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RESEARCH ARTICLE

Biological denitrification in a macrophytic lake: implications for macrophytes-dominated lake management in the north of China

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Abstract
Denitrification plays an important role in nitrogen (N) removal in freshwater ecosystems. Aquatic plants might have an impact on the sediment denitrification of water body, especially in macrophytes-dominated lake; however, there were different opinions about it. Our hypothesis was that the sediment denitrification rates differ significantly in different vegetation zones and seasons because of direct and indirect effect of the aquatic plants. Therefore, we studied sediment denitrification in Dongping Lake, a typical macrophytes-dominated lake located in the north of China. The acetylene inhibition technique was used to quantify the sediment denitrification rates (DRs) in the *Phragmites communis* (*P. communis*) zone, aquaculture zone, *Potamogeton crispus* (*P. crispus*) zone and mixed vegetation zone in July (summer), October (autumn), December (winter) of 2015 and March (spring) of 2016. The results showed that the average DRs were significantly higher in the *P. communis* zone (69.0 ± 91.6 μmol N m^{-2} h^{-1}) than the mixed vegetation zone (8.70 ± 5.44 μmol N m^{-2} h^{-1}), and the average DRs represented significant seasonal difference as in the order of winter (74.5 ± 88.3 μmol N m^{-2} h^{-1}) > autumn (15.7 ± 18.6 μmol N m^{-2} h^{-1}) ≈ summer (10.7 ± 5.90 μmol N m^{-2} h^{-1}) > spring (3.85 ± 1.29 μmol N m^{-2} h^{-1}). The DRs generally decreased with the increasing of depth; however, significant increase of DRs with depth were found in certain seasons at the vegetated zones except the non-vegetated zone (the aquaculture zone) indicating the possible rhizosphere effect of aquatic plants on denitrification. The higher DRs and cycling rates of nitrate in the *P. communis* zone might be related to the larger biomass and oxygen transporting capacity of *P. communis* than those of the other aquatic plants. Winter peaks of DRs might be attributed to the higher NO_3^- load and the absence of the plant uptake. The high cycling rates of nitrate in Dongping Lake indicated an enhanced internal N cycling by aquatic plants. Sediment denitrification could remove about 537.7 t N every year, which was about 26.5% of annual TN loading in Dongping Lake.

Keywords Denitrification · Sediment · Nitrogen removal capability · Cycling rate of nitrate · Aquatic plants · Macrophytic lake

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Introduction

Anthropogenic activities were responsible for 210 Tg N of reactive nitrogen to terrestrial and marine ecosystem, which was about half of global nitrogen fixation (Fowler et al. 2013), and this resulted in one-third of the world's population affected by nitrogen pollution (WWAP 2017). In the past 40 years, the nitrogen concentrations of lakes in China have increased dramatically, and the nitrogen concentrations of some lakes even have increased 32 times (Guan et al. 2011). High nitrogen loading can lead to water eutrophication, oxygen deficit, and water quality deterioration (Guan et al. 2011), so nitrogen pollution in aquatic ecosystem becomes a research hotspot (Camargo and Álvaro 2006; Shibata et al. 2015).

Nitrate is the main form of nitrogen in water body and has strong mobility and bioavailability, so its transportation and transformation have always been the focus. Nitrate in the aquatic ecosystem can be mainly removed by two ways. One way is to be assimilated by phytoplankton and other aquatic plants or microbes in water body and become an integral part of the organisms (O'brien et al. 2012). The other is to be transformed to N_2 or N_2O through denitrification and then transported into air (Burgin and Hamilton 2007; Scott et al. 2008; Beaulieu et al. 2011; Yin et al. 2015; Deng et al. 2019). Denitrification is the primary mechanism of nitrogen retention, followed by uptake of aquatic plants (Saunders and Kalff 2001). Furthermore, compared with the assimilation of nitrogen, denitrification is the more thorough way to remove nitrogen because its product, i.e., N_2 or N_2O, cannot be assimilated by most living creatures and can be discharged into atmosphere. Therefore, denitrification is of greatly important ecological significance to reduce the nitrogen load and remediate nitrogen pollution in water body.

Aquatic plants are an indispensable part of the lacustrine ecosystem, especially in the macrophytic lakes. There are different opinions on the effect of aquatic plants on nitrogen transformation. Liu et al. (2018) found that denitrification was controlled by physicochemical properties of water body, such as pH, Eh, temperature, concentration of nitrogen, etc. instead of the types of aquatic plants. Palacin-Lizarbe et al. (2020) found that nitrate concentration and temperature provided the most explanatory power for the actual denitrification rates in sediments of several lake types and habitats by using the multiple linear regression models. However, Zhang et al. (2017) found that aquatic plants could change physicochemical properties of water body and then affect denitrification indirectly. Eriksson and Weisner (1999) found that submersed vegetation (*Potamogeton pectinatus*) might greatly enhance the transformation of NH_4^+ to N_2 by the sequential action of nitrification and denitrification in NH_4^+-rich freshwater ecosystem. Nizzoli et al. (2014) also found that *P. pectinatus* would modify the N dynamics by enhancing N removal via assimilation and denitrification. Veraart et al. (2011) studied the effect of floating vegetation and submerged vegetation on denitrification rates using microcosm, and found that the oxygen production by photosynthesis of plants inhibited denitrification in the top layer of the sediment. Besides, aquatic plants can transport oxygen to the root zone and increase the oxygen supply to the root zone microbes (Sand-Jensen et al. 1982; Mi et al. 2008), which can stimulate the activities of the microbes and enhance the denitrification (Choi et al. 2009; Vila-Costa et al. 2016; Palacin-Lizarbe et al. 2020). Aquatic plants create an ideal environment for denitrification by increasing the supply of potentially limiting organic carbon and nitrate to denitrifying bacteria (Saunders and Kalff 2001). Moreover, when aquatic plants die and decay, they can release carbon and nitrogen which is to affect the denitrification rates (Bastviken et al. 2005; Li et al. 2014).

Dongping Lake is a typical macrophytes lake in the north of China. As a water collection center and a retention reservoir, the main role of Dongping Lake is to regulate and store floods of the Yellow River and the Dawen River. In addition, Dongping Lake serves important roles in the East Route of the South-to-North Water Diversion Project of China and water transmission from the west to the east of Shandong Province. One of the key potential water quality concerns in Dongping Lake is eutrophication (Yang et al. 2017), and NO_3^- as a non-carcinogen has health risk on human body. Although the non-carcinogenic risk values for NO_3^- were lower than the standard recommended by US EPA (Zhang et al. 2011), external nitrogen retention will increase the risk of eutrophication and the health risk of NO_3^-. We hypothesized that the sediment denitrification rates differ significantly in different vegetation zones and seasons because of direct and indirect effect of the aquatic plants. Taking the Dongping Lake as the study area, the objectives of this research are to (1) investigate the sediment denitrification rates in different aquatic vegetation zones and seasons, (2) identify the main factors that impact the denitrification rates, (3) compare the N cycling rates in different aquatic vegetation zones, and (4) calculate the capability of sediment to eliminate external nitrogen loading into the drinking water reservoir and examine the role of denitrification in controlling the nitrogen concentrations in the water body of different aquatic vegetation to make a suggestion for vegetation management.

Materials and methods

Study site

Dongping Lake (35° 30′~36° 20′ N, 116° 00′~116° 30′ E) is located in Dongping County, southwest of Shandong Province, China (Fig. 1). With an area of 134 km^2 retaining water all year round, Dongping Lake is the second largest freshwater lake in Shandong Province. The lake is a flat basin and its multi-annual mean water depth is 2~4 m (Yang et al. 2017). The area of Dongping Lake experiences a warm and semi-humid continental monsoon climate and has four distinct seasons, and the average annual precipitation and temperature were 601 mm and 13.3 °C respectively; and the average monthly water temperature varies between 4 °C (January) and 30 °C (August) (Compiling Team for Annals of Dongping County Shandong Province 2006). The average annual evapotranspiration water demand of Dongping Lake is 7.7×10^7 m^3 (Wang et al. 2014). Dongping Lake was at mesotrophic or eutrophic level (Yang et al. 2017) and the water quality was at moderate pollution level with TN as the predominant pollutant factor (He et al. 2010; Shi et al. 2011). Recharge to Dongping Lake relies mainly on surface runoff via the Dawen River which is about 10.6×10^8 m^3 a^{-1} and the East Route of the South-to-North Water Diversion Project (Yang et al. 2017). The

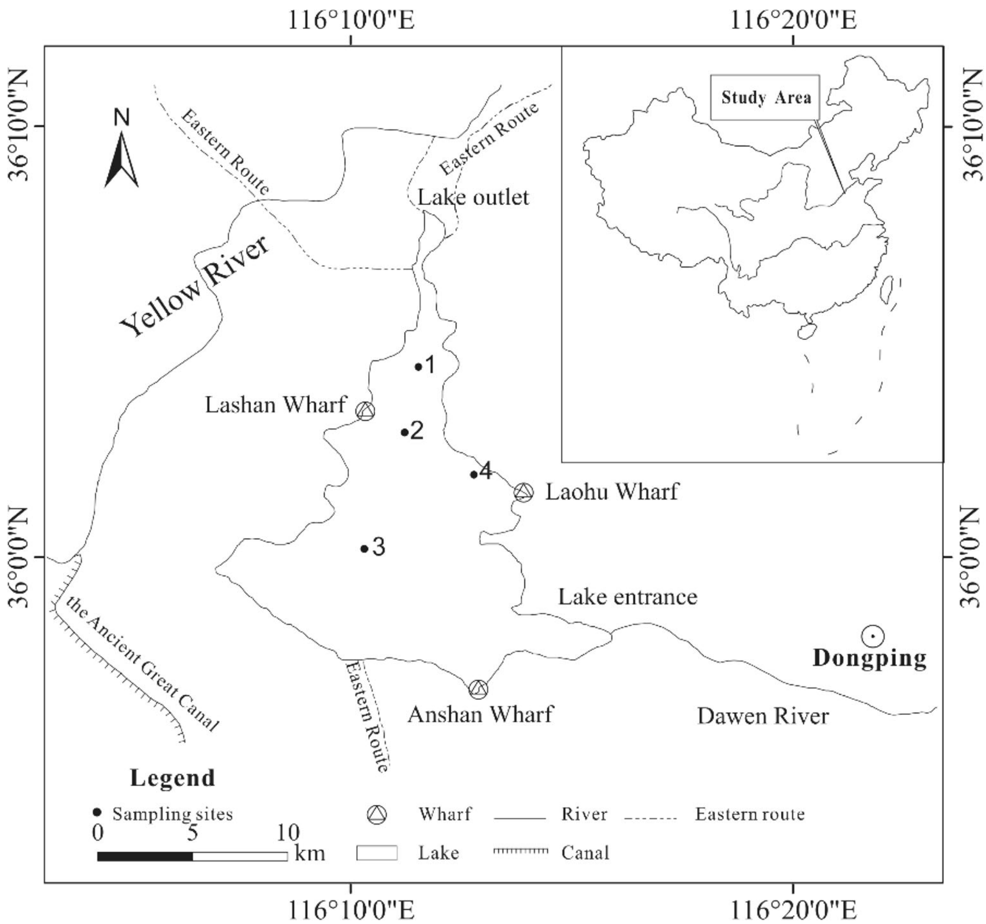

Fig. 1 Location of the sampling sites

water in the lake flows northward through the Xiaoqing River and the East Route of the South-to-North Water Diversion Project, finally entering the water-receiving area. In addition to the upstream water input, precipitation is the other main external source of nitrogen to the water body.

Dongping Lake has an abundance of vegetation types including *Phragmites*, *Potamogeton*, *Nymphaea*, *Typha*, *Cyperus*, etc. in which *Phragmites communis* (*P. communis*) and *Potamogeton crispus* (*P. crispus*) are the dominant species of the aquatic vascular plants (Shi et al. 2011). *P. communis* as an emergent plant shoots and grows in spring and summer, and it withers and decays in autumn and winter; while *P. crispus* as a submerged plant forms shoots in the autumn and grows in the winter and spring and it begins to die and decompose in early summer, which causes the deterioration of the aquatic ecological environment of Dongping Lake (Deng et al. 2016). According to the field investigation, *P. communis* is distributed mainly near estuary and outlet, while *P. crispus* is widely distributed in Dongping Lake except in estuary and outlet. A variety of aquatic plants including *P. communis*, *P. crispus*, *Nymphaea tetragona*, *Typha orientails Presl*, and *Cyperus rotundus* is found in the area near the lakeshore. Due to the intense aquaculture activities, the vegetation in the water body of the aquaculture zone were destroyed resulting in the less biomass of the vegetation. Considering the vegetation types and the impact of aquaculture activities, four sampling sites were established in Dongping Lake, which were *P. communis* zone (site 1), aquaculture zone (site 2), *P. crispus* zone (site 3), and the mixed vegetation zone (site 4) respectively (Fig. 1).

Sampling

Nine surface sediment cores (5-cm depth) were collected seasonally at each site in July (summer), October (autumn), December (winter) of 2015, and March (spring) of 2016 using the Perspex tubes (35 mm inner diameter × 350 mm long). Two caps with sealing washers inside were used to cover the two ends of each tube and the tubes were stored vertically in a large plastic box filled with the in situ water. Meanwhile, triplicate samples of surface water (150 ml ca. 10 cm depth) were collected and stored in a plastic bottle under ice. Temperature, pH, and dissolved oxygen (DO) concentrations of surface water were measured in situ by calibrated portable instruments, which were pH instrument (pH 400, SPECTRUM, USA) and DO instrument (HI9147-04, Italy). All the samples were then transported back to the laboratory as soon as possible.

Water and sediment analysis

All the collected water samples were processed in the laboratory within 6 h for inorganic nitrogen and soluble orthophosphorus (SOP) measurement. The concentrations of ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-), and SOP were analyzed using Nessler's reagent spectrophotometry, UV-Spectrophotometry, *N*-(1-naphthyl) ethylene diamine dihydrochloride spectrophotometry, and phosphomolybdate-blue spectrophotometry, respectively (SEPA (State Environmental Protection Administration) 2002).

Sediment cores were sliced into 1 cm layer subsamples, which were weighted to determine wet weight. A portion of each wet subsample was dried at 50 °C until constant weight. Water content was calculated by the ratio of the loss of wet subsample and the weight of dried subsample. Dry bulk density (BD) was calculated for each subsample according to the wet weight, water content, and volume. About 5 g of wet sediment sample was extracted by 2 mol L^{-1} KCl solution for determination of extractable nitrate (NO_3^-) and ammonium (NH_4^+). Extractable NO_3^- and NH_4^+ in sediments were measured by a standard colorimetric method (Grasshof et al. 1983). Dried sediment subsamples were ground and passed through a 60 mesh (250 μm) sieve for sediment organic matter (OM) content determination by $K_2Cr_2O_7$-H_2SO_4 oxidation method (Nelson and Sommers 1996).

Denitrification rate measurement and calculation

The measurement of the sediment denitrification rates was carried out as soon as the samples were sent to the laboratory. The acetylene inhibition technique is a relatively easy and rapid technique and has been one of the most frequently used methods for measuring denitrification in aquatic sediments. However, a potential drawback is that incomplete inhibition of N_2O reduction can occur or nitrification can be partially inhibited, which would underestimate the rates particularly in nitrate poor systems (Seitzinger et al. 1993). Nevertheless, this drawback is not essential in eutrophic lakes like Dongping Lake; the acetylene inhibition method was applied to measure denitrification in this study. About 6 g of each fresh sediment subsample was put into a 125 ml narrow mouth bottle. Each bottle was sealed with a rubber stopper and purged with 99.999% N_2 for 20 min to create anoxic environments by two needles which were inserted into bottle through rubber stopper, and then 20 ml gas sample was collected as initial sample, and then one needle was withdrawn. Fifteen milliliters of water which had been aerated with 99.999% N_2 for 30 min and 14 ml C_2H_2 were injected into the bottle through the remaining needle, and then the needle was withdrawn too. At the same time, three blank bottles were also treated in the same way and measured as the background. Next, all the bottles were incubated at the field temperature in the dark for 2 h (28 °C in summer, 13 °C in autumn, 4 °C in winter, and 19 °C in spring). At the end of incubation, 20 ml gas sample from each bottle was collected using a syringe and then injected into a gas chromatograph (GC) (Agilent 7890A, USA) equipped with an electron capture detector (ECD) for N_2O analysis. A 95:5 mixture of Ar/CH_4 was used as the carrier gas and the reference gas offered by National Institute of Metrology and National Research Center for Certified Reference Materials of China was analyzed for calibration. And the measurement precision was less than 1% relative standard deviation (six consecutive measurement) at approximately ambient concentrations (345.5 ppbv N_2O standard). N_2O concentrations in laboratory ambient air and in ultrahigh purity nitrogen gas (in case of any contamination) used in the incubations were also measured.

Denitrification rate was calculated by change of N_2O concentrations in the headspace of the bottle during the incubation, and the formula is as follows:

$$\mathrm{D_{Rate}} = \frac{C_{1m} \times (V_1 + V_{C_2H_2}) + C_{2m} \times V_2 - C_{3m} \times V_1}{W \times T} \quad (1)$$

where D_{Rate} is the denitrification rate in sediment (ng N g^{-1} h^{-1}), $C_{1\mathrm{m}}$ and $C_{2\mathrm{m}}$ are the N_2O concentration in N_2 and water in the bottle at the end of incubation (ng N·L^{-1}) respectively, $C_{2\mathrm{m}}$ is calculated as the product of α and $C_{1\mathrm{v}}$, α is the solubility coefficient of in water (Weiss and Price 1980; Wang et al. 2015), $C_{1\mathrm{v}}$ is the N_2O concentration in the bottle at the end of incubation (ppbv), $C_{3\mathrm{m}}$ is the N_2O concentration in N_2 in the headspace of the bottle at the beginning of incubation (ng N L^{-1}), V_1 and V_2 are the volume of N_2 and water in the bottle respectively (L), V_{C_2} H_2 is the volume of C_2H_2 injected into the bottle (L), W is the dry weight of the sediment sample in the bottle (g), and T is the incubation time (h).

Total denitrification rate of the 5-cm depth sediment per m^2 (D_{Rate/m^2}) was calculated according to the bulk density of the sediment sample, and the formula is as follows:

$$D_{\mathrm{Rate}/m^2} = \sum_{i=1}^{5} \left(\left(D_{\mathrm{Rate},i}/14 \right)/1000 \right) \times BD_i \times 10^6 \quad (2)$$

where D_{Rate/m^2} is the total denitrification rate in sediment per m^2 (μmol N m^{-2} h^{-1}), $D_{\mathrm{Rate},i}$ is the denitrification rate in sediment at *i*-cm depth (ng N g^{-1} h^{-1}), and BD_i is the dry bulk density of the sediment at *i*-cm depth (g cm^{-3}).

Quantifying of internal cycling rate and residence time of nitrate

The denitrification was affected by the diffusion flux of NO_3^- from the water column to the sediment (Liu et al. 2014); therefore, the denitrification rates could be used to represent the removal of nitrate from the water column and to quantify the internal cycling rate of nitrate if only denitrification was considered in the nitrogen removal process. Residence time in hydrodynamics was also used as a reference (Monsen et al. 2002), which was defined as the inverse of its cycling rate (Wu et al. 2019). Specifically, the cycling rate (CR) and residence time could be expressed as:

$$\mathrm{CR} = \mathrm{DR}/\mathrm{NO_3}^- \quad (3)$$

$$\text{Residence time} = \mathrm{NO_3}^-/\mathrm{DR} \quad (4)$$

where DR and NO_3^- represented the denitrification rate and nitrate concentration in sediment. This equation excludes the influence of other nitrogen removal processes like plant uptake, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (Anammox) which lead to underestimation of CR.

Statistical analyses

SPSS (18.0) was used for statistical analysis of the spatial and temporal difference of the denitrification rates, the correlation and multiple linear regression analysis between the denitrification rates and environmental factors, as well as the interaction effect of season, vegetation, and depth on denitrification rates at the $\alpha = 0.05$ level of significance. The data was first tested for the normality distribution. If the data was normally distributed, ANOVA was used to compare the difference; and if not, nonparametric test was performed. Specifically, the non-normal distributed data was first checked for difference using Kruskal-Wallis H test and then followed by Mann-Whitney U test for pairwise comparison. Before the correlation and regression analysis, variables were $\log_{10}$+1 transformed to reduce the influence of extreme values. The correlation between the denitrification rates and environmental factors was tested by Pearson correlation. For regression analysis, all log-transformed variables were further standardized to z-scores to obtain regression coefficients that are proportional to the influence of each explanatory variable, such as their relative importance can be immediately evaluated. The stepwise method was used to identify the factors driving the denitrification rates. The interaction effect of season, vegetation, and depth on denitrification rates was tested by three-way ANOVA.

Results and discussion

Physicochemical properties of water and sediment in different zones of Dongping Lake

As shown in Table 1, except NH_3 in the overlying water, there were no significant differences in the average concentrations of the other property index between the sites. The average concentration of NH_3 in the overlying water was significantly

Table 1 Physicochemical properties of the surface water and 0–5 cm sediment at different vegetation zones and in different seasons (average ± standard deviation)

Site[a]/ season	Overlying Water/(mg L^{-1})						Sediment/(mg kg^{-1})		
	T/°C	DO	NH_3-N	NO_3^--N	NO_2^--N	SOP	NH_4^+-N	NO_3^--N	OM/(g kg^{-1})
Site 1	15.7 ± 10.9 a	7.5 ± 3.1 a	1.09 ± 0.25 a	0.91 ± 0.45 a	0.012 ± 0.020 a	0.041 ± 0.028 a	97.7 ± 29.1 a	3.19 ± 2.36 a	47.1 ± 20.0 a
Site 2	15.2 ± 10.7 a	7.3 ± 2.9 a	1.11 ± 0.11 a	0.73 ± 0.61 a	0.007 ± 0.003 a	0.044 ± 0.027 a	80.3 ± 20.8 a	11.2 ± 7.37 a	36.1 ± 6.83 a
Site 3	14.8 ± 11.3 a	8.7 ± 2.6 a	1.13 ± 0.22 a	1.02 ± 0.78 a	0.007 ± 0.003 a	0.045 ± 0.034 a	81.3 ± 16.6 a	10.4 ± 7.01 a	30.4 ± 4.38 a
Site 4	16.8 ± 10.7 a	8.0 ± 0.9 a	1.35 ± 0.29 b	1.19 ± 0.60 a	0.017 ± 0.018 a	0.033 ± 0.039 a	68.3 ± 63.2 a	5.18 ± 5.16 a	18.9 ± 24.2 a
Summer	28 ± 0 .4 A	7.7 ± 3.3 A	1.07 ± 0.06 A	0.53 ± 0.20 A	0.004 ± 0.001 A	0.039 ± 0.019 A	116.8 ± 36.1 A	11.2 ± 6.31 A	48.5 ± 16.9 A
Autumn	13 ± 0.4 B	7.7 ± 3.4 A	1.19 ± 0.22 A	1.34 ± 0.33 BC	0.013 ± 0.004 B	0.044 ± 0.021 A	67.6 ± 35.5 B	8.06 ± 6.31 A	32.2 ± 21.8 A
Winter	2.2 ± 1.2 C	8.0 ± 1.6 A	1.24 ± 0.25 A	1.55 ± 0.36 C	0.021 ± 0.019 BC	0.007 ± 0.004 B	77.0 ± 25.1 AB	4.57 ± 3.96 A	30.7 ± 14.4 A
Spring	19.3 ± 2.3 D	8.2 ± 1.0 A	1.20 ± 0.07 A	0.48 ± 0.07 AD	0.006 ± 0.001 C	0.073 ± 0.017 C	66.1 ± 26.0 B	6.21 ± 4.37 A	21.0 ± 10.4 B

[a] Site 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively

Different small letters in the same column indicated the significant difference between the sites ($P < 0.05$), and different capital letters indicated the significant difference between the seasons ($P < 0.05$)

higher ($P < 0.05$) in the site 4 (the mixed vegetation zone) than those in the other sites. Furthermore, the average NH_3 concentrations in the overlying water all exceeded the limit (≤ 1.0 mg/L) for class III water body of the GB3838-2002 National Environmental Quality Standards for Surface Water. Significant seasonal difference was found in T, NO_3^-, NO_2^-, and SOP in the overlying water as well as NH_4^+ and OM in sediments. The NO_3^- concentrations in the overlying water were significantly higher in winter and autumn and lower in summer and spring. The average NO_2^- concentration was lowest in summer compared with the other seasons. Highest SOP concentration was found in spring, which was more than ten times of the average in winter. As for the sediment, the average concentration of extractable NH_4^+ was significantly higher in summer than in autumn and spring. The average concentration of OM in sediment was significantly lower in spring than in others seasons.

Sediment denitrification rates in different zones of Dongping Lake

The average denitrification rates in the top 5 cm sediment decreased from site 1 to site 4, which were 69.0 ± 91.6, 15.3 ± 17.2, 11.8 ± 15.5, and 8.70 ± 5.44 μmol N m^{-2} h^{-1} respectively; however, significant difference ($P < 0.05$) in denitrification rates was only found between site 1 (*P. communis* zone) and site 4 (the mixed vegetation zone). The average sediment denitrification rates in different seasons were in the order of winter (74.5 ± 88.3 μmol N m^{-2} h^{-1}) > autumn (15.7 ± 18.6 μmol N m^{-2} h^{-1}) > summer (10.7 ± 5.90 μmol N m^{-2} h^{-1}) > spring (3.85 ± 1.29 μmol N m^{-2} h^{-1}). There were significant seasonal difference ($P < 0.05$) in sediment denitrification rates except between autumn and summer. The variation of sediment denitrification rates by site and season were illustrated in Fig. 2. Significant seasonal and spatial differences were only found in site 2 and in autumn respectively. In aquaculture zone, the sediment denitrification rates in summer were significantly higher than in autumn and spring; while in autumn, the sediment denitrification rates in site 1 were significantly higher than in site 3.

The denitrification rates at each sediment layer in different vegetation zones were illustrated in Fig. 3. The significant decrease ($P < 0.05$) of sediment denitrification with increasing depth was found both in autumn and winter at site 1 and site 2, and also in spring at site 3 indicating that denitrification mainly occurred in the top layer sediment, which was similar to other water body (Liu et al. 2014; Fernandes et al. 2016; Wu et al. 2018). However, there were significant increase of sediment denitrification with depth in summer at site 1 and site 3, and in winter and spring at site 4; and the maximum of the denitrification rates occurred at the 3~5 cm sediment, which suggested the possible influence of aquatic plants compared with the non-vegetated site 2.

Analysis of factors affecting the sediment denitrification rates in Dongping Lake

Denitrification, the stepwise reduction of nitrate to N_2 under low-oxygen and anaerobic conditions, represents a permanent nitrogen removal from aquatic systems (Liu et al. 2014). We need to know the basic facts of denitrification, including its rates and controlling factors, if we want to enhance N removal through it. Using the correlation analysis, previous studies uncovered a group of factors that could exert influences on denitrification, including the nitrate concentration, temperature, oxygen, availability of organic matter, the presence/absence of aquatic vegetation, and so on (Wu et al. 2013; Liu et al. 2014; Li et al. 2018). These environmental factors were interrelated, in which nitrate concentration and

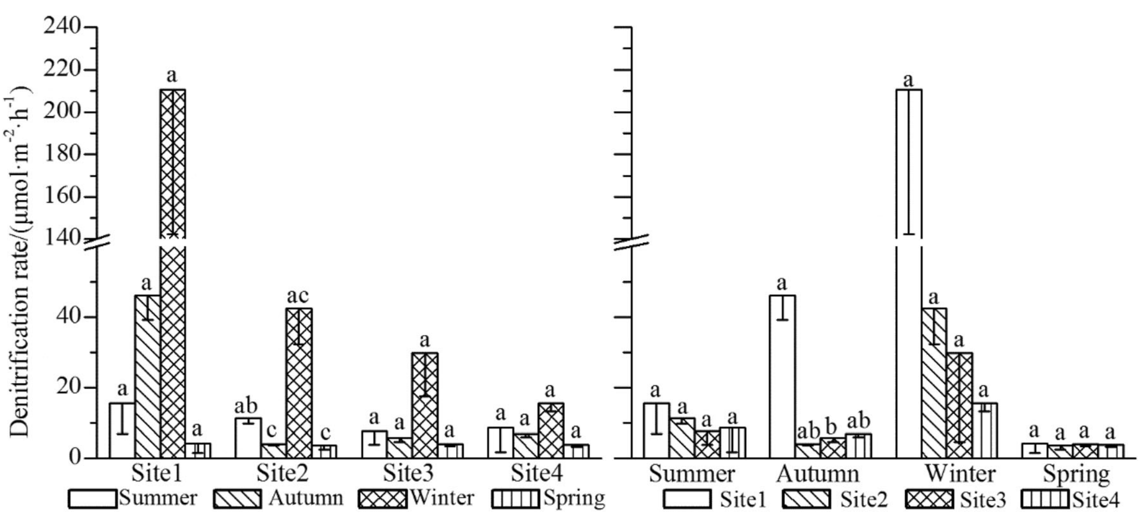

Fig. 2 The sediment denitrification rates in different seasons and at different vegetation zones in Dongping Lake (sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively. Error bar is the standard deviation, $n = 3$)

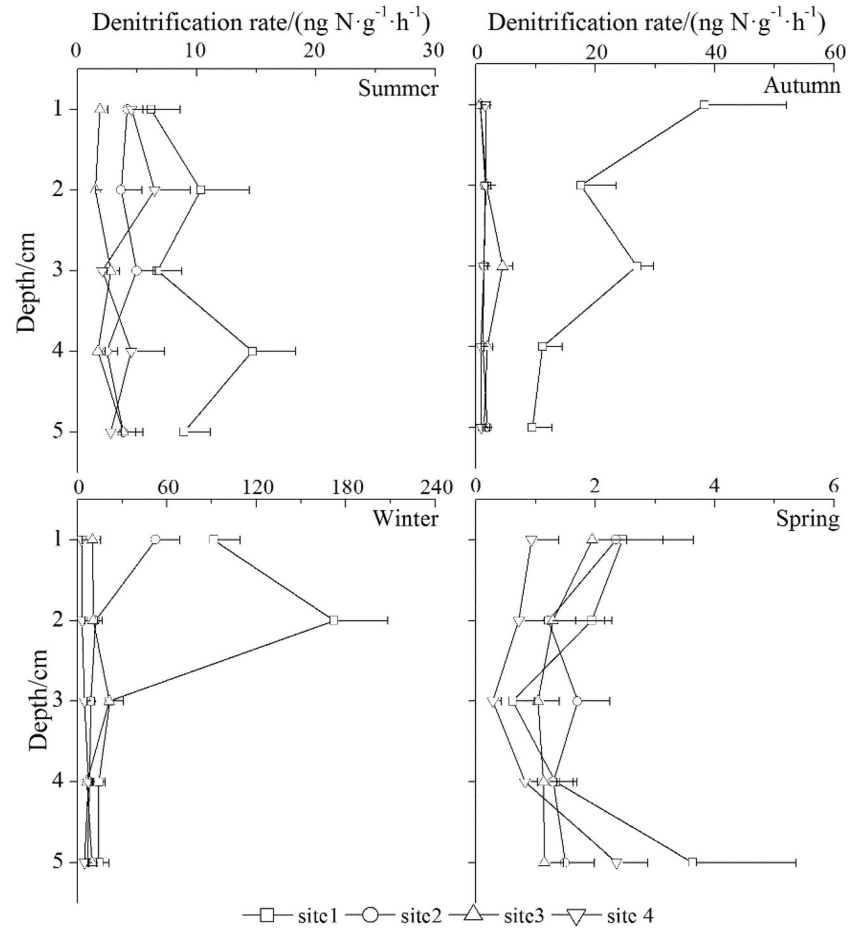

Fig. 3 The vertical profile of seasonal sediment denitrification rates of different vegetation zones in Dongping Lake (sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively. Error bar is the standard deviation, $n = 3$)

temperature were typically identified as the main drivers of denitrification (Zhong et al. 2020; Palacin-Lizarbe et al. 2018, 2020). Nevertheless, the influence of the two main drivers could be complex and the most limiting ones could vary under different environmental conditions (Zhong et al. 2010; Rissanen et al. 2011).

Pearson correlation analysis was conducted between the sediment denitrification rates (DRs) and the physicochemical properties of the overlying water and sediment, and the results were listed in Tables 2 and 3 respectively. In general, the DRs in Dongping Lake were significantly negatively correlated with the temperature (T) and the SOP concentrations in the surface water. However, for each site, these significant negative correlations only existed between the DRs and SOP at site 2 and site 3; furthermore, for each season, no significant correlations were found (Table 2). The DRs were positively correlated with the NO_3^- concentrations in the surface water, but the correlations were not significant ($P > 0.05$). On the whole, the DRs were significantly positively correlated with the NH_4^+ content and OM but negatively correlated with the NO_3^- content. The significant positive correlations between DRs and OM were also found in the other three seasons except spring. Otherwise, these significant correlations were only found in a certain site or season or at a certain layer sediment (Table 3).

The impact of the physicochemical properties of surface water and sediment on the DRs was further analyzed by the multiple linear regression. The optimal model for surface water was DR = − 0.578 × T-0.575 × NH_3-0.509 × DO-0.334 × SOP ($n = 16$, $P = 0.000$, $R^2 = 0.914$, Adj. $R^2 = 0.883$), while the optimal model for sediment was DR = 0.606 × OM-0.476 × NO_3^- ($n = 80$, $P = 0.000$, $R^2 = 0.388$, Adj. $R^2 = 0.372$). As all the variables were log-transformed and then scaled to *z*-scores, we could directly use the regression coefficients to evaluate the relative importance of the variables in the models. The influence of the properties of the surface water on DRs were decreased in the order of T > NH_3 > DO>SOP, and the model could explain 88.3% of the DR variation, while for sediment, OM had more impact on the DRs than NO_3^-; however, this regression model could only account for 37.2% of the DRs variation.

In most of studies (Wang et al. 2018; de Klein et al. 2017), temperature was a fundamental factor affecting the denitrification rates, although the correlations were not uniform. Wu et al. (2013) found that the denitrification rates were significantly positively related to temperature in the sediments due to the increased metabolic activity that occurs at higher temperatures. However, Luo et al. (2000) found that there was not a positive temperature effect on denitrification due to the wide

Table 2 Pearson correlation coefficients between the sediment denitrification rates and the physicochemical properties of the surface water

		T	DO	NH_3	NO_3^-	NO_2^-	SOP
Site[c] (for each site, $n = 4$)	Site 1	− 0.815	− 0.227	− 0.939	0.878	0.800	− 0.940
	Site 2	− 0.895	0.169	− 0.523	0.516	− 0.556	− 0.986[b]
	Site 3	− 0.931	− 0.113	0.722	0.800	− 0.355	− 0.990[a]
	Site 4	− 0.404	0.422	− 0.181	0.183	0.438	− 0.692
Season (for each season, $n = 4$)	Summer	0.166	− 0.824	0.485	0.366	− 0.734	0.166
	Autumn	− 0.839	− 0.781	− 0.335	− 0.263	− 0.795	− 0.839
	Winter	− 0.187	− 0.191	− 0.882	− 0.495	− 0.063	− 0.187
	Spring	− 0.435	0.176	− 0.823	− 0.050	− 0.663	− 0.435
All ($n = 16$)		− 0.620[b]	− 0.372	− 0.281	0.405	0.271	− 0.614[b]

[a] : the correlation is significant at 0.01 level (two-tailed)

[b] The correlation is significant at 0.05 level (two-tailed)

[c] Sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively

variation among environmental factors. Organic matter can provide essential carbon sources for the denitrification (Lin et al. 2018); therefore, positive relationship is often found between denitrification rates and organic matter or carbon contents of sediment (Bruesewitz et al. 2011; Yao et al. 2016). The significant positive correlation of the DRs with NH_4^+ and significant negative correlation with NO_3^- in sediment might indicate that nitrate from nitrification provide major N source for denitrification (Wang et al. 2017a; Wu et al. 2019); however, as both correlation coefficients were lower than 0.3 (Table 3) and moreover the sediment model could only explained less than 40% of the variation of DRs, the impact of sediment properties on DRs still needs further study.

Table 3 Pearson correlation coefficients between the sediment denitrification rates and the physicochemical properties of the sediment

		NH_4^+	NO_3^-	OM
Site [c] (for each site, $n = 20$)	Site 1	− 0.013	0.053	0.419
	Site 2	0.415	− 0.498[b]	0.264
	Site 3	0.420	− 0.240	0.307
	Site 4	0.530[b]	0.081	0.475[b]
Season (for each season, $n = 20$)	Summer	0.088	− 0.531 [b]	*0.610*[a]
	Autumn	0.445	− 0.084	0.535[b]
	Winter	0.233	− 0.347	0.552[b]
	Spring	0.016	− 0.330	0.204
Depth (for each depth, $n = 16$)	0–1 cm	0.248	− 0.384	0.513[b]
	1–2 cm	0.259	− 0.523 [b]	0.462
	2–3 cm	0.203	− 0.286	0.316
	3–4 cm	0.371	− 0.132	0.508[b]
	4–5 cm	0.439	− 0.090	0.431
all ($n = 80$)		*0.295*[a]	− 0.260[b]	*0.437*[a]

[a] The correlation is significant at 0.01 level (two-tailed)

[b] The correlation is significant at 0.05 level (two-tailed)

[c] Sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively

Based on the results of correlation and regression analysis, we categorized and summarized three factors which were season, vegetation, and the depth of the sediment according to their internal links. The factor of season represented the change of the temperatures. The growth of vegetation could change the physicochemical properties of surface water and sediment including nitrate concentrations, pH, oxygen, total organic carbon, and so on (Mi et al. 2008). The sediment at different depth also had different physicochemical properties such as the nitrate concentrations, Eh, and organic matter content.

The three-way multiplicative model is believed to be highly useful for parsimonious descriptions of three-way interactions. It is conceptually simple, easy to program, and with some extensions and precautions, it is also applicable to (mildly) incomplete data (Eeuwijk and Kroonenberg 1998). Many articles have been published describing three factors interactions (Singh and Agrawal 2005; Nelissen et al. 2014). In this study, the three-way multiplicative model was adopted to analyze the main effect and the interaction effect of the three factors, and the results were listed in Table 4. It could be found that season, vegetation, and depth had both significant main and interaction effect ($P < 0.01$) on the denitrification rates in Dongping Lake.

The winter peak of denitrification rates may be attributed to the higher NO_3^- load in surface water (Table 1), stimulating rates of sediment denitrification (Fear et al. 2005; Liu et al. 2014) and the absence of the plant uptake, resulting in lower competence for nitrate. In spring and summer, higher DRs were measured in deeper sediment layers of vegetated zones, pointing to a higher influence of oxygen root exudation, enhancing nitrification, which promoted denitrification during the growing season of aquatic plants (Yu et al. 2012; Liu et al. 2014).

Table 4 Difference analysis for factors affecting sediment denitrification rates of different vegetation zones

Factors	F	P	Partial eta square
Season	26.783	0.000***	0.334
Vegetation	24.575	0.000***	0.315
Depth	4.659	0.0014**	0.104
Season × vegetation	8.862	0.000***	0.333
Season × depth	4.390	0.000***	0.248
Vegetation × depth	4.376	0.000***	0.247
Season × vegetation × depth	4.251	0.000***	0.489

Level of significance: $^{**}P < 0.05$, $^{***}P < 0.001$

Different aquatic plants have different root system development. *P. communis* is a typical emergent plant, and a liquid film with thickness less than 1 mm filled with oxygen could be formed around its root hairs (Hupfer and Dollan 2003; Mi et al. 2008; Xing et al. 2008), and this effect was weaker for submerged plants such as *P. crispus* than *P. communis* (Xing et al. 2008). That might be the reason for the higher DRs in *P. communis* zones than in the other vegetated zones (Fig. 2).Higher DRs in summer at site 2 (the aquaculture zone) might be related to the dense aquaculture activities and the absence of the plant uptake for nitrate in the area. Nevertheless, the acetylene-block technique infra-estimates the denitrification coupled to nitrification, as it not only blocks the N_2O reduction but also blocks partially nitrification (Seitzinger et al. 1993) especially during the growing season of the aquatic plants when the root exudation effect is more intense. Besides, the slurry incubation approach could overestimate the in situ denitrification rates by enhancing the nitrate availability (Ambus 1993; Laverman et al. 2006); and this methodological artifact can be especially important in deeper sediment layers of the non-vegetated zone (site 2). Therefore, the method for the DR measurement needs to be improved in the future research (Laverman et al. 2006).

Internal N cycling rate and sediment N removal capacity in Dongping Lake

The cycling rates (CR) of nitrate were quantified by Eq. (3), while the residence time of nitrate was defined as the inverse of the CR and calculated by Eq. (4). As illustrated in Fig. 4, the cycling rates of nitrate were fastest in winter in all the sites, followed by autumn in sites 1, 3, and 4 but summer in site 2. Moreover, in the *P. communis* zone, the average residence time of nitrate was about 18.0 day, and the average cycling rate was about 20.4 to 31.4 times faster than those in the other vegetation zones where the residence time was about from 100.0 to 240.2 days.

In order to calculate the nitrogen removal capacity of sediment in Dongping Lake, it was necessary to determine the area of the different vegetation zones. Using the density segmentation and object-oriented method, Wang et al. (2017b) found the area of Dongping Lake retaining water all year round was 134.0 km^2, including 10.83 km^2 reed wetland. According to the notice of the People's Government of Dongping County on the centralized renovation of the cage and seine aquaculture in 2017, the area of aquaculture zone was 13.33 km^2. As the mixed vegetation zone was usually located near the lakeshore area, based on the reconnaissance and measurement of lakeshore line, the total area of the mixed vegetation zone was calculated and was about 7.06 km^2. The area of *P. crispus* zone was the total area minus the sum of the other area and is about 102.78 km^2.

Precipitation and inflow of rivers are the two main sources of nitrogen loading into Dongping Lake. Due to the lack of the TN concentrations in the tributary Dawen River, the TN concentrations of Dongping Lake were used to calculate the N input. From 2008 to 2013, the total nitrogen (TN) in water body of Dongping Lake was 1.98 ± 0.81 mg N L^{-1} (Shi et al. 2015). According to the annual volume of river water entering the lake (1.08×10^9 m^3 a^{-1}), there was 2.1×10^9 g N imported into reservoir annually. The average TN concentration in the precipitation near Dongping Lake was 1.95 mg N L^{-1} (Wang et al. 2006; Xu et al. 2016). Hence, based on the lake area and the average annual precipitation, the annual N input through precipitation was 2.5×10^6 g. As the

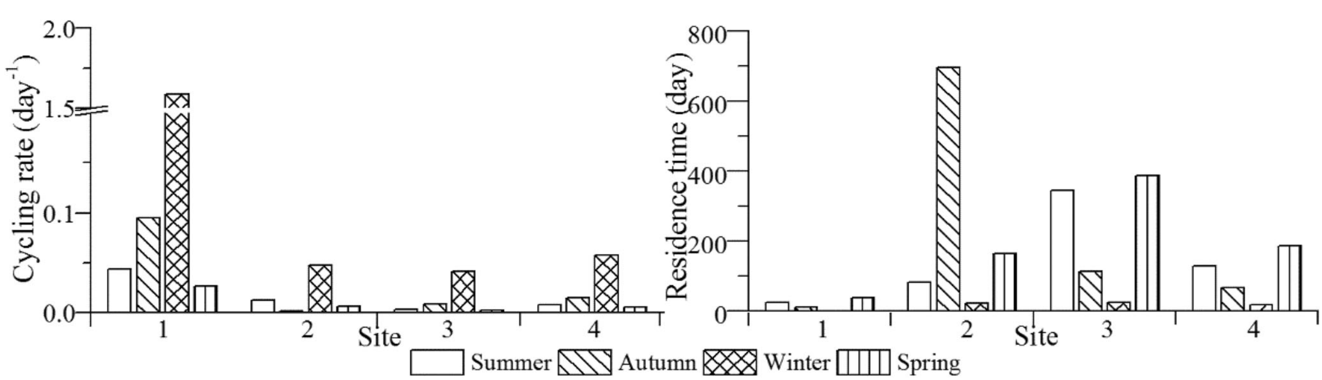

Fig. 4 The cycling rates and residence time of nitrate in different vegetation zones (sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively)

Table 5 Nitrogen removal capacity of sediment in different vegetation zones of Dongping Lake

Site[a]	Area/km^2	Summer	Autumn	Winter	Spring	N removal capacity/ t a^{-1}
Site 1	10.83	10.1	30.2	137.8	2.7	180.8
Site 2	13.33	9.0	3.2	34.2	2.9	49.3
Site 3	102.78	47.2	36.1	185.1	24.3	292.7
Site 4	7.06	3.7	3.0	6.6	1.6	14.9
N removal capacity/t	134.0	70.0	72.5	363.7	31.5	537.7

[a] Sites 1, 2, 3, and 4 were *P. communis* zone, aquaculture zone, *P. crispus* zone, and the mixed vegetation zone respectively

quality of inflowing and outflowing water in the Dongping Lake is required to meet the standards for class III water body of the GB3838-2002 National Environmental Quality Standards for Surface Water, and the inflow and outflow amount of water was equal; therefore, the nitrogen loading by the East Route of the south-to-north water diversion project was not considered in the calculation. At the same time, the TN standard value (1 mg L^{-1}) for Dongping Lake was used as the TN concentration of outgoing water by others rivers. Based on water balance equations, the effluent volume was 1.1×10^9 m^3 a^{-1}. Then, the nitrogen balance was calculated according to the balance principle, and the retention of nitrogen in Dongping Lake was about 2.03×10^9 g N a^{-1}.

Based on the hypothesis that the NO_3^- would be quickly exhausted at the surface of sediment, average sediment denitrification rates at the four sites were used to calculate the nitrogen removal capacity, and the results were listed in Table 5. Due to the highest denitrification rates in winter (Fig. 2), the nitrogen removal capacity was largest in the winter at all the sites. As the area of *P. crispus* zone was about 10 times of that of *P. communis* zone, the *P. crispus* zone had the largest nitrogen removal capacity despite of the low denitrification rates. On the whole, the denitrification of the sediment in Dongping Lake could remove about 537.7 t N every year, which was about 26.5% of the annual TN loading (2.03×10^9 g N a^{-1}). Therefore, there was still a potential increase of bioavailable nitrogen concentrations and eutrophication risk in Dongping Lake. Nevertheless, according to Table 1, the nitrogen concentrations in Dongping Lake had exceeded the TN standard value (1 mg L^{-1}), and using the standard value for calculation might overestimate the annual TN loading, which might result in the underestimation of the nitrogen removal ratio by denitrification.

Conclusions

The average sediment denitrification rates in the *P. communis* zone, the aquaculture zone, the *P. crispus* zone, and the mixed vegetation zone of Dongping Lake were 69.0 ± 91.6, 15.3 ± 17.2, 11.8 ± 15.5, and 8.70 ± 5.44 μmol N m^{-2} h^{-1} respectively. The DRs in the *P. communis* zone were significantly higher than in the mixed vegetation zone. The average sediment denitrification rates in different season were significantly decreased as follows: winter (74.5 ± 88.3 μmol N m^{-2} h^{-1}) > autumn (15.7 ± 18.6 μmol N m^{-2} h^{-1}) ≈ summer (10.7 ± 5.90 μmol N m^{-2} h^{-1}) > spring (3.85 ± 1.29 μmol N m^{-2} h^{-1}). The DRs generally decreased with the increasing of depth; however, there were significant increase of DRs in the 3~5 cm sediment in summer at the *P. communis* zone and the *P. crispus* zone, and in winter and spring at the mixed vegetation zones. The DRs in Dongping Lake were jointly controlled by factors of season, vegetation, and depth of sediment. Specifically, the winter peaks of denitrification rates and cycling rates of nitrate might be related to the higher NO_3^- load in winter, stimulating rates of sediment denitrification and the absence of plant uptake, resulting in lower competence for nitrate, while the highest DRs and cycling rates of nitrate in the *P. communis* zone might be attributed to the larger biomass and oxygen transporting capacity of *P. communis* than of the other aquatic plants. On the whole, the denitrification of the sediment could remove about 537.7 t N every year, which was about 26.5% of the annual TN loading (2.03×10^9 g N a^{-1}) in Dongping Lake. Considering the high sediment denitrification rates and nitrate cycling rates in the *P. communis* zone, we suggest that *P. communis* (reeds) should be the preferred plant for eutrophication prevention and ecological remediation in Dongping Lake. Results of this study might underestimate the effect of different aquatic plants and the total nitrogen removal capability of the sediment. Further investigation is needed to probe the influence mechanism of plants on sediment denitrification.

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