The simultaneous antibiotics and nitrogen removal in vertical flow constructed wetlands: Effects of substrates and responses of microbial functions

Yingrui Yuan\textsuperscript{a,1}, Baoshan Yang\textsuperscript{a,1}, Hui Wang\textsuperscript{a,1}, Xiaoshuang Lai\textsuperscript{a}, Feng Li\textsuperscript{a}, Mir Md Abdus Salam\textsuperscript{b}, Fuxia Pan\textsuperscript{c}, Yuqiang Zhao\textsuperscript{c}

\textsuperscript{a}School of Water Conservancy and Environment, University of Jinan, Jinan 250022, China
\textsuperscript{b}School of Forest Sciences, University of Eastern Finland, Yliopistokatu 7, P.O. Box 111, 80100 Joensuu, Finland
\textsuperscript{c}Jinan Environmental Research Academy, Jinan, Shandong 250102, China

\textbf{GRAPHICAL ABSTRACT}

\textbf{ARTICLE INFO}

Keywords: Manganese ore substrate, Constructed wetland, Antibiotic wastewater, Functional genes, Microbial composition

\textbf{ABSTRACT}

A vertical flow constructed wetland (VFCW) packed with the different substrates was designed to remediate the antibiotics in the wastewater. Zeolite (CW-Z) paralleled with Manganese (Mn) ore (CW-M) and biochar (CW-C) were used to enhance the synchronous removal of ciprofloxacin hydrochloride (CIPH), sulfamethazine (SMZ) and nitrogen (N) from the wastewater. The result indicated that CW-M had a significant potential to remove CIPH (93%), SMZ (69%), TN (71%), NH\textsubscript{4}\textsuperscript{+}-N (94%) and NO\textsubscript{3}\textsuperscript{-}-N (94%) across all the treatments. The abundance of amo\textsubscript{A}, nirK and nirS genes are dramatically higher in CW-M and CW-C, while CW-C inhibited the production of quinolone resistance genes. Results showed that different substrates could affect the microbial diversity and structure. The addition of Mn ore to the water led to an improved abundance of nitrogen-related phyla. Overall, Mn ore has a considerable potential to simultaneously remove antibiotics and N in VFCWs.

\textbf{1. Introduction}

Antibiotics are continuously releasing into the environment as a result of the frequent uses of antibiotics in clinical therapy, disease prevention, and the promotion of animal growth (Lien et al., 2016). Recently, the negative impacts of antibiotics dissemination have been a great concern due to the potential eco-environmental problems and hazard to human health (Zhang et al., 2015). Antibiotics can also...
promote the production and spread of antibiotic resistance genes (ARGs), which has been proved to be a global public health crisis (WHO, 2014). The small-scale wastewater treatment plants are the primary sources of antibiotics because the contaminated antibiotics discharged effluents are not appropriately managed in terms of environmental considerations (Osińska et al., 2019). Currently, there are no particular suitable technologies available to remove antibiotics from existing wastewater treatment plants (Cai et al., 2018). Therefore, it is crucial to find cost-efficient and sustainable, environmentally friendly solutions to overcome the problem of antibiotics in the wastewaters.

Constructed wetlands (CWs) have been designed and built based on the natural wetlands, which have been widely used to remove the antibiotics from the different sources of wastewater (Carvalho et al., 2014). However, antibiotic removal efficiency in the CWs is varied due to the complexity of CWs configurations and antibiotic species. Liu et al. (2019) reported that, the characteristics of the upper aerobic layer and the lower anoxic layer in the vertical flow constructed wetlands (VFCWs) provide a favorable environment for the bacteria to degrade the antibiotics. Thus, VFCWs are more pertinent in regard to antibiotic removal compared to the horizontal flow formed wetlands (HFCWs). Various antibiotics exhibit different removal properties. Liu et al. (2013) reported that quinolone and tetracycline antibiotics could be more easily degradable than sulfamidines and chloramphenicol. The main reason is that they could quickly adsorb through the substrate and photodegrade when exposing to ambient conditions. Antibiotics can persist a long time in the wastewaters and need to solve this issue more cost-efficiently. Although some helpful ecological techniques such as CWs have been used to remove antibiotics (Liu et al., 2019), but still, the knowledge of CWs is not updated, and it needs a proper evaluation to improve antibiotics removal efficiency from the complex wastewater. Furthermore, antibiotics rarely occur alone in the polluted waters. The co-occurrence of antibiotics and the excess amount of nutrients, especially N, in the sewage has been extensively detected at large-scale across the world (Tong et al., 2019). Due to low operation cost and convenient maintenance system, the CW method termed as a natural convenient maintenance system, the CW method termed as a natural environmental consideration (Osińska et al., 2019). Therefore, it is crucial to update the knowledge of CWs is not updated, and it needs a proper evaluation to improve the removal efficiency of antibiotics and N simultaneously is not explored yet.

Substrates of CWs are the critical components, which provide a reactive zone to remove pollutants and facilitate suitable habitat for the microorganisms that are actively involved to remove the contaminant from the wastewater (Wang et al., 2019; Xu et al., 2019). The substrates also accelerate physicochemical and biological processes that further aid to improve the synchronous removal of antibiotics and N. The study on N removals from the wastewater are ubiquitous, whereas the simultaneous extraction of antibiotics and N are still limited. In the CWs configuration, some cheap and environmentally friendly substrates such as manganese (Mn) ore and biochar were used to improve pollutant removal efficiency. These substrates also have other suitable characteristics such as large specific surface areas and porosity, which could provide a favorable site for biofilm adhesion and pollutant adsorption (Li et al., 2019; Liu and Zhang, 2009; Yang et al., 2019). Mn ore consisting of Mn oxides can be considered as a natural filter material, which not only plays a crucial role to adsorb contaminant but also effectively transforms and removes organic pollutants from the environment due to its high redox potential (Yang et al., 2019). However, although Mn (II), the reduction products of Mn oxides, is a hazardous metal, its concentration is lower than the drinking water standard in China after the treatment of CWs filled with Mn ore (Xie et al., 2018). Biochar is derived from the pyrolysis of waste biomass under limited oxygen condition, in which the incomplete pyrolysis of biomass can release dissolved organic carbon to provide a carbon source for microbial denitrification during N removal in CWs (Li et al., 2018). However, so far to our knowledge, the effects of biochar on the efficient simultaneous removal of antibiotics and N are still not studied. Therefore, consequences and mechanisms of both Mn ore and biochar as the preferred substrate in the configuration of CWs are needed to investigate for the simultaneous removal of antibiotics and N.

Substrates materials in CWs not only play a critical role removing pollutant, but also affect the formation of biofilm, resulting in the variation of functional microorganisms, which differ the removal efficiencies of N and organics (Wang et al., 2019). Biodegradation is the most crucial process in N removal in CWs (Huet et al., 2005). The previous studies have indicated that ammonia monoxygenase (amoa), nitrite oxidoreductase (nxaR), nitrite reductases (nirK/nirS), nitrous oxide reductase (nosZ) are critical functional genes during the N removal, among which gene amoa catalyzes NH4+ to NO3−, nxaR converts NO2− into NO3−, nirK and nirS involve the first step of denitrification which catalyze NO3− to N2O and N2 and eventually nosZ converts N2O to N2 (Wang et al., 2015). In addition, anammox 16S rRNA genes (Anmx) are the leading functional genes which can directly convert NH4+ and NO2− into N2 under anoxic conditions (Humbert et al., 2012). However, the presence of antibiotics can affect the genetic structure of microbial communities (Huang et al., 2017). Therefore, it is of considerable significance to explore the changes of the microbial community and function in CWs with different substrates configuration during the simultaneous removal of antibiotics and N.

In this study, we investigated the effects of substrates on the removal efficiencies of antibiotics (combined ciprofloxacin hydrochloride and sulfamethazine) and N in VFCWs. The resulting changes of microbial compositions and functions in the substrates of VFCWs were systematically explored via MiSeq sequencing and real-time polymerase chain reaction (RT-PCR). The objectives of the present study were (1) to compare the removal capacities of antibiotics and N in laboratory-scale VFCWs packed with different substrates; (2) to reveal the changes of bacterial community compositions and abundances of N-cycle functional genes resulting from different substrates in the VFCWs and the occurrence of ARGs; and (3) to identify the relationship between microbial communities and the simultaneous removal efficiencies of antibiotics and N in the VFCWs.

2. Materials and methods

2.1. Substrates

The five substrates, including cobblestone, zeolite, Mn ore, biochar, and quartz sand used in the experiment, were collected from Yuan Run Water Purification Material Co., Ltd (Zhengzhou, China). The physical characteristics of the used substrates were determined before packed in lab-scale wetlands. The diameters of cobblestone, zeolite, manganese ore and fruit stone biochar ranged 7–10 cm, 8–10 mm, 4–8 mm and 2–4 mm, respectively. The main characteristics of zeolite were as follows: BET specific surface area 1.22 m2/g, average pore diameter 22.11 nm, pore volume 5.65 mm3/g, and the corresponding physical characteristics of Mn ore and biochar were 77.59 m2/g, 4.46 nm, 6.70 mm3/g and 64.20 mm3/g, 5.44 nm, 6.28 mm3/g, respectively. Furthermore, the Mn content in Mn ore is more than 40%, and the soil used in the experiment was collected from farmland in Changqing District, Jinan, Shandong Province.

2.2. Experimental setup and VFCWs operation

As shown in Fig. 1, three parallel down-flow VFCWs with identical dimensions were designed using plexiglass with a length of 50 cm and a width of 40 cm. The total depth of the VFCWs was 60 cm with a working volume of 30 L according to the size of the container. All the VFCWs were wrapped with fiberglass mesh to prevent light from
penetrating the substrate. In this study, the three VFCWs were coded as CW-Z, CW-M, and CW-C, respectively. The bed of CW-Z was sequentially packed from bottom to top with 10 cm of cobblestone as a support layer, 30 cm of zeolite as the main substrate layer, which could provide adsorption sites for microorganisms and contaminants, 5 cm of quartz sand to isolate the soil from substrates, and 10 cm of the soil layer to grow plants. The substrates of CW-M and CW-C were the same as CW-Z besides the zeolite layer which was supplemented with Mn ore or fruit stone biochar, respectively in a volume ratio of 1:1.

Three VFCWs were set up in a greenhouse at the University of Jinan. The experiment was divided into two stages according to the water temperatures to explore the influence of temperature on the removal of pollutants in VFCWs with different substrates. Water temperatures were maintained to keep at 20 ± 2°C and 27 ± 2°C in the first stage (the early sixty days) and the second stage (the next sixty days), respectively. Before starting the experiment, a total of 90 L secondary wastewater collected from the China Everbright water treatment plant was synthetically created by adding 89 mg/L NH4Cl, 16 mg/L KNO3, 10 mg/L KH2PO4, 10 mg/L CaCl2, and 5 mg/L MgSO4 according to Zhi et al. (2015). Further, the resulted wastewater was pumped into the system in a down-flow mode at a hydraulic retention time (HRT) of 72 h after one month of planting Phragmites communis. Each system was manually drained after a cycle (72 h) and then re-filled with the new wastewater immediately, which formed the mode of the fills-and-draw batch with a hydraulic loading rate of 0.050 m3/(m2d). The duration of the wastewater treatment experiment was 120 days (April 2018 to July 2018). The polluted influent water was synthetic wastewater that was prepared with tap water and a mixture of 333 mg/L glucose, 89 mg/L NH4Cl, 16 mg/L KNO3, 10 mg/L KH2PO4, 10 mg/L CaCl2, and 5 mg/L MgSO4 according to Zhi et al. (2015). Further, the resulted mixture was added with a relatively high level of ciprofloxacin hydrochloride (CIPH) at the rate of 50 μg/L and sulfamethazine (SMZ) at the rate of 50 μg/L, respectively. We selected elevated levels of antibiotic resistance genes in the experiment to explore the microbial response towards antibiotics. The average contaminant concentrations in the influent are shown in Table 1. During the operation, the three VFCWs were implemented intermittent aeration with the airflow rate of 0.8 ± 0.2 L/min for 4 h (0–1, 6–7, 12–13 and 18–19 h) every day, as described by Lai et al. (2020).

2.3. Water sampling and analysis

The effluent samples were taken from the bottom of the microcosms simultaneously with the collection of the influent sample for the measurements of COD, NH4+-N, NO3--N, and TN according to protocols as described in APHA (2005). Antibiotics (CIPH and SMZ) concentrations in the effluents were detected every six days by high-performance liquid chromatography (Agilent 1260, USA), and the operating conditions were described in E-supplementary data. The dissolved oxygen (DO) values and water temperature of each sample were measured using a portable DO meter (Eutech, CyberScan DO100, USA) and the pH values were determined by a pH meter (DENVER UB-7, USA). All the tests of the target parameters were performed in triplicate for each sample.

2.4. DNA extraction and gene quantification

The rhizosphere soil and biofilms forming on the substrates were collected from each unit after running 120 days. The biofilms were eluted according to the methods described by Liu et al. (2014) and pooled with soil samples. Microbial DNA was extracted by DNeasy PowerSoil Kit (QIAGEN, Germany) according to the manufacturer’s protocols. Each sample had three replicates. The extracted DNA was stored at −20°C before further analysis.

Six functional genes for N transformation (amoA, nirK, nirS, nxrA, nosZ and Amx) and five antibiotic resistance genes (ARGs) including quinolone resistance genes (qnrA/qnrS), sulfonamide resistance genes (sulI/sulII) and the class 1 integron gene (intI) were quantified with RT-PCR system (BIO-RAD, CFX Connect, USA). The RT-PCR information on primer sequence, amplicon length, and annealing temperature are described in Table S3. Six functional genes were targeted using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). The 5′ ends of the primers were tagged with specific barcode per sample. Illumina MiSeq sequencing was conducted at LC-Bio Technology Co., Ltd (Hang Zhou, Zhejiang Province, China) to analyze the microbial communities. High-quality chimeric sequences were filtered using Vsearch software (v2.3.4). Operational Units (OTUs) were clustered using UPARSE (version 7.1) at 97% sequence identity.

2.5. Microbial community analysis

The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). The 5′ ends of the primers were tagged with specific barcode per sample. Illumina MiSeq sequencing was conducted at LC-Bio Technology Co., Ltd (Hang Zhou, Zhejiang Province, China) to analyze the microbial communities. High-quality chimeric sequences were filtered using Vsearch software (v2.3.4). Operational Units (OTUs) were clustered using UPARSE (version 7.1) at 97% sequence identity.

2.6. Statistical analysis

All statistical analyses were done using software SPSS 19.0 (SPSS
Inc., Chicago, USA). One-way analysis of variance (ANOVA) with multiple Turkey comparison tests was performed to assess the significant differences at \( p < 0.05 \) among water and microbial parameters in three VFCWs. To test the correlation between bacterial communities and removal rates of antibiotics and N, redundancy analysis (RDA) was performed by CANOCO 5.0. The absolute abundances of genes were expressed in the denary logarithm form. The Alpha diversity indices of the bacterial communities were conducted by QIIME (Version 1.8.0). All the figures were produced using Origin 9.0 (OriginLab Inc., USA).

3. Results and discussion

3.1. Effects of substrate configurations on antibiotics removal

The effluent concentrations and removal rates of the selected antibiotics CIPH and SMZ in three VFCWs compared here are depicted in Fig. 2. In general, the VFCWs showed a significant effect on CIPH removal (> 80%). The highest removal rates of pollutants were found in the CW-M treatment (93.70%), followed by CW-C (88.05%) and CW-Z (83.71%) respectively. The higher removal rates of CIPH in the CW-M and CW-C were attributed to the physical characteristics of larger specific surface area and more micropores in both Mn ore and biochar, which provided the possibility to attach contaminants and microbes and facilitated the degradation of antibiotics. However, all of the containers were wrapped with fiberglass mesh that might inhabit the photodecomposition process, which led to slightly reduce the overall removal rate of CIPH. In contrast, Li et al. (2019) and Tan et al. (2015) reported Mn ore contains many metal cations, which could improve the adsorption of amphoteric ciprofloxacin. (The average removal rates of SMZ in CW-Z, CW-M and CW-C were 48.85%, 69.38%, and 56.57%, respectively (Fig. 2A and 2B). The highest removal rate of SMZ was less than 70%, which is significantly lower as compared to the removal rate of CIPH pollutants across all treatments. These results support the findings of Liu et al. (2013), who indicated that the removal rates of sulfonamide antibiotics in CWs were much lower as compared to the removal rates of quinolone antibiotics. The highest removal rate of SMZ was also observed in the treatment CW-M. The reason may be described that Fe and Mn oxides in Mn ore have high redox potential, and they can actively participate in the degradation of antibiotics when the redox occurs (Xie et al., 2018). Another reason could be that sulfonamides tend to be adsorbed by electronegative clays because sulfonamides provided as cations at low pH levels (Kurwadkar et al., 2007). In the present study, the pH value was 6.6 in CW-M (Table 1), which may improve the removal rate of SMZ. In addition, Peiris et al. (2017) also found that biochar has strong adsorption to sulfonamide antibiotics at mildly acidic pH because the antibiotics dominantly exist as zwitterionic or cationic species. It was consistent with our result that the removal of SMZ in CW-C was higher than that in CW-Z although it was lower than CW-M.

3.2. Effects of substrate types on N removal

CWs can remove N through the interaction of microorganisms, substrates and plants (Li et al., 2018). In this study, the same amounts of Phragmites communis were planted in each VFCW. After harvesting, we didn’t find any significant differences between the total plant biomass production value in a VFCW and total plant biomass production value in another VFCW (these data can be found in E-supplementary data). This result would indicate that the plant growth didn’t affect the N removal process and plant took up an amount of N in equal measure in each VFCW. Compare to Filipa et al. (2019), who planted a considerable density of plants (80P. australis per microcosm) to investigate the effects of plants on CWs, Phragmites communis didn’t also take up an excessive amount of N in this study.

The water parameters and pollutant removal rates of three VFCWs are presented in Table 1. The removal rates of TN, NH\(_4\)\(^+\)-N, and NO\(_3\)\(^-\)-N fluctuated during the experimental period (Fig. 3). The average TN removal rates were significantly higher (\( p < 0.05 \)) in the CW-M (71.71%) and CW-C (62.98%) treatments compared to the CW-Z (58.23%) treatment. The results are consistent with the findings of the previous studies, which illustrated that the CW substrate with larger

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Effluent</th>
<th>Removal rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CW-Z</td>
<td>CW-M</td>
<td>CW-C</td>
</tr>
<tr>
<td>TN (mg L(^{-1}))</td>
<td>40.28 ± 0.77</td>
<td>17.13 ± 1.10</td>
<td>11.60 ± 1.03</td>
</tr>
<tr>
<td>NH(_4)(^+)-N (mg L(^{-1}))</td>
<td>30.56 ± 0.26</td>
<td>4.28 ± 0.55</td>
<td>1.57 ± 0.64</td>
</tr>
<tr>
<td>NO(_3)(^-)-N (mg L(^{-1}))</td>
<td>3.15 ± 0.38</td>
<td>0.16 ± 0.10</td>
<td>0.18 ± 0.10</td>
</tr>
<tr>
<td>COD (mg L(^{-1}))</td>
<td>307.93 ± 19.53</td>
<td>58.74 ± 14.48</td>
<td>49.79 ± 12.34</td>
</tr>
<tr>
<td>DO (mg L(^{-1}))</td>
<td>9.17 ± 0.12</td>
<td>2.21 ± 0.71</td>
<td>2.22 ± 0.75</td>
</tr>
<tr>
<td>pH</td>
<td>7.13 ± 0.30</td>
<td>7.33 ± 0.13</td>
<td>6.64 ± 0.18</td>
</tr>
</tbody>
</table>

All the data represent mean ± SD (n = 30). Different letters next to each parameter represent significant differences among the VFCWs (\( P < 0.05 \)).
N may be attributed to the gradually increased bio
5 mg/L and 50 mg/L for TN, NH4
(14.78 mg/L, 1.86 mg/L and 41.08 mg/L, respectively) could meet
(11.6 mg/L, 1.57 mg/L and 49.79 mg/L, respectively) and CW-C
NO3
significantly higher in CW-M than those of CW-C, although there was no statistically
nitri
However, according to the report of Zhai et al. (2016), the
SMZ (Fig. 2A and 2B). These results confirmed that both antibiotics
catalyze from NH4
2. Similar to the findings of Wang and Li (2015), the
NH4
–N and NO3
–N were increased by 7.47% and
3.3. Dissimilarity of N functional genes and ARGs in three VFCWs
Contributions of microorganisms to N removal are often reflected by the
abundances of amoA, nxxA, Amx, nirK, nirS and nosZ (Zhang et al.,
2018b). The absolute abundances of N transformation functional genes in three microcosms are shown in Fig. 4A. Ammonia monooxygenase and nitrite oxidase commonly have high-activities in aerobic conditions. Ammonia monooxygenase is coded by the amoA gene and can catalyze from NH4
–N to NO2
–N. Similarly, nitrite oxidase is encrypted by the
nxxA gene, which can catalyze from NO2
–N to NO3
–N (Dionisi et al.,
2002). The higher abundances of amoA and nxxA were found in CW-M and CW-C, while lower abundances of these genes were detected in CW-Z. The results showed that addition of Mn ore and biochar was beneficial to promote the growth and breeding of nitrifying bacteria that facilitated to improve removal efficiencies of NH4 + -N in CW-M and CW-C rather than CW-Z (Table 1). Amx is the representative gene involved in anammox (Humbert et al., 2012). Compared to CW-Z and CW-M, the copy numbers of Amx were significantly high in CW-C (Fig. 4). However, although CW-C showed higher abundances of Amx in CW-C but the higher NH4
–N removal rate was found in CW-M. This result implied that the growth of anaerobic bacteria was inhibited, and
the functions of anammox were not well exerted in CW-C. Another possible reason could be that biochar has a porous structure and allows the functions of anammox were not well exerted in CW-C. Another possible reason is that biochar releases carbon resources which could increase the abundances of functional genes contributing to removal of excess N from the wastewater.

Microbes may produce ARGs under the selective pressure of antibiotics, which is a drawback of all biological systems. In this part, the diversities of microbial communities were significantly lower in CW-C than CW-Z and CW-M (Table 2). This may be related to the various selection pressures of antibiotics to bacterial communities in different substrates in CWs. Huang et al. (2017) demonstrated that some bacteria that were highly sensitive to antibiotics were eliminated, and others were enriched under the chronic stimulus of antibiotics, which may cause the decline of bacterial diversity. Besides, the int1 gene abundance can reflect microbial susceptibility to antibiotics (Fonseca and Vicente, 2012). The number of int1 gene was the highest in CW-C (Fig. 4A), and this indicated that bacterial communities in CW-C were more sensitive to antibiotics.

The removal of pollutants by substrate microorganisms is the critical mechanism in CWs (Huett et al., 2005). After 120 days of operation, a total of 45 phyla were found in three VFCWs. The top 20 phyla of high relative abundance among the VFCWs were shown in Fig. 5. Proteobacteria (47.14%–62.26%) was the dominant phylum in all systems, followed by Candidatus Saccharibacteria (9.85%–22.67%), Actinobacteria (9.02%–12.69%), Acidobacteria (3.12%–7.60%), Bacteroidetes

![Fig. 4. The copy numbers (natural logarithm) of functional genes related to nitrogen metabolism (A) and six resistant genes (16S rRNA, int1, qnrA, qnrS, sulI and sulII) (B) in each system based on q-PCR analysis. Error bars are the standard errors.](image-url)

Table 2

<table>
<thead>
<tr>
<th>OUT number</th>
<th>Richness estimator</th>
<th>Diversity index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed species</td>
<td>Shannon Simpson</td>
</tr>
<tr>
<td>CW-Z</td>
<td>3061</td>
<td>2996.33</td>
</tr>
<tr>
<td>CW-M</td>
<td>3267</td>
<td>3248.33</td>
</tr>
<tr>
<td>CW-C</td>
<td>3008</td>
<td>2953.33</td>
</tr>
</tbody>
</table>

Different letters next to each parameter represent significant differences among the VFCWs.
1.79%–5%) and Firmicutes (0.84%–5.82%). Proteobacteria has been proved to be an essential member of the removal of nutrients and some organic pollutants (Yan et al., 2017). The highest abundance of Proteobacteria in CW-M may conduce to the best removal rates of antibiotics and N (Fig. 2 and Table 1). Candidatus Saccharibacteria has been frequently detected in activated sludge and plays an important role in the degradation of organic and sugar compounds (Kindaichi et al., 2016). However, the relative abundance of Candidatus Saccharibacteria in CW-M was considerably lower than that in CW-C, which was consistent with the lower removal efficiency of COD in CW-M than CW-C (Table 1). Furthermore, a previous study has shown that the abundances of Actinobacteria, usually not the dominant phylum in CWs, increased in the substrates exposed to antibiotics (Filipa et al., 2019). As a degrader of multifunctional hydrocarbon, Actinobacteria was proved to be a supporter for the growth of bacterial communities involved in the removal of antibiotics and N.

3.5. The correlation of the simultaneous removal of antibiotics and N and microbial community

Redundancy analysis (RDA) was conducted based on eight phyla with relative abundance greater than 1% and removal performances of NH₄⁺-N, NO₃⁻-N, TN, COD, SMZ and CIPH to investigate the relationship between microbial communities and environmental factors. As shown in Fig. 6, three VFCWs were obviously separated into three phases. The angle indicated that the removal rate of SMZ and CIPH were positively correlated with Proteobacterial, Actinobacterial, and Actinobacteria. Notably, CW-M was most conducive to the growth of Proteobacteria, while the Proteobacteria was positively correlated with the removal of CIPH and SMZ antibiotics and N. It has been reported that Proteobacteria can degrade fluoroquinolone antibiotics in sewage (Amorim et al., 2014). The RDA results also confirmed that the high removal rates of CIPH and SMZ in CW-M may be ascribed to Proteobacteria. In addition, the removal rates of NH₄⁺-N, NO₃⁻-N, and COD were positively with most of the phyla except for Gemmatimonadetes, Firmicutes, and Acidobacteria. A similar kind of result was found in the treatment CW-M and treatment CW-C (Fig. 6). Actinobacteria dominated the conversion of NH₄⁺-N and NO₃⁻-N. Our result was consistent with the findings of Chen et al. (2017), who reported that Actinobacteria played a vital role in denitrification, corresponding to the observed smaller angles with NH₄⁺-N and NO₃⁻-N (Fig. 6). The arrows of Planctomycetes, Candidatus Saccharibacteria and Bacteroidetes showed a positive correlation with the removal rate COD and they were distributed in CW-C, explaining that the highest COD removal rate in CW-C may be associated with the multiplication of functional microorganisms. Therefore, RDA results verified that substrate type in CW-M and CW-C had significant impacts on bacterial community compositions, which played a critical role in the high removal of antibiotics and N.

3.6. The application in practical engineering

This research shows that Mn ore has great potential in the removal of nitrate nitrogen and antibiotics and can be recommended for the use on full scale CWs especially for the treatment of medical and aquaculture wastewater, which have been confirmed to contain high concentrations of antibiotics (Lien et al., 2016). In addition, the results illustrate the potential application of biochar in wetland substrate to inhibit the production of quinolone resistance genes and should be further examined under different operational. Thus, it is critical for the screening of substrates from the various waste biomass for the future operation of constructed wetlands.

4. Conclusions

CW-M exhibited the optimal removal rates of ciprofloxacin hydrochloride, sulfamethazin and nitrogen, which was ascribed to higher affinity and redox condition, as well as more abundant functional microorganisms attached by Mn ore. Biochar inhibited the production of quinolone resistance genes and improved COD removal efficiency by increasing the abundances of related microorganisms. Both Mn ore and biochar increased the absolute abundances of the amoA, Aox, nirK, and nirS, thus enhancing the N removal efficiencies in CW-M and CW-C. In a word, both Mn ore and biochar reduced the negative effects on the simultaneous removal of antibiotics and N in VFCWs.

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (grant number 31870606, 41877424, 41801089), Natural Science Foundation of Shandong Province, China (grant number ZR2017MD022, ZR2018MD002) and the founding project of Jinan Environmental Research Academy (2017214).
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.123419.

References


