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RESEARCH ARTICLE

Enhanced nitrogen removal in biochar-added surface flow constructed wetlands: dealing with seasonal variation in the north China

Jing Li[1] · Jinlin Fan[2] · Daoxing Liu[3] · Zhen Hu[1] · Jian Zhang[1,4]

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Abstract

In the present study, the performance of surface flow constructed wetlands (SFCWs) added with different dosage of biochar (group A 0%, group B 10%, group C 20%; *v*/*v*) was investigated, to evaluate the effect of biochar on nitrogen removal of a constructed wetland. No significant difference was observed in NH_4^+-N removal among three groups even during different seasons. Labile organic carbon released from biochar distinctly enhanced denitrification process, which improved NO_3^--N removal efficiency by 4.58% in group B and 10.33% in group C. More importantly, compared with group A, biochar addition increased plant N removal by 82.24% and 192.11% in groups B and C, respectively. This result indicated that biochar could increase the accumulation of plant net biomass. In addition, TN removal of group A was much lower at low temperature (4.9 °C). However, no obvious influence of temperature on TN removal was observed in groups B and C with biochar addition. Microbial community analysis showed that, compared with that in group A, the total relative abundance of the main denitrification bacteria (*Proteobacteria*, *Firmicutes*, and *Bacteroidetes*) increased by 0.81% in group B and 13.63% in group C. These results provide a reasonable strategy for improving the performance of SFCWs under cold climate.

Keywords Biochar addition · Surface flow constructed wetlands · Plant uptake · Microbial community · Seasonal variation · Nitrogen removal

Introduction

Wastewater reclamation from sewage treatment plants (STPs) has gained considerable attention over the past 30 years in China (Lyu et al. 2016). However, discharge of sewage effluent containing a high level of nutrients such as nitrogen (N) has been a key restraint for the development of wastewater reclamation (Boonchai and Seo 2015). In China, the grade III national surface water standards (GB3838-2002) for total nitrogen (TN) was standardized to 1 mg L^{-1}. Therefore, it is urgent to achieve satisfactory N removal performance from sewage before being discharged into aquatic ecosystems.

Constructed wetlands (CWs) were acknowledged as a promising ecological technology to treat wastewater and widely applied in aquatic ecosystems due to their low cost, effective self-adaption, and eco-friendliness (De Rozari et al. 2016). N removal in CWs is a complex process that mainly includes substrate adsorption, plant absorption, and microbial immobilization. However, the treatment performance of CWs was subject to changes in seasonal temperature variation (Rai

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✉ Zhen Hu
huzhen885@sdu.edu.cn

✉ Jian Zhang
zhangjian00@sdu.edu.cn

[1] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan 250100, PR China

[2] National Engineering Laboratory of Coal-Fired Pollutants Emission Reduction, Shandong University, Jinan 250061, PR China

[3] Environmental Engineering Co., Ltd., Shandong Academy of Environmental Science, Jinan 250100, PR China

[4] State key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

et al. 2015). Previous studies have shown that N removal efficiency obviously declined in cold winter compared with that in warm seasons (Wang et al. 2012; Wang and Li 2015). This was mainly because most of the aquatic plants easily went into dormancy and decayed in winter, such as *Phragmites australis*, *Typha orientalis*, lotus, *Acorus calamus*, *Canna generalis*, and *Juncus effusus* (Fan et al. 2016). Moreover, microbial abundance and activities could also be inhibited when at low temperature (Wu et al. 2015). Thus, research on overwintering plant selection and sustainable nitrogen removal under seasonal temperature variation are crucial for the enhancement of treatment performance of CWs. *Oenanthe javanica* (*O. javanica*) is a typical macrophyte capable of rooting well and tolerating cold temperature in winter (Zhou et al. 2010). It can be applied to CWs for treating wastewater. Furthermore, little attention has been paid to determine the relationship between plants and substrate, and the influence of substrate on total biomass change and N accumulation in *O. javanica* is still unclear.

Biochar is a carbon-rich material pyrolyzed from biological residues under anoxic or anaerobic conditions (Manyà et al. 2012). Lately, it is of rising interest in biochar as a multifunctional adsorbent for wastewater treatment (Mohan et al. 2014). Biochar is also distinguished for its application of soil amendment. It can not only promote plants growth and reduce nutrient leaching and N_2O emission, but also impact soil microbial community and stimulate N transformation (Xu et al. 2014; Lehmann and Joseph 2015). However, few studies have been conducted to apply biochar as CWs substrate, and most of the studies only focused on improvement of nitrogen and phosphorus removal efficiencies (Gupta et al. 2015; De Rozari et al. 2016). Furthermore, no efforts have been made to estimate the influence of biochar on plants growth and N transformation under seasonal variation in CWs. Therefore, it is essential to develop biochar-added CWs concentrated on wetland plant growth characteristics and N removal processes under seasonal variation. On the one hand, the application of biochar produced from wetland plant residuals can significantly increase N retention in plants as nutrients. On the other hand, the characteristics of high porosity and large surface area of biochar can increase microbial attachment sites and then stimulate N transformation. The hypothesis of this study is that CWs combined with biochar addition could enhance plants growth and N transformation and then further enhance N removal under seasonal temperature variation.

In the present study, three groups of surface flow constructed wetlands (SFCWs) added with biochar and common sand were set up to evaluate the long-term treatment performance of STPs effluent. The main objectives of this study were as follows: (1) to compare N removal performance of three groups under seasonal temperature variation; (2) to investigate the effects of various amounts of biochar addition on plant growth characteristics and the corresponding N removal performance in SFCWs; and (3) to study the retention, transformation, and export of N in SFCWs by presence of biochar.

Materials and methods

Design and setup of batch-operated SFCWs

Three groups (each group has two parallels) were set up and performed under the transparent shelter in the central campus of Shandong University in Jinan, China (36° 40′ 53″ N, 117° 03′ 58″ E), where there has obvious seasonal change. Group A was packed with the sand substrate as control. Group B was packed with 10% biochar and 90% sand (*v*/*v*) substrate. Group C was packed with 20% biochar and 80% sand (*v*/*v*) substrate. Each group was 50 cm in height and 25 cm in diameter, and the substrate depth was 25 cm. Bed frame structure of substrate was divided into two layers. The main substrate layer was 20 cm filled with sand and biochar. The supporting layer was 5 cm filled with washed gravel. The schematic diagram of the experimental setup is shown in Fig. 1.

In the present study, *O. javanica* with mainly similar biomass was planted in the three groups, at a density of 12 rhizomes per reactor. Plants were transplanted into the SFCWs. Each group was kept flooded for about 2 months until the plants were well established. Biochar was prepared from the decayed wetland plant, *Arundo donax*, collected from Nansi Lake wetland, Shandong province, which was pyrolyzed under an anaerobic condition at 300 °C (Zheng et al. 2013). The reasons for selecting *A. donax* biochar were (1) environmental and renewable raw material; (2) high yield as well as developed aerenchyma; and (3) release of dissolved organic matter (DOM), which can provide essential carbon sources (Bruun et al. 2012).

Experiment procedure

The experimental CW microcosms were operated for 8 months at a temperature ranging from − 2 to 27 °C. The experiment involved the following two phases: phase I (with average temperature 4.9 °C) from 28 October 2016 to 11 March 2017 and phase II (with average temperature 19.4 °C) from 12 March 2017 to 15 May 2017. In order to minimize variability in the experiment, STP effluent (class IA) was simulated with synthetic wastewater. The detailed influent compositions are shown in Table 1. Due to poor treatment performance of CWs in the cold season (Spieles et al. 1999), the hydraulic retention time (HRT) of each group was 7 days in phase I and 3 days in phase II based on previous studies (Huang et al. 2000), corresponding to a hydraulic loading of 0.13 $m^3\ m^{-2}\ batch^{-1}$.

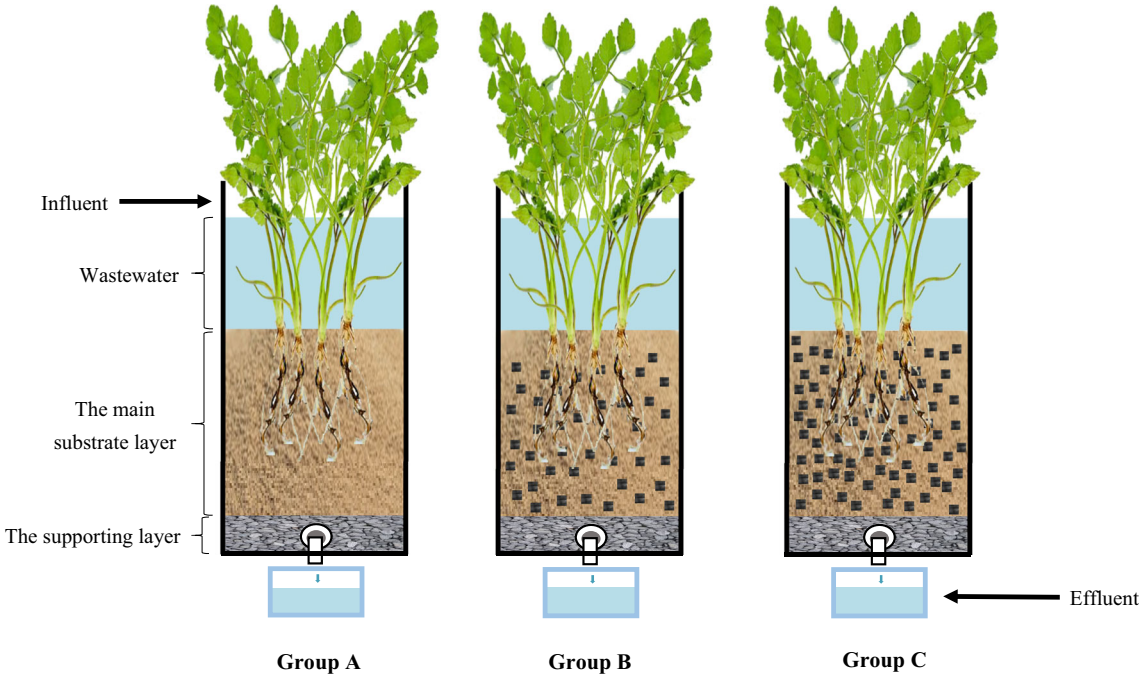

Fig. 1 Schematic diagram of experimental surface flow constructed wetlands (SFCWs)

Sampling and analysis

Water quality monitoring

In each batch, water samples were collected from the influent tank and the effluent of each group at 8:00–9:00. Chemical oxygen demand (COD), NH_4^+-N, NO_3^--N, and NO_2^--N were analyzed in the present study according to the methods described in APHA-AWWA-WPCF (2001). Dissolved oxygen (DO) and temperature were measured by a DO Meter (HQ30d, Hach, USA). The pH value was determined using a pH meter (SG2, METTLER TOLEDO, Switzerland).

Plant monitoring and analyses

Plant height was monitored during the whole experimental stage. Relative growth rate (RGR) per plant per day was calculated with Eq. (1):

$$\mathrm{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1) \quad (1)$$

where W_1 and W_2 are the initial dry biomass at t_1 (beginning of the experiment) and harvested dry biomass at t_2 (end of the experiment), respectively (Liu et al. 2012).

At the end of the experiment, plant aboveground and underground samples were separately harvested to estimate N retention in plants. The plant samples were washed with tap water to remove dust and then were dried in an oven at 65 °C for 72 h to obtain biomass dry weight. After grinding, all dried samples were sieved to pass through a 100-mesh sieve for performing the element content by the elemental analyzer (Vario EL cube, Germany).

The total N uptake (g m^{-2}) was estimated with Eq. (2):

$$U(\mathrm{gm}^{-2}) = (W_2 \times N_2 - W_1 \times N_1)/S \quad (2)$$

where N_1 and N_2 are the initial N content and ultimate N content in plant, respectively, and S (m^2) is the surface area of the SFCW.

The percentage of plant removal efficiency was calculated with Eq. (3):

$$R(\%) = U/(I\mathrm{n}_{\mathrm{load}} - \mathrm{Ef}_{\mathrm{load}}) \times 100\% \quad (3)$$

where U is total N uptake, and $\mathrm{In}_{\mathrm{load}}$ and $\mathrm{Ef}_{\mathrm{load}}$ are the N loads in influent and effluent.

Substrate sampling and analyses

Substrate samples of each group were collected for element content and microbial analysis. After collection, the substrate samples were dried at − 60 °C using a freeze dryer (Unicryo MC 2-L freeze dryer, Germany) for 36 h and then sieved (0.154 mm) and stored at − 20 °C for further analysis.

N_2O sampling and analyses

Closed chamber method was used to take gas samples. Gas samples were collected at 0, 8, 24, 32, 48, and 56 h in each typical cycle. The thermometers and gas fans were installed into the transparent shelter collection boxes (100 cm in height

Table 1 Water quality, pollutant concentrations in the influent and effluent, and the pollutant removal efficiencies of the surface flow constructed wetlands packed with biochar during the two experimental phases

Parameters	Phases I ($n = 22$)							
	Influent (mg L^{-1})	Effluent (mg L^{-1})			Removal efficiency (%)			
		Group A	Group B	Group C	Group A	Group B	Group C	Sig.
pH	7.52 ± 0.21	7.04 ± 0.16	6.98 ± 0.13	6.91 ± 0.11	/	/	/	/
DO	8.23 ± 0.47	2.46 ± 0.23	4.66 ± 0.27	5.21 ± 0.64	/	/	/	/
COD	57.21 ± 2.52	13.33 ± 1.81	14.86 ± 2.01	15.96 ± 1.75	76.32 ± 4.00	73.56 ± 4.67	71.64 ± 4.31	*
NH_4^+-N	6.66 ± 0.41	0.39 ± 0.18	0.42 ± 0.18	0.48 ± 0.19	94.28 ± 2.47	93.66 ± 2.54	92.85 ± 2.66	ns
NO_3^--N	11.44 ± 0.75	4.20 ± 1.48	2.02 ± 0.99	0.95 ± 0.65	62.18 ± 15.31	81.56 ± 9.76	91.27 ± 6.30	**
NO_2^--N	0	0.08 ± 0.02	0.05 ± 0.01	0.03 ± 0.02	/	/	/	/
TN	18.10 ± 1.13	4.66 ± 1.45	2.50 ± 0.98	1.47 ± 1.59	73.58 ± 9.64	85.72 ± 6.26	91.66 ± 3.77	**
Parameters	Phases II ($n = 25$)							
	Influent (mg L^{-1})	Effluent (mg L^{-1})			Removal efficiency (%)			
		Group A	Group B	Group C	Group A	Group B	Group C	Sig.
pH	7.57 ± 0.13	7.12 ± 0.19	7.11 ± 0.37	7.05 ± 0.25	/	/	/	/
DO	8.31 ± 0.36	2.52 ± 0.29	4.83 ± 0.43	5.48 ± 0.39	/	/	/	/
COD	57.21 ± 2.52	14.66 ± 1.03	12.87 ± 0.64	12.24 ± 0.59	74.49 ± 1.92	77.61 ± 1.26	78.71 ± 1.24	**
NH_4^+-N	11.78 ± 0.33	0.18 ± 0.07	0.18 ± 0.13	0.19 ± 0.09	95.33 ± 0.52	94.39 ± 1.08	94.26 ± 0.76	ns
NO_3^--N	20.68 ± 0.35	3.30 ± 0.44	2.50 ± 0.75	1.50 ± 0.61	84.04 ± 2.24	87.89 ± 3.78	92.72 ± 3.04	**
NO_2^--N	0	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	/	/	/	/
TN	32.45 ± 0.56	3.89 ± 0.44	3.16 ± 0.83	2.18 ± 0.66	88.01 ± 1.42	90.23 ± 2.65	93.26 ± 2.09	**

/Not determined. *Sig.* significant, *ns* not significant, *$p < 0.05$ significant, **$p < 0.01$ highly signification

and 28 cm in diameter). The air temperature outside and in the box was recorded simultaneously. All collected samples were measured by gas chromatography (GC, Agilent Technologies 7890A, USA) to detect N_2O concentration (Xia et al. 2013).

Microbial community composition

Quantitative polymerase chain reaction

PowerSoil™ DNA Isolation Kit (MOBIO) was used to extract the total genomic DNA from the collected substrate. Quantitative analysis was conducted on bacterial *16S rRNA* and nitrification functional genes (*amoA*, *NSR*) as well as denitrification functional genes (*nirS*, *nirK*, *narG*, *nosZ*) (Cheng et al. 2016).

High-throughput sequencing

To determine the microbial community of three groups, high-throughput sequencing was conducted at the Yuanxu Biotechnology Company (Shanghai, China). The sequences length shorter than 250 bp and quality score less than 30 were eliminated from the pyrosequencing-derived data sets.

Statistical analysis

All measurements were performed via the statistical program SPSS 11.0 (SPSS, Chicago, USA). The variance (ANOVA) was used to test the significance of the statistic. The results were considered to be statistically significant when $p < 0.05$ and no significant when $p > 0.05$.

Results and discussion

Water quality and N removal performance of SFCWs

Water quality

Table 1 shows the influent and effluent pH value and DO concentration throughout the experimental period. Similar effluent pH values were observed in three groups. It kept relatively stable between the two phases in each group, indicating that there was no obvious impact of biochar on pH values. The effluent DO concentrations of groups B and C were much higher than that of group A, indicating biochar addition facilitated oxygen diffusion. In phase II, the effluent DO concentrations of three groups were slightly increased compared with phase I. The possible reason may be that radial oxygen loss of wetland plants was slightly improved of the three groups as plants grow.

Nitrogen removal

The influent and effluent concentrations of various N form are shown in Table 1. The effluent concentrations of NH_4^+-N were all below 0.5 mg L^{-1} in three groups, which satisfied the standards of grade-III (NH_4^+-N 1 mg L^{-1}) national surface water. From Table 1, NH_4^+-N removal efficiencies of the three groups were all above 92%. There was no significant difference ($p > 0.05$) of NH_4^+-N removal efficiency of each group between the two phases, illustrating that SFCWs in the present study still keep satisfying NH_4^+-N removal performance when the season changed. In addition, NH_4^+-N removal efficiency (92.85–95.33%) in the present study was superior to other studies reported at similar operation condition (Jing et al. 2001; Gao et al. 2014).

In the present study, much higher NO_3^--N removal efficiency was observed in groups B and C. The NO_3^--N removal efficiencies of three groups were 62.18 ± 15.31%, 81.56 ± 9.76%, and 91.27 ± 6.30% in phase I and 84.04 ± 2.24%, 87.89 ± 3.78%, and 92.72 ± 3.04% in phase II ($p < 0.01$, Table 1). The present results demonstrated that biochar addition could significantly improve NO_3^--N treatment performance. As a result of promotion in nitrification and denitrification, the highest TN removal efficiency was obtained in group C (91.66%, 93.26%) at phases I and II, followed by group B (85.72%, 90.23%) and group A (73.58%, 88.01%). TN removal efficiencies of groups B and C were no obvious change between phases I and II. In phase I, however, TN removal declined by 16.40% in group A compared with phase II. These results demonstrated that biochar could be a promising choice for sustainable operation under seasonal temperature variation. TN removal efficiency in the present study was greater than other conventional and enhanced CWs (Vymazal et al. 2007; Zhi et al. 2014; Fan et al. 2016).

Plant growth

The individual plant height and the air temperature change with time are shown in Fig. 2. The plants in the SFCWs grew normally and without obvious symptom of toxicity in wastewater after domestication time has passed. It was observed that the growth of *O. javanica* was rather slow when the temperature dropped, but still showed good growth tendency, indicating *O. javanica* has the capacity to deal with seasonal temperature variation. Fast growth was also obtained in three groups as temperature increased. No significant difference was observed in the individual height ($p > 0.05$) of three groups. The net growth sizes were 79.70 cm, 88.05 cm, and 92.32 cm for plant height in three groups, suggesting that biochar was beneficial to the improvement of plants height.

RGR and plant net growth values were employed to investigate the influence of biochar on plants growth situation (Table 2). The RGR values of plant aboveground and

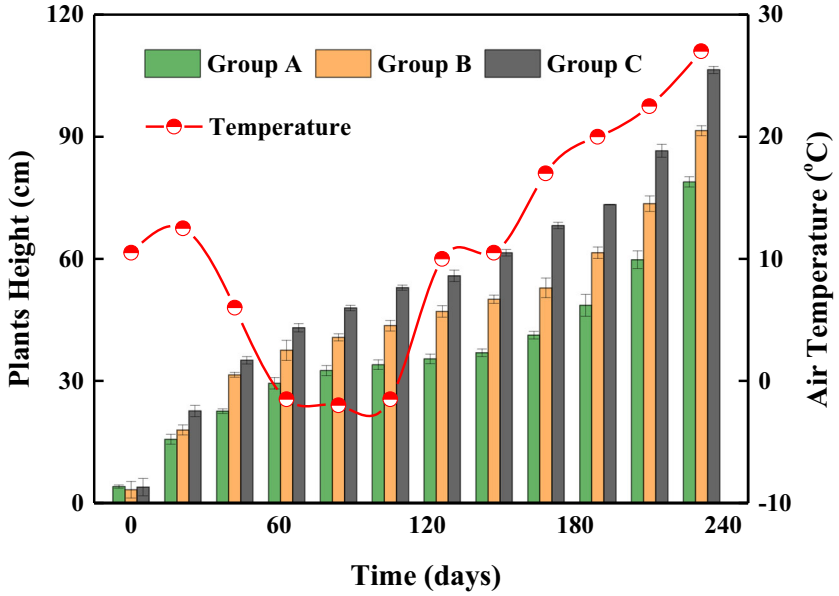

Fig. 2 Growth of plant height in three groups and air temperature during the experimental period

underground portion during the entire experimental period were 0.011 and 0.008 day^{-1} in group A, 0.013 and 0.009 day^{-1} in group B, and 0.014 and 0.010 day^{-1} in group C. The RGR was influenced by the plant portion as well as biochar addition, with the RGR order as follows: aboveground portion > underground portion; group A < group B < group C. These results reflected that biochar could accelerate plants growth, especially for an aboveground portion. RGR values in the present study were significantly superior to other studies obtained in winter (Gao et al. 2014) and even higher than other studies investigated under warm climate (Liu et al. 2012). Changes in plant biomass occurred with net growth, which was calculated by subtracting the initial measured value from the value measured at the end of the experiment. There was a significant difference ($p < 0.05$) of the plant net biomass accumulation among the three groups. Compared with group A, biochar addition increased plant biomass by 61.72% on average in group B and 104.43% in group C. This observable increase may be a function of the release of carbon and nitrogen elements from biochar to provide nutrients (Fig. 3). Furthermore, the net growth of aboveground biomass was larger than underground biomass in all groups, illustrating that nutrients mainly stored in aboveground portion via advanced root and mature plant, which was consistent with the results of RGR.

Microbial analysis

The quantity of nitrogen-related genes

The absolute abundance of bacterial *16S rRNA*, *amoA*, *NSR*, *narG*, *nirK*, *nirS*, and *nosZ* were quantified at the end of the experiment to determine the influence of biochar on microbial population and nitrogen transformation process in all groups (Fig. S5). The quantitative polymerase chain reaction (qPCR) data showed that biochar could slightly increase the absolute abundance of bacterial 16S rRNA ($p > 0.05$), demonstrating its stimulation of microbes. The *amoA*, *NSR*, *narG*, *nirK*, *nirS*, and *nosZ* genes were related to NH_4^+-N, NO_2^--N, and NO_3^--N transformation in the processes of nitrification and denitrification. The amounts of nitrification functional genes showed no significant difference among the three groups ($p > 0.05$),

Table 2 Plant relative growth rate (RGR), net biomass, N content, and contribution of plant uptake for N removal in three SFCW groups throughout the experimental period

Parameters	Group A		Group B		Group C	
	Aboveground	Underground	Aboveground	Underground	Aboveground	Underground
RGR (day^{-1})	0.011	0.008	0.013	0.009	0.014	0.010
Net biomass (g)	42.6	18.3	73.57	25.18	94.41	30.29
N content (%)	2.58	1.98	2.89	2.21	3.54	3.21
N uptake (g m^{-2})	22.80	7.60	43.60	11.80	68.20	20.60
N removal (%)	42.35	14.12	47.42	12.83	60.50	18.28

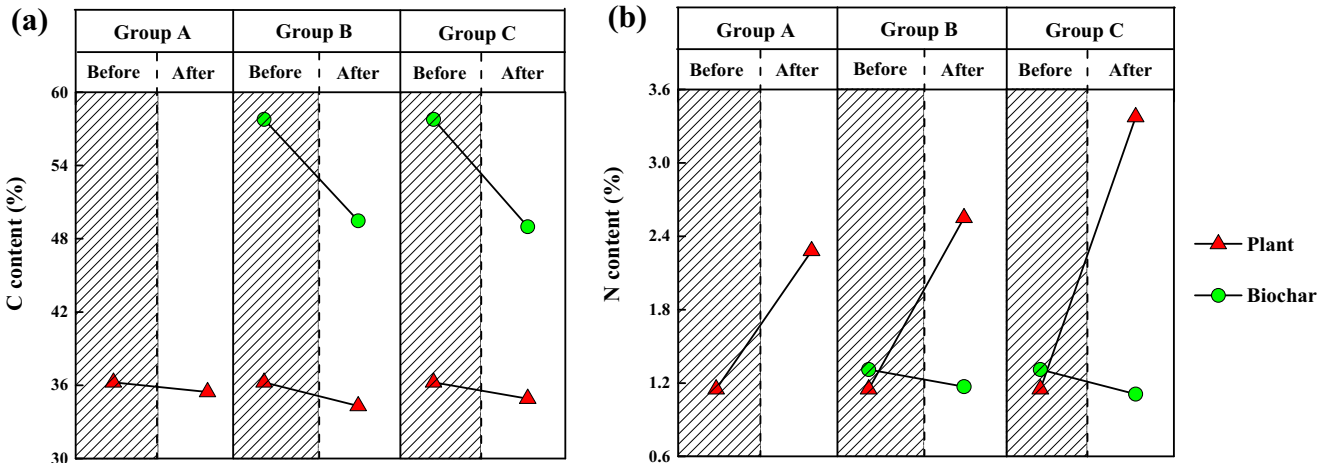

Fig. 3 Carbon (**a**) and nitrogen (**b**) content (%) in plant and biochar before and after the experimental period (240 days) in three SFCW groups

suggesting that biochar addition has no obvious impact on nitrification. The amounts of denitrification functional genes of group C were slightly higher than that of group B, and obviously higher than that of group A. The results indicated that biochar addition strengthened denitrification process. The performance was improved as the amounts of biochar increased in the present study. These results were consistent with the results of various forms of N removal. Therefore, biochar could meaningfully increase the absolute abundance of microbes and functional genes quantities and then enhance N removal. Furthermore, the microbial community structure also played a very important role in N transformation of CWs (Truu et al. 2009).

Bacterial community structure

Illumina high-throughput sequencing of the 16S rRNA gene of the three groups was adopted to further analyze the microbial mechanism. Each sample contained 52,173–83,753 reads with a read length of 409–410 bp. Based on the Good coverage index of 99.1 to 99.8%, the sequence number was capable to describe the bacterial community, with OTUs ranging from 1105 to 1216 (Table 3). According to Chao and ACE index, the addition of biochar in groups B and C could obviously increase the community richness. On the basis of community diversity estimators of the Shannon and Simpson index, the diversity followed the order of group B > group A > group C. These results indicated that the addition of biochar could have a positive influence on the bacterial community, which was consistent with N removal (Tables 1 and 2). Notably, the Shannon and Simpson index dropped slightly while the N removal performed well and the quantities of functional genes were larger than the other two groups in group C. The decrease demonstrated that larger amount of biochar addition promoted the development of the functional microbial community in SFCWs. Bacterial community composition was further investigated to explain this phenomenon.

Bacterial community composition is known as an important factor to evaluate N removal mechanism in SFCWs (Truu et al. 2009; Xu et al. 2018). Ten primary phyla were assigned to analyze bacterial community composition, which is shown in Fig. 5. *Proteobacteria* was the dominant phylum in three groups (with the relative abundance of 36.51%, 52.16%, and 30.20% for group A, group B, and group C, respectively), followed by *Firmicutes* (34.91%, 19.93%, and 50.41%, respectively), *Actinobacteria* (12.05%, 10.50%, and 5.33%, respectively), and *Bacteroidetes* (5.42%, 5.37%, and 6.70%, respectively). *Proteobacteria*, the major phylum in the three groups, was further analyzed under class level through sequencing. In the present study, the branches of alpha-, beta-, delta-, and gamma-*Proteobacteria* were particularly evaluated (Table 4). The percentage of alpha-*Proteobacteria* in group C increased to 19.68% compared to that in group A, suggesting that alpha-*Proteobacteria* was adapted well under biochar-added environment. The gamma- and beta-*Proteobacteria* were related to the nutrient biodegradation (Heylen et al. 2006; Manz et al. 1994). The proportions of gamma- and beta-*Proteobacteria* in group C increased by 76.22% and 19.07% compared to group A, respectively. The result implied that the changes in this relative abundance were likely

Table 3 Comparison of phylotype coverage, diversity, and richness estimators at a phylogenetic distance of 3%

Sample	OTUs	ACE	Chao	Shannon	Simpson	Good's coverage (%)
Group A	1105	1231.131	1414.75	6.768	0.919	99.1
Group B	1202	1323.667	1213.31	7.881	0.975	99.8
Group C	1216	1403.941	1343.449	5.568	0.806	99.1

Table 4 The relative sequence abundance (%) of *Proteobacteria* for three groups

Class	Group A	Group B	Group C
Alpha	13.50	13.79	19.68
Beta	14.26	10.34	16.98
Delta	13.63	0.91	2.41
Gamma	7.36	5.14	12.97
Total	48.75	30.18	52.04

responsible for the enhanced denitrification in SFCWs of group C. These results are consistent with the qPCR analysis.

N retention, transformation, and export

The present study focused on N retention, transformation, and export under seasonal temperature variation to clearly identify effects of biochar amounts on N processing in SFCWs. According to the "Nitrogen removal" section about N removal (Table 1), TN removal efficiencies varied among groups and seasons ranging from 73.58 ± 9.64% (group A, phase I) to 93.26 ± 2.09% (group C, phase II). This result could be attributed to the two factors.

On the one hand, plants uptake occupied the largest proportion of TN removal in three groups (more than 50%, Table 2). That is to say, most of N from influent and substrate was assimilated and stored in plants as nutrients (Table 2, Fig. 3). Table 2 illustrates the N content and N uptake of the plant after the whole experimental period in three groups. The initial N content of plant was 1.15%. At the end of the experiment, it increased by 11.84% in group B and 48.03% in group C compared with group A. A significant increase was benefited from biochar addition (Prendergast-Miller et al. 2014). Furthermore, plant N uptake was mainly determined by biomass and N content. On the basis of the highest biomass accumulation and N content, the plant N uptake in group C was 88.8 g m^{-2}, which was higher than that in group B (55.4 g m^{-2}), followed by that in group A (30.4 g m^{-2}). Higher plant N uptake usually led to more efficient N removal efficiency in SFCWs. The plant N uptake accounted for 56.47%, 60.25%, and 78.78% of mass N removal in three groups, respectively. Compared with non-biochar-added SFCWs, biochar increased N removal by 82.24% and 192.11% in groups B and C. This significant improvement highlights the direct effect of biochar on N retention in plants.

On the other hand, N transformation and export was influenced by nitrification-denitrification processes. Supporting results was obtained in Table 1. The export of NH_4^+-N was no significant difference among the three groups, even between two phases ($p > 0.05$). The results indicated that biochar did not show apparent adsorption for NH_4^+-N. Previous studies reported that nitrification was inhibited when the temperature was below 10 °C (Werker et al. 2002; Kuschk et al. 2003). While *O. javanica* in three groups retained favorable absorption capability for NH_4^+-N as well as enhanced oxygenation via the rhizosphere (Zhou et al. 2010), resulting in stable NH_4^+-N removal under seasonal temperature variation. The export of NO_3^--N varied according to biochar addition (higher in group A, lower in group C), which demonstrated that the NO_3^--N removal performance was positive correlated with the amount of biochar addition in the present study. This phenomenon could be supported by the results of COD effluent concentration, carbon content change of biochar, and N_2O emission in three groups (Table 1, Figs. 3 and 4). Firstly, there was no significant difference of COD effluent concentration among the three groups, while carbon element content of biochar reduced by 11.31% and 12.19% in group B and group C

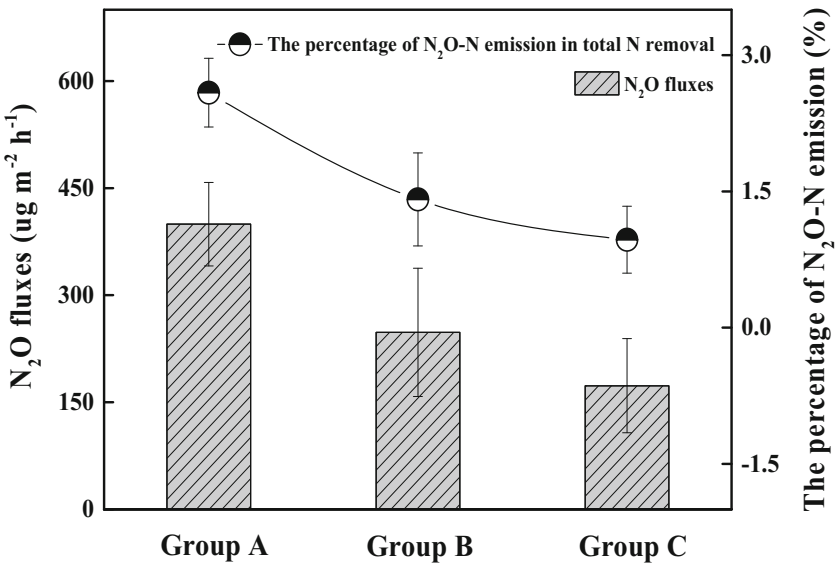

Fig. 4 The average N_2O fluxes and the percentage of N_2O-N emission in TN removal

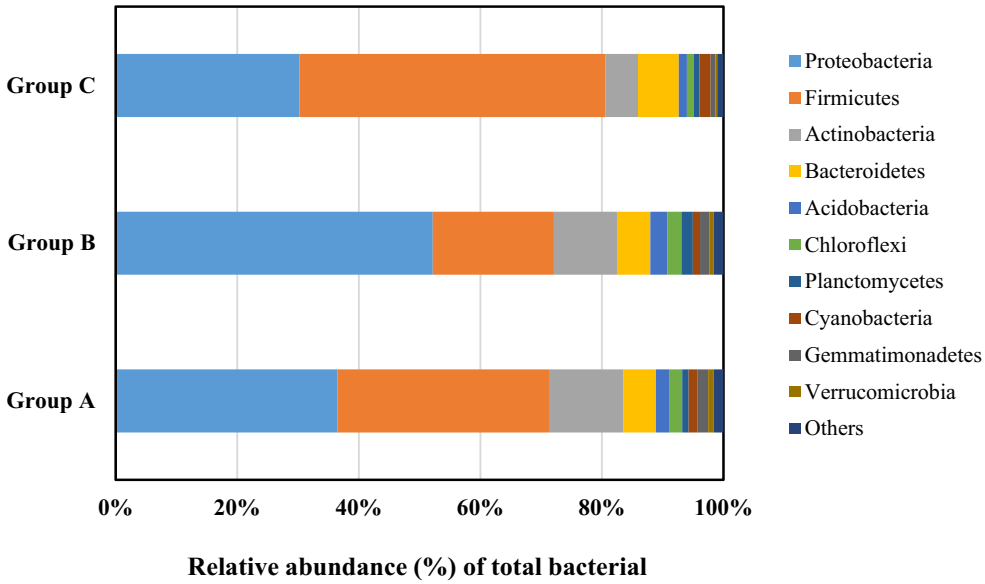

Fig. 5 Bacterial community composition at phylum level as revealed by high-throughput sequencing analyses in three SFCW groups

after the experiment. These results indicated the liable organic carbon release from biochar was consumed for denitrification (Saeed et al. 2012). Secondly, Fig. 4 shows the average N_2O fluxes and the percentage of N_2O-N emission in TN removal. The N_2O emission fluxes in group A was 1.6 and 2.3 times higher than that in groups B and C, indicating that denitrification was completely accomplished due to adequate carbon source released from biochar in groups B and C. Moreover, the characteristics of high porosity and large surface area of biochar could also create more microbial attachment sites for denitrification bacteria (Kizito et al. 2017). The previous study reported that the three typical phyla of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* strains were important for the denitrification process (Miao et al. 2015). As shown in Fig. 5, compared with group A, the total relative abundance values of the three phyla increase by 0.81% in group B and 13.63% in group C, respectively. The result suggested that biochar could increase many types of denitrification bacteria and then heightened denitrification process in SFCWs. Apart from enhanced denitrification process, NO_3^--N export was also influenced by plant presence. Nitrate is also one of the most essential N forms for plant assimilation (Kadlec and Knight 1996), so NO_3^--N produced from nitrification and in influent was converted into organic compounds serving as nutrients for plant growth.

Conclusion

Biochar played an important role in N processing (retention, transformation, and export) of constructed wetlands. It not only could increase the accumulation of plant net biomass, but also released the labile organic carbon source for denitrification bacteria to enhance TN removal. Moreover, the characteristics of high porosity and large surface area of biochar created more microbial attachment sites and correspondingly improved microbial abundance, which resulted in satisfactory N removal performance. During both summer and winter seasons, with the average temperature at 4.9 °C and 19.4 °C, respectively, TN removal efficiencies followed the order of group C > group B > group A. Group B and group C showed a stable treatment performance under seasonal temperature variation. Overall, for N removal from STP effluent, a combined biochar substrate-*Oenanthe javanica* microbe strategy was suitable for application in SFCWs over winter.

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