Transformation of nitrogenous compounds in the water-sediment-microbiological system from the Yangtze River

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Abstract. The water-sediment-microbiological system was constructed by flow-through technique in the laboratory using the undisturbed sediment cores collected from Nanjing section of Yangtze River to determine nitrification, denitrification and anaerobic ammonium oxidation (anammox) processes of nitrogenous compounds. The nitrification rates ranged from 11.95 to 19.29 μmol N m⁻² h⁻¹ in the sediments and the rates of denitrification and anammox ranged from 11.98 to 65.86 μmol N m⁻² h⁻¹ and 0.40 to 8.04 μmol N m⁻² h⁻¹, respectively. Denitrification dominated nitrogen removal process, especially non-coupling nitrification process. The decrease of total organic carbon (TOC) and electrical conductivity (EC) in sediments increased the activity of ammonia monooxygenase and promoted the rate of nitrification. The denitrification rates were related to NO₃⁻ concentrations in the overlying water, and TOC, pH and EC affected the activity of the nitrate reductase. The increase of TOC and clay content and the decrease of EC corresponded to the increase of nitrate reductase activity and anaerobic ammonia oxidation rate. The optimum temperature was 35°C for denitrification and 25°C for nitrification. The pH values influenced nitrogen conversion, denitrification occurred more easily in neutral environment, and the nitrogen conversion rates in the light were higher than in the dark.

1. Introduction

In recent decades, the fossil fuel combustion and the use of agricultural fertilizers have caused excess nitrogen to flow into streams and rivers, causing serious nitrogen pollution [1]. Nitrogen is a key limiting factor of water primary productivity and one of the reasons of the eutrophication [2]. Therefore, it is important to understand the transformation mechanism of nitrogen at sediment-water interface, which is of great significance for water environmental governance. As a region of rapid economic development, the Nanjing section of Yangtze River is the hardest-hit area of eutrophication [3]. The pathways of nitrogen removal from the water ecosystem, including nitrogen fixation, nitrification, denitrification, anaerobic ammonium (anammox) and dissimilatory nitrate reduction to ammonium (DNRA), have received a lot of attention.

In recent years, many researchers have studied the sources of nitrogen in rivers by using a stable isotope approach [1,4]. Ding et al found that the nitrification of N-containing organic materials was the important source of NO₃⁻ and nitrification played a more crucial role than assimilation for the decreasing ammonium concentrations in the Taige River in the East Plain Region of China [5].
Denitrification as the dominant nitrogen removal process and its nitrogen source comes mainly from nitrate nitrogen in overlying water and nitrification [6]. The water nutrients (e.g., concentrations of NO$_3^-$, organic carbon and NH$_4^+$) and environmental factors (e.g., pH, temperature and light) were found to be important factors to influence denitrification in the Ashtamudi estuarine sediments [7]. In addition, the effects of water quality, sediment properties and nutrients on denitrification were quantified by investigated nitrite reductase genes [8,9]. Environmental factors mainly influence the rate of nitrogen removal by affecting the enzyme activity in bacteria [10]. Light could stimulate benthic microorganisms to nitrify NH$_4^+$-N to NO$_x$-N, resulting in a decrease of NH$_4^+$-N flux and an increase of NO$_x$-N flux from sediment to overlying water [11].

The study of nitrogen transformation mechanism in sediment-water interface is an important way to understand the biogeochemical process of nitrogen. However, information on nitrogen conversion rates combined with related enzyme activities and N$_2$ release in sediment-water interface is limited. In this study, four columnar sediments samples were collected from Nanjing section of the Yangtze River, where nitrogen loadings were relatively high. Water-sediment-microbiological systems were constructed to investigate the nitrification, denitrification and anammox rates and to determine the influences of nutrients and environmental factors on nitrogen removal.

2. Materials and methods

2.1. Sampling sites and samples collections

Sediment and water samples were collected from four sites located Nanjing section of Yangtze River in September 2017 (figure 1). Columnar sediments (7.6 cm diameter, 20 cm deep) were collected from the shoals of each site by SS-H-02 sediment corer (EasySensor, Nanjing) that maintained the integrity of the sediment-water interface. The sediment samples shipped to the laboratory within 2 h and stored at 4°C. Forty liters of near-bottom water was collected using organic glass hydrophore from each station and put in the pre-washed polyethylene bottle for continuous cultivation. The surface sediments (about 200 g) were collected to use for the detection of sediment characteristics.

![Figure 1. Sampling sites in Nanjing section of Yangtze River.](image)

2.2. Nitrification

The in-situ sediments were transferred to the culture tubes (7.6 cm diameter, 20 cm deep), and the sediment height was adjusted to about 10 cm. The experimental groups were slowly injected 10% saturation acetylene (166.67 μL of C$_2$H$_2$ per mL) of the bottom water, avoiding disturbing sediment surfaces. The control groups were injected with the bottom water. The culture tube was sealed with a rubber plug to prevent leakage. And 3 replicates were prepared for each group. The incubations were performed at 25°C. After 4 h of undisturbed incubation, 60 mL of water sample was quickly extracted from the culture tube and filtered with a 0.45 μm mixed fiber filter membrane, and then determined the concentration of NH$_4^+$-N by the Nessler’s reagent spectrophotometry. The nitrification rate is represented by the reduction of ammonia nitrogen (μmol N m$^{-2}$ h$^{-1}$).
2.3. Denitrification and anammox

Incubations were conducted in a flow-through system in which bottom water was pumped over cores using a multi-channel peristaltic pump that maintained 1.5 mL min\(^{-1}\) flow. A rubber plug with inlet pipe (2 mm diameter, 8 cm length) and outlet pipe (2 mm diameter, 5 cm length) was inserted into each culture tube to ~5 cm from the sediment-water interface, which left 5 cm of overlying water. The cores were incubated in an artificial climate box, keeping the sampling temperature and normal light throughout the experiment. The 40 L overlying water was divided into three separate glass containers. One glass container added with \(^{15}\)N-KNO\(_3\) (99\%) to final concentrations of 3.30 mg L\(^{-1}\). The second glass container was enriched with \(^{15}\)N-NH\(_4\)Cl (99\%) to final concentrations of 2.29 mg L\(^{-1}\). The third glass container was remained in its original state as a control treatment. A 24 h pre-incubation was operated to reach equilibrium. Then, inflow and outflow samples were collected every 12 h from 0 to 48 h in the first and second cultivating device. The outlet pipe was inserted into the bottom of the headspace injection bottle (15 mL), and waiting for the outlet overflowed the bottle, and the saturated ZnCl\(_2\) (20 μL) was injected into the collected samples by the pipetting gun in order to inhibit the microbial activity, after that, the headspace injection bottle was quickly sealed, ensuring no bubble residue in the sample bottle. The samples were used to analyze the dissolved gases (\(^{29}\)N\(_2\) and \(^{30}\)N\(_2\)) by membrane inlet mass spectrometry (MIMS, Bay Instruments, Easton, MD, USA). The dissolved gas [12] was calculated according to the equation

\[
Flux(\mu\text{mol N m}^{-2}\text{ h}^{-1}) = (c_i - c_{i,0}) \times \frac{F}{A}
\]

Where \(Flux\) (\(\mu\text{mol N m}^{-2}\text{ h}^{-1}\)) is the \(N_2\) release rate, \(c_i\) (μM) represents the outflow concentration of \(N_2\), \(c_{i,0}\) (μM) is the inflow concentration of \(N_2\), \(F\) is the velocity of the peristaltic pump (L h\(^{-1}\)), and \(A\) is the surface area of the core (m\(^2\)).

The anammox of the \(^{14}\)NH\(_4\)\(^+\) and \(^{14}\)NO\(_3\)\(^-\) reported as \(A_{14}\), and it was calculated by the production of the \(^{29}\)N\(_2\) (\(p^{29}\text{NH}_4\)) from the \(^{15}\)N-NH\(_4\)Cl treatment, using the equations (2)-(4).

\[
A_{15} = p^{29}\text{NH}_4
\]

\[
A_{\text{total}} = \frac{p^{29}\text{NH}_4}{F} \times A
\]

\[
A_{14} = A_{\text{total}} \times A_{15}
\]

\(^{29}\text{NH}_4\) (μmol N m\(^{-2}\) h\(^{-1}\)) was the release rate of \(^{29}\)N\(_2\); \(A_{\text{total}}\) (μmol N m\(^{-2}\) h\(^{-1}\)) was the total anammox, and the \(F\) was the ratio of \(^{15}\text{NH}_4^+\) to total \(\text{NH}_4^+\) in the overlying water; \(A_{15}\) was the contribution of \(^{15}\text{NH}_4^+\) to anammox. Calculated denitrification based on the production of \(^{28}\)N\(_2\) (\(p^{29}\text{NH}_4\)) and \(^{30}\)N\(_2\) (\(p30\)) from the \(^{15}\)NO\(_3\)\(^-\) treatment and \(A_{14}\) that we figured out, using the equations (5)-(11)

\[
A_{14} = 2 \times A_{28} + A_{29}
\]

\[
r_{14} = \frac{A_{28}}{A_{29}}
\]

then, \(r_{14} = \frac{p^{29}\text{NO}_3}{2 \times p30} = \frac{p^{29} - A_{29}}{2 \times p30}
\]

\[
D_{15} = p^{29}\text{NO}_3 + 2 \times p30
\]

\[
D_{14} = D_{15} \times r_{14}
\]
The ammonia monooxygenase (AMO) activity was determined by measuring the concentration of TOC. The incubation temperatures were set as 15, 25 and 35°C. After incubation, the cells were disrupted at 4°C and 20 kHz for 3 min, then centrifuged at 4°C for 30 min. The supernatants were separated and used for enzyme activity analysis. The reaction system consisted of 600 μL of supernatants, 1.8 mL of phosphate buffered saline (0.01 M) and 1.6 mL of (NH₄)₂SO₄ (2 mM) was shaken for 30 min at 30°C, then the ammonia monooxygenase (AMO) activity was determined by measuring the concentration change of NH₄-N before and after reaction and expressed as μg kg⁻¹ h⁻¹. The reaction system consisted of 600 μL of supernatants, 1.8 mL of phosphate buffered saline (0.01 M) and 1.6 mL of NaNO₃ (2 mM) was shaken for 30 min at 30°C, then the activity of nitrate reductase (NAR, μg kg⁻¹ h⁻¹) was obtained by measuring the increment of NO₂⁻-N in the reaction system. The activity of nitrite reductase (NIR, μg kg⁻¹ h⁻¹) was determined by measuring the reduction of NO₂⁻-N in the reaction system.

\[
D_w = D_{15} \times \frac{^{15}NO_3^-}{^{14}NO_3^-} \quad (10)
\]
\[
D_n = D_{14} - D_w \quad (11)
\]

Where the \( A_{28} \) (μmol N m⁻² h⁻¹) and \( A_{39} \) (μmol N m⁻² h⁻¹) represent the production of \(^{28}N_2\) and \(^{29}N_2\) during the anammox, respectively; \( r_{14} \) is the ratio of \(^{14}NO_3^-\) and \(^{15}NO_3^-\) undergoing nitrate reduction; \( D_{14} \) (μmol N m⁻² h⁻¹) and \( D_{35} \) (μmol N m⁻² h⁻¹) are the contribution of \(^{14}NO_3^-\) and \(^{15}NO_3^-\) to denitrification; \( D_w \) (μmol N m⁻² h⁻¹) is the uncoupled nitrification-denitrification; \( D_n \) (μmol N m⁻² h⁻¹) is the coupled nitrification-denitrification.

2.4. Determination of enzyme activities related to nitrogen transformation

Surface sediment (25 g) was collected from the culture tubes after the incubation and then centrifuged for 3 min at 4000×g at 4°C, poured the supernatant and added the buffer (50 mM of phosphate buffered saline, pH=7.8, 5 mM MgCl₂) to 25 mL. The cells were disrupted at 4°C and 20 kHz for 2 min, then centrifuged at 4°C for 30 min. The supernatants were separated and used for enzyme activity analysis. The reaction system consisted of 600 μL of supernatants, 1.8 mL of phosphate buffered saline (0.01 M) and 1.6 mL of (NH₄)₂SO₄ (2 mM) was shaken for 30 min at 30°C, then the ammonia monooxygenase (AMO) activity was determined by measuring the concentration change of NH₄-N before and after reaction and expressed as μg kg⁻¹ h⁻¹. The reaction system consisted of 600 μL of supernatants, 1.8 mL of phosphate buffered saline (0.01 M) and 1.6 mL of NaNO₃ (2 mM) was shaken for 30 min at 30°C, then the activity of nitrate reductase (NAR, μg kg⁻¹ h⁻¹) was obtained by measuring the increment of NO₂⁻-N in the reaction system. The reaction system consisted of 600 μL of supernatants, 1.8 mL of phosphate buffered saline (0.01 M) and 1.6 mL of NaNO₃ (2 mM) was shaken for 30 min at 30°C, and the activity of nitrite reductase (NIR, μg kg⁻¹ h⁻¹) was determined by measuring the reduction of NO₂⁻-N in the reaction system.

2.5. Effects of environmental factors

Temperature, pH and light conditions were designated to investigate the effects of environmental factors on nitrogen transformation. The incubation temperatures were set as 15, 25 and 35°C, respectively. The pH values were adjusted to 5, 7 and 9 in the bottom water respectively. Moreover, nitrogen transformation experiments were completed simultaneously in the natural light and dark for comparison.

All the statistical analyses were performed with the SPSS 23.0. A one-way analysis of variance (ANOVA) following Dunnett’s t-test were carried out to determine the significant differences among different sampling sites or different environmental factors. Differences were considered to be significant at p < 0.05.

3. Results and discussion

3.1. In situ nitrogen transformation rates

The water quality and sediment characteristics of the Nanjing section of the Yangtze River were obtained by field sampling (table 1).

The NH₄⁺ concentration in the overlying water ranged between 0.17 and 1.92 mg L⁻¹, whereas NO₃⁻ concentration ranged between 1.19 and 1.80 mg L⁻¹. The pH was near neutral to alkaline and ranged from 7.41 to 8.23. Overlying water temperature (23.4 to 24°C) was similar across all sites. Ambient EC ranged between 247 and 336 μS cm⁻¹. The overlying water TOC concentration ranged from the lowest concentration at P4 (8.4 g kg⁻¹) to the highest concentration at P3 (15.3 g kg⁻¹). According to their size grade, the sediments were divided into clay (<2 μm), silt (2-20 μm) and sand (>20 μm), accounting for 4.69-6.14, 23.3-36.5 and 66.0-72.0%, respectively.
The nitrification rates in the sediments from different sampling sites ranged from 11.95 to 19.29 μmol N m⁻² h⁻¹ (table 2). The highest nitrification rate (19.29 μmol N m⁻² h⁻¹) was observed at P4, the activity of AMO was also the highest, while the activities of NAR and NIR were the lowest. Denitrification rates ($D_{\text{it}}$) exhibited a remarkable spatial variation, ranged from 11.98 μmol N m⁻² h⁻¹ in P1 to 65.86 μmol N m⁻² h⁻¹ in P3. Anammox rates ($A_{\text{it}}$) ranged from 0.40 μmol N m⁻² h⁻¹ (P4) to 8.04 μmol N m⁻² h⁻¹ (P3). The nitrification rate and AMO activity were the lowest in P3, whereas $D_{\text{it}}, A_{\text{it}},$ NAR and NIR activities in this site were significantly higher than the other sites. The activities of NAR ranged from 52.02 to 154.61 μg kg⁻¹ h⁻¹, and the activity of NIR was ranged from 0.82 to 1.42 μg kg⁻¹ h⁻¹. Compared to $D_{\text{it}}, D_{\text{it}}$ accounted for a great proportion of the nitrogen removal process at the study area, with the rate of 3.91-41.60 μmol N m⁻² h⁻¹.

Nitrification rates had distinct spatial variation at our respective sites. The highest nitrification rates were observed at P4. The nitrification increases with activity of AMO which is found in ammonia-oxidized bacteria [13]. It has been proved that many pivotal factors control the occurrence and rate of nitrification, including pH, temperature, NO$_3^-$-N, NH$_4^+$-N and EC [14,15]. The pH of sediments is related to the nitrification rate, and the pH affects the activity of enzymes in the related microorganisms, thus affecting the nitrification [16]. The optimum pH value on nitrification process was observed at 7.5 [17]. A previous study found that nitrification rates showed a significantly increasing trend with the temperature increased from 10 to 28°C, but obviously declined when temperature was up to 37°C [17]. However, we did not observe a remarkable temperature variation in our study area. Other factors than temperature were considered to be more important regulators of nitrification during our investigation. EC has a positive correlation with osmotic pressure, which lead to the inhibition of activity of AMO.

Denitrification, anammox, and dissimilatory nitrate reduction to ammonium (DNRA) are the most vital processes of dissimilatory nitrate reduction in aquatic environments. Yin et al measured the denitrification and anammox across the sediment–water interface of Jinpu Bay using continuous-flow experiments combined with a $^{15}$NO$_3^-$ tracing technique to determine their relative importance in this hypereutrophic coastal ecosystem [18]. $D_{\text{it}}$ was higher than $A_{\text{it}}$ at four sites, which proved that denitrification was the dominated nitrogen removal processes eliminating reactive nitrogen from aquatic environments [19]. It might be explained by examining water characteristics and sediments properties for the study area. Generally, the high organic matter deposition likely benefits denitrifiers over anammox bacteria since denitrification typically favours the sites with higher OC delivery versus
Anammox, which tends to occur in organic-poor conditions [12].

The essence of denitrification is a biochemical process of reducing NO$_3^-$ and NO$_2^-$ to N$_2$ or N$_2$O and NO in anaerobic under the involvement of denitrifying bacteria [20]. A large fraction of NO$_3^-$ that is denitrified to inert N$_2$ is coupled to nitrification ($D_n$) of NH$_4^+$ in porewater and relatively few NO$_3^-$, derived from the overlying water, was considered as external input which was uncoupled to nitrification ($D_o$) [12]. However, $D_o$ contributed a large part to denitrification (63-95%) in Nanjing section of the Yangtze River, except for P1 (32%). The research area is located in the industrial developed urban area. The discharge of domestic sewage and industrial waste makes the high concentration of NO$_3^-$ in the overlying water, resulting in higher $D_o$ than $D_n$ in the denitrification process. There is an artificial wetland park around P1 site that the fact can explained the difference of $D_o$ between P1 and other sites. After the treatment of sewage and industrial wastewater by artificial wetlands, the nitrogen is removed before it flows into the Yangtze River [21]. Therefore, the external input of NO$_3^-$ is low, and $D_o$ plays a dominant role in denitrification process at P1.

Anaerobic ammonia oxidation is a biologically mediated process in which ammonium oxidized with nitrite (NO$_2^-$) serving as electron acceptor under anaerobic condition [22]. Anammox had a remarkable district variation at our respective sites. Anammox activity and its relative contribution to total N$_2$ production were linked closely to the availability of organic carbon [23], which is in agreement with our result. TOC promoted the production of NO$_3^-$ by denitrifying bacteria, and NO$_3^-$ participated in the anammox process, thus increasing $A_{14}$ [18,22]. In addition, the anammox rates were observed to significantly positively relate to the concentration of NO$_3^-$ in overlying water in other study [24]. But $A_{14}$ had no correlation with NO$_3^-$ in our study. This proved that NO$_3^-$ concentration in the overlying water is not the limiting factor determining $A_{14}$ at the study area. Alternatively, we observed that the activity of NAR was closely linked with $A_{14}$. It may explained by the function of NAR which catalyzed denitrifying bacteria to reduce NO$_3^-$ to NO$_2^-$, promoting the process of $A_{14}$ [16].

What’s more, the anammox rates were observed to positively relate to sulfide concentrations in the sediments in other studies which imply that sulfide can enhance nitrite accumulation in the incomplete denitrification process via inhibiting nitrogen monoxide and nitrous oxide reductases, thus stimulating the anammox reaction indirectly [25,26]. There is no measurement of the effects of sulfide to the $A_{14}$ in our study, and more work needs to be done to verify the function of sulfide.

3.2. Effect of temperature

Nitrogen removal rates showed significant differences with temperature changes. $D_{14}$ and $D_o$ increased when increasing temperature and peaked at 58.07 μmol N m$^{-2}$ h$^{-1}$ and 54.54 μmol N m$^{-2}$ h$^{-1}$ at 35°C (figure 2). While the highest $D_o$ and $A_{14}$ rates (6.76 μmol N m$^{-2}$ h$^{-1}$ and 9.82 μmol N m$^{-2}$ h$^{-1}$) were observed at 25°C. The activity of NAR was the strongest at 25°C, and it showed was significant difference with AMO and NIR activities under all the temperature (p < 0.05). However the NAR and NIR activities did not show significant difference with temperature.
The process of denitrification was affected by various factors, in which temperature, pH and light affected was main factors. There was no significant temperature difference in activity of NAR, suggesting that the nitrification rate did not change significantly under the temperature gradient set in the experiment. We observed the optimal temperature for denitrification rates at 35°C, which was inconsistent with previous results from other Arctic sediments studies, where the optimal temperature values ranged from 20 to 28°C [27]. Denitrifying bacteria have different optimum temperatures in aquatic environment at different climatic conditions. It is important to note that the highest activity of NAR was occurred at 25°C and the activity of NIR was similar under the controlled temperature gradient. The diversity of nosZ communities which was contained in the N2O-reducing microbial assemblages that convert N2O to N2, increased with the increasing of soil temperature [28]. Those may explained by the fact that the NO3– reduction and NO2– reduction in denitrification process are not the key controlled stages to determine the denitrification rates, but the process of N2O reduction to N2 process dominates. The higher the activity of NAR, the more NO3– is produced by NO3– oxidation under the effect of NAR [16]. The NO3– produced is involved in anaerobic ammonia oxidation, which increases A14. We found that the highest rate of anaerobic ammonia oxidation occurred at lower temperatures comparing to denitrification. It was demonstrated that anammox bacteria may be more specialized for psychrophilic activity than denitrifying bacteria [27]. Therefore, the enhancement of nitrification rate could promote Dn and provided a way for river to eliminate internal pollution [12].

3.3. Effect of pH
The highest D14 and A14 were 33.45 μmol N m⁻² h⁻¹ and 1.81 μmol N m⁻² h⁻¹, respectively, under neutral conditions (figure 2), and maximum Dn (30.90 μmol N m⁻² h⁻¹) and Dn (7.28±4.17 μmol N m⁻² h⁻¹) were also observed at pH=7, although the changes were not significant with pH in most cases. The activities of AMO and NAR are the highest at pH=7, while NIR activity did not significantly change with pH values. The activity of NIR was consistently lower than AMO and NAR activities at different pH values. However, the activity of AMO was higher than NAR under acidic condition, while it was opposite under alkaline condition.

Denitrifying bacteria and anaerobic ammonia-oxidizing bacteria are sensitive to the changes of pH [28]. Previous studies have proved that soil pH imposed a general selective pressure on the microbial communities and that this results in changes in N2O emission potential [29]. The pH controls the nitrogen transformation process via limiting the activity of enzymatic related denitrification and anammox [30]. Excessively high or low pH values are not conducive to nitrogen conversion. The optimum pH values for biological reduction of NO3– are approximately 7.35 to 7.45 [30], which is lower than the optimal pH value measured in our study. The alkaline condition is more suitable for denitrification and anaerobic ammonia oxidation at the actual water-sediment interface. In addition, it was reported that genetic potential and transcriptional regulation also had effect when the pH changed [31]. The effects of pH on the composition and diversity of nitrogen-transformed microbial communities and the abundance of functional genes need to be further studied.
3.4. Effect of light

$D_{sa}$ in the light (65.86 µmol N m$^{-2}$ h$^{-1}$) was much higher than that in the dark (24.46 µmol N m$^{-2}$ h$^{-1}$), while $A_{sd}$ did not change significantly (figure 2). The proportion of $D_{sa}$ in denitrification was stimulated under the light treatment. The activity of NAR was significantly higher in the light than dark, however, AMO and NIR activities in the natural light did not differ from in the dark.

Light condition was found to be important factors affecting the nitrogen removing in lake and stream ecosystems by influencing the periphytic biomass and community [32,33]. The activities of AMO, NAR and NIR were inhibited in the dark might resulted in decreased denitrification and anammox rates. The abundance of nitrogen cycle related functional genes increased at the lower photosynthetically active radiation which was positively related to denitrification [34]. What’s more, light could effectively stimulate benthic microalgae to use N and promoted the sediment N release, thus influence on nitrogen cycling in ambient sediment-water interface [32,35]. However, light will inhibit the activity of anammox bacteria, resulting in the reduction of $A_{sd}$[36], which was consistent with our experimental results.

4. Conclusion

The transforming courses of nitrogenous compounds in sediment-water interface were simulated by dynamic lab-experiment and the nitrogen conversion rates at different sites in Nanjing section of the Yangtze River were determined. The nitrification rates were from 11.95 to 19.25 µmol N m$^{-2}$ h$^{-1}$, the denitrification rate were 13.92 to 89.21 µmol N m$^{-2}$ h$^{-1}$, and the anaerobic ammonia oxidation rates were 0.40 to 8.04 µmol N m$^{-2}$ h$^{-1}$. $D_{sa}$ dominated nitrogen removal process and $D_{sa}$ was always higher than $D_{sa}$. The optimum temperature was 35°C for denitrification 25°C for nitrification, respectively. Neutral environment and natural light were conducive to the transformation of nitrogenous compounds.

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