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External carbon addition increases nitrate removal and decreases nitrous oxide emission in a restored wetland

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ABSTRACT

To improve the ecosystem services of wetlands in response to global climate change, wetland restoration projects have become prevalent worldwide. Restored wetlands have the potential to remove external nitrogen through denitrification, but incomplete denitrification may increase nitrous oxide (N_2O) release. In order to comprehensively understand nitrogen removal and N_2O emission processes in restored wetlands, we conducted carbon (anhydrous sodium acetate) and nitrogen (sodium nitrate) addition experiments in the Fengxian coastal wetland site. These experiments explored the roles of external nitrogen, carbon sources, and wetland plants in nitrogen removal and N_2O emission. With the addition of nitrogen, the removal of external nitrogen was significant at the restored wetland site, but led to increasing N_2O release. However, by adding carbon sources, the restored wetland could not only enhance nitrogen removal efficiency through denitrification, but also significantly reduce N_2O emission. Our results demonstrate that carbon availability could improve the ecological functions of coastal restored wetlands by improving water quality and mitigating greenhouse gas emissions.

1. Introduction

Wetlands, the most productive ecosystems in the world, play an important role in fixing atmospheric carbon dioxide and removing nutrients from eutrophic waters (Brannon et al., 2016; He et al., 2016). Over the past few decades, due to urbanization, industrial development, and agriculture, wetland ecosystems have drastically declined and eutrophication has become more severe (Mitsch and Gosselink, 2000; Mitsch et al., 2005). Recently, more research has been dedicated to wetland restoration. Restored wetlands can regain almost all the ecological characteristics of natural wetlands, and have ecological benefits for human and natural ecosystems. Restored coastal wetlands can regulate external nitrogen (Ding et al., 2013) and promote biodiversity (Day et al., 2004). The nitrogen removal process of restored wetlands is more similar to natural wetlands than constructed wetlands because ecological restoration practices minimize engineering disturbance and maintain ecological services (Mitsch, 2014). Zedler and Kercher (2005) proposed that restoring wetlands was an important strategy for re-establishing ecosystem services and functions of deteriorated ecosystems.

Nitrogen removal is an essential ecosystem service, natural wetlands could remove 30–85% of the nitrate every year from the system (Seitzinger et al., 2002; Sutton et al., 2010). Unlike natural wetlands, restored wetlands can be regulated to achieve a specific goal for nitrogen removal. However, few studies have evaluated the nitrogen removal capacity of restored wetlands. Microbial-driven nitrification and denitrification, soil adsorption and filtration, plant uptake, and ammonia volatilization are the main mechanisms for wetland nitrogen cycling (Burgin and Hamilton, 2007; Byström et al., 2000; Hey et al., 2012; Burgin et al., 2011; Ballantine et al., 2014). Denitrification is regarded as the essential process for nitrogen removal in a restored coastal wetland ecosystem (Meuleman et al., 2003; Wallenstein et al.,

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2006; Ding et al., 2013; He et al., 2016). The NO_3^--N concentration influences the nitrogen removal in a restored wetland, since sufficient NO_3^--N is needed as an electron acceptor for denitrifying bacteria to complete respiration (Bowen et al., 2013). A carbon source is also a necessary energy material for denitrifying bacteria and dissimilatory nitrate reduction to ammonium (DNRA) bacteria (Giblin et al., 2013). Carbon sources can provide the carbonaceous material of microbial cells, but it can also be used to supply electron donors for microbial metabolism (Ray et al., 2014). Research on how carbon availability drives nitrogen removal is well developed in constructed wetlands, but research is lacking within restored wetlands. Ballantine et al. (2014) have reported that restored wetlands often have low denitrification rates, which may be due to the relatively low levels of soil C and microbial activity relative to natural wetlands. C:N ratio in the range of 3–8 is suitable for denitrification and DNRA processes in the restored wetland (Roberts et al., 2014).

Wetlands can emit a considerable amount of N_2O under high concentrations of nitrate (Seitzinger et al., 2006; Moseman-Valtierra et al., 2011; Mander et al., 2014; Zheng et al., 2017). This occurs when environmental conditions induce incomplete denitrification, where nitrite would be converted to N_2O. Higher N_2O fluxes have been shown to result from nitrogen additions (Brannon et al., 2016). Although denitrification is the primary process for nitrogen removal, denitrification may increase N_2O emissions. N_2O has a global warming potential (GWP) of 298 times greater than CO_2 and is now increasing globally at a rate of 0.2–0.3% per year (IPCC, 2007; Gao et al., 2017). Accordingly, N_2O emissions should be considered when using restored wetlands as a mechanism to remove nitrogen. N_2O emissions have been extensively studied in constructed wetlands (García-Lledó et al., 2011; Mander et al., 2015), but rarely in restored wetlands that were previously degraded by human disturbance. Wang et al. (2014) and Lyu et al. (2017) found under low C/N operations, constructed wetlands emitted more N_2O mainly due to the inhibition of N_2O reductase activity. However, the effects of external carbon addition on strengthening the nitrogen removal capacity and reducing N_2O production in the restored wetlands have not been reported. When implementing wetland ecological restoration strategies, a strong emphasis is placed on improving water quality, but not enough focus is on reducing greenhouse gas emissions (García-Lledó et al., 2011). It is essential to find an optimal solution to protect both ecological functions - improving nitrogen removal and reducing N_2O release.

In this study, nitrogen removal and N_2O emission experiments were conducted under simulated tidal conditions in a restored coastal wetland in Fengxian section of Hangzhou Bay, China. Then we discussed the effects of the main factors (nitrogen input, carbon source, and plants) on the wetland's denitrification ability, and proposed an approach to improve the nitrogen removal efficiency and reduce N_2O emission in the restored wetland. Consequently, this study helps to draw a comprehensive understanding on the processes of nitrogen regulation and N_2O release in restored wetlands.

2. Materials and methods

2.1. Study area

This study was conducted in the coastal restored wetland within Hangzhou Bay in the Fengxian District, Shanghai, China (N30°49′26.01″, W121°33′41.39″), with an area of about 6,000 m^2. Since 2013, the site was restored from previous mudflat mainly through amending the sediment, constructing an ecological dam to prevent erosion, and transplanting local plants onto the site. The dominant species was *Phragmites australis*. The salinity and pH of the wetland water were 5–10‰ and 6.5, respectively. This study was carried out in the summer of 2017, and water temperature was 32 ± 2 °C.

2.2. The nitrogen removal

2.2.1. Laboratory experiments

Before the *in-situ* experiment, we conducted the nitrogen removal experiment in the laboratory using airtight serum bottles (10 cm height). The bottles were filled 5 cm with sediment that was collected from Fengxian wetland site. The bottle was then filled with water (salinity of 10‰) that was collected from the wetland site. The lab experiment included two treatments, and three replicates for each treatment. One treatment (t1) contained additions of an external nitrogen source, and the other treatment (t2) included additions of external nitrogen and carbon sources into the water. The added nitrogen and carbon sources were sodium nitrate (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and anhydrous sodium acetate (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), respectively, the C/N was kept around 3 in t2 (Table 1).

After artificially adding the reagents, the porewater (collected at a soil depth of 3 cm) was sampled at 0 h and 4 h by the Rhizon soil moisture samplers (Rhizophere Research Products, Wageningen, Netherlands). The pore water samples were immediately tested for the key water nitrogen indicators, total soluble nitrogen (TDN), nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N).

2.2.2. In-situ experiment

This study used PVC plots, with a diameter of 50 cm and height of 70 cm (the height of soil was 40 cm inside), to complete the tide-simulation experiments. *Phragmites australis* was planted in the plots in 2016, the average height and density of the plant were 1.05 m and 318 (individuals/m^2) respectively during the experiment (July-September 2017). In the tide-simulation experiment, the on-site pump continuously pumped internal water from the restored wetland into the PVC plots. The pump was powered by solar energy and the solar power supply system controlled the pumping and stop time to better simulate the tidal flow. The inlet pipe over the soil surface 0–1 cm, and the overflow pipe (with a valve) was above the soil surface (10 cm), which was used to adjust the water level to ensure the water level was around 10 cm. Drain pipes were set up at the bottom of the PVC pipes so that the water could be discharged.

The experiment contained four treatments: plants without external nitrogen source (CK); plants with external nitrogen (T1); no plants with external nitrogen (T2); plants with external nitrogen and carbon (T3). There were three replicates for each treatment. The added nitrogen was sodium nitrate (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), and the added carbon was anhydrous sodium acetate (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), thus the C/N was kept around 3 in the external nitrogen and carbon treatment (Table 1). The reagents were added artificially into the water before the experiments. In the nitrogen addition treatment (T1, T2, T3), the initial concentration of NO_3^--N were higher than presupposed 2 mg/L, since the system water came from the interior of the restored wetland and had a background nitrogen concentration (Table 1).

In the Fengxian wetland, daily tidal flow lasted 4 h, so the experiment was conducted under a 4 h tidal cycle. The overlying water and the pore water were sampled at 0 h and 4 h. The Rhizon soil moisture samplers (Rhizophere Research Products, Wageningen, Netherlands) were used to sample the pore water in the 15–20 cm depth in the soil. The water samples were immediately placed in a cooler, and transported back to the lab where the samples were filtered and the nitrogen analysis was performed. The measured indicators included TDN, NO_3^--N and NH_4^+-N.

2.3. The analysis of water samples

The samples were placed in a cooler immediately after collection and were analyzed within a week. The water was filtered first, and then tested for key indicators in the ultraviolet and visible

Table 1
The setup of the laboratory and *in-situ* experiments.

	Treatments	External nitrogen (sodium nitrate, mg/L)	External carbon (anhydrous sodium acetate, mg/L)	Plant (*Phragmites australis*)	Salinity (‰)	pH	Background nitrogen concentrations of pore water (mg/L)
Laboratory	t1	2	/	/	10	6.47 ± 0.06	TDN 0.96 ± 0.045,
	t2	2	6	/			NO_3^--N 0.27 ± 0.02,
							NH_4^+-N 0.015 ± 0.0005
In-situ	CK	/	/	With	10	6.56 ± 0.11	TDN 1.04 ± 0.053,
	T1	2	/	With			NO_3^--N 0.29 ± 0.01,
	T2	2	/	/			NH_4^+-N 0.018 ± 0.0002
	T3	2	6	With			

spectrophotometer (UV-7504, Xinmao Co. Ltd., Shanghai, China). The concentrations of TDN, NO_3^--N and NH_4^+-N were analyzed by potassium persulfate oxidation method, zinc cadmium reduction method, hypobromite oxidation method, respectively.

The removal efficiencies of TDN, NO_3^--N and NH_4^+-N were calculated using the following equation:

$$\text{The removal efficiencies (\%)} = \frac{\text{0h concentration} - \text{4h concentration}}{\text{0h concentration}} \quad (1)$$

where 0 h concentration and 4 h concentration represent the concentrations of TDN, NO_3^--N and NH_4^+-N on the 0 h and 4 h after external solution add.

2.4. N_2O flux measurement

In-situ N_2O flux measurements within the restored wetland were carried out, adopting a closed static chamber-gas chromatograph (GC) technique. The static chamber is a cylindrical PVC column, with the inside diameter of 20 cm and the height of 1 m. An external pump was applied to circulate the gas in the static chamber, and a battery-powered fan was installed inside to mix interior gas. The gas samples were drawn into 60 mL nylon syringe at 0 min, 5 min, 10 min, 15 min, 20 min, and then were transferred to the 50 mL vacuum airbag (MBT41-0.1, Haide Technologies Co. Ltd., Dalian, China) (Brannon et al., 2016).

The concentration of N_2O was measured by the gas chromatography (7890A, Agilent Technologies Co. Ltd., California, USA) with the electronic capture detector. The column temperature and the detector temperature were set as 60 °C and 330 °C, respectively, and the carrier gas flow rate was 10 mL/min. After the concentration of the gas was extracted, the N_2O flux was calculated from a linear rate of change in gas concentrations (Martin and Moseman-Valtierra, 2015), as the following formula:

$$F = \frac{dC}{dT} \times \frac{PV}{RAT} \quad (2)$$

where F is the flux of N_2O (μmol/m^2/s^1); dc/dt is the changing concentration over time (μmol mol^{-1}); P is the air pressure, standard is 101223.7 (Pa); V is the effective volume of the static closed chamber (m^3); R is the gas constant, defaulted to 8.3144 (J/mol K); A is the base area of the chamber (m^2); T is the air temperature (K). When dc/dt had an R^2 value of less than 0.9, data were not included in the analysis.

2.5. Statistical analysis

The *in-situ* experiments, including nitrogen removal and N_2O flux measurements, were repeated three times during the summer season in 2017, and the experiments exhibited similar results. Wilcoxon Rank Sum test and T test were used to compare the impacts of nitrogen and organic carbon addition treatments of the lab experiment. Kruskal-Wallis Rank-Sums tests were used to compare the denitrification rates and the N_2O fluxes of each treatment for the *in situ* experiment. These tests were used to examine the effects of plants, external nitrogen and external carbon source on the measured nitrogen indicators (TDN, NO_3^--N and NH_4^+-N) and N_2O fluxes.

3. Results and discussion

3.1. The influence of external nitrogen on nitrogen removal

From the lab experiment, we found both t1 and t2 showed clear nitrogen removal, and t2 that contained an external carbon source had a higher nitrogen removal ability than t1. The average NO_3^--N removal efficiencies of t2, 17.6%, was significantly higher than the t1 4.2% removal efficiency, ($t(1) = 17.11$, $p = 0.0006$) (Fig. 1). After 4 h residence time, TDN concentrations of t1 and t2 decreased from an

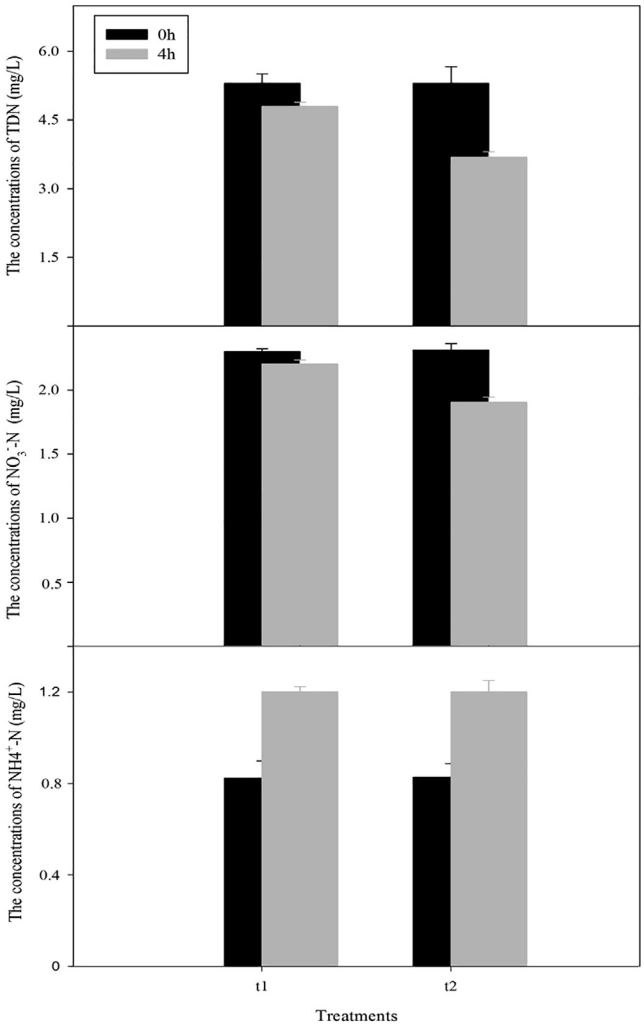

Fig. 1. The TDN, NO_3^--N, NH_4^+-N concentration changes of the treatments in the lab experiment. The dark and gray columns are the concentrations of TDN, NO_3^--N, NH_4^+-N after 0 h and 4 h, respectively.

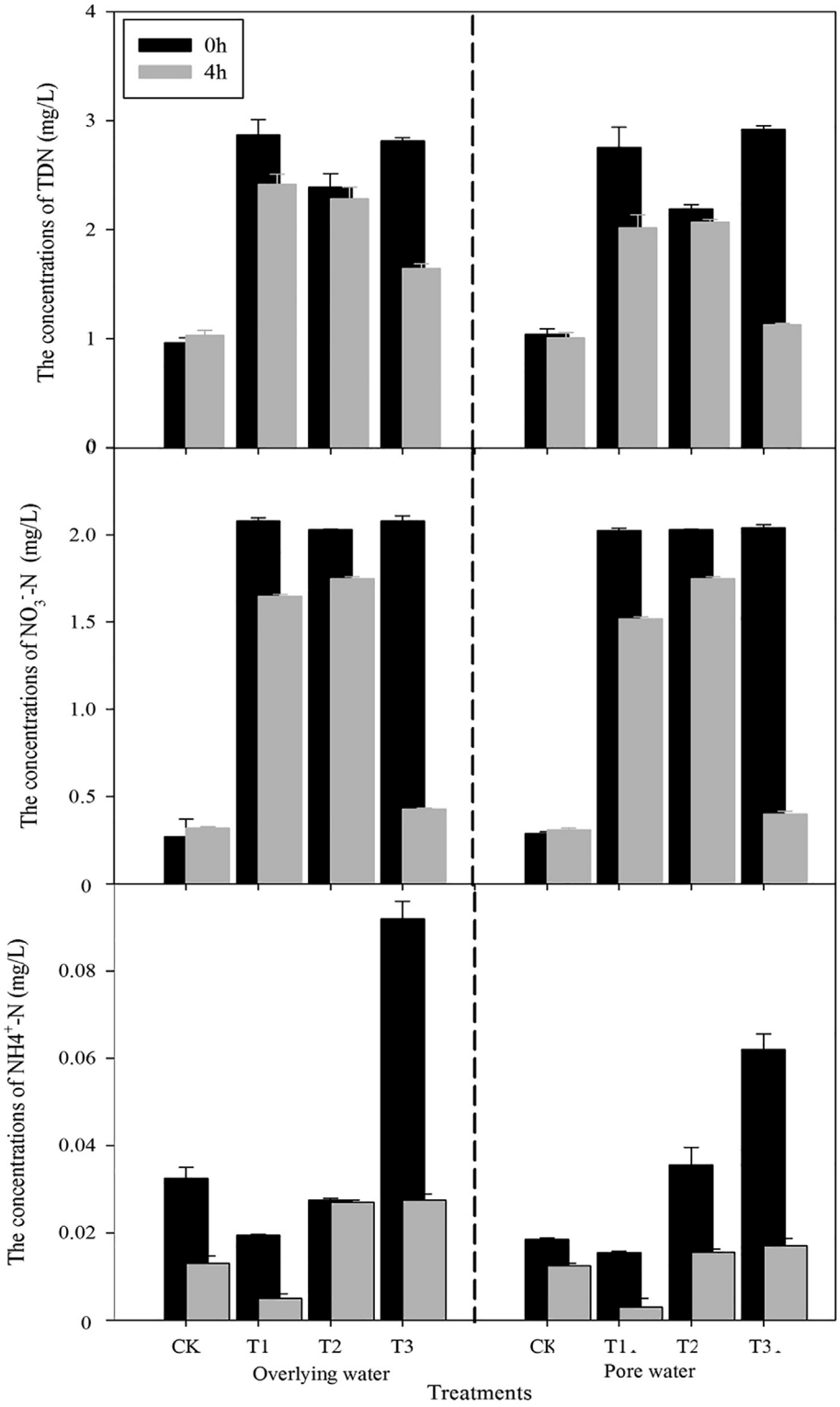

Fig. 2. The TDN, NO_3^--N, NH_4^+-N concentration changes of the treatments in the *in-situ* experiments. The dark and gray columns are the concentrations of TDN, NO_3^--N, NH_4^+-N after 0 h and 4 h, respectively.

average of 5.30 mg/L and 5.30 mg/L to an average of 4.80 mg/L and 3.69 mg/L, respectively. The final concentration TDN of the t2 treatment was significantly lower than t1 ($\chi^2(1) = 3.97$, $p = 0.0463$). The results showed the carbon source could effectively promote denitrification processes and nitrogen removal. These patterns of nitrogen removal were further supported in our *in-situ* experiment.

In the T1 (high nitrogen) group, TDN, NO_3^--N, and NH_4^+-N removal was apparent after 4 h residence time (Fig. 2). T1 showed significantly higher rates of nitrogen removal than the CK group in both overlaying and pore water samples of TDN and NO_3^--N (Table 2). There was no apparent nitrogen-reducing effect in the CK (low nitrogen) group after 4 h residence time, but rather a small increase in TDN and NO_3^--N concentrations of the CK group was seen (the concentrations of TDN increased 0.07 mg/L in the overlying water, as well

Table 2
The treatment comparison of the *in-situ* experiment.

Overlying water	Treatment comparison	Tukey Post-hoc p value	Pore water	Treatment comparison	Tukey Post-hoc p value
TDN Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156		TDN Model Statistic:	$\chi^2(3) = 10.42$, p = 0.0153	
TDN	CK-T1	0.0139	TDN	CK-T1	< 0.0001
	CK-T2	0.1954		CK-T2	0.0588
	CK-T3	0.0002		CK-T3	< 0.0001
	TI-T2	0.1978		TI-T2	< 0.0001
	T1-T3	0.0188		T1-T3	< 0.0001
	T2-T3	0.0018		T2-T3	< 0.0001
NO_3^--N Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156		NO_3^--N Model Statistic:	$\chi^2(3) = 10.57$, p = 0.0143	
NO_3^--N	CK-T1	< 0.0001	NO_3^--N	CK-T1	< 0.0001
	CK-T2	< 0.0001		CK-T2	< 0.0001
	CK-T3	< 0.0001		CK-T3	< 0.0001
	TI-T2	0.0013		TI-T2	< 0.0001
	T1-T3	< 0.0001		T1-T3	< 0.0001
	T2-T3	< 0.0001		T2-T3	< 0.0001
NH_4^+-N Model Statistic:	$\chi^2(3) = 10.42$, p = 0.0153		NH_4^+-N Model Statistic:	$\chi^2(3) = 10.42$, p = 0.0153	
NH_4^+-N	CK-T1	0.1255	NH_4^+-N	CK-T1	0.0814
	CK-T2	< 0.0001		CK-T2	0.0011
	CK-T3	< 0.0001		CK-T3	< 0.0001
	TI-T2	0.0004		TI-T2	0.0399
	T1-T3	< 0.0001		T1-T3	< 0.0001
	T2-T3	< 0.0001		T2-T3	< 0.0001

as NO_3^--N increased 0.05 and 0.02 mg/L in the overlying water and pore water, respectively). Compared with the CK group, the nitrogen removal rate notably increased after adding the nitrogen source in T1 (Table 2), indicating that the restored wetland could remove the added nitrogen through the denitrification processes of wetland microorganisms. While in the CK group, when the nitrogen concentration of the inlet was too low, the nitrogen would only maintain the nutrient requirement for microorganisms and plants in the system, and nitrogen-removal was less efficient in a short time frame compared with the high-nitrogen group.

The nitrogen removal efficiency of NH_4^+-N was significantly higher in the T1 group than that in the CK group of the overlying and pore water samples (Fig. 3; Table 3). We propose two potential reasons for this result. The first was that nitrification caused the conversion of NH_4^+-N to NO_2^--N or NO_3^--N; the second was the adsorption by sediment and plant roots to remove NH_4^+-N. Since the content of NH_4^+-N in the water was very low (ranged from 0.010 mg/L to 0.035 mg/L), DNRA may not be the main pathway of nitrogen removal; for this concentration gradient, root uptake was optimal and is likely the main cause of NH_4^+-N removal.

Generally, a positive relationship between nitrogen loading and nitrogen removal was found in the restored wetland; as nitrogen inputs increased, there was more nitrogen potentially available for denitrification (Seitzinger et al., 2006).

3.2. The influence of plants on nitrogen removal

Plants play an essential role within wetlands. Plant uptake of inorganic and organic nitrogen, which is utilized for plant growth is considered one of the major mechanisms of nitrogen removal in a wetland. Fig. 2 shows the influence of plants on nitrogen removal efficiency in the restored wetland.

We compared the nitrogen removal efficiencies of the T1 and T2 group. For the pore water samples the TDN, NO_3^--N and NH_4^+-N removal efficiencies in T1 was significantly higher than that in T2 under the same external nitrogen condition (Table 3) The TDN removal rate of T1 was 4.6 times than that of T2 for the pore water samples (Fig. 3). This result reflected the important role of plants in nitrogen removal within the restored wetland. A large plant root system could adhere more denitrifying microorganisms, and can facilitate increased oxygen transport to the roots. This process would then improve oxygenation of the plants' rhizosphere within the restored wetland system and provide suitable environmental conditions for the coupled nitrification–denitrification process performed by microorganisms (Dong et al., 2011; Li et al., 2013). During the growing period, the absorption and utilization of nitrogen by plants (i.e. plant assimilation) also promoted the efficiency of nitrogen removal in the restored wetland (Stottmeister et al., 2003). However, since the nitrogen content decreased rapidly just after 4 h, it was considered that the nitrogen removal was mainly due to the microbial denitrification, rather than plant assimilation (Zhang et al., 2016)

In the T2 group, under the high nitrogen and no plant coverage treatment, TDN and NO_3^--N were removed slightly after 4 h residence time, and the TDN and NO_3^--N removal rates of pore water and overlying water were similar (Fig. 3). This result indicates that in the absence of *Phragmites australis* root system, the abundance of microorganisms were less in soil–water interface, and consequently had less influence on reducing nitrogen. The lack of plant roots also eliminates the mechanism of plant assimilation to remove nitrogen.

3.3. The influence of external carbon on nitrogen removal

Fig. 2 shows the significant influence of external carbon on the nitrogen removal capacity in T3 (Table 2). By comparing the T1 group (without additional carbon) and T3 group (with additional carbon source), T3 had significantly higher removal efficiencies of TDN and NO_3^--N (Table 3), which shows the major impact of additional carbon availability to enhance denitrification. The removal efficiencies of TDN and NO_3^--N were 61.3% and 80.4% in T3 pore water, respectively, and 26.7% and 24.9% in T1 pore water, respectively (Fig. 3), indicating that the added carbon source could promote nitrogen removal. Lyu et al. (2017) also reported that adding carbon source can significantly enhance NO_3^--N removal, and the NO_3^--N removal efficiency reached 77.9% after 4 h retention in the constructed wetland, which was a little lower than that of T3 pore water in our results. External carbon source could improve C:N ratio in the restored wetland, and the high C:N might stimulate high denitrification rates in the presence of exogenous NO_3^-. This may be due to the release of electron donors from the carbon source as it is decomposed by microbes, which may lead to an increase in bacterial growth, and thereby enhance the denitrification efficiency (Tanner et al., 1995). Therefore, a carbon source is a key influencing factor for nitrate removal, and C:N ratio limitation would restrict the denitrification rate in the restored wetland (Hang et al., 2016; Ding et al., 2013).

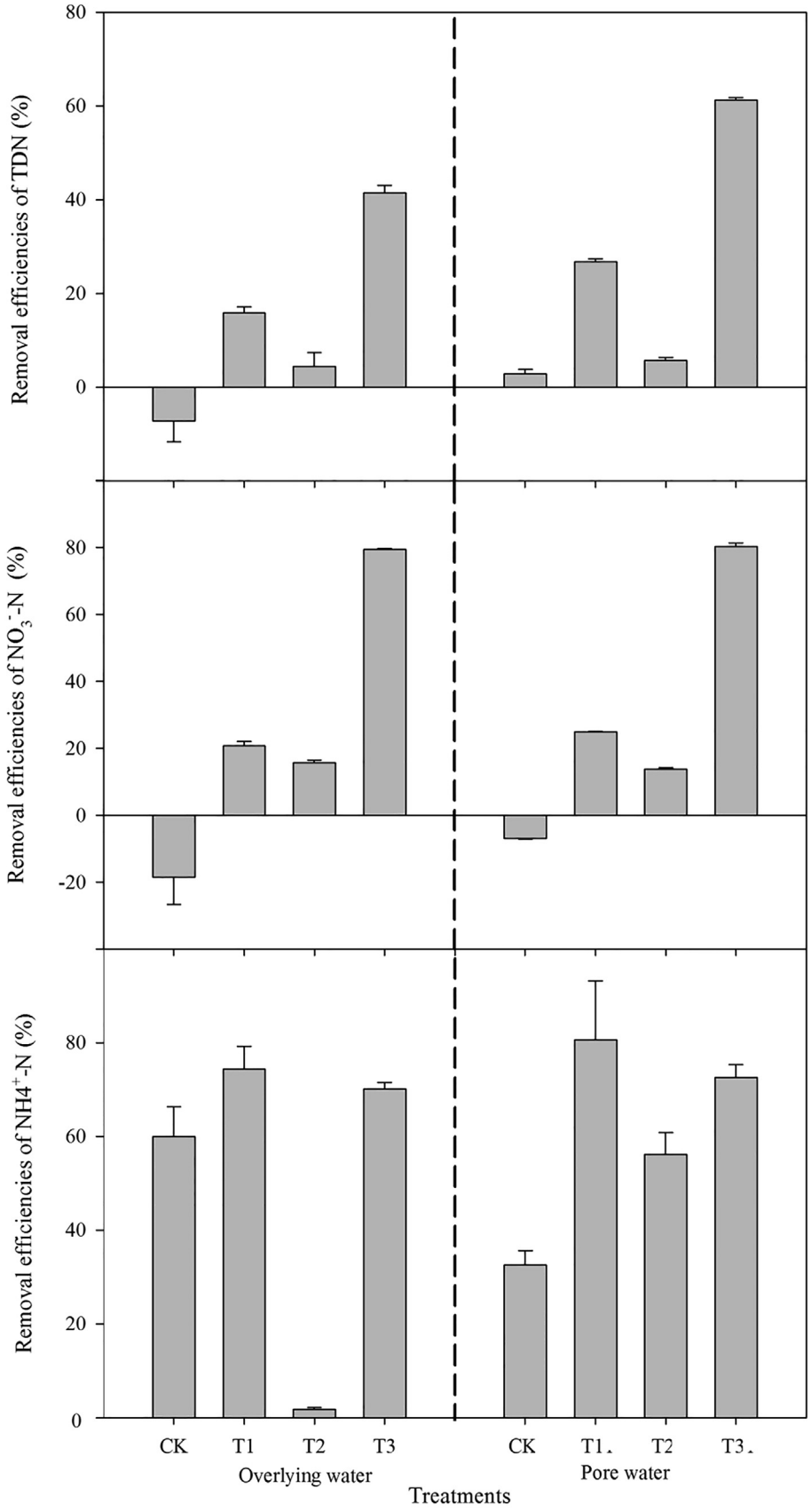

Fig. 3. The removal efficiencies of TDN, NO_3^--N, NH_4^+-N in the different treatments of the *in-situ* experiment.

Table 3
The treatment comparison of removal efficiencies of TDN, NO_3^--N, NH_4^+-N in the *in-situ* experiment.

Overlying water	Treatment comparison	Tukey Post-hoc p value	Pore water	Treatment comparison	Tukey Post-hoc p value
TDN Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156		TDN Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156	
TDN	CK-T1	< 0.0001	TDN	CK-T1	< 0.0001
	CK-T2	0.0045		CK-T2	0.0044
	CK-T3	< 0.0001		CK-T3	< 0.0001
	TI-T2	0.0053		TI-T2	< 0.0001
	T1-T3	< 0.0001		T1-T3	< 0.0001
	T2-T3	< 0.0001		T2-T3	< 0.0001
NO_3^--N Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156		NO_3^--N Model Statistic:	$\chi^2(3) = 10.38$, p = 0.0156	
NO_3^--N	CK-T1	< 0.0001	NO_3^--N	CK-T1	< 0.0001
	CK-T2	< 0.0001		CK-T2	< 0.0001
	CK-T3	< 0.0001		CK-T3	< 0.0001
	TI-T2	0.4865		TI-T2	< 0.0001
	T1-T3	< 0.0001		T1-T3	< 0.0001
	T2-T3	< 0.0001		T2-T3	< 0.0001
NH_4^+-N Model Statistic:	$\chi^2(3) = 9.67$, p = 0.0216		NH_4^+-N Model Statistic:	$\chi^2(3) = 9.46$, p = 0.0237	
NH_4^+-N	CK-T1	0.0103	NH_4^+-N	CK-T1	0.0001
	CK-T2	< 0.0001		CK-T2	0.0149
	CK-T3	0.0590		CK-T3	0.0005
	TI-T2	< 0.0001		TI-T2	0.0108
	T1-T3	0.6013		T1-T3	0.5200
	T2-T3	< 0.0001		T2-T3	0.0766

Generally, in the groups (T1, T2, T3), TDN and NO_3^--N were removed more quickly than the CK control group after 4 h residence time (Fig. 2; Tables 2–3), which indicated that denitrification had a short-term response to the specific environment (eg. the external nitrogen or carbon source). In the research of Gao et al. (2017), the NO_3^--N in the constructed wetland was removed 20% after 30 days, which was much slower than that in our results; and this difference may be because continuous effluent was discharged into the constructed wetland.

In the treatments, there was an insignificant trend showing that the TDN, NO_3^--N and NH_4^+-N removal rates of pore water were higher than that of the overlying water (Fig. 3), which is likely due to the strong nitrogen-removal capacity of the microorganisms adhered in the sediment and plant roots. Meanwhile, it was found that TDN and NO_3^--N also demonstrated significantly higher removal efficiencies in the overlying water in the T3 group compared to the CK control group (Table 3). This indicates that the addition of an organic carbon source (sodium acetate), which was soluble and easily used by microorganisms, could enhance denitrification in the overlying water. In addition, the NH_4^+-N removal effect in pore water of each treatment was significantly higher than the CK control group (Table 3), most likely due to the strong sediment adsorption. In the *in-situ* experiment, the NH_4^+-N removal coupled with NO_3^--N removal, suggested that anammox may have been responsible for the reduction of NO_3^-. NH_4^+ produced at the sediment bottom was assimilated and converted to nitrogen gas via anammox, or oxidized to NO_3^- at the sediment–water interface. However, the concentrations of NH_4^+-N in the lab experiment, which were about one order of magnitude higher than that in the *in-situ* experiment, increased after 4 h residence time. This may be because the lab experiment was under anaerobic conditions, and DNRA conserved nitrogen in the ecosystem as ammonium (Giblin et al., 2013). Contrarily, the *in-situ* experiment was an aerobic system with the simulated tide, and NH_4^+ could be easily oxidized as NO_3^- (Hou et al., 2006). The complex mechanism of ammonium regeneration needs to be further researched.

3.4. The influence of external carbon on N_2O emission

Although denitrification is the major approach for nitrogen removal, it can have a direct effect on global climate since the greenhouse gas N_2O is a product of incomplete denitrification (Seitzinger et al., 2006). In this study, there was a significant treatment effect of the N_2O emissions ($\chi^2(3) = 10.38$, p = 0.0156). The results showed the additional carbon did not only enhance the nitrogen removal rate, but also reduce the N_2O emission of the wetland. In T1, the N_2O flux rose to 61.92umol/m^2/d after the external nitrate was added, which was twice the N_2O flux of CK ($\chi^2(3) = 10.38$, p = 0.0156, Tukey post-hoc p < 0.0001) (Fig. 4). Moseman-Valtierra et al. (2011) found nitrate additions significantly increased N_2O flux in a *Spartina patens* marsh, which was consistent with our result, however, the N_2O flux of the Moseman-Valtierra et al. experiment (~163 μmol/m^2/d) was much higher than T1, which may be due to the higher additional nitrate level in their experiment. Conversely, the N_2O flux was 20.66 μmol/m^2/d in T3 with sufficient carbon source, which was significantly lower than that of T1 ($\chi^2(3) = 10.38$, p = 0.0156, Tukey post-hoc p < 0.0001) (Fig. 4). The flux in T3 was lower than the median N_2O flux (70.9 μmol/m^2/d) from the constructed wetlands (Mander et al., 2014).

In agreement with our results, Gao et al. (2017) reported that external carbon sources have dramatically enhanced the nitrogen removal efficiency with less N_2O emissions. This may be due to a limiting carbon source, which leads denitrifying bacteria to utilize the internal carbon for denitrification, and the denitrification process cannot be fully completed (lack of nitrous oxide reductase encoding nosZ gene), thus the NO_2^--N accumulated and the N_2O was released (Mander et al., 2015). Rather, the additional carbon sources can effectively provide an energy source for denitrifying bacteria, and then promote half reaction and improve NO_2^--N reduction to NO and N_2O, and final reduction to

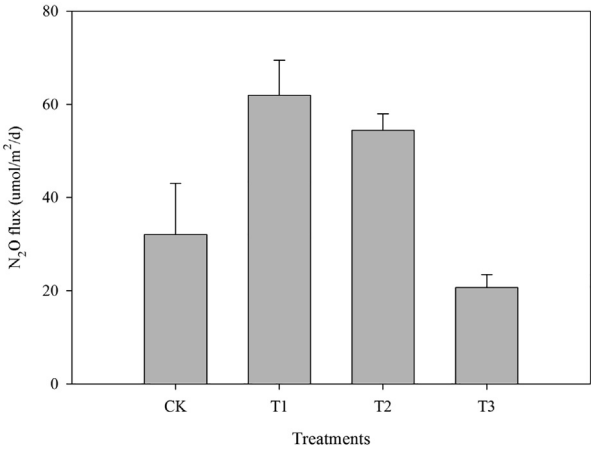

Fig. 4. The N_2O fluxes of the different treatments.

N_2. Through this method, high NO_3^--N could be removed from the wetland ecosystem to the atmosphere and would not cause an increase in emission of the greenhouse gas, nitrous oxide (Virdis et al., 2010).

3.5. Application for further implementation

The excess anthropogenic nitrogen input is the main cause of eutrophication in estuaries and coastal waters. This study suggests that restored wetlands have the ability to mitigate nitrogen pollution. This study explored the nitrogen removal potential, as well as the method to improve it, and then proposed feasible technology (adding sufficient carbon source) to control estuarine and coastal eutrophication. According to the NO_3^--N removal rate in T3 (Fig. 3), the nitrogen removal potential of the restored wetland could reach 0.68 g $N/m^2/d$ with sufficient carbon source supply, and therefore, this restored wetland could potentially remove 2.8 Kg N/d with the area of 6000 m^2. We believed this powerful nitrogen removal capacity could be embodied in other restored wetlands when carbon additions are included in the restoration process. In this study, we used anhydrous sodium acetate as dissolved organic carbon to test the significance of the carbon source during wetland restoration. However, adding anhydrous sodium acetate is high-cost and less environmentally friendly for wetland restoration engineering, thus we should find new organic carbon sources that are economical, non-toxic, efficient and practical, such as withered wetland plants and plant straw materials (Ding et al., 2013; Hang et al., 2016).

Moreover, nitrogen removal ability is also influenced by hydrological condition. Further studies should be conducted in the restored wetland site to develop engineering techniques to allow for equal tidal flow with optimal hydraulic retention time.

Meanwhile, restored wetlands should play an important role in reducing GWP in response to global climate change. The key goal of this study was to influence the mechanism of denitrification to favor N_2 production, rather than N_2O. Hence, the activities of NO reductase and N_2O reductase, which determine the final product of denitrification (Kampschreur et al., 2009), should be measured in future studies to reveal the mechanism of how carbon sources affect the nitrogen removal and N_2O emission. Since GWP also includes the important greenhouse gases CO_2 and CH_4, the further work should explore the CO_2 and CH_4 emissions to evaluate the GWP value in the restored wetland.

4. Conclusions

In a restored wetland, plants play a significant role in reducing nitrogen. With external high-nitrogen input, nitrogen removal could be enhanced in the restored wetland due to the increase in electron acceptor supply that can benefit denitrifying bacteria. However, external nitrate may increase the N_2O emission due to incomplete denitrification. In this study, we found that additional carbon sources could improve the nitrogen removal efficiency, and promote complete denitrification thus decreasing N_2O emission. Therefore, providing sufficient carbon sources is an effective bioengineering technique to enhance nitrogen removal and reduce greenhouse gas emission in restored wetland ecosystems.

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