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Riverine nitrate source identification combining $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ with $\Delta^{17}\text{O}\text{-NO}_3^-$ and a nitrification ^{15}N -enrichment factor in a drinking water source region

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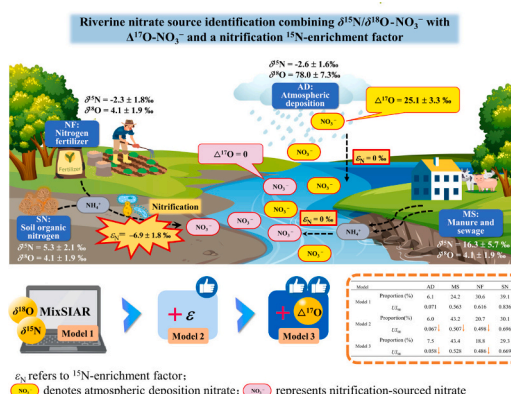
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HIGHLIGHTS

- Nitrification was identified as the major process controlling nitrogen dynamics.
- ^{15}N -enrichment factor in nitrification was determined as $-6.9 \pm 1.8\%$ by soil incubation experiment.
- MixSIAR model identified MS as the dominant nitrate source.
- Coupling $\delta^{15}\text{N}/\delta^{18}\text{O}/\Delta^{17}\text{O}\text{-NO}_3^-$ and ϵ_N reduced the uncertainty of source apportionment.

GRAPHICAL ABSTRACT



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ABSTRACT

Dual nitrate isotopes ($\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$) are an effective tool for tracing nitrate sources in freshwater systems worldwide. However, the initial $\delta^{15}\text{N}/\delta^{18}\text{O}$ values of different nitrate sources might be altered by isotopic fractionation during nitrification, thereby limiting the efficiency of source apportionment results. This study integrated hydrochemical parameters, site-specific isotopic compositions of potential nitrate sources, multiple stable isotopes ($\delta\text{D}/\delta^{18}\text{O}\text{-H}_2\text{O}$, $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ and $\Delta^{17}\text{O}\text{-NO}_3^-$), soil incubation experiments assessing the nitrification ^{15}N -enrichment factor (ϵ_N), and a Bayesian mixing model (MixSIAR) to reduce/eliminate the influence of $^{15}\text{N}/^{18}\text{O}$ -fractionations on nitrate source apportionment. Surface water samples from a typical drinking water

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source region were collected quarterly (June 2021 to March 2022). Nitrate concentrations ranged from 0.35 to 3.06 mg/L (mean = 0.78 ± 0.46 mg/L), constituting ~70 % of total nitrogen. A MixSIAR model was developed based on $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ values of surface waters and the incorporation of a nitrification ϵ_N (-6.9 ± 1.8 ‰). Model source apportionment followed: manure/sewage (46.2 ± 10.7 %) > soil organic nitrogen (32.3 ± 18.5 %) > nitrogen fertilizer (19.7 ± 13.1 %) > atmospheric deposition (1.8 ± 1.6 %). An additional MixSIAR model coupling $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ with $\Delta^{17}\text{O}\text{-NO}_3^-$ and ϵ_N was constructed to estimate the potential nitrate source contributions for the June 2021 water samples. Results revealed similar nitrate source contributions (manure/sewage = 43.4 ± 14.1 %, soil organic nitrogen = 29.3 ± 19.4 %, nitrogen fertilizer = 19.8 ± 13.8 %, atmospheric deposition = 7.5 ± 1.6 %) to the original MixSIAR model based on ϵ_N and $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$. Finally, an uncertainty analysis indicated the MixSIAR model coupling $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ with $\Delta^{17}\text{O}\text{-NO}_3^-$ and ϵ_N performed better as it generated lower uncertainties with uncertainty index (UI_{90}) of 0.435 compared with the MixSIAR model based on $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ ($UI_{90} = 0.522$) and the MixSIAR model based on $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ and ϵ_N ($UI_{90} = 0.442$).

1. Introduction

With the growth of population, accelerated urbanization and increasing intensity of human activities, a large amount of nitrogen (N) has been discharged into surface water, increasing from 34 to 64 Tg N/yr from 1990 to 2000 (Beusen et al., 2016). Accordingly, nitrate (NO_3^-), the dominant form of N in natural aerobic waters, has become a significant and escalating contaminant, especially in drinking water source watersheds (Jin et al., 2019b; Padula et al., 2021; Coffman et al., 2021). Nitrate is key to the occurrence of algal blooms through hydrodynamic transport and aggregation after being discharged from pollution sources, which greatly affects aquatic ecosystems (Burgis et al., 2020; Lao et al., 2023). Furthermore, high NO_3^- levels in drinking water can lead to increased risks to human health in the form of various cancers and methemoglobinemia in infants (Dellavalle et al., 2014; Coffman et al., 2021; Zumel-Marne et al., 2021). Therefore, taking effective practices to relieve NO_3^- contamination in drinking water source areas is urgently required for protecting environmental and human health. Riverine NO_3^- sources must be accurately identified firstly for nitrogen pollution management, because information regarding different source contributions can be used to guide prioritization of nitrogen reduction actions within watersheds. Nevertheless, how to identify the sources of riverine nitrate pollution and quantify contributions from different sources is a key technical challenge. Consequently, the purpose of this study is to develop a comprehensive isotope-based method for understanding the riverine nitrate sources. Information from our study is important to develop effective target nitrogen remediation strategies.

During the past decades, stable isotopes of NO_3^- ($\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$) have been successfully applied to trace NO_3^- sources in agricultural (Ji et al., 2017), urban (Chen et al., 2023), mining (Zhang et al., 2022c) and drinking water source areas (Shang et al., 2020) owing to different NO_3^- sources having distinctly different isotopic compositions (Kendall and McDonnell, 1998; Xue et al., 2009). However, it is important to acknowledge that initial $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$ values of nitrate sources might be altered by isotopic fractionations resulting from biological processes including nitrification and denitrification, hence obscuring the identification/apportionment of nitrate sources (Heaton, 1986; Denk et al., 2017; Nikolenko et al., 2018). Commonly, the enrichment factor is widely used to indicate the strength of isotopic fractionation effect (Hu et al., 2020; Ren et al., 2024). Pure bacterial culture experiments revealed that the ^{15}N -enrichment factor (ϵ_N) for denitrification and nitrification ranged from 5 ‰ to 40 ‰ and -35 ‰ to -5 ‰, respectively (Choi and Ro, 2003; Fukada et al., 2003; Mariotti et al., 1981; Yun and Ro, 2014).

A major limitation of most current studies is not fully incorporating $^{15}\text{N}/^{18}\text{O}$ -fractionation processes into nitrate source apportionment models. In general, if no clear evidence for denitrification is observed due to the absence of hypoxic/anoxic conditions, most studies assume negligible fractionation effects (Zhao et al., 2020; Wu et al., 2021a, 2021b; Zhao et al., 2022; Kim et al., 2023). However, it is worthy to note that microbial nitrification is a nearly ubiquitous watershed process for

the formation of nitrate because most nitrate pollution originates from nitrogen fertilizers (NF), organic soil nitrogen (SN) and manure/sewage (MS) comprised of organic/ammonium nitrogen forms (Xue et al., 2009; Mohanty et al., 2019). Correspondingly, to constrain the impact of isotopic nitrification-derived fractionation on nitrate source apportionment is critical for enhancing the efficiency of results in nitrification-dominated watersheds. Commonly, the $\delta^{15}\text{N}$ values of NF, SN and MS are directly analyzed by isotope ratio mass spectrometry and treated as the $\delta^{15}\text{N}$ values of the riverine nitrate derived from these sources (Zhang et al., 2018; Chen et al., 2023). Considering the ^{15}N -fractionation induced by nitrification, the “real” $\delta^{15}\text{N}$ values in nitrification-derived nitrate are typically smaller than the measured source values (Yu et al., 2021b). Therefore, ϵ_N is a key parameter to reduce or eliminate influence of ^{15}N -fractionation existing in nitrification. Recently, Yu et al. (2020) used soil incubation experiments to determine the ϵ_N for nitrification in Dagou River watershed of North China ($\epsilon_N = -8.7 \pm 2.7$ ‰), which improved the nitrate source identification in groundwater. However, using this approach to determine the nitrification-induced ϵ_N in drinking water source watersheds has not previously been attempted in Eastern China. As a result, nitrate source apportionment using $\delta^{15}\text{N}\text{-NO}_3^-$ contains inherent uncertainties.

$\delta^{18}\text{O}\text{-NO}_3^-$ can be used to distinguish atmospheric deposition nitrate (AD) and nitrification-derived nitrate from SN, NF, and MS. Similar to $\delta^{15}\text{N}\text{-NO}_3^-$, the $\delta^{18}\text{O}\text{-NO}_3^-$ isotopic signature is altered during nitrogen transformations in terrestrial ecosystems due to the isotopic fractionation effect. Additional shortcomings of the $\delta^{18}\text{O}\text{-NO}_3^-$ tracer approach include: (1) $\delta^{18}\text{O}\text{-NO}_3^-$ values in atmospheric deposition nitrate display a wide range from 25 ‰ to 75 ‰ (Durka et al., 1994; Mayer et al., 2001), thereby introducing significant uncertainty; and (2) direct measurement of microbially-produced $\delta^{18}\text{O}\text{-NO}_3^-$ from terrestrial sources is unattainable. As such, the estimation of $\delta^{18}\text{O}\text{-NO}_3^-$ produced by nitrification relies on the assumption that nitrifying bacteria incorporate two oxygen atoms from ambient water and one from atmospheric O_2 to produce nitrate (Andersson and Hooper, 1983; Boshers et al., 2019). However, this assumed ratio may vary under different conditions, leading to potential inaccuracies in estimating $\delta^{18}\text{O}\text{-NO}_3^-$ values for terrestrial sources (Snider et al., 2010; Venkiteswaran et al., 2019). The ^{18}O -enrichment factor from nitrification is difficult to obtain because of the complex oxygen atom sources of nitrate and fractionation occurs both inside and outside the bacterial cell during microbial nitrification (Casciotti, 2009; Fang et al., 2012). These limitations for $\delta^{18}\text{O}\text{-NO}_3^-$ pose challenges in accurately differentiating between AD and nitrification-derived nitrate from SN, NF, and MS.

A new tracer method known as the nitrate ^{17}O anomaly ($\Delta^{17}\text{O}\text{-NO}_3^-$) has been proposed to constrain AD-derived nitrate (Huang et al., 2020; Michalski et al., 2004). The majority of isotopic fractionation in nitrogen-cycling processes are mass balance fractionations which are dependent on the mass discrepancy of different isotopes within an element (e.g., ^{17}O , ^{18}O). Mass balance fractionation leads to a correlation between $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ being expressed as $\delta^{17}\text{O} = 0.52 \times \delta^{18}\text{O}$ (Michalski et al., 2003). In contrast, given photochemical production in

the atmosphere, oxygen isotope fractionation does not strictly obey mass-dependent fractionation related to O_3 , resulting in an anomalous ^{17}O excess and a deviation from mass-dependent fractionation that can be quantified as $\Delta^{17}O = \delta^{17}O - 0.52 \times \delta^{18}O$ (Hundey et al., 2016; Lim et al., 2022; Thiemens, 1999). ^{17}O -enriched O_3 provides oxygen atoms to atmospheric nitrate during NO_x oxidation process, resulting in the presence of ^{17}O anomaly in atmospheric nitrate. The $\Delta^{17}O-NO_3^-$ is positive in atmospheric deposition nitrate (range = 17–35 ‰), whereas the $\Delta^{17}O-NO_3^-$ approximately equals zero in nitrification-derived nitrate from SN, NF, and MS (Bostic et al., 2021; Kaushal et al., 2021; Michalski et al., 2004). Importantly, $\Delta^{17}O-NO_3^-$ is a reliable tracer that after atmospheric $\Delta^{17}O-NO_3^-$ deposit into terrestrial environment, atmospheric deposition $\Delta^{17}O-NO_3^-$ remains unchanged throughout terrestrial nitrogen-cycling processes such as denitrification and assimilation. For these reasons, $\Delta^{17}O-NO_3^-$ can reduce/eliminate the uncertainty in nitrate tracing results caused by fractionation of ^{18}O . However, few studies have applied $\Delta^{17}O-NO_3^-$ due to the lack of analytical capacity to routinely determine $\Delta^{17}O$.

Accurate identification of riverine nitrate sources is essential to control watershed nitrogen pollution. However, current nitrate pollution source apportionment method based on $\delta^{15}N/\delta^{18}O-NO_3^-$ needs to be improved due to the isotopic fractionation associated with N-cycling processes. The primary objective of this study was to constrain the impact of nitrification-induced fractionation on nitrate source apportionment by incorporating ϵ_N and $\Delta^{17}O-NO_3^-$ into our MixSIAR modeling

approach for obtaining refined contributions of multiple sources (e.g., AD, NF, SN, and MS) to riverine nitrate pollution. Specifically, we systematically collected surface water samples over four seasons, site-specific samples of potential nitrate sources (e.g., AD, NF, and SN), and watershed soils for incubation from a drinking water source watershed in Eastern China to: (1) identify the temporal and spatial characteristics of NO_3^- concentrations and isotopic compositions at the watershed scale, (2) determine the site-specific nitrification-induced ϵ_N through incubation experiments of field soils and application of the Rayleigh equation, and (3) enhance the quantitative assessment of different sources by integrating $\delta^{15}N/\delta^{18}O-NO_3^-$, ϵ_N , $\Delta^{17}O-NO_3^-$ and a Bayesian-based model MixSIAR. The novelty of this study is the application of $\Delta^{17}O-NO_3^-$ and nitrification-induced ϵ_N for the first time to derive more accurate nitrate apportionment results. This study contributes valuable information to advance our understanding of riverine nitrate pollution sources, as well as providing a theoretical foundation for water resource managers to develop nitrate pollution remediation strategies.

2. Materials and methods

2.1. Study area

The Shanxi drinking water source area (27°36'–27°50' N, 119°47'–120°15' E) is situated in Wenzhou, the third largest city (9.2

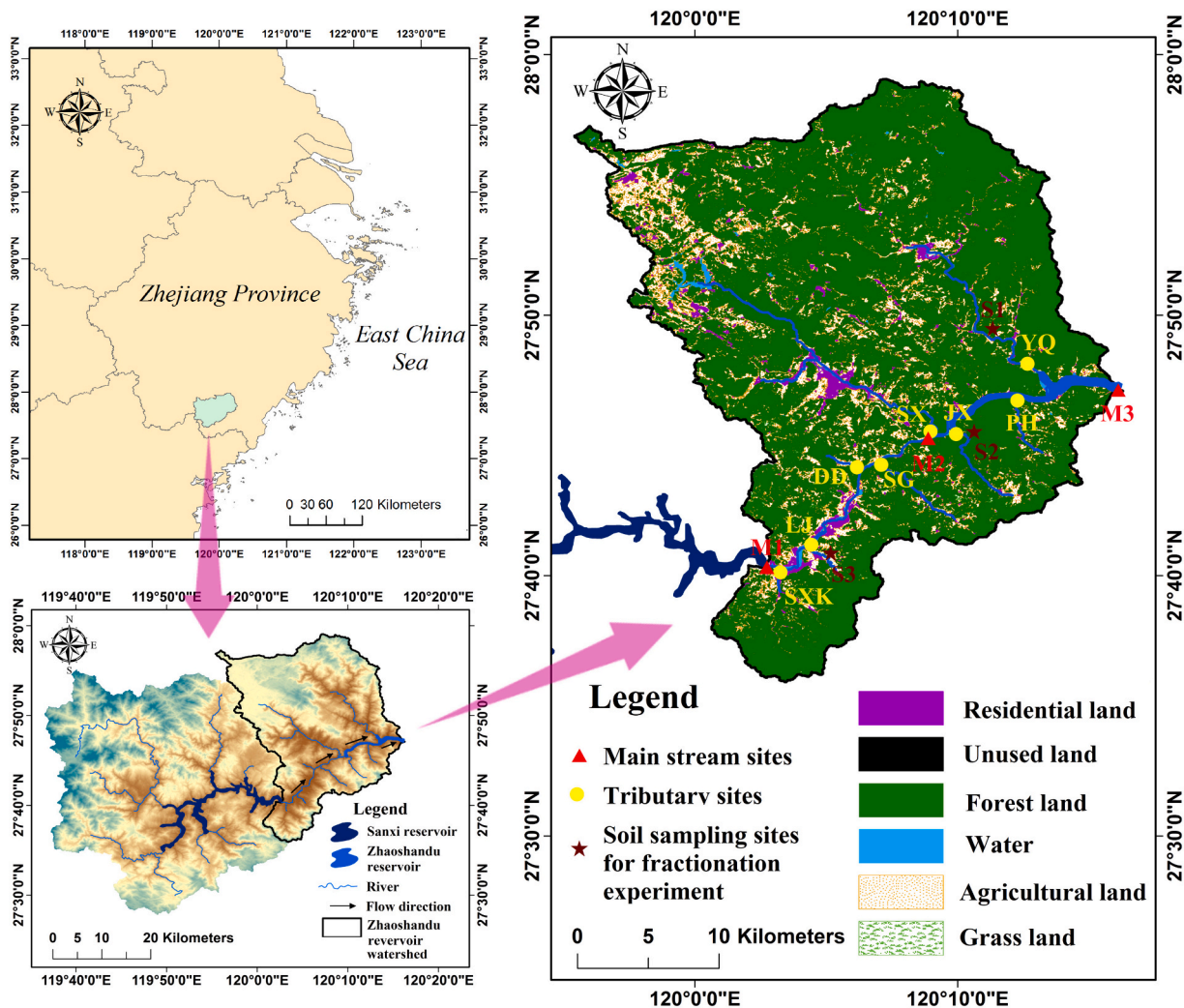


Fig. 1. Location of Zhaoshandu reservoir watershed and sampling sites.

million) in Zhejiang Province, Eastern China (Fig. 1). This drinking water source area covers an area of 2303 km² and is the primary drinking water source for surrounding domestic, agricultural and industrial uses. The Shanxi drinking water source watershed is characterized by towering and precipitous mountains, with the western region gradually transitioning from high terrain to lower elevations in the east. The middle and lower sections of the basin are dominated by moderately elevated hills, ranging from 600 to 1000 m above sea level, while the highest peak is located in the northwest, reaching an elevation of approximately 1362 m. The valley exhibits a pronounced “V” shape as it meanders towards the east through multiple mountain ranges. Moving downstream, the area gradually transitions into low-lying hills and plains, where elevations typically range from 200 to 600 m. The reservoir system can be divided into three sections: the upstream regulation reservoir (Shanxi reservoir), the downstream water diversion reservoir (Zhaoshandu reservoir), and the supporting delivery aqueduct (Huang et al., 2017). Zhaoshandu reservoir represents the integrated water quality of the entire drinking water source area because Shanxi reservoir and tributaries release to this reservoir. Hence, Zhaoshandu reservoir watershed was selected as the focus for this research.

The study area is located in a subtropical zone having a monsoon climate with a hot, rainy summer and a cold, dry winter. Mean annual precipitation and temperature are 1880 mm and 18 °C, respectively (Chinese Academy of Environmental Sciences, 2018). The majority of precipitation (~70 %) is concentrated during the wet period from April to September, and ~30 % rainfall falling during the dry period from October to March. Land use is dominated by forest, agriculture and residential classes with proportions of 75 %, 15 %, and < 5 %, respectively. The main agricultural crops include rice, sweet potato, pea, soybeans, tea, melons, oranges, bayberry and vegetables. The primary soil type is oxisols soil (Shang et al., 2020). The population within the watershed area is ~0.55 million, corresponding to a density of ~240 persons/km². Currently, statistic data provided by local Agricultural Bureau reveals that there are ~3000 pigs, ~1000 cows, ~6000 sheep, ~60,000 rabbits, ~60,000 chickens and ~50,000 ducks housed in the watershed (Wenzhou Eco-environment Bureau and Wenzhou Institute of Eco-environmental Sciences, 2021).

To improve drinking water safety, point source pollution was largely eliminated within the watershed area as polluting industries were removed from the water source conservation area by regulatory authorities. Thus, nonpoint sources are the primary NO₃⁻ inputs to this watershed, including AD, NF, SN and MS.

2.2. Sampling and analysis

For an effective watershed scale study in Zhaoshandu reservoir watershed, spatially distributed 11 sampling sites were selected based on characteristics of the river systems (Fig. 1). The study watershed contains eight major tributaries: Shanxikeng (SXX), Lijing (LJ), Dudu (DD), Shuanggui (SG), Sixi (SX), Jiuxi (JX), Pinghe (PH), and Yuquan (YQ). Accordingly, eight tributary sites were located in the river mouth before it flows into the mainstream to represent the tributary inputs. To represent the longitudinal flow of water through the study area, three sites were located along the mainstream of the Feiyunjiang River from upstream to midstream and downstream: outlet of Shanxi Reservoir (M1), Xuekou Bridge (M2) and outlet of Zhaoshandu reservoir (M3) (Fig. 1). M1 represents the input from upstream Shanxi Reservoir watershed. M2 reflects the water quality of midstream. M3 is the integrator site for the entire watershed. To examine seasonal variability, sampling was conducted in four seasons: June 2021 (summer), September 2021 (autumn), December 2021 (winter) and March 2022 (spring).

Surface waters were collected from a 30-cm depth at the midpoint of the stream channel. Samples (500 mL) were collected using a 2.5-L glass water sampler and stored in precleaned, high-density polyethylene bottles, which were rinsed three times with sampling water. Samples

were transported to the laboratory on ice on the day. Field measurements of pH, temperature (T), dissolved oxygen (DO), and electrical conductivity (EC) were measured with a multiparameter water-quality sonde (YSI-EXO2, USA) calibrated/verified at the beginning and end of the sampling day.

In addition, samples of potential NO₃⁻ sources were collected. A total of 37 soil samples were collected from vegetables, crops and forests close to the river system (Fig. S2). These sites were regarded as representative of soil nitrogen entering the river. Considering the loss of nitrogen was mainly from the surface soil, soil samples were collected within 10 cm of the soil surface by using a shovel. Three sub-samples were obtained at each site to create a single composite soil sample. Ten major nitrogen fertilizer samples, including ammonium and urea, were purchased from local farmers and markets. A total of 22 AD samples were collected on an event-basis from April 2021 to June 2021 (wet season, sample number = 14) and November 2021 to December 2021 (dry season, sample number = 8) using a funnel-bottle collector. To assure timely collection, the AD sampling site was at Wenzhou Medical University, approximately 50-km upwind from the study area (Fig. S3).

For the soil incubation experiment, soil samples (1–10 cm) were collected in June 2022 from three farmlands sites (Fig. 1; designated as S1, S2, and S3). Three soil cores were collected and composited from each site, avoiding field margins, roads, ditch margins, and area of abnormal terrain, as well as locations receiving recent compost. A 2-kg soil sample from each farmland site was secured in a cloth bag and transported to the laboratory on ice.

In the laboratory, all water samples for determination of dissolved ions, and isotope compositions were filtered through 0.45-μm membrane filters (Tengjin, Tianjin, China), whereas unfiltered samples were used for total nitrogen (TN) quantification. Both filtered and unfiltered samples were stored frozen until analysis. Concentrations of NO₃⁻, ammonium (NH₄⁺), nitrite (NO₂⁻), and TN (after alkaline persulfate digestion) were measured with a continuous-flow analyzer (Auto-analyser-3, Seal, Germany). Detection limits for NO₃⁻ and NH₄⁺ were 0.003 mg N/L, for NO₂⁻ was 0.001 mg N/L, and TN was 0.02 mg N/L. Total organic nitrogen (TON) was calculated as: TON = TN - NO₃⁻ - NH₄⁺ - NO₂⁻. Major anions (F⁻, SO₄²⁻, Cl⁻) were analyzed by ion chromatography (Compact IC plus 882, Metrohm, Switzerland), with a detection limit of ~0.1 mg/L. Major cations (K⁺, Na⁺, Ca²⁺, Mg²⁺) were determined by inductively coupled plasma-mass spectrometer (ICP-MS, Thermo XSERIES II, USA). The detection limits for K⁺/Ca²⁺ and Na⁺/Mg²⁺ were 1 × 10⁻⁴ mg/L and 1 × 10⁻⁵ mg/L, respectively. The HCO₃⁻ concentrations were determined using a dilute hydrochloric acid – methyl orange/phenolphthalein titration.

The δ¹⁵N/δ¹⁸O-NO₃⁻ and δ¹⁷O-NO₃⁻ values were analyzed at the Environmental Stable Isotope Laboratory, Chinese Academy of Agricultural Sciences (CAAS) (Beijing, China) and Institute of Applied Ecology, Chinese Academy of Sciences (CAS) (Shenyang, China), respectively. Nitrate isotopes were measured follow the bacteria denitrification method. In brief, nitrate was reduced to N₂O by denitrifying bacteria known as *Pseudomonas aureofaciens* (ACTT 13985, USA), which lack the reductase ability of N₂O. For δ¹⁷O-NO₃⁻ determination at Institute of Applied Ecology, CAS, the produced N₂O was loaded into a high temperature oven (875 °C) for thermal decomposition of N₂O to O₂ and N₂. Then, the produced O₂ was analyzed by a continuous-flow isotope ratio mass spectrometer (IsoPrime PrecisiON, IsoPrime Ltd., UK) for compositions of triple oxygen isotopes (i.e., ¹⁶O, ¹⁷O, ¹⁸O). Three international nitrate references (IAEA-N3, USGS34, and USGS35) were measured to calibrate the δ¹⁷O/δ¹⁸O-NO₃⁻ values. Finally, the Δ¹⁷O-NO₃⁻ values were calculated as Δ¹⁷O-NO₃⁻ = δ¹⁷O-NO₃⁻ - 0.52 × δ¹⁸O-NO₃⁻ (Liu et al., 2013). The analytical precision for Δ¹⁷O-NO₃⁻ was 0.5 ‰. For δ¹⁵N/δ¹⁸O-NO₃⁻ measurement at Environmental Stable Isotope Laboratory, CAAS, the produced N₂O gas was delivered to Tracegas preparation unit (Precon, Finnigan, Germany) through the Gilson autosampler. N₂O was extracted, purified, and trapped by Tracegas preparation unit. Afterwards, the δ¹⁵N/δ¹⁸O of N₂O were measured using an isotope ratio

mass spectrometer (Delta V plus, Finnigan, Germany). For the aim of quality control/assurance, two international calibrated standards (USGS32 and USGS34) were utilized to calibrate $\delta^{15}\text{N}$ values, and two international calibrated standards (USGS34 and USGS35) were utilized to calibrate $\delta^{18}\text{O}$ values. The analytical precision for $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- were 0.3 ‰ and 0.5 ‰, respectively.

$\delta^{18}\text{O}$ - H_2O and δD - H_2O were measured using a laser liquid water isotope analyzer (L2140-I, Picarro Santa Clara, USA) at Chinese Academy of Agricultural Sciences, CAAS. For each sample batch, two international standards VSMOW and SLAP were used to calibrate the data. $\delta^{18}\text{O}$ - H_2O and δD - H_2O accuracies were 0.3 ‰ and 0.5 ‰, respectively.

Fertilizer and soil samples were air-dried, homogenized and ground to pass a 149- μm nylon screen. The powdered samples were acidified with 0.5 M HCl and rinsed to a neutral pH with deionized water. First, an Elementar Vario (Elementar, UK) was applied to reduce the nitrogen from fertilizers and soils to N_2 via high temperature combustion, and then the resultant N_2 was sent to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime Ltd., UK) to determine the $\delta^{15}\text{N}$ values and calibrated with USGS 40 and USGS41, with an analysis accuracy of 0.2 ‰.

All the stable isotope ratios were expressed as follows:

$$\delta = \frac{R_{\text{SA}} - R_{\text{ST}}}{R_{\text{ST}}} \times 1000\text{‰} \quad (1)$$

where R_{SA} and R_{ST} are the isotope ratios (D/H , $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$ or $^{17}\text{O}/^{16}\text{O}$) of the samples and standards, respectively. The standard for nitrogen isotopes was atmospheric N_2 , whereas hydrogen and oxygen were reported in accordance with the VSMOW.

2.3. Isotopic-fractionation incubation experiment

To obtain an enrichment factor for the nitrification process, we conducted a laboratory soil incubation. The experiment was divided into two stages: preincubation and incubation. Preincubation was performed to reduce the effects of endogenous soil nitrogen transformations on the incubation results and restore the soil microbial activity. Soil was placed on glass plates, covered with a plastic wrap with holes to ensure gas exchange and then incubated in the dark for two weeks at 25 °C. Soil water content was adjusted to 70 % of water holding capacity (WHC) throughout the preincubation by adding ultrapure water every other day in accordance with weight loss.

For the soil incubation, we prepared 18 glass bottles (240 mL) for each soil (3 replicates $\times$ 6 sampling dates). A 20-g subsample of pre-incubated soil (dry basis) was added to each bottle and then amended with 1 mL of $(\text{NH}_4)_2\text{SO}_4$ to obtain a final soil N content of 120 mg N/kg (Yu et al., 2020). Each bottle was covered with a perforated cap to ensure gas exchange and incubated at 25 °C for 1, 4, 7, 14, 21 and 28 days. Three bottles were randomly selected at each sampling date for analysis of NH_4^+ and NO_3^- concentrations and $\delta^{15}\text{N}$ abundance of the residual NH_4^+ and NO_3^- . The soil water content was maintained at 70 % of WHC throughout the incubation period.

Fresh soil and the soil after preincubation and incubation were extracted with 1 M KCl using a soil/solution ratio of 1:5. Specifically, 20 g of soil (dry basis) was extracted with 100 mL of 1 M KCl by shaking at 220 rev/min and 25 °C for 1 h in a constant-temperature shaking incubator. The supernatant was transferred to 100-mL centrifuge tubes and centrifuged at 1500g for 10 min. Subsequently, the supernatant was filtered through a 0.45- μm membrane filter (Tengjin, Tianjin, China), and the filtrate used for analysis.

Concentrations of NH_4^+ and NO_3^- in the filtrate were measured by the same methods used for river water samples. The filtrate samples were transported to Environmental Stable Isotope Lab, CAAS (Beijing, China) for measurement of $\delta^{15}\text{N}$ - NH_4^+ and $\delta^{15}\text{N}$ - NO_3^- using an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime Ltd., UK). The $\delta^{15}\text{N}$ - NH_4^+ was analyzed using the hypobromite oxidation-azide reduction method.

USGS25 and USGS26 were used to calibrate $\delta^{15}\text{N}$ - NH_4^+ values. $\delta^{15}\text{N}$ - NO_3^- was determined by the cadmium-azide reduction method. USGS32 and USGS34 were used to calibrate $\delta^{15}\text{N}$ - NO_3^- . The analytical precision for $\delta^{15}\text{N}$ - NH_4^+ and $\delta^{15}\text{N}$ - NO_3^- was 0.2 ‰. The fresh soil characteristics for S1, S2 and S3 were as follows: WHC (%) of 36.3, 41.1, and 35.9; NH_4^+ (mg N/kg) of 5.9, 1.0 and 6.1; and NO_3^- (mg N/kg) of 6.0, 3.9 and 3.1. Following preincubation, S1, S2 and S3 N concentrations were as follows: NH_4^+ (mg N/kg) of 0.7, 0.4 and 0.4 and NO_3^- (mg N/kg) of 17.4, 13.7 and 18.1.

By convention, the isotopic fractionation factor (α_N) and ϵ_N were used to express the degree of fractionation during the nitrification process. The relationship between these parameters is shown as follows:

$$\alpha = \frac{R_p}{R_s} \quad (2)$$

$$\epsilon_N = (\alpha - 1) \times 1000 \quad (3)$$

where R_p and R_s represent the isotope ratios ($^{15}\text{N}/^{14}\text{N}$) of the product and substrate during a transformation process. The ϵ_N is more commonly used to express the degree of fractionation, and it can be calculated with the Rayleigh equation as follows (Xue et al., 2009; Denk et al., 2017):

$$\epsilon_N = \frac{\delta_t - \delta_0}{\ln f} \quad (4)$$

where δ_t and δ_0 are the $\delta^{15}\text{N}$ of the substrate at time t and zero, and f is the unreacted fraction of substrate at time t .

2.4. Bayesian isotope mixing model and uncertainty index

In this study, a MixSIAR model programmed in R software (version 3.6.3) was used to calculate the proportional contributions of NO_3^- sources in Zhaoshandu reservoir watershed. The model computes the proportional contributions of potential sources to the mixture (samples) through their tracers ($\delta^{15}\text{N}/\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$) based on mass conservation. The theoretical details of MixSIAR are reported in the literature (Stock et al., 2018; Cao et al., 2023). Three MixSIAR models (model 1: based on $\delta^{15}\text{N}/\delta^{18}\text{O}$ - NO_3^- , model 2: based on $\delta^{15}\text{N}/\delta^{18}\text{O}$ - NO_3^- and ϵ_N , model 3: based on $\delta^{15}\text{N}/\delta^{18}\text{O}/\Delta^{17}\text{O}$ - NO_3^- and ϵ_N) were constructed. Model 1 taken $\delta^{15}\text{N}/\delta^{18}\text{O}$ - NO_3^- as the target/end-member inputs with ϵ_N set to 0. Model 2 taken $\delta^{15}\text{N}/\delta^{18}\text{O}$ - NO_3^- as the target/end-member inputs, while ϵ_N was determined according to the soil incubation experiment results. Model 3 taken $\delta^{15}\text{N}/\delta^{18}\text{O}/\Delta^{17}\text{O}$ - NO_3^- as the target/end-member inputs with ϵ_N set as Model 2. During the MixSIAR modeling process, we set the MCMC run length = long, the Error structure = Residual*Process, and the Specify prior = uninformative. Given direct measurement of nitrification-sourced $\delta^{18}\text{O}$ - NO_3^- values is not feasible, $\delta^{18}\text{O}$ - NO_3^- values for NF, SN, and MS used in the MixSIAR model were theoretically estimated according to $\delta^{18}\text{O}$ - O_2 (23.5 ‰) and $\delta^{18}\text{O}$ in ambient water.

An uncertainty index (UI_{90}) was applied to characterize uncertainty based on the posterior distribution and then to interpret the uncertainty associated with the apportionment results (Ji et al., 2017; Torres-Martínez et al., 2021b; Zhang et al., 2022a). UI_{90} was calculated as:

$$UI_{90} = \frac{PC_{95} - PC_5}{90} \quad (5)$$

where PC_{95} and PC_5 denote the maximum and minimum proportional contribution values in the immediate increase segment with a 90 % cumulative probability. A larger UI_{90} value indicates high variability in apportionment results, whereas smaller values demonstrate that the apportionment is stable.

3. Results

3.1. Characterization of hydrochemistry

Hydrochemical parameters and other variables measured in the Zhaoshandu reservoir watershed are summarized in Table 1. Water temperature varied from 13.1 to 32.3 °C (mean ± SD = 22.1 ± 5.2 °C) throughout the study period. DO concentrations varied from 4.9 to 10.7 mg/L (mean ± SD = 8.2 ± 1.1 mg/L) with higher concentrations in the cool seasons (winter and spring). The higher DO concentrations in the cooler seasons likely results from the higher solubility of DO at lower temperatures (freshwater DO saturation is 10.1 versus 7.6 mg/L at 15 and 30 °C, respectively). The EC ranged from 35 to 138 μS/cm, with EC values of Lijing and Sixi higher than the other sites in every season, except for Shuanggui in autumn. The distinctly higher EC values suggest the existence of an anthropogenic input. The pH values ranged from 7.11 to 8.39 (mean ± SD = 7.79 ± 0.28), suggesting the river water was slightly alkaline.

Average cation concentrations for the four seasons followed the order: $\text{Ca}^{2+} > \text{Na}^+ > \text{K}^+ > \text{Mg}^{2+}$. Average anion concentrations during autumn followed $\text{HCO}_3^- > \text{SO}_4^{2-} > \text{Cl}^- > \text{F}^-$, whereas during the other three seasons they followed $\text{HCO}_3^- > \text{Cl}^- > \text{SO}_4^{2-} > \text{F}^-$. The highest values for Cl^- , SO_4^{2-} and HCO_3^- occurred during the dry winter period. Piper diagrams are useful to analyze changes in water chemistry types by allowing visualization of the distribution of major anions and cations in natural waters (Xiao et al., 2022). The dominant hydrochemistry type in Zhaoshandu watershed was Ca-HCO_3^- , followed by $\text{Na} + \text{K-HCO}_3^-$ (Fig. S4).

Concentrations of TN ranged from 0.47 to 3.92 mg N/L (mean ± SD = 1.12 ± 0.73 mg N/L) (Fig. S5), which exceeds the Class II water quality standard of China (1 mg N/L, GB3838-2002) (State Environment Protection Bureau of China, 2002). The TON concentrations account for >15 % of TN, ranging from 0.03 to 1.50 mg N/L (mean ± SD = 0.18 ± 0.21 mg N/L). Dissolved inorganic nitrogen concentrations followed $\text{NO}_3^- > \text{NH}_4^+ > \text{NO}_2^-$. The NO_3^- values ranged from 0.35 to 3.06 mg N/L (mean ± SD = 0.78 ± 0.46 mg N/L) with nitrate being the main nitrogen form, accounting for 69.8 % of TN. Thus, NO_3^- was considered the dominant nitrogen contaminant in Zhaoshandu watershed. The NH_4^+ concentrations ranged from <0.003 to 1.45 mg N/L (mean ± SD = 0.14 ± 0.25 mg N/L) and NO_2^- concentrations ranged from 0.003 to 0.16 mg N/L (mean ± SD = 0.03 ± 0.04 mg N/L).

Seasonally, NO_3^- concentrations were higher during summer, which

might be associated with leaching of soil/nitrogen fertilizer NO_3^- during the high precipitation-runoff season. The NO_3^- concentrations showed a slight increasing trend from upstream to downstream in the mainstream indicating that NO_3^- concentrations in the tributaries were collectively higher than those of the mainstream. The higher NO_3^- in tributaries might be due to the higher discharge intensity from nonpoint pollution sources in some tributary sub-watersheds, coupled with a smaller volume of water that reduces dilution and self-purification processes. Therefore, nitrogen pollution in tributary waters is generally more severe than that in the mainstream, especially in Lijing and Sixi (Fig. S5).

3.2. Stable isotopic composition of Zhaoshandu watershed

3.2.1. Isotopic composition of waters

The δD versus $\delta^{18}\text{O}$ of water is an effective tool for determining water recharge dynamics (e.g., soil runoff vs groundwater) as it provides climatic and environmental information (Fadhullah et al., 2020; Wu et al., 2021a, 2021b; Xie et al., 2023). The δD and $\delta^{18}\text{O}$ of surface waters ($n = 44$) ranged from −55.3 to −33.7 ‰ (mean ± SD = −40.9 ± 5.0 ‰) and from −8.0 to −5.6 ‰ (mean ± SD = −6.6 ± 0.5 ‰), respectively (Fig. S6). Moreover, the δD and $\delta^{18}\text{O}$ of precipitation ($n = 22$) varied from −60.16 to 3.61 ‰ (mean ± SD = −23.2 ± 20.6 ‰) and from −9.0 to −1.7 ‰ (mean ± SD = −4.7 ± 2.3 ‰). The regression line fit to these precipitation data was regarded as the local meteoric water line (LMWL): $\delta\text{D} = 8.88 \times \delta^{18}\text{O-H}_2\text{O} + 18.37$. The $\delta\text{D-H}_2\text{O}$ and $\delta^{18}\text{O-H}_2\text{O}$ data of surface waters were close to the LMWL (Fig. S6), suggesting that the river water was mainly recharged by recent precipitation (Lao et al., 2022; Li et al., 2022). The regression line fit to surface water data was considered as the evaporation line ($\delta\text{D} = 7.58 \times \delta^{18}\text{O-H}_2\text{O} + 8.95$). Fig. S6 shows that the slope of the evaporation line was similar to that of LMWL, demonstrating that evaporation had only a slight impact during the study period. This result can also be proved by that the mean d-excess value (11.7 ‰) of river samples was similar to that of global meteoric water line (d-excess = 10 ‰).

3.2.2. Isotopic composition of nitrate

The $\delta^{15}\text{N-NO}_3^-$ values ranged from 1.8 to 8.5 ‰ (mean ± SD = 5.7 ± 1.5 ‰) throughout the study period, with summer values remarkably lower than found in other seasons (Fig. S4). The $\delta^{18}\text{O-NO}_3^-$ values ranged from −7.8 to 11.6 ‰ (mean ± SD = 1.7 ± 4.5 ‰), with the average summer value considerably higher than in other seasons. The $\Delta^{17}\text{O-NO}_3^-$ value varied over a narrow range from −0.6 to 2.1 ‰ (mean ± SD = 0.4

Table 1
Summary statistics for hydrochemical parameters in the four seasons for Zhaoshandu reservoir watershed.

Parameters	2021-06 (summer)				2021-09 (autumn)				2021-12 (winter)				2022-03 (spring)			
	Min ^a	Max ^b	Mean	SD ^c	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
T (°C)	21.2	27.8	25.2	2.1	25.3	32.3	28.2	2.0	13.1	18.5	16.1	1.7	16.7	22.9	18.9	1.8
DO (mg/L)	7.1	8.2	7.8	0.4	4.9	8.1	7.2	0.9	6.2	10.7	8.9	1.2	7.1	10.1	8.9	0.9
EC (μS/cm)	35.0	107	61.4	22.0	38.0	138	68.9	32.5	37.0	117	55.6	30.2	36.0	132	58.3	27.8
pH	7.11	7.79	7.52	0.22	7.25	8.01	7.66	0.22	7.59	8.15	7.90	0.15	7.85	8.39	8.06	0.16
Chla (μg/L)	0.8	1.2	0.9	0.1	0.7	1.1	0.9	0.1	0.6	1.6	0.9	0.3	0.7	2.8	1.1	0.6
TN (mg N/L)	0.71	2.29	1.20	0.48	0.47	1.53	0.81	0.37	0.62	3.92	1.34	1.20	0.59	2.44	1.11	0.57
TON (mg N/L)	0.09	0.27	0.15	0.06	0.03	0.33	0.13	0.08	0.08	1.50	0.26	0.41	0.09	0.26	0.16	0.05
NH ₄ ⁺ (mg N/L)	0.04	0.21	0.09	0.06	0.03	0.32	0.11	0.09	<0.003	0.82	0.14	0.28	0.03	1.45	0.22	0.42
NO ₂ ⁻ (mg N/L)	0.01	0.05	0.02	0.01	0.01	0.11	0.02	0.03	0.004	0.16	0.03	0.05	0.003	0.11	0.02	0.04
NO ₃ ⁻ (mg N/L)	0.56	1.77	0.95	0.38	0.35	0.96	0.55	0.20	0.51	3.06	0.91	0.75	0.46	1.37	0.71	0.26
Cl ⁻ (mg/L)	2.15	5.56	3.64	1.19	1.37	5.29	3.10	1.21	2.00	11.56	3.99	2.85	2.20	5.95	3.39	1.30
F ⁻ (mg/L)	0.12	0.21	0.16	0.03	0.12	0.31	0.19	0.06	0.13	0.29	0.17	0.06	0.11	0.50	0.20	0.11
SO ₄ ²⁻ (mg/L)	2.21	7.36	3.63	1.41	2.17	8.23	3.39	1.82	2.36	10.04	3.81	2.36	2.25	6.40	3.35	1.23
Ca ²⁺ (mg/L)	1.11	7.83	3.72	1.93	1.07	6.56	3.64	2.86	3.20	11.49	5.27	2.69	2.62	11.66	4.96	2.46
Na ⁺ (mg/L)	0.77	4.26	2.19	1.02	1.18	5.50	2.86	1.27	2.14	9.07	3.59	2.06	1.71	6.33	3.26	1.18
K ⁺ (mg/L)	0.41	2.15	1.11	0.49	0.63	2.85	1.51	0.64	1.33	4.15	1.87	0.86	0.76	3.20	1.63	0.58
Mg ²⁺ (mg/L)	0.19	1.82	0.71	0.49	0.25	1.75	0.79	0.44	0.57	2.15	0.92	0.51	0.47	2.08	0.96	0.52
HCO ₃ ⁻ (mg/L)	14.8	35.3	27.1	5.2	17.1	45.5	28.2	7.9	22.8	58.6	29.5	11.3	20.1	49.1	27.9	8.4

^a Min is the minimum value.
^b Max is the maximum value.
^c SD denotes standard deviation.

± 0.9 ‰). The narrow range of $\Delta^{17}\text{O-NO}_3^-$ was beneficial to the identification of nitrate sources. For the mainstream, $\delta^{15}\text{N-NO}_3^-$ values for M1, M2 and M3 (mean $\pm$ SD) were 4.6 ± 1.2 ‰, 5.6 ± 1.4 ‰ and 5.8 ± 1.0 ‰, demonstrating an increasing trend from upstream to downstream. The tributaries exhibited higher $\delta^{15}\text{N}$ values than the mainstream contributing to the trend of increasing $\delta^{15}\text{N}$ along the mainstream (Fig. S7). In contrast, the mainstream exhibited higher $\Delta^{17}\text{O-NO}_3^-$ than the tributaries, indicating that the mainstream is more strongly affected by rainfall sources of nitrate. Larger surface water area of the mainstream compared to that of the tributaries might account for this phenomenon.

3.2.3. Isotopic composition of putative endmembers

In this study, the isotopic compositions of nitrate sources (i.e., AD, NF and SN) were obtained from field-collected samples, while those for MS were derived from Zhang et al. (2018) (Table 2). The $\delta^{18}\text{O-NO}_3^-$ values were calculated in accordance with the $\delta^{18}\text{O-H}_2\text{O}$ and $\delta^{18}\text{O-O}_2$ values obtained from the nearby International Atomic Energy Agency precipitation isotope monitoring station at Fuzhou, which is ~ 270 km from the study area. Additionally, the mean value of $\Delta^{17}\text{O-NO}_3^-$ for NF, SN and MS was set as the minimum value for riverine $\Delta^{17}\text{O-NO}_3^-$, with the standard deviation set as double the value of the analytical precision (Ji et al., 2022b). The $\Delta^{17}\text{O-NO}_3^-$ values of precipitation ranged from 21.0 to 31.4 ‰ (mean $\pm$ SD = 25.1 ± 3.2 ‰), which was higher than values previously reported for high-elevation regions (7.8–23.3 ‰, mean $\pm$ SD = 16.3 ± 3.9 ‰) (Xia et al., 2019). This discrepancy may result from the lower temperature, lower pressure and stronger UV radiation found in high-elevation regions, which ultimately lead to lower $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values in precipitation (Michalski et al., 2012; Xia et al., 2019).

3.3. $\delta^{15}\text{N}$ values and concentrations of soil inorganic nitrogen from soil incubation

The NH_4^+ concentrations during the 28-day soil incubation decreased from 120 mg N/kg to 0.43, 1.02 and 39.22 mg N/kg for S1, S2 and S3 soils, respectively (Fig. 2a). In contrast, NO_3^- concentrations increased to 143.9, 136.8 and 115.4 mg N/kg over the incubation period for S1, S2 and S3, respectively (Fig. 2b). As the low NH_4^+ concentrations (S1 = 0.43 ± 0.10 mg N/kg, S2 = 1.02 ± 0.23 mg N/kg) for samples collected at 28 days hindered the acquisition of $\delta^{15}\text{N}$ values, only $\delta^{15}\text{N}$ values for samples collected within the first 21 days were available. The $\delta^{15}\text{N}$ value of NH_4^+ exhibited an increasing trend during the incubation period, increasing from 4.5 to 55.5 ‰ (day 1 $\rightarrow$ 14) in S1, from 3.4 to 56.6 ‰ (day 1 $\rightarrow$ 21) in S2, and from 1.7 to 19.4 ‰ (day 1 $\rightarrow$ 21) in S3 (Fig. 2c). The value of $\delta^{15}\text{N-NO}_3^-$ decreased initially and was followed by an increase (Fig. 2d), which was similar to previous results by Yu et al.

Table 2

Mean and standard deviation for putative NO_3^- sources in Zhaoshandu reservoir watershed.

Source	$\delta^{15}\text{N}$ (‰)		$\delta^{18}\text{O}$ (‰)		$\Delta^{17}\text{O}$ (‰)	
	Mean	SD ^a	Mean	SD	Mean	SD
AD	−2.6	1.6	78.0	7.3	25.1	3.3
NF	−2.3	1.8	4.1 ^b	1.9 ^b	−0.6 ^c	1.0 ^d
SN	5.3	2.1	4.1 ^b	1.9 ^b	−0.6 ^c	1.0 ^d
MS	16.3 ^e	5.7 ^e	4.1 ^b	1.9 ^b	−0.6 ^c	1.0 ^d

AD: atmospheric deposition nitrate, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage.

^a SD denotes standard deviation.

^b Data are calculated according to $\delta^{18}\text{O-O}_2$ and $\delta^{18}\text{O-H}_2\text{O}$ from nearby Fuzhou station (about 270 km).

^c Set as the minimum value for $\Delta^{17}\text{O-NO}_3^-$ in river water.

^d Set as double the analytical precision value.

^e Data from Zhang et al. (2018).

(2021b). The reason of this phenomenon lies in that as the nitrification reaction progress, $^{14}\text{N-NH}_4^+$ was gradually consumed and ^{15}N was enriched in remaining NH_4^+ ; as such, more $\delta^{15}\text{N-NH}_4^+$ was involved in nitrification process (Nikolenko et al., 2018; Yu et al., 2021b).

3.4. Isotopic fractionation by nitrification in soil incubations

In real-world environments, ϵ_N resulting from nitrification is smaller than those obtained under pure bacterial cultures in the laboratory due to co-occurring processes (e.g., nitrogen fixation and nitrogen mineralization) and challenging environmental conditions (e.g., low soil pH, insufficient substrate, and presence of inhibitory substances) (Li et al., 2020; Liu et al., 2021a; Song et al., 2014). Therefore, the minimum value determined for each soil in the incubation experiments was considered as the ϵ_N from nitrification; S1, S2 and S3 were -7.40 , -8.89 and -4.50 ‰, respectively (Fig. 3). The three values were averaged to obtain an overall soil ϵ_N for the watershed of -6.93 ± 1.82 ‰. This ϵ_N value fell within the theoretical range of -35 ‰ to -5 ‰ and was consistent with the findings ($\epsilon_N = -8.7 \pm 2.7$ ‰) of Yu et al. (2020).

4. Discussion

4.1. Transformations of nitrate

Microbial nitrification is a multi-step oxidation process of ammonium nitrogen convert to nitrate nitrogen and thereby an oxygen-rich environment ($\text{DO} > 4$ mg/L) is benefit for the activity of nitrifying bacteria. Besides, activity of nitrifying bacteria is significantly influenced by the pH, with strongest bacterial activity often occurs at pH between 6.5 and 8 (Nikolenko et al., 2018). During the study period, surface water DO concentrations ranged from 4.9 to 10.7 mg/L and the pH varied between 7.11 and 8.39, indicating a favorable environment for microbial nitrification (Liu et al., 2021b). Theoretical $\delta^{15}\text{N-NO}_3^-$ values can be bounded by $\delta^{18}\text{O-O}_2$ (23.5 ‰) and $\delta^{18}\text{O-H}_2\text{O}$. Thus, the maximum $\delta^{18}\text{O-NO}_3^-$ can be calculated as $\delta^{18}\text{O-NO}_3^- = 2 \times [\delta^{18}\text{O-H}_2\text{O}]/3 + 1 \times [\delta^{18}\text{O-O}_2]/3$, and the minimum $\delta^{18}\text{O-NO}_3^-$ value was set by $\delta^{18}\text{O-H}_2\text{O}$ (Yu et al., 2021a; Zhang et al., 2022b). However, the $\delta^{18}\text{O}$ values of microbially-derived nitrate may be 5 ‰ higher than the calculated theoretical maximum value (Xue et al., 2009). Correspondingly, most of the isotopic values were within the theoretical range of nitrification, indicating that nitrification was a dominant process affecting $\delta^{18}\text{O-NO}_3^-$ and $\delta^{15}\text{N-NO}_3^-$ in the river system (Fig. 4).

Denitrification converts NO_3^- to N_2 , NO and/or N_2O under anaerobic conditions (Rivett et al., 2008; Xuan et al., 2022). During this process, light isotopes of NO_3^- are preferentially used by anaerobic microbes, resulting in an enrichment of the residual $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values as the NO_3^- concentrations decrease (Fukada et al., 2003; Ren et al., 2024). Previous studies demonstrated that the ratio of $\delta^{15}\text{N}$ to $\delta^{18}\text{O}$ in freshwater and terrestrial environments ranges from 1.3:1 to 2.1:1 as denitrification proceeds (Böttcher et al., 1990; Niu et al., 2022), which provides key evidence to distinguish the occurrence of denitrification. The values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in Zhaoshandu watershed did not present a significant correlation in the seasonal water samples (Fig. 5a). Similarly, a significant negative correlation was not observed between $\ln(\text{NO}_3^-)$ and $\delta^{15}\text{N-NO}_3^-$ (Fig. 5b), further confirming that in-stream denitrification was not a prevalent process. This inference is consistent with the aerobic nature of the surface waters having DO concentrations > 4 mg/L throughout the study period.

Appreciable ammonia volatilization from surface waters to the atmosphere may occur at pH values greater than ~ 9 (Yin et al., 2020; Shu et al., 2022). As lighter nitrogen isotopes are preferentially volatilized, the volatilization process leads to a large isotopic fractionation, resulting in ^{15}N enrichment (Kendall and McDonnell, 1998). Since the pH values of all water samples were < 9 , ammonia volatilization was deemed negligible in this study. Therefore, Zhaoshandu watershed is believed to be a nitrification-dominated watershed with in-stream denitrification

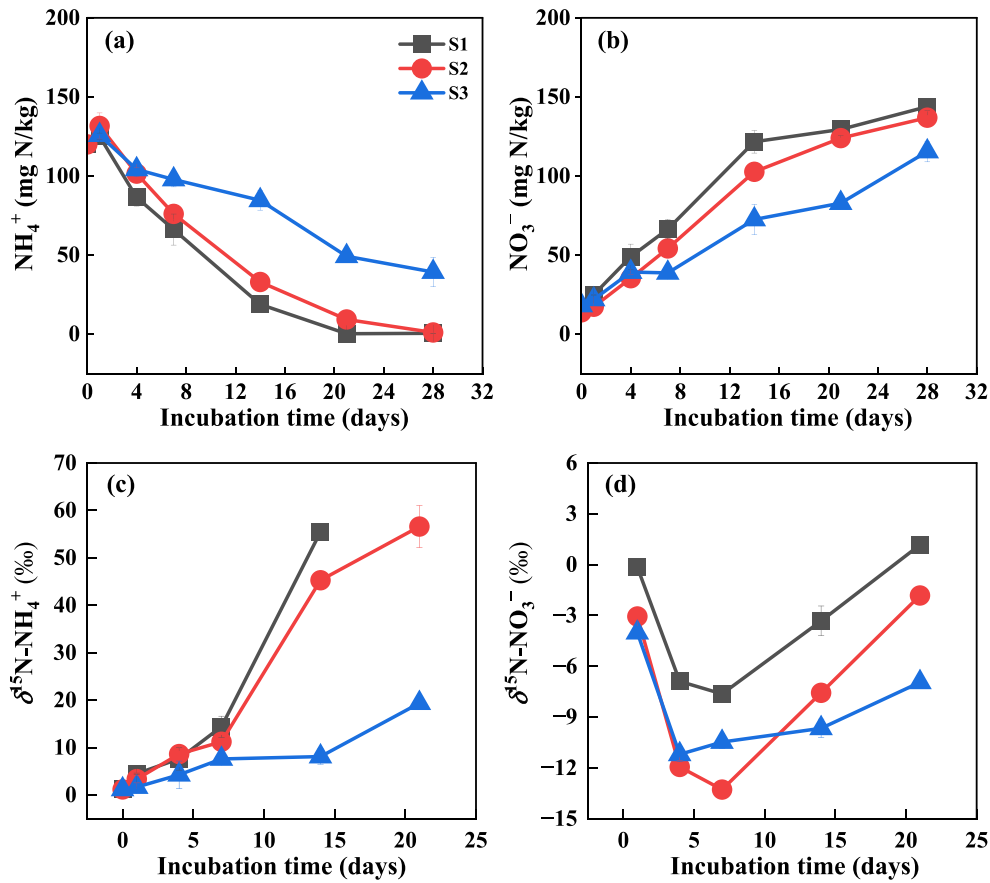


Fig. 2. Concentration changes (mean $\pm$ SD) of (a) NH_4^+ , (b) NO_3^- , (c) $\delta^{15}\text{N}\text{-NH}_4^+$, and (d) $\delta^{15}\text{N}\text{-NO}_3^-$ in soil incubation experiment.

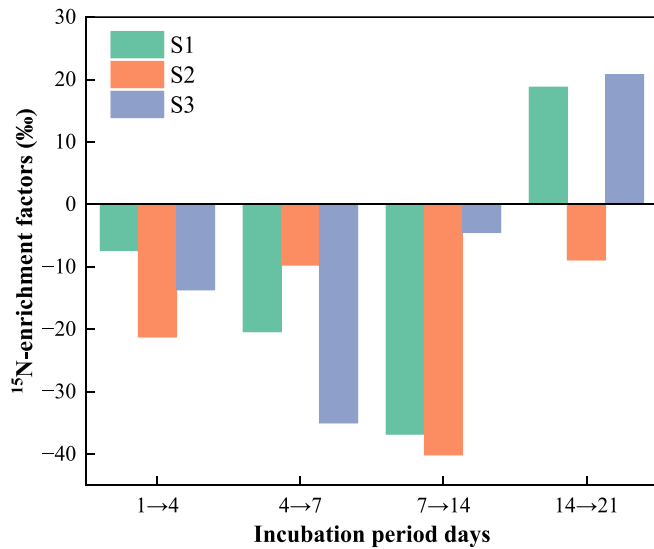


Fig. 3. ^{15}N -enrichment factors for soil nitrification of three soil samples (S1, S2, S3) at each incubation stage.

and volatilization deemed negligible, hence we incorporated the isotope fractionation due to nitrification into our source identification/apportionment modeling.

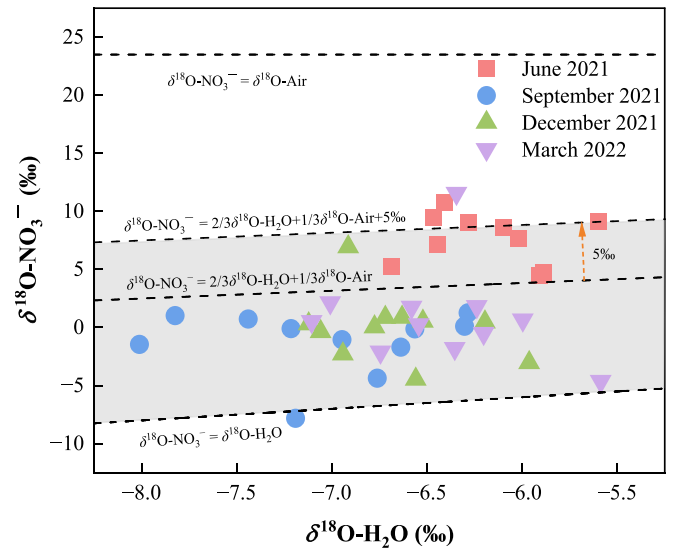


Fig. 4. Relationship between $\delta^{18}\text{O}\text{-H}_2\text{O}$ and $\delta^{18}\text{O}\text{-NO}_3^-$ in river waters. Gray area represents the theoretical range of $\delta^{18}\text{O}\text{-NO}_3^-$ formed by nitrification.

4.2. Identification of nitrate sources by hydrochemistry and $\delta^{15}\text{N}/\delta^{18}\text{O}\text{-NO}_3^-$

Chloride is often used as a tracer to identify NO_3^- sources in natural waters because it is a relatively physically, chemically and biologically inert element, thereby conservatively mixing among water sources (Chen et al., 2014; Torres-Martínez et al., 2021a). The relationship

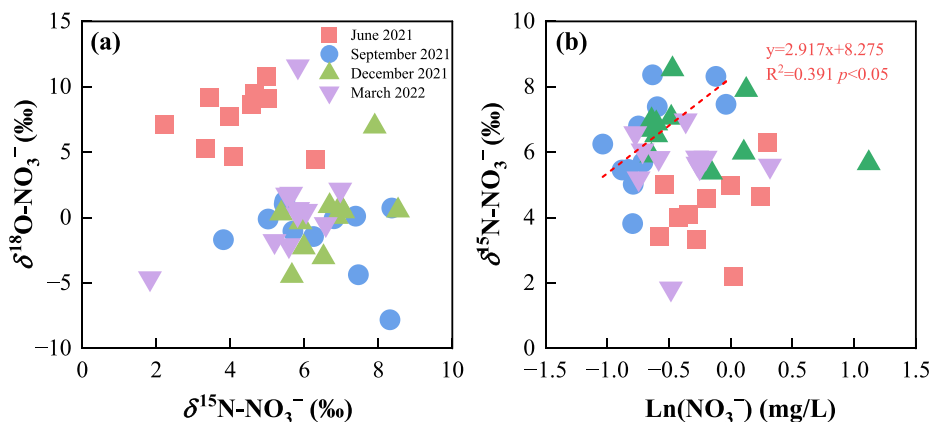


Fig. 5. Relationship between (a) $\delta^{18}\text{O-NO}_3^-$ and $\delta^{15}\text{N-NO}_3^-$ and (b) $\ln(\text{NO}_3^-)$ and $\delta^{15}\text{N-NO}_3^-$ in river waters. If there are no statistically significant regressions ($p > 0.05$), the regression line will not be displayed.

between Cl^- concentration and the $\text{NO}_3^-/\text{Cl}^-$ molar ratio is widely applied to identify anthropogenic NO_3^- sources in a watershed (Zhang et al., 2022c). Generally, NO_3^- is the predominant nitrogen component derived from agricultural activities due to its high mobility. Besides, agricultural sources (SN and NF) exhibit low level of Cl^- content. Nevertheless, domestic sewage typically includes elevated Cl^- concentrations and low NO_3^- concentrations. Correspondingly, a high $\text{NO}_3^-/\text{Cl}^-$ ratio and low Cl^- concentration indicate that NO_3^- is mainly contributed by agricultural activities, whereas a low $\text{NO}_3^-/\text{Cl}^-$ ratio and high Cl^- concentration indicate a predominance of MS sources (Shin et al., 2023). In Zhaoshandu watershed, the $\text{NO}_3^-/\text{Cl}^-$ ratios were relatively low, ranging from 0.27 to 0.86, and the Cl^- concentrations ranged from 0.04 to 0.33 mmol/L (mean $\pm$ SD = 0.10 ± 0.05 mmol/L) (Fig. S8). This combination of $\text{NO}_3^-/\text{Cl}^-$ ratios and Cl^- concentrations indicates a mixture of natural and anthropogenic sources. Moreover, the strong positive correlations between Cl^- and NO_3^- were indicative of a large proportion of nitrate derived from domestic sewage (Fig. S8). A $\delta^{15}\text{N-}$

NO_3^- versus $\delta^{18}\text{O-NO}_3^-$ biplot for each sampling date illustrates their relationship with local putative endmember nitrate sources (Fig. 6). Generally, most points fell in the overlapping range of NF, SN and MS, suggesting prevalent contributions from these sources (Li et al., 2022). The compact distribution of points throughout the study period suggests that the NO_3^- sources were similar among the four seasons. The $\delta^{18}\text{O-NO}_3^-$ values for river samples were much smaller than those for AD, demonstrating that AD was not the dominant NO_3^- source in Zhaoshandu watershed, despite the ~ 30 kg/ha/yr of atmospheric N deposition in this area (Yu et al., 2019). This phenomenon might be attribute to (1) a significant portion of NO_3^- derived from atmospheric deposition has the potential to infiltrate into soils and then accumulate in shallow sub-surface water or groundwater; and (2) some atmospherically-deposited NO_3^- might be assimilated by plants/microorganisms and/or undergoes substantial biological processing prior to being transported to receiving waters through runoff. Moreover, the average $\delta^{18}\text{O-NO}_3^-$ values were highest in summer indicating that the influence of summer rainfall

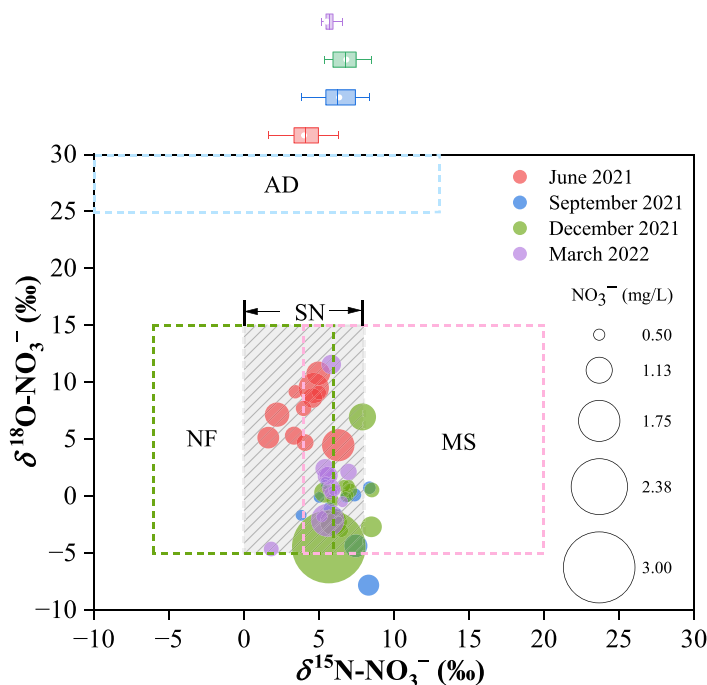


Fig. 6. Plot of $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values in river waters from Zhaoshandu reservoir watershed. AD: atmospheric deposition, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage. Box plots show the 25th, 50th and 75th percentiles with whiskers extending to the 5th and 95th percentiles for individual sampling periods; hollow circles in the box plots represent mean values.

provided a higher AD contribution. The $\delta^{15}\text{N}\text{-NO}_3^-$ values of autumn, winter and spring were higher than in summer, indicating that a nitrate source with higher $\delta^{15}\text{N}\text{-NO}_3^-$, such as MS, accounted for a greater proportion of nitrate inputs during these drier seasons.

4.3. Nitrate source apportionment combined with MixSIAR

To ascertain the apportionment of each nitrate source in Zhaoshandu watershed, we used a Bayesian mixing model MixSIAR to quantify the NO_3^- source contributions at the reservoir outlet (M3, spatial integrator site for the entire watershed). Since the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for AD were obtained directly from the determination of NO_3^- , the ϵ_N from AD was not considered. Notably, the fractionation effect strength associated with nitrification depends on the substrate pool size (Nikolenko et al., 2018). When the substrate is nearly depleted, it can be inferred that complete nitrification has occurred. In such cases of complete nitrification, the isotopic fractionation effect tends to be weak or absent (Nikolenko et al., 2018; Jin et al., 2019a). In Zhaoshandu reservoir watershed, the low content of riverine NH_4^+ (substrate for nitrification) and sufficient dissolved oxygen implied the occurrence of complete nitrification during the in-stream process. Hence, ϵ_N for MS was set as zero since most MS was discharged directly to the river, where complete nitrification was assumed to occur. For SN and NF, nitrification commonly occurred within the soils, which sufficient substrate can result in significant nitrification fractionation. Therefore, ϵ_N obtained from soil incubation was used for SN and NF sources. Accordingly, we set the ϵ_N of NF and SN as $-6.9 \pm 1.8\text{‰}$ based on the soil incubation results. According to Markov Chain Monte Carlo statistical theory, MixSIAR model is capable of providing a complete posterior probability distribution to show the uncertainties inherent in the proportional contribution of each pollution source (Ji et al., 2017; Ju et al., 2022). In this study, box plots were used to demonstrate the modelled contributions of different sources by MixSIAR model, reflecting the uncertainties caused by spatio-temporal variations of isotopic composition of nitrate sources, isotopic fractionation, and multiple NO_3^- sources.

The model output showed MS was the most significant contributor of nitrate comprising $46.2 \pm 10.7\%$, followed by SN ($32.3 \pm 18.5\%$), NF

($19.7 \pm 13.1\%$) and AD ($1.8 \pm 1.6\%$) over the entire study period (Fig. 7). The proportional contributions of each putative source during the four seasons are shown in Fig. 8. Contributions from the nitrate sources during the four seasons exhibited the same order: $\text{MS} > \text{SN} > \text{NF} > \text{AD}$. The contribution of AD, NF, SN and MS in the four seasons followed: $6.0 \pm 1.8\%$, $20.7 \pm 14.0\%$, $30.0 \pm 19.7\%$ and $43.2 \pm 14.0\%$ in summer; $0.8 \pm 0.8\%$, $17.6 \pm 15.2\%$, $20.6 \pm 22.0\%$ and $61.0 \pm 20.1\%$ in autumn; $0.80 \pm 0.8\%$, $16.7 \pm 13.3\%$, $21.9 \pm 19.2\%$ and $60.6 \pm 16.6\%$ in winter; and $0.8 \pm 0.8\%$, $17.8 \pm 14.4\%$, $24.9 \pm 22.5\%$ and $56.5 \pm 18.9\%$ in spring. The highest contribution of AD ($6.0 \pm 1.8\%$) was observed in summer owing to this being the highest rainfall period. Similarly, the summer season had the highest contributions of NF ($20.7 \pm 14.0\%$) and SN ($30.0 \pm 19.7\%$), indicating that precipitation was an important factor driving soil and nitrogen fertilizer runoff/leaching. NF showed a near uniform seasonal contribution suggesting that chemical fertilizer was largely taken up by crops and/or experienced a lag effect in its transport to the river (Van Meter et al., 2018; Wang et al., 2019). Alternatively, the NH_4^+ and NO_3^- from chemical fertilizer could be immobilized by microbial biomass and hence become organic N via cycling through the microbial loop, which could subsequently leach water-soluble organic N to the river along with surface runoff (Niu et al., 2022). The elevated nitrogen loads in the watershed from SN may be due to accelerated soil erosion (Ren et al., 2015). Unreasonable tillage type (e.g., downslope tillage, long term rotary tillage) and timing (summer period of high rainfall) can appreciably increase soil erosion. MS had a higher contribution in autumn and winter when lower rainfall/runoff provides a lower input of AD, NF and SN to the total nitrate load. Many villages located near the river have minimal sewage treatment facilities, resulting in large amount of untreated domestic wastewater being discharged directly into the river or leaking from sewer systems. This untreated domestic sewage is believed to be the primary contributor of MS pollution as livestock numbers are currently limited (see Section 2.1) and the amount of produced N from livestock effluent is significantly lower compared to that generated by domestic sewage (~ 80 ton N/yr vs. ~ 1800 ton N/yr). Several studies reported similar NO_3^- source apportionment results for drinking water source regions. For example, Li et al. (2019) found NO_3^- mainly originated from SN, NF, and MS in the Xijiang river of Southern China, and those anthropogenic inputs (MS and NF) increased in the dry season. Similarly, Fadhullah et al. (2020) found that MS (37%) was the dominant source for a reservoir in the northern state of Peninsular Malaysia.

With the aim of elucidating the spatial variation of riverine nitrate pollution sources, contributions of different sources for each sampling site were calculated by a MixSIAR model with ϵ_N . As shown in Fig. S9, low to moderate spatial variations of the nitrate source contributions were observed in Zhaoshandu reservoir watershed. Source contributions for all other sampling sites were found to be similar to M3 with the ranking followed $\text{MS} > \text{SN} > \text{NF} > \text{AD}$. Progressive increase in MS contributions together with a corresponding rise in NO_3^- concentrations from M1 $\rightarrow$ M2 $\rightarrow$ M3, implying the accumulation effect of MS pollution along the water flow path in the mainstream. The contributions of MS source showed higher proportion in Sixi and Lijing compared to other tributaries, which was consistent with the spatial variation of nitrate content. Lijing sampling site is located downstream from a wastewater treatment plant. Sixi is a tributary that flows through the county seat of Wencheng County having a population of $>100,000$. Consequently, the high N content likely originates from wastewater discharge.

To determine whether the incorporation of nitrification ϵ_N into the model improved the traceability efficiency, we calculated source apportionment without the nitrification ϵ_N . When ignoring ϵ_N , the contribution of NF and SN increased, whereas that of MS decreased (Fig. 7). In the absence of ϵ_N consideration, SN became the dominant nitrate source, which conflicts with the quantitative identification results from the chloride method and field investigations conducted by the local government (Wenzhou Eco-environment Bureau and Wenzhou Institute of Eco-environmental Sciences, 2021). Combining the export

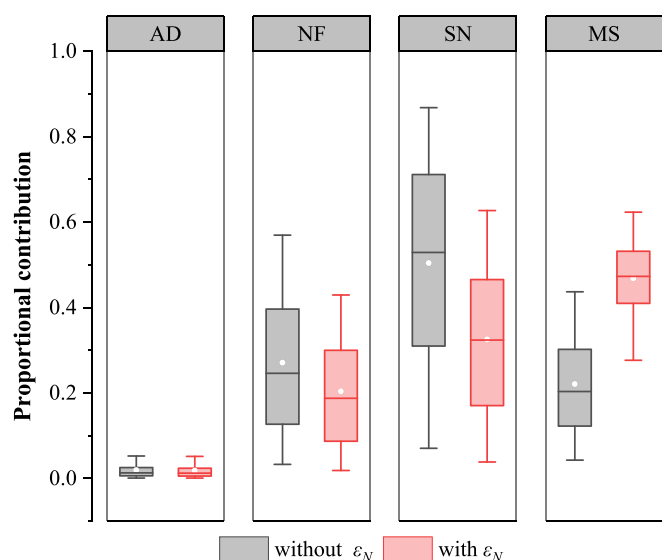


Fig. 7. Box plot showing the proportional contribution of putative NO_3^- sources in the Zhaoshandu reservoir watershed based on MixSIAR model results with and without nitrification ^{15}N -enrichment factor (ϵ_N) consideration. Box plots show the 25th, 50th and 75th percentiles with whiskers extending to the 5th and 95th percentiles; the hollow circles represent mean values. AD: atmospheric deposition nitrate, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage.

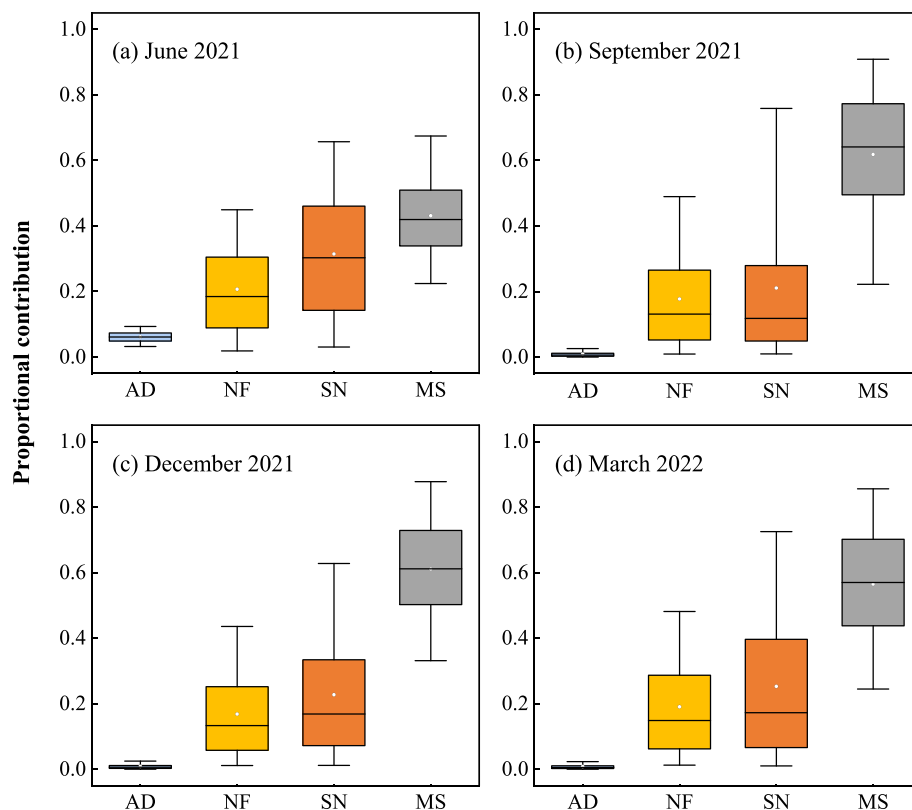


Fig. 8. Box plots showing the proportional contribution of putative NO_3^- sources in the Zhaoshandu reservoir watershed. Box plots show the 25th, 50th and 75th percentiles with whiskers extending to the 5th and 95th percentiles; the hollow circles represent mean values. AD: atmospheric deposition nitrate, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage.

coefficient method recommended by the First National Survey of Pollution Sources in China and field investigations, Wenzhou Eco-environmental Bureau reported that AD, NF, SN, and MS contributed 1 %, 26 %, 30 %, and 43 % of riverine nitrogen, respectively, in the Shanxi drinking water source region. This obtained result was in agreement with the output of the MixSIAR model considering ε_N , implying that MS was the primary nitrogen sources. Hence, applying the nitrification ε_N to the different nitrate sources was concluded to theoretical improve the efficiency of NO_3^- source apportionment result, consistent with a previous study by Liu et al. (2021b) and Yu et al. (2020).

Furthermore, we applied UI_{90} to evaluate the uncertainties of the results with and without ε_N . The average UI_{90} of these two models were

0.385 (with ε_N) and 0.492 (without ε_N) (Fig. 9). By way of comparison, the average UI_{90} incorporating ε_N was higher than it in the La Paz aquifer system (Mexico, UI_{90} range = 0.03–0.41), but lower than it in the Changle River watershed (China, UI_{90} range = 0.08–0.67) (Torres-Martínez et al., 2021b; Ji et al., 2017). Notably, UI_{90} values for AD, MS, NF and SN in the MixSIAR models with vs. without ε_N were 0.053 vs. 0.056, 0.389 vs. 0.433, 0.451 vs. 0.591 and 0.648 vs. 0.887, respectively. This uncertainty analysis indicates that the modeling results were more stable when considering ε_N , as found in several previous studies (Jiang et al., 2021; Ji et al., 2022a; Ren et al., 2022).

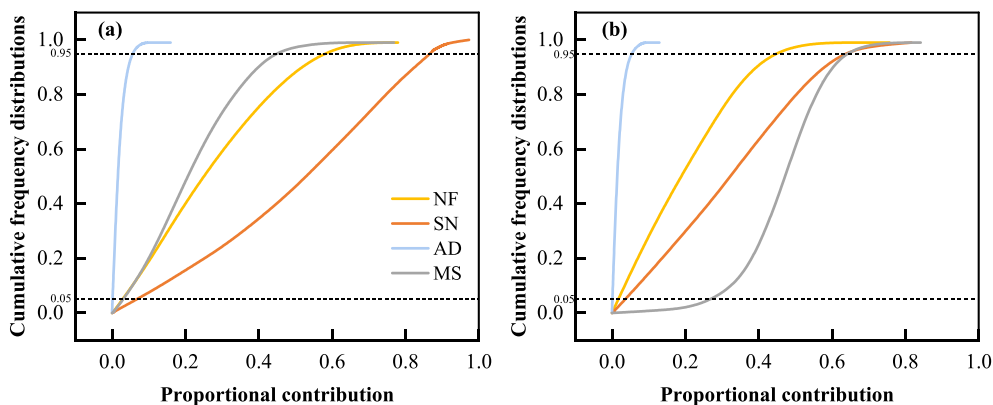


Fig. 9. Cumulative probability distributions for proportional contributions of putative NO_3^- sources in the Zhaoshandu reservoir watershed based on outputs of (a) MixSIAR model without the nitrification ^{15}N -enrichment factor and (b) with the nitrification ^{15}N -enrichment factor. AD: atmospheric deposition nitrate, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage.

4.4. Utilization of the $\Delta^{17}\text{O-NO}_3^-$ isotopic signature to improve and validate atmospheric deposition nitrate contribution

A difficulty in tracing N pollution sources is the alteration of N/O isotopic composition by microbial transformations. These processes often obscure the isotopic composition of the original nitrate sources by simultaneously modifying the oxygen and nitrogen isotopic ratios, typically by isotopic enrichment of the residual nitrate pool (Kendall and McDonnell, 1998). Although $\delta^{15}\text{N-NO}_3^-$ with ϵ_N provides relevant information about terrestrial pollution sources, ^{18}O -fractionation cannot be disregarded for distinguishing between atmospheric deposition nitrate and nitrate formed by terrestrial sources (Liu et al., 2013). Herein, we introduced $\Delta^{17}\text{O-NO}_3^-$ to estimate the potential nitrate source contributions for reducing the uncertainty caused by the wide range of $\delta^{18}\text{O-NO}_3^-$ values and ^{18}O -fractionation during the N-cycling. A MixSIAR model combining $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$, ϵ_N and $\Delta^{17}\text{O-NO}_3^-$ for the June 2021 water samples was developed as this season had the highest AD contribution. The modeling results applying $\Delta^{17}\text{O-NO}_3^-$ showed a similar source contribution ranking of MS ($43.4 \pm 14.1\%$) > SN ($29.3 \pm 19.4\%$) > NF ($19.8 \pm 13.8\%$) > AD ($7.5 \pm 1.6\%$); however, the AD contribution ($7.5 \pm 1.6\%$) was higher than results obtained from $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$ and ϵ_N ($6.0 \pm 1.8\%$) model (Table 3).

UI_{90} was used to evaluate whether the addition of $\Delta^{17}\text{O-NO}_3^-$ improved the uncertainties. The probability contribution and UI_{90} for each source during June 2021 is shown in Table 3. The average UI_{90} values decreased from 0.522 to 0.442 and 0.435 in the three models (model 1: based on $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$, model 2: based on $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$ and ϵ_N , model 3: based on $\delta^{15}\text{N}/\delta^{18}\text{O}/\Delta^{17}\text{O-NO}_3^-$ and ϵ_N), suggesting a small improvement in the uncertainty. The traceability results of MixSIAR model with $\Delta^{17}\text{O-NO}_3^-$ and ϵ_N had lowest uncertainties due to (1) incorporating ϵ_N to MixSIAR model is benefit for reducing the uncertainty caused by the ^{15}N -fractionation existing microbial nitrification processes; (2) The direct $\Delta^{17}\text{O-NO}_3^-$ measurements eliminate the ^{18}O -fractionation uncertainty produced by oxygen atom sources during nitrification (Wang et al., 2022); and (3) the isotopic range of $\Delta^{17}\text{O-NO}_3^-$ (range = 21.0–31.4 ‰) was much narrower than that of $\delta^{18}\text{O-NO}_3^-$ (range = 67.6–95.0 ‰), which is beneficial for tracing nitrate sources (Xia et al., 2019).

4.5. Implications

This study provided a comprehensive nitrate source apportionment method by combining $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$ with ϵ_N and $\Delta^{17}\text{O-NO}_3^-$. Findings obtained from this study have multiple impacts: firstly, it serves as an important baseline for studies regarding nitrate pollution source evaluation in other river systems. Secondly, valuable isotopic data (e.g., ^{15}N -enrichment factor during nitrification, site-specific isotope compositions

of pollution sources, $\Delta^{17}\text{O-NO}_3^-$ values of atmospheric deposition and rivers in Eastern China) obtained here can be used as crucial parameters for researchers engaged in nitrogen source assessment to improve the efficiency of $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$ method in other studies. Thirdly, the findings of this study have significant implications for local water management authorities in utilizing quantitative information in terms of riverine nitrate sources to develop effective target nitrogen remediation strategies, thereby improving regional nitrogen management to sustain the health of river ecosystem and safeguard the drinking water supply.

Herein, sewage was demonstrated to be the most important nitrate source in Zhaoshandu reservoir watershed. Thus, there is an urgent need for enhanced collection and treatment of sewage effluent, including expanding the sewage pipeline networks, constructing new wastewater treatment plants, improving wastewater treatment processes to reduce the nitrogen loads in discharge of wastewater treatment plant, as well as developing in-situ treatment measures (e.g., constructed wetlands) in the rural zone, to mitigate the nitrate pollution derived from sewage containing high nitrogen concentrations. Moreover, SN also contributed a considerable amount of nitrate in Zhaoshandu reservoir watershed. As a result, it is recommended to implement best management practices like buffer strips and riparian plantings to prevent soil nitrogen loss to nearby rivers.

5. Conclusion

This study evaluated the riverine NO_3^- concentration and isotope characteristics, nitrification-induced ϵ_N , and then successfully resolved the evolution of nitrate sources with the help of a MixSIAR model in a drinking water source watershed of Eastern China. This is a first study to integrate the MixSIAR model, traditional dual nitrate isotopes, nitrification-derived ϵ_N , and $\Delta^{17}\text{O-NO}_3^-$ to calculate the contributions of different potential nitrate sources. Results showed that water quality in Zhaoshandu reservoir was impaired for drinking water utilization (Class-III standard) by NO_3^- levels. The tributaries exhibited higher nitrate concentrations than the mainstream. In addition, elevated nitrate concentrations were found during summer in comparison to other seasons. The $\delta^{15}\text{N-NO}_3^-$, $\delta^{18}\text{O-NO}_3^-$, and $\Delta^{17}\text{O-NO}_3^-$ values of collected river samples ranged from 1.8 to 8.5 ‰, from −7.8 to 11.6 ‰, and from −0.6 to 2.1 ‰, respectively. Hydrochemical and isotopic evidence revealed that nitrification was the primary nitrogen transformation process influencing NO_3^- concentrations and isotopic composition. Incubation of watershed soils revealed an ϵ_N for the nitrification of $-6.9 \pm 1.8\%$. Incorporation of the nitrification ϵ_N in the MixSIAR model remarkably improved the efficiency of NO_3^- source apportionment. At the watershed outlet, MS was the largest contributor of NO_3^- ($46.2 \pm 10.7\%$), followed by SN ($32.3 \pm 18.5\%$), NF ($19.7 \pm 13.1\%$) and AD ($1.8 \pm 1.6\%$). Higher contributions of AD ($6.0 \pm 1.8\%$) and SN ($30.0 \pm 19.7\%$) occurred in summer, whereas a higher contribution of MS occurred in the drier winter and autumn periods. With incorporation of $\Delta^{17}\text{O-NO}_3^-$ values for summer samples into the model with ϵ_N and $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$, the contributions of MS, SN, NF and AD to riverine nitrate during summer were $43.4 \pm 14.1\%$, $29.3 \pm 19.4\%$, $19.8 \pm 13.8\%$, and $7.5 \pm 1.6\%$, respectively. The average uncertainty index was smaller when incorporating $\Delta^{17}\text{O-NO}_3^-$ into the model with nitrification ϵ_N and $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$.

These results highlight the efficacy of using $\Delta^{17}\text{O-NO}_3^-$ and nitrification ϵ_N to improve nitrate source identification and apportionment in riverine systems. However, it should be acknowledged that the determination of the isotope enrichment factor was conducted through soil incubation experiments in a controlled laboratory setting rather than in field experiments, which may introduce uncertainties associated with the estimation results. Furthermore, the limited frequency of sampling events poses challenges for a comprehensive assessment of nitrogen sources within the watershed since the large temporal variability in watershed processes. Currently, determining the isotope enrichment factor existing N-cycling via in field experiment remains challenging, so

Table 3

Proportion and uncertainty index (UI_{90}) for the four putative NO_3^- sources as determined by different model formulations for the June 2021 samples.

Model		AD	MS	NF	SN
Model 1	Proportion	6.1 ±	24.2 ±	30.6 ±	39.1 ±
	(%)	1.9	15.8	17.2	23.9
	UI_{90}	0.071	0.563	0.616	0.836
Model 2	Proportion	6.0 ±	43.2 ±	20.7 ±	30.1 ±
	(%)	1.8	14.0	14.0	19.7
	UI_{90}	0.067	0.507	0.498	0.696
Model 3	Proportion	7.5 ±	43.4 ±	19.8 ±	29.3 ±
	(%)	1.6	14.1	13.8	19.4
	UI_{90}	0.058	0.528	0.486	0.669

Model 1: MixSIAR model based on $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$.

Model 2: MixSIAR model based on $\delta^{15}\text{N}/\delta^{18}\text{O-NO}_3^-$ and ^{15}N -enrichment factor.

Model 3: MixSIAR model based on $\delta^{15}\text{N}/\delta^{18}\text{O}/\Delta^{17}\text{O-NO}_3^-$ and ^{15}N -enrichment factor.

AD: atmospheric deposition nitrate, NF: nitrogen fertilizer, SN: soil organic nitrogen, MS: manure and sewage.

that future research is warranted to address this knowledge gap. Besides, more samples from river water and pollution sources across different temporal scales are necessary to capture variability under varying climate and hydrological conditions, thereby improving model estimation accuracy. Moreover, inclusion of $\delta^{11}\text{B}$ is recommended as it can aid in separating riverine nitrate sourced from manure and sewage, thus providing a better understanding regarding the impacts of watershed pollution sources on river nitrate pollution.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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CRediT authorship contribution statement

Lielin Shu: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Wenli Chen:** Formal analysis, Investigation. **Yinli Liu:** Investigation. **Xu Shang:** Conceptualization. **Yue Yang:** Investigation, Supervision. **Randy A. Dahlgren:** Conceptualization, Methodology, Supervision, Writing – original draft. **Zheng Chen:** Supervision. **Minghua Zhang:** Funding acquisition, Supervision. **Xiaoliang Ji:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.170617>.

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