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Bioaugmented constructed wetlands for denitrification of saline wastewater: A boost for both microorganisms and plants

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ABSTRACT

The inhibition of salt stress on plant and microbial functions has led to the reduction of nitrogen removal capacity of constructed wetlands (CWs) under saline conditions. The mechanisms and effectiveness of bioaugmented CW (Bio-CW) microcosms with a salt-tolerant microbial inoculum were evaluated for nitrogen removal at different salinity levels. The results showed that the denitrification capacity of CWs was improved under saline conditions by adding the salt-tolerant microbial inoculum. At an EC of 15 mS/cm, the removal percentages of ammonia nitrogen (NH_4^+-N) and total nitrogen (TN) in Bio-CW microcosms (95.7% and 99.4%) on Day 5 were significantly ($p < 0.05$) higher than that in unbioaugmented CW (un-Bio-CW) microcosms (68.5% and 76.4%), respectively. The high throughput sequencing data of substrate samples indicated that the microbial community in the CWs was changed by the addition of the salt-tolerant microbial inoculum and the frequency of bacteria with nitrogen removal function was increased in the CWs. Furthermore, both growth and the TN accumulation capacity of plants in Bio-CW microcosms were promoted compared with the un-Bio-CW microcosms. In conclusion, the addition of the salt-tolerant microbial inoculum can enhance the nitrogen removal efficiency of CWs under saline condition via boosting the function of both microorganisms and plants.

1. Introduction

With the rapid development of coastal high-density aquaculture, high volumes of mariculture wastewater, a main source of saline wastewater, have been produced (Liang et al., 2018a). In addition, high volumes of saline wastewater can also be produced from agricultural drainage in saline areas, various industrial sectors (e.g., winery and pharmacy, etc.), and other secondary sources like the concentrated wastewater from membrane and electro dialysis equipment (Ioannou et al., 2015; Ng et al., 2016; Shi et al., 2015). High salinity has a negative impact on normal activities and survival of aquatic plants, animals and microorganisms (Liang et al., 2017b). Excluding the potential damage on aquatic survival caused by soluble salts in saline wastewater, the ecological and environmental hazard of excessive nitrogen in saline wastewater is also extremely serious (Lyu et al., 2019; Yang et al., 2015). The discharge of nitrogen-laden saline wastewater such as mariculture wastewater can promote the eutrophication of coastal water, which provides a suitable ecological environment for red-tide organisms by accelerating their reproduction, and then inducing harmful algal blooms (HABs) (Tan et al., 2017; Wurtsbaugh et al., 2019). The frequent occurrence of HABs is a severe threat to economic development and public health, and is of widespread concern on internationally (Schmale et al., 2019).

As a lower cost, high efficiency and energy saving water remediation technology with no production of secondary pollutants, constructed wetlands (CWs) have been widely applied in wastewater treatment (Liu et al., 2019b; Sun et al., 2012). The presence of soluble salts in saline wastewater can inhibit the microbial activity in CWs and consequently limit the purification capacity of CWs (Fu et al., 2019b; Leung et al., 2016). To overcome this obstacle, others have proposed isolating and enriching CWs with salt-tolerant bacteria to promote treating saline wastewater (Fu et al., 2019b). Salt-tolerant microorganisms have a variety of metabolic pathways existing under saline conditions, in which contribute to both their survival in an environment and their high-efficiency to degrade multiple pollutants (e.g., nitrogen) (Ahmadi et al., 2017; Soltani et al., 2013). A few studies have

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demonstrated the potential of bioaugmented CWs (Bio-CWs) for treating saline wastewater after introducing exogenous bacterial into the CWs. For example, a pilot-scale subsurface CW with the inoculation of salt-tolerant microorganisms can improve the removal efficiencies of chemical oxygen demand (COD) as higher as 83.6%under saline conditions (1.5% NaCl) (Karajic et al., 2010). Similar to the removal of COD, the removal of nitrogen in CWs mainly relies on the participation of microorganisms, especially those in biofilms on the surface of substrates and plant rhizosphere (Adrados et al., 2014; Xu et al., 2015). However, intensification strategies for nitrogen removal in saline wastewater by CWs are rare and there is an urgent need to develop some Bio-CWs for nitrogen removal under saline conditions. Furthermore, the inoculation of CWs with exogenous microorganisms might markedly change the microbial community structure (Zhao et al., 2016). Thus, we hypothesize that the inoculation of CWs with a salt-tolerant denitrifying bacterium might enhance the nitrogen removal efficiency due to not only the function of this exogenous bacterium but also the enhancement of some aboriginal functional microorganisms, e.g., some aboriginal nitrifying and denitrifying bacteria, and even some microorganisms that are beneficial to the growth of emergent plants.

Therefore, in order to fill these technical and knowledge gaps, this study aims to develop a salt-tolerant strain based Bio-CWs for enhancing nitrogen removal efficiency under saline conditions and to explore the relevant mechanisms. There are three specific objectives: (1) evaluate the effectiveness of Bio-CWs inoculated with a salt-tolerant microbial inoculum (i.e., strain *Alishewanella* sp. F2) for nitrogen removal of saline wastewater; (2) reveal the response of microbial community structure in CWs with the addition of strain *Alishewanella* sp. F2; and (3) quantify the growth and nitrogen accumulation capacity of emergent plants in CWs as affected by the inoculation of strain *Alishewanella* sp. F2. This study will provide a new solution on nitrogen removal in CWs under saline conditions, which is beneficial for broadening the application scope of CWs to various types of wastewater treatment. The study will also provide a new insight on the interaction between aboriginal and exogenous microorganisms in CWs.

2. Materials and methods

2.1. Preparation of salt-tolerant microbial inoculum

The salt-tolerant microbial inoculum used in this study was made from the strain *Alishewanella* sp. F2 (Genbank registration number: MN396708), which was isolated as a salt-tolerant denitrifying bacterium from seawall muddy water in Dalian City, Liaoning Province, China (39°38′31″ N, 122°58′19″ E). The concentrated bacteria were obtained by centrifuging the bacterial culture at 5000 rpm for 15 min. To obtain approximately the same concentration of the inoculum for each treatment, a 1.5 g (wet weight) of the concentrated bacteria were used to configure 1000 mL of bacterial suspension by sterile deionized water. The salt-tolerant microbial inoculum was used immediately after preparation.

2.2. The design of CW microcosms

A total of eighteen CW microcosms were established in the greenhouse at Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences located in Changchun, China. The mean temperature and relative humidity of the greenhouse were 23–28 °C and 64–86%, respectively.

Each CW microcosm was a column made of nontransparent Polyethylene material (6 mm in thickness) with a dimension of 18 cm in diameter and 40 cm in height (Fig. 1). The outlet pipes were located at a height of 2, 15, and 30 cm from the bottom, respectively. The substrate of each CW microcosm was divided into two layers, i.e., a fine gravel (30 cm in thickness) layer at the bottom and a soil (7 cm in thickness) layer on the top. Fine gravel (diameter of 5–10 mm) was purchased and washed continuously with tap water to avoid the influence of impurities adsorbed on their surface. The soil was collected from the topsoil of a natural wetland with a sterile spatula to support the growth of emergent plant. The *Canna indica* (*C. indica*), a widely used plant species in CWs, was selected as a wetland plant species for the CW microcosms because it can grow under both slight-saline and non-saline conditions, enabling the CWs to treat saline wastewater with a wide range of salinity levels. The seedlings of *C. indica* with similar size (~35 cm in height and fresh weight of 35.71 ± 3.23 g/plant) were transplanted into the CW microcosms (one plant per CW microcosm) in June 2019. Before transplanting, the entire plants were meticulously washed with deionized water until the soil residue on their surface was completely removed. The CW microcosms were then supplied with 1/8 Hoagland's solution until the formal experiment started in 60 days after the transplantation. During the 60 days of acclimatization, the microorganisms in CW microcosms adapted to the wetland environment, and a macroscopic biomembrane appeared on the surface of substrate.

2.3. Experimental design and operation of the CW microcosms

Synthetic wastewater was used in this study, and prepared as follows: NH_4Cl (37.5 mg/L), $Ca(NO_3)_2$ (29.0 mg/L), KH_2PO_4 (13.0 mg/L) and CH_3COONa (13.0 mg/L), respectively, and the pH was 8. The total nitrogen (TN) concentration of synthetic wastewater was 16.59 ± 0.49 mg/L, including 9.52 ± 1.15 mg/L of ammonia nitrogen (NH_4^+-N) and 6.25 ± 0.14 mg/L of nitrate nitrogen (NO_3^--N), which was similar to the reported characteristics of the real mariculture wastewater in China (Li et al., 2017). Three salinity levels, i.e., electrical conductivity (EC) of ~0.51, 15 and 30 mS/cm, equivalent to 0%, 0.75% and 1.75% of NaCl concentration at 25 °C, respectively, were designed and prepared by adding a designated amount of NaCl into the described synthetic wastewater based on our previous study (Liang et al., 2017a). The designed salinity levels were ~0%, ~14% and ~50% of the salinity levels in seawater, respectively (Song et al., 2020). Especially, the EC of 15 and 30 mS/cm treatments could represent the salinity levels of most mariculture wastewaters as reported in previous studies (Van Den Hende et al., 2014; Zhao et al., 2010). The wastewater was prepared conveniently and treated safely in the laboratory.

The denitrification capacity of strain *Alishewanella* sp. F2 was tested under saline conditions in a pre-experiment with 500 mL Bunsen beakers. Results suggested that strain *Alishewanella* sp. F2 was efficient in denitrification under saline condition (See Text S1 and Text S2 in supplemental materials). In the formal experiment, eighteen CW microcosms were separated into six treatments with three replicates for each treatment. Three of the treatments (referred to as 0-N, 15-N, and 30-N) were unbioaugmented CW (un-Bio-CW) microcosms without inoculation of any exogenous microorganisms operating at salinity levels of EC at ~0.51, 15 and 30 mS/cm, respectively. The other three treatments (referred to as 0-F2, 15-F2, and 30-F2) were Bio-CW microcosms inoculated with the salt-tolerant microbial inoculum prepared by strain *Alishewanella* sp. F2 operating at salinity levels of EC at ~0.51, 15 and 30 mS/cm, respectively. A sequencing fill and draw batch mode was used during the operation, with 5 d for each Trial. A total of six trials were conducted. In each trial, the salt-tolerant microbial inoculums and the synthetic wastewater were evenly mixed before being supplied to the Bio-CW microcosms with an inoculation size of 5% (v/v). It was verified in our previous study that strain *Alishewanella* sp. F2 exhibited the highest denitrification efficiency when the inoculation size was 5% (data not presented). Influent water was supplied vertically (down from the top to the bottom) into each CW microcosm to a depth of 37 cm. A 100 mL water sample was collected daily from the bottom drainage outlet of each CW microcosm. By the end of the last trial, top fine gravel samples at the same height (30 cm in height) were collected from each CW microcosm with 50 mL sterilized centrifuge tubes. The plant (both above and below ground biomass) was harvested in each CW microcosm for the analysis of total biomass and TN concentrations.

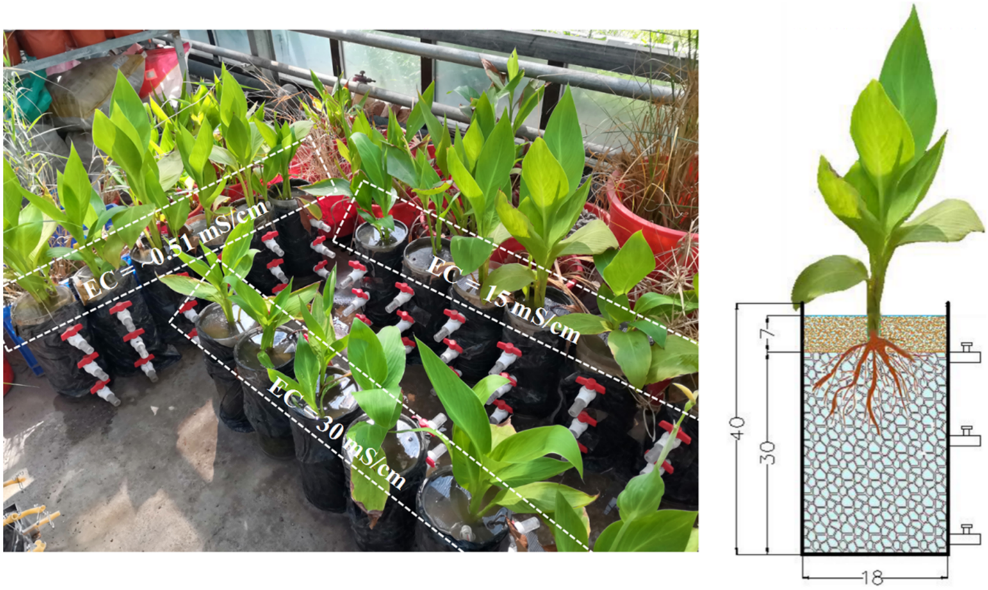

Fig. 1. A photo and schematic diagram of CW microcosms (Unit: centimeter). EC: electrical conductivity.

2.4. *Samples analysis*

The EC, pH and the nitrogen (i.e., NH_4^+-N, nitrite nitrogen (NO_2^--N), NO_3^--N and TN) concentrations of water samples and the TN concentrations in leaves, stems and roots of plants were measured based on the methods described in previous studies (Liang et al., 2017a). The fine gravel samples were sent to Sangon Biotech Co., Ltd. (China) to quantify the polymerase chain reaction and analyze the microbial communities by high-throughput sequencing techniques using the Silva database (*http://www.arb-silva.de/*).

2.5. *Statistical analysis*

Statistical analysis of all experimental data was performed by SPSS statistics 22.0 software for Windows system. All graphic designs were performed by Origin 9.1 (USA) software for Windows system. The data in all figures were presented as means ± standard deviation. Means between different treatments were compared by one-way ANOVA with *Tukey*'s test at a significance level of 0.05.

3. Results

3.1. *Denitrification efficiency of the Bio-CW microcosms*

The NH_4^+-N removal efficiency was significantly ($p < 0.05$) suppressed by high salinity (i.e., EC of 15 and 30 mS/cm) in un-Bio-CW microcosms (Fig. 2a). The NH_4^+-N was effectively removed (96.4%) on Day 5 under a non-saline condition (i.e., EC of ~0.51 mS/cm). However, when the EC was increased to 15 and 30 mS/cm, the NH_4^+-N removal efficiencies were significantly ($p < 0.05$) reduced compared with EC of ~0.51 mS/cm treatment, with the removal percentages of 68.5% and 51.8% for EC of 15 and 30 mS/cm treatments, respectively. After the addition of the salt-tolerant microbial inoculum (i.e., strain *Alishewanella* sp. F2), the NH_4^+-N removal efficiency was not significantly improved at EC of ~0.51 and 30 mS/cm treatments. However, for EC of 15 mS/cm treatment, the NH_4^+-N removal percentage on Day 5 was 95.7% in Bio-CW microcosms, which was 27.2% significantly ($p < 0.05$) higher than that in un-Bio-CW microcosms (68.5%).

The NO_2^--N was not prepared in the synthetic wastewater in this study, but the NO_2^--N, which was helpful for revealing the important process of nitrogen removal in CWs, was quickly generated as an intermediate product of nitrification in CW microcosms. The peak values of NO_2^--N concentration (0.19–1.49 mg/L) were observed on Day 1 for all the treatments (Fig. 2b). Thereafter, the NO_2^--N concentration gradually decreased with the increase of operation time. High salinity led to excessive accumulation of NO_2^--N on Day 1 and the NO_2^--N concentration in both un-Bio-CW and Bio-CW microcosms at EC of 30 mS/cm treatment was significantly ($p < 0.05$) higher than that at EC of ~0.51 mS/cm treatment. After the inoculation of strain *Alishewanella* sp. F2, there was no significant difference in the effluent NO_2^--N concentration between un-Bio-CW and Bio-CW microcosms.

The removal percentages of NO_3^--N in all CW microcosms on Day 5 were in the range of 93.7–96.6%, and there was no significant difference in the NO_3^--N removal percentages between Bio-CW and un-Bio-CW microcosms (Fig. 2c). However, after the addition of the salt-tolerant microbial inoculum, the removal efficiency of NO_3^--N was improved in CW microcosms with EC of 15 and 30 mS/cm treatments on Day 1. When the EC was 15 mS/cm, the NO_3^--N removal percentage (80.1%) was 17.3% higher in Bio-CW microcosms on Day 1 than 62.8% in un-Bio-CW microcosms.

The removal efficiency of TN in un-Bio-CW microcosms was inhibited by salt stress (i.e., EC of 15 and 30 mS/cm) similar to NH_4^+-N removal. At a non-saline condition (i.e., EC of ~0.51 mS/cm), the removal percentage of TN in un-Bio-CW microcosms on Day 5 was 99.0%. However, when the EC increased to 15 and 30 mS/cm, the TN removal percentages decreased to 76.4% and 64.7%, which were 22.6% and 34.3% lower than that at EC of ~0.51 mS/cm treatment, respectively. After the addition of the salt-tolerant microbial inoculum, the TN removal efficiency was improved in CW microcosms for EC of 15 and 30 mS/cm treatments (Fig. 2d). When the EC was 15 mS/cm, the TN removal percentage in Bio-CW microcosms on Day 5 was significantly ($p < 0.05$) increased compared with the un-Bio-CW microcosms. The TN removal percentage in Bio-CW microcosms on Day 5 was 99.4%, which was 23.0% higher than 76.4% in un-Bio-CW microcosms. At an EC of 30 mS/cm treatment, although not statistically significant, the TN removal percentage increased from 64.7% in un-Bio-CW microcosms to 70.2% in Bio-CW microcosms on Day 5. Furthermore, as an important indicator to characterize the salinity of wastewater, the effluent EC values in Bio-CW microcosms under saline conditions (i.e., EC of 15 and 30 mS/cm) were lower than those in un-Bio-CW microcosms. The effluent EC reduction percentages in Bio-CW microcosms increased by 5.5% and 1.1% compared with the un-Bio-CW microcosms for EC of 15 and 30 mS/cm treatments, respectively (Fig. 3).

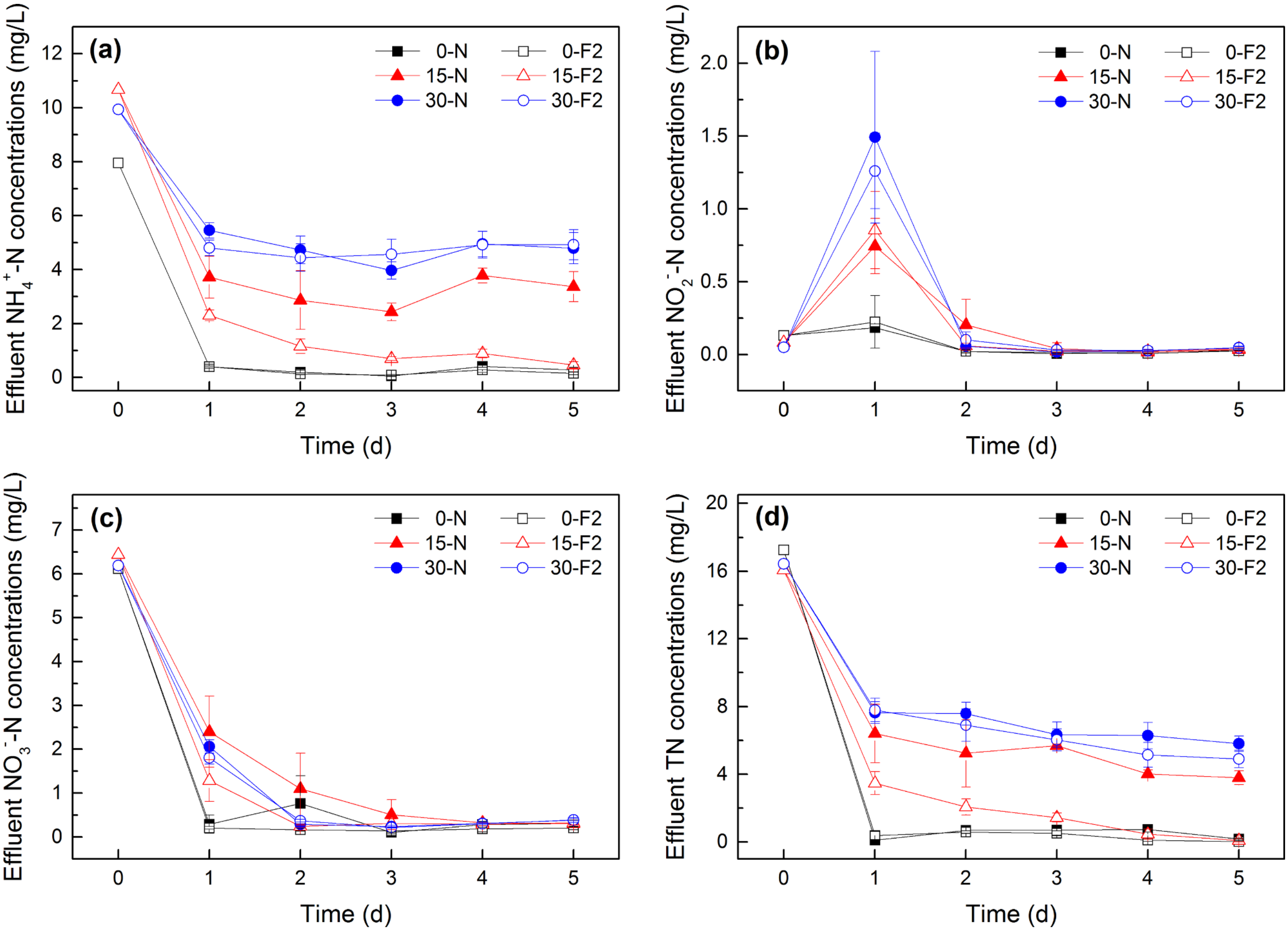

Fig. 2. Removal efficiency of nitrogen (a: NH_4^+-N; b: NO_2^--N; c: NO_3^--N; d: TN) in CW microcosms at different salinity levels. 0-N, 15-N and 30-N: un-Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively; 0-F2, 15-F2 and 30-F2: Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively. Values represent the mean of three replicates and error bars represent the standard deviation.

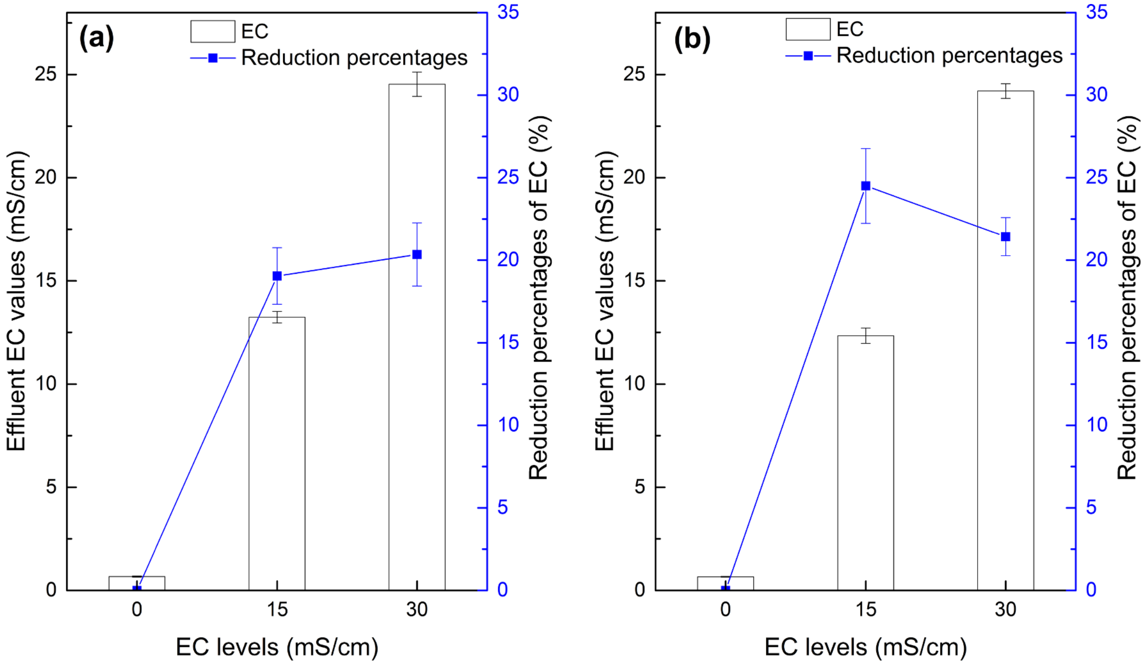

Fig. 3. Effluent EC values of CW microcosms (a: the un-Bio-CW microcosms; b: the Bio-CW microcosms) at different salinity levels. EC: electrical conductivity. Values represent the mean of three replicates and error bars represent the standard deviation.

Table 1
Continuous operation efficiency of CW microcosms for TN removal at different salinity levels on Day 5 (mean ± SD).

Influent salinity	Sample ID	The TN removal percentage (%)						
		Trial 1 (n = 3)	Trial 2 (n = 3)	Trial 3 (n = 3)	Trial 4 (n = 3)	Trial 5 (n = 3)	Trial 6 (n = 3)	Average value (n = 18)
EC = ~0.51 mS/cm	0-N	99.0 ± 0.3a	90.7 ± 2.9a	95.5 ± 0.9a	95.7 ± 0.8a	83.8 ± 1.3a	82.4 ± 2.5a	91.2 ± 6.5a
	0-F2	99.8 ± 0.1a	92.0 ± 0.7a	96.1 ± 0.9a	96.1 ± 0.4a	88.4 ± 2.3a	88.0 ± 2.7a	93.4 ± 4.6a
EC = 15 mS/cm	15-N	76.4 ± 2.5b	88.3 ± 1.1a	94.9 ± 1.2a	93.3 ± 0.4a	58.5 ± 1.2b	32.8 ± 8.9c	74.0 ± 22.5b
	15-F2	99.4 ± 0.6a	91.9 ± 0.6a	95.8 ± 1.3a	96.6 ± 0.6a	85.8 ± 10.4a	57.7 ± 0.3b	87.9 ± 14.8ab
EC = 30 mS/cm	30-N	64.7 ± 2.7c	53.2 ± 12.3b	66.1 ± 0.8b	52.2 ± 5.9b	42.6 ± 4.0b	23.4 ± 3.5c	50.4 ± 15.7c
	30-F2	70.2 ± 3.1bc	74.6 ± 3.3a	70.9 ± 8.7b	59.4 ± 1.3b	51.0 ± 3.9b	26.9 ± 0.3c	58.8 ± 16.9c

Note: 0-N, 15-N and 30-N: un-Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively; 0-F2, 15-F2 and 30-F2: Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively. Columns containing different letters indicate significant differences among treatments at $p = 0.05$.

To further verify the nitrogen removal efficiency of Bio-CW microcosms, another five trials were continuously conducted after the completion of trial 1. TN is a sum for NH_4^+-N, NO_2^--N, NO_3^--N, and organic nitrogen (organic-N), and is the main indicator of eutrophication in water (Liu et al., 2020). Therefore, the TN removal percentages in CW microcosms operated for six trials are mainly described in this study, while, the NH_4^+-N and NO_3^--N removal percentages of the six trials are described in Text S3, Tables S1 and S2 of the supplemental materials. As presented in Table 1, the mean removal percentages of TN on Day 5 were improved in CW microcosms after the addition of the salt-tolerant microbial inoculum. The mean removal percentages of TN in Bio-CW microcosms operated for six trials at EC of 15 and 30 mS/cm were 87.9% and 58.8%, which were 13.9% and 8.4% higher than 74.0% and 50.4% in un-Bio-CW microcosms, respectively. Among the six trials, the TN removal percentages were significantly ($p < 0.05$) increased at 99.4%, 85.8% and 57.7% in Bio-CW microcosms in Trial 1, 5 and 6, respectively, at EC of 15 mS/cm compared with the un-Bio-CW microcosms (76.4%, 58.5% and 32.8%, respectively). Although not significantly different, the TN removal percentages were all insignificantly higher on Day 5 in Bio-CW microcosms in Trial 2, 3 and 4 compared to those in un-Bio-CW microcosms.

3.2. Nitrogen absorption capacity by plants in Bio-CW microcosms

As shown in Fig. 4a, the growth of plants in un-Bio-CW microcosms was significantly ($p < 0.05$) inhibited by EC of 30 mS/cm treatment compared with EC of ~0.51 mS/cm. The addition of the salt-tolerant microbial inoculum into CW microcosms exhibited no influence on plant growth compared with the un-Bio-CW microcosms for EC of ~0.51 mS/cm treatment. However, the dry weights of plants were 31.13 and 12.09 g dry weight/microcosm in Bio-CW microcosms for EC of 15 and 30 mS/cm treatments, which were 9.35 and 2.97 g dry weight/microcosm higher than those in un-Bio-CW microcosms (21.78 and 9.12 g dry weight/microcosm), respectively. The TN concentration of plant tissues (i.e., leaves, stem and root of *C. indica*) was affected by salinity levels but not the salt-tolerant microbial inoculums (Fig. 4b). For treatments with EC of ~0.51 and 15 mS/cm, the tissue TN concentrations of *C. indica* were leaves > stem > root in both Bio-CW and un-Bio-CW microcosms. However, the tissue TN concentrations of *C. indica* were root > stem > leaves in both Bio-CW and un-Bio-CW microcosms for EC of 30 mS/cm treatment. At EC of 30 mS/cm treatment, the TN concentration of leaves was significantly ($p < 0.05$) decreased in Bio-CW and un-Bio-CW microcosms compared with EC of ~0.51 mS/cm treatment, although there was no statistical significance in TN concentration of stems and roots among different salinity levels. Although only a slight influence of salt stress was observed on the unit mass concentration of nitrogen in plant, due to the boost of plant growth in Bio-CW microcosms, the consequent TN content absorbed by the entire plant was increased (Fig. 4c). The TN content absorbed by the entire plant in Bio-CW microcosms with EC of 15 and 30 mS/cm were 401.18 and 165.08 mg dry weight/microcosm, which were 61.61 and 44.17 mg dry weight/microcosm higher than 339.57 and 120.91 mg dry weight/microcosm in un-Bio-CW microcosms, respectively.

3.3. Characteristics of microbial community structure in Bio-CW microcosms

The top substrate samples near the rhizosphere of plants were collected and analyzed for microbial community structure. The operational taxonomic units (OTUs), Chao 1 richness estimator, Shannon-wiener diversity index, Simpson diversity index and Coverage indices were selected for assessing the microbial diversity and community richness in top substrate of each respective CW microcosm (Table 2). The mean number of OTUs for all treatments ranged from 4599 to 5811, with the highest mean number of OTUs recorded in Bio-CW microcosms at EC of ~0.51 mS/cm treatment. The lowest and highest mean Chao 1 richness estimators were observed at EC of 30 and 15 mS/cm treatments in Bio-CW microcosms, with the values of 7573.76 and 9430.10, respectively. Both the Shannon-wiener diversity index and Simpson diversity index were changed in Bio-CW microcosms compared with those in un-Bio-CW microcosms, suggesting the different microbial diversity between Bio-CW and un-Bio-CW microcosms. To be specific, after the addition of the salt-tolerant microbial inoculums, the Shannon-wiener diversity index decreased and the Simpson diversity index was increased in CW microcosms for EC of ~0.51 and 30 mS/cm treatments, while both indexes exhibited an opposite trend those at EC of 15 mS/cm treatment. The mean Coverage indices were higher for all samples in all CW microcosms than 94.0%, indicating a good coverage of the sample library.

The relative abundances of microbial community at phylum, class and genus levels in top substrate samples of each respective CW microcosm are presented in Fig. 5. As shown in Fig. 5a, the top 5 dominant phyla in all CW microcosms were the same, which were *Proteobacteria*, *Chloroflexi*, *unclassified-Bacteria*, *Bacteroidetes* and *Acidobacteria*, respectively. With the increasing influent salinity levels in both un-Bio-CW and Bio-CW microcosms, the frequencies of *Proteobacteria*, *Chloroflexi* and *Bacteroidetes* increased, however, the frequencies of *unclassified-Bacteria* and *Acidobacteria* decreased. In this study, the phylum of the salt-tolerant microbial inoculum (i.e., strain *Alishewanella* sp. F2) was *Proteobacteria*, and the frequency of *Proteobacteria* was the highest in all CW microcosms. The phylum *Proteobacteria* was more abundant in Bio-CW microcosms than that in un-Bio-CW microcosms for EC of ~0.51 and 30 mS/cm treatments, but there was no statistical significance. The species of microbial community at class level were basically the same among all CW microcosms but different frequencies of microbial community at class level were observed (Fig. 5b). In particular, the frequency of class *Actinobacteria* was significantly ($p < 0.05$) decreased in un-Bio-CW microcosms at increasing salinity levels (i.e., EC of 15 and 30 mS/cm). In Bio-CW microcosms, the frequency of *Actinobacteria* was increased under all salinity treatments compared with the un-Bio-CW microcosms, therein, the class *Actinobacteria* showed a significantly ($p < 0.05$) higher frequency in Bio-CW

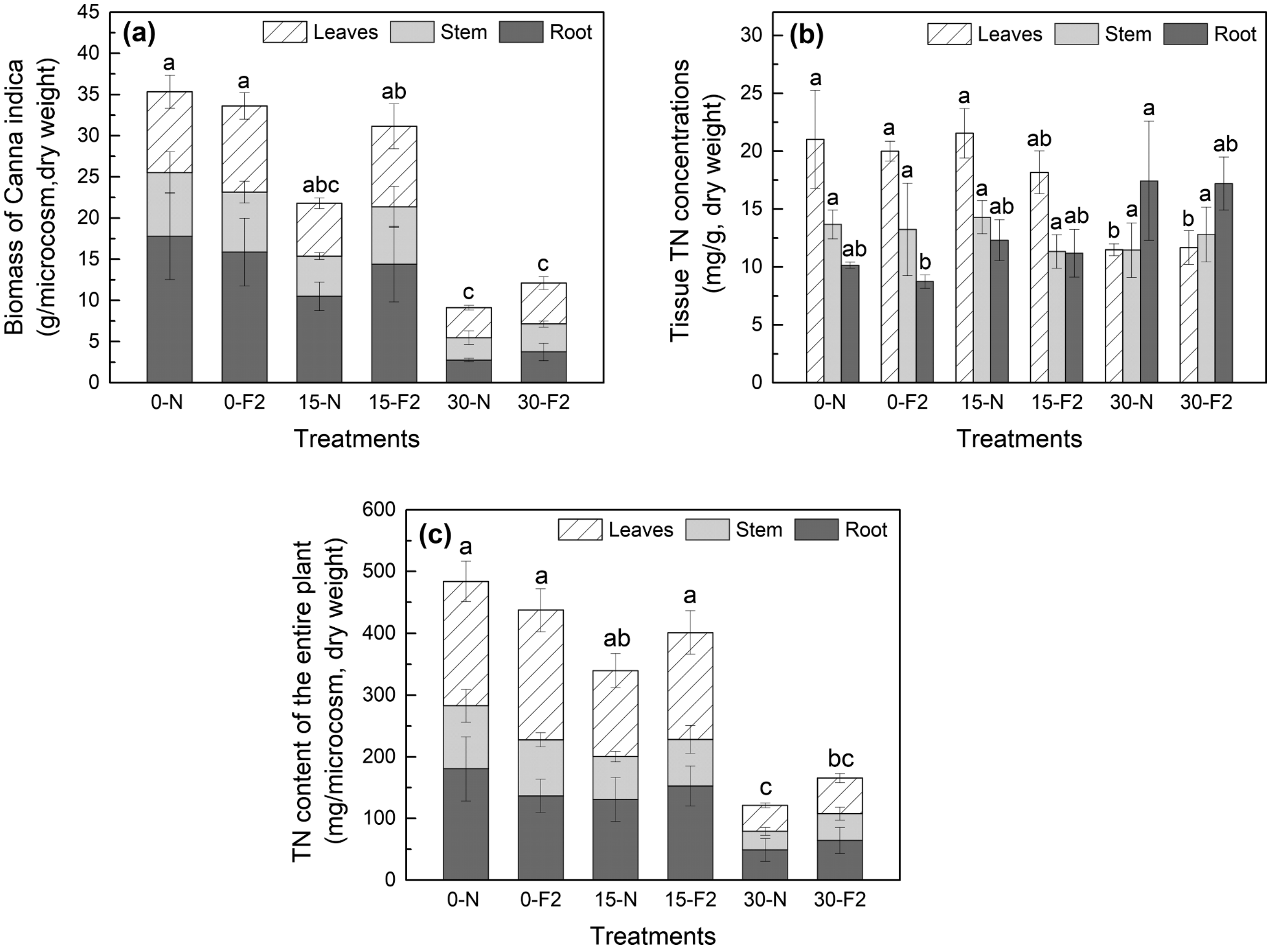

Fig. 4. Nitrogen absorption capacity by plants in CW microcosms at different salinity levels. a: the biomass of *C. indica*; b: tissue TN concentrations in *C. indica*; c: TN content absorbed by the entire plant. 0-N, 15-N and 30-N: un-Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively; 0-F2, 15-F2 and 30-F2: Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively. Values represent the mean of three replicates and error bars represent the standard deviation. Columns containing different letters indicate significant differences among treatments at $p = 0.05$.

microcosms than in un-Bio-CW microcosms at EC of 15 mS/cm treatment. The microbial genera detected in all CW microcosms are shown in Fig. 5c. All CW microcosms had the same species of microbial community at genus level, but the frequency of each species in six treatments was different. Under saline conditions (i.e., EC of 15 and 30 mS/cm), the frequencies of *Thioclava*, *Pseudomonas* and *Anaerolinea* in CW microcosms were increased compared with treatment at an EC of ~0.51 mS/cm. However, the frequency of *Arthrobacter* exhibited an opposite trend and was lower than that under a non-saline condition (i.e., EC of ~0.51 mS/cm). At EC of 15 and 30 mS/cm treatments, the genera *Thioclava* and *Arthrobacter* in Bio-CW microcosms were more abundant than those in un-Bio-CW microcosms. Although the added salt-tolerant bacterium (i.e., strain *Alishewanella* sp. F2) did not reach the frequencies of top 20 genera, it was detected in all Bio-CW microcosms and its frequency was included in the "other" section in Fig. 5c.

4. Discussion

4.1. Inhibition of salinity on nitrogen removal capacity of CWs

In this study, the inhibitive effects of salt stress on NH_4^+-N, NO_3^--N and TN removal were observed in CW microcosms (Fig. 2), which was consistent with the results of previous studies (Fu et al., 2019a; Liang et al., 2017a). The species, abundance and metabolism of

Table 2
Summary statistics of microbial community diversity indices in CW microcosms.

Influent salinity	Sample ID	OTUs	Chao 1	Shannon	Simpson	Coverage
EC = ~0.51 mS/cm	0-N	5278	8463.68	7.00	0.0061	0.940
	0-F2	5602	8920.90	6.71	0.0141	0.946
EC = 15 mS/cm	15-N	5185	8346.45	6.69	0.0074	0.954
	15-F2	5811	9430.10	6.98	0.0067	0.945
EC = 30 mS/cm	30-N	5074	8171.22	6.89	0.0045	0.954
	30-F2	4599	7573.76	6.41	0.0096	0.957

Note: OTUs: the operational taxonomic units. 0-N, 15-N and 30-N: un-Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively; 0-F2, 15-F2 and 30-F2: Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively. Values represent the mean of three replicates.

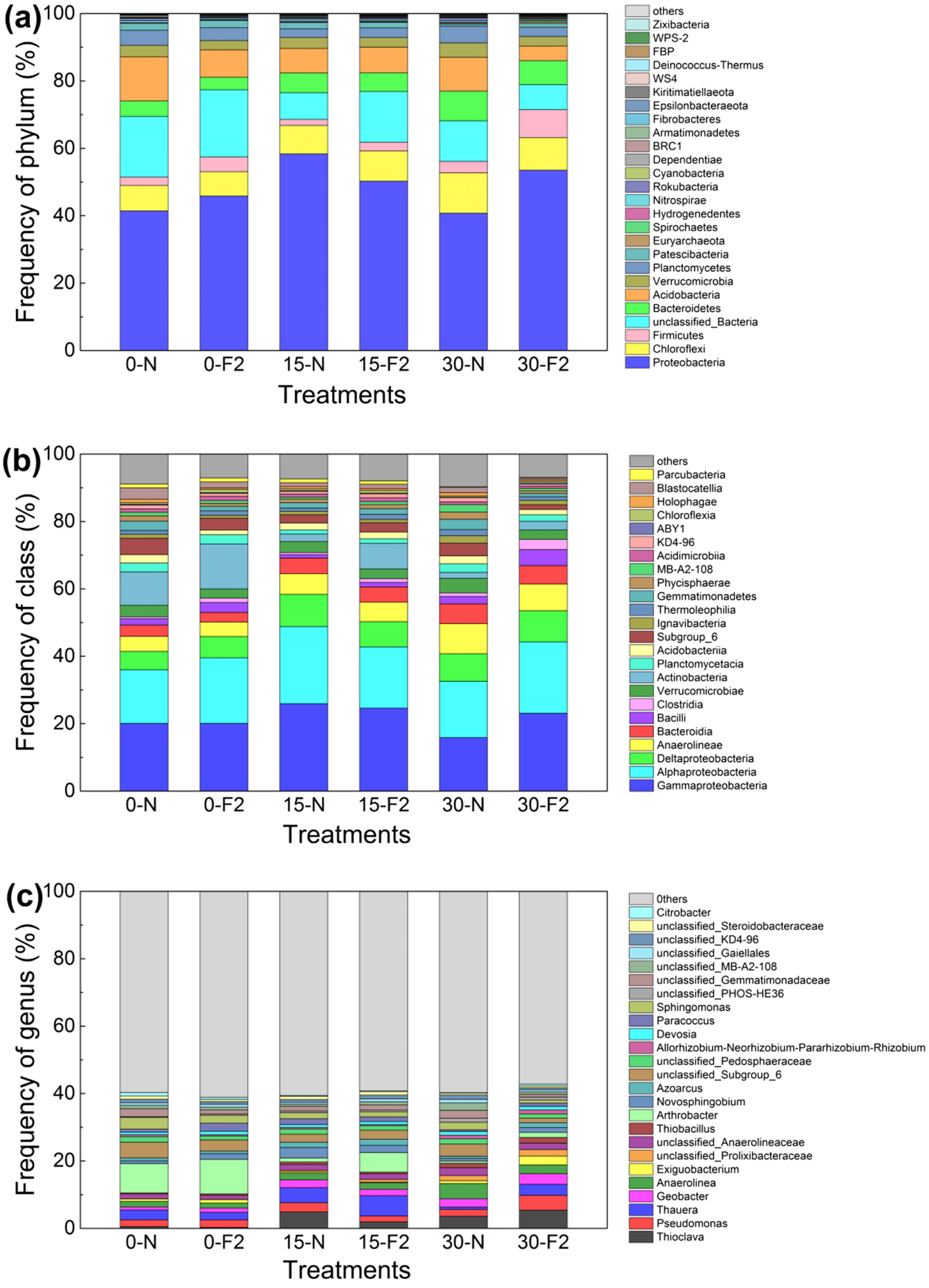

Fig. 5. Taxonomic classifications of the bacterial communities in all CW microcosms with different salinity treatments at phylum (a), class (b) and genus (c) levels. 0-N, 15-N and 30-N: un-Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively; 0-F2, 15-F2 and 30-F2: Bio-CW microcosms with EC of ~0.51, 15 and 30 mS/cm, respectively. Values represent the mean of three replicates.

microorganisms have an impact on the degradation process of nitrogen in CWs (Liu et al., 2019a). Salt stress can cause high osmotic pressure, which has a toxic effect on the microorganisms, causes cell dehydration and deactivation, then reduces the survival rate of microorganisms and the microbial enzyme activity, and the denitrification capacity is consequently debased (He et al., 2017; Wang et al., 2015). According to the indices of OTUs and Chao 1 richness estimator observed in this study (Table 2), the number of microbial species in CW microcosms decreased with increasing influent salinity levels, demonstrating that high salinity led to a negative impact on the microbial species richness in CWs. This observation partly explained the inhibitive effect of salt stress on nitrogen removal in this study. Additionally, the accumulation of NO_2^--N on Day 1 also increased with increasing salinity in CW microcosms (Fig. 2b). Excessive NO_2^--N indicates that the existence of inhibitory factors (i.e., salinity in this study) leads to an inefficient denitrification in CWs. Denitrifying bacteria are sensitive to salinity, while ammonia oxidizing bacteria are less affected by salinity. Therefore, only short-cut nitrification can be achieved in CWs under salt stress, which causes a large accumulation of NO_2^--N (Aslan and Simsek, 2012; Hong et al., 2013).

Besides the negative impact on microorganisms, the inhibition of salt stress on plants also explains the lower nitrogen removal efficiency in CWs. A series of physiological changes in plants can occur in a saline environment (Wang et al., 2019a). Due to salt stress in plants, including ion toxicity, osmotic imbalance and nutritional energy imbalance, etc., the growth of many plants can be inhibited in saline environments (Liang et al., 2018b). With the increase of influent salinity levels, the osmotic stress of plants was increased, resulting in the decrease of plant leaf area and stomata density, the decrease of transpiration and other morphological and physiological characteristics (Rao et al., 2007).

Consequently, the activity of rhizosphere microbes was affected and the denitrification capacity of CWs was decreased. Although these indicators were not tested in this study, the decreased biomass of plants under salt stress observed in this study reflected the inhibitive effect of salt stress on wetland plants (Fig. 4a). The toxicity of Na^+ ions to plants gradually increased with exposure time until it reached the most severe status (Cheng et al., 2018). Due to the toxicity of Na^+ ions and the consequent lower biomass of plants, the TN content absorbed by the entire plant consequently decreased in CW microcosms, resulting in the unsatisfactory nitrogen removal capacity under salt stress in CW microcosms (Fig. 4c). The substrate adsorption might have been gradually close to saturation after the operation of a few trials, and substrate adsorption function in CWs is limited (Zhu et al., 2011). In addition, with the increase of trials, the activity and quantity of some microorganisms with nitrification and denitrification capacity gradually decreased and were finally eliminated under salt stress. Therefore, the TN removal capacities of CW microcosms in Trial 6 under salt stress (i.e., EC of 15 and 30 mS/cm) were sharply decreased in this study (Table 1).

Although the nitrogen removal capacity of CWs was affected by high salinity, some nitrogen in the synthetic wastewater was still removed in CW microcosms at EC of 15 and 30 mS/cm treatments (Fig. 2a, b and c). Based on the microbial community structure data observed in CW microcosms, some bacteria with salt-tolerance and denitrification capacity can survive under saline conditions (i.e., EC of 15 and 30 mS/cm), and the frequencies of these bacteria were higher than under a non-saline condition (i.e., EC of ~0.51 mS/cm) (Fig. 5). For example, the phylum *Bacteroidetes* is salt-tolerant and commonly found in CWs (Guo et al., 2015). The classes *Gemmatimonadetes* and *Bacteroidia*, which were generally observed in saline environments, have demonstrated halophilic characteristics (Sivasankar et al., 2019). In this study, the frequency of *Bacteroidia* was higher at EC of 15 and 30 mS/cm treatments than that at EC of ~0.51 mS/cm, and the frequency of *Gemmatimonadetes* was the highest in all CW microcosms (Fig. 5b). The nitrogen removal efficiency of both Bio-CW and un-Bio-CW microcosms were highly sensitive to salinity (Fig. 2a, c and d). The increasing salinity inhibited the nitrogen removal by limiting the nitrification process, rather than the denitrification process in both Bio-CW and un-Bio-CW microcosms (Fig. 2a and c). During the nitrification process, there is a threshold value for the salt-tolerance of different microorganisms in CWs. In this study, some aboriginal microorganisms could not survive in CWs when the salinity increased to 30 mS/cm. The surviving salt-tolerant microorganisms that maintained the nitrogen removal efficiency were domesticated in a saline environment, while other microorganisms with low salt-tolerance were gradually eliminated in CWs (Wang et al., 2019a). In conclusion, although the nitrogen removal efficiency of CWs was inhibited under salt stress, some nitrogen was still removed by the denitrification of those salt-tolerant microorganisms that survived in CWs.

4.2. Intensification effectiveness of the Bio-CWs and its mechanisms

Although the added strain *Alishewanella* sp. F2 did not become one of the dominant bacteria in the Bio-CW microcosms, the denitrification capacity of the CW microcosms based on strain *Alishewanella* sp. F2 was effectively improved in salt-stressed environments. As discussed above, the decrease of nitrogen removal efficiency of CWs by high salinity is mainly due to the inhibition of microorganisms and plant functions. Therefore, we speculate that the promising effectiveness of the Bio-CWs is related to the enhancement of microorganisms and/or plant functions by the addition of strain *Alishewanella* sp. F2.

Microbial nitrification and denitrification are considered the dominant nitrogen removal mechanisms in CWs (Yu et al., 2019). In this study, a rich diversity of nitrifying and denitrifying bacteria that have nitrogen removal capacity in wastewater treatment was observed in all CW microcosms. For example, *Thauera* is an important genus for denitrification in wastewater treatment (Iorhemen et al., 2019). The genus *Pseudomonas* is a typical heterotrophic nitrifying bacterium which exhibited an efficient heterotrophic nitrification-aerobic denitrification capacity (Yang et al., 2019). The growth substrate of *Pseudomonas* can be inorganic nitrogen (inorganic-N) or organic-N. *Pseudomonas* can ammonify organic-N to NH_4^+-N and then convert NH_4^+-N to NO_2^--N, or even directly convert organic-N to NO_3^--N (Richardson et al., 1998). Moreover, the genus *Pseudomonas* is reported to be salt-tolerant and can survive in a saline environment (Castillo-Carvajal et al., 2014). The frequencies of both *Thauera* and *Pseudomonas* in Bio-CW microcosms for EC of 30 mS/cm treatment increased compared with the un-Bio-CW microcosms (Fig. 5c), indicating that the inoculation of strain *Alishewanella* sp. F2 boosted the abundance of *Thauera* and *Pseudomonas*. Consequently, besides the denitrification function of strain *Alishewanella* sp. F2, the contribution of both *Pseudomonas* and *Thauera* promoted by *Alishewanella* sp. F2 also explained the increasing denitrification capacity of the Bio-CW microcosms.

As we have hypothesized in the introduction section, the inoculation of an exogenous strain into a system might boost the abundance of some aboriginal functional microorganisms that even contain bacteria that are beneficial to the growth of plants (Compant et al., 2010). The recorded data of plant biomass and microbial community structure obtained in this study have proved our hypothesis (Figs. 4 and 5). The genera of both *Pseudomonas* and *Arthrobacter* were reported to have the ability to promote plant growth (Banaei-Asl et al., 2015; Wang et al., 2019b). The abundance of *Arthrobacter* in Bio-CW microcosms was significantly ($p < 0.05$) increased at EC of 15 mS/cm treatment (Fig. 5). Additionally, the phylum *Proteobacteria* contains many species of bacteria at genus level that can also promote plant growth (Chao et al., 2016). The abundance of all these bacteria was boosted after the inoculation of strain *Alishewanella* sp. F2 at EC of 30 mS/cm treatment (Fig. 5). Therefore, the plant growth in Bio-CW microcosms was better than that in un-Bio-CW microcosms and the TN contents absorbed by the entire plant in Bio-CW microcosms were consequently 18.1% and 36.5% higher than those in un-Bio-CW microcosms at EC of 15 and 30 mS/cm, respectively (Fig. 4c). This boosted plant function can also partly explain the promotive effectiveness of denitrification capacity in Bio-CW microcosms. It is noteworthy that the boosted microorganisms and plant functions were not independent of each other, and there is an internal relationship and interaction between the promotion of plant growth and functional microbial abundance (Qin et al., 2016). A better plant growth provides more favorable rhizospheric environments for microorganisms (Zhuang et al., 2019), meanwhile, the promotive functional microbial abundance can sometimes in turn be beneficial for plant growth, which was observed in this study.

4.3. Future prospect of Bio-CWs for denitrification in a saline environment

Bioaugmentation technology makes full use of bacteria that have been screened for special tracts from a natural environment and utilize them for the restoration of degraded environment (Herrero and Stuckey, 2015). The addition of exogenous microbial inoculum can be regarded as a method for fast start-up of CWs (Zhao et al., 2019). The addition of an exogenous microbial inoculum can also be beneficial for maintaining the microbial activity of CWs for long-term operation. Importantly, bioaugmentation is an effective technology to overcome the severe/extreme environmental stress (e.g., low-temperature and high-salt environment) of CWs and improve the purification efficiency for CWs, which has been fully confirmed by many studies, including this study (Ying et al., 2010; Zhao et al., 2016). However, despite the many advantages of bioaugmentation technology, it is still necessary to improve its water research and real application. For example, a series of complex relationships and mechanisms exist between plant growth and functional microorganisms in CWs after the inoculation of exogenous microbial inoculum (i.e., strain *Alishewanella* sp. F2 in this study). In this study, although the low abundance of strain *Alishewanella* sp. F2 improved the denitrification capacity of CWs under salt stress, the salt-tolerant microbial inoculum was added to CWs at the beginning of each trial. Therefore, the development of strategies for improving the durability of denitrification capacity in Bio-CWs is a point of concern. Additionally, the ecological risk caused by the introduction of exogenous microorganisms into ecosystems is also a noticeable problem and the biosafety of adding exogenous microorganisms into an ecosystem is a wide concern of all sectors of society (Keswani et al., 2019). In these regards, the following future studies are recommended: (1) investigate the internal mechanism of the promotive effectiveness of strain *Alishewanella* sp. F2 on plant growth in CWs under salt stress; (2) optimize strategies for adding strain *Alishewanella* sp. F2 or inoculate with multiple salt-tolerant microorganisms to improve the durability of Bio-CWs for treating saline wastewater; and (3) analyze the potential toxicity of exogenous salt-tolerant microorganisms to the ecological security of Bio-CWs.

5. Conclusions

This study demonstrated that the addition of a salt-tolerant microbial inoculum (i.e., strain *Alishewanella* sp. F2) can effectively improve the nitrogen removal capacity of CWs under saline conditions. The NH_4^+-N and TN removal percentages of 95.7% and 99.4% in Bio-CW microcosms were obtained, respectively, when EC was 15 mS/cm. The salt-tolerant microbial inoculum not only possesses denitrification capacity itself, but also can survive and boost the function of both microorganisms and plants in CWs. After the addition of the salt-tolerant microbial inoculum, the microbial community structure changed and the abundance of some aboriginal salt-tolerant microorganisms increased in CWs. In Bio-CWs, the inhibition of plant growth by salinity was alleviated and the TN content absorbed by the entire plant increased. This study provides both a new strategy for enhancing nitrogen removal from saline wastewater and a new understanding on the interaction between exogenous and aboriginal microorganisms in CWs.

CRediT authorship contribution statement

Xinyi Wang: Methodology, Investigation, Writing - original draft. **Hui Zhu:** Resources, Writing - review & editing, Project administration, Supervision, Funding acquisition. **Baixing Yan:** Resources. **Brian Shutes:** Writing - review & editing. **Gary Bañuelos:** Writing - review & editing. **Huiyang Wen:** Investigation.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105628.

References

Adrados, B., Sanchez, O., Arias, C.A., Becares, E., Garrido, L., Mas, J., Brix, H., Morato, J., 2014. Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. Water Res. 55, 304–312. https://doi.org/10.1016/j.watres.2014.02.011.

Ahmadi, M., Jorfi, S., Kujlu, R., Ghafari, S., Soltani, R.D.C., Haghighifard, N.J., 2017. A novel salt-tolerant bacterial consortium for biodegradation of saline and recalcitrant petrochemical wastewater. J. Environ. Manage. 191, 198–208. https://doi.org/10.1016/j.jenvman.2017.01.010.

Aslan, S., Simsek, E., 2012. Influence of salinity on partial nitrification in a submerged biofilter. Bioresour. Technol. 118, 24–29. https://doi.org/10.1016/j.biortech.2012.05.057.

Banaei-Asl, F., Bandehagh, A., Uliaei, E.D., Farajzadeh, D., Salzata, K., Mustafa, G., Komatsu, S., 2015. Proteomic analysis of canola root inoculated with bacteria under salt stress. J. Proteomics 124, 88–111. https://doi.org/10.1016/j.jprot.2015.04.009.

Castillo-Carvajal, L.C., Sanz-martín, J.L., Barragán-huerta, B.E., 2014. Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review. Environ. Sci. Pollut. Res. Int. 21 (16), 9578–9588. https://doi.org/10.1007/s11356-014-3036-z.

Chao, Y.Q., Liu, W.S., Chen, Y.M., Chen, W.H., Zhao, L.H., Ding, Q.B., Wang, S.Z., Tang, Y.-T., Zhang, T., Qiu, R.-L., 2016. Structure, variation, and co-occurrence of soil microbial communities in abandoned sites of a rare earth elements mine. Environ. Sci. Technol. 50 (21), 11481–11490. https://doi.org/10.1021/acs.est.6b02284.

Cheng, X.W., Zhu, H., Bañuelos, G., Yan, B.X., Shutes, B., Liang, Y.X., Chen, X., 2018. Saline-alkaline tolerance of hygrophilous plant species during their asexual propagation and continued growth stages. S. Afr. J. Bot. 118, 129–137. https://doi.org/10.1016/j.sajb.2018.07.005.

Compant, S., Clément, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42 (5), 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024.

Fu, G.P., Han, J.Y., Yu, T.Y., Huangshen, L.K., Zhao, L., 2019a. The structure of denitrifying microbial communities in constructed mangrove wetlands in response to fluctuating salinities. J. Environ. Manage. 238, 1–9. https://doi.org/10.1016/j.jenvman.2019.02.029.

Fu, G.P., Zhao, L., Huangshen, L.K., Wu, J.F., 2019b. Isolation and identification of a salt-tolerant aerobic denitrifying bacterial strain and its application to saline wastewater treatment in constructed wetlands. Bioresour. Technol. 290. https://doi.org/10.1016/j.biortech.2019.121725.

Guo, X.C., Miao, Y., Wu, B., Ye, L., Yu, H.Y., Liu, S., Zhang, X.-X., 2015. Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics. Bioresour. Technol. 197, 99–105. https://doi.org/10.1016/j.biortech.2015.08.049.

He, H.J., Chen, Y.J., Li, X., Cheng, Y., Yang, C.P., Zeng, G.M., 2017. Influence of salinity on microorganisms in activated sludge processes: A review. Int. Biodeterior. Biodegrad. 119, 520–527. https://doi.org/10.1016/j.ibiod.2016.10.007.

Herrero, M., Stuckey, D.C., 2015. Bioaugmentation and its application in wastewater treatment: A review. Chemosphere 140, 119–128. https://doi.org/10.1016/j.chemosphere.2014.10.033.

Hong, J.M., Li, W.B., Lin, B., Zhan, M.C., Liu, C.D., Chen, B.-Y., 2013. Deciphering the effect of salinity on the performance of submerged membrane bioreactor for aquaculture of bacterial community. Desalination 316, 23–30. https://doi.org/10.1016/j.desal.2013.01.015.

Ioannou, L.A., Li Puma, G., Fatta-Kassinos, D., 2015. Treatment of winery wastewater by physicochemical, biological and advanced processes: A review. J. Hazard. Mater. 286, 343–368. https://doi.org/10.1016/j.jhazmat.2014.12.043.

Iorhemen, O.T., Hamza, R.A., Sheng, Z., Tay, J.H., 2019. Submerged aerobic granular sludge membrane bioreactor (AGMBR): Organics and nutrients (nitrogen and phosphorus) removal. Bioresour. Technol. Rep. 6, 260–267. https://doi.org/10.1016/j.biteb.2019.03.015.

Karajic, M., Lapanje, A., Razinger, J., Zrimec, A., Vrhovsek, D., 2010. The effect of the application of halotolerant microorganisms on the efficiency of a pilot-scale constructed wetland for saline wastewater treatment. J. Serb. Chem. Soc. 75 (1), 129–142. https://doi.org/10.2298/jsc1001129k.

Keswani, C., Prakash, O., Bharti, N., Vilchez, J.I., Sansinenea, E., Lally, R.D., Borriss, R., Singh, S.P., Gupta, V.K., Fraceto, L.F., de Lima, R., Singh, H.B., 2019. Re-addressing the biosafety issues of plant growth promoting rhizobacteria. Sci. Total Environ. 690, 841–852. https://doi.org/10.1016/j.scitotenv.2019.07.046.

Leung, J.Y.S., Cai, Q., Tam, N.F.Y., 2016. Comparing subsurface flow constructed wetlands with mangrove plants and freshwater wetland plants for removing nutrients and toxic pollutants. Ecol. Eng. 95, 129–137. https://doi.org/10.1016/j.ecoleng.2016.06.016.

Li, Z.W., Chang, Q.B., Li, S.S., Gao, M.C., She, Z.L., Guo, L., Zhao, Y.G., Jin, C.J., Zheng, D., Xu, Q.Y., 2017. Impact of sulfadiazine on performance and microbial community of a sequencing batch biofilm reactor treating synthetic mariculture wastewater. Bioresour. Technol. 235, 122–130. https://doi.org/10.1016/j.biortech.2017.03.113.

Liang, Y.X., Cheng, X.W., Zhu, H., Shutes, B., Yan, B.X., Zhou, Q.W., Yu, X.F., 2018a. Historical evolution of mariculture in China during past 40 years and its impacts on eco-environment. Chinese Geogr. Sci. 28 (3), 363–373. https://doi.org/10.1007/s11769-018-0940-z.

Liang, W.J., Ma, X.L., Wan, P., Liu, L.Y., 2018b. Plant salt-tolerance mechanism: a review. Biochem. Biophys. Res. Commun. 495 (1), 286–291. https://doi.org/10.1016/j.bbrc.2017.11.043.

Liang, Y.X., Zhu, H., Banuelos, G., Yan, B., Shutes, B., Cheng, X.W., Chen, X., 2017a. Removal of nutrients in saline wastewater using constructed wetlands: Plant species, influent loads and salinity levels as influencing factors. Chemosphere 187, 52–61. https://doi.org/10.1016/j.chemosphere.2017.08.087.

Liang, Y.X., Zhu, H., Banuelos, G., Yan, B.X., Zhou, Q.W., Yu, X.F., Cheng, X.W., 2017b. Constructed wetlands for saline wastewater treatment: A review. Ecol. Eng. 98, 275–285. https://doi.org/10.1016/j.ecoleng.2016.11.005.

Liu, F.-F., Fan, J.L., Du, J.H., Shi, X., Zhang, J., Shen, Y.H., 2019a. Intensified nitrogen transformation in intermittently aerated constructed wetlands: removal pathways and microbial response mechanism. Sci. Total Environ. 650, 2880–2887. https://doi.org/10.1016/j.scitotenv.2018.10.037.

Liu, L.L., Dong, Y.C., Kong, M., Zhou, J., Zhao, H.B., Tang, Z., Zhang, M., Wang, Z.P., 2020. Insights into the long-term pollution trends and sources contributions in Lake Taihu, China using multi-statistic analyses models. Chemosphere 242, 125272.

Liu, Y., Liu, X.H., Li, K., Lu, S.Y., Guo, X.C., Zhang, J., Xi, B.D., 2019b. Removal of nitrogen from low pollution water by long-term operation of an integrated vertical-flow constructed wetland: Performance and mechanism. Sci. Total Environ. 652, 977–988. https://doi.org/10.1016/j.scitotenv.2018.10.313.

Lyu, Y.-H., Zhou, Y.-X., Li, Y., Zhou, J., Xu, Y.-X., 2019. Optimized culturing conditions for an algicidal bacterium *Pseudoalteromonas* sp. SP48 on harmful algal blooms caused by Alexandrium tamarense. Microbiologyopen 8 (8). https://doi.org/10.1002/mbo3.803.

Ng, K.K., Shi, X.Q., Ong, S.L., Lin, C.F., Ng, H.Y., 2016. An innovative of aerobic bio-entrapped salt marsh sediment membrane reactor for the treatment of high-saline pharmaceutical wastewater. Chem. Eng. J. 295, 317–325. https://doi.org/10.1016/j.cej.2016.03.046.

Qin, Y., Druzhinina, I.S., Pan, X.Y., Yuan, Z.L., 2016. Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. Biotechnol. Adv. 34 (7), 1245–1259. https://doi.org/10.1016/j.biotechadv.2016.08.005.

Rao, A.R., Dayananda, C., Sarada, R., Shamala, T.R., Ravishankar, G.A., 2007. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour. Technol. 98 (3), 560–564. https://doi.org/10.1016/j.biortech.2006.02.007.

Richardson, D.J., Wehrfritz, J.M., Keech, A., Crossman, L.C., Roldan, M.D., Sears, H.J., Butler, C.S., Reilly, A., Moir, J.W.B., Berks, B.C., Ferguson, S.J., Thomson, A.J., Spiro, S., 1998. The diversity of redox proteins involved in bacterial heterotrophic nitrification and aerobic denitrification. Biochem. Soc. Trans. 26 (3), 401–408. https://doi.org/10.1042/bst0260401.

Schmale, D.G., Ault, A.P., Saad, W., Scott, D.T., Westrick, J.A., 2019. Perspectives on harmful algal blooms (HABs) and the cyberbiosecurity of freshwater systems. Front. Bioeng. Biotechnol. 7, 7. https://doi.org/10.3389/fbioe.2019.00128.

Shi, X., Ng, K.K., Li, X.-R., Ng, H.Y., 2015. Investigation of intertidal wetland sediment as a novel inoculation source for anaerobic saline wastewater treatment. Environ. Sci. Technol. 49 (10), 6231–6239. https://doi.org/10.1016/j.jenvman.2019.109398.

Sivasankar, P., Poongodi, S., Seedevi, P., Sivakumar, M., Murugan, T., Loganathan, S., 2019. Bioremediation of wastewater through a quorum sensing triggered MFC: A sustainable measure for waste to energy concept. J. Environ. Manage. 237, 84–93. https://doi.org/10.1016/j.jenvman.2019.01.075.

Soltani, R.D.C., Rezaee, A., Godini, H., Khataee, A.R., Jorfi, S., 2013. Organic matter removal under high loads in a fixed-bed sequencing batch reactor with peach pit as carrier. Environ. Prog. Sustain. Energy 32 (3), 681–687. https://doi.org/10.1002/ep.11685.

Song, W.L., Lee, L.Y., You, H., Shi, X.Q., Ng, H.Y., 2020. Microbial community succession and its correlation with reactor performance in a sponge membrane bioreactor coupled with fiber-bundle anoxic bio-filter for treating saline mariculture wastewater. Bioresour. Technol. 295. https://doi.org/10.1016/j.biortech.2019.122284.

Sun, G.Z., Zhu, Y.F., Saeed, T., Zhang, G.X., Lu, X.G., 2012. Nitrogen removal and microbial community profiles in six wetland columns receiving high ammonia load. Chem. Eng. J. 203, 326–332. https://doi.org/10.1016/j.cej.2012.07.052.

Tan, E., Hsu, T.C., Huang, X.F., Lin, H.J., Kao, S.J., 2017. Nitrogen transformations and removal efficiency enhancement of a constructed wetland in subtropical Taiwan. Sci. Total Environ. 601, 1378–1388. https://doi.org/10.1016/j.scitotenv.2017.05.282.

Van Den Hende, S., Claessens, L., De Muylder, E., Boon, N., Vervaeren, H., 2014. Microalgal bacterial flocs originating from aquaculture wastewater treatment as diet ingredient for Litopenaeus vannamei (Boone). Aquac. Res. 47 (4), 1075–1089. https://doi.org/10.1111/are.12564.

Wang, Q., Cao, Z.F., Liu, Q., Zhang, J.Y., Hu, Y.B., Zhang, J., Xu, W., Kong, Q., Yuan, X.C., Chen, Q.F., 2019a. Enhancement of COD removal in constructed wetlands treating saline wastewater: Intertidal wetland sediment as a novel inoculation. J. Environ. Manage. 249, 109398. https://doi.org/10.1016/j.jenvman.2019.109398.

Wang, Q., Ma, L.Y., Zhou, Q.Y., Chen, B., Zhang, X.C., Wu, Y.J., Pan, F.S., Huang, L.K., Yang, X.E., Feng, Y., 2019b. Inoculation of plant growth promoting bacteria from hyperaccumulator facilitated non-host root development and provided promising agents for elevated phytoremediation efficiency. Chemosphere 234, 769–776. https://doi.org/10.1016/j.chemosphere.2019.06.132.

Wang, Z.C., Gao, M.C., She, Z.L., Wang, S., Jin, C.J., Zhao, Y.G., Yang, S.Y., Guo, L., 2015. Effects of salinity on performance, extracellular polymeric substances and microbial community of an aerobic granular sequencing batch reactor. Sep. Purif. Technol. 144, 223–231. https://doi.org/10.1016/j.seppur.2015.02.042.

Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdisciplinary Rev.-Water 6 (5), 27. https://doi.org/10.1002/wat2.1373.

Xu, Q.L., Hunag, Z.J., Wang, X.M., Cui, L.H., 2015. Pennisetum sinese Roxb and Pennisetum purpureum Schum. as vertical-flow constructed wetland vegetation for removal of N and P from domestic sewage. Ecol. Eng. 83, 120–124. https://doi.org/10.1016/j.ecoleng.2015.06.011.

Yang, L., Wang, X.H., Xiao, Q., Ren, Y.X., Chen, N., Cui, S., 2019. Kinetic characteristics and N_2O production of a heterotrophic nitrifying bacterium *Pseudomonas putida* YH capable of tolerating adverse environmental conditions. J. Chem. Technol. Biotechnol. 94 (12), 3941–3950. https://doi.org/10.1002/jctb.6195.

Yang, Y.F., Chai, Z.Y., Wang, Q., Chen, W.Z., He, Z.L., Jiang, S.J., 2015. Cultivation of seaweed *Gracilaria* in Chinese coastal waters and its contribution to environmental improvements. Algal Res. 9, 236–244. https://doi.org/10.1016/j.algal.2015.03.017.

Ying, G., Xing, Y., Li, Z., Pan, J., Kuang, X., 2010. Advantages of psychrophiles in improving bio-treatment efficiency of small size constructed wetlands during cold weather. Environ. Prog. Sustain. Energy 29 (1), 25–33. https://doi.org/10.1002/ep.10354.

Yu, G.L., Peng, H.Y., Fu, Y.J., Yan, X.J., Du, C.Y., Chen, H., 2019. Enhanced nitrogen removal of low C/N wastewater in constructed wetlands with co-immobilizing solid carbon source and denitrifying bacteria. Bioresour. Technol. 280, 337–344. https://doi.org/10.1016/j.biortech.2019.02.043.

Zhao, G., Mehta, S.K., Liu, Z., 2010. Use of saline aquaculture wastewater to irrigate salt-tolerant Jerusalem artichoke and sunflower in semiarid coastal zones of China. Agric. Water Manage. 97 (12), 1987–1993. https://doi.org/10.1016/j.agwat.2009.04.013.

Zhao, X.Y., Bai, S.W., Li, C.Y., Yang, J.X., Ma, F., 2019. Bioaugmentation of atrazine removal in constructed wetland: Performance, microbial dynamics, and environmental impacts. Bioresour. Technol. 289. https://doi.org/10.1016/j.biortech.2019.121618.

Zhao, X.Y., Yang, J.X., Bai, S.W., Ma, F., Wang, L., 2016. Microbial population dynamics in response to bioaugmentation in a constructed wetland system under 10 °C. Bioresour. Technol. 205, 166–173. https://doi.org/10.1016/j.biortech.2016.01.043.

Zhu, W.L., Cui, L.H., Ouyang, Y., Long, C.F., Tang, X.D., 2011. Kinetic Adsorption of Ammonium Nitrogen by Substrate Materials for Constructed Wetlands. Pedosphere 21 (4), 454–463. https://doi.org/10.1016/S1002-0160(11)60147-1.

Zhuang, L.-L., Yang, T., Zhang, J., Li, X.Z., 2019. The configuration, purification effect and mechanism of intensified constructed wetland for wastewater treatment from the aspect of nitrogen removal: A review. Bioresour. Technol. 293. https://doi.org/10.1016/j.biortech.2019.122086.