Investigating the spatial–temporal variation of nitrogen cycling in an urban river in the North China Plain

J. Wang, Y. S. Pei, K. J. Zhang, G. Gao and Z. F. Yang

ABSTRACT

Urban rivers are essential in retaining nutrients, but little is known about nitrogen cycling in these rivers in semiarid areas. We measured chemical and isotopic compositions of ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) to investigate spatial–temporal variation of nitrogen cycling in the Fuhe River in the North China Plain. Nitrogen pollution in the river was mainly induced by extra NH₄⁺-N inputs which come from the discharges of urban sewage and effluents of wastewater treatment plants in upstream. NH₄⁺-N obtained from decomposing organic matter of sediments can diffuse into the overlying water. Intense nitrification then occurs at the terrestrial–aquatic interface. Due to less vegetation in spring and autumn, loss of NH₄⁺-N is mainly caused by nitrification. In contrast, significant NH₄⁺-N is absorbed by plants in summer. NO₃⁻-N generated from nitrification can be denitrified during the study period. The highest NO₃⁻-N loss (about 86.3%) was observed in summer. The contribution of NO₃⁻-N loss due to denitrification is 44.6%. The remaining 55.4% is due to plant uptake. The results suggested that nitrogen cycling in the river is related to temperature and dry–wet cycles. And vegetation restoration along the river could benefit the incremental improvements to the aquatic ecosystem.

Key words | nitrogen isotope, nitrogen pollution, seasonal variation, transformation, urban river

INTRODUCTION

Biogeochemical cycling in a river is governed by a variety of processes including physical–chemical processes, biological processes, and hydrologic processes. McClain et al. (2003) considered the flux and rate of nitrogen cycling as spatial and temporal variables. In a river, besides the processes occurring in the river channel, enhanced fluxes of nutrients and oxygen could occur at the terrestrial–aquatic interfaces with water exchanges (Triska et al. 1993). Antagonistic processes, i.e. denitrification and nitrification, can simultaneously occur at the interfaces where both aerobic and anoxic zones exist. Thus nitrogen cycling is active at the interface which are named as ‘hot spots’ of aquatic systems (McClain et al. 2003; Chen et al. 2009). Temporal variation of nitrogen cycling is also investigated, which is connected with moisture-related dry–wet cycles or temperature-related seasonal cycles (Chen et al. 2009).

To quantitatively capture the influence of internal processes on nitrogen removal monitoring only concentrations of nitrogenous substances and river discharge is not sufficient. In recent years, the stable nitrogen isotopic signatures have increasingly been used for scaling internal biochemical processes responsible for ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) turnover in rivers and groundwater (Rock & Mayer 2006). The isotopic compositions of NH₄⁺-N and NO₃⁻-N can be altered by different microbial nitrogen transformations (Böttcher et al. 1990). The degree of isotopic signature variation depends on specific processes (Robinson 2001; Sebilo et al. 2006). Chen et al. (2009) successfully quantified the denitrification process in different seasons in the Beijiang River using the dual isotopes of NO₃⁻-N. Sebilo et al. (2006) also investigated the extents of nitrification and denitrification in the Seine River based on the isotopic compositions of NH₄⁺-N and NO₃⁻-N. As nitrogen compounds of different origins often exhibit different ¹⁵N signatures, these signatures can be used to identify the sources of nitrogen inputs (Robinson 2001).

The Fuhe River receives urban sewage and effluents of wastewater treatment plants (WWTP) from the Baoding City. The large amount of nutrient inputs through the river leads to the serious eutrophication status in the downstream...
lake, i.e. the Baiyangdian Lake, which is the largest natural freshwater body in the North China Plain. As the river is located in the semi-arid area, fresh surface water seldom flows into the river, and enhancement of nitrogen retention in river channel is especially crucial for the downstream ecosystem. However, to date, there are no studies on nitrogen cycling occurring in the North China Plain. And little nitrogen isotope work has been done for urban rivers to investigate nitrogen processing in semi-arid areas. Using chemical and isotopic techniques, the objectives of this study were the following: (1) to identify the sources of nitrogen contaminants in the Fuhe River; (2) to investigate spatial–temporal variations of nitrogen cycling in the river, especially at the terrestrial–aquatic interface of the river bank.

MATERIALS AND METHODS

Study area and sampling

The Baiyangdian Lake is located in 38°43′–39°02′N and 115°38′–116°07′E with an area of 366 km² (Figure 1), with a temperate continental and semi-arid climate. The Fuhe River is the only inflow river that has perennial flows. More than 100,000 m³ sewage and treated wastewater with higher nutrients enters the lake every day and 45.24% is attributed to the Fuhe River. Two contaminant sources, i.e. urban sewage and WWTP effluents in upstream are identified. Urban sewage from the Baoding City is discharged into the river at site 1, while effluents from the secondary treatment plants enter the river at site 2.

Measurements of NH₄⁺-N and NO₃⁻-N concentrations and isotopic compositions were carried out along 6 profiles of the river in May, August and October 2009 (Figure 1). The average flow velocity in August was 0.32 m/s, which was higher than that in May and October (ca. 0.26 and 0.29 m/s, respectively). The hydraulic retention time in May was about 2 days; longer than that in August and October. Site 7 was in the north of the lake to represent the lake environment. River water was collected from the middle of river at a depth of 30 cm, and then stored at 4 °C. Water temperature (T), dissolved oxygen (DO) and pH were measured on site (Thermo Orion, America). Surface sediments (the upper 10 cm layer) in the river bank were collected and homogenized. Small parts were stored at 4 °C, the residuals were centrifuged at 5,000 rpm for 30 min to get interstitial water for the following analysis.

Laboratory analysis

NH₄⁺-N and NO₃⁻-N concentrations in water were analyzed according to standard methods reported in AWWA (1999). Total organic carbon (TOC) and total organic nitrogen (TON) of sediments were measured using an Elemental Analyzer (EA, Vario EL, Elementar, Germany). To extract mineral N from sediments, 5 g of fresh sediment was shaken in 25 mL 1 mol/L KCl for 0.5 h. NH₄⁺-N and NO₃⁻-N concentrations in filtered extracts were determined using standard colorimetric methods.

To determinate the isotopic composition, filtered water samples were passed through a cation exchange resin (3 mL of Biorad AG 50W-X8, H⁺ form) and subsequently through
an anion exchange resin (5 mL Dowex 1-X8, Cl⁻ form) with a rate of 6 mL/min. The anion exchange resins containing the NO₃⁻ were stored at 4 °C until further experiments. NO₃⁻ was eluted and converted to AgNO₃ according to the method presented by Silva et al. (2000). For determination of nitrogen isotope ratios of NH₄⁺, samples were filtered and processed based on the techniques described in Lehmann et al. (2001). Nitrogen isotope ratios (δ¹⁵N (‰)) are given by

\[
\delta^{15}N = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} - 1\right] \times 1000
\]

(1)

where \(R\) is the abundance ratio of ¹⁵N to ¹⁴N. Nitrogen isotope ratios were determined on N₂ after thermal decomposition of samples using a DELTA plus XP mass spectrometer connected with a CE EA1112 C/N/S analyzer. International standard IAEA-N3 was used for δ¹⁵N calibration. The reproducibility of duplicate analyzers was better than 0.2‰.

The isotopic composition of the substrate (δₛ) can be calculated from the following relation, which is known as the Rayleigh equation.

\[
\delta_s = \delta_0 + \varepsilon(1 - f) = \delta_0 + \varepsilon(\ln N - \ln N_0)
\]

(2)

where \(\varepsilon\) is the enrichment factor, \(\delta_0\) and \(\delta_s\) are the substrate isotopic compositions at initial and final time, \(f\) is the fraction of substrate converted, and \(N_0\) and \(N\) are the substrate concentrations at initial and final time, respectively. The δ¹⁵N of the accumulated product (δₚ) is given by

\[
\delta_p = \varepsilon(1 - f)/[\ln(1 - f)]/f
\]

(3)

RESULTS AND DISCUSSION

Extra input of nitrogen pollutants

Due to the typical characteristics of the climate and geologic structure of the study area, the nonpoint pollution caused by runoff was assumed to be no significant contribution to nutrient loading in the river. Thus, urban nitrogen loading of the river was mainly contributed by the effluents discharged into the upstream, especially those from WWTPs, which still contain higher NH₄⁺-N. Accordingly, the isotopic composition of NH₄⁺-N in the river (about 4–5‰) is similar to that of industrial and domestic wastewater (δ¹⁵N-NH₄⁺ < 10‰) (e.g. Kendall 1998; Li et al. 2005). Since NO₃⁻-N concentration in WWTPs effluents is below the detection limit, the higher NO₃⁻-N concentration observed at site 2 was mainly due to nitrification. The concentration and δ¹⁵N of NH₄⁺-N at site 7 represented values in the lake which are much lower than that of the river. The δ¹⁵N-NH₄⁺, varying from 0.79 to 2.07‰, is close to that of runoff and natural water (e.g. Kendall 1998; Robinson 2001). The results confirmed that the nitrogen loading in the river was mainly generated from anthropogenic pollution.

Variation of NH₄⁺-N dynamics in the river

Seasonal variation of NH₄⁺-N dynamics

Generally, NH₄⁺ in solution is in equilibrium with NH₃ at a pH of 9.3 (Korom 1992). In the river, pH ranged between 7.16 and 8.25, thus loss of NH₄⁺ due to volatilization could be negligible. In upstream, effluents are sources of both chemical compounds and microorganisms, which can be activated in the river. The input of nitrifying bacteria results in rapid nitrification in the river. During nitrification, the residual nitrogen isotope of NH₄⁺-N is progressively increasing over time. Thus in May, nitrification might occur along the river, mainly from site 1 to site 2, resulting in the decline of NH₄⁺-N concentrations (from 36.92 to 20.02 mg/L), together with an increase in δ¹⁵N of the residual NH₄⁺-N (Table 1). However, at site 6, the NH₄⁺-N concentration decreased to 14.27 mg/L, while δ¹⁵N-NH₄⁺ reduced to 1.55‰. The δ¹⁵N values varied between that measured in the river and the lake. As site 6 is located at the river mouth, the variation of δ¹⁵N-NH₄⁺ indicates the mixing process of river water and lake water occurred so that the concentration of NH₄⁺-N and δ¹⁵N values decreased simultaneously.

The most important factors influencing nitrification are light intensity, substrate, and DO. DO is considered as the inhibiting factor for nitrification, since the light intensity and NH₄⁺-N concentration are not limiting factors in the study river. Oxygen deficiency is routinely observed during summer at the higher temperature, which is a typical feature of the river. Thus, in summer the lower DO (less than 2 mg/L) from site 1 to site 4 inhibits the activity of nitrifying bacteria. While δ¹⁵N-NH₄⁺ remained almost constant, indicating that nitrification contributed little to the NH₄⁺-N reduction. In August the aquatic plants biomass reached the maximum, and reeds dominated the vegetative belts. They can assimilate a considerable amount of nitrogen from the river. The assimilation of reeds has little effects on ¹⁵N enrichment, especially with mature plants (Lund et al. 2000). As the preferred form of inorganic nitrogen for most biota, plant uptake might contribute mostly to NH₄⁺-N reduction during summer. Thus restoration of vegetation distribution could be the key to
releases eutrophic status of the aquatic system. In autumn, reeds were harvested, and the longitudinal variation of $\delta^{15}$N-NH$_4^+$ was similar to that in spring (except for site 5), which indicated that nitrification in the river became actively due to the increase of DO.

**Variation of NH$_4^+$-N dynamics at the interface**

Compared with river water, the NH$_4^+$-N concentrations of interstitial water were higher (Table 1), due to the decomposition processes in the anaerobic area. NH$_4^+$-N obtained from decomposition of organic matter entered the interstitial water, which was supported by linear relationships between NH$_4^+$-N concentration of interstitial water and the concentrations of NH$_4^+$-N and TON of the sediments in spring and summer (Figure 2). The deficit of NH$_4^+$-N ($\Delta$NH$_4^+$-N) at the interface was determined based on the difference between NH$_4^+$-N concentrations of interstitial water and river water. According to the Fick’s formula, the NH$_4^+$-N flux was almost positive, indicating a potential diffusion from the sediment to the overlying water. Since DO is one factor that controls nutrient fluxes, a linear decrease relationship was observed for NH$_4^+$-N flux versus DO concentration (in May, August and October, $R^2$ were 0.5654, 0.3193 and 0.6328, respectively). Thus a much higher NH$_4^+$-N flux was observed in upstream, where DO was lower compared to that in downstream.

When NH$_4^+$-N of interstitial water diffused to the interface an intense nitrogen cycle might occur. Nitrification will result in the production of NO$_3^-$-N accompanied by the

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**Table 1** Physical characteristics, concentrations and $\delta^{15}$N values of NH$_4^+$ and NO$_3^{-}$/CO$_2$ in the river water and interstitial water (*) of the Fuhe River

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Indicators</th>
<th>Sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>1</td>
</tr>
<tr>
<td>May</td>
<td>C (mg/L)</td>
<td>36.92</td>
</tr>
<tr>
<td></td>
<td>C* (mg/L)</td>
<td>39.10</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N* (%o)</td>
<td>7.22</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>C (mg/L)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>C* (mg/L)</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>–6.69</td>
</tr>
<tr>
<td>August</td>
<td>NH$_4^+$</td>
<td>17.98</td>
</tr>
<tr>
<td></td>
<td>C (mg/L)</td>
<td>68.68</td>
</tr>
<tr>
<td></td>
<td>C* (mg/L)</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>4.76</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>C (mg/L)</td>
<td>2.87</td>
</tr>
<tr>
<td></td>
<td>C* (mg/L)</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>–2.82</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N* (%o)</td>
<td>4.60</td>
</tr>
<tr>
<td>October</td>
<td>NH$_4^+$</td>
<td>19.08</td>
</tr>
<tr>
<td></td>
<td>C (mg/L)</td>
<td>36.26</td>
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<tr>
<td></td>
<td>C* (mg/L)</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>2.83</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>C (mg/L)</td>
<td>5.92</td>
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<tr>
<td></td>
<td>C* (mg/L)</td>
<td>4.66</td>
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<tr>
<td></td>
<td>$\delta^{15}$N (%o)</td>
<td>8.55</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}$N* (%o)</td>
<td>6.89</td>
</tr>
</tbody>
</table>

*Concentrations and $\delta^{15}$N values of NH$_4^+$ and NO$_3^-$ in the interstitial water.
Equations (1) and (3), one gets: 

\[ \delta^{15}N \text{ obtained from NH}_4^+ \text{. However, } \delta^{15}N-\text{NO}_3^- \text{ at site 2 was 8.56\% in May, much higher than } \delta^{15}N-\text{NH}_4^+ \text{ (about 4\%), which demonstrated that nitrification and denitrification occurred simultaneously in the river. Isotopic fractionation during denitrification is controlled by microbial reduction rate and varies proportionally with the initial NO}_3^-\cdot N concentration and inversely with the temperature and organic reductant concentration. Because organic donors are relatively abundant in this river, isotopic fractionation reached the minima during summer at the higher temperature and lower NO}_3^-\cdot N concentration, while it reached the maxima in autumn (Table 1). Isotopic fractionation in May was intermediate as the temperature and NO}_3^-\cdot N concentration varied between that in summer and autumn. 

As the activity of nitrifying bacteria was inhibited in summer, except for site 2, NO}_3^-\cdot N formed in nitrification was assumed negligible. We supposed that the river did not receive any other significant NO}_3^-\cdot N input during this period; the denitrification process can be simulated as a Rayleigh process Equation (2). As shown in Figure 3(a), the linear increase of \( \delta^{15}N-\text{NO}_3^- \), corresponding to the logarithmic decrease of the NO}_3^-\cdot N concentration, indicates the reduction of NO}_3^-\cdot N was caused by denitrification. To calculate the degree of denitrification, the lowest \( \delta^{15}N-\text{NO}_3^- \) at site 2, −3.52\%, was chosen as the initial isotopic composition. The corresponding isotopic enrichment factor (\( \epsilon \)) was −4.16\%, which is lower than that for denitrifying systems based on laboratory experiments (varying from −17\% to −29\%) (Lund et al. 2000). While, it is similar to factors for denitrification derived in various natural aquatic systems, such as ponds, river riparian zones, and reed belts around lakes (Lund et al. 2000; Dhondt et al. 2005; Fukuhara et al. 2007). The discrepancy between the observed \( \epsilon \) value in the river and the laboratory measurements might be caused due to the assimilation in plants and microorganisms.

Using the method suggested by Lund et al. (2000), literature value for the isotopic enrichment factor of denitrification (\( \epsilon^D \)) is −17\% (Blackmer & Bremner 1977), and that of assimilation (\( \epsilon^A \)) is 0\%. As shown in Figure 3(b), because the final NO}_3^-\cdot N concentration represents a loss of 86.5\% (remaining fraction of 0.137), about 44.6\% (0.385/0.863) of the NO}_3^-\cdot N loss was attributed to denitrification, and the remaining 55.4\% (0.478/0.863) was due to assimilation. However, the use of the denitrification related curve might underestimate the proportion of NO}_3^-\cdot N loss arising from denitrification (Lund et al. 2000). Thus, denitrification might play a more important role in the NO}_3^-\cdot N reduction, especially in the periods with less vegetation (e.g. autumn).

enrichment of \( ^{15}N-\text{NH}_4^+ \) in the overlying water. If sufficient nitrogen is available, i.e. \( f \) approaches zero, combining Equations (1) and (3), one gets:

\[ \epsilon = 1,000[(\delta_{\text{sink}} - \delta_{\text{source}})/(1 + \delta_{\text{sink}}/1,000)] = \delta_{\text{sink}} - \delta_{\text{source}} = \Delta \]  (4)

here ‘substrates' and ‘products' are replaced by more relevant ‘sources' and ‘sinks'. We assumed that the overlying water was the sink for \( \text{NH}_4^+\cdot N \), thus \( \epsilon \) \( (\text{NH}_4^+) \) ranged from −7.41 to −0.42\% in downstream which was often larger than that in upstream. This indicates that nitrification was more likely to occur in downstream, due to the spatial changes of DO in the river.

**Variation of NO}_3^-\cdot N dynamics in the river**

**Seasonal variation of NO}_3^-\cdot N dynamics**

NO}_3^-\cdot N in the river was mainly caused from nitrification. If only nitrification occurred, the final \( \delta^{15}N-\text{NO}_3^- \) would approach to the \( \delta^{15}N \) obtained from \( \text{NH}_4^+ \). However, \( \delta^{15}N-\text{NO}_3^- \) at site 2 was 8.56\% in May, much higher than \( \delta^{15}N-\text{NH}_4^+ \) (about 4\%), which demonstrated that nitrification and denitrification occurred simultaneously in the river. Isotopic fractionation during denitrification is controlled by microbial reduction rate and varies proportionally with the initial NO}_3^-\cdot N concentration and inversely with the temperature and organic reductant concentration. Because organic donors are relatively abundant in this river, isotopic fractionation reached the minima during summer at the higher temperature and lower NO}_3^-\cdot N concentration, while it reached the maxima in autumn (Table 1). Isotopic fractionation in May was intermediate as the temperature and NO}_3^-\cdot N concentration varied between that in summer and autumn.

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As shown in Table 1, large variation of \( \delta^{15}\text{N-NO}_3^-/C_0 \) (from \(-1.67\) to \(14.87\)‰) in autumn was induced by the intensive denitrification with less plant uptake.

**Variation of NO\(_3\) -N dynamics at the interfaces**

The seasonal and spatial variations of NO\(_3\) -N concentration in interstitial water were not significant (Table 1). Compared with \( \Delta N\text{H}_4^+ - N \), the deficit of NO\(_3\) -N (\( \Delta \text{NO}_3^- - N \)) was much lower and negative in upstream and positive in downstream are identified. This shows that NO\(_3\) -N can diffuse from the overlying water to the sediment in upstream, indicating that there is a potential denitrification in this area. Since the release processes are not only influenced by the molecular diffusion, but also by the hydrologic processes. The result obtained from Fick’s formula might underestimate nutrient fluxes in some cases. However, the relationship of nutrient flux with the impacted factors still valid hold in this research which facilitates the estimate of nutrient fluxes across the terrestrial–aquatic interface.

During the year, \( \delta^{15}\text{N-NO}_3^- \) of interstitial water varied from 1.85 to 13.78‰ (Table 1). \( \delta^{15}\text{N-NO}_3^- \) of interstitial water were not analyzed in May, due to a lack of samples. In August, \( \delta^{15}\text{N-NO}_3^- \) increased from 3.57 to 13.78‰ along the river, while it resides between 1.85 to 8.14‰ with fluctuations in October. The \( ^{15}\text{N-NO}_3^- \) of the interstitial water was higher than that of adjacent river water in most of the sampling sites. This indicates that the interfaces stimulated denitrification, compared to the river channel. Generally, denitrification depends on the supply of easily degradable organic carbon, and it can be inferred that this carbon can be provided by the high autochthonous primary production in soils. Thus the differences of \( ^{15}\text{N-NO}_3^- \) were larger in summer, resulting from the efficient supply of DOC in the river.

### CONCLUSIONS

Nitrogen pollution in the Fuhe River was mainly caused by extra NH\(_4^+ - N \) inputs coming from urban sewage and treated wastewater. NH\(_4^+ - N \), formed in decomposing sediment organic matter, can also diffuse to the overlying water, and the intense nitrogen cycle then occurs at the interface. Compared to the river channel, the interfaces easily stimulate denitrification. Despite the upstream area favoring denitrification, nitrification was most likely to occur in downstream. This might be caused by the variation of DO in the river. Seasonal variation of nitrogen processes is closely related to temperature and dry–wet cycles. Due to less vegetation in spring and autumn, loss of nutrients is caused by the nitrification and denitrification along the river. In summer, NH\(_4^+ - N \) was mainly absorbed by plants. The loss rate of NO\(_3\) -N is 86.3%, and 44.6% nitrate loss is caused by denitrification and the remainder was due to plant uptake. Thus, restoration of vegetation distribution along the river benefits the incremental improvements to the aquatic ecosystem.

### ACKNOWLEDGEMENTS

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