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Effect of salinity on the performance of constructed wetlands treating mariculture wastewater with different halophytes and its molecular biological mechanism

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ABSTRACT

Constructed wetlands (CWs) have been widely applied in mariculture effluents disposal, however, the pollutants removal performance of mariculture wastewater with different salinity, especially the effect of different halophytes in CWs is rarely discussed. In this study, the nutrients removal efficiencies and absolute abundance of nitrogen (N) transformation microbes in CWs microcosms were investigated. With the increase of the salinity, both *Phragmites australis* (*P. australis*) and *Suaeda salsa* (*S. salsa*) planted CWs showed a significant reduction in chemical oxygen demand removal performance ($p < 0.05$), while no significant restriction on the nitrogen removal rates of all groups was obtained. The N removal efficiency was even promoted under 1.4% in these two kinds of CWs, respectively. And total phosphorus removal efficiency declined at the salinity of 1.4% and 2.1% for *P. australis* and *S. salsa* planted CWs, respectively. Further analysis showed that the presence of nitrification and denitrification was the major approaches of N removal. The absolute abundance of genes related with N transformation genes was significantly influenced by fluctuation of salinity, which affected the treatment efficiency of wastewater. The two different CWs presented high possibility of application on mariculture treatment in proper salinity.

Keywords: Mariculture effluent; Constructed wetland; Functional gene; Nitrogen removal

1. Introduction

Mariculture is a kind of production way breeding and reproducing marine economic animals and plants, which mostly based on shallow sea, beach, harbor, etc. It is one of the most significant ways to utilize marine living resources and develop oceanic fishery with the advantages of short production cycle and high output per unit area. Recent estimations showed that the world aquaculture production by marine environment has increased at an average annual growth rate of 3.81%, from 15.2 million tons in 2005 to 21.3 million tons in 2014 [1]. At the same time, the rapid development of mariculture leads to a large amount of salinity wastewater discharge, which contains high concentrations of suspended solids, inorganic nitrogen (N), and phosphate (P) pollutants, and need high oxygen demand due to low utilization rate of

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feeds [2]. The high nutrient in mariculture effluent has direct and serious influences on the marine environment, especially inorganic N and active P, exceeding more than 31.2% and 14.6% of upper limit in nearshore sea areas, respectively [3]. Extra N and P pollutants have been shown to be capable of promoting algae growth rapidly, which result in the decrease of dissolved oxygen (DO) concentration and light necessary for other organisms [4].

Nutrients in mariculture wastewater can be removed by the physical precipitation and adsorption, and chemical technologies, such as catalytic ozonation [5], foam separation [6], and poly(allylamine hydrochloride) (PAA·HCl) polymer hydrogel methods [7]. However, those physical and chemical methods still have problems of bad maneuverability, high operation, and maintenance cost, as well as some secondary pollution. Constructed wetlands (CWs), as an ecological wastewater treatment system, have been shown to remove the main pollutants in mariculture wastewater successfully [8] and realized high efficiencies with low cost since 1980s. Previous studies [9–11] demonstrated satisfactory pollutant removal efficiency have been achieved in different types of CWs treating mariculture wastewater only with low concentration of salinity (below 1%). Therefore, studies are needed to address the challenges of increasing salinity, and improve the treating performance of CWs through utilizing adaptive plants and microorganisms.

Plants have important structural and biological component of CWs and play a key role in obtaining better treatment efficiency of contaminants. Because plants not only assimilate the inorganic compounds in wastewater, but they can also provide oxygen and organic compounds (such as sugars and vitamins) for microorganisms in rhizosphere [12]. Several researchers reported that the activity and quantity of nitrate reducers and denitrifiers were stimulated by rhizosphere effect [13–16]. However, mariculture is conducted with low salinity of 3 parts per thousand (ppt) in the inland areas and high salinity of 30 parts per thousand (ppt) in coastal areas [17,18]. High-salt stress in mariculture effluents could restrict the growth and productivity of plants by decreasing the amount of water and increasing concentrations of salts in the cytosol [19,20]. Some studies indicated that, *Phragmites australis* (*P. australis*), *Suaeda salsa* (*S. salsa*), and *Salicornia europaea* (*S. europaea*) have great potential for disposal of mariculture wastewater in CWs [9,21,22]. Especially, *S. salsa* can grow well with high salinity with 400 mM NaCl due to its superior salt tolerance [23]. Whereas, *S. salsa* is still less extensively applied to treat mariculture wastewater in CWs in comparison with other halophytes such as *P. australis* and cattail species [24]. The lack of mariculture treatment performance of *S. salsa* planted CWs limits our ability to optimize treatment processes and to reliably predict specific effluent quality.

Despite the fact that the CWs are effective and environmentally friendly process with low operating and maintenance costs [25], poor N removal performance caused by different requirements for oxygen and organic carbon for nitrification and denitrification has been described in many studies [26–29]. Lack of DO is one of the most common issues in CWs on account of short oxygen transfer efficiency of the surface air and substrate [27]. Diverse and complex microbial community plays a key role in the degradation performance of nitrogen removal in CWs, such as nitrifiers, denitrifying bacteria, and anaerobic ammonium oxidation (anammox) bacteria [30]. NH_4–N is converted to NO_2–N or NO_3–N by nitrifiers with O_2 as the electron acceptor, while NO_2–N or NO_3–N are converted to gaseous N by denitrifiers with NH_4–N as the electron donor under anoxic conditions [31]. Furthermore, anammox is also one of the most important biological nitrogen removal technologies, in which NH_4–N is anaerobically converted to N_2 using NO_3–N as an oxidant [32]. However, it is so difficult to apply practically due to its requirement of a long start-up period and strict anaerobic conditions [33]. Salinity may cause cell plasmolysis and death of bacteria due to its biotoxicity in conventional wastewater treatment process [34]. Therefore, the effect of salinity on microbe, especially nitrogen related microbe, need further study.

This study explored the operational adaptability and feasibility of two different halophytes planted CWs for treating mariculture wastewater under different salinity, as well as to quantitatively understand their underlying molecular mechanisms. The objective of this study was to evaluate the chemical oxygen demand (COD), N, and P treatment performance, and to quantify the absolute abundance of the functional genes involved in nitrogen removal.

2. Materials and methods

2.1. Microcosms setup

Twelve lab-scale CW microcosms were established under a transparent rain shelter in Bai Hua Park, Jinan, Shandong province. As shown in Fig. 1, each microcosm was a plastic drum with 34 cm in diameter and 45 cm height. The bottom of the CWs was covered with 22 cm thick layer of sand and 15 cm depth of water. The hydraulic loading of each system was 0.287 $m^3/(m^2 \cdot batch)$, and hydraulic retention time was 6 d. Those microcosms were divided into four groups: S (*S. salsa*), SS (*S. salsa* + salt), P (*P. australis*), and PS (*P. australis* + salt), and each group contained three parallels.

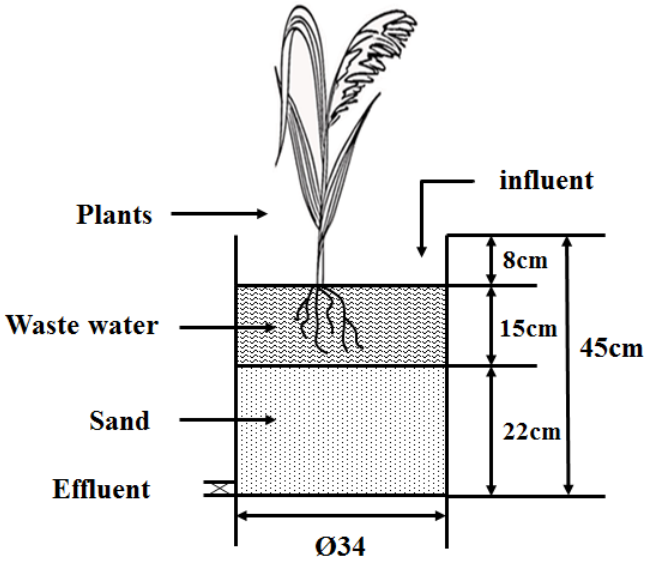

Fig. 1. Simulated diagram of the constructed wetlands.

2.2. Experimental design

The experiment was carried out from April 2016 to October 2016. *S. salsa* and *P. australis* were cultivated with freshwater for 2 weeks once planted and then cultivated with simulated wastewater for 2 weeks before the beginning of the experiments. To minimize variability of mariculture wastewater, artificial wastewater (Table S1) was prepared according to the artificial seawater formulated by Kester et al. [35]. Artificial wastewater was made from tap water (Jinan groundwater) and the concentration of COD, NH_4–N, NO_3–N, and total phosphorus (TP) was 60, 20, 5, and 10 mg/L, respectively [36–38]. As shown in Table 1, after stabled for more than 3 weeks, the salinity of simulated mariculture effluents was increased. And there was only one control group data and named S and P of *S. salsa* and *P. australis* CWs, respectively.

2.3. Water parameters

Water samples were taken at the influent and effluent sites of the microcosms. Water temperature, DO, and pH levels were determined in situ using a pH analyzer (PHS-3C, Leici, Shanghai) and DO analyzer (HQ30d 53LEDTM, Hach, USA). Water salinity was measured by portable electrical conductivity meter (DDBJ-350, Leici, Shanghai). Water samples were taken every 2 d to test dissolved nutrients: NH_4–N, NO_2–N, NO_3–N, and TP. Samples were collected in acid-washed 500 mL plastic jars and filtered (GF/C Whatman). Triplicate nutrient concentration was measured according to the standard methods [39]. TP was analyzed according to Bray [40].

The calculation methods of nitrogen removal rate (NRR) referred to previous research [41].

Nutrient removal efficiency (RE) was calculated as follows [42]:

$$RE = (C_i - C_0) \times 100/C_i$$

where C_i is the concentration of influent (mg/L); C_0 is the concentration of effluent (mg/L). The results were standardized by dividing these parameters by the surface area so as to define the values obtained per square meter.

2.4. DNA extraction and real-time quantitative polymerase chain reaction

Three replicates of DNA for each microcosm were extracted from 500 mg substrate samples using the power soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) following the manufacturer's instructions. The concentration of DNA was measured using microspectrophotometer (K5500, KAIAO Technology, China) according to the instructions. The value of OD260/280 measured after DNA extraction ranged between 1.8 and 2.0 to guarantee the quality of DNA.

To explore the effect of salinity on N removal performance, the real-time quantitative polymerase chain reaction (qPCR) was conducted with a selection of *Nitrospira* (*nobL*), anammox bacterial 16S rRNA (*anammox*), ammonia monooxygenase (*amoA*), membrane-bound nitrate reductase (*narG*), copper-containing nitrite reductase (*nirK*), and cd1-containing nitrite reductase (*nirS*). Table S2 lists the information of the primers selected for amplification of the 16S rRNA fragment of *Nitrospira* (*nobL*) and anammox (*anammox*), and the target fragments of the following functional genes: ammonia monooxygenase (*amoA*). The qPCR was carried out in LightCycler 480 with Sequence Detection Software v1.4 (Applied Biosystems, USA). Each qPCR mixture (20 μL) was composed of 10 μL of SYBR Premix Ex Taq™ II (2×), 0.4 μL of each forward and reverse primers, 8.2 μL ddH_2O and 1.0 μL of template DNA (TaKaRa Biotechnology, Japan) and the amplification program was performed as Yun et al. [43]. The temperature programs of qPCR were shown in Table S3. All the standard curves showed very good correlations between the DNA template concentration and the crossing point with high coefficients of determination ($R^2 > 0.99$). qPCR efficiency was in the range from 80.66% to 98.52%.

2.5. Data analysis

Abundance of tested genes was normalized by log transformation. A one-way analysis of variance was performed using SPSS v20 to access the significant differences of all parameters between samples. The results were considered to be statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Water parameters and removal performance

There were no significant differences in DO concentration and pH among all groups. The DO concentration on the water surface ranged from 1.5 to 4.8 mg/L, and pH value averaged at 7.8 ± 0.8. And the salinity in inflow and effluent were almost the same in different groups (data not shown). Although the salinity showed no effect on those parameters, the pollution removal performance showed much difference with the increasing salinity and *P. australis* was more sensitive than *S. salsa* (Figs. 2–4).

As a common water quality, COD removal performance is shown in Fig. 2. At the salinity of 0.7%, COD removal efficiencies of *P. australis* planted CWs were 21.86% less than those of *S. salsa* planted CWs. However, no significant

Table 1
Salinity of experiment wastewater

Date	S (%)	SSA (%)	SS (%)	P (%)	PSA (%)	PS (%)
5 May 2015 to 5 June 2015	0	0.35	0.35	0	0.35	0.35
6 June 2015 to 20 July 2015	0	0.7	0.7	0	0.7	0.7
21 July 2015 to 25 August 2015	0	1.4	1.4	0	1.4	1.4
25 August 2015 to 8 October 2015	0	2.1	2.1	0	2.1	2.1

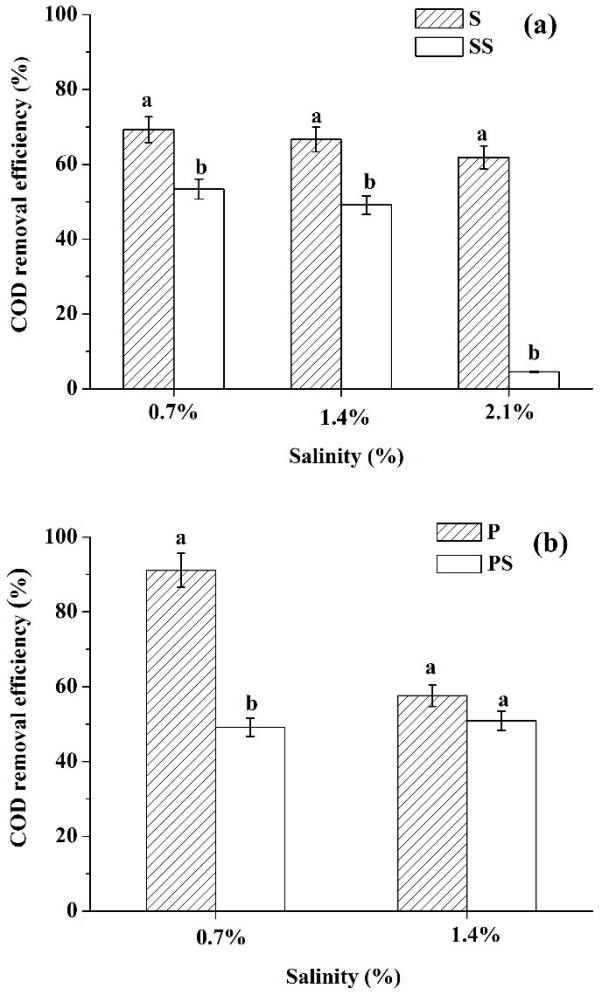

Fig. 2. COD removal efficiency in treating wastewater with different salinity in (a) *S. salsa* and (b) *P. australis* planted CWs. Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$).

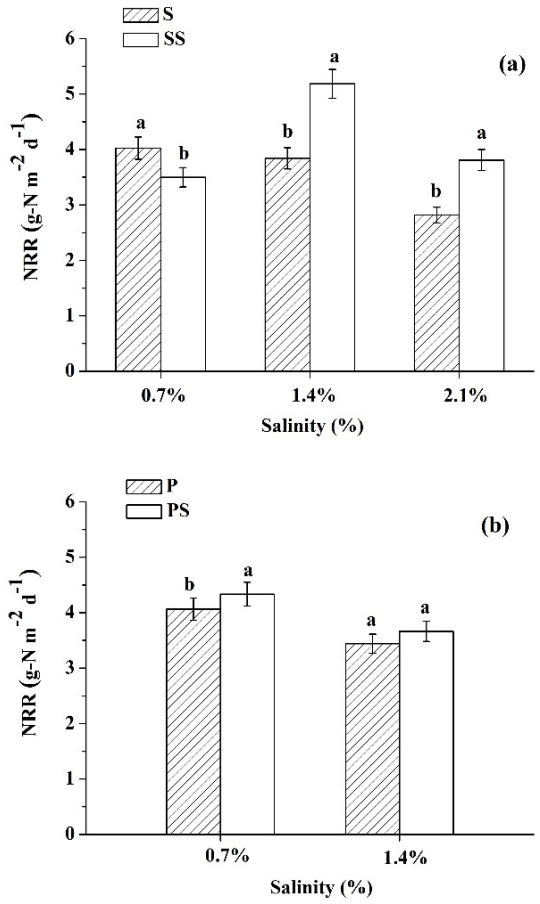

Fig. 3. Inorganic nitrogen removal rates (NRRs) in treating wastewater with different salinity in (a) *S. salsa* and (b) *P. australis* planted CWs (no salt addition in S and P). Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$).

difference between two different halophytes planted CWs were observed at salinity of 1.4%. For *S. salsa* planted CWs, as the salinity increased from 1.4% to 2.1%, the COD removal efficiency of SS significantly decreased from 49.12% to 4.53%. For *P. australis* planted CWs, the COD removal efficiencies were similar (49.15% and 50.88%) under different salinity. The result with the salinity of 2.1% was not shown due to the unsatisfactory growth of *P. australis* in CWs. Above all, COD removal efficiencies of *S. salsa* planted CWs were lower than that in *P. australis* plated CWs in control groups (P and S). The results were in accordance with previous study, in which, *P. australis* has better ability for COD removal due to its developed root system [44]. The well-developed root system tends to improve uptake of nutrients and provide an ideal habitat for the microorganisms. However, the COD removal efficiency declined significantly with the salinity addition ($p < 0.05$), resulting in worse treatment performance in *P. australis* plated CWs. When the salinity was lower than 2.1%, the COD removal efficiencies maintained 40%–50%, whereas it suddenly dropped when salinity was higher than 2.1%. The results indicated that excess salt in mariculture wastewater could inhibit the halophytes absorption of COD [45,46].

Opposed to COD, all the microcosms showed high efficiency in NRR. The NRR in treating wastewater under different salinity is shown in Fig. 3. In the main, the removal efficiency of N was accelerated by salinity in almost all microcosms. In *S. salsa* planted CWs, NRR of *S. salsa* CWs reached the maximum of 5.18 g N/m^2/d for SS under 1.4%. The NRR of SS was much higher than those of control groups without salinity addition and there was distinctly difference between group SS and S under different salinity ($p < 0.05$). For *P. australis* planted CWs, the elimination of N was less affected by salinity and there was almost no significant difference among groups with and without salt. Given all that, the performance of N removal was promoted by *S. salsa* and had no effect of *P. australis* under saline environments. There were no significant differences in NRR under different salinity ($p > 0.05$), whereas the maximum value of NRR of *S. salsa* planted CWs was slightly higher than that in *P. australis* planted CWs. The differences might be related to salt tolerance mechanism of two different kinds of halophytes. As the salinity increased, concentration of Na^+ ions in *P. australis* roots increased [47], while *S. salsa* roots could absorb Na^+ into the vacuole [48]. Genes

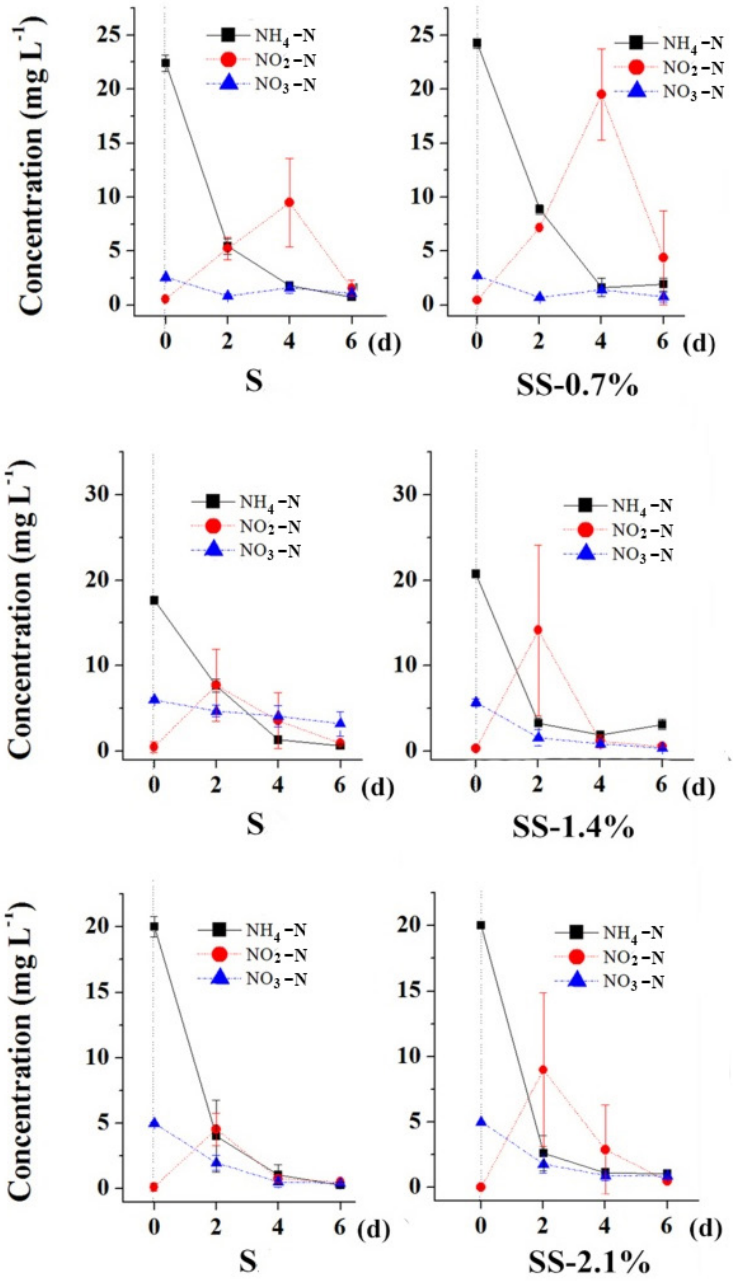

Fig. 4. Concentration variation of NH_4–N, NO_2–N, and NO_3–N in *S. salsa* planted CWs with different salinity.

linked to salt tolerance in *S. salsa* may help to regulate proper osmotic pressure in vacuole, so that the optimum salinity for its growth is 200 mM NaCl [23]. Therefore, *S. salsa* performed better in enduring different salinity than *P. australis*, and could promote the N removal of saline wastewater.

The concentrations of NH_4–N, NO_2–N, and NO_3–N in different groups of a typical cycle are shown in Figs. 4 and 5. In different groups, the variations of the three different forms of nitrogen were similar. In *S. salsa* planted CWs (Fig. 4), the residual NH_4–N in the effluent significantly declined and the NO_2–N was significantly accumulated with increasing salinity after 2 or 4 d treatment. The first process of ammonia oxidation (from NH_4–N to NO_2–N) was attributed to NH_4–N removal. Magalhaes et al. [49] found that nitrification rates (calculated by the difference between NH_4–N production measured in treatments) were promoted when increased the salinity properly (from 0 to 3%) in the intertidal sandy sediments. In addition, no obvious accumulation of NO_3–N was observed with the decline of NO_2–N (Fig. 4), suggesting that nitrite oxidation was restrained by salinity. This was because nitrite oxidation process is more sensitive to salinity than ammonium oxidation process [50]. Ultimately, little NO_2–N and NO_3–N were accumulated in effluent, indicating that denitrification was sufficient to completely remove nitrogen from the wastewater with no obvious influence of salinity [49]. In *P. australis* planted CWs (Fig. 5), the abovementioned

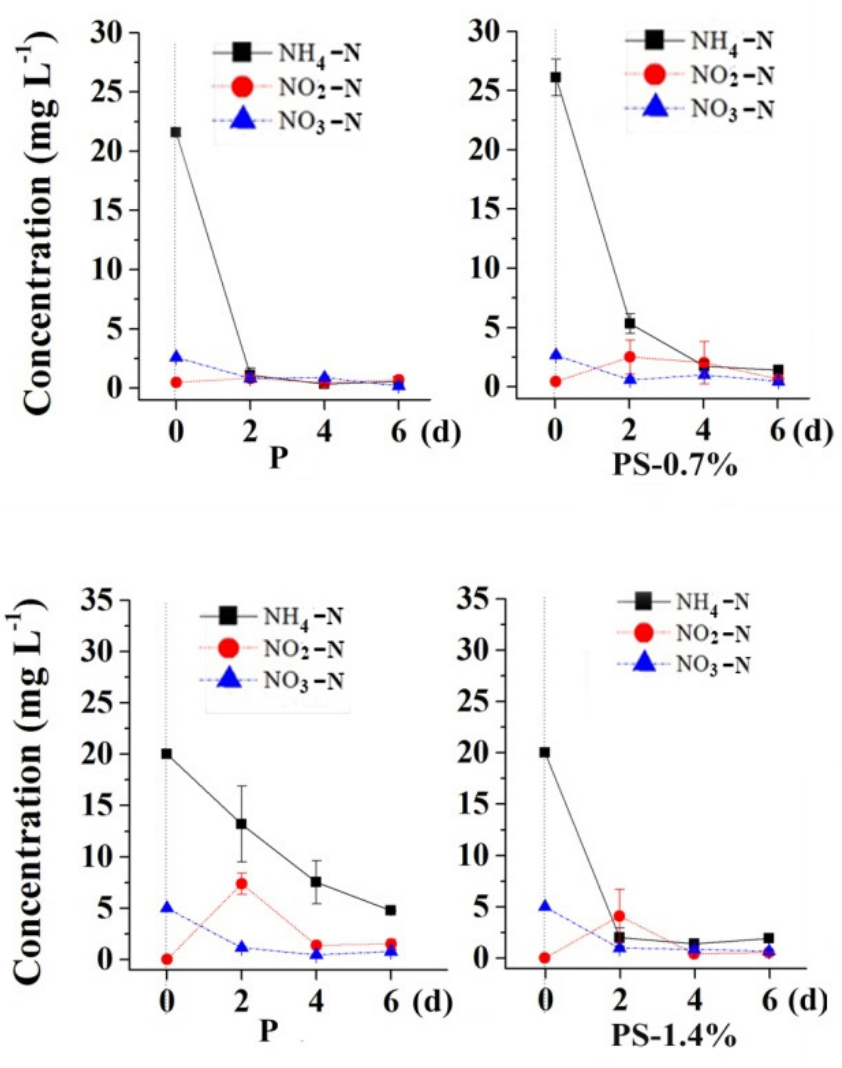

Fig. 5. Concentration variation of NH_4–N, NO_2–N, and NO_3–N in *P. australis* planted CWs with different salinity.

variation of NO_2–N was not obvious. The pollutant absorption capacity of *P. australis* roots might explain the result, which could absorb more nitrogen [44].

Fig. 6 shows TP removal performance in all microcosms. In general, the treatment efficiency of TP was roughly the same in two kinds of CWs. In *S. salsa* planted CWs, the salinity had little effect on TP removal at 1.4% and below. However, when the salinity arrived 2.1%, there showed statistic difference between two groups ($p < 0.05$), in which S group had a higher removal efficiency, followed by SS. In *P. australis* planted CWs, the TP removal efficiencies of two groups had little difference under 0.7%, and TP removal efficiency of P was much higher than PS when the salinity increased to 1.4% ($p < 0.05$). Although salinity could decrease the removal efficiency of TP, *P. australis* presented more obvious than *S. salsa* under much lower concentration. The two kinds of halophyte CWs performed likewise in TP removal. *S. salsa* planted CWs performed the best and reached the maximum value at the salinity of 1.4%. TP removal efficiencies of *P. australis* planted CWs were lower than those of *S. salsa* planted CWs, and the value under 0.7% and 1.4% were similar. This also indicated that the salt tolerance of *S. salsa* was better than *P. australis*, and the Na^+, K^+ could promote the adsorption and removal of P [51].

3.2. The absolute abundance of nitrogen transfer related gene

Given that the removal efficiency of N with different salinity, the absolute abundance of N related gene in the substrate of different microcosms was further investigated. Figs. 7 and 8 give the absolute abundance of six kinds of N transformation genes, including *amoA*, *nobL*, *anammox*, *nirG*, *nirS*, and *nirK*. The S and P groups represented the background values of microbes in CW sediments.

For *S. salsa* planted CWs (Fig. 7), the increase in salinity led to significant decrease in the absolute abundance of *amoA* and *nobL* comparing with control groups in all samples at all salinity ($p < 0.05$). However, the performance of *anammox* was more variable. The significant difference ($p < 0.05$) between S and SS gradually decreased as salinity continued rising, and even resulting in no difference under 2.1% salinity with the groups without salinity addition ($p > 0.05$). As for related

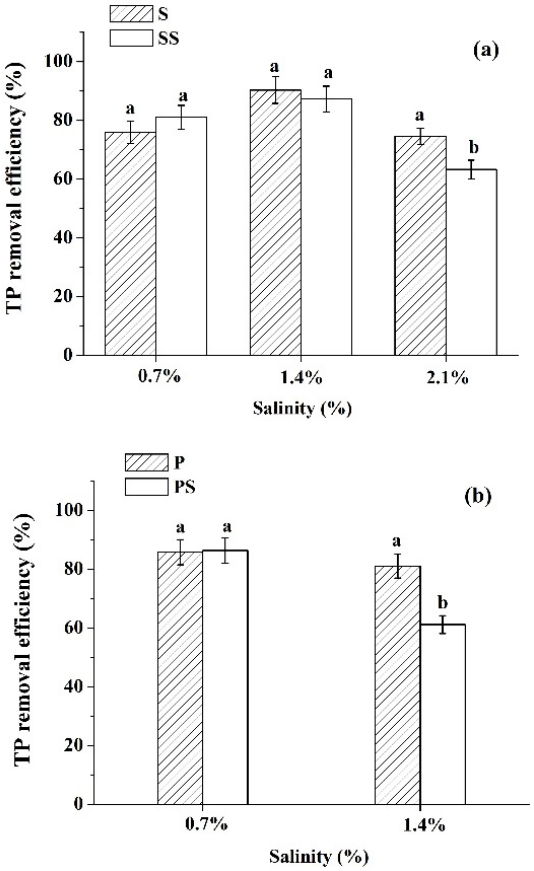

Fig. 6. Variation of TP removal efficiency in CWs with different salinity in (a) *S. salsa* and (b) *P. australis* planted CWs. Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$).

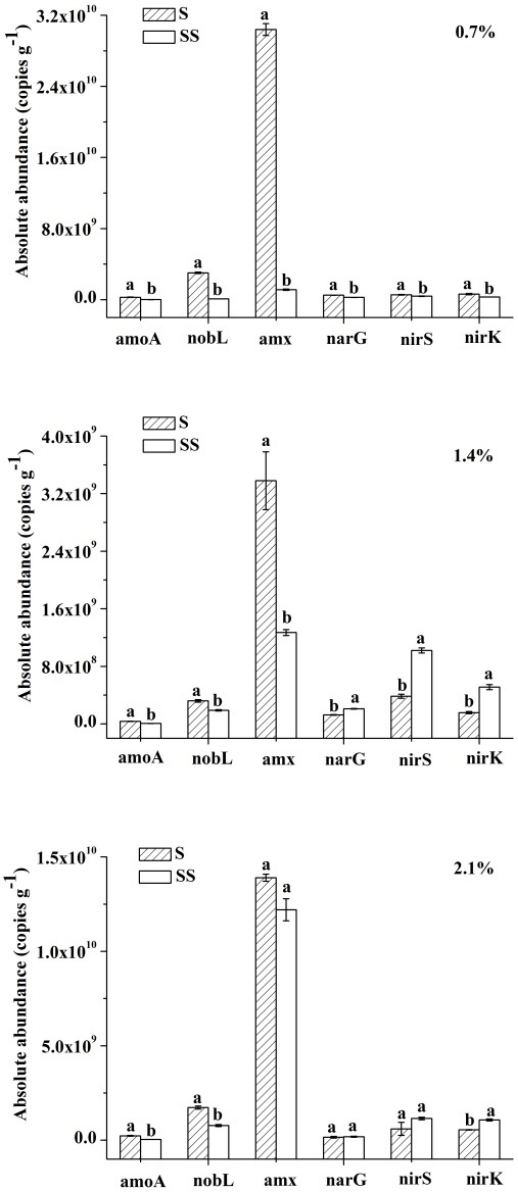

Fig. 7. Microbial abundance of *S. salsa* constructed wetlands with salinity of 0.7%, 1.4%, and 2.1%. Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$).

genes in microbial denitrification, the absolute abundance of *narG*, *nirS*, and *nirK* showed similar trends. After a significant decline at 0.7% salinity ($p < 0.05$), the quantities of those three kinds of genes increased above those of S sample, and showed significant difference at 1.4% and 2.1% salinity ($p < 0.05$). The results indicated that the salinity would inhibit microbial nitrification. However, microbial denitrification was potentially stimulated under 1.4% and 2.1% salinity, with anammox presented no difference at the salinity of 2.1%.

For *P. australis* planted CWs (Fig. 8), the performance of those six types of genes showed similar trend with that of *S. salsa* planted CWs. The quantities of two genes involved in microbial nitrification were inhibited under all salinity. The quantity of three kinds of genes related with denitrification was also demonstrated the inhibition effect under 0.7% and stimulation at 1.4%. As for *anammox*, there showed some difference with *S. salsa* planted CWs, its absolute abundance was lower at the salinity of 0.7% and then increased and even kept up with those in control at the salinity of 1.4% ($p > 0.05$).

According to the above results, the number of *amoA* and *nobL* declined with the increase of salinity. It might be caused by salinity stress on ammonia oxidizing bacteria and nitrite oxidizing bacteria in accordance with previous studies, in which, nitrification efficiency dropped gradually with increasing salt [52,53]. Especially for *anammox*, with the increasing concentration of salt stress, the numbers of salt addition groups gradually increased, which was due to a good adaptability of anammox to the salinity concentration up to 30 g NaCl/L [54]. As for the related genes involved in denitrification, our results were similar to a recent study that a pulse of 0.7% salinity did influenced denitrification adversely, but the intermediate salinity pulse (1.4%) practically promoted denitrification [55]. It suggested that appropriate salt addition could promote the performance of denitrification and anammox after a period of orientation.

Wetlands microcosms with *S. salsa* and *P. australis* performed well in removing nutrient in mariculture effluents, especially *S. salsa* planted CWs. These halophytes were low-cost and high-efficiency, which provided the potential candidates for treatment of wastewater from marine farms.

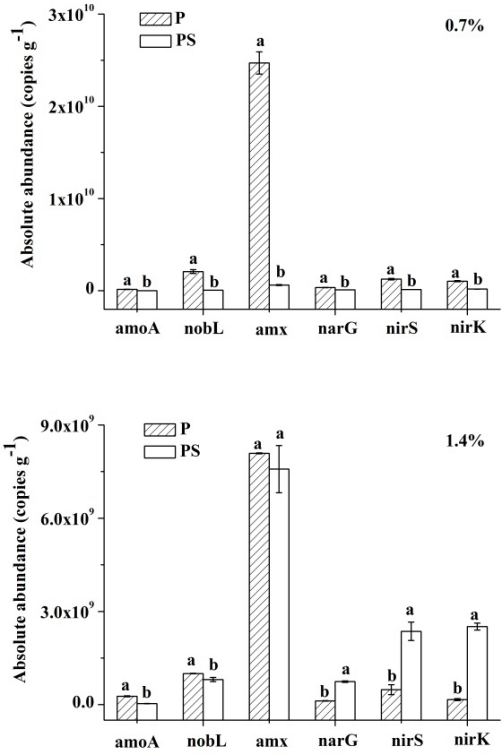

Fig. 8. Microbial abundance of *P. australis* constructed wetlands with salinity of 0.7% and 1.4%. Values followed by the same letter are not significantly different according to LSD test ($p < 0.05$).

Some questions should be explored in further studies such as enlarging the lab-scale to pilot-scale CWs, applying more halophytes to the CWs, and enhancing different microbes. We would selectively plant appropriate halophytes in CWs to treat mariculture effluents of different salinity and nutrient concentrations in the near future.

4. Conclusions

The nutrient removal efficiency of mariculture wastewater with different salinity and different halophytes in CWs was established. In particular, it was found that two kinds of CWs showed a significant reduction in COD removal efficiency under low salinity concentration of 0.7%–1.4%. Additionally, our results showed that no significant differences were observed in NRR under different salinity. Furthermore, the absolute abundances of six N related genes changed constantly because of different salinity. Appropriate salt addition could promote the absolute abundance of denitrifiers and anammox bacteria after a period of orientation. The two different CWs presented high possibility of application on mariculture treatment in proper salinity. *S. salsa* performed better in enduring different salinity than *P. australis*, and could promote the N removal of saline wastewater.

Acknowledgments

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Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors. All authors declare that they have no conflict of interest.

References

[1] FAO, FAO Yearbook, Fishery and Aquaculture Statistics, 2014, 2016.
[2] C.E. Boyd, Guidelines for aquaculture effluent management at the farm-level, Aquaculture, 226 (2003) 101–112.
[3] MEP, Report on the State of the Environment in China 2014, Ministry of Environment Protection of the People's Republic of China, 2014.
[4] K. Havens, T. Frazer, Rethinking the Role of Nitrogen and Phosphorus in the Eutrophication of Aquatic Ecosystems, Sea Grant, 2015.
[5] B. Legube, N.K.V. Leitner, Catalytic ozonation: a promising advanced oxidation technology for water treatment, Catal. Today, 53 (1999) 61–72.
[6] L.K. Wang, Y.T. Hung, N.K. Shammas, Advanced Physicochemical Treatment Processes, Handbook of Environmental Engineering, Vol. 4, 2006.
[7] D.R. Kioussis, F.W. Wheaton, P. Kofinas, Reactive nitrogen and phosphorus removal from aquaculture wastewater effluents using polymer hydrogels, Aquacult. Eng., 23 (2000) 315–332.
[8] Y.-F. Lin, S.-R. Jing, D.-Y. Lee, Y.-F. Chang, Y.-M. Chen, K.-C. Shih, Performance of a constructed wetland treating intensive shrimp aquaculture wastewater under high hydraulic loading rate, Environ. Pollut., 134 (2005) 411–421.
[9] Y.-F. Lin, S.-R. Jing, D.-Y. Lee, T.-W. Wang, Nutrient removal from aquaculture wastewater using a constructed wetlands system, Aquaculture, 209 (2002) 169–184.
[10] Y. Liang, H. Zhu, G. Bañuelos, B. Yan, B. Shutes, X. Cheng, X. Chen, Removal of nutrients in saline wastewater using constructed wetlands: plant species, influent loads and salinity levels as influencing factors, Chemosphere, 187 (2017) 52–61.
[11] J.M. Jesus, A. Cassoni, A.S. Danko, A. Fiúza, M.-T. Borges, Role of three different plants on simultaneous salt and nutrient reduction from saline synthetic wastewater in lab-scale constructed wetlands, Sci. Total Environ., 579 (2017) 447–455.
[12] U. Stottmeister, A. Wießner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R. Müller, H. Moormann, Effects of plants and microorganisms in constructed wetlands for wastewater treatment, Biotechnol. Adv., 22 (2003) 93–117.
[13] S. Henry, S. Texier, S. Hallet, D. Bru, C. Dambreville, D. Chèneby, F. Bizouard, J. Germon, L. Philippot, Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates, Environ. Microbiol., 10 (2008) 3082–3092.
[14] J. Woldendorp, The quantitative influence of the rhizosphere on denitrification, Plant Soil, 17 (1962) 267–270.
[15] E. Benizri, O. Dedourge, C. Dibattista-Leboeuf, S. Piutti, C. Nguyen, A. Guckert, Effect of maize rhizodeposits on soil microbial community structure, Appl. Soil Ecol., 21 (2002) 261–265.
[16] D. Cheneby, S. Perrez, C. Devroe, S. Hallet, Y. Couton, F. Bizouard, G. Iuretig, J. Germon, L. Philippot, Denitrifying bacteria in bulk and maize-rhizospheric soil: diversity and N_2O-reducing abilities, Can. J. Microbiol., 50 (2004) 469–474.
[17] R. Boopathy, C. Bonvillain, Q. Fontenot, M. Kilgen, Biological treatment of low-salinity shrimp aquaculture wastewater using sequencing batch reactor, Int. Biodeterior. Biodegrad., 59 (2007) 16–19.

[18] E. Marinho-Soriano, S.O. Nunes, M.A.A. Carneiro, D.C. Pereira, Nutrients' removal from aquaculture wastewater using the macroalgae *Gracilaria birdiae*, Biomass Bioenergy, 33 (2009) 327–331.
[19] S.I. Allakhverdiev, A. Sakamoto, Y. Nishiyama, M. Inaba, N. Murata, Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp., Plant Physiol., 123 (2000) 1047–1056.
[20] U. Deinlein, A.B. Stephan, T. Horie, W. Luo, G. Xu, J.I. Schroeder, Plant salt-tolerance mechanisms, Trends Plant Sci., 19 (2014) 371–379.
[21] J.M. Webb, R. Quinta, S. Papadimitriou, L. Norman, M. Rigby, D.N. Thomas, L. Le Vay, Halophyte filter beds for treatment of saline wastewater from aquaculture, Water Res., 46 (2012) 5102–5114.
[22] J.J. Brown, E.P. Glenn, K.M. Fitzsimmons, S.E. Smith, Halophytes for the treatment of saline aquaculture effluent, Aquaculture, 175 (1999) 255–268.
[23] J. Song, B. Wang, Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model, Ann. Bot., 115 (2015) 541–553.
[24] Y. Liang, H. Zhu, G. Bañuelos, B. Yan, Q. Zhou, X. Yu, X. Cheng, Constructed wetlands for saline wastewater treatment: a review, Ecol. Eng., 98 (2017) 275–285.
[25] J. Vymazal, Constructed wetlands for wastewater treatment: five decades of experience, Environ. Sci. Technol., 45 (2011) 61–69.
[26] D.O. Huett, S.G. Morris, G. Smith, N. Hunt, Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands, Water Res., 39 (2005) 3259–3272.
[27] L.-y. Zhang, L. Zhang, Y.-d. Liu, Y.-w. Shen, H. Liu, Y. Xiong, Effect of limited artificial aeration on constructed wetland treatment of domestic wastewater, Desalination, 250 (2010) 915–920.
[28] Y. Ding, X. Song, Y. Wang, D. Yan, Effects of dissolved oxygen and influent COD/N ratios on nitrogen removal in horizontal subsurface flow constructed wetland, Ecol. Eng., 46 (2012) 107–111.
[29] F. Li, L. Lu, X. Zheng, H.H. Ngo, S. Liang, W. Guo, X. Zhang, Enhanced nitrogen removal in constructed wetlands: effects of dissolved oxygen and step-feeding, Bioresour. Technol., 169 (2014) 395–402.
[30] B. Adrados, O. Sánchez, C.A. Arias, E. Becares, L. Garrido, J. Mas, H. Brix, J. Morató, Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters, Water Res., 55 (2014) 304–312.
[31] L. Kuai, W. Verstraete, Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system, Appl. Environ. Microbiol., 64 (1998) 4500–4506.
[32] S.Q. Ni, B.Y. Gao, C.C. Wang, J.G. Lin, S. Sung, Fast start-up, performance and microbial community in a pilot-scale anammox reactor seeded with exotic mature granules, Bioresour. Technol., 102 (2011) 2448–2454.
[33] I. Tsushima, Y. Ogasawara, T. Kindaichi, H. Satoh, S. Okabe, Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors, Water Res., 41 (2007) 1623–1634.
[34] D. Jang, Y. Hwang, H. Shin, W. Lee, Effects of salinity on the characteristics of biomass and membrane fouling in membrane bioreactors, Bioresour. Technol., 141 (2013) 50–56.
[35] D.R. Kester, I.W. Duedall, D.N. Connors, R.M. Pytkowicz, Preparation of artificial seawater, Limnol. Oceanogr., 12 (1967) 176–179.
[36] V.H. Riveramonroy, L.A. Torres, N. Bahamon, F. Newmark, R.R. Twilley, The potential use of mangrove forests as nitrogen sinks of shrimp aquaculture pond effluents: the role of denitrification, J. World Aquacult. Soc., 30 (1999) 12–25.
[37] C. Schulz, J. Gelbrecht, B. Rennert, Treatment of rainbow trout farm effluents in constructed wetland with emergent plants and subsurface horizonal water flow, Aquaculture, 217 (2003) 207–221.
[38] W. Zhi, L. Yuan, G. Ji, C. He, Enhanced long-term nitrogen removal and its quantitative molecular mechanism in tidal flow constructed wetlands, Environ. Sci. Technol., 49 (2015) 4575–4583.
[39] APHA, Standard Methods for the Examination of Water and Wastewater, Vol. 4, 14th ed., American Public Health Association, 1971, p. 113.
[40] R. Bray, Determination of total, organic and available forms of phosphorus in soils, Soil Sci., 59 (1944) 39–46.
[41] J. Liu, Z. Yang, W. Yu, J. Hu, H. Xie, S. Wang, J. Zhang, Relationship between nitrogen transformation and its related genes: comparison among riparian, marsh, and full-scale constructed wetlands, Desal. Wat. Treat., 57 (2016) 21806–21816.
[42] M. Shpigel, D. Ben-Ezra, L. Shauli, M. Sagi, Y. Ventura, T. Samocha, J.J. Lee, Constructed wetland with *Salicornia* as a biofilter for mariculture effluents, Aquaculture, 412–413 (2013) 52–63.
[43] J. Yun, G. Zhuang, A. Ma, H. Guo, Y. Wang, H. Zhang, Community structure, abundance, and activity of *methanotrophs* in the Zoige wetland of the Tibetan Plateau, Microb. Ecol., 63 (2012) 835–843.
[44] J. Wang, Y.S. Zhang, J.Q. Qin, Y.X. Miao, X.Y. Zeng, H.F. Ren, Influence of seawater salinity on purification efficiency of subsurface flow constructed wetland, China Water Wastewater, 25 (2009) 9–11.
[45] J. Qiu, J. Wang, Y. Zhang, Applicability of constructed wetlands to the treatment of wastewater with high salinity and its research progress, Ind. Water Treat., 29 (2009) 1–3.
[46] Y. Liu, Y.J. Liu, Biosorption isotherms, kinetics and thermodynamics, Sep. Purif. Technol., 61 (2008) 229–242.
[47] S.M.S. Algarni, Increasing NaCl – salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis, Am.-Eurasian J. Agric. Environ. Sci., 1 (2006) 119–126.
[48] L. Zheng, C. Chen, F.Y. Zhang, Development of water quality model in the Satilla River Estuary, Georgia, Ecol. Modell., 178 (2004) 457–482.
[49] C.M. Magalhaes, S.B. Joye, R.M. Moreira, W.J. Wiebe, A.A. Bordalo, Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal, Water Res., 39 (2005) 1783–1794.
[50] C. Cortés-Lorenzo, M. Rodríguez-Díaz, D. Sipkema, B. Juárez-Jiménez, B. Rodelas, H. Smidt, J. González-López, Effect of salinity on nitrification efficiency and structure of ammonia-oxidizing bacterial communities in a submerged fixed bed bioreactor, Chem. Eng. J., 266 (2015) 233–240.
[51] R. Chitrakar, S. Tezuka, A. Sonoda, K. Sakane, K. Ooi, T. Hirotsu, Adsorption of phosphate from seawater on calcined MgMn-layered double hydroxides, J. Colloid Interface Sci., 290 (2005) 45–51.
[52] S. Liu, F. Yang, Z. Gong, Z. Su, Assessment of the positive effect of salinity on the nitrogen removal performance and microbial composition during the start-up of CANON process, Appl. Microbiol. Biotechnol., 80 (2008) 339–348.
[53] A.R. Dinçer, F. Kargi, Salt inhibition of nitrification and denitrification in saline wastewater, Environ. Technol., 20 (1999) 1147–1153.
[54] B. Kartal, M. Koleva, R. Arsov, d.S.W. Van, M.S. Jetten, M. Strous, Adaptation of a freshwater anammox population to high salinity wastewater, J. Biotechnol., 126 (2006) 546–553.
[55] B.M. Marks, L. Chambers, J.R. White, Effect of fluctuating salinity on potential denitrification in coastal wetland soil and sediments, Soil Sci. Soc. Am. J., 80 (2016) 516.

Supplementary material

Table S1
Nutrient and salinity composition of the influent [1]

Nutrient composition			
COD (mg/L)	NH_4^+–N (mg/L)	NO_3^-–N (mg/L)	TP (mg/L)
60	20	5	10
Salt[a] weighted			
Salt	Molecular weight (wt)	Concentration *G* (kg)	
NaCl	58.44	23.926	
Na_2SO_4	142.04	4.008	
KCl	74.56	0.677	
$NaHCO_3$	84.00	0.196	
KBr	119.01	0.098	
H_3BO_3	61.83	0.026	
NaF	41.99	0.003	
Salt[a] soluted			
Salt	Molecular weight (wt)	Mole (kg)	Concentration (M)
$MgCl_2{\cdot}6H_2O$	203.33	0.05327	1.0
$CaCl_2{\cdot}2H_2O$	147.03	0.01033	1.0
$SrCl_2{\cdot}6H_2O$	266.64	0.00009	0.1

[a]Salt was diluted by water to 1,000.000 g.

Table S2
Primers of target genes used in qPCR

Primers	Amplification size (bp)	Primer sequences (5′–3′)	Target genes	Reference
338F	180	ACTCCTACGGGAGGCAGCAG	Bacterial 16S rRNA	[2]
518R		ATTACCGCGGCTGCTGG		
amo598f	120	GAATATGTTCGCCTGATTG	Ammonia monooxygenase (*amoA*)	[3]
amo718r		CAAAGTACCACCATACGCAG		
AMX809F	257	GCCGTAAACGATGGGCACT	Anammox 16S rRNA (*anammox*)	[4]
AMX1066R		AACGTCTCACGACACGAGCTG		
NSR1113f	119	CCTGCTTTCAGTTGCTACCG	*Nitrospira* 16S rRNA (*nobL*)	[3]
NSR1264r		GTTTGCAGCGCTTTGTACCG		
1960m2f	100	TA(CT)GT(GC)GGGCAGGA(AG)AAACTG	Membrane-bound nitrate reductase (*narG*)	[5]
2050m2r		CGTAGAAGAAGCTGGTGCTGTT		
nirK583F	326	TCA TGGTGCTGCCGCGKGACGG	Copper-containing nitrite reductase (*nirK*)	[6]
nirK909R		GAA CTTGCCGGTKGCCCAGAC		
nirScd3aF	425	GT(C/G)AACGT(C/G)AAGGA(A/G)AC(C/G)GG	Cd1-containing nitrite reductase (*nirS*)	[7]
nirSR3cd		GA(C/G)TTCGG(A/G)TG(C/G)GTCTTGA		

IUPAC degenerate bases: R, A + G; S, G + C; Y, C + T.

Table S3
Program of target genes used in qPCR analysis [8]

Target gene	Programs
Bacterial 16S rRNA	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min
Ammonia monooxygenase (*amoA*)	Pre-heating at 50°C for 2 min (one cycle), pre-denaturation at 95°C for 10 min (one cycle), denaturation at 95°C for 15 s, annealing at 56°C for 45 s, and extension at 72°C for 30 s (40 cycles)
Anammox 16S rRNA (*anammox*)	Pre-heating at 50°C for 2 min (one cycle), pre-denaturation at 95°C for 10 min (one cycle), denaturation at 95°C for 15 s, annealing at 56°C for 45 s, and extension at 72°C for 30 s (40 cycles)
Nitrospira 16S rRNA (*nobL*)	Pre-heating at 50°C for 2 min (one cycle), pre-denaturation at 95°C for 10 min (one cycle), denaturation at 95°C for 15 s, annealing at 58°C for 45 s, and extension at 72°C for 30 s (40 cycles)
Membrane-bound nitrate reductase (*nar*G)	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 58°C for 45 s, and extension at 72°C for 30 s
Copper-containing nitrite reductase (*nirK*)	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 64°C for 40 s, and extension at 72°C for 30 s
Cd1-containing nitrite reductase (*nirS*)	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s

References

[1] D.R. Kester, I.W. Duedall, D.N. Connors, R.M. Pytkowicz, Preparation of artificial seawater, Limnol. Oceanogr., 12 (1967) 176–179.

[2] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol., 59 (1993) 695–700.

[3] H.M. Dionisi, A.C. Layton, G. Harms, I.R. Gregory, K.G. Robinson, G.S. Sayler, Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR, Appl. Environ. Microbol., 68 (2002) 245–253.

[4] I. Tsushima, T. Kindaichi, S. Okabe, Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR, Water Res., 41 (2007) 785–794.

[5] Z. Chen, J. Liu, M. Wu, X. Xie, J. Wu, W. Wei, Differentiated response of denitrifying communities to fertilization regime in paddy soil, Microb. Ecol., 63 (2012) 446–459.

[6] T. Yan, M.W. Fields, L. Wu, Y. Zu, J.M. Tiedje, J. Zhou, Molecular diversity and characterization of nitrite reductase gene fragments (nirK and nirS) from nitrate- and uranium-contaminated groundwater, Environ. Microbiol., 5 (2003) 13–24.

[7] I.N. Throbäck, K. Enwall, A. Jarvis, S. Hallin, Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE, FEMS Microbiol. Ecol., 49 (2004) 401–417.

[8] J. Liu, J. Wang, C. Zhao, A.G. Hay, H. Xie, J. Zhan, Triclosan removal in wetlands constructed with different aquatic plants, Appl. Microbiol. Biotechnol., 100 (2016) 1459–1467.