



American Journal of Current & Applied Research in Microbiology

Research Article

Relationship between Spatial Variations in Microbial Community and Surface Water Quality in the Xiantao Artificial Wetland, China -

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Submitted: 02 February 2019; **Approved:** 25 April 2019; **Published:** 29 April 2019

Citation this article: Song SF, Gao XF, Yang F, Ma LA. Relationship between Spatial Variations in Microbial Community and Surface Water Quality in the Xiantao Artificial Wetland, China. American J Current App Res Microbiol. 2019;1(1): 001-008.

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ABSTRACT

Artificial wetlands are useful for wastewater treatment; however, relatively little is known of the effects of sewage on artificial wetland microbial community structure. Therefore, we assessed the effect of municipal sewage on microbial community diversity in surface water throughout an artificial wetland (Xiantao artificial wetland) treating municipal sewage. We analyzed the relationship between physicochemical parameters of surface water (i.e., Chemical Oxygen Demand (COD), Total Nitrogen (TN), Total Phosphorus (TP), and $\text{NH}_4^+\text{-N}$) with microbial community structure (Illumina MiSeq sequencing followed by abundance indices). The results showed that the total microbial community in surface water was significantly correlated with COD, TN, TP, and $\text{NH}_4^+\text{-N}$ ($r = 0.764, 0.897, 0.883, 0.839, P < 0.05$). In addition, the most abundant taxa were significantly correlated with COD ($r = 0.803, P < 0.05$). The relative abundance of rare operational taxonomic units in the more purified water farther downstream was higher than in the polluted area, suggesting that rare groups were more sensitive to physicochemical parameters than abundant groups, and that the abundance of some bacteria could indirectly indicate the degree of aquatic pollution. Our results indicate that the responses of microorganisms in artificial wetlands to environmental conditions should be considered to ensure efficient treatment.

Keywords: Artificial wetland; Microbial diversity; Microorganism; Sewage treatment; Surface water

INTRODUCTION

Water is vital to human survival; however, socioeconomic development, industrialization, and human activity have exerted tremendous pressure on the aquatic environment, resulting in widespread water pollution, which has serious consequences on human health and development [1]. As such, effective methods for preventing aquatic pollution are necessary.

Artificial wetlands represent one pollution remediation method, and are particularly useful for effective organic matter removal. They are operated continuously, and comprise sedimentation ponds, oxidation ponds, and soil infiltration beds. Moreover, they are planted with beneficial aquatic plants, such as reeds, cattails, and grasses. Insoluble organic matter particles suspended in water can be trapped via filtration, resulting in the sedimentation of the substrate in the wetland, which can then be utilized by certain facultative or anaerobic microorganisms. Meanwhile, soluble organic matter is removed via adsorption onto biofilm formed on substrate and plant root surfaces and decomposition via metabolism of the microbes within the biofilm [2-3].

Microorganisms have a vital role in maintaining the stability of aquatic ecosystems, including artificial wetlands [4], via microbial assimilation and subsequent degradation of pollutants. However, microorganisms are sensitive to changes in their external environment, making them useful as indicators to monitor and reflect water quality. Thus, it is essential to understand the spatial characteristics of microbial communities in artificial wetlands during domestic sewage treatment to clarify the relationship with the history, migration, and transformation of pollutants.

The rapid development and improvements to high-throughput sequencing technology in recent years [5] have enabled the rapid and accurate simultaneous detection of multiple microbial taxa, including both dominant rare/unknown species. For instance, high-throughput sequencing has been applied widely to studies of the microbial community structures of rivers, lakes [6], reservoirs [7], and oceans [8].

At present, there are many studies of pollutant removal via artificial wetlands in terms of law and policy, selection of wetland plants, development and combination of substrate materials, and their overall structure. In addition, research has focused on improving the efficacy of artificial wetland sewage treatment [9-11]. However, there are no studies of the microbial population and quantitative changes in domestic sewage and surface water in the treatment of flood diversion channels.

Therefore, the purpose of this study was to clarify the relationship between surface water microbial communities and artificial wetland operation. In particular, we (1) investigated the spatial changes in the structure and composition of the microbial community in the surface water of an artificial wetland along a horizontal gradient and (2) assessed the relationships of microbial community with various environmental factors. To this end, we investigated microbial diversity using molecular biology techniques and high-throughput sequencing. From the results, the changes in the microbial population in the surface water environment were discussed at a microcosmic level. These results provide theoretical support to ensure the efficient operation of artificial wetlands based on microbial community structure.

MATERIALS AND METHODS

Study site and sample collection

We collected samples in the Xiantao artificial wetland, China, for the present study. This artificial wetland is coupled with a municipal sewage treatment plant, and is accessed via the G318 National Road. It comprises two $95 \text{ m} \times 50 \text{ m} \times 5 \text{ m}$ sedimentation ponds, two $450 \text{ m} \times 130 \text{ m} \times 4 \text{ m}$ oxidation ponds, and two $350 \text{ m} \times 6000 \text{ m}$ ecological soil infiltration bed separated by a 3-m-wide road at the along the banksends. Among ecological soil infiltration bed, 100,000 reeds, 50,000 cattails, and $7.5 \times 10^4 \text{ m}^2$ aquatic bitter grass plants have been artificially planted. For a more detailed description of the experimental area and sampling sites, please refer to Song et al. [12].

Sampling was performed on October 14, 2014. Surface water samples were collected at one site downstream of the sewage plant but before the artificial wetland (site J1: 200 m), four sampling points downstream of the sewage treatment plant along the artificial wetland (J2: 400 m, J3: 600, J4: 3000 m, J5: 5000 m), and at one point 2000 m upstream of the artificial wetland/sewage treatment plant (JC: -2000 m) (Figure 1). Table 1 provides the details and physicochemical parameter values of these six sites.

At each site, surface water samples were collected by placing a sampler (QCC15; Beijing Purity Instrument Co. Ltd., Beijing, China) 0.10–0.30 m below the water surface. At least two samples were collected at each site, and at least 1 L was collected for each parallel sample, and the collected water samples were kept below 4°C and transported to the laboratory.

Physicochemical parameter measurement

Aquatic pollution is commonly measured as the Chemical

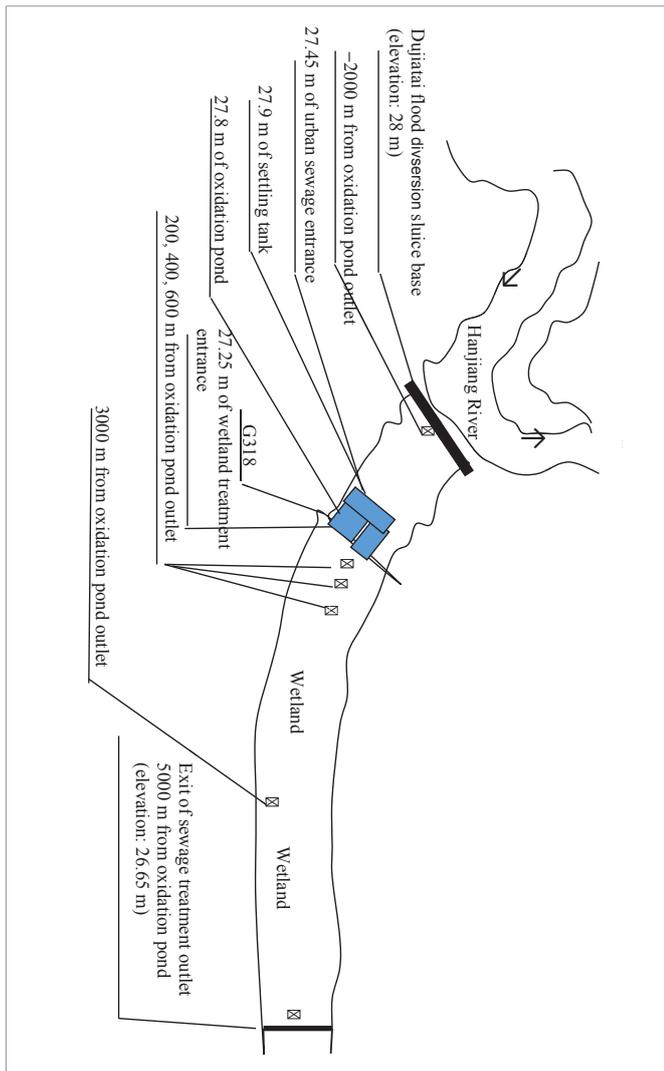


Figure 1: Map (azimuthal projection) of the Xiantao artificial wetland (30°04'N-30°32'N, 112°55'E-113°49'E). Star, surface water sampling point

Table 1: Physicochemical results of at surface water sampling sites at the Xiantao artificial wetland.

Sampling site	JC	J1	J2	J3	J4	J5
Location (m) [*]	-2000	200	400	600	3000	5000
COD _{Cr} (mg/L)	31.50	203.52	182.53	151.66	111.16	38.59
TP (mg/L)	0.08	5.22	5.14	4.98	3.45	0.15
NH ₄ ⁺ -N (mg/L)	0.50	9.10	8.81	8.43	6.55	0.80
TN (mg/L)	1.85	21.11	19.17	16.51	14.21	2.28

^{*}Distance from the sewage plant; positive (negative) values are downstream (upstream) of the plant

Note: COD_{Cr}: Chemical Oxygen Demand; TP: Total Phosphorus; NH₄⁺-N: Ammonium Nitrogen; TN: Total Nitrogen.

Oxygen Demand (COD_{Cr}), which is an indicator of the amount of reductive substances in water; these predominantly comprise organic matter, but also include nitrites, sulfides, and ferrous salts. As such, higher COD_{Cr} values are indicative of more serious organic pollution, and COD_{Cr} can be used as a comprehensive index of the relative content of organic matter in a water body, which can directly reflect the degree of pollution [13-14]. Thus, we measured the COD_{Cr} of the water samples via the potassium dichromate titration method

(Chinese Standard GB/T 11914-1989). In addition, we measured Total Nitrogen (TN) via alkaline potassium persulfate digestion and ultraviolet spectrophotometry (Chinese Standard HJ 636-2012), Total Phosphorus (TP) via potassium persulfate digestion with ammonium molybdate spectrophotometry (Chinese Standard GB/T 11893-1989), and ammonium nitrogen (NH₄⁺-N) using a Nessler's reagent-based colorimetric method (Chinese Standard GB/T 7479-1987). At least two samples were collected at each site, and each parameter was measured in triplicate for each sample.

DNA isolation and sequencing

Upon arriving at the laboratory, the samples were filtered through 0.22-µm microfiltration membranes (Mixed Cellulose Microporous Filtration Membrane; Shanghai Xingya Purification Material Factory, Shanghai, China) to collect microorganisms, which were then stored at -80°C. DNA was extracted from the samples using the Water DNA Kit (OMEGA Bio-tek, Norcross, GA, USA) and amplified with a specific set of 515F and 909R primers. Finally, the amplified products were sent to the Chengdu Institute of Biology, Chinese Academy of Sciences (Chengdu, China) for high-throughput sequencing on their Illumina MiSeq platform.

Sequencing analysis

Paired-end raw sequences from the original DNA fragments were merged using FLASH [15]. Paired-end read sequences were assigned to each sample according to a unique barcode, primers and low-quality sequences were deleted, and the optimized sequences were analyzed [16]. Next, we used the UCLUST algorithm to define Operational Taxonomic Units (OTUs), using a sequence similarity cut-off of 97% [17]. Representative sequences were selected for each OTU; they were aligned using PyNAST [18] and FastTree was applied to construct the phylogenetic tree. The OTUs were analyzed using alpha diversity (within samples) indices, including the Chao1 index (a measure of community species richness), Shannon-Weiner index (community heterogeneity and evenness), equitability index (evenness, distribution of phylotypes), and phylogenetic distance of the whole tree (phylogenetic closeness within a subset of phylotypes). Following previous studies [19-21], samples with OTU abundances above 1% and less than 0.01% were defined as abundant and rare taxa, respectively. To assess differences in microbial community composition, the collected data underwent analysis of relevance using the SPSS software package (SPSS ver. 20.0; IBM Corp., Armonk, NY, USA), and cluster analysis and principal coordinate analysis were conducted using the Past3 package (<https://folk.uio.no/ohammer/past/>). In addition, the Past3 package was used to perform the Mantel test to assess the correlations between community structure and physicochemical parameters.

Nucleotide sequence accession numbers

The original sequences are deposited in the Sequence Read Archive (NCBI) (accession no. SRS3045895; BioProject: SRP135735).

RESULTS

Analysis of physicochemical parameters

The results of the physicochemical parameters at each sampling site are shown in table 1. In China, municipal sewage treatment plant pollutant discharge standards are specified in the Chinese Standard GB 18918-2002. Based on these standards, the three downstream sites closest to the treatment plant (J1, J2, and J3) were seriously polluted (COD_{Cr} > 120 mg/L), whereas the next site, J4, reached the

second-level standard ($100 \text{ mg/L} < \text{COD}_{\text{Cr}} < 120 \text{ mg/L}$) and both the upstream reference site (JC) and the site farthest downstream (J5) reached the primary standard ($\text{COD}_{\text{Cr}} < 50 \text{ mg/L}$). The TP, $\text{NH}_4^{+}\text{-N}$, and TN concentrations at all sampling sites were $0.08\text{-}5.22 \text{ mg/L}$, $0.5\text{-}9.10 \text{ mg/L}$, and $1.85\text{-}21.11 \text{ mg/L}$, respectively; all of these parameters were highly significantly positively correlated with COD_{Cr} ($r = 0.974$, $P = 0.001$; $r = 0.971$, $P = 0.001$; $r = 0.986$, $P = 0.0003$, respectively) (Table 1). Among these three indicators, TN had the most significant contribution to COD_{Cr} .

Spatially, the concentrations of pollutants decreased with increasing distance downstream of the treatment plant, and at 3000 m downstream of the treatment plant, the concentrations of pollutants reached the GB 18918-2002 standard. Moreover, at 5000 m downstream, the concentrations of pollutants were similar to those of the upstream control area. These findings suggest that the treatment of domestic sewage with an artificial wetland has no detrimental effect on domestic water for residents farther than 5000 m downstream of the initial treatment plant.

Microbial diversity of the artificial wetland

After quality filtering of the raw reads, a total of 80,524 effective sequences with 7777-24,785 sequences (mean: 13,421) and 730.8-966.7 OTUs (mean: 868) were recovered from all six sampling sites. The diversity indices of all sampling sites were as follows: Shannon–Wiener index, 4.6-5.6; whole-tree phylogenetic distance, 53.5-60.8; Chao1 index, 2393.2-3092.1 (Table 2). Across all sampling sites, the dominant phyla (relative abundance > 1%) were Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, Verrucomicrobia, Firmicutes, Acidobacteria, and Chloroflexi, (Figure 2), of which Proteobacteria was the most abundant phylum, accounting for > 79% of the total sequence reads.

A total of 14-20 OTUs in the surface water samples were classified as abundant. These abundant OTUs accounted for 6.01-18.45% of the total OTUs in the samples, and had relative abundances of 64.66-76.60% at each sampling site. In contrast, 0-139 rare OTUs were classified, which accounted for 0-59.66% of the total OTUs, and had relative abundances of 0-6.49% at each sampling site (Table 3). The most abundant OTUs were Bacteroidetes and Proteobacteria, which accounted for 29.61-66.4% and 5.05-31.66% of the total sequence reads in the samples (Table 4).

The analysis revealed that sampling sites with similar physicochemical parameter values had similar microbial structures. For instance, cluster analysis revealed that the three seriously polluted sites (J1, J2, and J3; $\text{COD}_{\text{Cr}} > 120 \text{ g/L}$) were grouped into one distinct cluster (Figure 3), and were dominated by sequences related to the bacterial classes Epsilonproteobacteria, Flavobacteriia,

Gammaproteobacteria, and Betaproteobacteria. The one site within the second-level standard (J4; $100 \text{ mg/L} < \text{COD}_{\text{Cr}} < 120 \text{ mg/L}$) was dominated by sequences related to the classes Epsilonproteobacteria, Flavobacteriia, and Betaproteobacteria. Finally, the site that met the primary standard (J5; $\text{COD}_{\text{Cr}} < 50 \text{ mg/L}$) was dominated by Actinobacteria, Flavobacteriia, Alphaproteobacteria, and Betaproteobacteria. For comparison, the upstream site (JC) formed a separate cluster that was dominated by Actinobacteria, Flavobacteriia, and Betaproteobacteria (Table 5).

Table 6 presents the relative abundances (> 2%) of genera and species within the samples. Six dominant bacterial genera were identified in samples J1–J3: *Methylothera*, *Flavobacterium*, *Arcobacter*, *Pseudomonas*, *Methylophaga*, and *Malikia*. Because of the high values of the physicochemical indicators at these sites, the emergence of these dominant bacteria may have been related to eutrophication. Meanwhile, there were seven dominant bacterial taxa at J5: *Albidiferax*, *Candidatus Planktophila*, marine metagenome, *Phalacroma mitra*, hgcl clade, *Pseudarcicella*, and *Fluviicola*. All physicochemical indicators at J5 met the discharge standards; therefore, the emergence of these bacteria may have been driven by the highly purified water (Table 6).

CCA analysis was conducted to examine samples in response to the environmental variables (Figure 5). As showed by figure 5, COD, TP, $\text{NH}_4^{+}\text{-N}$ and TN were positively correlated, and they had a great influence on the structure of microbial community. Between environmental factors and samples, J1, J2, J3, J4 and J5 were most affected by environmental factors, while JC was least affected by environmental factors. J2, J3, J4 and J1 environmental factors have a small difference, but the distribution distance is far, which is possibly determined by other (not measured) factors lead to a greater difference between J2, J3, J4 and J1.

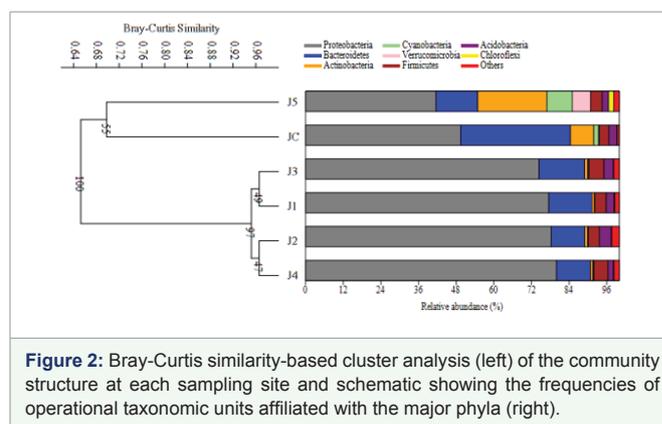


Figure 2: Bray-Curtis similarity-based cluster analysis (left) of the community structure at each sampling site and schematic showing the frequencies of operational taxonomic units affiliated with the major phyla (right).

Table 2: Alpha diversity of the microbial community in surface water in the Xiantao artificial wetland.

Sampling site*	Total reads	Observed OTUs	Simpson	Shannon–Wiener	Whole-tree PD	Good's Coverage	Chao1
JC	7777	860.7	0.994	8.7	53.8	0.65	2985.9
J1	8910	912.8	0.984	8.4	58.4	0.62	3092.1
J2	8237	966.7	0.987	8.7	59.3	0.61	3055.5
J3	11,839	937	0.989	8.7	60.8	0.63	2638.8
J4	24,785	730.8	0.975	7.6	57.6	0.7	2535.6
J5	18,976	802.6	0.994	8.7	53.5	0.7	2393.2

*Sampling sites are the same as those in table 1

Note: OUT: Operational Taxonomic Unit; PD: Phylogenetic Diversity.



Correlation between physicochemical parameters and microbial community

Statistical analysis further confirmed the relationship between the physicochemical parameters and microbial community structure. There was a significant correlation between the total community structure of surface water samples and COD_{Cr} based on Mantel ($r = 0.764, P < 0.05$) tests (Figure 3) (Table 7). Furthermore, the abundance of microbial communities in surface water was significantly correlated with COD_{Cr} based on Mantel ($r = 0.803, P < 0.05$) tests (Figure 4) (Table 7). Finally, TP ($r = 0.897$ and 0.924 , respectively), NH₄⁺-N ($r = 0.883$ and 0.912), and TN ($r = 0.839$ and 0.875) were significantly related to the total and abundant groups of microbial communities based on the Mantel test (Table 7).

DISCUSSION

Consistent with our findings that Proteobacteria was the dominant phylum in all samples, followed by Bacteroidetes (except J5), the results of Dou [22] and Li [23] showed that Proteobacteria and Bacteroidetes were the most abundant phyla in municipal sewage. Moreover, in the present study, the spatial distributions of the diversity/richness indices and microbial taxa revealed more complex, albeit different, microbial community structures in the seriously polluted versus primary standard surface waters, where the site with high-quality water (J5) had higher biological diversity (Figure 2). For comparison, Yang et al. [24] used a terminal restriction

Table 3: Abundance estimates of operational taxonomic units (OTUs) divided into abundant and rare OTUs at the surface water sampling sites.

Sampling site*	Abundant OTUs†	Abundant OTU relative abundance (%)	Rare OTUs‡	Rare OTU relative abundance (%)
JC	19 (18.45%)	76.6	0 (0.00%)	0
J1	14 (9.93%)	67.29	38 (26.95%)	3.49
J2	15 (10.42%)	64.79	0 (0.00%)	0
J3	17 (9.29%)	64.66	37 (20.22%)	2.47
J4	14 (6.01%)	74.05	139 (59.66%)	6.49
J5	20 (10.05%)	67.96	92 (46.23%)	5.27

*Sampling sites are the same as those in table 1

†Total number (number of abundant OTUs/total OTUs in each sample; %)

‡Total number (number of rare OTUs/total OTUs in each sample; %)

Table 4: Relative abundance (%) of abundant OTUs in dominant phyla (abundance > 1%) at each surface water sampling site at the Xiantao artificial wetland.

Phylum*	JC	J1	J2	J3	J4	J5
Proteobacteria	38.8	59.44	58.21	54.8	66.4	29.61
Bacteroidetes	31.66	6.34	5.05	8.39	6.14	5.95
Actinobacteria	6.15	0	0	0	0	19.71
Cyanobacteria	0	0	0	0	0	5.95
Verrucomicrobia	0	0	0	0	0	4.61
Firmicutes	0	1.52	1.53	1.47	1.52	1.06
Chloroflexi	0	0	0	0	0	1.06

Note: Sampling sites are the same as those in table 1. Values of 0 are indicative of relative abundances of 0

*The phyla are sorted from highest to lowest abundance in the samples.

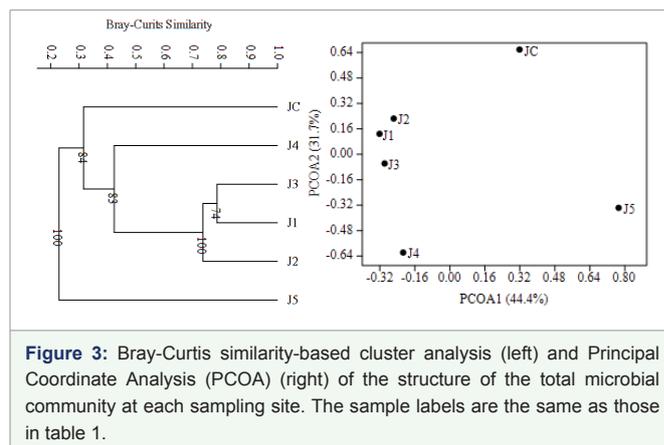


Table 5: Relative abundance (%) of the top ten classes in surface water at the Xiantao artificial wetland.

Class*	JC	J1	J2	J3	J4	J5
Betaproteobacteria	42.27	58.75	60.19	54.3	57.47	32.58
Flavobacteriia	31.04	8.77	6.25	7.99	5.7	5.17
Actinobacteria	7.45	0.64	0.91	0.87	0.73	22.03
Epsilonproteobacteria	0.12	6.38	4.94	6.02	15.5	0.11
Gammaproteobacteria	2.67	9.83	9.71	10.33	3.56	2.66
Alphaproteobacteria	4.03	2.3	2.78	2.54	1.59	5.95
Bacteroidia	0	3.95	2.67	4.95	3.85	0.13
Sphingobacteriia	2.23	0.64	1.48	1.4	0.89	4.73
Bacilli	2.17	2.48	2.56	2.41	1.17	3.16
Melainabacteria	0	0	0	0.07	0.05	0.13

Note: Sampling sites are the same as those in table 1

*The classes are sorted from highest to lowest abundance in the samples.

fragment length polymorphism full-scale processing method to dynamically track and monitor the microbial community in a real dye wastewater biological treatment system, and found that, over the course of the system operation and wastewater treatment, the diversity of bacteria and archaea in the sludge increased. Meanwhile, Li [25] reported the microbial community structures of activated sludge from different sources of wastewater (dye/textile, enzyme preparation, and pharmaceutical wastewater) by polymerase chain reaction–denaturing gradient gel electrophoresis. The author found a richer microorganism community in the aerobic treatment stage than in the anaerobic treatment stage of dye/textile wastewater, the highest abundance in the early stage of anaerobic treatment for the enzyme preparation wastewater, and a gradual increase in microbial diversity over the course of treatment for pharmaceutical wastewater. The findings of previous studies and the present study indicate that microbial diversity tends to be higher in later stages of biological wastewater treatment.

Interestingly, in our study, Actinobacteria, Cyanobacteria, Verrucomicrobia, and Chloroflexi accounted for a significantly greater proportion of the total microbial population of site J5 than J1–J4. Actinobacteria is a prokaryotic phylum, comprising mainly aerobic heterotrophic species with strong decomposition abilities. As such, they have important applications in steroid conversion, petroleum dewaxing, and wastewater treatment [23]. At J5, Actinobacteria had a relative abundance of 22.07%, second only to Proteobacteria (41.57%). This suggests that it has an important role

in sewage treatment. In addition, the abundance of Cyanobacteria was 0 in J1–J2, but the highest abundance was found at J5 (8.09%; Figure 2). Similarly, Xing et al. [26] studied the dynamic changes in microbial community in a bio-hydrogen production reactor, and found that the sludge microbial community changed continually over the duration of the reactor operation, including increases/declines in the original community, as well as strengthening of secondary succession. The results of Xing et al. [26] and those of this study indicate that microorganisms might have been newly introduced later in the treatment process; as such, the effects of sewage treatment and stage as related to the needs of this taxon should be further studied. Verrucomicrobia is a relatively newly identified bacterial phylum, with few culturable species. Verrucomicrobia species have been mainly found in water, soil, and human feces, with as-yet unknown functions [27]. The abundance of Verrucomicrobia in surface water was very low at J1–J4 (< 0.27%; Figure 2) and relatively high at J5 (5.82%; Figure 2), suggesting an important role in the surface water system. Chloroflexi species typically exist in sludge bacteria floc, and in the form of floc skeleton, which provides structural support to aerobic granular sludge [28]. The abundances at J1–J4 were below 0.23% (Figure 2), but that at J5 was relatively higher (1.66%; Figure 2). Kragelund et al. [29] found that Chloroflexi species exhibited a relatively good biological phosphorus removal ability. Thus, the increased abundance of Chloroflexi at J5 suggested that it may have had a role in the decrease in TP at this stage (Figure 2).

At the genus level, there were differences in the most abundant genera in highly polluted surface water samples versus samples that met the standard. Of note, the relative abundance of some genera were similar to background environmental abundances. For example, the relative abundances of *Albidiferax*, *Candidatus Planktophila*, marine metagenome, *Phalacroma mitra*, hgcl clade, *Pseudarcicella*, and *Fluviicola* in wetland surface water environment increased with decreasing aquatic pollution, of which the relative abundance of *Albidiferax* differed significantly among all six samples ($P <$

0.05). However, there are few studies on these bacteria; therefore, they may be newly discovered microorganisms. By contrast, the relative abundances of *Methylotenera*, *Flavobacterium*, *Arcobacter*, *Hydrogenophaga*, *Pseudomonas*, *Sulfurimonas*, *Methylophaga*, and *Malikia* decreased with decreasing aquatic pollution (Table 6). Overall, these changes in surface water microbial community structure suggest that artificial wetland treatment of domestic sewage has a significant impact on microbial community structure.

The spatial variation among the environmental factors and wetland water samples was observed in this study. CCA analysis of samples from different sampling sites after wetland treatment showed that there was a strong correlation between samples and environmental factors, and environmental factors such as COD, TP, $\text{NH}_4^+\text{-N}$ and TN had a great influence. Between environmental factors and samples, J1, J2, J3, J4 and J5 were most affected by environmental factors, while JC was least affected by environmental factors.

It is also worth noting that the relative abundances of both rare and abundant species were lower at the seriously polluted sampling sites (J1–J3) than in the areas that met various water standards (J4, J5) (Tables 3 and 4), where the response of rare communities was more obvious than that of abundant communities (demonstrated by larger relative abundance). This indicates that rare taxa may exhibit more restrictive distributions than abundant taxa (in terms of relative abundance changes). This could be explained by the fact that abundant taxa may use diverse resources more efficiently with a higher overall viability and probability of propagation. By contrast, rare taxa could inhabit niches less-suited to surface water environments high in nutrients, making them more susceptible to environmental conditions than abundant groups. As such, rare taxa may be more sensitive than abundant taxa to growth conditions. This finding is similar to that of Yang et al. [21].

This study was limited to one sampling point at five horizontal surface-water sampling sites at various distances downstream of the

Table 6: Relative abundance (%) of abundant genera or species within different families across the studied samples.

Taxon	J1	J2	J3	J4	J5	JC	P value
<i>Methylotenera</i>	37.71	34.53	33.23	37.82	1.73	3.91	0.016
<i>Flavobacterium</i>	8.04	5.57	6.89	4.81	0.93	30.42	0.08
<i>Albidiferax</i>	3.45	4.32	3.11	1.52	14.12	5.9	0.032
<i>Arcobacter</i>	4.82	3.58	4.21	10.07	0.06	0	0.055
<i>Candidatus Planktophila</i>	0.14	0.17	0.27	0.05	8.52	0.25	0.311
<i>Polynucleobacter</i>	0.37	0.62	0.43	0.42	0.89	4.72	0.136
marine metagenome	0	0	0	0	4.61	0	0.363
<i>Phalacroma mitra</i>	0	0	0	0	4.15	0	0.363
<i>Hydrogenophaga</i>	1.1	1.02	0.8	0.97	0.76	3.91	0.036
hgcl clade	0.09	0.17	0.07	0.05	3.42	0	0.308
<i>Sulfurimonas</i>	0.87	0.68	1.1	3.25	0	0	0.101
<i>Pseudomonas</i>	3.17	2.44	2.41	0.85	0.47	0.62	0.017
<i>Methylophaga</i>	2.11	1.87	2.84	0.46	0	0	0.058
<i>Pseudarcicella</i>	0.09	0	0	0	2.61	1.74	0.174
<i>Fluviicola</i>	0	0	0	0.04	2.49	0.62	0.252
<i>Malikia</i>	2.16	1.36	1	0.7	0.61	2.3	0.006
<i>Sulfuricurvum</i>	0.32	0.45	0.57	2.03	0.06	0.12	0.104

Note: Sampling sites are the same as those in table 1.

sewage inlet, which could only reflect the mechanism of wetland decontamination to some extent. Therefore, future research should extend sampling to different points across the width of the artificial wetland, as well as various distances downstream of the sewage inlet, and analyze the pollutant removal mechanism of constructed wetlands following a three-dimensional sampling procedure.

Regardless of this limitation, our findings show that the physicochemical parameters of surface water at the Xiantao artificial wetland affect microbial community diversity, including number and species of microorganisms. As such, the variations in and abundance of certain bacterial taxa could be used to indirectly indicate the degree of aquatic pollution or purification. Moreover, this study demonstrated the utility and power of applying high-throughput sequencing technology for rapid classification of microorganisms at the family and genus levels to study the relationship between microorganisms and environmental factors. Such studies can clarify the operation mechanism of artificial wetlands and surface water pollution from a microbial perspective. Therefore, the findings of this study provide theoretical and practical support for follow-up work.

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 grateful to Professor He Ji Zheng the Center for Ecological Environment Research (Chinese Academy of Sciences) for his help in designing the experiment.

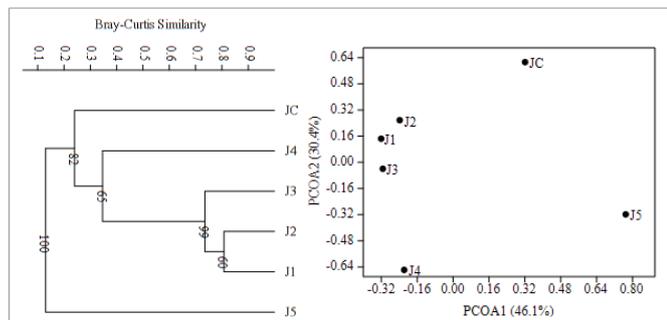


Figure 4: Bray-Curtis similarity-based cluster analysis (left) and Principal Coordinate Analysis (PCOA) (right) of the structure of the abundant microbial community at each sampling site. The sample labels are the same as those in table 1.

Table 7: Correlation (Pearson coefficients based on Mantel test) between surface water microbial community structure similarity and environment parameters at the Xiantao artificial wetland.

Environmental parameter	All OTUs	Abundant OTUs
COD _{Cr}	0.764*	0.803*
TP	0.897*	0.924*
NH ₄ ⁺ -N	0.883*	0.912*
TN	0.839*	0.875*

Note: Pearson's coefficients were calculated and their significances were tested based on 999 permutations. OUT: Operational Taxonomic Unit; COD_{Cr}: Chemical Oxygen Demand; TP: Total Phosphorus; NH₄⁺-N: Ammonium Nitrogen; TN: Total Nitrogen
*P < 0.05.

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