N$: 4 to 25‰, $\delta^{18}O$: -5 to 10‰) (Jin et al., 2015), 3) soil nitrate (SN) ($\delta^{15}N$: 0 to 8‰, $\delta^{18}O$: 0 to 15‰) (Kendall et al., 2007), and 4) chemical fertilizers (CF) ($\delta^{15}N$: -5 to 5‰, $\delta^{18}O$: 17 to 25‰) (Bu et al., 2016). The Bayesian SIAR (Stable Isotope Analysis in R) mixing model has been proved to be effective in estimating quantitatively the contributions of different nitrate sources (Liu et al., 2018; Yue et al., 2017).

It is well known that surface and underground water is rich in dissolved inorganic carbon (DIC) in karst region as a result of extensive carbonate weathering. Yang et al. (2016) found that phytoplankton biomass was positively correlated with DIC concentration in the Pearl River, indicating the DIC fertilization effect on aquatic photosynthesis. Thus, lake ecosystem is expected to respond more sensitively to N addition in karst region than in non-karst region. This highlights the importance of identifying accurately nitrate sources, which provides guidance for formulating effective nitrogen pollution control strategies. However, quantitative investigations on nitrate sources to lakes in karst

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region are limited until now (Husic et al., 2020; Yue et al., 2018). In this study, Caohai Lake, a typical karst lake, is selected to carry out a comprehensive investigation on the δ^{15}N-NO_3^- and δ^{18}O-NO_3^- with the aims of: 1) revealing the spatial-temporal distribution of δ^{15}N-NO_3^- and δ^{18}O-NO_3^-; 2) identifying quantitatively the nitrate sources in different seasons; and 3) proposing effective measures for nitrate pollution control.

2. Materials and methods

2.1. Study site

Caohai Lake (26°47′ − 26°52′ N, 104°10′ − 104°20′ E) is located in northwestern Guizhou Province, Southwest China. It is the largest karst lake in Guizhou Province, with an area of 25 km^2. The maximum depth is 5.0 m, and the average depth is only 1.5 m. Caohai Lake belongs to a typical lake wetland ecosystem (Ramsar Convention Secretariat, 2006). It is also the most important wintering place for many migratory birds, including black necked crane (Peng et al., 2018). The annual average inflow and outflow are 0.48×10^8 m^3 and 0.45×10^8 m^3, respectively. The annual precipitation is 950 mm, most of which falls in summer. The annual precipitation and evaporation of the lake surface are 0.24×10^8 m^3 and 0.27×10^8 m^3, respectively (Wang and Dou., 1998). The storage capacity of Caohai Lake is 0.6×10^8 m^3, and the hydraulic retention time is 85.6 days (Sun et al., 2020). Caohai Lake is characterized by the subtropical monsoon climate. Average annual temperature and relative humidity are 10.6℃ and 79 %, respectively. Precipitation is the primary supply water source to Caohai Lake, followed by groundwater (Cao et al., 2016; Zhu et al., 2013). The main inflowing rivers include Sha River (R1), Zhong River (R2), Luoze River (R3), Dongshan River (R4), and Maojia Haizi River (R5), among which R4 has the highest discharge. The only outlet (R6) is located at the northwest bank of the lake (Fig. 1). The bedrock is mainly sedimentary carbonate rock. Soil in the catchment is dominated by the yellow brown soil, marked by high relative humidity and rich organic matter (Zhang et al., 2014). The lake water has high concentrations of dissolved inorganic carbon (DIC), with pH values ranging normally from 8 to 9.

2.2. Sampling

Water samples in the lake (S1 − S28) and rivers (R1 − R6) were collected in October 2018 (normal season), April 2019 (dry season) and June 2019 (wet season) (Fig. 1). All samples were filtered through 0.45 μm cellulose-acetate membrane. Temperature (T), dissolved oxygen (DO), electrical conductivity (EC) and pH of each site were measured using an automated multi-parameter profiler (model YSI EXO-2).

The DIC usually consists of HCO_3^-, CO_3^{2-} and CO_2. The pH values of lake water range from 8 to 9, so DIC exists mainly in the form of HCO_3^-. The HCO_3^- concentration was used to represent DIC concentration, determined by in situ titration using a MColortest alkalinity test kit, with an accuracy of 0.05 mM L^{-1}. Concentrations of Cl^- and NO_3^- in water were determined by Dionex ion chromatography (IC) system 90 (Dionex Corp., Sunnyvale, CA, USA) with a precision of ≤5%. The NH_4^+-N concentrations were determined by spectrophotometry after distillation and treatment using Nessler reagent spectrophotometry (HJ535 − 2009, China).

Samples of SN, CF, AP, M and SW were collected to represent different nitrate sources. Soil samples (1 cm–10 cm depth) were taken from unfertilized plots (about 1 km away from the lake), evenly distributed around Caohai Lake. Precipitation samples were collected from four stations around the lake in dry and wet seasons. Seven sewage samples were obtained from the domestic sewage outfalls and rivers (Fig. 1). Chemical fertilizer samples were collected from surrounding farmers. Manure samples were obtained at the farms of pigs and cattle. The precipitation and sewage were filtered by 0.45 μm cellulose acetate membrane, and stored at 4 ℃ until further analyses. The soil, fertilizer and manure samples were preserved in polyvinyl chloride bags and refrigerated (4 ℃). 60 g soil, 40 g chemical fertilizer and manure samples were put into 250 mL polyethylene bottles, leached overnight with deionized water (200 mL). After decanting the clear liquid, the slurry was centrifuged for maximum recovery of the nitrate solution. The leaching liquor is filtered with 0.45 μm cellulose acetate membrane, and stored at 4 ℃ in the dark (Xing et al., 2001).

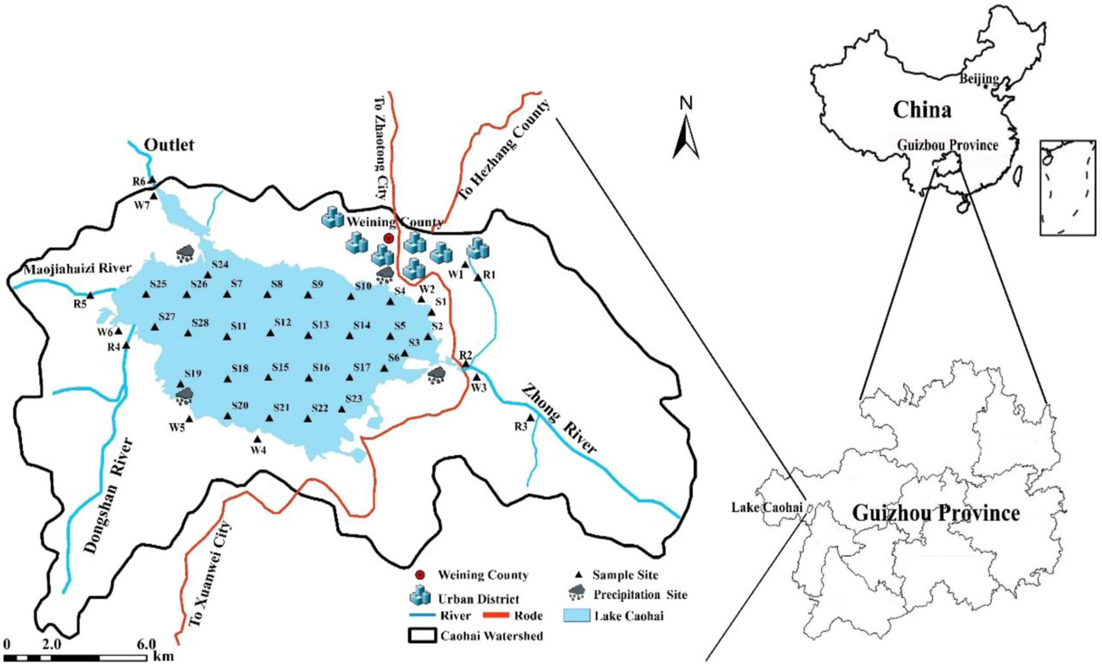

Fig. 1. Location of the sampling sites.

2.3. Analysis of nitrate concentration and stable isotopes

$\delta^{15}N$ and $\delta^{18}O$ were measured using the denitrified method (Panno et al., 2006). One strain of denitrifying bacteria that lacked nitrous oxide reductase activity was used to convert nitrate into N_2O gas, and the ratios of isotope N and O were analyzed in an isotopic N_2O analyzer by isotope ratio mass spectrometer (IRMS) at the Institute of the Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences. $\delta^{15}N$ was calibrated with USGS32 (180.0 ± 1.0‰ for $\delta^{15}N$), USGS34 (-1.8 ± 0.2‰ for $\delta^{15}N$) and IAEA N3 (4.7 ± 0.2‰ for $\delta^{15}N$), and $\delta^{18}O$ was calibrated with USGS34 (-27.8 ± 0.4‰ for $\delta^{18}O$), IAEAN3 (25.6 ± 0.4‰ for $\delta^{18}O$) and USGS35 (56.8 ± 0.3‰ for $\delta^{18}O$) (Casciotti et al., 2002). The analytical precisions of $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$ are ± 0.2‰ and ± 0.5‰, respectively. Isotope ratios are expressed as δ values and defined as:

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

where R_{sample} and $R_{standard}$ represent $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the sample and standard, respectively. N_2 in air and Vienna Standard Mean Ocean Water (V-SMOW) are references for the $^{15}N/^{14}N$ and $^{18}O/^{16}O$, respectively.

All δD and $\delta^{18}O$ of water samples were analyzed by high-precision laser spectroscopy (LWIA-24d, Los Gatos Research, USA) at the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences. $\delta^{18}O$ were calibrated by the V-SMOW and standard light Arctic precipitation (SLAP). The precisions of δD and $\delta^{18}O$ are ± 1‰ and ± 0.3‰, respectively.

$$\delta D‰ = [(\delta D / \delta H_{sample} - \delta D / \delta H_{standard}) / \delta D / \delta H_{standard}] \times 1000 \quad (2)$$

$$\delta^{18}O‰ = [(\delta^{18}O / \delta^{16}O_{sample} - \delta^{18}O / \delta^{16}O_{standard}) / \delta^{18}O / \delta^{16}O_{standard}] \times 1000 \quad (3)$$

2.4. Quantification of nitrate source contributions

The Bayesian SIAR (Stable Isotope Analysis in R) mixing model was used to quantify the contribution of nitrate sources (Parnell et al., 2010).

$$\begin{aligned} X_{ij} &= \sum_{k=1}^{k} pk(S_{jk} + C_{jk}) + \varepsilon_{ij} \\ S_{jk} &\sim N(\mu_{jk}, \omega_{jk}^2) \\ C_{jk} &\sim N(\lambda_{jk}, \tau_{jk}^2) \\ \varepsilon_{ij} &\sim N(0, \sigma_j^2) \end{aligned} \quad (4)$$

where X_{ij} is the isotope value j of the mixture i, in which I = 1, 2, 3, …, N and j = 1, 2, 3, …, J; S_{jk} refers to the source value k on isotope j (k = 1, 2, 3, …, K) and is normally distributed with mean μ_{jk} and standard deviation ω_{jk}; p_k is the proportion of source k, which must be estimated using the SIAR model; C_{jk} is the fractionation factor for isotope j on source k and is normally distributed with mean λ_{jk} and standard deviation τ_{jk}; and ε_{ij} is the residual error which represents the additional unquantified variation between individual mixtures and is normally distributed with mean 0 and standard deviation σ_j. More detailed descriptions of the model can be found in Moore and Semmens (2008) and Parnell et al. (2010).

3. Results

3.1. Hydrochemical characteristics

The temperature, DO, pH and EC of the lake and river waters in different seasons were shown in Table 1. The average concentration of $NO_3{}^-$ in lake waters was 8.6 μmol L^{-1}, much higher than $NH_4{}^+$-N (1.4–1.7 μmol L^{-1}) in all seasons, which indicates $NO_3{}^-$ was the main form of nitrogen in Caohai Lake. The concentrations of Cl^- were fairly constant (from 0.4 to 0.6 mmol L^{-1}) throughout the sampling period. The concentrations of $NO_3{}^-$ (7.8–292.0 μmol L^{-1}) and Cl^- (between 0.1 and 0.8 mmol L^{-1}) in river waters varied widely during different periods, while the $NH_4{}^+$-N concentrations were relatively constant (Table 1). As a result of extensive carbonate weathering in karst region, the lake water had high DIC concentrations (about 1.2–1.9 mmol L^{-1}).

3.2. Isotopic compositions of $NO_3{}^-$

The average $\delta^{15}N\text{-}NO_3{}^-$ value of the lake water in dry season was 12.7‰, higher than in other seasons (Fig. 2). The $\delta^{15}N\text{-}NO_3{}^-$ in normal season had the second highest value among the three seasons, with a mean value of 6.6‰. The average $\delta^{18}O\text{-}NO_3{}^-$ value of the lake water was 30.0‰ in dry season, slightly higher than that in normal season (26.7‰). The lake water had the lowest $\delta^{18}O\text{-}NO_3{}^-$ value in wet season (Table 2). The average values of $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$ in river waters were 7.6‰ and -8.0‰ respectively, during wet season, slightly lower than the normal and dry seasons (Fig. 3).

The $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$ of precipitation lie generally within the range of reported data (Wang et al., 2020b), with $\delta^{15}N\text{-}NO_3{}^-$ from -17.0 to 0.5‰ and $\delta^{18}O\text{-}NO_3{}^-$ from 45.1 to 57.3‰. In comparison to AP, SW and SN had much narrower ranges of $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$. The $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$ of SW were from 6.0 to 12.1‰ and -4.0 to 0.8‰, and that of SN fluctuated from 0 to 5.9‰ and 1.6 to 8.2‰, respectively. The $\delta^{15}N\text{-}NO_3{}^-$ and $\delta^{18}O\text{-}NO_3{}^-$ of CF ranged from 0.4 to 5.2‰ and 0 to 14.9‰, while the values of M varied from 6.0 to 11.2‰ and 9.4 to 12.0‰, respectively (Table 3).

3.3. Hydrogen and oxygen isotopic compositions of water

The δD-water and $\delta^{18}O$-water of precipitation have been widely used to trace the water vapor source of atmospheric precipitation, and the correlation between them represents the meteoric water line (Darling and Bowes, 2016;Freyberg, 2017). The δD-water (-52.9 ± 21.7‰) and $\delta^{18}O$-water (-7.1 ± 3.5‰) in the lake water were lower in wet season than that in dry season (-15.5 ± 2.9‰ and -0.9 ± 0.6‰, respectively) (Table 2). The LMWL (the local meteoric water line) in Caohai Lake ($\delta D = 8.82 \times \delta^{18}O + 22.07$) was calculated according to available data collected from the nearest monitoring station in GNIP (Global Network of Isotopes in Precipitation, IAEA). A significant correlation was found between the δD-water and $\delta^{18}O$-water in Caohai Lake in wet season ($\delta D = 6.15 \times \delta^{18}O - 9.55$, $R^2 = 0.994$) and dry season ($\delta D = 6.01 \times \delta^{18}O - 9.81$, $R^2 = 0.992$). Variations of δD and $\delta^{18}O$ in Caohai Lake were close to the LMWL and GMWL (the Global Meteoric Water Line), suggesting that the lake water was mainly derived from atmospheric precipitation. Moreover, δD-water and $\delta^{18}O$-water fell below the LMWL slightly, indicating that lake water was affected by evaporation to some extent (Fig. 4).

4. Discussion

4.1. Indication of hydrochemical characteristics on the sources of nitrate

Cl^- is a good indicator of human activities (fertilization and swage discharge), because it is not subject to physical, chemical and biological processes (Liu et al., 2006). Potential sources of Cl^- include natural sources (dissolution of minerals), chlorine detergent, animal manure, chemical fertilizer, and so on (Ding et al., 2014). In general, high Cl^- concentration is considered to be from M and SW (Liu et al., 2006). High $NO_3{}^-/Cl^-$ ratios usually result from intensive agricultural activities, such as fertilization and agricultural land-use (Liu et al., 2006; Zeng et al., 2019). The relationship between $NO_3{}^-/Cl^-$ ratios and Cl^- concentrations showed that the major sources of $NO_3{}^-$ varied among different seasons. High $NO_3{}^-/Cl^-$ ratios and Cl^- concentrations

Table 1
Hydrochemical parameters of the lake water and river waters in different seasons.

		T (°C)	DO (mg L^{-1})	EC (mS cm^{-1})	pH	Cl$^-$ (mmol L^{-1})	NO$_3^-$ (μmol L^{-1})	NH$_4^+$-N (μmol L^{-1})	DIC (mmol L^{-1})
Normal Season	Lake	15.4 ± 0.7	7.6 ± 1.1	0.3 ± 0.1	8.9 ± 0.4	0.4 ± 0.1	13.0 ± 22.4	1.4 ± 0.1	1.3 ± 0.4
	Rivers	12.7 ± 0.7	6.7 ± 2.1	0.5 ± 0.2	8.3 ± 0.2	0.1	51.4 ± 27.4	7.4 ± 13.4	2.9 ± 1.4
Dry Season	Lake	20.3 ± 1.7	7.8 ± 2.7	0.4 ± 0.1	8.4 ± 0.4	0.6 ± 0.1	0.7 ± 0.3	1.7 ± 0.1	1.9 ± 0.4
	Rivers	19.3 ± 2.5	5.7 ± 2.6	0.5 ± 0.2	8.2 ± 0.3	0.8 ± 0.7	7.8 ± 9.0	1.7 ± 0.1	2.3 ± 1.1
Wet Season	Lake	21.4 ± 0.8	12.8 ± 1.9	0.3 ± 0.1	9.1 ± 0.6	0.5 ± 0.1	12.2 ± 41.1	1.6 ± 0.4	1.2 ± 0.5
	Rivers	19.2 ± 2.2	5.4 ± 0.3	0.5 ± 0.2	8.2 ± 0.3	0.3 ± 0.1	292 ± 191	1.7 ± 0.1	2.3 ± 1.3

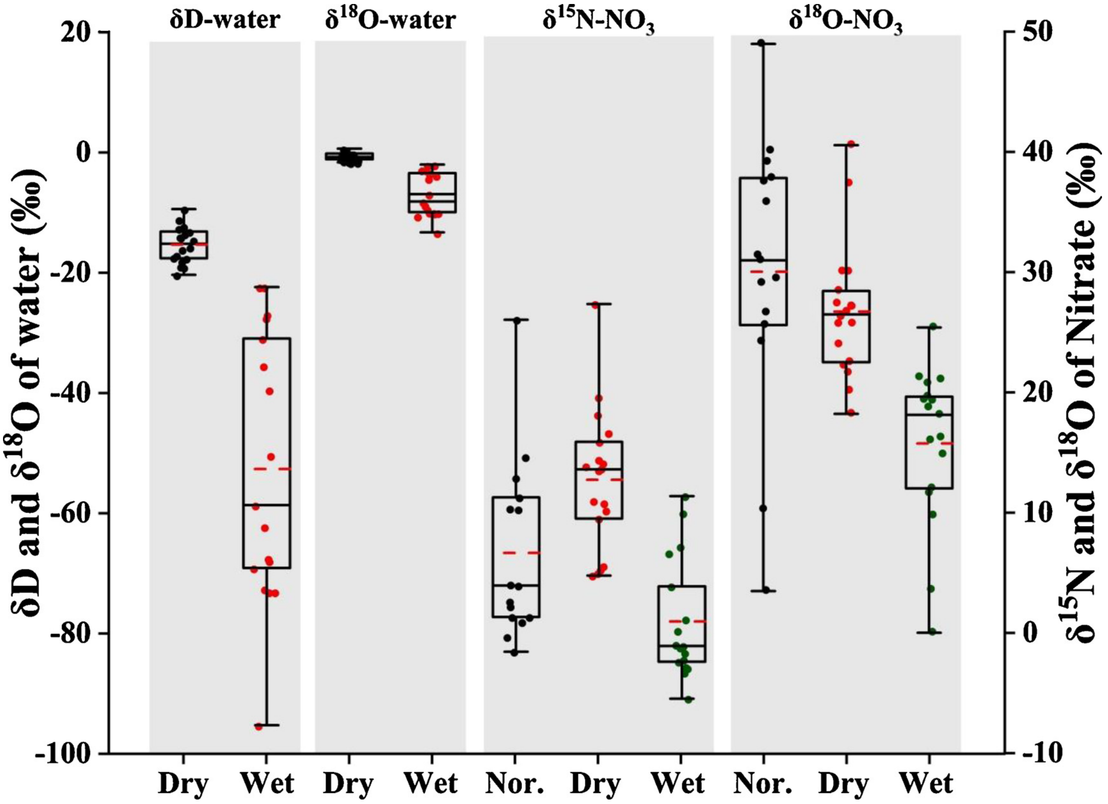

Fig. 2. δD-water, δ^{18}O-water, δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ of the lake water.

Table 2
δD-water, δ^{18}O-water, δ^{15}N-NO$_3^-$, and δ^{18}O-NO$_3^-$ of the lake water and river waters in different seasons.

		δD-water (‰)	δ^{18}O-water (‰)	δ^{15}N-NO$_3^-$ (‰)	δ^{18}O-NO$_3^-$ (‰)
Normal Season	Lake	—	—	6.6 ± 7.2	30.0 ± 11.1
	Rivers	—	—	9.7 ± 3.8	−0.1 ± 3.5
Dry Season	Lake	−15.5 ± 2.9	−0.9 ± 0.6	12.7 ± 5.7	26.7 ± 5.4
	Rivers	−30.5 ± 33.1	−3.5 ± 5.5	7.7 ± 3.5	2.0 ± 5.0
Wet Season	Lake	−52.9 ± 21.7	−7.1 ± 3.5	0.9 ± 4.9	15.8 ± 6.4
	Rivers	−58.5 ± 16.5	−8.0 ± 2.8	7.6 ± 4.6	−8.0 ± 2.8
	Precipitation	−56.5 ± 5.9	−7.6 ± 1.1	−9.6 ± 5.8	49.0 ± 5.0

— Not determined.

indicate that the agricultural sources are dominant, and conversely, suggesting SW and M sources are dominant (Yu et al., 2018). The relationship between NO$_3^-$/Cl$^-$ ratios and Cl$^-$ concentrations showed that the major source of NO$_3^-$ was AP in normal season (Fig. 5). High NO$_3^-$/Cl$^-$ ratios of some water samples in normal and wet seasons suggest that CF may have an important contribution to nitrate in Caohai Lake.

4.2. Source and biogeochemical cycle of nitrate: evidences from nitrogen and oxygen isotopes

During normal season, the dual nitrate isotopic signatures of the lake water fell within the range of AP source category, indicating that AP might be the major NO$_3^-$ source (Fig. 6). In wet season, the isotope signatures of the lake water were primarily consistent with the CF source category, suggesting that CF was the major nitrate source. This may result from unreasonable agricultural activities and excessive use of fertilizers in wet season in order to improve soil fertility (Ding et al., 2014; Yu et al., 2020). The δ^{18}O-NO$_3^-$ of the lake water in dry season

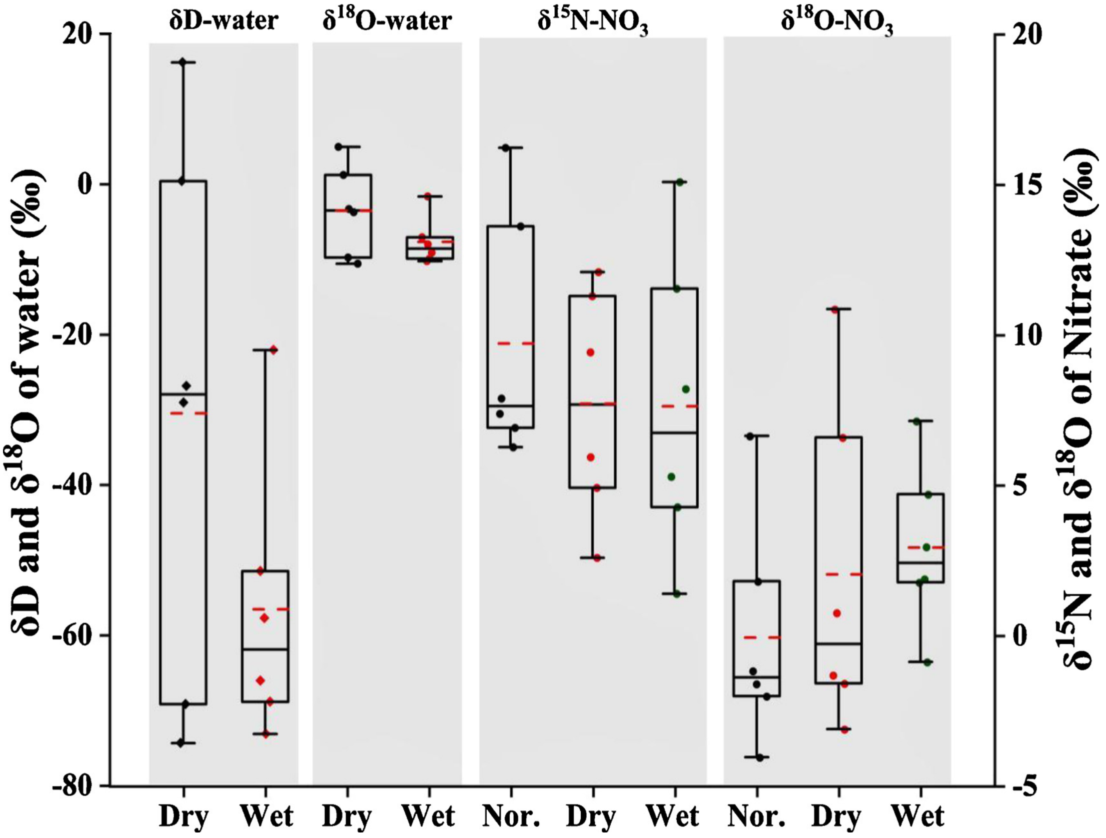

Fig. 3. δD-water, δ^{18}O-water, δ^{15}N-NO_3^- and δ^{18}O-NO_3^- of river waters.

Table 3
Nitrogen and oxygen stable isotopic ratios (‰) of different sources of nitrate.

Sources	δ^{15}N-NO_3^- (‰)	Mean (δ^{15}N-NO_3^-)	δ^{18}O-NO_3^- (‰)	Mean (δ^{18}O-NO_3^-)
AP (n = 4)	−17 − 0.5	−7.1 ± 6.7	45.1 − 57.3	51.1 ± 5.7
SW (n = 7)	6.0 − 12.1	7.6 ± 2.3	−4.0 − 0.8	−1.8 ± 1.4
CF (n = 4)	0.4 − 5.2	3.1 ± 2.0	0 − 14.9	9.0 ± 6.8
M (n = 3)	6.0 − 11.2	8.0 ± 2.3	9.4 − 12.0	10.6 ± 1.1
SN (n = 6)	0 − 5.9	2.7 ± 2.2	1.6 − 8.2	4.1 ± 2.8

was similar to that in normal season, while the lake water had higher δ^{15}N in dry season. The δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in river waters varied slightly in different seasons, and fell mainly in M and SW source categories, indicating that nitrate in the rivers was primarily derived from SW and M.

In summary, the relationships between δ^{15}N-NO_3^- and δ^{18}O-NO_3^- of the lake water were distinctly different from that in river waters. Lake reclamation was extensive in the catchment of Caohai Lake in the past, and there is large amount of farmland around the lake. Thus, the nitrate derived from agricultural activities in the farmland can be directly conveyed into the lake by surface runoff, not through the rivers. Because of the low river discharge of Caohai Lake, lake water is mainly supplied by the precipitation. The precipitation brings 0.24 × 10^8 m^3 water to the lake each year, accounting for about half of the annual average inflow (0.48 × 10^8 m^3) (Wang and Dou, 1998). Therefore, precipitation may contribute considerable nitrate to the lake, while has no significant influence on isotope composition of the river waters. This may explain the significant isotopic differences between the lake water and river waters.

Although δ^{15}N-NO_3^- and δ^{18}O-NO_3^- depend mostly on their sources, they could be modified by biological processes during nitrogen transformation such as nitrification, denitrification, nitrogen fixation, diffusion, and volatilization (Kendall and McDonnell, 2012). The NH_4^+-N concentration is quite low in Caohai Lake, so the influence of NH_4^+ volatilization on the isotopic composition can be neglected (Sebilo et al., 2006). NH_4^+ may convert to NO_3^- by microorganisms during nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$). In this process, one oxygen atom in nitrate comes from dissolved O_2 in water, and the other two oxygen atoms come from water (δ^{18}O-NO_3^- = 1/3 δ^{18}O-O_2 + 2/3 δ^{18}O-H_2O) (Xing and Liu, 2016). δ^{18}O-H_2O in the lake ranged between -7.1‰ and -0.9‰ in dry and wet seasons (Table 2). The theoretical range of δ^{18}O-NO_3^- in different periods could be calculated as 3.1–7.2 ‰ according to the atmospheric δ^{18}O-O_2 value of 23.50‰ (Xue et al., 2009), much lower than the δ^{18}O-NO_3^- in lake water (15.8–30.0‰) (Table 2). This suggested that nitrification was negligible in lake water. The δ^{18}O-NO_3^- values in river waters (from -8.0 to 2.0‰) mostly fell within the theoretical range of nitrification, indicating that nitrification might occur in river waters during dry and wet seasons.

During denitrification, microorganisms transform NO_3^- into N_2O and N_2, and light isotopes are preferentially used in these processes. Consequently, the δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values of water increase with decreasing NO_3^- concentrations when denitrification occurs. When the δ^{15}N/δ^{18}O ratios of NO_3^- are within the range of 1.3–2.1, denitrification is inferred to have occurred (Chen et al., 2009; Xue et al., 2012). The δ^{15}N/δ^{18}O ratios of NO_3^- in Caohai Lake were out of the range of denitrification during normal and wet seasons. The linear regression analysis showed that there were no negative correlations between ln(NO_3^-/Cl^-) and δ^{15}N-NO_3^- during the dry and wet seasons (Fig. 7), indicating that the denitrification process was not significant in Caohai Lake. This is supported by the aerobic condition in lake water (DO > 7 mg L^{-1}). The minimum and maximum concentrations of DO in the lake water and river waters were 7.6 and 12.8 mg L^{-1}, respectively (Table 1). The effect of denitrification on the isotope fractionation was expected to be limited in Caohai Lake. Therefore, the nitrate sources in the lake water could be quantitatively traced using the SIAR

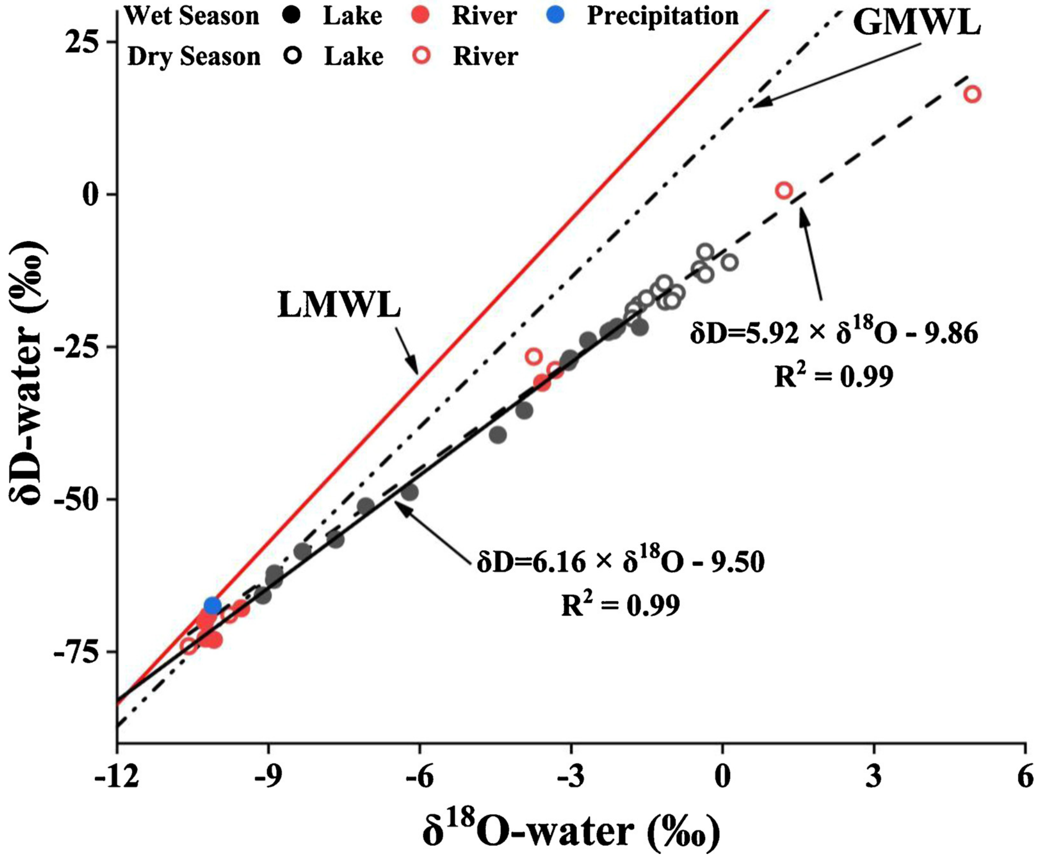

Fig. 4. Relationship between δD and $\delta^{18}O$ in water.

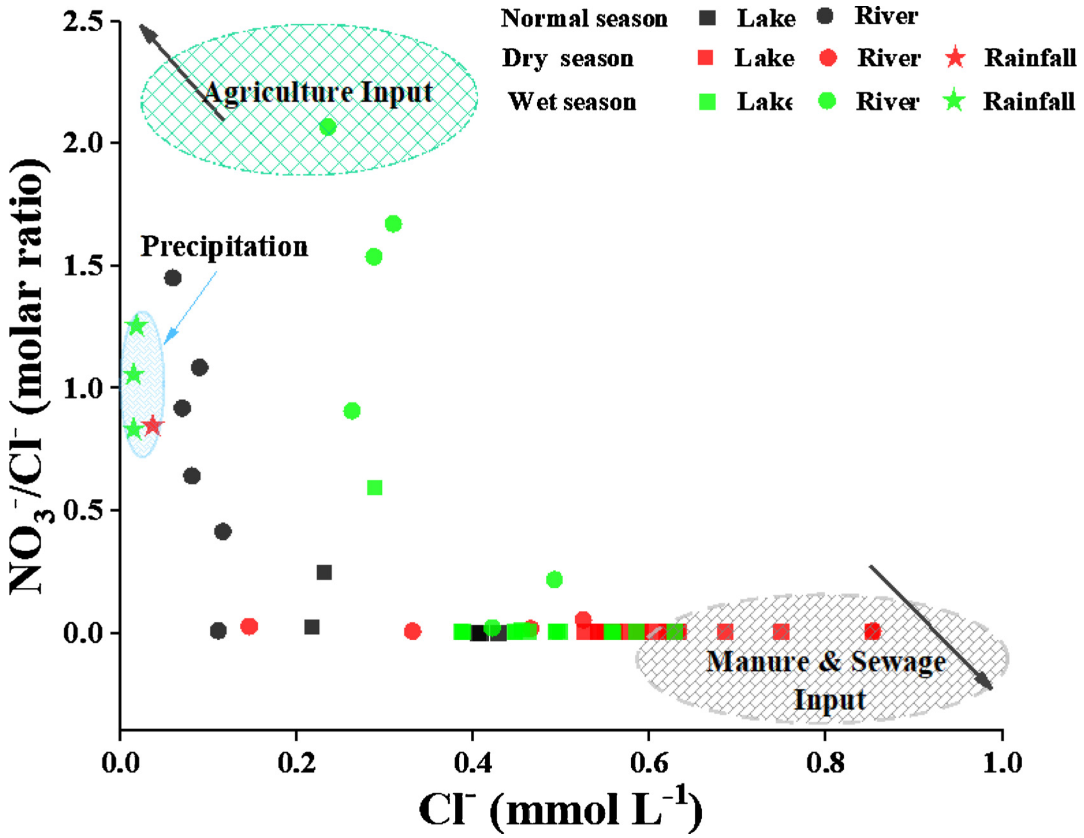

Fig. 5. Relationship between Cl– and NO_3–/Cl– in Caohai Lake.

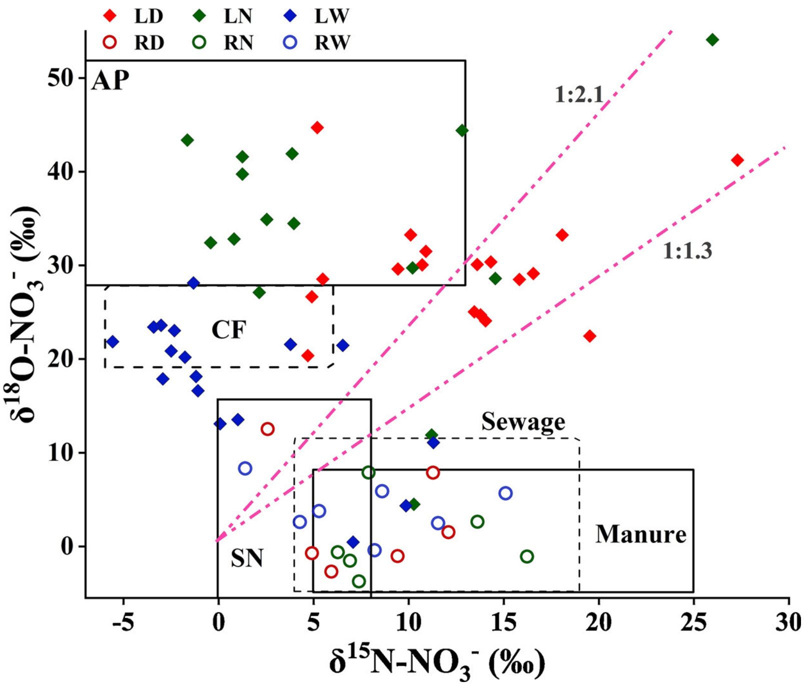

Fig. 6. δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ of different source categories and water samples.

model, and the fractionation coefficient (C_{jk}) was set as 0 (Eq. 4).

4.3. Quantitative estimation of different nitrate source contributions

The contribution of different sources to nitrate could be quantitatively estimated according to δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ using the SIAR mode. The results showed that AP contributed about 50 % of the nitrate in lake water during normal and dry seasons, but only 23 % in wet season (Fig. 8). In wet summer, the application amount of chemical fertilizer and manure increase greatly as a result of intensive agriculture activities. Therefore, the contribution of AP may be diluted by the massive input from agriculture activities in wet season. AP accounted for a larger proportion in normal and dry seasons than in wet season. The nitrate contribution of AP was significantly higher in Caohai Lake than other lakes (Liu et al., 2018; Soto et al., 2019).

Due to the incomplete facilities of domestic sewage pipe network and the limited capacity of sewage treatment, the wastewater with high concentration of NO$_3^-$ is discharged directly into the lake and may have a distinct impact on the lake ecosystem. In wet season, a large amount of domestic sewage entered the lake by surface runoff. Thus, the highest contribution of SW was found in wet season, significantly higher than in normal and dry seasons.

The nitrate contribution of CF was 18 % during wet season, higher than in normal and dry seasons (11 % and 10 %, respectively). This reflects the excessive nitrate input to the lake as a result of unreasonable agricultural fertilization.

Manure contributed to 18 % of the nitrate during wet season, followed by 17 % in normal season and 16 % in dry season. A considerable number of domestic animals such as cattle, pigs and chickens, are raised in surrounding area of Caohai Lake, and a large number of migratory birds live there in winter (Peng et al., 2018). Thus, large amounts of feces had been accumulated around Caohai Lake.

Nitrate contribution of SN varied from 19 % in wet season to 9% in normal and dry seasons. High concentrations of SN in wet season could be caused by heavy precipitation, when more soil particles and nutrients were transferred into the lake through surface runoff in karst peak-cluster depression area (Wang et al., 2019; Zhang et al., 2019; Wang et al., 2020c).

Total contributions of nitrate from AA (including CF, M and SN) were 37 %, 35 %, and 55 % during normal, dry and wet seasons,

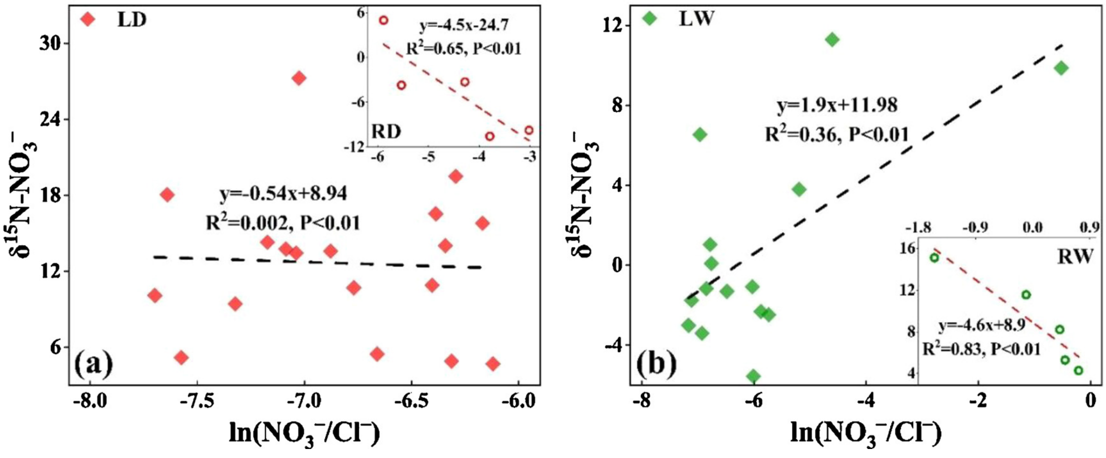

Fig. 7. Relationship between δ^{15}N-NO$_3^-$ and ln(NO$_3^-$/ Cl$^-$) of water during dry and wet seasons.

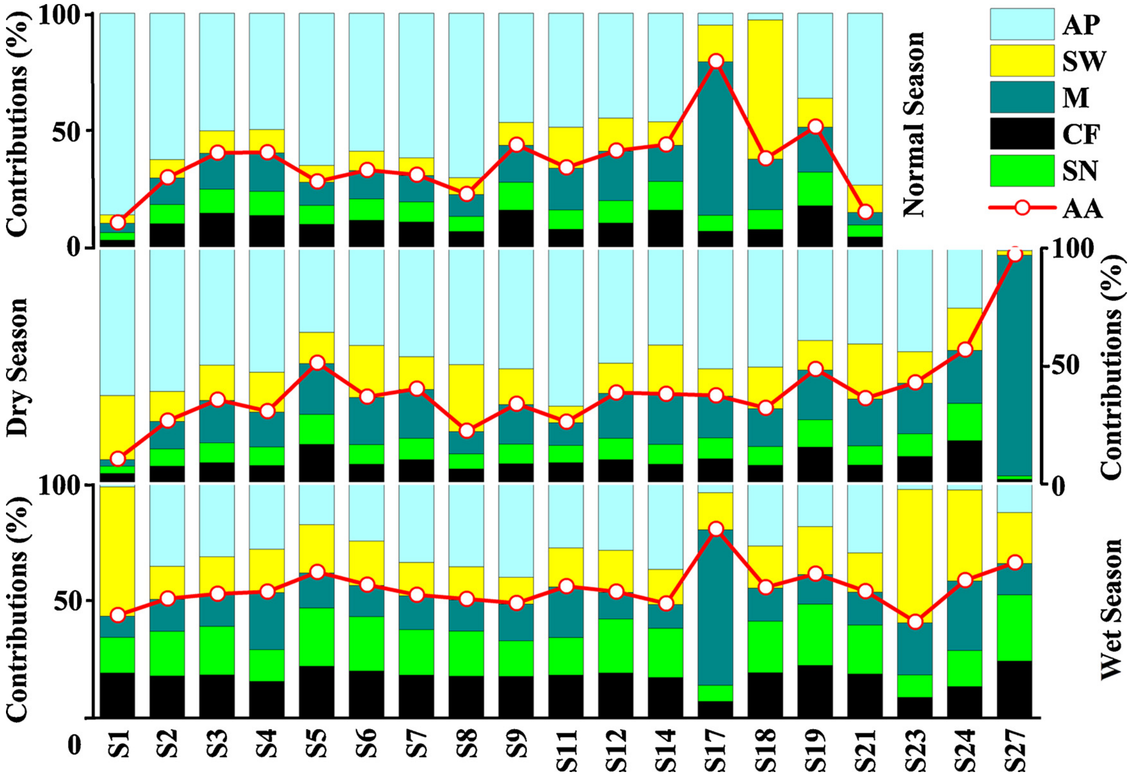

Fig. 8. Contribution of different nitrate sources to Caohai Lake in different seasons.

Table 4
Contribution percentages of CF, SN, M, SW and AP to nitrate in Caohai Lake.

	CF (%)	SN (%)	M (%)	SW (%)	AP (%)	AA (%)
Normal Season	11 ± 4	9 ± 3	17 ± 13	13 ± 12	50 ± 21	37 ± 15
Dry Season	10 ± 3	9 ± 3	16 ± 6	16 ± 6	49 ± 12	35 ± 10
Wet Season	18 ± 4	19 ± 5	18 ± 12	22 ± 13	23 ± 12	55 ± 8

AA: sum of CF, SN and M.

respectively (Table 4), with the average contribution reaching up to 42 % (M = 17 %; CF = 13 %; SN = 12 %). In general, the agricultural sources were the most important nitrate source in Caohai Lake. Thus, strict measures should be taken to prohibit unreasonable agricultural activities and to improve nutrient use efficiency through optimized fertilization and irrigation management in the catchment as far as possible. The average contribution percentages of nitrate from AP and SW were 41 % and 17 %, respectively.

4.4. *Implications for nitrogen control in karst lakes*

In karst region, lake water is generally rich in DIC which can be used in photosynthesis by an array of carbon concentrating mechanisms (CCMs) to promote the growth of aquatic plants (Liu et al., 2010). This mechanism has evolved in most groups of aquatic phototrophs including phytoplankton and higher plants (such as submerged plants) (Meyer and Griffiths, 2013; Yang et al., 2016). For example, cyanobacteria can utilize DIC to promote its reproduction, even in low N/P concentration (Suganya et al., 2016). Therefore, the lake ecosystem is expected to be more sensitive to N/P addition in karst region as a result of the DIC fertilization.

In this study, AA was demonstrated to be the most important nitrate source, and SW also contributed a considerable amount of nitrate in Caohai Lake. China consumed one third of global fertilizers (Wang et al., 2020a). Considering the high DIC background, N/P addition from AA and sewage discharge is more easily to cause water quality deterioration in Caohai Lake than other lakes. Therefore, reducing the N loading from agricultural activities is crucial for karst lakes. Furthermore, the urban sewage pipe network should be improved to assure effective collection of sewage. Considering the sensitivity of karst lake ecosystem to N/P addition, centralized sewage treatment with higher discharge standard requirement is necessary for restoring water ecosystem and maintaining good water quality.

5. Conclusions

NO_3^- was the predominant form of inorganic nitrogen in Caohai Lake. The Cl^- concentrations and NO_3^-/Cl^- ratios indicated that the water quality of Caohai Lake was significantly affected by human activities and precipitation. The $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ of the lake water displayed significant seasonal variations, with the lowest value in wet season.

As the primary sources of nitrate, agricultural activities resulted in high nitrate levels in Caohai Lake, with an average contribution percentage of 42 %. Stricter measures should be taken to prohibit unreasonable agricultural activities and to improve nutrient use efficiency through optimized fertilization and irrigation management. The average contribution percentages of nitrate from AP and SW were 41 % and 17 %, respectively. Considering the sensitivity of karst lake ecosystem to N/P addition, centralized sewage treatment with higher discharge standard requirement is necessary for restoring water ecosystem and maintaining good water quality.

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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