



Effects of Sewage Treatment on Microbial Community Structure of Surface Water in Xiantao Wetland

Shufen Song¹, Fan Yang², Li-An Ma^{1*}, Xiu-Fang Gao^{3*}

¹College of Life Science, Yangtze University, Jingzhou, China

²Wuhan Yihui Far Biotechnology Co. Ltd., Wuhan, China

³College of Resources and Environment, Yangtze University, Wuhan, China

Email: 760100785@qq.com, *malian@yangtzeu.edu.cn, *gxf2017@yangtzeu.edu.cn

How to cite this paper: Song, S.F., Yang, F., Ma, L.-A. and Gao, X.-F. (2018) Effects of Sewage Treatment on Microbial Community Structure of Surface Water in Xiantao Wetland. *Open Access Library Journal*, 5: e4583.

<https://doi.org/10.4236/oalib.1104583>

Received: April 8, 2018

Accepted: April 26, 2018

Published: April 29, 2018

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Abstract

[Objective] The aim of study is to research the treatment of urban sewage by the semi natural wetland method in the high connectivity water system of the surface water and surface water in the inland waterway. **[Methods]** Microbial diversity and community structure in the surface water of eight samples on the Xiantao artificial wetland were investigated by using the Illumina Miseq sequencing technique and the resulting microbial data were statistically analyzed in combination with environmental variabl. **[Results]** The results showed that COD concentration had a very significant positive correlation with total microbial communities ($r = 0.700$, $P < 0.01$), and TP, $\text{NH}_4^+\text{-N}$ and TN were significantly positively correlated ($r = 0.509$, 0.517 and 0.508 , $P < 0.05$). In addition, COD concentration was positively correlated with abundant groups ($r = 0.488$, $P < 0.05$), and TP, $\text{NH}_4^+\text{-N}$ and TN were significantly positively correlated ($r = 0.537$, 0.356 and 0.360 , $P < 0.05$). For rare groups, they may be more sensitive to the environment than their abundant groups. **[Conclusion]** For rare taxa, it is likely to be more sensitive to the environment than their rich taxa. The relative abundance of rare taxa of samples collected when buried deep 5 m is greater than that of surface water. Therefore, the environmental conditions of microbial responses (rare taxa) should be carefully evaluated in the future.

Subject Areas

Environmental Sciences

Keywords

Artificial Wetland, Surface Water, Microorganism, Diversity

1. Introduction

Wetland is at the intersection of land and water, and it is the intermediate zone between land and aquatic ecosystem. Because of its strong purification capacity, wetlands are favored by scientists and engineers, and constructed wetlands are used for purifying various kinds of sewage [1] [2] [3]. There are a large number of aerobic, anaerobic and facultative anaerobic microbial communities in wetland, which are widely distributed in the soil matrix and plant root surface of wetland system [4].

As an important index of water quality evaluation, microorganism can reflect the changes of the whole aquatic environment in time [5]. Water pollution will affect the composition and diversity of microbial communities [6] [7]. In recent years, the research on microbial community structure and distribution, microbial degradation mechanism and other aspects in wetland ecosystem is attracting more and more attention from researchers both at home and abroad [8] [9] [10].

In this study, a simple and low-cost surface flow constructed wetland was used to decompose and control organic pollutants through microbial communities [11] [12] [13] [14], affecting and regulating domestic sewage, purifying water quality, and to a certain extent, reflecting the characteristics of water pollution and the evolution of ecological function. This study explored the structure and diversity of microbial community in surface water of wetland treated by wetland, which is significant for understanding the dynamic changes and ecological functions of community structure, elucidates the specific relationship between communities and habitats, and explores the operation mechanism of constructed wetlands.

2. Methods and Materials

2.1. Introduction of Xiantao Wetland Method for Domestic Sewage Treatment Test

Dujiatai Flood Diversion Sluice Downstream Xiantao Hanjiang River wetland is located at about 2.5 km. Since the Dujiatai Flood Diversion project built from April 1956, a total of 21 times is the flood, flood utilization rate of most engineering gate is the highest, it each time less than a week of flood period in the idle state long-term implementation. South to North Water Diversion Project in December 2014 after the official implementation, downstream water decreased sharply in Hanjiang River, underground water level decrease, so the implementation of the Hanjiang River downstream from Changjiang River water diversion project. The future probability of Dujiatai Flood Diversion Project of flood is very small, the possibility is almost zero. In accordance with the provisions of the floodway construction, application and get approved, built in 2004 in Dujiatai floodway by wetland ecosystem and operation capacity of sewage treatment for the sewage treatment plant 60 thousand tons of scale. The construction of natural wetland conditions (without impervious layer treatment) has rich biodiversity and has formed a unique ecological environment, which has great social

and economic value and scientific research value. The sewage treatment scale of 60 thousand tons per day, the oxidation pond process using the pretreatment, the main process of 2 sedimentation tank, 2 oxidation pond, and then enter the length of about 5 km, width of 600 m in the narrow region to calamus, reed wetland plants for artificial wetland mainly, through the fall way gravity (Figure 1).

2.2. Determination of Sampling and Environmental Factors of Water Samples

Autumn 2014, Groundwater and shallow groundwater samples were collected from Xiantao wetland downstream, its detection results are shown in Table 1 (surface water samples directly put the sampler under the surface of water for 0.1 - 0.3 meters). DNA extraction were firstly collected into sterile Polyethylene plastic bottle (volume is at least 1 L) using a sterilizing sampling bottle. The collected water samples are then stored in a box filled with sufficient ice to keep the sample temperature below 4°C during transit. When we arrived in the laboratory, we used microporous membrane (0.22 m) to filter water samples, filtered pore size filter membrane, stored in -80°C refrigerator, DNA was extracted with Water DNA Kit kit from OMEGA Company, The extracted DNA was amplified using a specific set of bar-code 515F and 909R. Purification was recovered by Beijing Kang for century general DNA purification recovery Kit, and finally sent to the Chinese Academy of Sciences, Chengdu Institute of biology sequenced by using an Illumina Miseq platform DNA. In addition, Determination and analysis of water physical and chemical indexes: surface water (COD_{Cr}) was determined

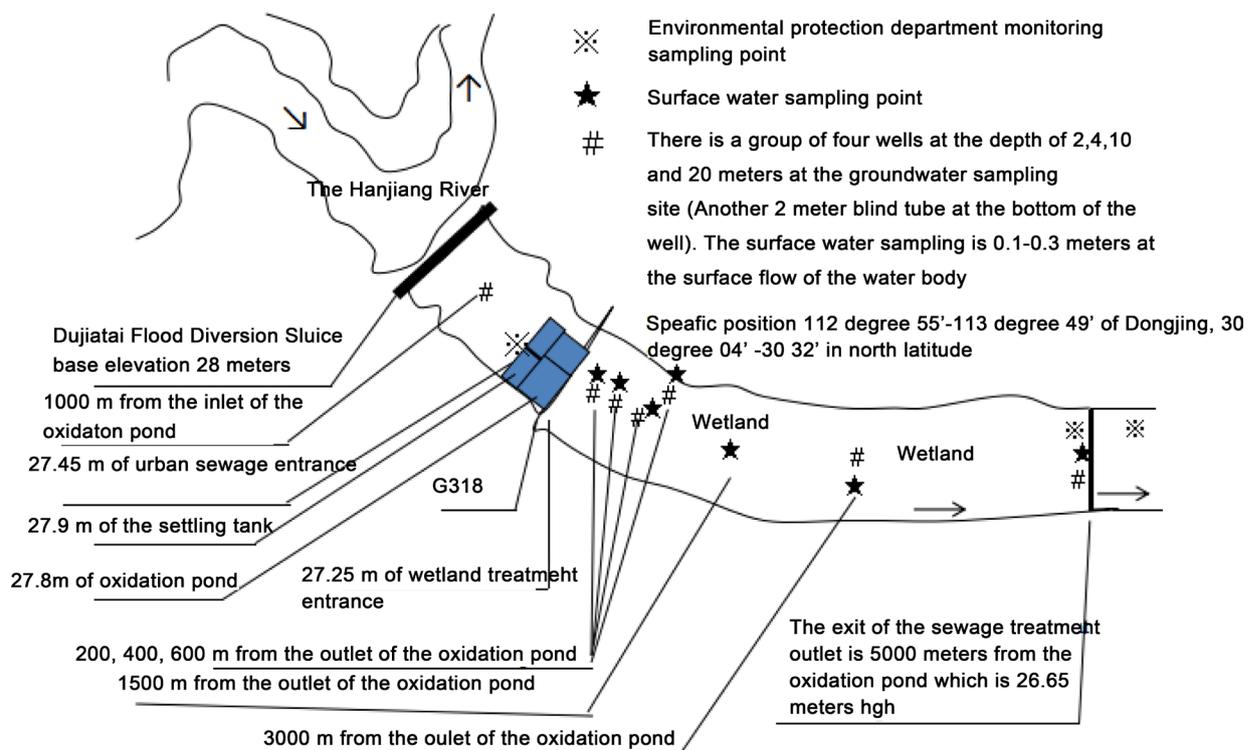


Figure 1. Xiantao wetland azimuth map.

by potassium dichromate titration, The groundwater (COD_{Mn}) was determined by acid high potassium acid method, TN by alkaline potassium persulfate digestion UV spectrophotometry spectrophotometric method, TP using potassium persulfate digestion ammonium molybdate spectrophotometric method, NH_4^+ -N using Nessler's reagent colorimetric method.

2.3. Sequencing Analyses

The raw data obtained by sequencing need to be fused with dual end sequences using Flash software, and returned to the corresponding sample sequence split by adding bar-code, primers and low quality sequences were removed, then the optimized sequence is analyzed [15]. Operational taxonomic units (OTUs) were defined at 97% sequence similarity by using UCLUST algorithm [16]. The use of Usearch clustering software platform, using RDP Classifier Bias algorithm for analysis of the taxonomic similarity level of 97% representative sequences OTU [17], Composition and statistics of each sample in each classification level of the community, a variety of alpha diversity indices were calculated including Chao1 (a measure of richness, namely the estimated number of phylotypes), Shannon (includes both richness and evenness), Equitability (evenness, distribution of phylotypes), and phylogenetic distance whole tree (phylogenetic closeness across the entire tree in a subset of phylotypes). In addition to analyzing the different members of a community and their responses to environmental factors, Yang jian have extracted abundant and rare taxa based on OTU relative abundance following previous studies [18] [19], the OTUs with relative abundance higher than 1% and lower than 0.01% within one sample were defined as abundant and rare taxa [20]. In order to assess the differences in structure of community, principal coordinate analysis (PCoA) was conducted in the PAST.

3. Result and Analysis

3.1. Analysis of Physical and Chemical Indexes of Wastewater

According to the state regulations, the treated sewage should reach the standard of pollutant discharge of GB 18918-2002 "municipal wastewater treatment plant" [21], **Table 1** shows that the F1 (400 m) and F3 (600 m) are high concentration ($\text{COD}_{\text{Cr}} > 120 \text{ mg/L}$); F5 (3000 m) had achieved the two level standard of "municipal wastewater treatment plant" ($100 \text{ mg/L} < \text{COD}_{\text{Cr}} < 120 \text{ mg/L}$). The F2, F4 and F6 were reach the first level standard ($\text{COD}_{\text{Cr}} < 50 \text{ g/L}$). The TP of these samples were 0.03 - 5.14 mg/L, negatively correlated with COD_{Cr} ($r = 0.982$, $P < 0.0001$). NH_4^+ -N were 0.96 - 8.81 mg/L, negatively correlated with COD_{Cr} ($r = 0.987$, $P < 0.0001$). TN were 1.28 - 19.17 mg/L, negatively correlated with COD_{Cr} ($r = 0.980$, $P < 0.0001$). As can be seen from **Table 1**, along with the level of wetland before treatment, the environmental factors gradually decreased. There is no phosphorus in the groundwater quality standard (GB/T 14848-2017). It is generally believed that the concentration of phosphorus in the groundwater is very low, but the phosphorus in the groundwater is present, and the amount

of the phosphorus is large, and the pollution of phosphorus cannot be ignored. The characteristics of the highly connected water system of surface water and groundwater are fully reflected here. From the surface water to the vertical direction of groundwater, that is, the environmental indicators from surface water and groundwater depth 5 meters are also decreasing.

3.2. Microbial Diversity of the Researched Constructed Wetland

A total of 80,524 high-quality sequences with 7030 - 24,785 sequences (mean = 15,546.5) and 730.4 - 1077.1 OTUs (mean = 859.6) for eight water samples were obtained. The diversity index, including Shannon (4.9 - 6.0), phylogenetic distance of a whole tree (50.9 - 68.5), and Chao 1 (2306.8 - 3165.8) were shown in **Table 2**. The dominant phyla (the relative abundance of more than 1%) in the studied sediments were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes*, *Acidobacteria*, and *Chloroflexi* (**Figure 2**). *Proteobacteria* is the most abundant phylum (more than 82% of total sequence reads).

Among the retrieved OTUs, a total of 8-20 OTUs were classified as abundant

Table 1. Detection results of surface water in constructed wetland.

Sample	Location		COD _{Cr} (mg/L)	TP (mg/L)	NH ₄ ⁺ -N (mg/L)	TN (mg/L)
FC1	-2000	Surface water	38.59	0.04	1.96	2.03
FC2	-2000	Deep of 5 m	2.89	0.03	1.73	1.28
F1	400 m	Surface water	182.53	5.14	8.81	19.17
F2	400 m	Deep of 5 m	3.67	0.18	1.85	2.94
F3	600 m	Surface water	151.66	4.98	8.43	16.51
F4	600 m	Deep of 5 m	2.94	0.05	1.33	2.87
F5	3000 m	Surface water	111.16	3.45	6.55	14.21
F6	3000 m	Deep of 5 m	2.92	0.04	0.96	1.77

Table 2. Alpha-diversity of the surface water in the studied Xiantao constructed wetland.

Sample	Total reads	Observed OTUs	Simpson	Shannon_Wiener	PD_whole_tree	Good's Coverage	Chao1
FC1	18,976	802.6	0.994	8.7	53.5	0.7	2393.2
FC2	21,167	735.1	0.972	7.8	50.9	0.7	2306.8
F1	8237	966.7	0.987	8.7	59.3	0.6	3055.5
F2	11,825	1077.1	0.992	9.2	68.5	0.6	3165.8
F3	11,839	937	0.989	8.7	60.8	0.6	2638.8
F4	20,513	897	0.993	8.8	55.3	0.6	3029.1
F5	24,785	730.8	0.975	7.6	57.6	0.7	2535.6
F6	7030	730.4	0.977	7.7	51.7	0.7	2419.5

OTUs. These abundant OTUs accounted for 5.63% - 12.37% of total OTUs and represented 64.66% - 80.85% relative abundance of sequence reads in the studied samples. In contrast, a total of 0 - 139 rare species were identified and they accounted for 0% - 64.19% of total OTUs and 0% - 6.98% relative abundance of sequence reads in the studied samples (Table 3). Most abundant OTUs belonged to *Proteobacteria* and *Actinobacteria*, which the abundant OTUs accounted for 29.61% - 68.14% and 0% - 19.71% of total sequence reads in the studied samples (Table 4).

Studies have shown that water samples with similar physicochemical values have similar microbial structure patterns. For example, cluster analysis shows that the surface water of F1, F3 and F5 (COD > 110 mg/L) wetlands is clustered into a cluster, different from other water samples (Figure 3). The horizontal direction Surface water of groundwater in Constructed Wetlands (FC1, F1, F3 and F5) were dominated by sequences affiliated with *Betaproteobacteria*, *Gamma-proteobacteria*, *Epsilonproteobacteria*, *Actinobacteria*. The depth is 5 meters

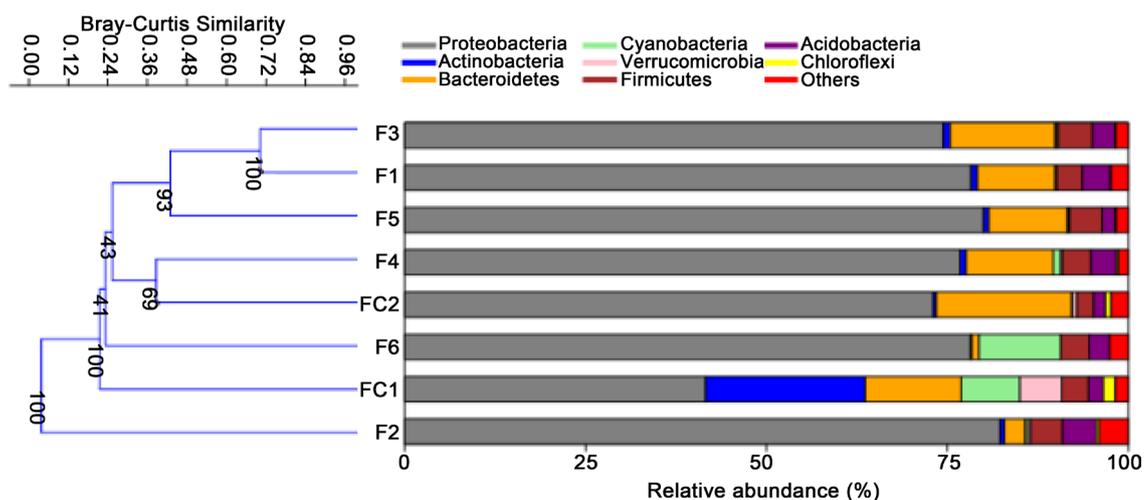


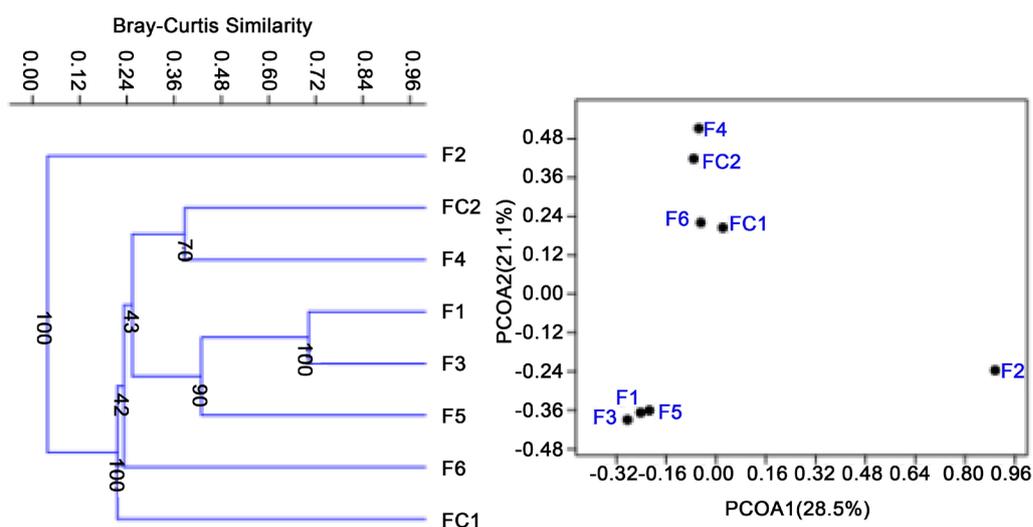
Figure 2. Bray-Curtis similarity-based cluster analysis (left) of structure of community in the studied samples and schematic figures (right) showing the frequencies of OTUs affiliated with major phyla in this study.

Table 3. Abundance estimates of the abundant and rare OTUs in the studied surface water samples in this study.

Sample	Abundant OTUs (Percentage of abundant OTUs/total OTUs in each sample)	Abundant OTU relative abundance (%)	Rare OTUs (Percentage of rare OTUs/total OTUs in each sample)	Rare OTU relative abundance (%)
FC1	20 (10.05%)	67.96	92 (46.23%)	5.27
FC2	17 (7.91%)	77.93	138 (64.19%)	6.98
F1	15 (10.42%)	64.79	0 (0.00%)	0
F2	8 (5.63%)	66.82	36 (25.35%)	3.27
F3	17 (9.29%)	64.66	37 (20.22%)	2.47
F4	12 (6.45%)	73.03	93 (50.00%)	5.03
F5	14 (6.01%)	74.05	139 (59.66%)	6.49
F6	12 (12.37%)	80.85	0 (0.00%)	0

Table 4. Relative abundance of abundant OTUs within different phyla across the studied samples in this study.

Phylum	<i>Proteobacteria</i>	<i>Actinobacteria</i>	<i>Bacteroidetes</i>	<i>Cyanobacteria</i>	<i>Verrucomicrobia</i>	<i>Firmicutes</i>	<i>Chloroflexi</i>
FC1	29.61	19.71	5.95	5.95	4.61	1.06	1.06
FC2	63.30	0.00	14.64	0.00	0.00	0.00	0.00
F1	58.21	0.00	5.05	0.00	0.00	1.53	0.00
F2	65.37	0.00	0.00	0.00	0.00	1.45	0.00
F3	54.80	0.00	8.39	0.00	0.00	1.47	0.00
F4	63.21	0.00	8.76	0.00	0.00	1.06	0.00
F5	66.40	0.00	6.14	0.00	0.00	1.52	0.00
F6	68.14	0.00	0.00	11.18	0.00	1.53	0.00

**Figure 3.** Clustering and principal coordinates analysis of total MCC among the studied samples based on Bray-Curtis similarity.

(FC2, F2, F4, and F6) were dominated by sequences affiliated with *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Melainabacteria*, *Sphingobacteriia*. The vertical of 400 m (F1 and F2) were dominated by sequences affiliated with *Betaproteobacteria*. 600 m (F3 and F4) were dominated by sequences affiliated with *Betaproteobacteria*, *Gammaproteobacteria*, *Sphingobacteriia*. 3000 m (F5 and F6) were dominated by sequences affiliated with *Betaproteobacteria*, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Melainabacteria*. The contrast samples (FC1 and FC2) were dominated by *Betaproteobacteria*, *Bacteroidia*, *Actinobacteria* (Table 5).

3.3. Statistical Analyses.

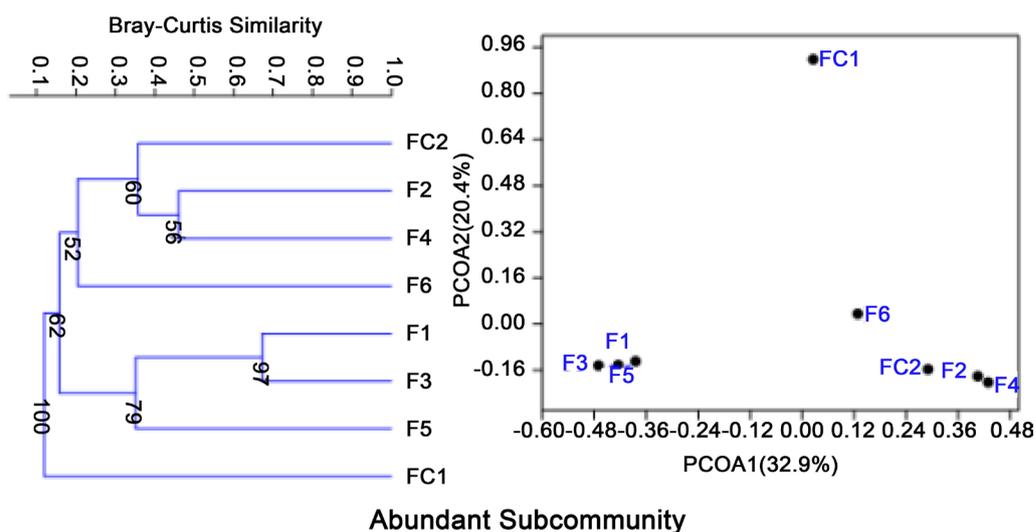
Mantel test showed that there was a significant correlation between the community structure of domestic sewage treated by wetland and CODcr ($r = 0.488$, $P < 0.05$) (Table 6). In addition, the similarity of Bray Curtis based microbial communities was positively correlated with CODcr ($r = 0.700$) (Figure 4). Similarly, Mantel test showed that the abundance of communities was significantly correlated

Table 5. Relative abundance (%) of top 10 classes in the surface water of the studied Xiantao constructed wetland.

Top10 classes	Betaproteo bacteria	Bacilli	Gammaproteo bacteria	Alphaproteo bacteria	Flavo bacteria	Epsilonproteo bacteria	Bacteroidia	Melaina bacteria	Sphingo bacteria	Actino bacteria
FC1	32.58	3.16	2.66	5.95	5.17	0.11	0.13	0.13	4.73	22.03
FC2	60.24	1.24	7.68	1.42	0.56	0.13	13.16	0.04	1.06	0.42
F1	60.19	2.56	9.71	2.78	6.25	4.94	2.67	0.00	1.48	0.91
F2	74.35	1.95	4.54	2.72	0.86	0.32	0.00	0.23	1.82	0.64
F3	54.30	2.41	10.33	2.54	7.99	6.02	4.95	0.07	1.40	0.87
F4	36.31	2.55	37.74	1.90	0.65	0.09	0.75	0.95	10.53	0.88
F5	57.47	1.17	3.56	1.59	5.70	15.50	3.85	0.05	0.89	0.73
F6	59.76	3.22	13.55	4.59	0.21	0.21	0.00	11.18	0.58	0.00

Table 6. Mantel test showing the correlation between structure of community similarity and environment parameters of the studied surface water in this study.

	All OTUs	Abundant OTUs
COD	0.488*	0.700**
TP	0.537*	0.509*
NH ₄ ⁺ -N	0.356*	0.517*
TN	0.360*	0.508*

**Figure 4.** Cluster analyses and principal coordinates analyses of abundant structure of community among the studied samples based on Bray-Curtis similarity.

with COD_{Cr} ($r = 0.700$) (Table 6). In addition, the Mantel test also showed that the total amount of the community and the rich group structure were significantly related to the other three indicators, TP ($r = 0.537$ and 0.509), NH₄⁺-N ($r = 0.356$ and 0.517), TN ($r = 0.360$ and 0.508) (Table 6).

4. Discussion

Microbial diversity represents the stability of microbial communities, and also reflects the influence of environmental and ecological mechanisms on commun-

ities. It can also be defined as the richness of microbial life. The comprehensive effect of microbial monitoring can not only reflect the various factors of pollution [22], but also reflect the historical status of the environmental pollution, become the most sensitive monitoring indicators of exposure and effects of pollutants, it is of great significance for the early prediction of toxicity and risk assessment of environmental pollutants long-term.

In this study, the effects of sewage treatment on the surface water biota were found. Through the diversity index, richness index and the distribution of microbial groups, we can see that the surface water of the wetland and the surface water of deep 5 m detected the complex flora, but the composition of the microbial community was different. The abundance of *Proteobacteria* is the main advantage in all samples, which is consistent with the findings of Dou Na Sha [23] and Li Simin [24]. *Bacteriobacteria* is a common dominant group in the surface water samples. *Cyanobacteria* is a common dominant group with deep 5 m. Compared with the surface water samples of wetland 400 m (0%), the abundances of *Cyanobacteria* at the depth of 5 m at 3000 m depth were higher (11.29%), but the abundance of the *Verrucomicrobia* and the *Chloroflexi* in the 3000 m depth 5 m was 0 (Figure 2). In the study of the species, abundance and ecophysiology of Chloroflexi in activated sludge, Kragelund also found that *Chloroflexi* has a better biological phosphorus removal effect [25]. The change of phosphorus from 400 m to 3000 m also revealed that the reason for the reduction of TP in this stage is the role of *Chloroflexi*.

The different nutrition level and the physical and chemical conditions of the artificial wetland are different, forming their own “small environment”. Their respective “small environment” is suitable for the growth of different microbial communities, producing different dominant bacteria groups. In this study, the surface water of wetland increased the relative abundance of species [26] and the increasing trend of the rare species as the flow direction and depth increased (Table 3, Table 4). The relative abundance of 400 m surface water and 3000 m depth 5 m in wetland was 0, indicating that the rare communities showed more obvious response to the environment than the rich communities (proved by the trend of relative abundance), which showed that the rare groups may have more restrictive distribution than the rich groups. Abundant taxa can use abundant resources, so they have low extinction probability and high transmission probability. In addition, rare taxa may occupy less suitable niche in higher or lower levels of physical and chemical values, which are more vulnerable to environmental conditions than abundant taxa. Therefore, the rare taxa may be more sensitively to other environmental conditions than other abundant taxa.

5. Conclusion

The microbial community diversity of the surface water in the sewage treatment by wetland method is related to the amount and species of microorganism. The abundance changes of some genera can indirectly indicate the degree of water

pollution or purification. Rare taxa are more sensitive to environment than their abundant counterparts.

Acknowledgements

This research was supported by grants from the National Natural Science Foundation of China (Grant No. 41371464), supported by the study abroad fund of Yangtze University.

We are very grateful to Professor He Jizheng of the Chinese Academy of Sciences, the center for ecological environment research, Jian Yang teacher analysis of data.

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