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Nitrate dynamics during impoundment and flood periods in a subtropical karst reservoir: Hongfeng Lake, Southwestern China

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Nitrogenous species, particularly nitrate, are some of the most significant contaminants in freshwater rivers and lakes in China, posing a significant threat to human and ecosystem health. To identify the major nitrate sources and transformation processes in a subtropical karst lake (Hongfeng, HF) in Southwest China, two sampling campaigns involving three lake profiles were conducted during the impoundment period (April) and flood period (August). Hydro-chemistry parameters, concentration of nitrogenous species, and dual isotopes of nitrate were analyzed. Nitrate was the major nitrogenous species in HF lake with higher proportion in April than August. The concentrations of NH_4^+ , NO_2^- and dissolved organic nitrogen were below the detection limit in April, while NH_4^+ increased with depth in August, which may be a result of mineralization. Nitrification was detected during the impoundment period and from the surface to a depth of 10 m during the flood period. Denitrification was detected in the lake bottom waters during the flood period with isotopic fractionation of -10.7‰ for $\delta^{15}\text{N}-\text{NO}_3^-$ and -4.7‰ for $\delta^{18}\text{O}-\text{NO}_3^-$. The Rayleigh distillation demonstrated denitrification showed spatial variation (53% and 89% of nitrate in the southern and northern region of the lake, respectively). The Bayesian modelling results suggest that organic nitrogen degradation and sediment make the largest contributions to nitrate (51% and 38% in April and 33% and 24% in August, respectively) to the lake. Tributaries contributed more nitrate in August (37%) than in April (10%). The results highlight that modified endogenous nitrogen contributed a high proportion of nitrate sources within the lake system during the two periods.

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Environmental significance

Globally, lakes are important drinking water sources, but gradually elevated nitrate concentration in lakes poses a challenge to water quality, human health and ecosystem balance. This work used dual nitrate isotopes and an isotope mixing model to identify nitrate seasonal dynamics and calculate the contribution of sources to a karst lake in Southwestern China. Nitrification was the main process controlling nitrate dynamics, whereas denitrification was only detected at the bottom of the lake during the flood period. The mixing model showed that endogenous contributions (ON degradation and sediment) contributed the majority of nitrate to the lake, particularly in the impoundment period, which should be considered for water quality management of the lake system.

Introduction

Nitrogen, one of the elements essential to life, has increased in terrestrial and aquatic ecosystems during the last few decades, which poses a threat to human health and ecosystem diversity.¹ Human industrial, agricultural and pastoral activities are the primary anthropogenic influences on the Earth's nitrogen cycle.^{2,3} Nitrate is one of the dominant compounds of nitrogen

entering riverine environments and many researchers have focused on the detrimental effect of high levels of nitrate on aquatic ecosystem sustainability.⁴⁻⁶

Lakes and reservoirs, widely distributed in Southwest China and an important supply of drinking water, are important reactive nitrogen sinks, contributing 33% to total global nitrogen removal.^{5,7} Previous studies had observed high levels of nitrate in karst underground and surface streams and eutrophication in lakes had been reported.⁸⁻¹⁰ Constraining the sources of nitrogen is hence a first step in understanding lake ecosystems and water quality.

However, there is a diverse range of nitrogen sources in aquatic systems including atmospheric precipitation, synthetic nitrogenous fertilizer, soil organic nitrogen, sewage and manure.¹⁰⁻¹² Additionally, the numerous conversion processes are complex

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which include physicochemical processes like nitrification, assimilation, denitrification, source mixing, and internal biogeochemical cycles, posing challenges to understanding major sources.¹² As one research approach, stable isotopes of nitrate ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$) have been used to elucidate nitrate sources and transformations in complex lake environments.^{13–18}

The nitrate dual isotope approach ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$) is one approach to elucidating nitrate sources and transformations in lake environments and has successfully been applied in numerous watersheds.^{19–22} For example, biological processes change the isotopic composition of nitrate, such as the assimilation process maintains the $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ ratio at 1 : 1,^{18,23} while denitrification causes the $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ ratio to be approximately 2 : 1.^{18,24} Combined with stable isotope mixing model analysis, such as Bayesian modelling which considers multiple sources and isotopic fractionation, nitrate dual isotopes can provide a powerful tracer of source contribution.

There are 2693 large lakes or reservoirs (area > 1.0 km²) in China and about 59% are distributed on the Tibetan Plateau. Lakes in karst areas, particularly in SW China (Yunnan-Guizhou Plateau and Guangxi Autonomous Region) have an area of about 1240.3 km².²⁵ The karst region of SW China experiences water shortages despite high precipitation event frequency and the dense water network. Thus, research on nitrogen sources and transformations in the karst area is particularly important. HF is a typical karst lake and many studies have been carried out here on nutrient biogeochemical cycling and eutrophication processes.^{15,26,27} In the present study, nitrogenous species concentrations and nitrate dual isotopes during the wet season were analyzed to (1) understand the sources and transformation of nitrate in HF lake, (2) calculate the extent of denitrification in the water column, and (3) assess endogenous and exogenous contribution based on the Stable Isotope Analysis in R (SIAR) method.

Materials and methods

Study area

Hongfeng Lake (26°24'–26°34' N, 106°20'–106°26' E) is located in central Guizhou Province, SW China, with a capacity of

$7.53 \times 10^8 \text{ m}^3$ and a lake surface area of 57.2 km². This lake was constructed in 1958 and supplies drinking water for more than 3 million inhabitants in Guiyang city, the capital of Guizhou province. It consists of two sub-lakes: the northern lake (site NL) and southern lake (site SL), with an average depth of 25 m and 16 m, respectively (Fig. 1). The big dam (site BD, depth ~ 22 m) was built on the northern lake. There are six main tributaries (R1, R2, R3, R4, R5, and R9) which recharge water to the lake (Fig. 1). Average annual precipitation in the local area generally ranges from 1000 to 1300 mm and rainfall in the wet season (May to October) accounts for most of the annual precipitation.²⁸

This catchment has a complex terrain with different types of land use, including intensive agriculture, industrial facilities, domestic accommodation and patches of semi-natural silviculture.²⁹ Due to the rapid development of the local area, HF lake experienced several severe eutrophication events in the early 1990s. Based on the water level of HF lake, there are four different annual hydrological periods: the low flow period (December–March), the impoundment period (April and May), the flood period (June–August) and the fluctuation period (September–November).²⁹ The concentration of nitrogen is relatively low and stable during the fluctuation period and low flow period,²⁷ whereas during the impoundment period and the flood period when there are significant changes in the water level, the nitrogen concentration varies significantly, which is in the focus of this study.

Sampling and analysis

Samples were collected in the HF lake in April and August 2013 including river water (R, $n = 9$), precipitation (P, $n = 18$) and three water profiles sampled in two-meter increments from SL, NL and BD (Fig. 1). The nitrogen input from tributaries to HF lake is known to be low in the impoundment period, so nitrate isotopes were only measured from the lake profile samples. Nitrate isotopes of tributary and lake profile samples were all measured in the flood period.

At each sampling site, the electrical conductivity (EC), dissolved oxygen (DO), pH and temperature (T) were obtained with

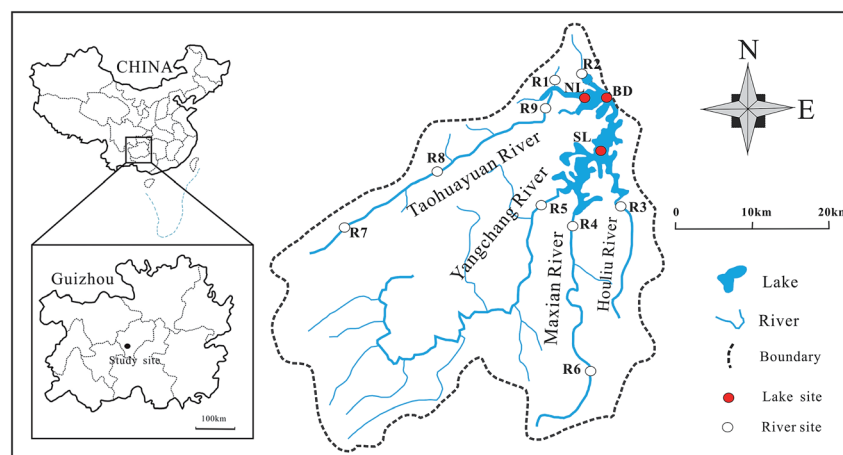


Fig. 1 Location of Hongfeng lake and water sampling sites.

a multi-parameter monitoring instrument (Yellow Springs Instruments: YSI 6600 v2). All water samples were filtered through 0.45 μm cellulose-acetate filters and transferred to pre-acid-washed polyethylene bottles, and then sealed and protected from light in an ice pack incubator in the field. The collected samples were stored in a refrigerator on their return to the laboratory until analyses for nutrients, anions and isotope values were carried out.

Nitrogenous concentrations (NO_2^- , NH_4^+ and NO_3^-) were analyzed using an automatic flow analyzer (SKALAR Sans Plus Systems). DON was calculated by subtracting DIN (NH_4^+ and NO_3^-) from TDN. Samples from two of the profiles in each period were analyzed for nitrate dual isotopes. Nitrate isotopes were measured by the denitrifying bacteria method, in which nitrate and nitrite are reduced to nitrous oxide (N_2O) quantitatively, and N_2O is analyzed using an isotope ratio mass spectrometer (IsoPrime, GV, UK).³⁰ Each sample was measured in duplicate and four international nitrate standards (USGS-32, USGS-34, USGS-35 and IAEA-N3) were used to calibrate the measured data. The standard errors for $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ were 0.3‰ and 0.5‰, respectively. All these measurements were conducted at the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Science.

Isotope ratios are given in δ units defined as:

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

where R_{sample} and R_{standard} represent the ratio of $^{15}\text{N}/^{14}\text{N}$, and $^{18}\text{O}/^{16}\text{O}$ in samples and standards, respectively. The $^{15}\text{N}/^{14}\text{N}$ standard is N_2 in air and the $^{18}\text{O}/^{16}\text{O}$ data are standardized to Vienna standard mean ocean water (V-SMOW).

Isotopic fractionation follows the Rayleigh distillation equation as follows:³¹

$$\delta_r = \delta_0 + \varepsilon \ln(f)$$

where δ_r is the isotope value of the residual nitrate, δ_0 is the initial isotope value of nitrate, and f and ε are the remaining fraction of nitrate and the isotopic enrichment factor, respectively.

SIAR based on the Bayesian mixing model was developed to estimate the proportional contribution of different sources. Deviations in isotopic composition caused by fractionation can be incorporated into the calculation, and it can therefore be used to quantify the contributions of different sources.³² The mixing model can be expressed as follows:

$$X_{ij} = \sum_{k=1}^K F_k (S_{jk} + c_{jk}) + \varepsilon_{ij}$$

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

$$c_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2)$$

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

where X_{ij} is the measured isotope value j ($j = 1, 2, 3, \dots, J$) of the mixture I ($i = 1, 2, 3, \dots, N$), and S_{jk} is the source value k ($k = 1, 2, 3, \dots, K$) on isotope j and is normally distributed with mean μ_{jk} and standard deviation ω_{jk} ; F_k is the proportion of source k estimated by 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 representing the additional unquantified variation between individual mixtures and is normally distributed with mean 0 and standard deviation σ_j . Detailed description of this model is given in numerous papers.^{32,33}

Results

Hydrological chemical parameters

There was no significant difference in temperature at the same depth between the two sampling periods. Temperatures decreased gradually from the surface to the lake bottom and the depth of the thermocline was 6 to 10 meters (Fig. 2). The DO concentration gradually increased with depth from the surface to the top of the thermocline at 6 m, then decreased from the thermocline and finally became stable at about 4.0 mg L^{-1} (except one site of BD at 18 m) in the impoundment period. However, the DO concentration ranged from 6.0–9.3 mg L^{-1} in surface water (<10 m below surface), and then decreased sharply at 10 meters depth because of restricted photosynthesis and consumption by degradation. However, the discontinuity of temperature along with depth would restrict the water exchange, which would result in a decrease of DO concentration by degradation of rich organic matter in the bottom of the lake.¹⁶ Finally, the DO concentration approached zero at the bottom of the lake in the flood period. The variation of pH in the impoundment period and the flood period was not significantly different (not shown), and all samples were alkaline with a pH range of 8.0 to 9.4.

Concentration of nitrogenous species

Nitrate was the main component of dissolved inorganic nitrogen in the profile samples, accounting for 91% and 79% in the impoundment period and flood period respectively. The highest NO_3^- -N concentration occurred in the impoundment period with a value of 1.76 mg L^{-1} , which is lower than the highest value (4 mg L^{-1}) of the previous studies.²⁷ The nitrate concentration in the three profiles showed similar trends from the surface to the bottom during each period, but slightly varied between the two periods (Fig. 3 and 4). There was spatial variation of NO_3^- -N concentration in three profiles in the impoundment period, but no significant spatial variation in the flood period. Low NO_3^- -N concentrations were observed in the surface water, gradually increasing with depth and then remaining stable from 12 m to the lake bottom during the impoundment period (Fig. 3a). During the flood period, the NO_3^- -N concentration remained stable from the surface to 8 m depth at sites BD and NL, then gradually increased with depth until the highest values were encountered at 12 m, and then decreased with depth. At site SL, the NO_3^- -N concentration remained relatively stable, varying slightly from the surface to 10 m depth and decreased with depth

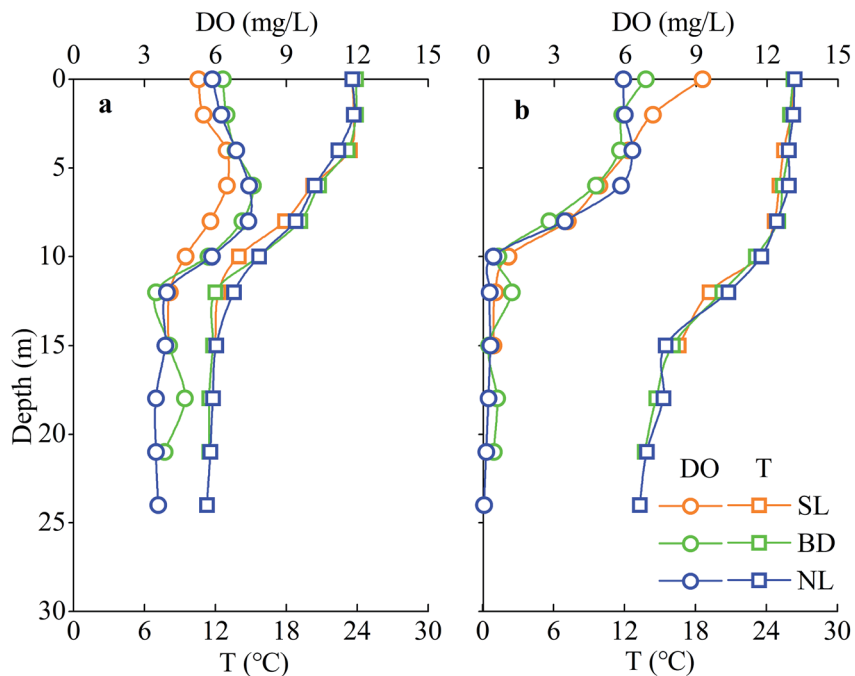


Fig. 2 Water column profiles of DO and T during the (a) impoundment period and (b) flood period in HF lake during 2013.

from 10 m to the bottom (Fig. 4a). The DON concentration in the flood period ranged from 0.02 to 0.78 mg L⁻¹, which is higher than that (in a range of 0.01 to 0.47 mg L⁻¹) during the impoundment period. Most samples in the impoundment period had low DON concentrations (<0.2 mg L⁻¹), except one sample in profile NL at a depth of 4 m. The DON concentration of the three profile sites in the flood period showed an increasing trend from the surface to the lake bottom. All samples in lake water had low NH₄⁺-N and NO₂⁻-N concentrations and some were below the detection limits during both sampling periods.

During the two sampling periods, river samples had a similar range of NO₃⁻-N, from 0.54 to 4.12 mg L⁻¹, and 0.27 to

4.98 mg L⁻¹ in the impoundment period and flood period respectively, which is higher than that of the lake water samples, but a spatial variation of NO₃⁻-N concentration in tributaries was found. For example, the average NO₃⁻-N concentration (0.82 ± 0.88 mg L⁻¹) in the tributaries (R3, R4 and R5) of SL is lower than the average NO₃⁻-N concentration (3.4 ± 1.4 mg L⁻¹) in the other three tributaries (R1, R2 and R9) of NL during the impoundment period. Higher NH₄⁺-N was observed during the impoundment period than the flood period, which ranged from 0.03 to 0.86 mg L⁻¹, with a mean value of 0.2 mg L⁻¹ in river samples. The DON concentration in river samples during the flood period (0.53 ± 0.89 mg L⁻¹) had

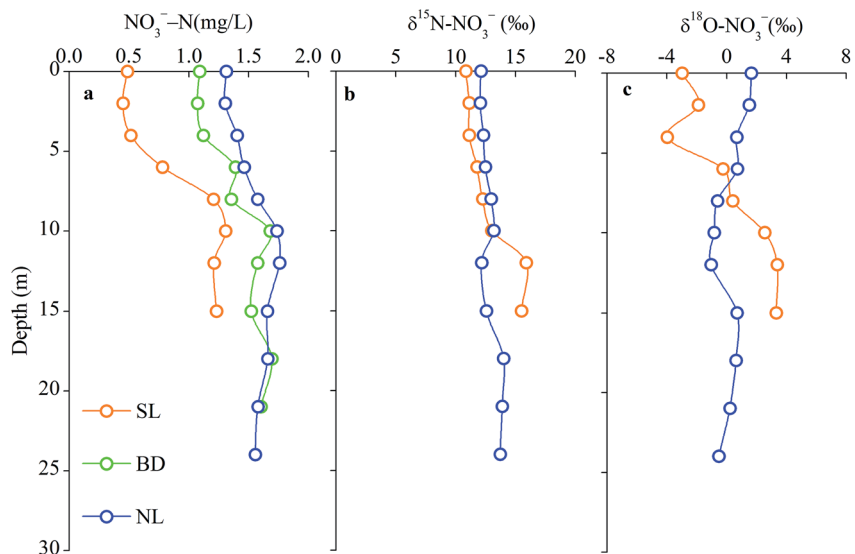


Fig. 3 Water column profiles of dissolved (a) NO₃⁻-N, (b) δ¹⁵N-NO₃⁻, and (c) δ¹⁸O-NO₃⁻ in HF lake during the impoundment period in 2013.

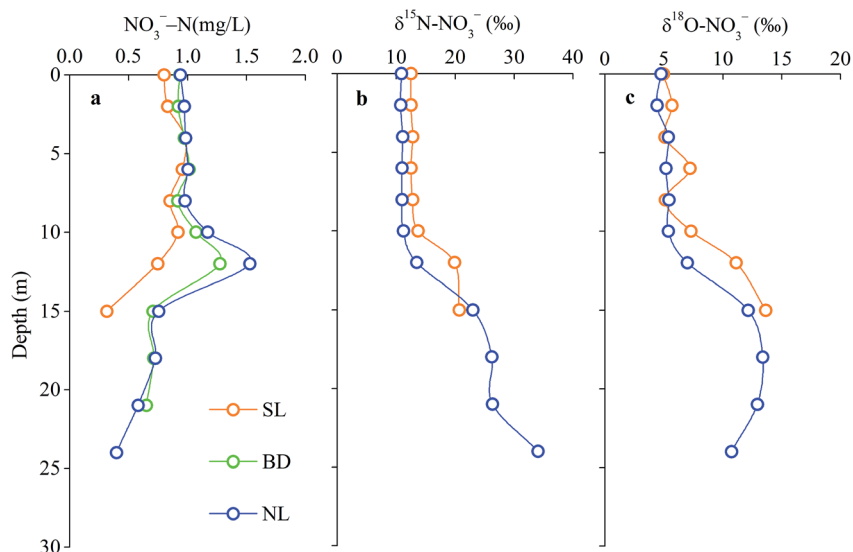


Fig. 4 Water column profiles of (a) dissolved NO_3^- -N, (b) $\delta^{15}\text{N}$ - NO_3^- and (c) $\delta^{18}\text{O}$ - NO_3^- in HF lake during the flood period in 2013.

a higher average value than in the impoundment period ($0.25 \pm 0.61 \text{ mg L}^{-1}$). Precipitation samples had higher NH_4^+ -N concentrations than river and lake samples with an average value of $0.89 \pm 0.6 \text{ mg L}^{-1}$, and low NO_3^- -N concentrations ranging from 0.07 to 0.71 mg L^{-1} .

Nitrate dual isotopes

The $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- values of HF lake in the impoundment period ranged from +10.9 to +15.9‰, with a mean value of $+12.8 \pm 1.3$ ‰, and from -4.0 to +3.4‰, with a mean value of $+0.3 \pm 1.9$ ‰, respectively (Fig. 3b and c). There was no obvious difference in $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- values from the surface to the bottom in the sampling site NL, but $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- values gradually increased with depth in the sampling site SL during the impoundment period. In addition, the $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- values of NL and SL in the flood period ranged from +10.8 to +34.1‰, with a mean value of $+15.6 \pm 6.4$ ‰, and from +4.4 to +13.7‰, with a mean value of $+7.7 \pm 3.0$ ‰, respectively (Fig. 4b and c). The two profiles showed the same vertical variation with the water with low $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values being located at the surface of the lake and high values being located below 12 m depth.

Nitrate isotopes in river samples ranged from +8.1 to +22.1‰, with a mean value of $+11.6 \pm 4.0$ ‰ (mean ± 1 SD) for $\delta^{15}\text{N}$ - NO_3^- and from +0.9 to +6.0‰, with a mean value of $+3.8 \pm 1.9$ ‰ for $\delta^{18}\text{O}$ - NO_3^- . The highest $\delta^{15}\text{N}$ - NO_3^- value (+22.1‰) and lowest $\delta^{18}\text{O}$ - NO_3^- value (+0.9‰) were detected at the same site (R5). The $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- values of precipitation samples ranged from -3.2 to +3.4‰, and +48.2 to +74.6‰, with mean values of -0.6 ± 1.6 ‰ and $+60.3 \pm 7.9$ ‰, respectively.

Discussion

Factors influencing the nitrogenous species and concentrations of HF lake water

Biological organisms involved in the nitrogen cycle obtain energy from the processes of mineralization and nitrification. In an

optimal aquatic ecosystem, the primary or unique nitrogenous species should be nitrate.³⁴ Under aerobic conditions, *e.g.* during the impoundment period, organic nitrogen can convert to NH_4^+ by mineralization and subsequently to NO_3^- by nitrification, species which can all be used by aquatic organisms.³⁵ However, the continuous input of reductive species, like NH_4^+ and sediments from the catchment, will consume and decrease the concentration of DO, which will result in incomplete mineralization and nitrification. Comparing the two periods, the lower levels of DON, NH_4^+ -N and NO_2^- -N concentrations in all three profiles during the impoundment period suggest less input of these species from tributaries or complete nitrification. The DON concentration of the three profile samples showed a vertically increasing trend during the flood period, which might be the result of incomplete decomposition of sinking phytoplankton or particles from the upper lake water due to the consumption of DO.³⁶ At the BD site, mineralisation at the bottom of the lake may cause a slight decline in NH_4^+ -N concentration with depth. The spatial variation of NO_3^- -N was larger during the impoundment period than the flood period, which suggests that nitrate sources and transformations varied between the two periods.²⁷ A low average NO_3^- -N concentration in tributaries of the SL site was observed during the impoundment period, which is one possible reason for the lower NO_3^- -N concentration at site SL than at site NL (Fig. 3a). Additionally, the limited exchange between the two regions of the lake (NL and SL) also is a potential reason for spatial variation.

Fate of nitrogen in karstic lake based on nitrate isotopes

Biogeochemical processes and source mixing not only impact aquatic nitrogenous levels but can also have an influence on the isotopic composition discrepancy between lake water and its riverine sources.³⁷ The temporal variation of nitrate concentration and isotope values (Fig. 3 and 4) suggests that different processes control the lake nitrate dynamics during the impoundment period and flood period. Mineralization and nitrification convert organic nitrogen to inorganic nitrogen in

the aquatic system. The increasing NO_3^- -N concentration from the lake surface to 10 m suggests assimilation or production from DON by mineralization and nitrification at depth during the impoundment period. In the lake system, aquatic plants can take up nitrogen from water, which converts inorganic nitrogen into organic nitrogen. High chlorophyll concentrations indicating assimilation are mostly found in near surface water.²⁷ The relatively stable hydrological condition during the impoundment period may facilitate formation of an active microbial community.³⁸ However, the slight variation observed in dual nitrate isotopes means that there is uncertainty as to whether assimilation is a dominant process.

Previous research found that oxygen atoms in nitrate derived from nitrification originate from surrounding oxygen and water oxygen with a ratio of 1 : 2.³⁹ However, other research found that more oxygen atoms are derived from water by oxygen exchange with nitrite during nitrification.⁴⁰ The $\delta^{18}\text{O}$ - H_2O of HF lake in this study ranged from -7.5 to -5.3‰ , giving a theoretically calculated nitrate $\delta^{18}\text{O}$ range from $+2.8$ to $+4.3\text{‰}$. This calculated $\delta^{18}\text{O}$ range is higher than the measured values during the impoundment period and lower than during the flood period. In addition, Swart *et al.* (2014) found a concentration dependence for the fractionation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ during macroalgal assimilation of NO_3^- . High concentrations of NO_3^- (more than $100 \mu\text{M}$) can enrich ^{15}N in the residual NO_3^- , and result in a 1 : 1 ratio between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$.⁴¹ This suggests that the low $\delta^{18}\text{O}$ - NO_3^- values during the impoundment period may result from oxygen exchange during nitrification and the high $\delta^{18}\text{O}$ - NO_3^- values during the flood period may result from denitrification and assimilation. In addition, the relatively lower nitrate concentration and lower dual nitrate isotope values at the SL site than the NL site from the surface to 10 m preclude the assimilation process, which also demonstrates that the gradually increasing nitrate concentration with depth is produced by nitrification. Degradation and conversion to nitrate of sinking particulate organic nitrogen (PON) could explain the increasing NO_3^- -N concentration from 8 to 12 m during the flood period. The aerobic conditions during the impoundment period exclude the possibility of denitrification, but the low DO concentration (near to 0 mg L^{-1}) in bottom waters during the flood period creates suitable conditions for denitrification. Denitrification is then a plausible explanation for the decrease in NO_3^- -N concentration from 12 m to the lake bottom during the flood period.

Aquatic plants fix nitrogen through photosynthesis from the atmosphere into aquatic environments with little isotopic fractionation. Consequently, bio-immobilized nitrogen isotope values should be close to atmospheric N_2 isotope values (0‰).¹² Ammonia oxidation and nitrite oxidation are the two steps of nitrification, with the finally produced nitrate having similar isotope values to the original substrate (0‰).⁴² If significant NH_4^+ from mineralization is converted to NO_3^- by nitrification under aerobic conditions, $\delta^{15}\text{N}$ - NO_3^- will have a low value at the commencement of the process and increase with continuing nitrification.⁴³ Conversely, low NH_4^+ concentrations suggest complete nitrification. High $\delta^{15}\text{N}$ values ($>11\text{‰}$) and a positive relationship between NO_3^- -N concentration and $\delta^{15}\text{N}$

values ($\delta^{15}\text{N} = 2.7 \times [\text{NO}_3^- - \text{N}] + 8.5$ $R^2 = 0.97$ in NL and $\delta^{15}\text{N} = 2.1 \times [\text{NO}_3^- - \text{N}] + 10.0$ $R^2 = 0.93$ in SL) in the upper layer (0–10 m) suggest enrichment of ^{15}N in NH_4^+ , resulting in high isotope values in nitrate subsequently produced by nitrification. The highest value of NO_3^- -N concentration at 10 m depth may indicate that nitrification occurred to a greater extent at the bottom of the thermocline due to the sinking of aquatic organisms from the upper layer. However, higher $\delta^{18}\text{O}$ - NO_3^- values can be observed at the same time, which suggests enrichment of ^{18}O in O_2 involved in this process.

Denitrification as one of the major sink processes in the nitrogen cycle can consume nitrate to produce N_2O or N_2 , depending on the bacterial enzyme.¹² Denitrification is strongly influenced by redox conditions, especially at the water–sediment interface of lakes. There is significant temperature variation between rivers, karst underground water, and lake water, and between the upper and lower parts of the water column due to thermal heating by solar radiation. This results in the occurrence of a thermal zone, which may also result in a redox transition zone in the lake system, facilitating the removal of nitrate. Although a heated surface zone can be observed during both periods, the redox transition zone during the flood period is more pronounced than during the impoundment period, suggesting that denitrification is likely to occur during the flood period. In closed systems, such as deep groundwater or lakes, the nitrate dual isotopes during denitrification have been observed to increase with a ratio of 2 : 1 with decreasing nitrate concentration.^{44,45} The isotopic fractionation during denitrification is equal to the slope of the relationship between $\delta^{15}\text{N}/\delta^{18}\text{O}$ and $\ln(\text{NO}_3^-)$ in the Rayleigh equation. The coupled increase in isotopic values suggests that denitrification may be occurring, particularly at the lake bottom during the flood period. The linear relationship between $\delta^{15}\text{N}/\delta^{18}\text{O}$ and $\ln(\text{NO}_3^-)$ at the lake bottom suggests isotopic fractionation effects of -10.7‰ for $\delta^{15}\text{N}$ - NO_3^- and -4.7‰ for $\delta^{18}\text{O}$ - NO_3^- (Fig. 5b).

To calculate the extent of denitrification by the Rayleigh distillation equation, initial isotopic composition values and isotopic enrichment factors are required. We used average nitrate isotope values of the upper layers (0–10 m) as the initial isotopic composition values. Thus, the initial $\delta^{15}\text{N}$ - NO_3^- values for the sites of SL and NL were assigned as $+12.6\text{‰}$ and $+10.9\text{‰}$, respectively. The calculated results suggest that denitrification removed up to 53% of nitrate in SL and 89% of nitrate in NL from a depth of 12 meters to the bottom of the lake during the flood period. The size of the redox transition zone is larger in NL than in SL, which resulted in favorable anaerobic conditions for complete denitrification. This spatial variation in the extent of denitrification between SL and NL may be caused by the difference in the exposure time of nitrate in the depth-induced anoxic zone.⁴⁶

Nitrate source contribution based on SIAR

The nitrate isotope values measured in this study are substantially different to 0‰ , so the contribution of algal nitrogen fixation to the lake nitrogen budget is probably negligible.

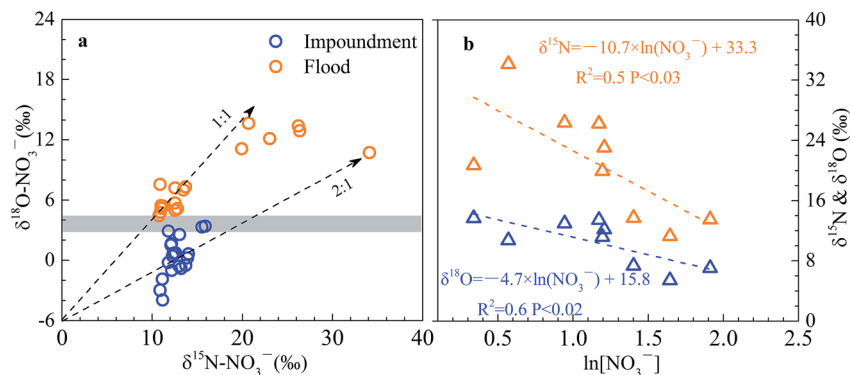


Fig. 5 The relationship between (a) $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ values of lake samples during the two sampling periods and (b) the linear relationships between the natural log of nitrate concentration and nitrate isotope values ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of bottom waters (below 12 m) during the flood period in 2013. The gray shade area represents theoretical $\delta^{18}\text{O}$ ranges produced by nitrification.

Although exchange between the bottom of the lake system (including water and sediment) and upper water is limited during periods of thermal stratification, the exchange process can occur and release nitrate sources from the bottom of the lake system when thermal stratification is removed. Most oxidized nitrogen species can be removed by denitrification, but 85% of the nitrogen in sediments of HF lake was organic, which can nitrify and increase oxidized nitrogen species again.²⁶ Sediment is also one of the potential endogenous nitrogen sources in river and lake systems.^{47,48} Nutrients may be continuously added to the surface layer from the interface by active nitrogen cycling across the interface between sediment and bottom waters in HF lake.²⁶ The water–sediment interface and lower layer (two depths at the bottom of the lake) can hence be thought of as one contributor, which is an internal source.

The isotopic composition of nitrate in the tributaries varied, indicating the major nitrate sources in each river may vary. Although the tributaries exhibited different nitrate isotopic signatures ($+11.6 \pm 4.0\text{‰}$ for $\delta^{15}\text{N}$ and $+3.8 \pm 1.9\text{‰}$ for $\delta^{18}\text{O}$), they can be defined as a single external source relative to HF lake. Precipitation is also a potential nitrate source with higher $\delta^{18}\text{O}-\text{NO}_3^-$ values than the other sources (tributaries and water–sediment interface). Additionally, ON degradation and subsequent nitrification had an obvious influence on the nitrate composition, so can also be defined as an endogenous source in the lake system. Here we choose the $\delta^{15}\text{N}-\text{PON}$ value (11.4‰) from the same lake and the $\delta^{18}\text{O}-\text{NO}_3^-$ values of the first three sites of SL during the impoundment period, which showed an obvious nitrification signature, to represent the ON source values in calculations.¹⁵

Table 1 The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of endmembers in HF lake

Potential sources	$\delta^{15}\text{N}$ -avg (‰)	$\delta^{15}\text{N}$ -std (‰)	$\delta^{18}\text{O}$ -avg (‰)	$\delta^{18}\text{O}$ -std (‰)
Tributary	11.8	5.3	3.9	2.1
ON degradation ¹⁵	11.4	0.0	-2.9	1.0
Internal source	14.8	1.1	1.6	2.0
Precipitation	-0.6	1.6	60.3	7.9

This study found that four sources contributed to the nitrate measured in HF lake, using the nitrogen and oxygen isotope values of HF lake and the isotopic values of each source (Table 1), and the source contribution can be calculated by SIAR. In order to improve the accuracy of SIAR results, the isotopic effect of denitrification for dual nitrate isotopes (calculated in Fig. 5b) was used during the flood period. The results demonstrate that the primary nitrate source of the lake came from ON degradation and the water–sediment interface contributed 51% and 38% (Fig. 6a), and

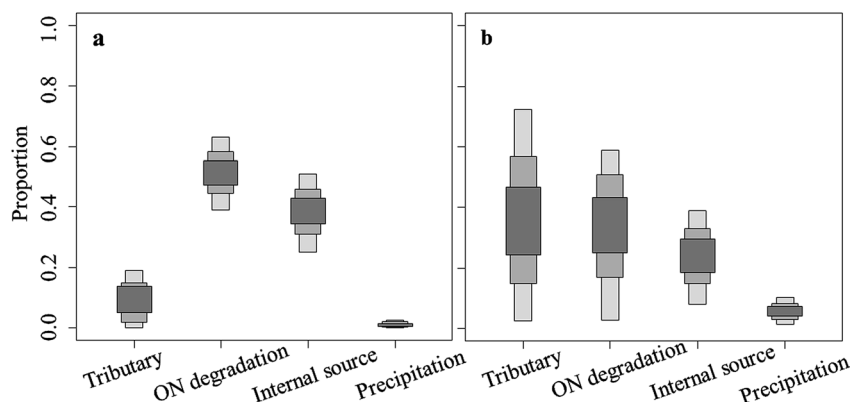


Fig. 6 Calculated contribution of each nitrate source by SIAR of the (a) impoundment period and (b) flood period.

33% and 24% (Fig. 6b) of nitrate during the impoundment period and flood period, respectively. The tributary contributed more nitrate to the lake during the flood period (37%) than the impoundment period (10%) and precipitation made a low contribution to nitrate concentrations during the two sampling periods. Source apportionment showed that internal sources make a higher contribution during the impoundment period than the flood period, suggesting a high contribution from the water–sediment interface release under aerobic conditions.

The nitrate source apportionment conducted in this study could provide guidance for the conservation of HF lake and set a clear target for controlling the nitrate input. The endogenous contribution (ON degradation and the water–sediment interface) should be the first considered factor for water quality management. However, the tributaries should also be considered as the highest exogenous contributor to HF lake. Previous studies concluded that chemical fertilizer and manure constituted the main loss of nitrogen from the adjacent karst agricultural catchment.^{8,49} So, we conclude that nitrate derived from agriculture may constitute the majority of nitrate introduced by tributaries to HF lake.

Conclusion

Water sampling from the HF lake catchment in the karst area of SW China was carried out for over two periods representative of the two major flow conditions encountered in this region. Water chemistry and the concentration of different species of nitrogen were analyzed. The results indicate that the main component of nitrogen in the HF lake was nitrate accounting for 91% and 79% in the impoundment period and flood period, respectively. Nitrification is thought to be the main process that controlled nitrate during the impoundment period and in shallow water (0–10 m) in the flood period. A lower degree of assimilation may occur at SL during the flood period but this doesn't have a significant influence on nitrate concentration. Denitrification was detected at the bottom of the lake during the flood period, and the results from the Rayleigh distillation equation showed that 53% and 89% of nitrate were removed by denitrification at the bottom of the SL and NL, respectively. The nitrate contribution from precipitation, ON degradation, the water–sediment interface, and the tributaries was calculated using SIAR. The results suggest that endogenous contribution (ON degradation and water–sediment interface derived nitrate) input was the main nitrate source to HF lake, contributing the majority of nitrate to the lake (51% and 38%, and 33% and 24% during the impoundment period and flood period respectively). The tributary made a greater contribution to the lake during the flood period (37%) than the impoundment period (10%).

Conflicts of interest

There are no conflicts to declare.

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References

- 1 L. Fewtrell, Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion, *Environ. Health Perspect.*, 2004, **112**, 1371–1374.
- 2 D. E. Canfield, A. N. Glazer and P. G. Falkowski, The evolution and future of Earth's nitrogen cycle, *Science*, 2010, **330**, 192–196.
- 3 J. N. Galloway, A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger and M. A. Sutton, Transformation of the nitrogen cycle: recent trends, questions, and potential solutions, *Science*, 2008, **320**, 889–892.
- 4 A. H. W. Beusen, A. F. Bouwman, L. P. H. Van Beek, J. M. Mogollón and J. J. Middelburg, Global riverine N and P transport to ocean increased during the 20th century despite increased retention along the aquatic continuum, *Biogeosciences*, 2016, **13**, 2441–2451.
- 5 J. C. Finlay, G. E. Small and R. W. Sterner, Human influences on nitrogen removal in lakes, *Science*, 2013, **342**, 247–250.
- 6 N. Ohte, I. Tayasu, A. Kohzu, C. Yoshimizu, K. I. Osaka, A. Makabe, K. Koba, N. Yoshida and T. Nagata, Spatial distribution of nitrate sources of rivers in the Lake Biwa watershed, Japan: Controlling factors revealed by nitrogen and oxygen isotope values, *Water Resour. Res.*, 2010, **46**, W07505.
- 7 J. A. Harrison, R. J. Maranger, R. B. Alexander, A. E. Giblin, P.-A. Jacinthe, E. Mayorga, S. P. Seitzinger, D. J. Sobota and W. M. Wollheim, The regional and global significance of nitrogen removal in lakes and reservoirs, *Biogeochemistry*, 2009, **93**, 143–157.
- 8 F. J. Yue, S. L. Li, C. Q. Liu, Y. C. Lang and H. Ding, Sources and transport of nitrate constrained by the isotopic technique in a karst catchment: an example from Southwest China, *Hydrol. Processes*, 2015, **29**, 1883–1893.
- 9 Y. J. Jiang, Y. X. Wu and D. X. Yuan, Human impacts on karst groundwater contamination deduced by coupled nitrogen with strontium isotopes in the Nandong underground river system in Yunan, China, *Environ. Sci. Technol.*, 2009, **43**, 7676–7683.
- 10 C. Q. Liu, S. L. Li, Y. C. Lang and H. Xiao, Using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values to identify nitrate sources in karst ground

- water, Guiyang, Southwest China, *Environ. Sci. Technol.*, 2006, **40**, 6928–6933.
- 11 S. L. Li, C. Q. Liu, J. Li, Z. C. Xue, J. Guan, Y. C. Lang, H. Ding and L. B. Li, Evaluation of nitrate source in surface water of southwestern China based on stable isotopes, *Environ. Earth Sci.*, 2013, **68**, 219–228.
 - 12 C. Kendall, Tracing anthropogenic inputs of nitrogen to ecosystems, in *Stable Isotopes in Ecology and Environmental Science*, ed. R. H. Michener and K. Lajtha, 2nd edn, Blackwell, Oxford, 2007, pp. 375–449.
 - 13 F. J. Yue, S. L. Li, C. Q. Liu, K. M. G. Mostofa, N. Yoshida, S. Toyoda, S. L. Wang, S. Hattori and X. L. Liu, Spatial variation of nitrogen cycling in a subtropical stratified impoundment in southwest China, elucidated by nitrous oxide isotopomer and nitrate isotopes, *Inland Waters*, 2018, **8**, 186–195.
 - 14 U. Tsunogai, T. Miyauchi, T. Ohyama, D. D. Komatsu, M. Ito and F. Nakagawa, Quantifying nitrate dynamics in a mesotrophic lake using triple oxygen isotopes as tracers, *Limnol. Oceanogr.*, 2018, **63**, S458–S476.
 - 15 X. L. Liu, S. L. Li, Z. L. Wang, B. L. Wang, G. L. Han, F. S. Wang, L. Bai, M. Xiao, F. J. Yue and C. Q. Liu, Sources and key processes controlling particulate organic nitrogen in impounded river–reservoir systems on the Maotiao River, southwest China, *Inland Waters*, 2018, **8**, 167–175.
 - 16 C. B. Wenk, C. H. Frame, K. Koba, K. L. Casciotti, M. Veronesi, H. Niemann, C. J. Schubert, N. Yoshida, S. Toyoda, A. Makabe, J. Zopfi and M. F. Lehmann, Differential N₂O dynamics in two oxygen-deficient lake basins revealed by stable isotope and isotopomer distributions, *Limnol. Oceanogr.*, 2016, **61**, 1735–1749.
 - 17 Z. F. Jin, X. Qin, L. X. Chen, M. T. Jin and F. L. Li, Using dual isotopes to evaluate sources and transformations of nitrate in the West Lake watershed, eastern China, *J. Contam. Hydrol.*, 2015, **177–178**, 64–75.
 - 18 B. Mayer and L. I. Wassenaar, Isotopic characterization of nitrate sources and transformations in Lake Winnipeg and its contributing rivers, Manitoba, Canada, *J. Great Lakes Res.*, 2012, **38**, 135–146.
 - 19 C. Li, Y. B. Jiang, X. Y. Guo, Y. Cao and H. B. Ji, Multi-isotope (¹⁵N, ¹⁸O and ¹³C) indicators of sources and fate of nitrate in the upper stream of Chaobai River, Beijing, China, *Environ. Sci.: Processes Impacts*, 2014, **16**, 2644–2655.
 - 20 S. Q. Wang, C. Y. Tang, X. F. Song, R. Q. Yuan, Q. X. Wang and Y. H. Zhang, Using major ions and delta¹⁵N-NO₃⁻ to identify nitrate sources and fate in an alluvial aquifer of the Baiyangdian lake watershed, North China Plain, *Environ. Sci.: Processes Impacts*, 2013, **15**, 1430–1443.
 - 21 S. L. Li, C. Q. Liu, J. Li, X. L. Liu, B. Chetelat, B. L. Wang and F. S. Wang, Assessment of the sources of nitrate in the Changjiang River, China using a nitrogen and oxygen isotopic approach, *Environ. Sci. Technol.*, 2010, **44**, 1573–1578.
 - 22 D. M. Xue, J. Botte, B. De Baets, F. Accoe, A. Nestler, P. Taylor, O. Van Cleemput, M. Berglund and P. Boeckx, Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater, *Water Res.*, 2009, **43**, 1159–1170.
 - 23 J. Granger, D. M. Sigman, M. M. Rohde, M. T. Maldonado and P. D. Tortell, N and O isotope effects during nitrate assimilation by unicellular prokaryotic and eukaryotic plankton cultures, *Geochim. Cosmochim. Acta*, 2010, **74**, 1030–1040.
 - 24 C. B. Wenk, J. Zopfi, J. Blees, M. Veronesi, H. Niemann and M. F. Lehmann, Community N and O isotope fractionation by sulfide-dependent denitrification and anammox in a stratified lacustrine water column, *Geochim. Cosmochim. Acta*, 2014, **125**, 551–563.
 - 25 R. H. Ma, G. S. Yang, H. T. Duan, J. H. Jiang, S. M. Wang, X. Z. Feng, A. N. Li, F. X. Kong, B. Xue, J. L. Wu and S. J. Li, China's lakes at present: Number, area and spatial distribution, *Sci. China: Earth Sci.*, 2010, **54**, 283–289.
 - 26 Y. C. Wang, *Biogeochemical effects of nutrients (P, N and C) in the sediment-water interface of Hongfeng Lake and Baihua Lake from Guizhou Province*, University of Chinese Academy of Sciences, China, Guiyang, 2001.
 - 27 X. L. Liu, *The effects of cascade reservoirs on biogeochemical cycling of nitrogen in river reservoir system take wujiang river and its branch maotiao river in SW China for example*, University of Chinese Academy of Sciences, Guiyang, 2010.
 - 28 H. W. Xiao, H. Y. Xiao, A. M. Long, Y. L. Wang and C. Q. Liu, Chemical composition and source apportionment of rainwater at Guiyang, SW China, *J. Atmos. Chem.*, 2013, **70**, 269–281.
 - 29 F. X. Tao, Air–water CO₂ flux in an algae bloom year for Lake Hongfeng, Southwest China: implications for the carbon cycle of global inland waters, *Acta Geochim.*, 2017, **36**, 658–666.
 - 30 M. R. McIlvin and K. L. Casciotti, Technical Updates to the Bacterial Method for Nitrate Isotopic Analyses, *Anal. Chem.*, 2011, **83**, 1850–1856.
 - 31 J. Böttcher, O. Stöbel, S. Voerkelius and H. L. Schmidt, Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer, *J. Hydrol.*, 1990, **114**, 413–424.
 - 32 A. C. Parnell, R. Inger, S. Bearhop and A. L. Jackson, Source partitioning using stable isotopes: coping with too much variation, *PLoS One*, 2010, **5**, e9672.
 - 33 A. L. Jackson, R. Inger, S. Bearhop and A. Parnell, Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008), *Ecol. Lett.*, 2009, **12**, E1–E5.
 - 34 G. A. E. Ramakrishnan Vv, Nitrogen Sources and Cycling in the Ecosystem and its Role in Air, Water and Soil Pollution: A Critical Review, *Journal of Pollution Effects & Control*, 2015, **03**, 2375–4397.
 - 35 C. A. Francis, J. M. Beman and M. M. Kuypers, New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation, *ISME J.*, 2007, **1**, 19–27.
 - 36 X. B. Liang, J. M. Zhu, C. Q. Liu, Z. Q. Wei, F. S. Wang, G. J. Wan and R. G. Huang, Enzymatic and microbial

- degradation of organic matter in Lake Hongfeng, Guizhou Province, China, *Chin. J. Geochem.*, 2004, **23**, 81–88.
- 37 S. L. Wang, K. M. Yeager, G. J. Wan, C. Q. Liu, Y. C. Wang and Y. C. Lü, Carbon export and fate in carbonate catchments: A case study in the karst plateau of southwestern China, *Appl. Geochem.*, 2012, **27**, 64–72.
- 38 A. F. Tonetto, R. Cardoso-Leite, M. C. Novaes and R. Guillermo-Ferreira, The relationship between macroalgal morphological complexity and hydraulic conditions in stream habitats, *Hydrobiologia*, 2015, **747**, 33–41.
- 39 B. Mayer, S. M. Bollwerk, T. Mansfeldt, B. Hütter and J. Veizer, The oxygen isotope composition of nitrate generated by nitrification in acid forest floors, *Geochim. Cosmochim. Acta*, 2001, **65**, 2743–2756.
- 40 C. Buchwald and K. L. Casciotti, Oxygen isotopic fractionation and exchange during bacterial nitrite oxidation, *Limnol. Oceanogr.*, 2010, **55**, 1064–1074.
- 41 P. K. Swart, S. Evans, T. Capo and M. A. Altabet, The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate, *Biogeosciences*, 2014, **11**, 6147–6157.
- 42 J. W. Moore and B. X. Semmens, Incorporating uncertainty and prior information into stable isotope mixing models, *Ecol. Lett.*, 2008, **11**, 470–480.
- 43 K. L. Casciotti, D. Sigman and B. B. Ward, Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria, *Geomicrobiol. J.*, 2003, **20**, 335–353.
- 44 C. B. Wenk, J. Zopfi, W. S. Gardner, M. J. McCarthy, H. Niemann, M. Veronesi and M. F. Lehmann, Partitioning between benthic and pelagic nitrate reduction in the Lake Lugano south basin, *Limnol. Oceanogr.*, 2014, **59**, 1421–1433.
- 45 L. I. Wassenaar, 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.
- 46 X. F. Chen, L. Y. Yang, L. Xiao, A. J. Miao and B. D. Xi, Nitrogen removal by denitrification during cyanobacterial bloom in Lake Taihu, *J. Freshwater Ecol.*, 2012, **27**, 243–258.
- 47 X. H. Xia, S. B. Zhang, S. L. Li, L. W. Zhang, G. Q. Wang, L. Zhang, J. F. Wang and Z. H. Li, The cycle of nitrogen in river systems: sources, transformation, and flux, *Environ. Sci.: Processes Impacts*, 2018, **20**, 863–891.
- 48 J. Y. Li, E. T. Brown, S. A. Crowe and S. Katsev, Sediment geochemistry and contributions to carbon and nutrient cycling in a deep meromictic tropical lake: Lake Malawi (East Africa), *J. Great Lakes Res.*, 2018, DOI: 10.1016/j.jglr.2017.12.001.
- 49 Z. J. Wang, F. J. Yue, J. Zeng and S. L. Li, The influence of urbanization on karst rivers based on nutrient concentration and nitrate dual isotopes: an example from southwestern China, *Acta Geochim.*, 2017, **36**, 446–451.