

## Article

# Treatment Effect of Long-Term Subsurface-Flow Constructed Wetland on Mariculture Water and Analysis of Wetland Bacterial Community

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**Abstract:** To improve the quality of natural seawater to meet the needs of aquaculture production, a large-scale subsurface-flow constructed wetland (HSFCW) was constructed and operated stably for 2 years to understand the reasons for its purification effect on natural seawater. The results of the study showed that the system could maintain a high purification effect on natural seawater during aquaculture; the average removal rates of COD, TSS, TN, NH<sub>4</sub><sup>+</sup>-N, and DON (organic nitrogen) were 22.29%, 49.33%, 36.94%, 10.88%, and 44.08%, respectively. Additionally, the HSFCW could effectively remove harmful algae such as Cyanobacteria; the removal rate of dominant algae species in Cyanobacteria was 90.33–97.93%. The pyrosequencing of 16S ribosomal DNA revealed that *Proteobacteria*, *Nitrospirae*, and *Chloroflexi* were the main and key bacterial phyla in the system. Members of these key gates are regarded as playing important roles in resisting water purification. The study results suggest that the subsurface-flow wetland system can effectively improve seawater quality and reduce the density of harmful algae cells.

**Keywords:** subsurface-flow wetland; aquaculture water; purification; algal removal; bacterial community



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## 1. Introduction

The global aquaculture industry produced around 82 million tons in 2018, with 30.8 million tons from marine aquaculture, which accounted for 37.5% of global aquaculture production [1]. Experts have predicted significant future growth in the percentage of aquaculture coming from marine areas [2]. Meanwhile, land-based and nearshore aquaculture systems have widely been accepted and promoted [3]. Seawater is usually extracted from offshore areas for aquaculture. However, in recent decades, offshore seawater quality has deteriorated, such as from eutrophication, and influent water from offshore areas needs to be properly treated before entering the aquaculture system [4].

As an ecofriendly technology, constructed wetlands (CWs) have the advantages of less secondary pollution, low construction cost, and simple management and operation [5–7]. Therefore, CWs are widely used in treating water pollution from agricultural nonpoint sources. CWs rely on the simultaneous occurrence of several complicated physical, chemical, and biological processes for removing pollutants, including sorption and sedimentation, photolysis, hydrolysis, volatilization, plant uptake and accumulation, plant exudation, and microbial degradation [6,8,9]. Wu, et al. [10] conducted long-term monitoring of secondary effluent treatment from wastewater treatment plants by a continuous large-scale constructed wetland system. The average removal efficiency of COD and NH<sub>4</sub><sup>+</sup>-N reached 53% and 72%, respectively. At that time, most of the existing research was based on freshwater

constructed wetlands, and there was a lack of long-term tracking analysis of large-scale seawater constructed wetland systems.

Microorganisms play a crucial role in constructed wetland systems inorganic pollutant degradation, nutrient fixation, and transformation [11]. In the past, the research on microbial community structure in different functional blocks of CWs mainly focused on the short-term operation of CWs, which lacked study of the long-term operation of CWs, especially seawater CWs [12].

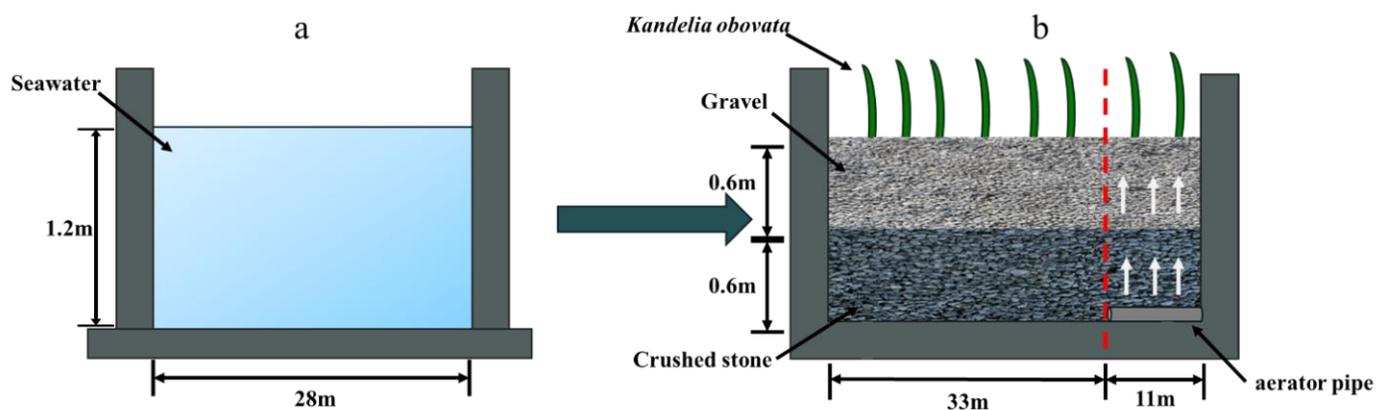
Therefore, a large-scale seawater constructed wetland system that had been operating stably for 2 years was monitored in this study. The specific objectives were to (1) evaluate the removal efficiency of conventional pollutants and algae in low-polluted seawater by long-term operation of large-scale wetland systems; (2) explore the spatial distribution of bacterial communities in various areas of the wetland system; and (3) analyze the relationship between bacterial community and pollutant removal.

## 2. Materials and Methods

### 2.1. Subsurface-Flow Wetland System

The wetland was built to meet the demand for aquaculture water in the Yongxing Base of Zhejiang Marine Aquaculture Research Institute (Wenzhou, China). The total land area of this base is approximately 18.5 hectares. The Base mainly conducts *Litopenaeus vannamei* breeding and shellfish nursery work.

A large horizontal subsurface-flow constructed wetland (HSFCW) was built in August 2017 with an area of 1224 m<sup>2</sup>. The wetland mainly treated natural seawater from Wenzhou Bay, with a daily treatment capacity of 1800 m<sup>3</sup>. The hydraulic retention time (HRT) was around 12 h, and the hydraulic load (HL) was 1.47 t/m<sup>2</sup>·d<sup>-1</sup>. The system was divided into two functional zones: an influent sedimentation area and a filler treatment area (Figure 1). The UPVC pipe with a diameter of 20 mm was laid at the bottom of the filler treatment area for aeration, and the aeration area accounted for one-third of the total filler treatment area. The aeration zone was aerated throughout the day, and the aeration intensity was 12.5 m<sup>3</sup>/min. The filling area was filled with gravel (particle size 3–5 cm, filling height 0.6 m) and gravel (particle size 6–8 cm, filling height 0.6 m) from bottom to top, and the inlet water passed through 20–30 cm of surface layer substrate. In this constructed wetland, mangrove *Kandelia obovata* was the main plant with a planting density of 30 plants/m<sup>2</sup> (Figure 1).



**Figure 1.** The plane structure of the subsurface-flow wetland: (a) inlet sedimentation area; (b) filler treatment area.

The experiment was operated from April to December 2020 (production and breeding activities were not carried out from January to March due to low temperature), and the system had been operating stably for 2 years. During the operation of the system, the water

temperature was 11.5–32.5 °C, pH was  $8.14 \pm 0.17$ , salinity was  $19.4 \pm 0.3\%$ , and dissolved oxygen (DO) was 4.89–7.59 mg /L.

## 2.2. Water Sampling and Analytical Methods

The effect of the constructed wetland on purifying natural seawater was evaluated by monitoring influent (S-1) and effluent (S-2) water quality. Chemical indexes of water quality included total nitrogen (TN), ammonia nitrogen ( $\text{NH}_4^+$ -N), nitrite-nitrogen ( $\text{NO}_2^-$ -N), nitrate-nitrogen ( $\text{NO}_3^-$ -N), and chemical oxygen demand (COD). All chemical indicator tests were based on standard methods [13]. Total suspended solid (TSS) was tested by the gravimetric method. The species and density of phytoplankton were determined according to Hu's method [14]. Water quality was tested bimonthly, and four groups of parallel sampling were used for water quality analysis at each sampling point.

To study the microbial community status of the subsurface wetland system, intermediate water was collected from the water inlet (S-1) and outlet (S-2); additionally, substrates were collected from the aerobic (J-O) and anaerobic (J-H) zones in the packed bed during August 2020. Three samples were randomly selected in each designated area and mixed for microbial analysis. The biofilms on the samples were separated by an ultrasonic cleaner, and then the suspensions were filtered with 0.22  $\mu\text{m}$  filter membranes. After filtration, the sample material remaining on the filter membrane was the biofilm. Finally, the filter papers were stored in an ultralow temperature refrigerator at  $-80$  °C for subsequent DNA extraction experiments.

## 2.3. DNA Extraction and PCR Amplification

The extraction of biofilm DNA was performed using a water sample genomic DNA rapid extraction kit, and then the extracted genomic DNA was detected by 1% Agarose gel electrophoresis. The V4–V5 region of the 16S rRNA gene was amplified by PCR using the primers 515F: 5'-GTGCCAGCMGCCGCGG-3' and 907R: 5'-CCGTCAATTCMTTRAGTTT-3'. Then, the amplified PCR fragments were detected by 2% Agarose gel electrophoresis. Finally, the PCR products were quantified by the QuantiFluor™-ST blue fluorescence quantitative system (Promega Company, Beijing, China). According to the sequencing requirements for each sample, the corresponding proportion was mixed to construct the Miseq gene library.

The high-throughput sequencing platform was Illumina Miseq PE250. The PE reads obtained by Miseq sequencing were spliced according to the overlap relationship, and the sequence quality was controlled and filtered. After distinguishing the samples, OTU clustering analysis and species taxonomy analysis were performed. When analyzing the composition of species structure, in order to prevent chlorophyll interference, the OTUs were counted after removing cyanobacteria and rare species.

## 2.4. Data Analysis

Origin 11.0 and SPSS18.0 were used for water quality data analysis. QIIME PE250 software was used to analyze  $\alpha$ -biodiversity.

### Average Removal Amount

$$\text{Average removal amount (ARA)} = [(C_{\text{in}} \times Q_{\text{in}}) - (C_{\text{out}} \times Q_{\text{out}})] / W_{\text{area}}$$

where the ARA is in a unit of  $\text{g}/\text{m}^2 \cdot \text{d}^{-1}$ , where  $W_{\text{area}}$  is the area of the subsurface-flow wetland system ( $\text{m}^2$ );  $C_{\text{in}}$  and  $C_{\text{out}}$  are the average mass concentration of influent and effluent ( $\text{mg}/\text{L}$ ); and  $Q_{\text{in}}$  and  $Q_{\text{out}}$  are the average flow rate of influent and effluent ( $\text{m}^3 \cdot \text{d}^{-1}$ ).

## 3. Results and Discussion

### 3.1. Variation in Water Quality

The purification effect of organic matter and suspended particulate matter in the subsurface-flow wetland is shown in Figure 2. The average concentration of COD in the

influent was  $3.25 (\pm 0.51)$  mg/L. According to China's water standard GB 3097-1997 as a reference [15], the seawater belonged to the third category of water quality, which was not suitable for aquaculture without treatment (Table 1). The average concentration of COD in the effluent treated by the subsurface-flow wetland was  $2.60 (\pm 0.50)$  mg/L, and the water quality was upgraded to the second category. The average removal amount was  $0.96 (\pm 0.11)$  g/m<sup>2</sup>·d<sup>-1</sup>, and the average removal rate was  $20.29 (\pm 2.88)$  %. In the subsurface-flow wetland system, the physical processes of precipitation, matrix adsorption, filtration, and microbial biodegradation were the main ways organic matter in seawater was removed [16]. As an important evaluation index of water quality monitoring, COD could reflect the organic pollutants in water. Because the influent COD content was low, the system treatment effect was not obvious.

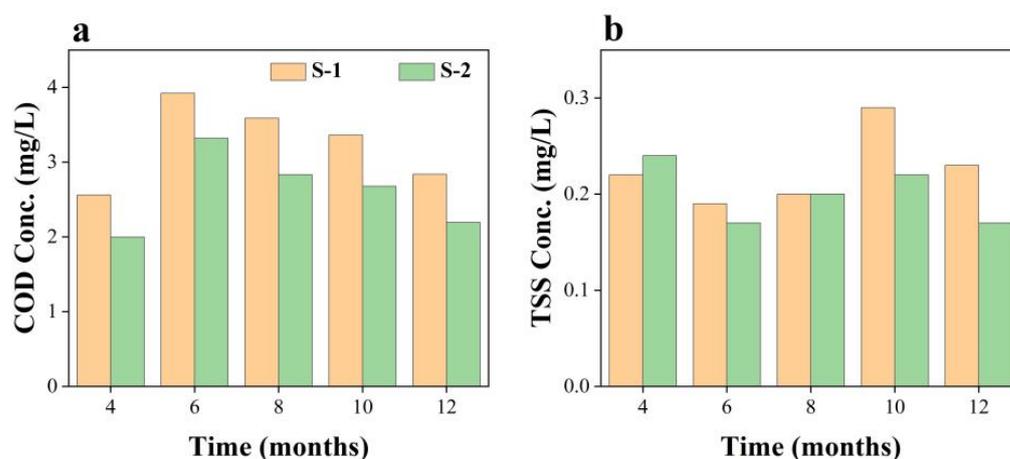


Figure 2. Removal effect on COD (a) and TSS (b) in the HSFCW (S-1: inlet, S-2: outlet).

Table 1. Seawater quality standard.

Grade	COD <sub>≤</sub> (mg/L)	DIN <sub>≤</sub> (mg/L)	Applicable Site
I	2	0.20	Marine fishery waters
II	3	0.30	Aquaculture areas
III	4	0.40	Industrial water function zone
IV	5	0.50	Ocean port waters

The clogging problem has always been an important factor affecting the long-term stable operation of wetland systems [17]. Therefore, to prevent the reduction in its water conductivity and removal effect, the system had been cleaned and maintained regularly every year since its establishment and operation. Previous studies have suggested that the maximum TSS load of a wetland system should be  $15 \text{ g/m}^2 \cdot \text{d}^{-1}$  [18]. The removal amount of TSS was  $12.27 (\pm 2.74)$  g/m<sup>2</sup>·d<sup>-1</sup>, the average removal rate was  $49.33 (\pm 12.75)$  %, and the average concentration of effluent was less than 10 mg/L. The removal amount was 14.91 and 15.21 g/m<sup>2</sup>·d<sup>-1</sup> in June and October, respectively.

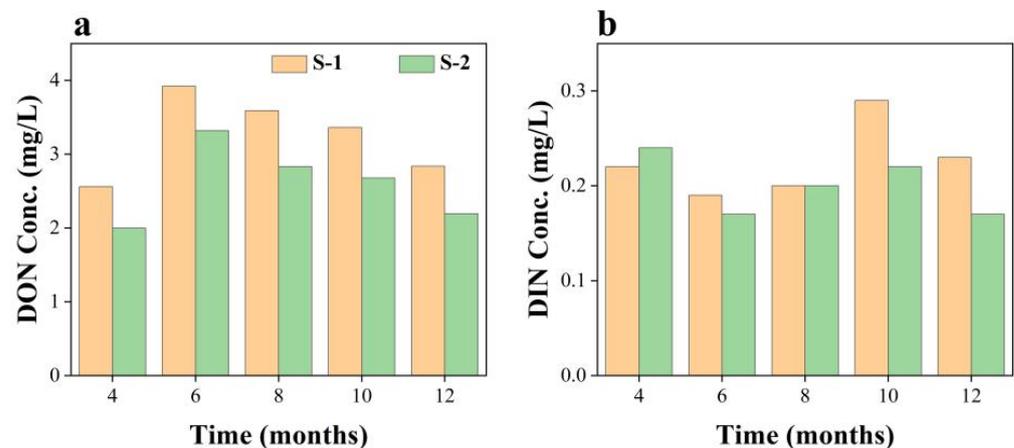
Excessive nitrogen may affect the normal physiology and behavior of aquatic organisms [19]. It can be seen from Table 2 that the concentration of TN in the influent and effluent was 0.92–2.06 mg/L and 0.72–1.15 mg/L, respectively. The average removal amount was  $0.78 (\pm 0.43)$  g/m<sup>2</sup>·d<sup>-1</sup>, and the average removal rate was 36.94%. The concentration of DIN (dissolved inorganic nitrogen) in the influent was 0.19–0.29 mg/L, and the effluent was 0.17–0.24 mg/L. The average removal amount and removal rate of DIN was  $0.04 \text{ g/m}^2 \cdot \text{d}^{-1}$  and 10.88%. The concentration of DON (dissolved organic nitrogen) in the influent and the effluent was 0.69–1.77 mg/L and 0.51–0.93 mg/L. The average

removal amount was  $0.74 \text{ g/m}^2\text{-d}^{-1}$ , and the average removal rate was 41.82%. The removal rate of DON in the subsurface-flow wetland characterizes the mineralization intensity of organic nitrogen, and the removal rate was positively correlated with temperature [20]. In April, the total nitrogen concentration of the influent and effluent of the system was  $0.92 (\pm 0.02) \text{ mg/L}$  and  $0.75 (\pm 0.05) \text{ mg/L}$ , respectively, and the removal rate was only 17.8%. Due to higher temperatures and more active microbial activity from June to October, the TN removal rate remained at a high level, with the highest removal rate at 44.08%. The main ways of nitrogen removal in the subsurface-flow wetland system were ammonia volatilization, denitrification, deposition, and adsorption [21,22]. Related studies have shown that subsurface-flow wetland systems are suitable for the removal of nitrogen pollutants in rivers and lakes, and could maintain a high TN removal rate even under long-term operation [23].

**Table 2.** The removal effect of nitrogen nutrients by the HSFCW.

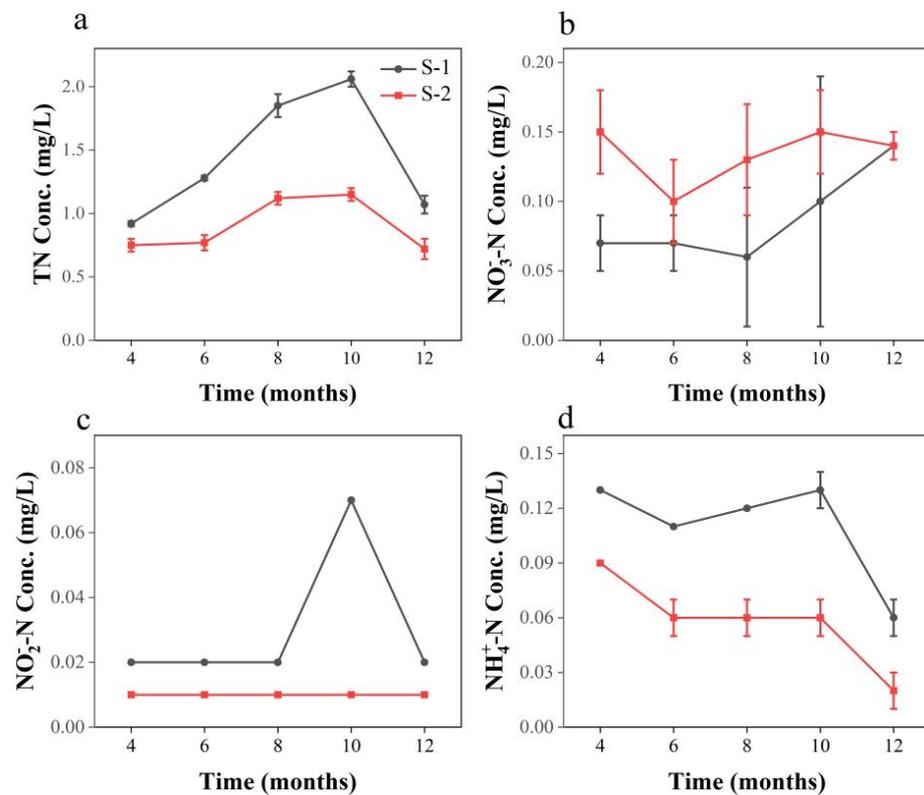
Index	Average Inlet (mg/L)	Average Outlet (mg/L)	Average Removal Amount ( $\text{g/m}^2\text{-d}^{-1}$ )	Average Removal (100%)
TN	$1.44 \pm 0.41$	$0.90 \pm 0.17$	$0.78 \pm 0.43$	36.94
$\text{NO}_3^-$ -N	$0.09 \pm 0.03$	$0.13 \pm 0.02$	$-0.10 \pm 0.08$	-52.02
$\text{NO}_2^-$ -N	$0.03 \pm 0.02$	$0.01 \pm 0.00$	$0.03 \pm 0.02$	78.54
$\text{NH}_4^+$ -N	$0.11 \pm 0.02$	$0.06 \pm 0.02$	$0.07 \pm 0.02$	45.07
DIN	0.23	0.20	0.04	10.88
DON	1.21	0.70	0.74	41.82

The form of nitrogen in the influent was mainly DON, which accounted for 75.68–89.26%, while DIN accounted for 10.74–24.32% (Figure 3). After subsurface-flow wetland treatment, the proportion of organic nitrogen decreased, the proportion of inorganic nitrogen increased, and DIN in the effluent was mainly  $\text{NO}_3^-$ -N. The subsurface-flow wetland system had the most significant effect on the removal of DON, while for DIN,  $\text{NH}_4^+$ -N decreased from  $0.11 (\pm 0.03) \text{ mg/L}$  to  $0.06 (\pm 0.02) \text{ mg/L}$  and  $\text{NO}_3^-$ -N increased from  $0.09 (\pm 0.03) \text{ mg/L}$  to  $0.14 (\pm 0.02) \text{ mg/L}$ . It is generally believed that  $\text{NO}_2^-$ -N has high biological toxicity to aquatic organisms. Elevated  $\text{NO}_2^-$ -N can hinder biological growth and even induce death in aquatic organisms [24].  $\text{NO}_2^-$ -N decreased from  $0.028 (\pm 0.021) \text{ mg/L}$  to  $0.006 (\pm 0.003) \text{ mg/L}$  during the monitoring period, and the average removal rate reached 78.54% (Figure 4).



**Figure 3.** Changes in DON (a) and DIN (b) in the HSFCW (S-1: inlet, S-2: outlet).

Statistical analyses showed that the wetland system significantly reduced the concentrations of TSS, COD, TN, DON,  $\text{NH}_4^+$ -N, and  $\text{NO}_2^-$ -N in seawater ( $p < 0.05$ ).



**Figure 4.** Removal effect on TN (a); NO<sub>3</sub><sup>-</sup>-N (b); NO<sub>2</sub><sup>-</sup>-N (c); and NH<sub>4</sub><sup>+</sup>-N (d) in the HSFCW (S-1: inlet, S-2: outlet).

### 3.2. Variation in Phytoplankton Community Structure

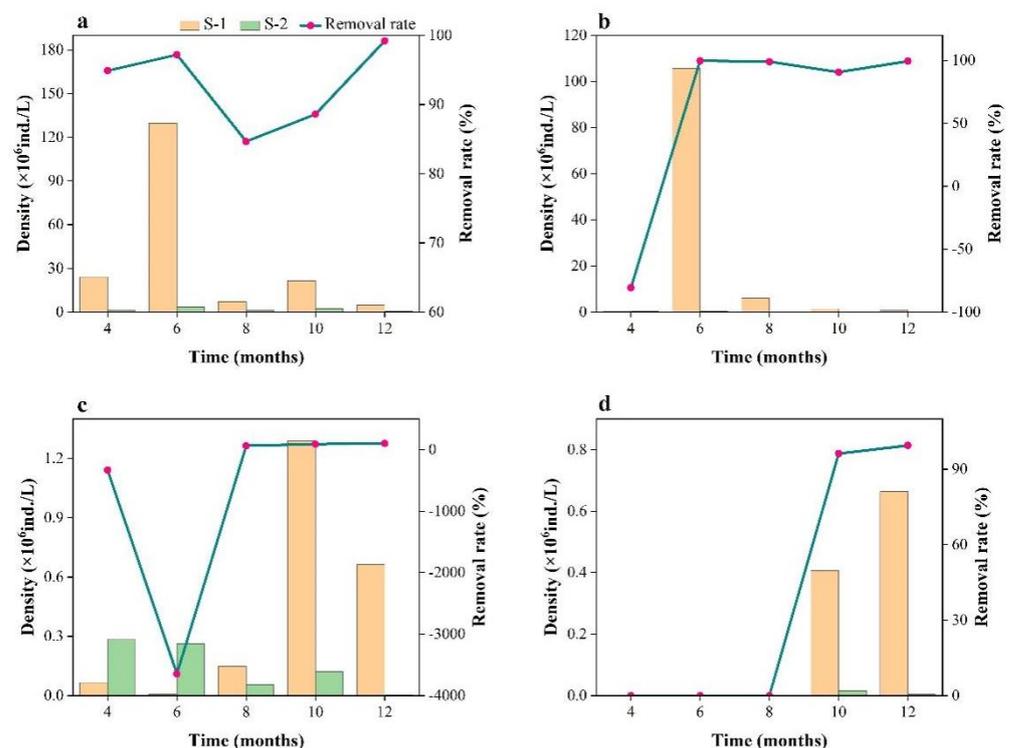
Because various cyanobacteria and dinoflagellates released some toxic secondary metabolites (e.g., microcystins, nodularins), this process affected the taste and odor of the cultured organisms.

The subsurface-flow wetland system mainly achieved the removal of phytoplankton through precipitation, matrix adsorption, physical interception, flocculation precipitation, and microbial degradation [25]. The dominant species in influent and effluent were *Microcystis* ( $30,577.29 \times 10^3$  ind./L), *Synechocystis* ( $2673.70 \times 10^3$  ind./L); *Phaeodactylum* ( $24,030 \times 10^3$  ind./L), *Melosira* ( $135.30 \times 10^3$  ind./L), and Chlorophyta *Dunaliella* ( $148.69 \times 10^3$  ind./L) (Table 3).

**Table 3.** Variation in the main algae density in influent and effluent.

Phyla	Genera	Frequency	Inlet Abundance ( $\times 10^3$ ind./L)	Outlet Abundance ( $\times 10^3$ ind./L)	Average Removal (100%)
Cyanophyta	<i>Microcystis</i>	6	30,577.29	2957.55	90.33
	<i>Synechocystis</i>	5	2673.70	65.94	97.53
	<i>Stamieria</i>	4	77.33	1.60	97.93
	<i>Oscillatoria</i>	4	2.40	13.22	-450.87
Bacillariophyta	<i>Phaeodactylum</i>	5	24,030.00	74.64	99.69
	<i>Melosira</i>	5	135.30	25.55	81.12
	<i>Cylindrotheca</i>	5	40.12	3.33	91.70
	<i>Navicula</i>	6	39.73	12.39	68.81
	<i>Stephanopyxis</i>	6	38.98	13.66	64.96
Chlorophyta	<i>Dunaliella</i>	4	148.69	691.80	-365.25
	<i>Chlamydomonas</i>	3	6.87	4.80	30.10
	<i>Ulothrix</i>	4	2.96	8.98	-203.16
	<i>Platymonas</i>	1	19.60	3.60	81.63
	<i>Alexandrium</i>	2	346.94	15.28	95.60

The effect of the subsurface-flow wetland system on algae removal is shown in Figure 5. The wetland system had a significant removal effect on Cyanophyta and Pyrrophyta, and the average removal rates were 92.90% and 97.85%, respectively. The density of Cyanophyta in the influent increased first and then decreased with the seasons, while the removal rate of Cyanophyta in the subsurface-flow wetland system was stable. The density of Cyanophyta in the influent reached its highest value in June,  $129.60 \times 10^6$  ind./L, and decreased to  $3.64 \times 10^6$  ind./L after treatment by the subsurface-flow wetland (Figure 5a). The density of Pyrrophyta in the influent was low. In October and December, during the treatment by the subsurface-flow wetland system, the densities of Pyrrophyta were  $0.41 \times 10^6$  ind./L and  $0.66 \times 10^6$  ind./L, which were reduced to  $0.02 \times 10^6$  ind./L and  $0.003 \times 10^6$  ind./L, respectively (Figure 5b). The density of Bacillariophyta in the influent increased first and then decreased with the change in seasons. The density of algae was the highest in June, with an average of  $105.61 \times 10^6$  ind./L. The density of Bacillariophyta decreased to  $0.26 \times 10^6$  ind./L after subsurface-flow wetland treatment. When the Bacillariophyta density in the influent was low, the Bacillariophyta density in the effluent increased. When the density of Bacillariophyta in the influent was high, the removal efficiency of Bacillariophyta in the subsurface-flow wetland system was stable, and the average removal rate was 97.24% from June to December (Figure 5c). The density of Chlorophyta in the influent was low. From April to June, the density of Chlorophyta in the influent was less than  $0.10 \times 10^6$  ind./L. After treatment in the subsurface-flow wetland, the density of Chlorophyta increased. After subsurface-flow wetland treatment, the density of Chlorophyta clearly decreased, and the average removal rate was 84.17% (Figure 5d).



**Figure 5.** Removal effect on main algae: (a) Cyanophyta; (b) Bacillariophyta; (c) Chlorophyta; and (d) Pyrrophyta in the HSFCW (S-1: inlet, S-2: outlet).

Currently, chemical measures are usually used to regulate the planktonic state of the water, such as the addition of copper sulfate [26,27]. Although the treatment effect is good, there are chemical residues and cost problems. From the point of view of treatment effect, a constructed wetland is a better alternative scheme, which has a better removal effect on cyanobacteria and greatly reduces the operation cost. However, since the removal of

harmful algae involved a series of biological and physicochemical processes, it is difficult to determine which pathway plays a major role in the system.

### 3.3. Characteristics of Microbial Communities

#### 3.3.1. Richness and Diversity of Microbial Communities

Table 4 shows the different indices of microbial communities among different sampling points in the constructed wetland. The Coverage index of all samples reached more than 0.98, indicating that the measured OTUs (operational taxonomic units) represented the actual situation of the microorganisms in each sample, and the data were comparable. The Shannon index reflected the microbial diversity of samples. Chao 1 and Ace reflected the microbial richness of the samples [28]. The significance of the sample Shannon index was J-H > J-O > S-2 > S-1, and a similar value tendency was found for the Chao 1 and Ace indices. The results showed that the microbial diversity and richness of the substrate were significantly greater than those measured for the water sample. Under anaerobic conditions, the species diversity and richness of the epiphytic bacteria were higher. After the aquaculture water was treated by the subsurface-flow wetland, the species diversity and richness increased.

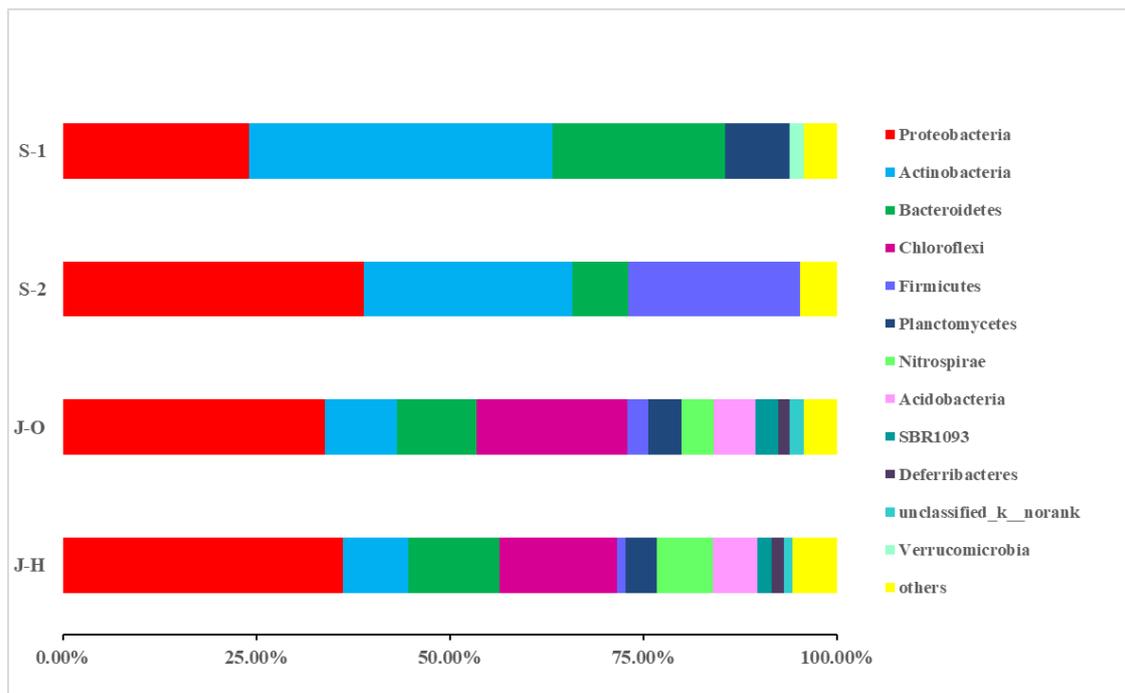
**Table 4.** Comparison of species diversity, richness, and coverage index in water samples and substrates (S-1: inlet, S-2: outlet, J-O: aerobic zone, J-H: anaerobic zone).

Sample	Shannon	Ace	Chao1	Coverage
S-1	3.489	1315.87	1147.28	0.99
S-2	4.43	1903.07	1860.98	0.99
J-O	6.25	3200.59	3200.45	0.98
J-H	6.56	3782.29	3778.42	0.98

#### 3.3.2. Composition and Distribution of Microbial Communities

To further analyze the bacterial community structure of the system matrix and water samples, the sample differences were compared based on phylum, family, and genus classification. Figure 6 shows that the microbial community in the subsurface-flow wetland had a high diversity at the phylum level, while the bacterial community in the aquaculture water had a low diversity at the phylum level. The matrix aerobic zone and anaerobic zone in the subsurface-flow wetland had similar microbial community composition. Proteobacteria, Chloroflexi, Firmicutes, and Nitrospira were the main bacterial phyla. Among them, Proteobacteria had the greatest abundance, accounting for 33.82% and 36.22% of the bacterial communities in the aerobic and anaerobic zones, respectively. Proteobacteria were mostly obligate or facultative anaerobic metabolic bacteria, which were the dominant phylum in the subsurface-flow wetland system. Nitrifying bacteria and most denitrifying bacteria play an important role in wetland nitrogen removal [29,30]. Studies have shown that Proteobacteria is the dominant phylum in wetlands, usually accounting for 30–40% of all flora [31]. In the matrix microbial community composition in this study, Proteobacteria accounted for more than 30%, which is consistent with previous studies [32]. The microorganisms from the phylum Chloroflexi accounted for a large proportion of the system flora, but its value in the system is not yet clear.

*Firmicutes* are common in anaerobic nitrification reactors similar to constructed wetlands, where functional bacteria capable of degrading macromolecular organic matter exist [33]. *Nitrospira*, comprising aerobic autotrophic nitrite-oxidizing bacteria, plays an important role in the nitrification process. The richness in the distribution of *Nitrospira* is the main reason for the removal of nitrogen nutrients [34]. In the microbial community structure of water samples S-1 and S-2, the microbial communities in natural seawater were mainly composed of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Planctomycetes*. The subsurface-flow wetland might promote the growth of *Actinobacteria*, *Bacteroidetes*, and *Planctomycetes*, but inhibit the growth of *Proteobacteria* and *Firmicutes*.

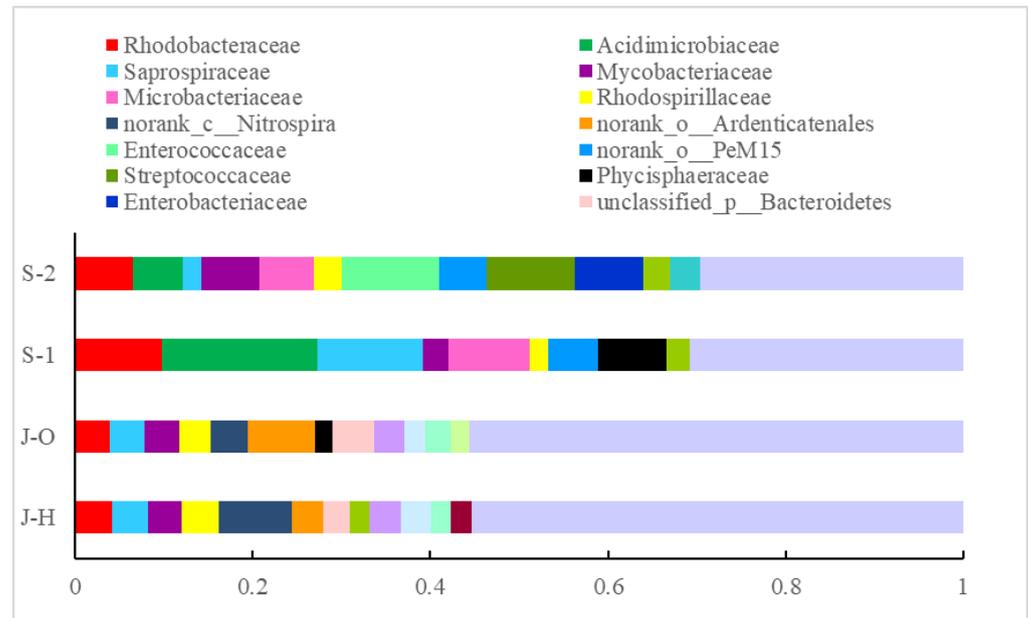


**Figure 6.** Community structure composition of different samples (phylum level) (S-1: inlet, S-2: outlet, J-O: aerobic zone, J-H: anaerobic zone).

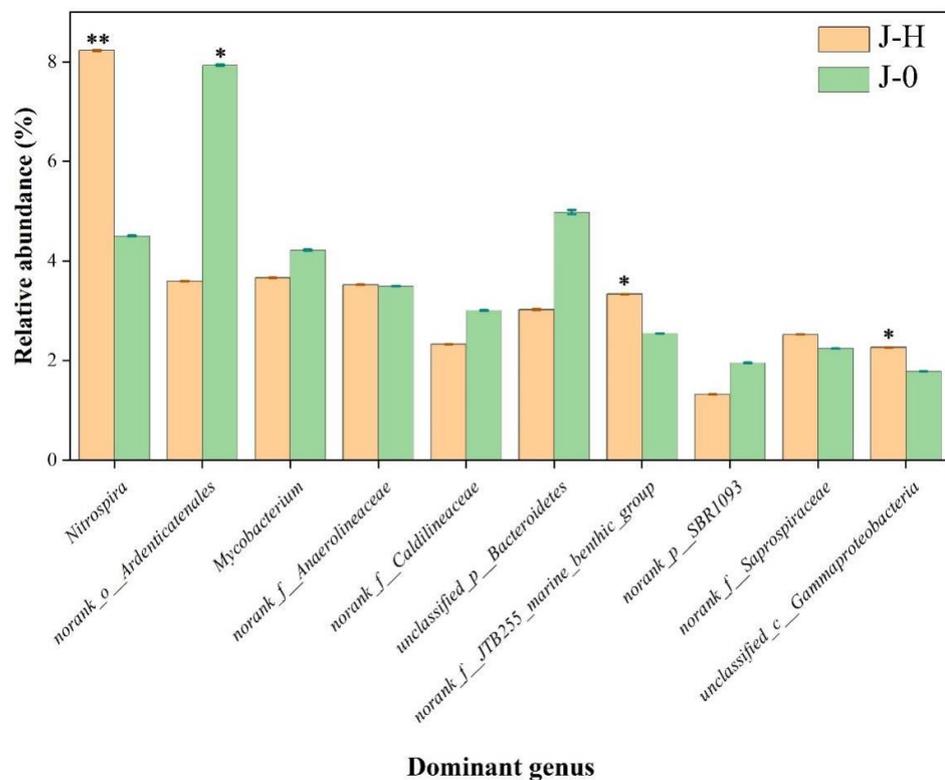
A total of 305 families were identified from the database, including 21 dominant families with relative abundance greater than 2%, as shown in Figure 7. The dominant bacteria filling the matrix were *Nitrospiraceae*, *Saprospiraceae*, *Anaerolineaceae*, and *Flavobacteriaceae*. *Nitrospiraceae* was the only established family in *Nitrospirae*, including *Nitrospira*, *Leptospirillum*, and *Thermodesulfovibrio*. Only *Nitrospira* was detected in the subsurface system. *Nitrospira* is widely distributed in aerobic wetland systems. It is the main bacteria known in nature that catalyzes nitrite oxidation and is crucial to the biogeochemical nitrogen cycle [35]. *Saprospiraceae* is often detected in the activated sludge of wastewater treatment systems. Its isolated and in situ strains have the ability to hydrolyze and utilize carbon sources. The bacteria of this family play an important role in the decomposition of complex organic matter in the environment [36]. *Saprospiraceae* is the dominant family of bacteria on biofilms in the presence of AOM (algal organic matter), capable of hydrolyzing proteins [37] and usually coexisting with *Flavobacteria* [38]. In this system, *Saprospiraceae* and *Flavobacteriaceae* were the dominant families. *Anaerolineaceae* is widely found in natural ecosystems and is an important organic degradation bacteria under anaerobic conditions [39]. *Rhodobacteraceae* is often detected in marine aquaculture biofilters and macroalgae-associated microorganisms. Related studies have reported that *Rhodobacteraceae* and *Flavobacteriaceae* are key groups in biological flocculation systems [40]. *Rhodospirillaceae* is a large family of  $\alpha$ -proteobacteria, and 12 genera were identified in the subsurface-flow wetland system. This family of bacteria is widely reported to have biohydrogen production capacity [41,42]. Dominant bacteria basically have an important pollutant degradation function, and may play a synergistic purification effect.

The top 10 genera in the matrix were analyzed for differences among groups, and the results are shown in Figure 8. The dominant genera in the microbial community of the subsurface-flow wetland were *Nitrospira*, norank\_o\_\_Ardenticatenales, *Mycobacterium*, etc. Among them, the relative abundance of *Nitrospira*, norank\_o\_\_Ardenticatenales, TB255\_marine\_benthic\_group, and unclassified\_c\_\_Gammaproteobacteria were significantly different ( $p < 0.05$ ). The relative abundance of *Nitrospira* in the anaerobic zone (8.23%) was significantly higher than that in the aerobic zone (4.25%) ( $p < 0.05$ ). The relative abundance of norank\_o\_\_Ardenticatenales in the anaerobic zone (3.6%) was

significantly lower than that in the aerobic zone (7.5%) ( $p < 0.05$ ). Previous studies suggested that nitrification is catalyzed by AOB (ammonia-oxidizing bacteria) or AOA (ammonia-oxidizing archaea) and NOB (nitrite-oxidizing bacteria), and recent studies have found that *Nitrospira* is also a key component in the nitrogen cycling microbial community, with the global distribution of *Nitrospira* representing the most diverse known NOB group [43].



**Figure 7.** Community structure composition of different samples (Class level) (S-1: inlet, S-2: outlet, J-O: aerobic zone, J-H: anaerobic zone).



**Figure 8.** The relative abundances of the top 10 dominant genera from the J-O and J-H bacterial communities (J-O: aerobic zone, J-H: anaerobic zone, \*\*  $p < 0.01$ , \*  $p < 0.05$ ).

#### 4. Conclusions

In this study, the pollutant removal capacity and bacterial community status of the seawater subsurface-flow constructed wetland with stable operation for 2 years were investigated. It was found that the system had a good removal effect on conventional pollutants (TSS, COD, TN, DON,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$ ) and potentially harmful algae such as cyanobacteria during the stable operation of the subsurface-flow wetland system. Studies found that *Nitrospiraceae*, *Saprospiraceae*, *Anaerolineaceae*, *Rhodobacteraceae*, *Flavobacteriaceae* played synergistic roles in degradation in the removal of pollutants. In general, the seawater subsurface-flow wetland could significantly improve the water quality and met the demand of aquaculture water.

The abuse of antibiotics has always been a pain point in the aquaculture industry. If the large-scale constructed wetland system is used for the treatment of mariculture wastewater, the removal of antibiotics may be a good evaluation standard.

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