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Effects of Different Submerged Macrophytes on the Water and Sediment in Aquaculture Ponds with Enrofloxacin Residues

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Abstract: Submerged macrophyes have been widely used to restore aquaculture ponds in recent years. Yet, whether the residual antibiotics in ponds will affect the remediation effect of submerged macrophyes, and the effect of different submerged macrophyes on the water and sediment in aquaculture ponds with antibiotic residues, is unclear. A microcosm experiment was carried out to study the interaction between three kinds of submerged macrophytes and their growing environment with antibiotic residues. *Ceratophyllum demersum* L. with no roots, *Vallisneria spiralis* L. with flourish roots, and *Hydrilla verticillata* L with little roots were chosen to be planted in the sediment added with enrofloxacin (ENR). The growth of submerged macrophytes, the changes of the overlying water and sediment characteristics, and the microbial community in the sediment were analyzed. The results showed that according to the growth rate and nutrients accumulation ability, *V. spiralis* with flourish roots performed best among the three submerged macrophytes. The concentrations of TOC, TP, NH_4^+-N, and TN in the overlying water were 25.0%, 71.7%, 38.1%, and 24.8% lower in the *V. spiralis* treatment comparing with the control, respectively. The richness and diversity of the microorganisms in the sediment of *V. spiralis* treatment were significantly higher than those in the control, but this advantage was not obvious in the *H. verticillata* treatment. *V. spiralis* promoted the growth of *Proteobacteria* (22.8%) and inhibited the growth of *Acidobacteria* (32.1%) and *Chloflexi* (31.7%) in the rhizosphere sediment with ENR residue. The effects of the three submerged macrophytes on the removal of ENR from sediment were not reflected due to the limitation of water depth. Compared with *C. demersum* and *H. verticillata*, *V. spiralis* was more suitable for the remediation of the aquaculture ponds with ENR residue.

Keywords: aquaculture pond; submerged macrophytes; remediation; sediment; enrofloxacin residues

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1. Introduction

Aquaculture has become the main source of aquatic protein for humans [1]. Its rapid development faces challenges, such as disease control and environmental pollution, especially in high-density aquaculture farms [2]. The negative effects of nutrients released by aquaculture to aquatic ecosystems are increasingly attracting human attention. On the one hand, dissolved inorganic nutrients such as ammonia and phosphate produced by aquaculture can be absorbed by phytoplankton and macroalgae, which can stimulate their growth. The enhanced production may lead to harmful algal blooms [3]. On the other hand, antibiotics are added into aquaculture ponds, one side for diminishing disease appearance, another for increasing the survival and mean weight of animals [4], while aquaculture ponds polluted by over-used antibiotics are considered to be a notorious source of antibiotic resistance genes (ARGs), which are harmful for the environment and humans and have been recognized as one of the most critical public health concerns of the 21st century by the World Health Organization [5,6]. Xu et al. [7] demonstrated that antibiotics significantly changed the microbial composition of the rhizosphere and had a profound effect on the nitrogen and carbon cycling in the microenvironment formed by the plant–microbe system. Antibiotics also can be taken up by aquatic organisms and retained in their bodies. Currently, antibiotics have been detected in various aquatic organisms, such as fish, bivalves, gastropods, crustaceans, and macroalgae [8,9]. Ecotoxicological studies indicate that antibiotics may cause genotoxicity, phototoxicity, and oxidative stress in aquatic organisms [6]. Previous studies showed that the administration of antibiotics to fish can have great impact on gut microbial community by reducing their diversity and number [10]. ENR affected the carbon fixation and assimilation processes of algae (*Scenedesmus obliquus*) by damaging their photosystem [11], and high concentration of ENR triggered metabolic disturbance in rice seedlings [12].

To date, the treatment of wastewater from aquaculture has received widespread attention. Lots of physical, chemical, and biological technologies such as constructed wetlands, microalgae, submerged macrophytes, ultraviolet photolysis, and immobilized microbial granules were researched for the removal of nutrients or antibiotics from aquaculture [11,13–16]. Submerged macrophytes are an environmentally friendly, effective, and low-cost restoration technology. They can remove nutrients (especially N and P) from both water and sediment [14]. A previous study showed that a subtropical aquacultural lake was restored successfully by submerged macrophytes through controlling the internal phosphorus loading [17]. As a result, more and more aquaculture farms choose planting submerged macrophytes in aquaculture ponds to control the water and sediment quality.

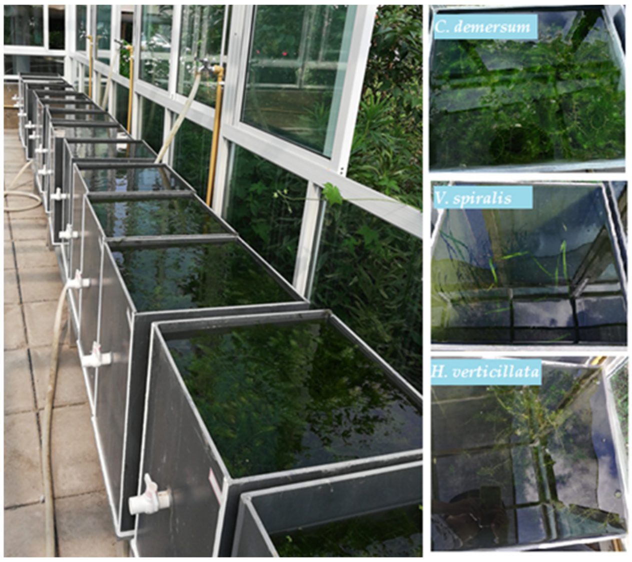
The residue antibiotics in the aquaculture ponds may influence the growth of submerged macrophytes, leading to different remediation effects. In a recent review, antibiotics were concluded to increase the root activities and exudates of plants in constructed wetlands [18]. Xu et al. [7] showed that low-dose antibiotics (sulfamethoxazole and ofloxacin) increased the root activity of *Cyperus involucratus*, but high-dose antibiotics (higher than 1 mg/L) produced toxic stress effects. The effects of ENR on *V. spiralis* also show the pattern of "low-dose-promotion and high-dose-inhibition" [14,19]. Thus, the effects of antibiotics on plants may be related to antibiotic concentrations and plant species. The growth and water purification efficiencies of different submerged macrophytes need to be compared when being used to remediate the aquaculture ponds with antibiotic residues. In turn, the growth and nutrients absorption of submerged macrophytes also changed the physi-chemical condition and microbial community in the aquaculture ponds, which may affect the pollutants (including antibiotics) removal from the water and sediment. Thus, the interaction among the submerged macrophytes, water and sediment characteristics, and the microbial community in the aquaculture ponds with antibiotics, needs more research.

As a synthetic third generation quinolone broad-spectrum antibiotic, ENR has been reported to be used in 5 among the 6 top aquaculture-producing countries [20]. So, it was chosen in this study. The main objectives of this study were to: (1) evaluate the effects of ENR on the growth of submerged macrophytes with distinct root morphology; (2) assess the changes of the overlying water and sediment characteristics; and (3) explore the microorganism in the sediment influenced by the plant species and ENR residues.

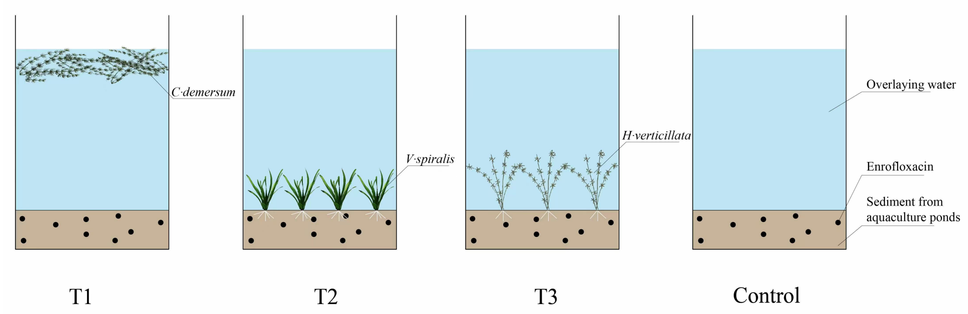
2. Material and Methods

2.1. Experimental Mesocosms

Twelve PVC tanks (0.4 m $\times$ 0.4 m $\times$ 0.6 m) were used for the experiment in a transparent glass greenhouse located in the downtown area of Shanghai, China (Figure 1a). Four treatments were set up with three replicates for each: (1) T1: planted with *Ceratophyllum demersum* L. (*C. demersum*); (2) T2: planted with *V. spiralis*; (3) T3: planted with *Hydrilla verticillata* L. (*H. verticillata*) and (4) unplanted treatment (control). These three kinds of submerged macrophytes were common and easily obtained, but they have a distinct morphology (Figure 1b). The roots of *V. spiralis* are fine and flourish. The stems of *H. verticillata* can survive and grow new roots. *C. demersum* has no root. One hundred g fresh weight of submerged macrophytes were planted in each tank.



(**a**)



(**b**)

Figure 1. (**a**) The experimental images. (**b**) Schematic of the experimental set-up.

The top 10 cm of the sediment in a shrimp pond of Fengxian in Shanghai was collected and mixed thoroughly. The sediment characteristics were as follows, total nitrogen (TN) 2.36 ± 0.18 g/kg DW, total organic carbon (TOC) 25.0 ± 2.95 g/kg DW, total phosphorus (TP) 1.66 ± 0.21 g/kg dry weight (DW), zinc (Zn) 141.5 ± 12.8 mg/kg DW, copper (Cu) 70.1 ± 6.01 mg/kg DW, lead (Pb) 28.6 ± 2.37 mg/kg DW, chromium (Cr) 40.4 ± 4.23 mg/kg DW, cadmium (Cd) 0.35 ± 0.03 mg/kg DW and arsenic (As) 6.38 ± 0.47 mg/kg DW, respectively. A 6 cm layer of sediment (about 7.5 kg of wet weight) was laid in each tank, and then 50 mL of ENR solution with a concentration of 100 mg/L was added and mixed with the sediment evenly. Then, the tanks were filled with tap water and which was replenished weekly to compensate for the evaporated water. During the experimental period, the water temperature was between 19.2 and 33.9 °C with an average value of 26.8 °C.

2.2. Sampling and Analysis

The experiment was carried out from 1 June to 20 September 2018. Water samples were collected and analyzed every 4–14 days. The mixture of the wet sediment samples from the center and four corners of each tank were collected at the beginning and end of the experiment. Part of the sediment was freeze-dried for the characteristics determined, and the rest was stored at −80 °C until microbial community analysis. Detailed determination methods for water (TOC, TP, TN and NH_4^+-N) and sediment (TN, TP, TOC, ENR and CIP) samples were described in our previous study [14]. All of the submerged macrophytes were harvested and weighed at the end of the experiment. The samples were powdered after freeze-drying (FD-1A-50, Hanno, Shanghai, China). Then, the N and P concentrations were determined according to Zhang et al. [14].

2.3. DNA Extraction and High throughout Sequencing

The analysis of microbial community in the sediment samples was using High Throughput Sequencing technology. DNA extraction and Illumina MiSeq sequencing were conducted by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). DNA extraction was performed using E.Z.N.A. Soil DNA Kit (Omega, Bio-tek, Norcross, GA, USA). The DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The 16S rRNA V3 and V4 regions were amplified with two universal primers: 338F primer (ACTCCTACGGGAGGCAGCAG) and 806R primer (GGACTACHVGGGTWTCTAAT) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, Los Angeles, CA, USA). Sequencing was performed using the Illumina MiSeq system (Illumina, San Diego, CA, USA), following the manufacturer's instructions. The data were analyzed on the free online platform of Majorbio I-Sanger Cloud Platform (cloud.majorbio.com (accessed on 20 November 2019)). The detailed analysis methods in this section were introduced in Nie et al. [21].

2.4. Statistical Analysis

Differences in the growth of the three kinds of submerged macrophytes and the characteristics of the sediment were evaluated by one-way ANOVA. Two-way ANOVA at the level of 0.05 with treatments and sampling time as factors was performed to compare the water parameters among the four treatments. All the statistical analysis and graphing were carried out using SPSS 24.0 and Origin 8.0.

3. Results and Discussion

3.1. Growth of Submerged Macrophytes

Fresh weight and relative growth rate (RGR) are important indicators for plant growth. It can be seen from Figure 2A that the harvested fresh weight and RGR of *V. spiralis* (1.76 ± 0.37 kg/m^2, 0.0093 ± 0.0020 d^{-1}, respectively) were significantly higher than those of the other two species ($p < 0.05$). Comparing the nutrients accumulation of the three submerged macrophytes, the accumulated N of *V. spiralis* (2.93 ± 0.73 g/m^2) and *H. verticillata* (3.10 ± 0.39 g/m^2) were significantly higher than that of *C. demersum* (1.29 ± 0.30 g/m^2, $p < 0.05$). The P accumulated ability of *V. spiralis* (2.64 ± 0.55 g/m^2) was also much higher than that of *C. demersum* (1.37 ± 0.48 g/m^2, Figure 2B). The results may be explained by the morphological differences among the three submerged macrophytes. On one hand, the roots of *V. spiralis* and *H. verticillata* can absorb nutrients directly from sediment, while *C. demersum* has no root. On the other hand, a previous study showed that the submerged macrophytes with flat-leaf type (*V. spiralis* and *H. verticillata*) possessed a greater photosynthetic compared with the needle-leaf types (*C. demersum*) [22]. Greater photosynthetic may lead to more N and P absorption. Thus, according to the growth rate and nutrients accumulated ability, *V. spiralis* performed best in the three submerged macrophytes.

3.2. Overlying Water Quality

The decrease of TOC concentration in the overlying water of *C. demersum* treatment was obvious in the early 50 days of the experiment (Figure 3a). *C. demersum* has no root and floated in the top layer of water, the photosynthesis of which may be obviously higher than the other two species in this period because of the stronger light illumination, but this advantage disappeared when the leaves of *V. spiralis* and *H. verticillata* grew to flourish (50 days later). The mean concentrations of TOC in the overlying water of the three planted treatments were 26.6%, 24.3% and 22.1% lower than that of the Control 50 days later, respectively. The submerged macrophytes changed the redox environment of the water by increasing dissolved oxygen (DO) concentration, while the removal rates of organic matter in water depend on the DO levels available to the bacteria [23]. This can explain the lower TOC concentrations in the three planted treatments.

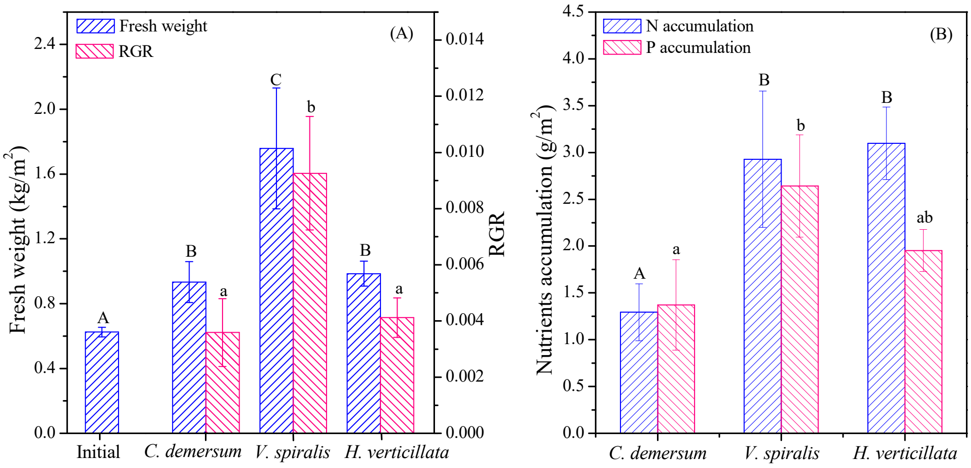


Figure 2. Growth parameters of the three submerged macrophytes ((**A**) Fresh weight and RGR of the submerged macrophytes; (**B**) N and P accumulation of the submereged macrophytes). Significant difference ($p < 0.05$) between treatments indicated by different letters (A, B, C, a, b) on the bars.

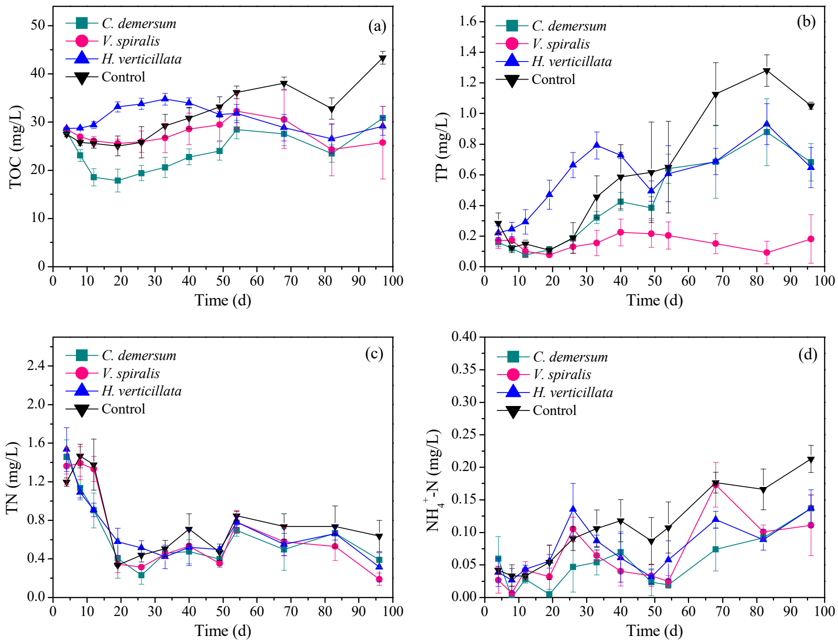


Figure 3. Characteristics of overlying water: (**a**) TOC, (**b**) TP, (**c**) TN and (**d**) NH_4^+-N concentrations.

TP concentrations in the control increased gradually with time, indicating the continuous release of P from the sediment (Figure 3b). The effect of *V. spiralis* on reducing TP concentrations in the overlying water was obvious. The average TP concentration was 0.16 ± 0.04 mg/L in *V. spiralis* treatment, which was significantly lower than that of the *C. demersum* treatment (0.39 ± 0.08 mg/L), the *H. verticillata* treatment (0.56 ± 0.04 mg/L) and the control (0.55 ± 0.11 mg/L, $p < 0.05$, Figure 3b). On the one hand, more biomass of *V. spiralis* could absorb more P from the water, which can be seen from Figure 2B. On the other hand, submerged macrophytes can inhibit the release of P from the sediment into the overlying water effectively, which depended on the DO level in the bottom water [17]. The study of Zhu et al. [23] showed that photosynthesis of *V. spiralis* changed the redox environment of the water by increasing DO concentration. Thus, much more biomass of *V. spiralis* may absorb more P from the water; meanwhile, decreased has more P released from the sediment. The TOC and TP concentrations in the overlying water were significantly higher in the *H. verticillata* treatment (early 50 days) than those in the other three treatments ($p < 0.05$, Figure 3a,b). It was probably due to the fact that the stems of *H. verticillata* were cut into sections before planting, some of which decayed, causing the increase of organic matter and P concentrations in the overlying water.

The concentrations of TN decreased in the early 20 days and later on, basically remained stable in the four treatments. At the end of the experiment, the TN removal efficiencies from overlying water were 73.4%, 86.0% 79.6% and 46.7% for *C. demersum*, *V. spiralis*, *H. verticillata* and the control, respectively (Figure 3c). NH_4^+-N concentrations in the overlying water basically increased in the four treatments. The average value was 0.051 ± 0.014 mg/L in the *C. demersum* treatment, which was significantly lower than that in the control (0.102 ± 0.011 mg/L, $p < 0.05$, Figure 3d).

To sum up, *V. spiralis* performed best on the overlying water quality among the three submerged macrophytes grown in the sediment with ENR. The concentrations of TOC, TP, TN and NH_4^+-N were 25.0%, 71.7%, 24.8% and 38.1% lower in the *V. spiralis* treatment than those in the control, respectively. A previous study of Liu et al. [22] found that the flat-leaf types (*V. spiralis* and *H. verticillata*) performed better than the needle-leaf types (*C. demersum*) in the improvements of underwater light conditions and water quality. Moreover, much more biomass and higher RGR made *V. spiralis* perform better than *H. verticillata* in the present study.

3.3. Effects of Submerged Macrophytes on the Sediment Characteristics

The concentration of TOC in the sediment of the *V. spiralis* treatment was slightly lower than those of the other treatments, which may be related to the developed roots of *V. spiralis* (Figure 4a). The roots of *V. spiralis* displayed strong oxidation potential, its growth increased O_2 concentration in the sediment and which was mainly due to the radial oxygen loss (ROL) from the root system [24]. The rates of radial oxygen loss (ROL) from the roots of *V. spiralis* were calculated, ranging between 58.3 and 658 μmol m^{-2} h^{-1} [25,26]. Gu et al. [27] showed that the plants with extensive aerenchyma tissue transported excess oxygen to roots and diffused into their surroundings, forming an oxidation-reduction micro-environment in the rhizosphere of constructed wetlands. They also found that the plants with higher ROL exhibited better nutrients and organic matter removal than did the other plant species. Aerobic degradation of organic matter by microorganisms contributed to the change of organic matter content in the sediment [28]. The oxygen secretion and exudates of the roots was beneficial to the reproduction of aerobic bacteria.

TN and TP concentrations in the sediment of the four treatments changed little at the end of the experiment compared with the background values (Figure 4b). On the one hand, better remediation performance under a high background concentration of TN and TP in the sediment may need a longer time [14]. This may be the main reason. TN and TP concentrations in the sediment reached several g/kg DW in this study. On the other hand, the removal of N and P may be inhibited by the ENR and CIP in the sediment. Ohore et al. [18] showed that the N and P removal efficiencies were decreased 22% and 9.3% by antibiotics in the constructed wetlands. It concluded that the impact of antibiotics on microbes affected the N removal directly in constructed wetlands [18].

The total removal efficiencies of ENR and CIP were 12.3%, 40.8%, 41.9% and 61.0% in the sediment of the four treatments (Figure 4). The removal ability of the control was the highest, followed by the two rooted submerged macrophytes treatments, and *C. demersum* with no root was the lowest. Lin et al. [29] reported that natural irradiation plays a major role in the degradation of ENR and CIP in the water and sediment, with biodegradation playing just a minor role. The depth of the water in this study was no more than 60 cm, which may facilitate the photodegradation of ENR and CIP in the sediment of the control. The covering of submerged macrophytes hindered the photodegradation of ENR and CIP in the sediment of the three planted treatments. Especially for the *C. demersum*, it has the greatest blocking effect on the water, leading to the lowest ENR and CIP removal. It can be speculated that photodegradation by solar radiation contributed more to the ENR and CIP removal than submerged macrophytes and microbial degradation in the present study. On the other hand, plant absorption and diffusion into the overlying water also contributed to the decrease of ENR and CIP in the sediment of the planted treatments [14,30].

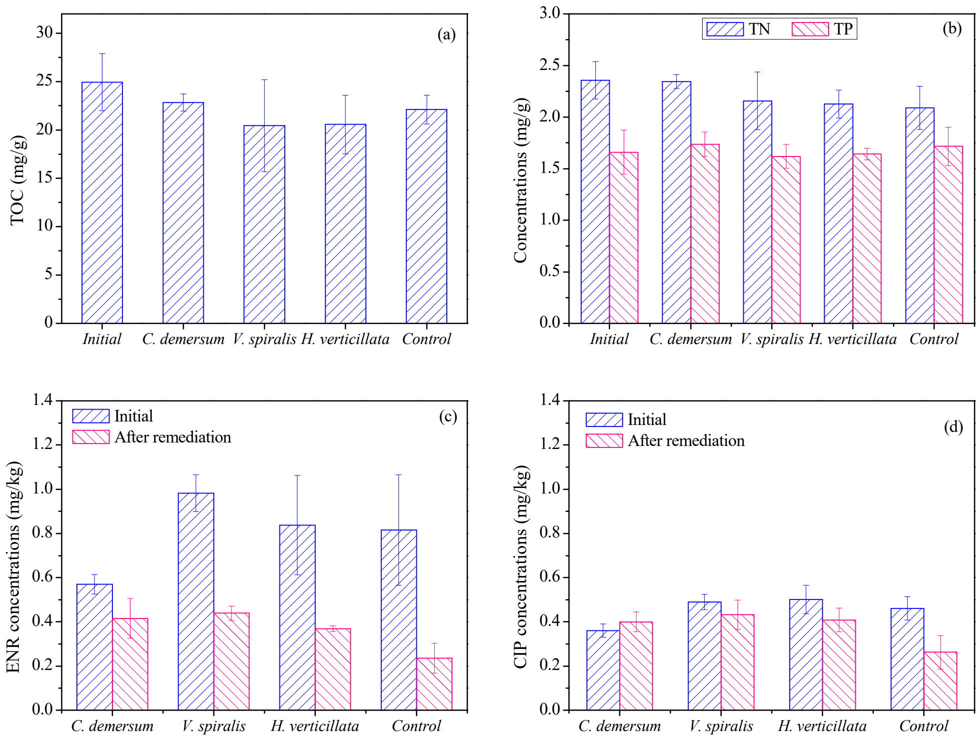


Figure 4. Variations of (**a**) TOC, (**b**) TN, TP, (**c**) ENR and (**d**) CIP concentrations in the sediment.

3.4. Effects of Plant Species on the Microorganism in Sediment with ENR

Table 1 showed the Alpha Diversity Index for the sediment samples from the four treatments. The values of Good's coverage index were all higher than 0.984, suggesting that the depth of sequencing covered the majority of species in the samples. Table 1 shows that the number of OTUs in the sediment of the three planted treatments was more than that of the control, especially the sample of the *V. spiralis* treatment ($p < 0.05$). *V. spiralis* treatment contained more unique OTUs (144) than those in the other three treatments (59, 39 and 27, respectively). The Chao1 and ACE index of microbial communities in the sediment suggested that the richness and diversity of the microorganisms in the planted treatments were also significantly higher than those in the control, except for the *H. verticillata* treatment, in which the Chao1 and ACE indexes were comparable to the control (Table 1). Compared with the Control, *V. spiralis* induced an increase of 21.7% for the number of OUTs, 17.8% for Chao1 index and 19.3% for the ACE index of the microbial community in the sediment with ENR residues. There was also no significant difference, but higher numbers of OTUs and higher values of the Chao1 estimator, ACE and Shannon indexes were observed for bacterial communities in the *H. verticillata* microcosms compared to the *C. demersum* microcosms in a previous study [31]. The author explained that it was due to the direct influence of rooted *H. verticillata* on the sediment microbial community by oxygen release and organics secretion. This was consistent with our view that the roots promoted the microbial communities. We speculated that the inhibitory effect of ENR on microorganisms in the sediment may lead to the opposite results in this research. Therefore, the planting of submerged macrophytes, especially *V. spiralis*, increased the richness and diversity of the microbial communities in the sediment with ENR and CIP, which also played an important role in the construction of the unique microbial population [23]. The results of Man et al. [32]

also showed that wetland plants formed the stability of rhizosphere microorganisms and increased their ability to tolerate antibiotics stress.

Table 1. The richness and diversity indices of microbial communities for sediment samples.

Sample	Good's Coverage (%)	OTUs	ACE	Chao1	Shannon	Simpson ($\times 10^{-3}$)
C. demersum	98.4 ± 0.15	2034 ± 117 [b]	2543.1 ± 164.4 [b]	2528.7 ± 195.0 [b]	5.92 ± 0.19	10.5 ± 3.3
V. spiralis	98.4 ± 0.07	2150 ± 70 [b,c]	2638.8 ± 72.9 [b,c]	2598.7 ± 103.7 [b,c]	6.07 ± 0.14	9.5 ± 4.8
H. verticillata	98.6 ± 0.03	1859 ± 230 [a,b]	2303.5 ± 189.1 [a,b]	2265.5 ± 195.1 [a,b]	5.89 ± 0.32	9.8 ± 4.2
Control	98.6 ± 0.10	1767 ± 57 [a]	2212.7 ± 98.3 [a]	2205.4± 116.1 [a]	5.83 ± 0.10	9.8 ± 1.2

Notes: OTUs were defined with a similarity of 97%. Average values and standard deviations (n = 3) are displayed. Significant difference (p < 0.05) between treatments indicated by different letters ([a], [b], [c]).

Apart from bacterial community richness and diversity, the microbial community structure of the sediment in the four treatments was also analyzed at the phylum and genus level (Figure 5). The development of submerged vegetation significantly influenced the bacterial community structure in the sediment [33]. *Proteobacteria*, *Acidobacteria* and *Chloflexi* were the dominated phylotypes in the sediment of this study, accounting for 70.8–72.8% of the microbial community, which was similar with the results of Chao et al. [34]. The relative abundance of *Proteobacteria* in *V. spiralis* treatment (47.9%) was higher than that in the *C. demersum* treatment (43.5%), *H. verticillata* treatment (38.1%) and control (39.0%). Similar to this result, Ohore et al. [35] found that CIP caused an increase in the relative abundance of *Proteobacteria* in the epiphytic biofilm on *V. spiralis*, while the relative abundance of *Acidobacteria* and *Chloflexi* were lower in *V. spiralis* treatment compared with the other three treatments (Figure 5). Thus, *V. spiralis* promoted the growth of *Proteobacteria* and inhibited the growth of *Acidobacteria* and *Chloflexi* in the rhizosphere sediment with ENR.

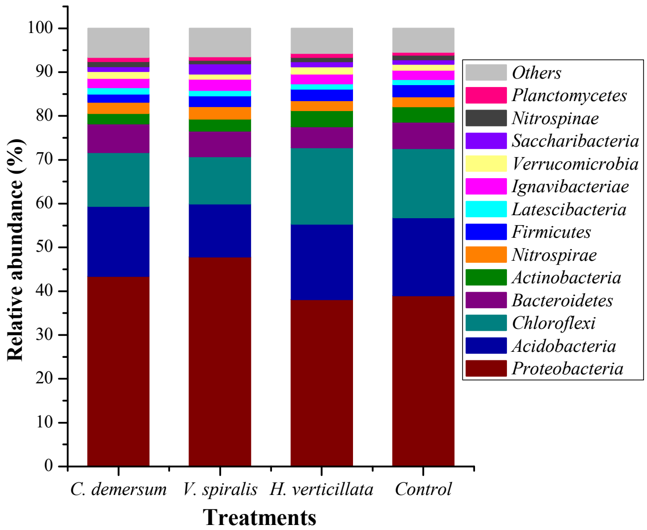


Figure 5. Relative abundance of the main bacterial phyla in sediment samples.

At the genus level, the relative abundance of *norank_f_Anaerolineaceae* and *norank_c_Acidobacteria* in the *V. spiralis* treatment was lower than that in the other three treatments. Wang et al. [33] showed that *norank_c_Acidobacteria* and *norank_f_Anaerolineaceae* which are related to organic carbon degradation, were significantly lower during the high macrophyte biomass period. Submerged macrophytes recruited and selected bacteria instead of randomly obtaining bacteria from surrounding environments. Although bacteria from surrounding environments could randomly meet the root surface of submerged macrophytes, the hydrophytes determined and derived who would dwell and develop in epiphytic microbiomes [36]. The oxygen released by the roots of *V. spiralis* promotes a more oxidized rhizosphere environment [26]. The changed environmental condition reduced the growth and reproduction of anaerobic bacteria in the root region. *Pseudomonas* is a genus that was endemic to the *V. spiralis* and *C. demersum* treatments, but not to the other two (Figure 6). *Pseudomonas* was identified as a typical denitrifying heterotroph; the sufficient carbon source was beneficial for their growth and reproduction [37,38]. It has been well known that the root exudates and decaying tissues of aquatic plants can supply organic matters for the growth of heterotrophic bacteria which help them outcompete the slow-growing autotrophic bacteria [39]. As a resistant bacterium, *Pseudomonas* was widely distributed in the antibiotic contaminated environment and exhibited multidrug resistance towards antibiotics [40]. Thus, more organic matters produced by well-developed aquatic vegetation and the resistance towards antibiotics were beneficial for the growth of *Pseudomonas* at the rhizosphere of submerged macrophytes.

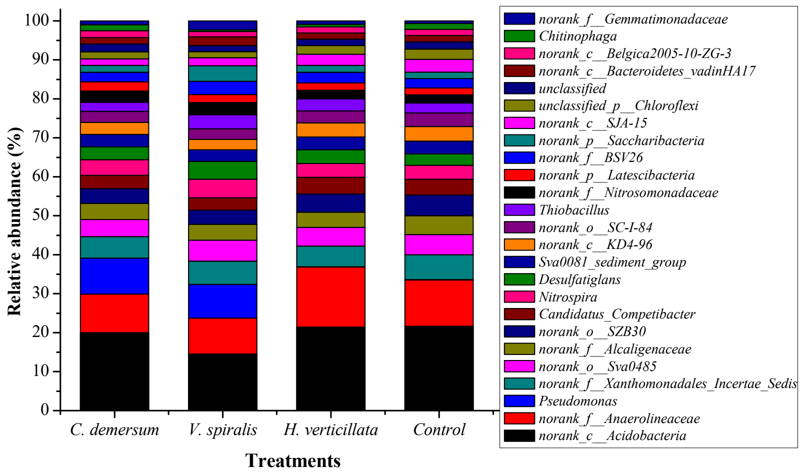


Figure 6. Relative abundance of different genus in sediment samples.

In general, the results above concluded that compared with the other two submerged macrophytes, *V. spiralis* showed a more significant effect on the diversity and structure of microbial communities in the sediment with ENR and CIP.

4. Conclusions

Three kinds of submerged macrophyte with distinct root morphology (flourish roots, little roots and no roots) were chosen to restore the aquaculture ponds with enrofloxacin (ENR) residues. According to the growth rate, nutrients accumulation and water purification ability, *V. spiralis* with flourish roots performed best among the three submerged macrophytes. The TOC, TP, NH_4^+-N and TN concentrations in the overlying water were decreased by *V. spiralis* with 25.0%, 71.7%, 38.1% and 24.8%, respectively. The planting of *V. spiralis* also increased the richness and diversity of the microorganisms in the sediment with 21.7% for the number of OUTs, 17.8% for Chao1 index and 19.3% for ACE index. *V. spiralis* promoted the growth of *Proteobacteria* and inhibited the growth of *Acidobacteria* and *Chloflexi* in the rhizosphere sediment. Compared with *C. demersum* and *H. verticillata*, *V. spiralis* was more suitable for the restoration of the aquaculture ponds with ENR residue. Our results add to understanding the influences of submerged macrophytes on nutrients removal and the microbial community in aquaculture ponds with antibiotic residues. However, due to the limitations of the water depth in this study, the effect of submerged macrophytes on the removal of ENR was not clear. Further studies are needed to explore the ENR removal efficiencies by different submerged macrophytes and related mechanisms such as the effect of released oxygen and exudates from the roots on the removal of antibiotics.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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