ISSN 1001-0742
CN 11-2629/X

JES

JOURNAL OF ENVIRONMENTAL SCIENCES

April 1, 2015 Volume 30
www.jesc.ac.cn

MBR in Wastewater Reclamation

ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

Journal of Environmental Sciences Volume 30 2015

www.jesc.ac.cn

Highlight articles

Review articles

Regular articles

CONTENTS

Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

JOURNAL OF ENVIRONMENTAL SCIENCES
www.jesc.ac.cn

Assessment of the sources and transformations of nitrogen in a plain river network region using a stable isotope approach

Jingtao Ding[1], *Beidou Xi*[2,*], *Qigong Xu*[2], *Jing Su*[2], *Shouliang Huo*[2], *Hongliang Liu*[2], *Yijun Yu*[3], *Yanbo Zhang*[3]

1. *School of Environment, Beijing Normal University, Beijing 100875, China. E-mail: dingjingtao@163.com*
2. *Chinese Research Academy of Environmental Sciences, Beijing 100012, China*
3. *Changzhou Environmental Monitoring Center, Jiangsu 213001, China*

ARTICLE INFO

Article history:
Received 27 June 2014
Revised 9 October 2014
Accepted 9 October 2014
Available online 31 January 2015

Keywords:
Nitrate
Stable isotopes
Nitrification
Assimilation
Plain river network region

ABSTRACT

The great spatial and temporal variability in hydrological conditions and nitrogen (N) processing introduces large uncertainties to the identification of N sources and quantifying N cycles in plain river network regions. By combining isotopic data with chemical and hydrologic measurements, we determined the relative importance of N sources and biogeochemical N processes in the Taige River in the East Plain Region of China. The river was polluted more seriously by anthropogenic inputs in winter than in summer. Manure and urban sewage effluent were the main nitrate (NO_3^-) sources, with the nitrification of N-containing organic materials serving as another important source of NO_3^-. In the downstream, with minor variations in hydrological conditions, nitrification played a more important role than assimilation for the decreasing ammonium (NH_4^+-N) concentrations. The N isotopic enrichment factors (ε) during NH_4^+ utilization ranged from –13.88‰ in March to –29.00‰ in July. The ratio of the increase in $\delta^{18}O$ and $\delta^{15}N$ of river NO_3^- in the downstream was 1.04 in January and 0.92 in March. This ratio indicated that NO_3^- assimilation by phytoplankton was responsible for the increasing $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in winter. The relationships between $\delta^{15}N$ of particulate organic nitrogen and isotopic compositions of dissolved inorganic nitrogen indicated that the phytoplankton in the Taige River probably utilized NH_4^+ preferentially and mainly in summer, while in winter, NO_3^- assimilation by phytoplankton was dominant.

Introduction

Nitrogen (N) contamination in freshwater systems is a worldwide environmental problem. High levels of N not only pose a potential threat to human health but also stimulate water eutrophication (Kendall, 1998; Lee et al., 2008). External supplies of N to river water are generally associated with inputs from anthropogenic activities (*e.g.*, municipal and industrial wastewater, livestock wastewater, atmospheric deposition and agricultural runoff). N usually undergoes numerous transformations, such as nitrification, assimilation, and denitrification; the occurrence and extent of these processes depend on a variety of environmental variables (Middelburg and Nieuwenhuize, 2001). Identification of the sources and forms of N, as well as their changes with time, is an important step in improving the management practices associated with maintaining water quality in rivers (Sugimoto et al., 2011; Li et al., 2010).

* *Corresponding author*. E-mail: xibeidou@263.net (Beidou Xi).

http://dx.doi.org/10.1016/j.jes.2014.10.006
1001-0742/

Many studies have shown that stable isotope techniques are useful in understanding N sources and cycling in terrestrial and aquatic ecosystems (Wells and Krothe, 1989; Feast et al., 1998; Mayer et al., 2002; Curt et al., 2004). Dual isotope analysis of nitrate (δ^{15}N-NO_3^- and δ^{18}O-NO_3^-) has been frequently used to differentiate NO_3^- sources because of the distinct isotopic characteristics of the main NO_3^- sources, such as rain, chemical fertilizers, and NO_3^- derived from nitrification (Xue et al., 2009; Nestler et al., 2011). To provide valuable information for identifying NO_3^- sources and the processes during NO_3^- retention and transport in watersheds, scientists have used this dual-isotope technique in combination with physicochemical water quality data, precipitation data, hydrological data, land use data, and other tracers (*e.g.*, boron isotopes, chloride) in forested (Burns et al., 2009; Piatek et al., 2009), agricultural (Deutsch et al., 2006; Johannsen et al., 2008), urban, and mixed watersheds (Kaushal et al., 2011; Chen et al., 2012).

Zhushan Bay is a semi-closed lacustrine bay in the north of Taihu Lake, the third largest freshwater lake in the East Plain Region in China (Fig. 1). Zhushan Bay suffers from steadily worsening eutrophication caused by the inflow of nutrients from the Taige River (Yan et al., 2011; Zhang et al., 2011). The river catchment basin is flat, with intersecting rivers and water flowing slowly (annual mean flow velocity is 0.13 m/sec). Due to the complexity of rivers and the channel network of rivers, it is difficult to identify the sources of nutrients, especially the sources of nitrogen and its biogeochemical processes in the Taihu Lake watershed. To our knowledge, few works on N isotopes have been completed in the context of the region, probably resulting from the fact that isotopic signatures of N sources in such regions are obscured by complicated hydrographic connections and the mixing of multiple N sources with overlapping isotopic composition as well as *in situ* biogeochemical processes.

The present study analyzed the chemical parameters and isotopic compositions of NO_3^-, NH_4^+, and particulate organic matter (POM) in the water in the plain river network region. The specific goals of the study were to (1) evaluate the spatial variations in dissolved inorganic nitrogen (DIN) concentrations and isotopic compositions as well as the dominant sources of river NO_3^-, (2) evaluate the relative role of NO_3^- and NH_4^+ in the origin of POM in the river using DIN isotopic data, and (3) investigate possible seasonal shifts in nutrient sources (NO_3^- or NH_4^+) for phytoplankton in the river.

1. Materials and methods

1.1. Study site

Known as the main channel between Gehu Lake and Zhushan Bay, the Taige River empties into the northern part of Zhushan Bay of Taihu Lake (Fig. 1). The river is about 22.4 km long and 40 m wide on average. The slope ratio is 1:3. The mean annual discharge and velocity are 10.8 m^3/sec and 0.13 m/sec, respectively. Fourteen main tributaries are directly connected to the Taige River. At most times, the flow direction is from north to south, but the direction is occasionally unsteady because of the variation in water levels and sluice gates of the tributaries. The river catchment is located in the Wujin District in Jiangsu Province. This area, with a population of about 0.18 million people and an area of 179.06 km^2, is a typical plain river network region in the east of China, where the economy is developed and water pollution is severe. The area is a mixed land use watershed, which mainly serves agriculture (38% of land area in crops), residential, and industrial purposes. Many villages and townships characterized by high population density, urbanization, and economic development are situated along the banks of the upstream water catchment area of the Taige River. Water pollutants in the Taige River originate from different pollution sources and combinations of pollutants.

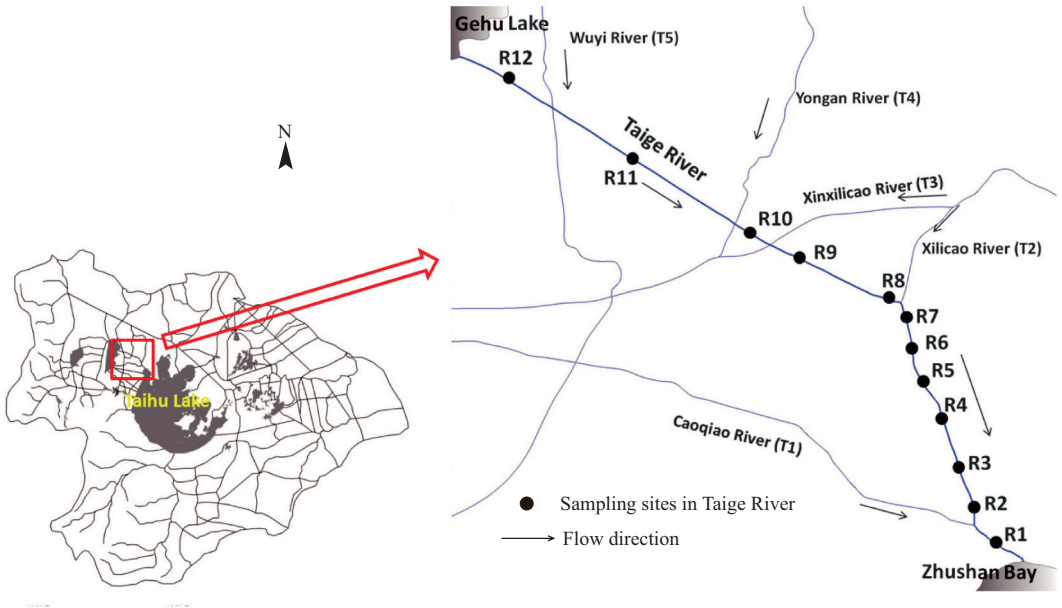

Fig. 1 – Sampling sites in the main stream of the Taige River.

1.2. Sampling

Twelve sites in the main stream of the Taige River were chosen for water sample collection (Fig. 1). Samplings in the main stream were performed on 9 January, 6 March, 7 May and 9 July in 2013. The discharge and flow velocity in each site was determined using an Acoustic Doppler Current Profiler. All sampling equipment was pre-cleaned with deionized water. Water temperature and dissolved oxygen (DO) were measured using a portable instrument (YSI 650MDS, Xylem, Yellow Springs, Ohio, USA) *in situ*. Water samples at 20 cm depth in the middle of the river were collected by boat and stored in pre-cleaned polyethylene bottles. About 300 mL of collected water samples was filtered on site through pre-combusted (450°C, 12 hr) and individually pre-weighed glass fiber filters (Whatman, GF/F, 47 mm in diameter). Filtrate and filter samples were stored at −30°C until needed for further treatments. In addition, 0.6 L of water samples was immediately carried back to the laboratory for nutrient concentration determination, within 24 hr at most.

1.3. Analytical methods

Water samples were filtered through 0.45 μm cellulose-acetate filter paper into polyethylene bottles and stored at 4°C until analysis. NH_4^+-N concentrations were analyzed on a continuous flow analyzer. NO_3^--N, nitrite (NO_2^--N), and chloride analyses were carried out by ion chromatography.

Isotopic analyses of N and oxygen of NO_3^- were carried out using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) based on the isotopic analysis of the nitrous oxide (N_2O) produced by denitrifying *Pseudomonas aureofaciens*. The N_2O was concentrated and purified on a Tracegas system, and the isotopic composition was determined using an isotope ratio mass spectrometer (IRMS; Isoprime100, Isoprime, Cheadle, UK) calibrated with ultra-high purity N_2 gas against air N (Xu et al., 2013). During measurement of $\delta^{15}N$, USGS34 potassium nitrate (KNO_3, $\delta^{15}N = -1.8‰ \pm 0.2‰$) and USGS32 KNO_3 ($\delta^{15}N = +18‰ \pm 1‰$) standards were used to correct the values obtained. For $\delta^{18}O$ measurement, the samples were referenced using USGS34 ($\delta^{18}O = +27.9‰ \pm 0.6‰$) and USGS35 sodium nitrate ($NaNO_3$, $\delta^{18}O = +57.5‰ \pm 0.6‰$) standards. The stable isotope ratios are expressed in delta (δ) units and a per mil (‰) notation relative to an international standard:

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

where, R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratios of the sample and standard for $\delta^{15}N$ and $\delta^{18}O$, respectively. Values of $\delta^{15}N$ are reported relative to N_2 in atmospheric air, and $\delta^{18}O$ values are reported relative to Vienna Standard Mean Ocean Water. The analytical precision of the $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- are ±0.2‰ and ±0.5‰, respectively.

Determination of $\delta^{15}N$-NH_4^+ values was carried out using the ammonia distillation method of Velinski et al. (1989). The analytical precision of the $\delta^{15}N$-NH_4^+ samples was ±0.5‰. For the determination of the POM concentration as well as the $\delta^{13}C$-POC and $\delta^{15}N$-PN values, the GF/F filters were treated as follows in the laboratory. The filter samples were slightly wetted by hydrochloric acid (0.6 mol/L) and put in a desiccator overnight with hydrochloric acid (0.6 mol/L) fumes to remove inorganic carbon. Subsequently, the filters were rinsed with deionized water to remove chloride, dried using a vacuum freeze dryer for 24 hr, and weighed to calculate POM concentration by weight difference. Then, $\delta^{13}C$-POC, $\delta^{15}N$-PN, and C/N ratios were measured in an elemental analyzer (vario PYRO cube, Elementar, Hanau, Germany) connected online to an IRMS. Values are reported relative to atmospheric N_2 ($\delta^{15}N$) and Vienna PeeDee Belemnite ($\delta^{13}C$). The analytical precision for both stable isotope ratios was ±0.2‰.

2. Results and discussion

2.1. Hydrological features and seasonal variation of water chemistry

The flow quantity (Q) and velocity (V) of the Taige River in the study period were 21.75 ± 13.42 m^3/sec and 0.28 ± 0.16 m/sec (mean ± standard deviation (SD)), respectively. The factor that significantly influenced the hydrological condition of the river was the flow input from the Xilicao River (T2; Q = 24.30 ± 8.75 m^3/sec), which divides the Taige River into two sections with different hydrological features (Fig. 2). The Q of the upstream (before site R8), with lower Q (7.17 ± 2.52 m^3/sec) and V (0.11 ± 0.05 m/sec) than the downstream (Q = 33.90 ± 0.57 m^3/sec, V = 0.42 ± 0.04 m/sec), was strongly influenced by the Wuyi River (T5), Yongan River (T4), and Xinxilicao River (T3). In the downstream, the Q was almost constant and the V decreased slowly because of the backwater region of Zhushan Bay.

During the sampling period, NH_4^+-N, NO_3^--N, NO_2^--N and Cl^- concentrations ranged from 0.35 to 4.01 mg/L, from 0.42 to 3.75 mg/L, from 0.06 to 0.32 mg/L and from 56.1 to 130.2 mg/L, respectively. In order to get a better understanding of the temporal variability of water chemistry in the river water, it was helpful to divide the data into two seasonal periods, winter (January and March, average temperature = 9.2°C) and summer (May and July, average temperature = 25.5°C). NH_4^+-N, NO_3^--N and Cl^- concentrations in winter were higher than in summer, while NO_2^- concentrations were higher in summer

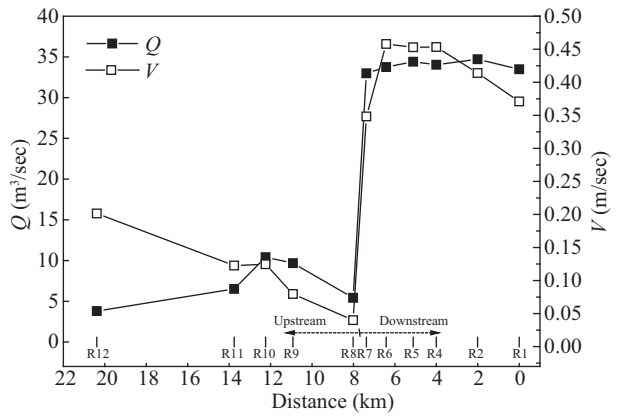

Fig. 2 – Longitudinal plots of averaged flow quantity (Q) and velocity (V) in the Taige River referenced to kilometers downstream from site R1.

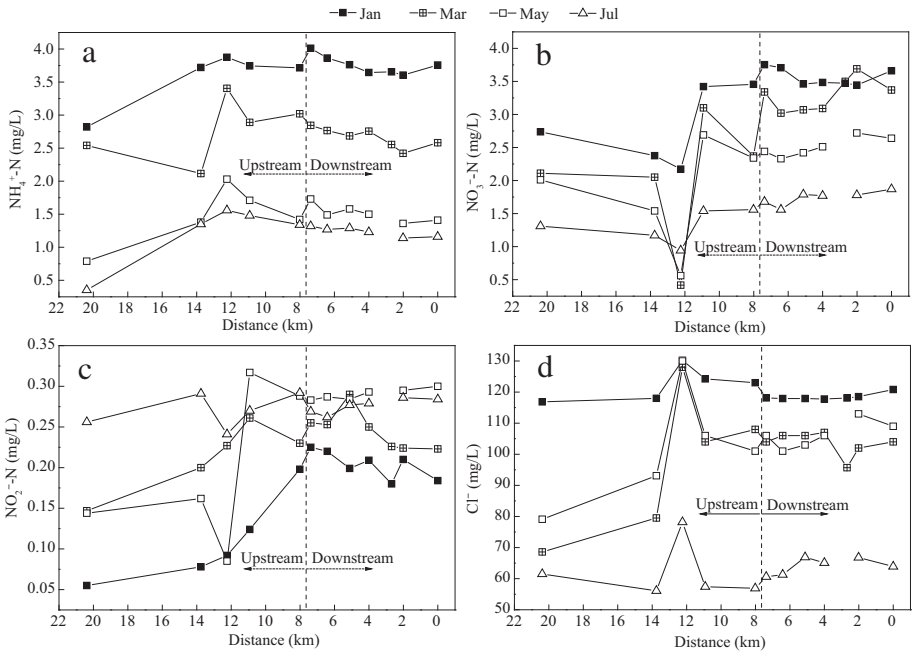

Fig. 3 – Longitudinal plots of (a) NH_4^+-N, (b) NO_3^--N, (c) NO_2^--N, and (d) Cl^- concentrations referenced to kilometers downstream from site 1. NH_4^+-N: ammonium; NO_3^--N: nitrate nitrogen; NO_2^--N: nitrite nitrogen; Cl^-: chloride.

(Fig. 3). This finding can be attributed to the dilution by the rain in the rainy season (June to July) in the study area.

Following the trends of the hydrological features, NH_4^+-N, NO_3^--N, NO_2^--N and Cl^- concentrations in the upstream varied more extensively than in the downstream. At site R10 in the upstream, NH_4^+-N and Cl^- concentrations increased and NO_3^--N concentration decreased sharply because of the input water of the Yongan River (T4), which drained urbanized areas (the city of Changzhou) and was polluted by domestic sewage and livestock wastewater with additional high NH_4^+-N and Cl^- concentrations (Fig. 3a, b and 3d). The mean concentration of DO measured at Yongan River was 1.2 mg/L, which was ideal for denitrification (DO concentration <1 mg/L to 2 mg/L) (Rivett et al., 2008) and caused the low content of NO_3^--N in the Yongan River and in R10. In the downstream, NH_4^+-N concentrations decreased slowly, while NO_3^- concentrations increased. The Cl^- concentrations had little change.

2.2. Spatial and temporal isotopic composition of NO_3^-, NH_4^+ and POM

The isotopic compositions of the NO_3^- in the Taige River were found to range from +4.56‰ to +8.79‰ (n = 45, mean value = +6.83‰) for $\delta^{15}N$ and from −0.24‰ to +8.17‰ (n = 44, mean value = +3.18‰) for $\delta^{18}O$. The $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-POM values ranged from −0.96‰ to +12.82‰ (n = 45, mean value = +4.58‰) and from −3.83‰ and +0.66‰ (n = 44, mean value = −1.46‰), respectively. The $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values in winter (mean ± SD = +7.36‰ ± 0.98‰, +4.66‰ ± 1.25‰, respectively) were higher than those in summer (mean ± SD = +6.23‰ ± 1.02‰, +1.39‰ ± 1.02‰, respectively) (Fig. 4a and b). The $\delta^{15}N$-POM values were also higher in winter (Fig. 4d), but the $\delta^{15}N$-NH_4^+ values in winter (mean ± SD = +2.60‰ ± 1.84‰) were lower than those in summer (mean ± SD = +6.85‰ ± 2.59‰) (Fig. 4c). The reasons for temporal variation of isotopic composition are discussed in Section 2.3.

Longitudinal variability in isotopic composition in the Taige River was significantly controlled by shifting hydrologic conditions. The isotopic composition of NO_3^-, NH_4^+ and POM in the upstream varied more extensively because of the inputs of various pollution sources with different isotopic composition in tributaries in the upstream. The Yongan River (T4), which was seriously polluted with high NH_4^+ and low DO content (see Section 2.1), had high $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values (mean ± SD = +16.21‰ ± 3.81‰, +11.00‰ ± 3.09‰, respectively). The input of T4 resulted in the increased $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values at the site R10 in the Taige River (Fig. 4a and b). At the site R9, the $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-POM values increased sharply and then decreased (Fig. 4c and d). This was caused by the Xinxilicao River (T3) input with high $\delta^{15}N$-NH_4^+ (mean ± SD = +9.40‰ ± 3.81‰) and $\delta^{15}N$-POM values (mean ± SD = −0.88‰ ± 0.59‰). In the downstream of the Taige River, the $\delta^{15}N$-NO_3^-, $\delta^{18}O$-NO_3^- and $\delta^{15}N$-POM values showed a clear increasing tendency (The reasons are discussed in Section 2.3) (Fig. 4a, b and d), while $\delta^{15}N$-NH_4^+ values showed no such clear development (Fig. 4c).

2.3. Nitrate source identification determined by dual-isotopic approach

The following sources possibly contributed to the NO_3^- budget in the Taige River in winter: precipitation, chemical fertilizer, nitrification of N-containing organic materials, livestock, and urban sewage effluent. The boxes in Fig. 5 show typical ranges of $\delta^{15}N$ and $\delta^{18}O$ for various natural and anthropogenic sources of NO_3^-.

The contribution of NO_3^- from precipitation was probably negligible for the waters of the Taige River considering the low $\delta^{15}N$-NO_3^- (−13‰ to +13‰, close to 0‰) and high $\delta^{18}O$-NO_3^-

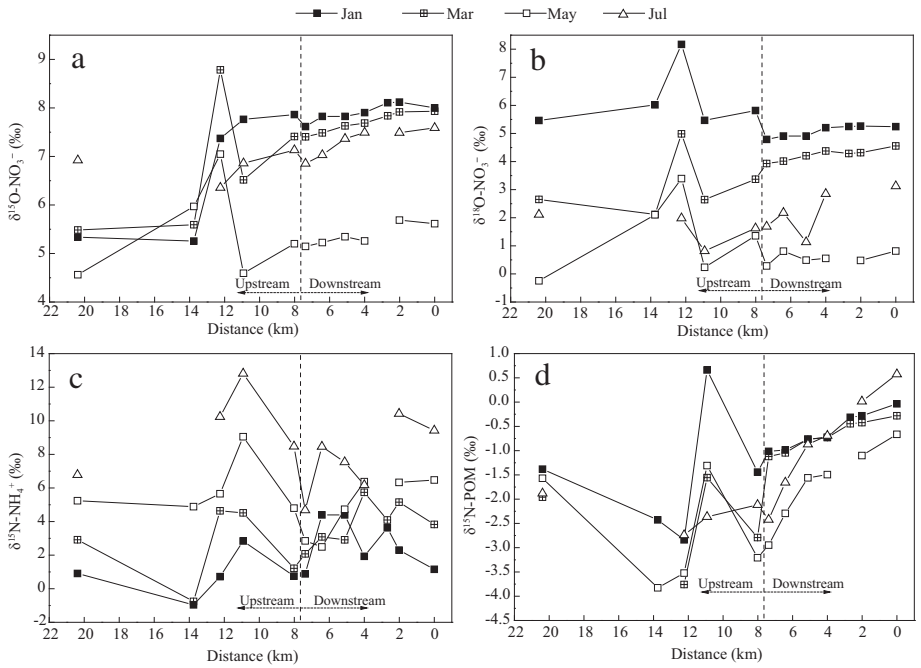

Fig. 4 – Longitudinal plots of (a) δ^{15}N-NO_3^-, (b) δ^{18}O-NO_3^-, (c) δ^{15}N-NH_4^+, and (d) δ^{15}N-POM values referenced to kilometers downstream from site 1. NH_4^+-N: ammonium; NO_3^--N: nitrate nitrogen; POM: particulate organic matter.

values (+25‰ to +75‰) in rain (Wassenaar, 1995; Durka et al., 1994; Kendall, 1998). In this district, fertilizer N was almost all in its reduced form, *i.e.*, urea and ammonium, and the NO_3^- form was negligible. Ammonium fertilizer was not directly distributed into the rivers at the time of sampling because of its low δ^{15}N-NO_3^- values (−6‰ to +6‰). However, the isotopic composition of NO_3^- from chemical fertilizer would be modified by biological processes during the flow of water into rivers from the soil and unsaturated zone (Mengis et al., 2001). Manure and sewage are enriched with ^{15}N relative to other N sources, and the δ^{15}N values of NO_3^- range from +4‰ to +25‰ (Xue et al., 2009). In this study, the δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values of the water samples were in the range of "soil N" and "manure and sewage" source boxes, thus

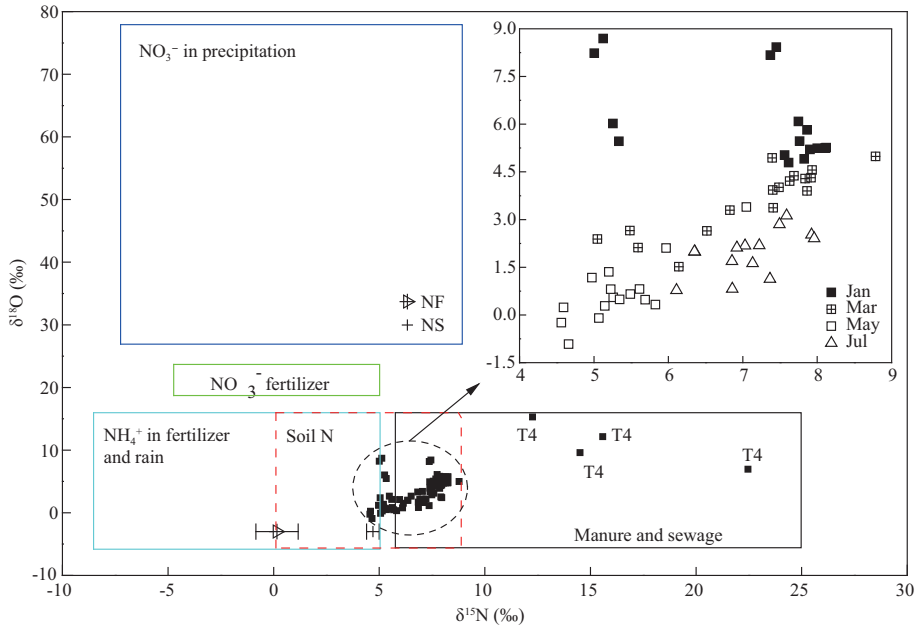

Fig. 5 – Cross plots of δ^{18}O-NO_3^- *versus* δ^{15}N-NO_3^- in water samples. The box diagram shows the isotopic composition of various sources (adapted from Kendall, 1998; Xue et al., 2009; Nestler et al., 2011). NS represents NO_3^- derived from soil organic matter nitrification; NF represents NO_3^- derived from chemical fertilizer nitrification. The δ^{15}N and δ^{18}O-NO_3^- values of NS and NF are expressed by mean values with error bars (Ding et al., 2014). NO_3^-: nitrate.

indicating that manure, urban sewage effluent, and nitrification of N-containing organic matter (including "modified fertilizer") were likely the major sources of NO_3^- in the Taige River. These results are in good agreement with those by Townsend-Small et al. (2007), Chen et al. (2012), and Xing et al. (2001), who used the NO_3^- isotopic method and found that sewage-derived N makes a dominant contribution to the rivers to the northwest of Taihu Lake.

The $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values in the Yongan River (T4) were located in the "manure and sewage" source box. This was due to wastewater discharge from many livestock and poultry farms along the riverside of the Yongan River, and these NO_3^- sources are known to have high $\delta^{15}N$-NO_3^- values that can reach +14‰ and sometimes even +20‰ (Heaton, 1986). Furthermore, other biochemical processes (mostly denitrification) in the Yongan River could also elevate the $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values.

Fig. 5 also indicates that the $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values in winter were higher than those in summer. In winter, the low flow and anthropogenic inputs (manure and urban sewage effluent) resulted in more serious NO_3^- pollution in the Taige River (Fig. 4b). This was supported by NO_3^--N *versus* Cl^- concentration relationships in the Taige River. Chloride is a good indicator of sewage impact and dilution because it is not subject to physical, chemical, and biological processes (Liu et al., 2006). High Cl^- content mainly resulted from domestic sewage and livestock wastewater. NO_3^--N *versus* Cl^- concentrations in river water showed that river water was more significantly affected by manure and urban sewage effluent, with higher $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values in winter than in summer (Fig. 6). In summer, the $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values of the Taige River were close to the low $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values of soil organic matter and chemical fertilizer in the study area (Fig. 5). This indicated that the intensified microbial nitrification of the soil organic matter and chemical fertilizer resulted in low $\delta^{15}N$ and $\delta^{18}O$-NO_3^- in river water as climate warming and fertilizing activities took place in summer. Furthermore, dilution caused by precipitation in the rainy season could also result in low isotopic composition for NO_3^- in river water.

2.4. Assessment of the transformations of nitrogen in the downstream

According to the distributions of hydrological and water chemistry features, the mixing process of the three guest rivers (Wuyi River, Yongan River, and Xinxilicao River) with different $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values caused the distinction between isotopic compositions in the upstream and the downstream of the Taige River. To further understand the biogeochemical N process in the Taige River, the following analysis focuses on the downstream of the Taige River, which has only minor variations in hydrological conditions.

During the sampling period, a decrease in NH_4^+-N concentrations was observed in the downstream (Fig. 3a). This decrease could be attributed to ammonia volatilization, nitrification, and uptake by biota. The $\delta^{15}N$-NH_4^+ values in the downstream were small and could not be interpreted as the result of a ^{15}N enrichment of residual NH_4^+ resulting from volatilization (Chang et al., 2002; Sebilo et al., 2006). Either microbial nitrification or NH_4^+ assimilation by algae could result in increased $\delta^{15}N$-NH_4^+ values and decreased NH_4^+-N concentration. The negative correlation between $\delta^{15}N$-NH_4^+ values and the natural logarithm of NH_4^+-N concentration in the downstream indicated that nitrification and assimilation probably occurred simultaneously in the downstream of the Taige River (Fig. 7).

Although NH_4^+ assimilation in aquatic algae occurred (as discussed below), we believe that nitrification played a more important role in the decreasing NH_4^+-N concentrations. First, coupled with the decreasing NH_4^+-N concentrations, the NO_3^--N concentrations increased in the downstream, indicating the occurrence of nitrification (Fig. 3a, b). Furthermore, in our study, the N isotopic enrichment factors (ε) during ammonium utilization in the downstream ranged from −13.88‰ in March to −29.00‰ in July (except in January, when the water temperature was low and microbial nitrification was restricted, $r^2 = 0.09$, $p > 0.05$) (Fig. 7). These factors (ε) were in the range of the N isotopic enrichment factors (ε) during nitrification (−12‰ to −29‰) (Kendall, 1998).

To better understand the nitrogen assimilation in the Taige River, we analyzed not only the relationship between $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- but also the relationships between $\delta^{15}N$-POM and isotopic compositions of DIN.

In addition to the increase in NO_3^- $\delta^{15}N$ and $\delta^{18}O$ values (Fig. 4a, b), we found a significant correlation between NO_3^- $\delta^{15}N$ and $\delta^{18}O$ values in the downstream in January ($r^2 = 0.81$, $p < 0.01$) and in March ($r^2 = 0.75$, $p < 0.01$) (Fig. 8). Algae/biota

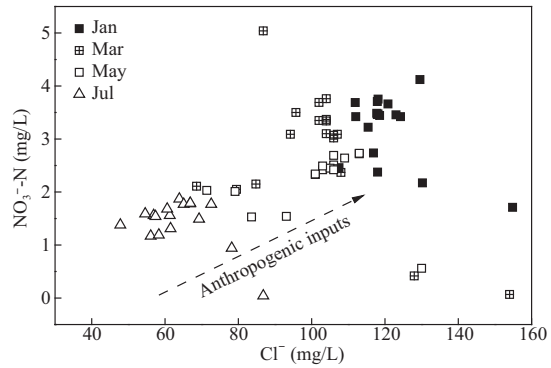

Fig. 6 – NO_3^--N *versus* Cl^- concentrations in river water. NO_3^--N: nitrate nitrogen; Cl^-: chloride.

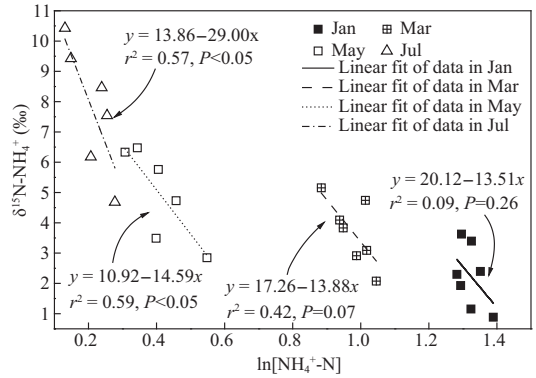

Fig. 7 – Dependence of $\delta^{15}N$-NH_4^+ values on the natural logarithm of NH_4^+-N concentration (ln[NH_4^+-N]) in the downstream of the Taige River, with the *lines* indicating linear regression fitting. NH_4^+-N: ammonium.

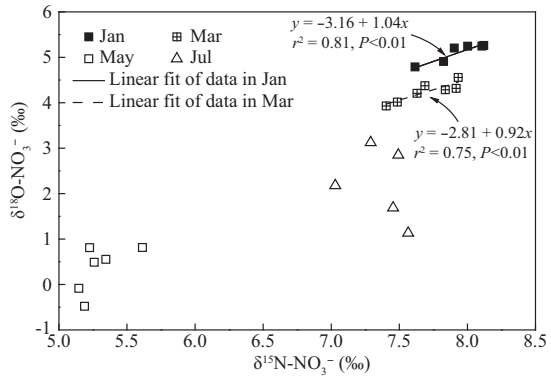

Fig. 8 – $\delta^{15}N\text{-}NO_3^-$ *versus* $\delta^{18}O\text{-}NO_3^-$ in the downstream water samples from the Taige River shown by a linear regression line. NO_3^-: nitrate.

generally prefer the uptake of light isotopes of NO_3^-, which would lead to enrichment with heavy isotopes in residual NO_3^- (Battaglin et al., 2001; Johannsen et al., 2008). The ratio of the increase in $\delta^{18}O$ and $\delta^{15}N$ of river NO_3^- in the downstream was 1.04 in January and 0.92 in March, which were closer to the ~1:1 increase reported for NO_3^- assimilation of marine phytoplankton (Granger et al., 2004) than to the 1:1.3 or the 1:2.1 increase reported for denitrification in freshwaters (Böttcher et al., 1990; Kendall, 1998; Mengis et al., 1999; Burns et al., 2009; Kaushal et al., 2011). The results indicated that NO_3^- assimilation by phytoplankton was responsible for the increasing $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in winter.

In the study area, many laboratory and field studies have indicated that the dominant N source responsible for the growth of phytoplankton was NH_4^+ (McCarthy et al., 2007; Paerl et al., 2011; Zhou et al., 2013). Our study demonstrates that the presence of POM in the Taige River was mainly derived from aquatic phytoplankton without terrigenous organic matter playing a role (Fig. 9). Thus, the relationships between $\delta^{15}N$-POM and isotopic compositions of DIN could help us to understand the nitrogen assimilation. Fig. 10a shows two positive linear correlations existing between $\delta^{15}N$-POM and $\delta^{15}N\text{-}NO_3^-$ values in winter, which supported NO_3^- assimilation by phytoplankton in winter, as discussed before using NO_3^- $\delta^{15}N$ and $\delta^{18}O$ values. Three other positive linear correlations were found between $\delta^{15}N$-POM and $\delta^{15}N\text{-}NH_4^+$ values from March to July (Fig. 10b), which indicated that NH_4^+ assimilation in aquatic algae occurred and was partly responsible for the decreasing NH_4^+-N concentrations. Therefore, we concluded that the phytoplankton in the Taige River probably utilized NH_4^+ preferentially and mainly in summer, while in winter, NO_3^- assimilation by phytoplankton was dominant. The results were supported by a laboratory study and field monitoring in Taihu Lake conducted by Zhou et al. (2013). They found *Microcystis* always assimilated NH_4^+ preferentially, but when NH_4^+-N concentration exceeded 2 mg/L, their growth rate declined sharply. In our study, NH_4^+-N concentrations of the Taige River were above 2 mg/L in winter and were below 2 mg/L in summer (Fig. 3a), which could partly explain the assimilation of different nitrogen forms in winter and in summer. Further systematic study should be performed to clearly understand N cycling in the area.

3. Conclusions

In our study of the Taige River, we found the river was polluted more seriously by anthropogenic inputs in winter than in summer, based on DIN concentrations. Manure and urban sewage effluent, as well as the nitrification of N-containing organic materials, were the major sources of N. Efforts to reduce N exported from livestock waste, urban wastewater and soil should be a priority. In the downstream of the Taige River, nitrification and assimilation probably occurred simultaneously. Nitrification played a more important role than assimilation in the decreasing NH_4^+-N concentrations. Nitrification strengthened as the water temperature increased. Based on the data of the isotopic composition of DIN and POM, we also found that the phytoplankton in the Taige River probably utilized NH_4^+ preferentially and mainly in summer, while in winter, NO_3^- assimilation by phytoplankton was dominant. Our study demonstrates that the dual-isotope approach ($\delta^{15}N$ and $\delta^{18}O$ of NO_3^-), combined with analyses of NH_4^+ and POM isotopic compositions, is a helpful tool in investigating sources of NO_3^- and N transformation processes along rivers in the plain river network region.

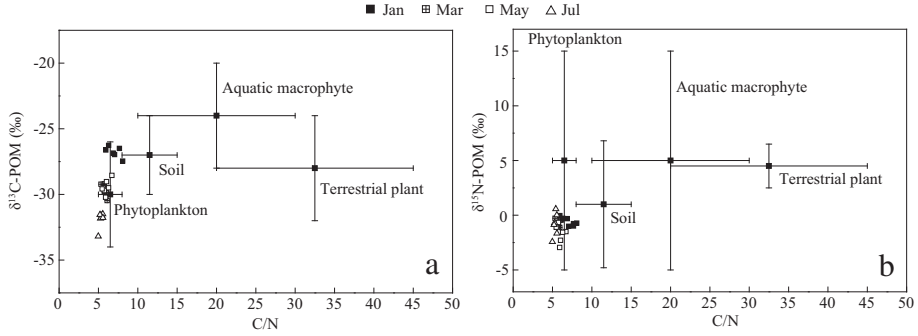

Fig. 9 – Relationships between (a) $\delta^{13}C$-POM and C/N and (b) $\delta^{15}N$-POM and C/N ratios in the Taige River, including the ranges of the potential sources of organic material.
Adapted from Hamilton and Lewis (1992), Angradi (1994), Thorp et al. (1998), Kendall (1998), Vuorio et al. (2006), Ogrinc et al. (2008), and Tang and Zhang (2010).

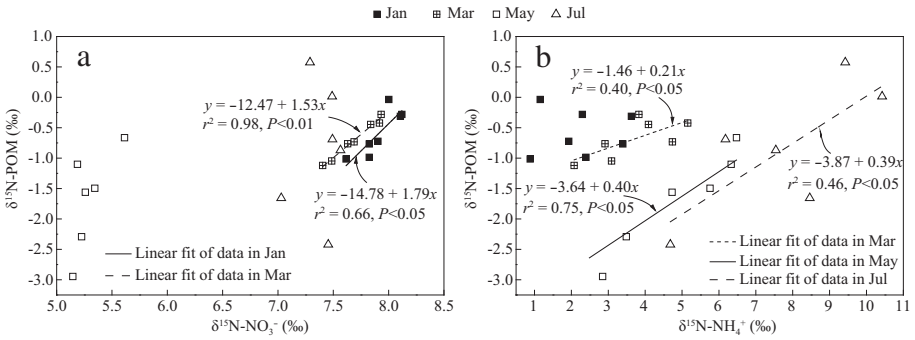

Fig. 10 – Relationships between (a) δ^{15}N-POM and δ^{15}N-NO_3^- values and (b) δ^{15}N-POM and δ^{15}N-NH_4^+ values in the Taige River. POM: particulate organic matter; NO_3^-: nitrate; NH_4^+: ammonium.

Acknowledgments

This research was supported by the Mega-projects of Science Research for Water Environment Improvement (No. 2012ZX07101). The authors are grateful to the associated persons for their hard work in conducting the field investigations and samplings. The authors also thank professor Li for his practical assistance in the laboratory.

REFERENCES

Angradi, T.R., 1994. Trophic linkages in the lower Colorado River: multiple stable isotope evidence. J. N. Am. Benthol. Soc. 13, 479–495.

Battaglin, W.A., Kendall, C., Chang, C.C.Y., Silva, S.R., Campbell, D.H., 2001. Chemical and isotopic evidence of nitrogen transformation in the Mississippi River, 1997–98. Hydrol. Process. 15, 1285–1300.

Böttcher, J., Strebel, O., Voerkelius, S., Schmidt, H.L., 1990. Using isotope fractionation of nitrate-nitrogen and nitrate–oxygen for evaluation of microbial denitrification in a sandy aquifer. J. Hydrol. 114, 413–424.

Burns, D.A., Boyer, E.W., Elliott, E.M., Kendall, C., 2009. Sources and transformations of nitrate from streams draining varying land uses: evidence from dual isotope analysis. J. Environ. Qual. 38, 1149–1159.

Casciotti, K.L., Sigman, D.M., Galanter, H.M., Böhlke, J.K., Hilkert, A., 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal. Chem. 74, 4905–4912.

Chang, C.C.Y., Kendall, C., Silva, S.R., Battaglin, W.A., Campbel, D.H., 2002. Nitrate stable isotopes: tools for determining nitrate sources among different land uses in the Mississippi river basin. Can. J. Fish. Aquat. Sci. 59, 1874–1885.

Chen, Z.X., Liu, G., Liu, W.G., Lam, H.W., Liu, G.J., Yin, X.B., 2012. Identification of nitrate sources in Taihu Lake and its major inflow rivers in China, using δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values. Water Sci. Technol. 66 (3), 536–542.

Curt, M.D., Aguado, P., Sánchez, G., Bigeriego, M., Fernández, J., 2004. Nitrogen isotope ratios of synthetic and organic sources of nitrate water contamination in Spain. Water Air Soil Pollut. 151, 135–142.

Deutsch, B., Mewes, M., Liskow, I., Voss, M., 2006. Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate. Org. Geochem. 37, 1333–1342.

Ding, J.T., Xi, B.D., Gao, R.T., He, L.S., Liu, H.L., Dai, X.L., Yu, Y.J., 2014. Identifying diffused nitrate sources in a stream in an agricultural field using a dual isotopic approach. Sci. Total Environ. 484, 10–18.

Durka, W., Schulze, E.D., Gebauer, G., Voerkelius, S., 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. Nature 372, 765–767.

Feast, N.A., Hiscock, K.M., Dennis, P.F., Andrews, J.N., 1998. Nitrogen isotope hydrochemistry and denitrification within the Chalk aquifer system of north Norfolk. J. Hydrol. 211, 233–252.

Granger, J., Sigman, D.M., Needoba, J.A., Harrison, P.J., 2004. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol. Oceanogr. 49 (5), 1763–1773.

Hamilton, S.K., Lewis Jr., W.M., 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. Geochim. Cosmochim. Acta 56, 4237–4246.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem. Geol. 59, 87–102.

Johannsen, A., Dähnke, K., Emeis, K., 2008. Isotopic composition of nitrate in five German rivers discharging into the North Sea. Org. Geochem. 39, 1678–1689.

Kaushal, S.S., Groffman, P.M., Band, L.E., Elliott, E.M., Shields, C.A., Kendall, C., 2011. Tracking nonpoint source nitrogen pollution in human-impacted watersheds. Environ. Sci. Technol. 45 (19), 8225–8232.

Kendall, C., 1998. Tracing Sources and Cycling of Nitrate in Catchments. Elsevier, Amsterdam.

Lee, K.S., Bong, Y.S., Lee, D., Kim, Y., Kim, K., 2008. Tracing the sources of nitrate in the Han River watershed in Korea, using δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values. Sci. Total Environ. 395, 117–124.

Li, S.L., Liu, C.Q., Li, J., Liu, X.L., Chetelat, B., Wang, B.L., Wang, F.S., 2010. Assessment of the sources of nitrate in the Changjiang River, China using a nitrogen and oxygen isotopic approach. Environ. Sci. Technol. 44, 1573–1578.

Liu, C.Q., Li, S.L., Lang, Y.C., Xiao, H.Y., 2006. Using δ^{15}N and δ^{18}O values to identify nitrate sources in Karst ground water, Guiyang, Southwest China. Environ. Sci. Technol. 40, 6928–6933.

Mayer, B., Boyer, E.W., Goodale, C., et al., 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern US: isotopic constraints. Biogeochemistry 57 (58), 171–197.

McCarthy, M.J., Lavrentyev, P.J., Yang, L.Y., Zhang, L., Chen, Y.W., Qin, B.Q., Gardner, W.S., 2007. Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China). Hydrobiologia 581, 195–207.

Mengis, M., Schiff, S.L., Harris, M., English, M.C., Aravena, R., Elgood, R.J., MacLean, A., 1999. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. Ground Water 37 (3), 448–457.

Mengis, M., Walther, U., Bernasconi, S.M., Wehrli, B., 2001. Limitations of using $\delta^{18}O$ for the source identification of nitrate in agricultural soils. Environ. Sci. Technol. 35, 1840–1844.

Middelburg, J.J., Nieuwenhuize, J., 2001. Nitrogen isotope tracing of dissolved inorganic nitrogen behaviour in tidal estuaries. Estuar. Coast. Shelf Sci. 53, 385–391.

Nestler, A., Berglund, M., Accoe, F., Duta, S., Xue, D.M., Boeckx, P., Taylor, P., 2011. Isotopes for improved management of nitrate pollution in aqueous resources: review of surface water field studies. Environ. Sci. Pollut. Res. 18, 519–533.

Ogrinc, N., Markovics, R., Kanduč, T., Walter, L.M., Hamilton, S.K., 2008. Sources and transport of carbon and nitrogen in the River Sava watershed, a major tributary of the River Danube. Appl. Geochem. 23, 3685–3698.

Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., Gardner, W.S., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. Water Res. 45, 1973–1983.

Piatek, K.B., Christopher, S.F., Mitchell, M.J., 2009. Spatial and temporal dynamics of stream chemistry in a forested watershed. Hydrol. Earth Syst. Sci. 13, 423–439.

Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W., Bemment, C.D., 2008. Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. Water Res. 42, 4215–4232.

Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, J., Mariotti, A., 2006. Assessing nitrification and denitrification in the Seine River and estuary using chemical and isotopic techniques. Ecosystems 9, 564–577.

Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., Böhlke, J.K., 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal. Chem. 73, 4145–4153.

Sugimoto, R., Kasai, A., Fujita, K., Sakaguchi, K., Mizuno, T., 2011. Assessment of nitrogen loading from the Kiso-Sansen Rivers into Ise Bay using stable isotopes. J. Oceanogr. 67, 231–240.

Tang, Y.L., Zhang, G.X., 2010. Identifying sources of particles organic matter in surface water based on stable isotope tracing on basin scale. China Environ. Sci. 30 (9), 1257–1267.

Thorp, J.H., Delong, M.D., Greenwood, K.S., Casper, A.F., 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. Oecologia 117, 551–563.

Townsend-Small, A., McCarthy, M.J., Brandes, J.A., Yang, L.Y., Zhang, L., Gardner, W.S., 2007. Stable isotopic composition of nitrate in Lake Taihu, China, and major inflow rivers. Hydrobiologia 581, 135–140.

Velinski, D.J., Pennock, J.R., Sharp, J.H., Cifuentes, L.A., Fogel, M.L., 1989. Determination of the isotopic composition of ammonium–nitrogen at the natural abundance level from estuarine waters. Mar. Chem. 26, 351–361.

Vuorio, K., Meili, M., Sarvala, J., 2006. Taxon-specific variation in the stable isotope signature ($\delta^{13}C$ and $\delta^{15}N$) of lake phytoplankton. Freshw. Biol. 51, 807–822.

Wassenaar, L., 1995. Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. Appl. Geochem. 10, 391–405.

Wells, E.R., Krothe, N.C., 1989. Seasonal fluctuation in $\delta^{15}N$ of groundwater nitrate in a mantled Karst aquifer due to macropore transport of fertilizer-derived nitrate. J. Hydrol. 112, 191–201.

Xing, G.X., Cao, Y.C., Shi, S.L., Sun, G.Q., Du, L.J., Zhu, J.G., 2001. N pollution sources and denitrification in waterbodies in Taihu Lake region. Sci. China B 44 (3), 304–314.

Xu, C.Y., Li, Y.Z., Li, Q.Z., Dong, Y.W., Fang, F.L., Guo, Z.C., 2013. Measurement of ^{15}N and ^{18}O isotope abundance of nitrate using denitrifier method on Tracegas-Isotope Ratio Mass Spectrometry. Adv. Mater. Res. 726–731, 1346–1349.

Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., Van Cleemput, O., Berglund, M., Boeckx, P., 2009. Present limitations and future prospects of stable isotope methods for nitrate source identification in surface and groundwater. Water Res. 43, 1159–1170.

Yan, S.W., Yu, H., Zhang, L.L., Xu, J., Wang, Z.P., 2011. Water quantity and pollutant fluxes of inflow and outflow rivers of Lake Taihu, 2009. J. Lake Sci. 23 (6), 855–862.

Zhang, Y.M., Zhang, Y.C., Gao, Y.X., Zhang, H.H., Cao, J.Y., Cai, J.B., Kong, X.J., 2011. Water pollution control technology and strategy for river-lake systems: a case study in Gehu Lake and Taige Canal. Ecotoxicology 20, 1154–1159.

Zhou, T., Li, Z.K., Feng, L.L., 2013. The different roles of ammonium and nitrate in the bloom self-maintenance of Lake Taihu. China Environ. Sci. 33 (2), 305–311.

Editorial Board of Journal of Environmental Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

环境科学学报(英文版)

www.jesc.ac.cn

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Journal of Environmental Sciences (Established in 1989)
Volume 30 2015

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
	Tel: 86-10-62920553; http://www.jesc.ac.cn	Foreign	Elsevier Limited
	E-mail: jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	X. Chris Le	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742