Environmental Science and Pollution Research (2018) 25:32631–32639
https://doi.org/10.1007/s11356-018-3242-1

RESEARCH ARTICLE

Using dual isotopes and a Bayesian isotope mixing model to evaluate sources of nitrate of Tai Lake, China

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Received: 11 April 2018 / Accepted: 13 September 2018 / Published online: 21 September 2018

Abstract

Identification and quantification of sources of nitrate (NO_3^-) in freshwater lakes provide useful information for management of eutrophication and improving water quality in lakes. Dual $\delta^{15}N$- and $\delta^{18}O$-NO_3^- isotopes and a Bayesian isotope mixing model were applied to identify sources of NO_3^- and estimate their proportional contributions to concentrations of NO_3^- in Tai Lake, China. In waters of Tai Lake, values for $\delta^{15}N$-NO_3^- ranged from 3.8 to 10.1‰, while values of $\delta^{18}O$ ranged from 2.2 to 12.0‰. These results indicated that NO_3^- was derived primarily from agricultural and industrial sources. Stable isotope analysis in R called SIAR model was used to estimate proportional contributions from four potential NO_3^- sources (agricultural, industrial effluents, domestic sewage, and rainwater). SIAR output revealed that agricultural runoff provided the greatest proportion (50.8%) of NO_3^- to the lake, followed by industrial effluents (33.9%), rainwater (8.4%), and domestic sewage (6.8%). Contributions of those primary sources of NO_3^- to sub-regions of Tai Lake varied significantly ($p < 0.05$). For the northern region of the lake, industrial source (35.4%) contributed the greatest proportion of NO_3^-, followed by agricultural runoff (27.4%), domestic sewage (21.3%), and rainwater (15.9%). Whereas for the southern region, the proportion of NO_3^- contributed from agriculture (38.6%) was slightly greater than that contributed by industry (30.8%), which was similar to results for nearby inflow tributaries. Thus, to improve water quality by addressing eutrophication and reduce primary production of phytoplankton, NO_3^- from both nonpoint agricultural sources and industrial point sources should be mitigated.

Keywords Nitrate · Sources · Dual isotopes · Bayesian isotope mixing model · Eutrophication · Tai Lake

Highlights

1. Dual isotopes and a Bayesian mixing model were applied to identify NO_3^- sources.
2. Agriculture and industry are main contributors to NO_3^- sources in Tai Lake.
3. Industrial source (35.4%) was the largest nitrate contributor to northern lake.
4. Agricultural source (38.6%) was the largest nitrate contributor to southern lake.
5. The results will help evaluate remediation efforts and strategies in the region.

Responsible editor: Philippe Garrigues

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Introduction

Eutrophication, which is often related to anthropogenic activities, is a severe environmental problem in many freshwaters, and has caused ever increasing concern worldwide (Carpenter 2005; Chen et al. 2015; Zhu et al. 2015). Eutrophication occurs when excess nutrients, including primarily phosphorus (P), but also nitrogen (N) and sometimes trace elements, such as Iron (Fe) are available in aquatic ecosystems, particularly lakes. Eutrophication greatly reduces transparency and dissolved oxygen and thus can impair overall water quality (Kendall et al. 2007; Nestler et al. 2011; Vitousek et al. 1997). Strategies for reduction of eutrophication are generally focused on limiting allochthonous sources of P to lakes. Phosphorus is normally the critical, limiting nutrient in freshwaters and generally easier to control than input of N (Chen et al. 2015; Feng et al. 2016; Liu et al. 2016; Liu et al. 2017). Nitrogen can be present in many forms, some of which are exchangeable with the atmosphere (Griffin et al. 2002; He et al. 2014). Thus there can be multiple sources of N, including fixation of atmospheric nitrogen with loading to lakes from precipitation, discharges from municipal sewage treatment plants and industrial effluents and runoff of agricultural fertilizers (Xue et al. 2009). Amounts of nitrate (NO_3^-), which is the most readily available form of N, varies greatly among lakes (Xue et al. 2009). Therefore, identification of sources of NO_3^- and quantitative estimation of relative contributions from different sources is critical to efficiently control input of NO_3^- during efforts to remediate eutrophication and restore water quality in lakes.

Since various sources of NO_3^- have distinct ratios of isotopes of nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$), it is possible to use the dual isotope approach of ^{15}N- and ^{18}O-NO_3^- to identify sources of NO_3^- and allocated relative proportions among those sources (Ji et al. 2017; Wankel et al. 2006; Xue et al. 2012; Xue et al. 2009). For example, NO_3^- derived from rain and chemical fertilizer generally has lesser ^{15}N-NO_3^- values ranging from − 6 to + 6‰ and from − 13 to + 13‰, respectively, than that from urban sewage (from + 4 to + 19‰) and livestock manure (from + 5 to + 29‰) (Mayer et al. 2002; Xue et al. 2009). Also, ^{18}O signatures in atmospheric NO_3^- (from 25 to 75‰) and NO_3^- produced by microbes in soil (from 0 to 15‰) differ significantly (Kendall et al. 2007; Pardo et al. 2004; Xue et al. 2009). Furthermore, over the last two decades, $\delta^{15}N$- and $\delta^{18}O$- NO_3^- have been widely used to quantify relative contributions of NO_3^- from various sources, by use of mass balance mixing (Davis et al. 2015; Kim et al. 2015; Li et al. 2010; Liu et al. 2006). A mass balance model, based on stable isotopes of N and O in NO_3^- was successfully applied to estimate relative contributions of three sources of NO_3^- (agricultural wastewater, atmospheric deposition, and leaching and runoff from soils) in the Baltic Sea catchment (Fry, 2003). Also, it was reported that the dual isotope approach could be used to identify relative proportions of NO_3^- in riverine environments from atmospheric deposition, agricultural soils, and groundwater, by use of a mass balance model (Deutsch et al., 2006). These observations provided insight into sources of N and served as an effective tool to optimize control of nitrogen. However, this mixing model does not take into account of several substantial sources of uncertainty due to temporal and spatial variability in $\delta^{15}N$- and $\delta^{18}O$- NO_3^- in NO_3^-, and fractionation during denitrification (Moore and Semmens, 2008). Also too many NO_3^- sources contributing to the mixture (i.e., number of sources > number of isotope + 1) would made the model impossible to quantify the sources of nitrate (Moore and Semmens 2008; Xue et al. 2009). To fully incorporate those sources of uncertainty, a Bayesian stable isotope mixing model called SIAR was developed and implemented (Moore and Semmens 2008; Parnell et al. 2013). SIAR uses a Bayesian framework to determine the probability distribution of contributions of each source to a mixture (Parnell et al. 2013). This model has been applied to quantify contributions of various sources to mixtures in surface water, ground water, and the atmosphere (Divers et al. 2014; Lockhart et al. 2014; Xue et al. 2012; Zong et al. 2017).

The objective of this study was to apply the SIAR model for determination of relative contributions of NO_3^- from various sources to Tai Lake. Distinct isotopic characteristics of NO_3^- from various sources, including agricultural runoff, industrial discharges, domestic sewage, and rain to rivers in the catchment of Tai Lake were evaluated. Based on the multiple isotope' values and concentrations of NO_3^- in water of Tai Lake, a Bayesian, numerical mixing model that included four sources was developed and applied to understand sources of NO_3^- as well as nitrification and denitrification in the lake. The Bayesian, numerical mixing model was then used to determine relative contributions of various sources from agricultural, industrial, domestic sewages, and rainwater to the NO_3^- in the lake so that the most effective management could be applied in restoration of Tai Lake.

Materials and methods

Site description

Tai Lake (Ch: Taihu), which located between 30° 55′ to 31° 32′ N latitude and 119° 52′ to 120° 36′E longitude, is the largest freshwater lake in the Yangtze River Delta, China. It covers 0.4% of the total area of China with a surface area of 2338 km^2, average depth of less than 2 m, and total storage capacity of 4760 million m^3. The watershed is mainly controlled by a subtropical monsoon climate, which provides an annual, mean precipitation of 1100–1150 mm, primarily from April to October (Chen et al. 2012a; Qin et al. 2007).

The whole lake connects Jiangsu and Zhejiang provinces which accounted for more than 14% of China's gross domestic production. The lake was surrounded by boarders Wuxi city in the north, Suzhou city in the east, Huzhou city in the south and Yixing city in the west. As an important water resource in the lower reaches of the Yangtze River, Tai Lake supplies drinking water to these surrounding cities and is simultaneously utilized for navigation, aquaculture, irrigation, flood control, and tourism. Thus, the northwestern and southern parts of the lake are dominated by high density urban areas, where are featured with hyper-eutrophic and annual cyanobacteria blooms.

Collection and analysis of samples

In order to determine pollution sources in the area, a systematic investigation was carried out in end-member waters and various regions of the Tai Lake watershed. The end-member water samples were collected from four major sources (agricultural, industrial, domestic sewage, and atmospheric sources). Six water samples of agricultural runoff were collected from the western, northern, and southern lakeshore farmland, respectively. Five samples of industrial sources were collected from two industrial parks in Suzhou and Huzhou along the shore of Tai Lake. Five domestic sewage samples were collected from outfalls of urban domestic sewage treatment plants in Suzhou and Huzhou. And one atmospheric deposition sample was collected from rainwater above the surface of the Suzhou area of Tai Lake. Mixed water samples were collected from 16 selected sample sites in lake and major inflow rivers by 2.5-L Plexiglass water sampler on May 2011 (Fig. 1). These sampling sites were classified as representative of three sub-regions: inflow rivers (R1 to R5), northern lake (NL-1 to NL-5), and southern lake (SL-1 to SL-6). All of the sampling equipment were pre-cleaned with deionized water. Water samples were collected by boat in the middle of the inflow stream and lake at a depth of ~20 cm and stored in pre-cleaned polyethylene bottles. Collected samples were filtered on site through pre-combusted (450 °C, 12 h) and individually pre-weighed glass fiber filters (Whatman, GF/F, 47 mm in diameter). Filtrate samples were stored at −30 °C until required for further treatments.

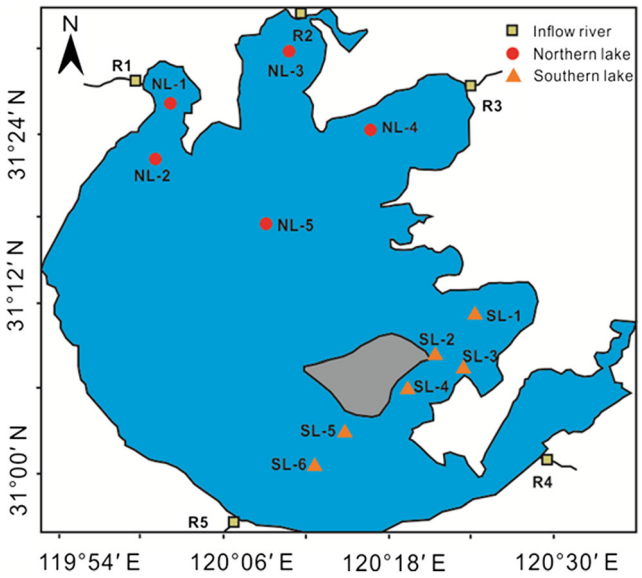

Fig. 1 Location of the 16 sampling points in both inflow rivers and Tai Lake. Inflow rivers (R1 to R5), northern lake (NL-1 to NL-5), and southern lake (SL-1 to SL-6)

Concentrations of NO_3^- were determined by ion chromatography by use of a Dionex model 90 with a precision of 5%. Nitrate was collected by resins (Bio-Rad AG50W-X4 and Bio-Rad AG1-X8) and subsequently converted to $AgNO_3$ (Silva et al. 2000). Resin columns were stored cool until further preparation at the laboratory. Nitrate was eluted from the anion exchange resin by use of 3 mol/L HCl. After SO_4^{2-} and PO_4^{3-} were removed by precipitation with $BaCl_2$, filtered eluate was then passed through cation exchange resin. An excess of Ag_2O was then used to remove Cl^- and the solution was neutralized to pH of 5–6, after which, the solution of $AgNO_3$ was freeze-dried for isotopic analysis. Isotopic compositions of N and O in NO_3^- were determined by use of elemental analysis-isotope ratio mass spectrometry (EA-IRMS), as δ notation per mil (‰) relative to N_2 in air (Li et al. 2010; Voss et al. 2006) and Vienna Standard Mean Ocean Water (VSMOW), respectively (Eq. 1).

$$\delta_{\text{sample}}(‰) = \left(\left(R_{\text{sample}}/R_{\text{standard}}\right)-1\right) \times 1000 \quad (1)$$

where R_{sample} and R_{standard} are the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio of the sample and standard for $\delta^{15}N$ and $\delta^{18}O$, respectively. The analytical precisions of $\delta^{15}N$- NO_3^- and $\delta^{18}O$-NO_3^- are ±0.2‰ and ±0.5‰, respectively.

Statistics

To check whether there was a significant difference between values of $\delta^{15}N$ or $\delta^{18}O$ among sampling sites, and the difference of proportional contributions among locations, the Tukey HSD (Tukey Honest Significant Difference) test was applied to perform a multiple comparison.

A Bayesian isotope mixing model

To quantify proportional contributions of potential sources of NO_3^- to surface waters of Tai Lake, a Bayesian isotope mixing model (Stable Isotope Analysis in R, SIAR) was applied. By defining a set of N mixture measurements on J isotopes with K source contributors, the mixing model can be expressed

(Eq. 2) (Parnell et al. 2010; Parnell et al. 2013; Phillipsdonald et al. 2014).

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

where X_{ij} is the isotope value j of the mixture I, in which $i = 1, 2, 3,..., N$ and $j = 1,2,3,..., J$; S_{jk} is the source value k based on isotope j ($k = 1, 2, 3, \ldots, K$) and is normally distributed with mean μ_{jk} and standard deviation ω_{jk}; p_k is the proportion of source k, which needs to be 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. A detailed description of this model can be found in Parnell et al. (2010), Moore and Semmens (2008), and Phillips et al. (2005).

Results and discussion

Sources and controlling factors for NO_3^- in lake waters

The concentrations of NO_3^- and δ^{15}N- and δ^{18}O- NO_3^- data of the 16 sampling points were compared between different parts of lakes and inflow rivers. Concentrations of NO_3^- in Tai Lake ranged from 4.9 to 29.1 mg/L with a mean of 19.9 mg/L. The NO_3^- concentrations in inflow rivers ranged from 5.6 to 23.3 mg/L. The δ^{15}N- and δ^{18}O- NO_3^- values of the mixing water samples in Tai Lake are provided in Fig. 2. There was a narrow range of δ^{15}N- NO_3^- from 6.8 to 10.1‰ with a mean value of 8.6‰ in Tai Lake. For inflow rivers, δ^{15}N- NO_3^- values ranged from 8.4 to 9.9‰, which were relatively greater than those in lakes. Mean δ^{15}N- NO_3^- value for the northern portion of Tai Lake was relatively lesser than other parts, which, ranged from 6.8 to 9.4‰. The δ^{15}N- NO_3^- values for the southern portion of Tai Lake were intermediate, ranging from 7.4 to 10.1‰. This result of δ^{15}N- NO_3^- values is consistent with previous reported by Townsend-Small et al. (2007) and Chen et al. (2014), who also investigated the isotopic composition of N in Tai Lake. Values of δ^{18}O in NO_3^- ranged from 2.2 to 12.0‰ with a mean value of 8.2‰. ^{18}O- NO_3^- Values for inflow rivers were least, ranging from 2.2 to 7.7‰. Values of δ^{18}O-NO_3^- for the northern portion of Tai Lake varied from 10.6 to 12‰, while in the southern portion of Tai Lake, δ^{18}O-NO_3^- value ranged from 6.0 to 11.4‰. These results were also consistent with observations reported previously (Chen et al. 2014; Li et al. 2010). These revealed that isotopic composition of NO_3^- in Tai Lake was rather heterogeneous, and inflow rivers might contribute the greatest proportion to values of δ^{15}N and δ^{18}O in Tai Lake.

There was no distinct positive or negative relationship between contents and isotopic values of NO_3^- for the lake waters (Fig. 3), suggesting that there were multiple sources and factors affecting distribution of NO_3^- in Tai Lake. The Tai Lake watershed has several sources of NO_3^-, of which inputs are vulnerable to seasonal, climatic, geographical, and human factors. In general, sources of NO_3^- include organic nitrogen in soil, fertilizers, human and animal excreta, industrial wastewater, domestic sewage, and rainwater (Xue et al. 2009). Thus, the classical, dual isotope bi-plot approach (δ^{15}N- and δ^{18}O- NO_3^-) was used to identify predominant sources of NO_3^- at various locations in Tai Lake, as well as inflow rivers (Fig. 4). Isotope signatures of those sites mainly fall into the "manure and swage" and "Soil N" source windows, which indicate that most of the loading of NO_3^- is from soil, manure and sewage near Tai Lake and inflow rivers. Thus, the predominant source of NO_3^- to Tai Lake was attributed to soil N, manure and domestic sewage, and agricultural and industrial wastewater.

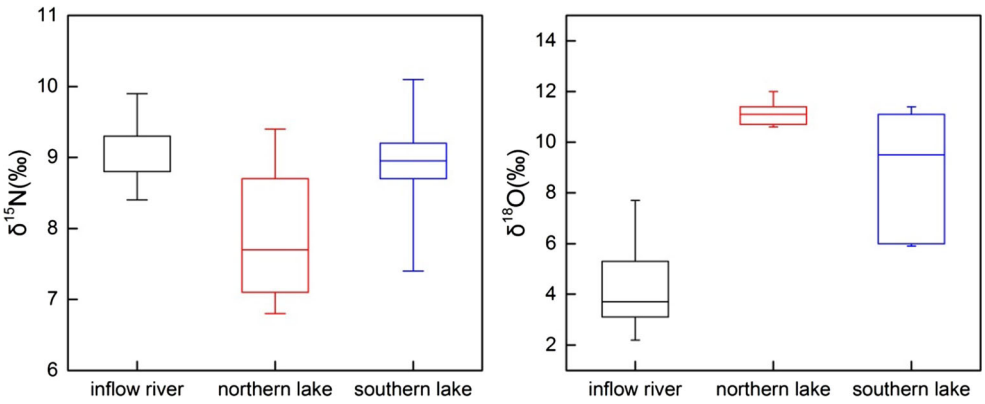

Fig. 2 Boxplots of δ^{15}N- NO_3^- (**a**) and δ^{18}O- NO_3^- (**b**) observed in inflow rivers, northern lake, and southern lake regions in Tai Lake. Boxplots illustrate the 25th, 50th, and 75th percentiles and whiskers indicate the 5th and 95th percentiles

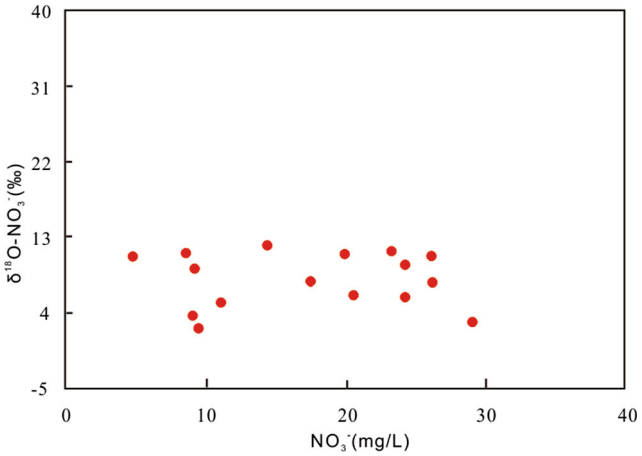

Fig. 3 Relationship between concentrations of NO_3^- and values of $\delta^{18}O$-NO_3^- for water in Tai Lake

There are several processes that affect the fate of NO_3^- in the lake. These include denitrification, accumulation by phytoplankton and nitrification of organic matter. The NO_3^- in inflows (sites R1 to R5) had $\delta^{18}O$-NO_3^- values that were less than that from the lake, which indicates that NO_3^- measured in Tai Lake originated primarily from industrial point pollution and nitrification of organic matter. Bacteria and algae generally preferentially use lighter isotopes during denitrification and assimilation, which would lead to enriching heavier isotopes in NO_3^-. The northern part of the lake is hyper-eutrophic and characterized by annual blooms of cyanobacteria (Chen et al. 2012a), which could explain locally greater proportions of heavier isotopes of N in NO_3^- at locations NL-1 to NL-5. Nitrate in waters from other regions of Tai Lake might be affected primarily by runoff from aquaculture

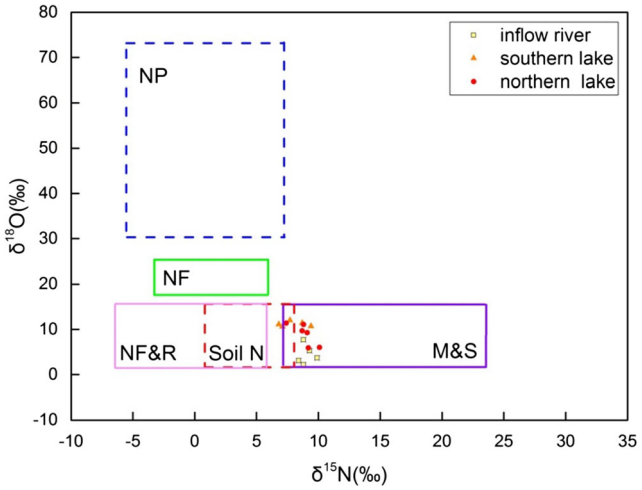

Fig. 4 Mean $\delta^{15}N$- and $\delta^{18}O$- NO_3^- values of the mixing water samplings in Tai Lake. Ranges of isotopic compositions for five potential sources are taken from by Xue et al. (2009) and indicated by boxes: NO_3^- in precipitation (NP), NO_3^- fertilizer (NF), NH_4^+ in fertilizer and rain (NF&R), soil N (Soil N) and manure and sewage (M&S)

and denitrification, especially in the eastern portion of Tai Lake, which lacks major inflows from rivers (Qin et al. 2007). It has been reported that sediments can be important in dynamics of denitrification in Tai Lake (Mccarthy et al. 2007). It has also been reported that conventional denitrification plays a significant role in removal of NO_3^- from cyanobacteria as an organic carbon source (Chen et al. 2012a). Thus, in this study, denitrification needs to be further checked whether it is an important factor in isotope fractionation in NO_3^- in Tai Lake.

Conclusive information regarding all predominant NO_3^- sources cannot be obtained using the bi-plot approach. Therefore, a more quantitative technique based on a dynamic model of isotopic mixing was deemed to be necessary to be applied for identification of sources and determination of their relative contributions to concentrations in Tai Lake.

End-members of $\delta^{15}N$ and $\delta^{18}O$ designated for use in mixing models used to estimate sources of NO_3^-

Before calculating the proportional contributions from potential sources of NO_3^-, values of $\delta^{15}N$- and $\delta^{18}O$- NO_3^- of four potential NO_3^- sources to Tai Lake were measured (Table 1). Among the four potential sources of NO_3^- to Tai Lake, the six agricultural sources had $\delta^{15}N$ values ranging from 3.6 to 4.9‰ and $\mu \pm \sigma$ was 4.1 ± 0.5; their $\delta^{18}O$ values ranged from 16.3 to 24.2‰ and $\mu \pm \sigma$ was 20.4 ± 3.0‰. For the four industrial sources, $\delta^{15}N$ values ranged from 11.1 to 18.3‰ and $\mu \pm \sigma$ was 15.3 ± 2.7‰; $\delta^{18}O$ values ranged from 2.0 to 3.9‰ and $\mu \pm \sigma$ was 3.1 ± 0.7‰. For the five domestic sewage sources, $\delta^{15}N$ values ranged from 19.3 to 23.9‰ and $\mu \pm \sigma$ was 22.2 ± 2.2‰; $\delta^{18}O$ values ranged from -2.7 to -1.2‰ and $\mu \pm \sigma$ was -2.1 ± 0.6‰. For atmospheric deposition (rainwater), the $\delta^{15}N$ and $\delta^{18}O$ values were 1.3 and 33.2‰, respectively.

Using δ values of N and O isotopes as the horizontal and vertical coordinates, respectively, a coordinate system for δ values for these isotopes in NO_3^- pollution sources of Tai Lake was established (Fig. 5). To determine the sources of NO_3^- based on values of stable isotopes, the δ values of stable isotopes from different sources should have significant differences after correcting for potential fractionation. Inappropriate choices of potential sources of NO_3^- put into the model can affect the solutions to the equations and thus the accuracy of apportionment among potential sources. The values of isotopic ratios for N and O in NO_3^- from agricultural sources fell within ranges of values for $\delta^{15}N$ and $\delta^{18}O$ reported for NO_3^- in fertilizer, which indicates that agriculture was a source of NO_3^- from fertilizers. δ values for N and O in rainwater from the Tai Lake region, were consistent with ranges of NO_3^- reported for precipitation in other areas (Kendall et al. 2007). Values of δ for N and O isotopes in industrial and domestic sewages observed during this study were consistent with those

Table 1 End-members of $\delta^{15}N$ and $\delta^{18}O$ designated for the mixing models

Sources	$\delta^{15}N$	$\delta^{18}O$
Agriculture	4.1 ± 0.5	20.4 ± 3.0
Industry	15.3 ± 2.7	3.1 ± 0.7
Domestic sewage	22.2 ± 2.2	−2.1 ± 0.6
Rain	1.3	33.2

previously observed for manure and septic waste in other areas. The dual isotopic pattern indicated that NO_3^- in Tai Lake was derived primarily from a mixture of agricultural and industrial sources as well as municipal sewage. In the coordinate system, values of δ of NO_3^- in water from the same source were similar and centered, while δ values of the various sources were different and did not overlap (Fig. 5). Consequently, proportions of isotopes of N and O in NO_3^- of in mixed lake water from Tai Lake were distinct, and can be used to estimate proportional contributions of NO_3^- from various sources.

Estimation of proportional contributions of NO_3^- from various sources

Denitrification is a process that leads to greater value of $\delta^{15}N$- and $\delta^{18}O$- NO_3^-. A linear relationship with an enrichment of ^{15}N relative ^{18}O by factors of 1.3:1 and 2.1:1 is indicative of denitrification (Fukada et al. 2003; Xue et al. 2009). In the current study, results of which are presented here, enrichment of $\delta^{15}N$ and $\delta^{18}O$ were not observed for NO_3^- in water from Tai Lake (Fig. 5), which indicated that no obvious denitrification occurred during the period of study. Also, denitrification requires anaerobic or hypoxic conditions, where dissolved

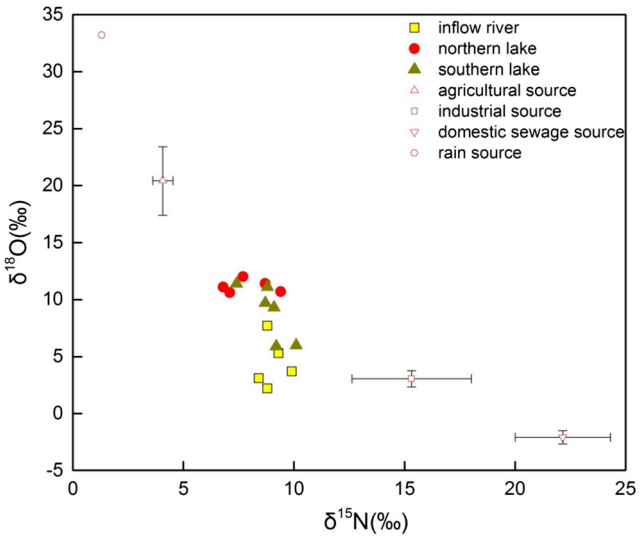

Fig. 5 Ranges of values of $\delta^{15}N$ and $\delta^{18}O$ in samples of water from end-members, inflow rivers and Tai Lake

oxygen concentrations are not greater than 2 mg/L (Rivett et al. 2008). However, the mean dissolved oxygen concentration measured in the sampling sites was 6.6 mg/L, which is not deal for denitrification. Thus, contributions of denitrification to differences in $\delta^{15}N$- and $\delta^{18}O$ of NO_3^- in waters of Tai Lake can be ignored. Indeed, many previous studies have also reported that denitrification rarely occurs in surface waters (Ding et al. 2014; Xing and Liu 2016; Xue et al. 2012).

SIAR modeling was applied to estimate proportional contributions of four potential sources of NO_3^-, agricultural, industrial, domestic sewage, and rainwater to Tai Lake. Results of SIAR modeling showed that contributions varied significantly ($p < 0.05$) among sources. Ranges of proportions contributed by each source to NO_3^- in water are shown in Fig. 6a. For the whole lake, the greatest contribution, with a mean value of 50.8%, was from agriculture, followed by industrial (33.9%), rainwater (8.4%), and domestic sewage (6.8%). This revealed that nonpoint source pollution from agriculture and point source pollution from industry are predominant NO_3^- source in Tai Lake, which could be attributed to intensive agriculture and industrialized urban areas such as the cities of Changzhou, Suzhou, and Wuxi that surround Tai Lake. Sources of NO_3^- estimated by use of SIAR were consistent with qualitative results of analyses based on dual isotopes. Results of this study were also consistent with those reported previously (Yi et al., 2017), which demonstrated nonpoint sources from fertilizer/soils constituted the greatest proportion of total nitrogen loaded to Tai Lake during the high flow season of summer. However, proportional contributions of precipitation observed during this study were less than previously reported during summer (Chen et al. 2012b; Yi et al. 2017). One possible explanation might be that there was less rainfall during our sampling period than during the contemporaneous monsoon. The speculation was supported by the lesser precipitation of 45.3 mm in the Tai Lake watershed during May 2011 (Shi and Mao 2013). It can be concluded that sources and cycling of nitrogen was closely associated with the hydrological regime in drainage basins (Yi et al. 2017). Another possible explanation is due to the fact that the end-member isotope signatures of potential sources in this study were based on field collection and measurements, while most previous studies were referred to literature rather than direct measurements (Xue et al. 2012; Yi et al. 2017).

SIAR was also applied to estimate proportions of NO_3^- contributed by various sources in each sub-region including inflow rivers (R1 to R5), the north lake locations (NL-1 to NL-5) and southern lake regions (SL-1 to SL-6). The SIAR mixing model outputs revealed a high variability in contributions of the four potential sources of NO_3^-. Contributions of these four sources of NO_3^- to sub-regions are shown in Fig. 6. Sources apportionment was similar between the southern region of the lake and inflow rivers (Fig. 6b, d). Contributions from agriculture to the rivers and southern portion of the lake

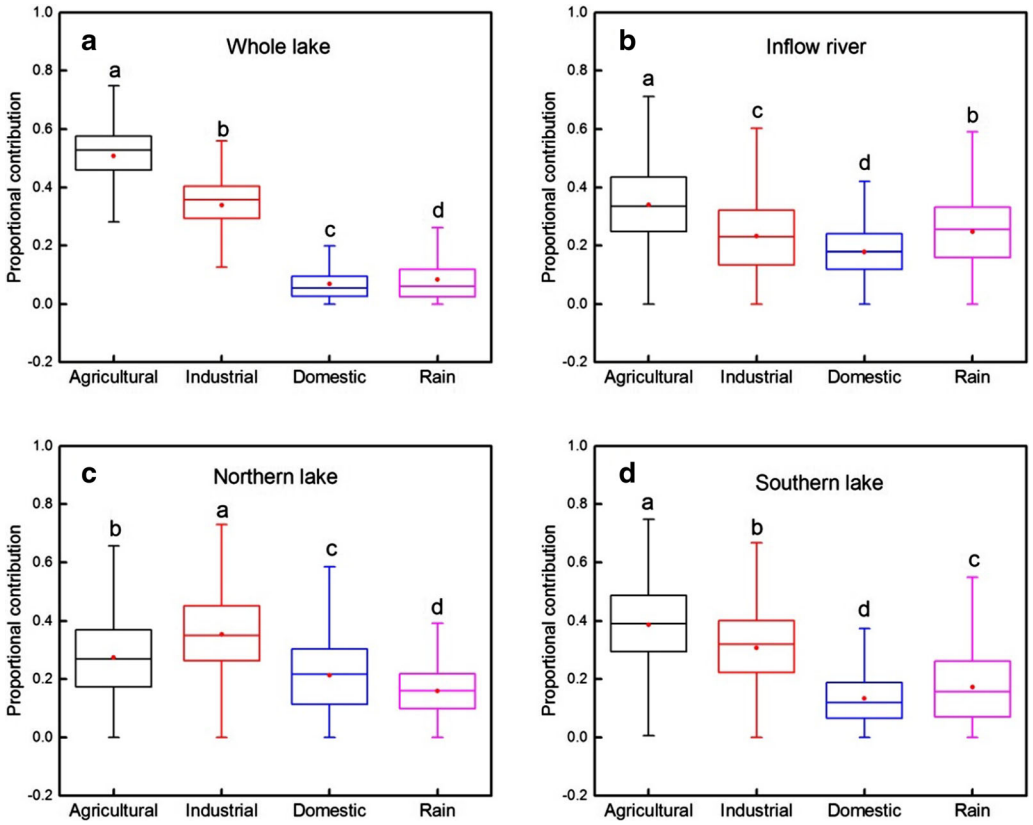

Fig. 6 Proportional contribution of four potential sources of NO_3^- for the whole lake (**a**) and sub-region including inflow river (**b**), northern Tai Lake (**c**) and southern Tai Lake (**d**). The four potential sources are agricultural, industrial, domestic, and rain sources. Boxplots illustrate the 25th, 50th, and 75th percentiles; the whiskers indicate the 5th and 95th percentiles; the red circles indicate the mean percentiles. Lowercase letters above the upper whiskers represent a significant difference between the two sources by ANOVA results

were greatest (34.0% and 38.6%, respectively), while the contributions of domestic sewage were least (17.9% and 13.3%, respectively), and contributions from the two other sources were intermediate (industrial were 23.2% and 30.8, respectively; rain were 24.8% and 17.3%, respectively). In the northern portion of the lake, the greatest proportion of NO_3^- was contributed by industrial effluents (Fig. 6c), which accounting for 35.4% during the sampling period, followed by agriculture (27.4%), domestic sewage (21.3%), and rainfall (15.9%). The result that greater contributions from industry to the northern region of Tai Lake was likely due to the more industrialized urban areas such as Changzhou, Wuxi, and Yixing surrounded. The greater contribution from agriculture to the southern region of Tai Lake might be attributed to the livestock, farm pesticides, and fertilizers widely used in this region. These results from SIAR calculations for the southern and northern portions of Tai Lake are consistent with previous reports (Chen et al. 2014), where it was suggested that sewage and manure were dominant sources of NO_3^- to western Tai Lake (named north lake in our study), and sources in the eastern portion of Tai Lake (named south lake in this study) were likely derived from soil organic nitrogen. However, contributions of NO_3^- from various inflow rivers observed by Chen et al. (2014), suggested that NO_3^- in feeder rivers was mainly from sewage and manure. This could be attributed to the location of sampling sites of inflow rivers. In the current study, sampling sites of inflow rivers were from both north and south, while only north rivers were included in the study of Chen et al. (2014). From this it can also be concluded that agricultural sources contribute most of the nitrates to southern rivers. Outputs of the SIAR model based on three sub-regions were consistent with analysis results based on the whole lake. In summary, agricultural and industrial sources were the primary contributors of NO_3^- to Tai Lake.

Through rational partitioning, the isotopic method effectively revealed various sources of NO_3^- to Tai Lake. Meanwhile, some uncertainties were evaluated by use of the SIAR mixing model in this study. Since it was difficult to distinguish NO_3^- derived from manure and sewage, as well as NO_3^- derived from NH+ 4 fertilizer, nitrate fertilizer, rain and soil N, potential sources were classified as agricultural, industrial, domestic, and rain. Thus, the overlapping between various sources in the nitrogen and oxygen stable isotope coordinate system

can be avoided. Additionally, end-member isotopic signatures of these four potential sources of NO_3^- are directly measured using end-member water samples rather than using values from the literature. Even though, uncertainties also remain in the calculation of relative contributions of sources. For example, calculations of posterior distributions of outputs have relatively large variations among seasons. However, seasonal variations in absolute and relative concentrations of concentrations of NO_3^- were not measured in this study. As a consequence, multiple uncertainties associated with the mixing model should be acknowledged, and the mixing model results should be interpreted with caution (Kaushal et al. 2011).

Implications for nitrogen reduction in Tai Lake

In this study, the SIAR mixing model was parameterized and then used to calculate more accurate contributions of four different sources (i.e., agricultural, industrial, domestic sewage and rainwater) of NO_3^- to Tai Lake. Despite limitations of the approaches and uncertainties in the data, results obtained could provide guidance for reduction of loadings of nitrogen to Tai Lake. Results revealed that proportional contributions of agriculture were greatest among the four potential source classes of NO_3^-. Industrial effluents were another significant source of NO_3^- to Tai Lake. Thus, substantial control of nonpoint sources from agriculture and point sources from industries would significantly decrease the sources of NO_3^- to Tai Lake. Sources of NO_3^- released by agricultural activities include applications of fertilizer and manure. Thus, optimization and control of use of chemical fertilizers is a useful strategy for controlling nitrogen pollution. Moreover, extensive management of livestock farms and advanced processing technology of manure can effectively prevent contamination from releases of excrement from livestock. Additionally, for control of nonpoint sources or diffuse point source pollution, potential for denitrification in the lake catchment should be enhanced. Water zones can provide possible buffers for retention of nitrogen, and is useful for nitrogen removal (Burns et al. 2009; Yu et al. 2016). Ecological engineering practices, such as wetlands, ponds, and grass channels in riverine areas could play significant roles in reduction of loadings by nitrogen, as a long-term strategy (Yi et al., 2017). For industrial point source pollution, a strict standard for discharge of wastewater should be critical for the nitrogen reduction in the whole basin. As we all know, the strategy has been launched in Tai Lake basin since 2008, however, nitrogen pollution from industrial was still an important source for Tai Lake. Thus, control of sources of nitrogen still needs additional, long-term effort. Also, multiple efforts on reasonable plant layout, industrial structure would be beneficial for reduction of concentrations of nitrogen in Tai Lake.

Funding information The work was supported in part by the National Natural Science Foundation of China (Nos. 41807372, 41630645, 41603113, and 41573126) and China Postdoctoral Science Foundation (2017M622280 and 2016M591227).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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