

Article

Effect of Sewage and Industrial Effluents on Bacterial and Archaeal Communities of Creek Sediments in the Taihu Basin

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Abstract: Different characteristics of wastewater have different effects on the diversity and abundance of bacteria and archaea in rivers. There are many creeks around Taihu Lake, and they receive a large volume of industrial wastewater and domestic sewage, which is discharged into these creeks, and finally into Taihu Lake. The present study determined Illumina reads (16S rRNA gene amplicons) to analyze the effects of industrial wastewater and domestic sewage on the bacterial and archaeal communities at the different sampling sites along two creeks. The bacterial and archaeal diversity of the creek receiving sewage was higher than that of the creek receiving industrial waste. *Proteobacteria* dominated the microbial communities of all samples in both creeks. *Betaproteobacteria* dominated in the sewage creek, and its abundance declined along the creek. Certain pollutant-resistant classes were more abundant at the site near to the pollution source of the industry creek (e.g., *Epsilonproteobacteria* and *Flavobacteria*). *Halobacteria* belonging to the phylum *Euryarchaeota* was the dominant archaea at all sites in both creeks, while *Methanobacteria* was more abundant in the industry creek. The bacterial community was more affected by the distance between the sampling site and the pollutant source than the archaeal community, indicating that bacterial diversity and abundance could be a good index to distinguish domestic and industrial pollution, especially when the main pollution sources are difficult to identify.

Keywords: bacterial community; archaeal community; Illumina Miseq; sewage and industrial effluents; creek

1. Introduction

Microbes are an important part of the ecological system, and the population structure of microorganisms changes with variations in the environment. Previous studies of microbes in the environment have mainly been conducted using culture-based technique, but most microorganisms cannot be purified and separated in the laboratory, making it difficult to understand the differences in the microbial population structure under different habitats [1]. With the rapid development of molecular biotechnology, PLFA (phospholipid fatty acids), spectrogram analysis of phospholipid fatty acids [2,3], the Biolog method [4], polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) [5,6], and high-throughput sequencing [7], have been widely applied to investigate microbial communities. Because of its high efficiency and accuracy, as well as its other characteristics [8], high-throughput sequencing has been widely used in the study of microbial

communities in soil [9], freshwater ecosystems [10], and marine ecosystems [11]. Some studies have shown that the microbial community changed in soil polluted by heavy metals [12]. Moreover, changes in the microbial community were reported in tropical river sediment with the duration of pollution [13]. The river ecological system is an important component of the surface-water environment, however, economic development has led to increased amounts of pollutants being discharged into rivers [14]. Such pollutants mainly originate from synthetic nitrogenous fertilizers, animal manure, sewage, and industrial wastewater [15]. At present, constructed wetland systems are one of the main technologies for pollution control along rivers, and many studies have focused on the microbial communities of these systems [16–18]. Because of the high technical requirements and large areas that they require, constructed wetland systems cannot be widely applied to controlling river pollution. Indeed, rivers are fed by many small streams in their basins, and these are often impacted by large numbers of residents and factories along their banks. The wastewater produced by these local residents and factories is directly discharged into creeks, and the concentrations of contaminants decrease as they travel through the creeks because of retention and self-purification. However, changes in the microbial population structures of creeks receiving different pollutant sources are not well understood. Zhihugang River, which is located in the Taihu Lake basin, has a total length of 20.1 km, an average width at the bottom of 30 to 40 m, an average velocity of 0.1 m/s, and an average flow rate of 10 m³/s. In 2007, the population around Zhihugang River was 399,000, including an urban population of 151,000 people, and there were a total of 2476 factories around the river [19]. The industrial facilities around Zhihugang River primarily include the chemical industry, printing and dyeing, electroplating, food, and paper factories. In recent years, Chinese researchers have studied the pollution sources [20,21], nutrient inputs [22], and the distribution of heavy metals in the sediments of rivers flowing into Taihu Lake [23]. Additionally, some researchers have focused on the macrobenthos, phytoplankton [24], and zooplankton [25,26] populations of Taihu Lake. However, no studies have investigated the microbial population structures of the creeks around Zhihugang River, or changes in the microbial population structure of creeks caused by different pollution sources.

To explore the differences in the microbial communities caused by the sewage and industrial effluents, two creeks located around Zhihugang River were investigated in this study. Samples were collected from 21 to 25 September, 2014, and the bacterial and archaeal community structures were analyzed by Illumina Miseq.

2. Materials and Methods

2.1. Study Area

A creek located in Duangou and another in Jixian were investigated. The creek in the Duangou village is mainly polluted by domestic sewage (hereafter referred to as the sewage creek), while the one in Jixian is primarily polluted by industrial effluents (hereafter referred to as the industry creek) from a machinery factory and a chemical fiber factory. The machinery factory is primarily engaged in the production and processing of all kinds of mechanical parts. The chemical fiber factory is mainly engaged in the production of high-emulsion chemical fiber. The locations of the two creeks are shown in Figure 1. Water with pollutants flows from each pollutant source to D1/J1, D2/J2, and then D3/J3. The distances between the pollutant source of domestic sewage and D1, D2, and D3 were 10 m, 85 m, and 160 m, respectively. The distances between the industrial pollution sources and J1, J2, and J3 were 10 m, 160 m, and 560 m, respectively. Hydrological data of the two creeks are shown in Table 1.

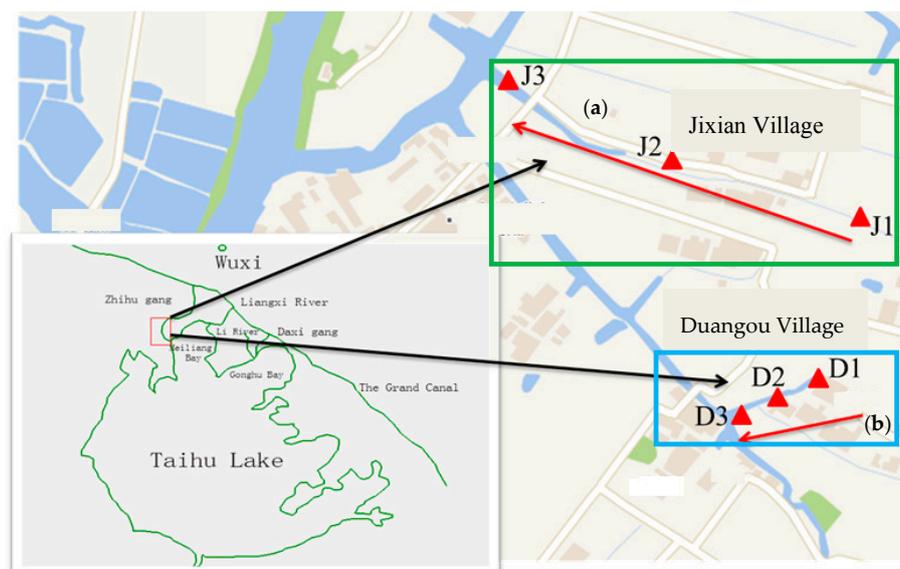


Figure 1. Map of the creeks and sample sites. (a) Creek polluted by sewage (in blue rectangle); (b) creek polluted by industrial effluent (in green rectangle).

Table 1. Hydrological data of two creeks.

Creek Name	Length (m)	Width (m)	Depth (m)	Sediment (m)	SD (m)	Flow (m ³ /s)
Sewage creek	150	8.4	0.68	0.10	0.56	0.026
Industry creek	560	6.7	0.72	0.10	0.24	0.026

2.2. Sample Collection and Measurement

About 1 L of overlying water was collected using an organic glass hydrophore, and the temperature (T), pH, and dissolved oxygen (DO) were determined directly in situ using a multi-parameter water quality monitoring instrument (Mettler Toledo, SevenGo Duo pro, SG68, SC, USA). All sensors were calibrated before taking measurements. Ammonium (NH₄⁺-N) was measured with Nessler's reagent, while nitrate nitrogen (NO₃⁻-N) and total nitrogen (TN) were determined by ultraviolet spectrophotometry and the alkaline potassium persulfate oxidation-UV spectrophotometric method, respectively. Total Organic Carbon (TOC) concentrations were determined using a TOC-V Analyzer (Shimadzu-TNM-1, Kyoto, Japan), with an estimated detection limit of 0.05 µg/L. Chemical oxygen demand (COD) was measured using the alkaline potassium permanganate method, while TP was measured using the potassium persulfate oxidation method. About 500 g of surface sediment samples were collected from all sites using a surface sediment sampler (Van Veen, HYDRO-BIOS, Kiel, Germany), and were then stored at −70 °C until use. The concentrations of Cr, Ni, Cu, Zn, As, Cd, and Pb in sediments were measured as previously described [27]. Water transparency (SD) was determined using a Secchi disk (diameter = 20 cm). The Chl_a was determined using the standard methods [28]. Chloride (Cl⁻) was measured by silver nitrate titration. Sulfate (SO₄⁻) was measured using the spectrophotometric method [29].

2.3. DNA Extraction and Illumina MiSeq Sequencing

The DNA was extracted from 0.5 g samples of wet sediment using a Power soil DNA isolation kit according to the manufacturer's protocols (Mo Bio, Carlsbad, CA, USA). The bacterial and archaeal 16S rRNA genes of all samples were amplified by barcoded primers to construct a community library by high-throughput pyrosequencing. The primers and amplification conditions are shown in Table 2. PCR amplification was conducted using an ABI9700 thermocycler (ABI, Foster City, CA, USA) with TransStart Fastpfu DNA polymerase (TransGen, Beijing, China). Technically triplicate positive PCR

products were purified with an AxyPrep DNA Gel Extraction Kit (Axygen, Tewksbury, MA, USA). DNA concentrations of the purified PCR products were measured using QuantiFluor™-ST (Promega, Madison, WI, USA). The Illumina Miseq platform was used to sequence the 16S rRNA gene fragments of the bacterial and archaeal communities at Personalbio (Beijing Haocheng Mingtai Technology Co. LTD, Beijing, China).

Table 2. PCR primers and amplification conditions.

Target Gene	Primer Sequence (5'-3')	Thermal Programme	Reference
Archaeal 16S rRNA	Arch519F: CAGCCGCCGCGGTAA	5 min at 98 °C, 28 cycles at 98 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min.	[30]
	Arch 915R: GTGCTCCCCGCCAATTCCT		
Bacterial 16S rRNA	338F: ACTCCTACGGGAGGCAGCA	5 min at 95 °C, 30 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min	[31]
	806R: GGACTACHVGGGTWCTAAT		

2.4. Sequencing Data Processing and Statistical Analysis

All raw paired-end reads of samples were merged using Fast length adjustment of short reads FLASH [32], and the low quality reads were removed by Trimmomatic [33]. After strict filtration, the remaining 16S rRNA sequences were clustered into operational taxonomic units (OTUs) by setting 97% similarity, after which classification of the taxonomic groups was assigned using the Ribosomal Database Project (RDP) classifier Bayesian Algorithm via the Silva database with a confidence threshold of 0.7 [34–36]. The diversity statistics, including library coverage, Chao 1 value, Shannon index, and abundance-based coverage estimator (ace), were obtained for each sample after OTUs had been clustered using Mothur [37]. Based on the Bray–Curtis distances, principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS), and hierarchical clustering were determined and plotted using the R program [38]. Hierarchical cluster analysis was performed using the squared Euclidean distance with Ward's method to evaluate the pollution intensity of nutrients and heavy metals in the two creeks. Because the longest detrended correspondence analysis (DCA) eigenvalues of bacteria and archaea (0.360 and 0.388), respectively were <3, the correlations between environmental factors and microbial OTUs composition were analyzed by redundancy analysis (RDA) using CANOCO 4.5 [39]. Monte Carlo permutations were used to construct the models of the microbe–environment relationships. All reads have been archived at the NCBI Sequence Read Archive (SRA) under accession number SRR5246806 for bacteria and SRR5247037 for archaea.

3. Results

3.1. Water Quality and Sediment Heavy Metal

The water quality of the two creeks is shown in Table 3. The pH of the sewage creek and industry creek ranged from 7.13 to 7.26 (mean 7.17) and from 7.35 to 7.65 (mean 7.48), respectively. The DO of the sewage creek and industry creek ranged from 5.66 to 6.97 mg/L (mean 6.10 mg/L) and 7.27 to 8.06 mg/L (mean 7.26 mg/L), respectively. The concentrations of inorganic phosphorus (IP), total phosphorus (TP), NH₄⁺-N, NO₃⁻-N, and total nitrogen (TN) declined by 86.8%, 69.3%, 41.7%, 41.1%, and 64.7% in the sewage creek, and by 30.7%, 47.4%, 14.4%, 4.3%, and 3.6% in the industry creek, respectively. These results indicate that the self-purification ability of the sewage creek was stronger than that of the industry creek because the hydraulic staying time of the sewage creek was shorter (Table 1).

Table 3. Water quality of the creeks (mg/L).

Sample Site	DO	T	pH	Chl _a	TP	TOC	COD	NH ₄ ⁺ -N	NO ₃ ⁻ -N	TN	SO ₄ ²⁻	Cl ⁻
Sewage Creek												
D1	5.66	19.6	7.13	0.075	0.49	22.2	22.3	2.28	3.36	7.14	66.7	166.1
D2	5.68	19.8	7.14	0.023	0.18	16.9	23.0	1.05	1.37	4.51	59.2	133.5
D3	6.97	20.2	7.26	0.020	0.15	17.4	21.7	0.68	1.21	2.52	55.6	123.7
Mean	6.10	19.9	7.17	0.039	0.27	18.8	22.3	1.33	1.98	4.72	60.5	141.1
Industry Creek												
J1	7.27	19.8	7.44	0.003	0.19	14.7	6.33	0.83	1.60	3.36	176.8	131.2
J2	6.47	19.4	7.35	0.003	0.15	15.1	5.55	1.06	1.38	3.66	129.6	100.5
J3	8.06	19.6	7.65	0.006	0.10	14.6	4.79	0.71	1.53	3.24	66.5	60.7
Mean	7.26	19.6	7.48	0.004	0.15	14.8	5.56	0.86	1.50	3.42	124.3	97.4

The concentrations of heavy metals in the sediments of the two creeks are shown in Table 4. The concentrations of Cr, Cu, As, Cd, and Pb in the sediments of the industry creek were higher than those of the sewage creek and the background values of the rivers around Taihu Lake [40–42].

Table 4. Content of heavy metals in the creek sediments (mg/kg).

Sample Site	Cr	Ni	Cu	As	Cd	Zn	Pb
Sewage Creek							
D1	44.17	30.83	38.12	9.04	0.62	131.78	102.58
D2	41.46	24.78	29.51	8.22	1.06	116.21	53.13
D3	40.45	24.89	30.98	7.35	0.91	137.92	10.25
Mean	42.02	26.83	32.87	8.20	0.86	128.63	55.32
Industry Creek							
J1	45.82	29.68	51.13	12.79	1.55	197.35	92.51
J2	45.47	25.82	47.82	10.46	0.38	115.14	89.32
J3	41.99	23.10	21.75	10.14	1.21	70.70	52.96
Mean	44.42	26.20	40.23	11.13	1.05	127.73	78.26

As shown in Table 3, a trophic state index (TSI) was used to evaluate the trophic state of the two creeks. The TSI of each site was calculated based on the method described by Wang et al. [43]. TSI < 30 indicates an oligotrophic, $30 \leq \text{TLI} \leq 50$ a mesotropher, TLI > 50 a eutropher, $50 < \text{TLI} \leq 60$ a light eutropher, $60 < \text{TLI} \leq 70$ a middle eutropher, and TLI > 70 a Hyper eutropher. As shown in Table 4, the potential ecological risk (RI) was used to evaluate the degree of heavy metal pollution in the sediment of the two creeks. The RI of each site was calculated based on the method described by Hakanson [44]. RI < 150 indicates slight risk, $150 < \text{RI} < 300$ a middle risk, $300 \leq \text{RI} < 600$ a high risk, and $\text{RI} \geq 600$ an extraordinary risk. The result of the TSI and RI values are shown in Table 5.

The order of the TSI of the two creeks was D1 > D2 > D3 > J1 > J2 > J3. The potential contribution to eutrophication of Taihu Lake was higher for the sewage creek than for the industry creek. The order of RI was J1 > J2 > J3 > D2 > D3 > D1, and the potential ecological risk of heavy metals in the sediment of the industry creek was higher than that of the sewage creek. These findings indicated that the heavy metals pollution of the sediment in the industry creek posed a greater ecological risk to Taihu Lake than that of the sewage creek.

Table 5. Trophic state index and ecological risk of the two creeks.

Sample site	TSI	Level	RI	Level
Sewage Creek				
D1	76	Hyper	189	Middle
D2	69	Middle	277	Middle
D3	68	Middle	234	Middle
Industry Creek				
J1	67	Middle	409	High
J2	63	Middle	364	High
J3	57	Light	310	High

3.2. Bacterial Diversity of the Creeks

Using Illumina MiSeq pyrosequencing, six libraries of bacterial 16S rRNA genes were constructed. A total of 18,265–47,498 high quality reads for the six samples of the two creeks were obtained from 263,713 raw reads of bacteria after filtering the low quality reads. After trimming adaptor and barcode primers, an average sequence length of 440–444 nt was obtained. The number of OTUs, ACE, Chao1, Coverage, and Shannon indices were estimated for the six samples (Table 6). The Coverage of the six samples ranged from 98 to 99%, indicating that the libraries of the six samples could cover the diversity of the bacterial community well. The Shannon indices ranged from 5.12 to 5.96 for the six samples from the two creeks, with the highest diversity being found at D3 of the sewage creek and J3 of the industry creek. Based on an independent *T*-test, the Shannon indices between D2 and D3 had no significant difference, but there were significant differences between D1 and D2 ($p < 0.05$), and D1 and D3 ($p < 0.05$). The Shannon indices between J2 and J3 has no significant difference, but significant differences were observed between J1 and J2 ($p < 0.05$) and between J1 and J3 ($p < 0.05$). Therefore, the diversity of each creek was $D3 \approx D2 > D1$ and $J3 \approx J2 > J1$, respectively. Additionally, the diversity of the two creeks was $D3 \approx D2 > J3 \approx J2 > J1 > D1$. The Shannon indices of sediment in the same creek increased as the distance from the pollution source increased.

Table 6. Diversity indices of bacteria and archaea of the two creeks.

Sample	Reads	OTUs	Ace	Chao1	Coverage	Shannon
Bacterial 16S rRNA						
D1	34,091	1207	1302	1302	0.99	5.12
D2	21,115	1144	1248	1275	0.99	5.91
D3	34,233	1332	1382	1391	0.99	5.96
J1	47,498	1192	1301	1320	0.99	5.32
J2	31,031	1328	1402	1421	0.99	5.85
J3	18,265	1115	1256	1273	0.98	5.75
Archaeal 16S rRNA						
D1	32,255	690	748	733	0.98	4.27
D2	61,300	652	735	750	0.99	3.79
D3	36,363	590	721	703	0.99	3.86
J1	46,435	585	614	608	0.99	3.54
J2	39,875	523	679	694	0.98	3.53
J3	52,445	547	619	615	0.99	3.63

There are 41 phyla in the filtered sequences across six samples in total, including 31 at D1, 34 at D2, and 39 at D3 of the sewage creek, and 33 at J1, 39 at J2, and 37 at J3 of the industry creek. The relative abundance of sequences could be classified into known groups, but those with a relative abundance <1% were assigned to 'others'. The bacterial phyla of the samples are shown in Figure 2a. The dominant phyla of the two creeks were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Chloroflexi*, which were present with average percentages of 39%, 24%, 13%, and 8.1% in the sewage creek and 42%, 20%, 13%, and 9.1% in the industry creek, respectively. The classes of each sample in the phylum *Proteobacteria* were different. *Betaproteobacteria* in the *Proteobacteria* phylum was the dominant class in all samples of the sewage creek, but the dominant classes of different samples from the industry creek were different. The dominant class was *Epsilonproteobacteria* at J1, which was the site nearest to the industrial pollution sources, while *Betaproteobacteria* and *Deltaproteobacteria* were dominant at J2 and J3, respectively, which were farthest from the industrial pollution source. *Nitrospira* was found in all three samples of the sewage creek, with a relative abundance of more than 1%, but at J1 and J2 of the industry creek, the relative abundance of *Nitrospira* was less than 1%.

As shown in Figure 3, the number of detected bacterial genera was 381 at D1, 396 at D2, and 406 at D3 of the sewage creek, and 391 at J1, 414 at J2, and 391 at J3 of the industry creek. The bacterial genera are shown in Figure 3 (with an abundance of more than 0.01%). The number of detected genera with an abundance of more than 1% in the sewage creek was 19 at D1, 27 at D2, and 22 at D3, while

Clustering of the samples from the two creeks is shown in Figure 4a. The result showed that clusters reflecting sites nearest to the pollution source (D1 and J1) were farthest away from each other, indicating that the different pollution sources had different effects on microbial diversity. The NMDS (see Supplementary Materials, Figure S1a) illustrated that J1 was far from J2 and J3, indicating a lower similarity of bacterial communities between these three sites and that the bacterial community was determined by the distances from the pollution source.

The shared OTUs of the samples from the same creek were analyzed using a Venn diagram (Figure 5a,b). Reads in the shared OTUs of genes among the three samples from the same creek are summarized in Table S1 (see Supplementary Materials). *Anaerolineae* shared the most OTUs among the six sites, and the reads of OTUs were more than 1%. The number of shared OTUs of the sewage creek was 933, while that of the industry creek was 838, indicating that the similarity of the bacterial community of the industry creek was lower among sites. The unique OTUs of each sample from the same creek were analyzed using a Venn diagram. At D1 there were 36 OTUS, while there were 62 at D2, 87 at D3, 46 at J1, 82 at J2, and 78 at J3. Sites further from the pollution source had more unique OTUs, indicating that the microbial communities changed with the distance from a pollution source.

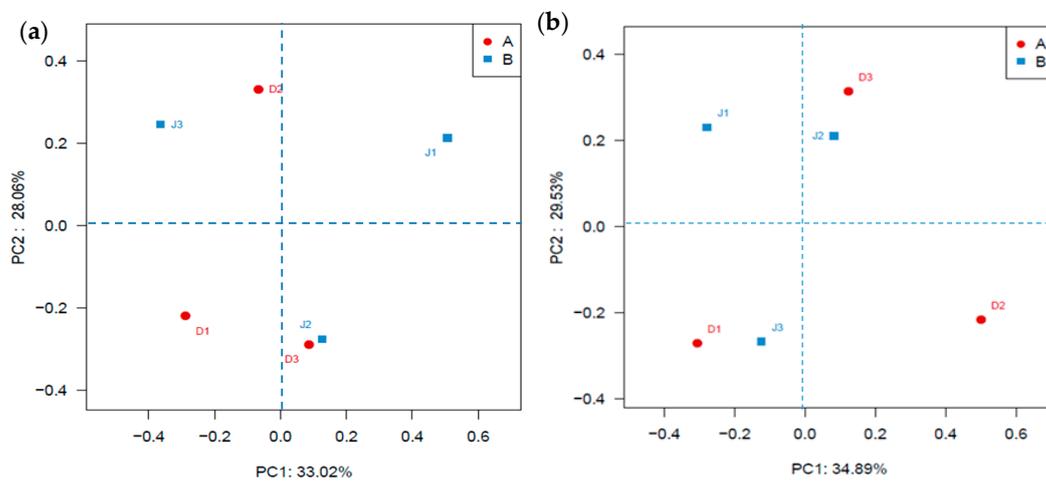


Figure 4. Community analysis based on PCoA on the Bra—Curtis distances of bacteria. (a) Bacterial genes; (b) Archaeal genes.

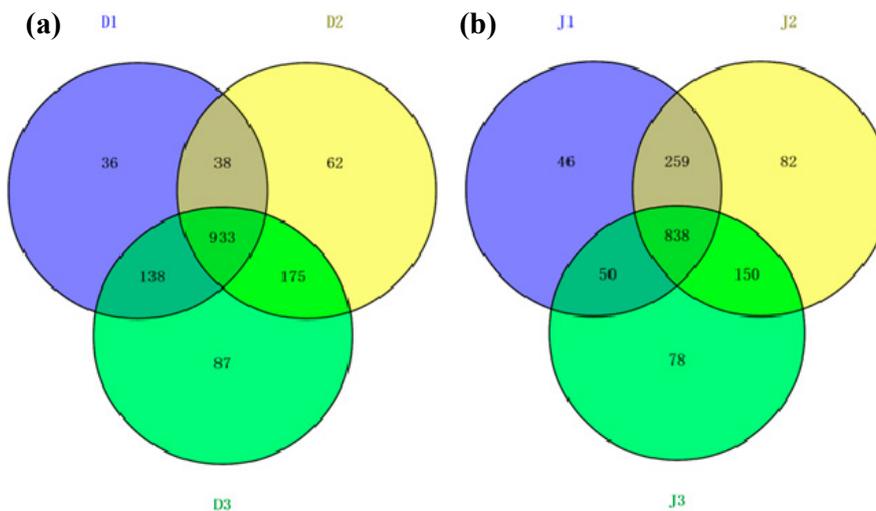


Figure 5. Shared OTUs analysis of bacterial 16S rRNA gene libraries of the samples in (a) sewage creek; (b) industry creek.

3.3. Archaeal Diversity of the Creeks

Six libraries of archaeal 16S rRNA genes were constructed using Illumina MiSeq pyrosequencing. A total of 32,255–61,300 high quality reads for the six samples of the two creeks were obtained from 334,439 raw reads of bacteria after filtering the low quality reads. After trimming the adaptor and barcode primers, an average sequence length of 399–403 nt was obtained. The number of OTUs, as well as the ACE, Chao1, Coverage, and Shannon indices were estimated for the six samples (Table 6). The Coverage of the six samples ranged from 98 to 99%, indicating that the libraries of the six samples could cover the diversity of the archaeal community well. As shown in Table 6, the Shannon indices ranged from 3.54 to 4.27 for the six samples from the two creeks, and the bacterial diversity of the two creeks was not significantly different. The highest level of diversity was found at D1 of the sewage creek and J3 of the industry creek. Independent T-tests revealed that there were no significant differences in Shannon indices between D2 and D3, while those between D1 and D2 ($p < 0.05$) and D1 and D3 ($p < 0.05$) differed. The Shannon indices of J2 and J3, as well as those of J1 and J3 ($p < 0.05$) also differed significantly ($p < 0.05$), while there was no significant difference between J1 and J2. These findings indicated that the diversity was similar between D2 and D3, as well as between J1 and J2. The order of diversity of each creek was $D1 > D2 \approx D3$ and $J3 > J2 \approx J1$, respectively, indicating that the Shannon indices of the samples in the sewage creek decreased with increasing distance from the pollution source, while they increased with distance from the pollution source in the industry creek. The results of the independent T-test showed that the influence of sewage pollution on archaeal diversity was higher than that of industrial pollution. The Shannon index between D1 and D3 was significantly different, but no significant difference was observed between J1 and J2, and for the same distance between J1 and J2 as D1 and D3 (160 m away from the pollution source).

There were three phyla including 19 classes in the filtered sequences across six samples in total, including 17 at D1, 15 at D2, and 15 at D3 of the sewage creek, and 15 at J1, 15 at J2, and 15 at J3 of the industry creek. The relative abundance of sequences could be classified into known groups, and those with a relative abundance $< 0.04\%$ were assigned to 'others'. The archaeal phyla of the samples are shown in Figure 2b. The dominant phyla of the two creeks were *Euryarchaeota*, *Thaumarchaeota*, and *Crenarchaeota*, which were present with average percentages of 70.4% and 1.9% in the sewage creek, and 67.3%, 31.8%, and 0.77% in the industry creek, respectively. *Halobacteria* from the *Euryarchaeota* phylum was dominant in all the samples. Additionally, *Soil_Crenarchaeotic_GroupSCG* belonging to the *Thaumarchaeot* phylum was the second most abundant class at sites D1 and D2, which were near the sewage source. Furthermore, *Miscellaneous_Crenarchaeotic_Group* in the *Thaumarchaeot* phylum was the second most abundant class at site D3, which was far away from the pollution source. In the industry creek, the *Miscellaneous_Crenarchaeotic_Group* belonging to the *Thaumarchaeot* phylum was the second most abundant class at J1, which was closest to the pollution source, while *Soil_Crenarchaeotic_GroupSCG* of *Thaumarchaeot* phylum was the second most abundant class at D3, which was far away from the pollution source. *Miscellaneous_Crenarchaeotic_Group* was one of the most abundant microbial groups in the environment, such as lakes [45] and terrestrial hot springs [46].

At the genus level, the number of detected archaeal genera was 42 at D1, 43 at D2, and 46 at D3 of the sewage creek and 48 at J1, 45 at J2, and 41 at J3 of the industry creek. The archaeal genera are shown in Figure 6. The dominant genera in all the sites were significantly different. The number of detected genera with an abundance $> 1\%$ was 8 at D1, 10 at D2, and 14 at D3 in the sewage creek, but 14 at J1, 13 at J2, and 9 at J3 in the industry creek. The dominant genus was *Halobacterium* in all the samples. The main genera of sites nearest to the sewage source were *Candidatus_Nitrososphaera*, *Methanobacterium*, and *Methanobacteriaceae*, while they were *Candidatus_Parvarchaeum* and *Methanobacterium* at sites far away from the pollution source. The main genera of sites nearest to the industrial source were *Methanobacterium* (at J1 and J2), while they were *Candidatus_Nitrososphaera* and *Candidatus_Nitrosoarchaeum* at sites far away from the pollution source.

As shown in Figure 4b, clear clustering of the samples from the two creeks was observed. The result showed that the sites (D1) nearest to the pollution source were far away from the other sites

in the same creek, but the distance of the sites of the industry creek was closer than that of the sewage creek, indicating that the sewage and industrial effluents had different effects on the archaeal diversity. The MNDS analysis (see Supplementary Materials, Figure S1b) illustrated that the site of the same creek located in different quadrants, indicating a lower similarity of the archaeal community among three sites and the distances from a pollution source, determined the archaeal community.

The shared OTUs of the samples from the same creek were analyzed using a Venn diagram (Figure 7). The reads in the shared OTUs of genes among the three samples of the same creek are summarized in Table S2. *Halobacteria* shared the most OTUs among the six samples, and the reads of the OTUs were more than 30% in the sewage creek and 40% in the industry creek. The number of shared OTUs of the sewage creek was 278, while it was 249 for the industry creek, indicating that the similarity of the archaeal community in the industry creek was lower than that of the sewage creek. The unique OTUs of each sample from the same creek were analyzed via a Venn diagram. The unique OTUs at D1 were 226, while they were 126 at D2, 109 at D3, 171 at J1, 81 at J2, and 155 at J3, respectively. Sites further from the pollution sources had less unique OTUs, indicating that the distance from a pollution source had an effect on the archaeal communities in the environment.

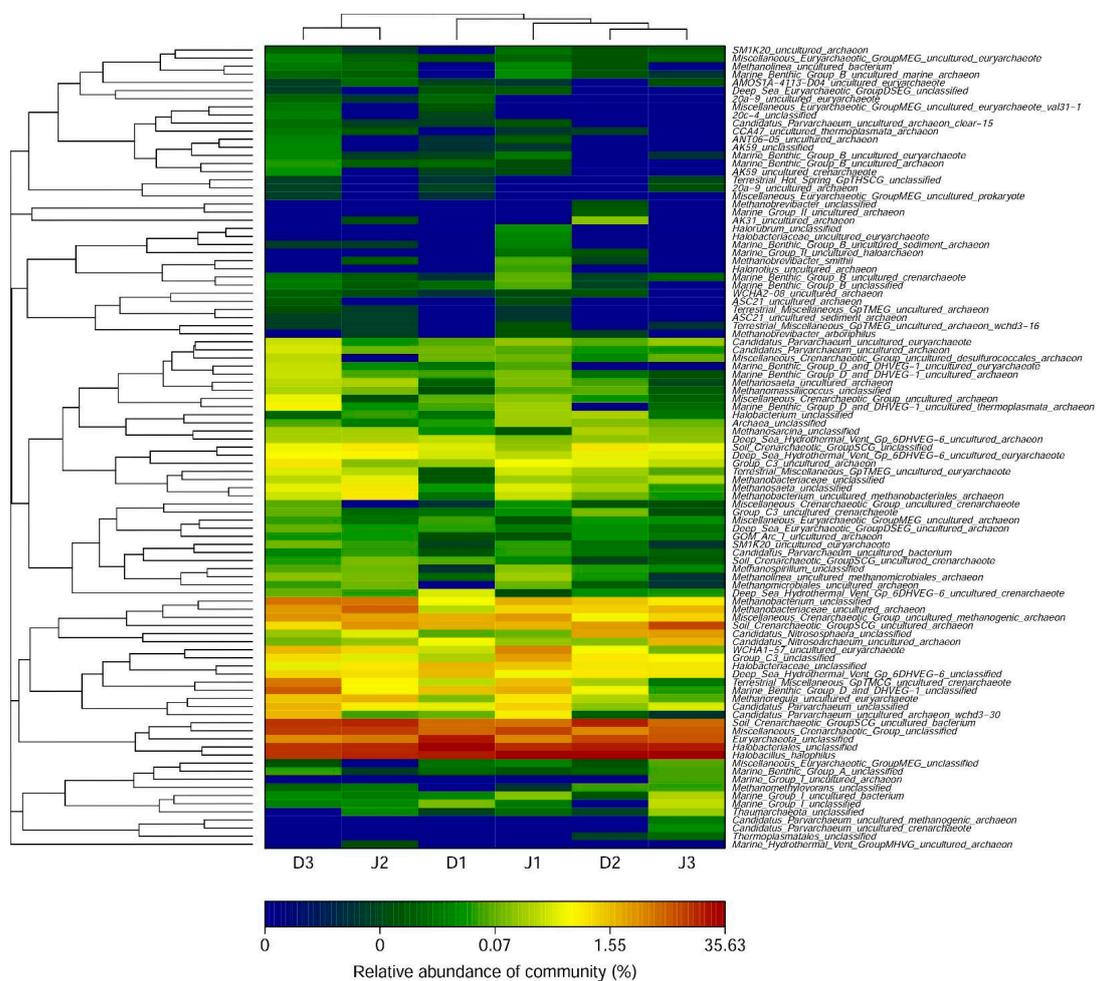


Figure 6. Archaeal genera of samples in the creeks (with relative abundances >0.01%). The heat map was plotted using the R program.

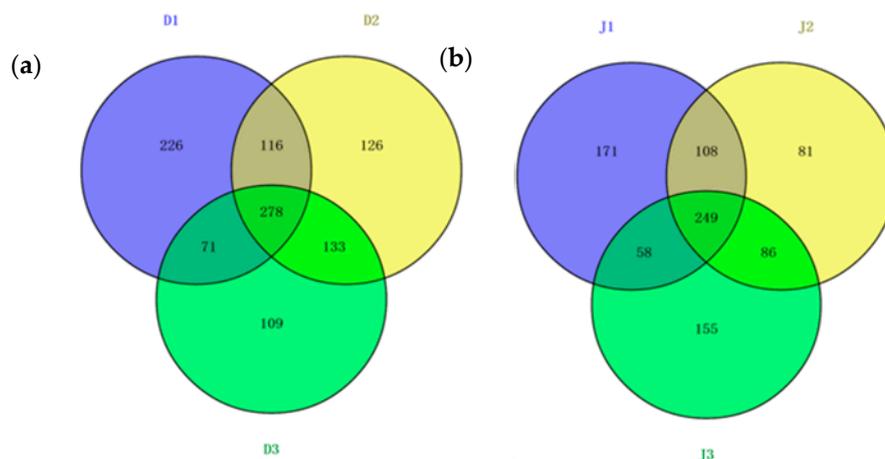


Figure 7. Shared OTUs of the archaeal 16S rRNA gene libraries of samples from (a) sewage creek; (b) industry creek.

4. Discussion

Human activities generally decrease environmental quality, diversity, and microbial abundance. Because of the different physicochemical properties and toxicity of sewage and industrial effluents, they have different impacts on microbial diversity and microbial abundance [47,48].

The six sampling sites were classified into four distinct clusters based on RI, TSI, SO_4^{2-} , Cl^- , NH_4^+ -N, and NO_3^- -N (Figure 8). Cluster 1 was formed by D2 and D3, cluster 2 by D1, and cluster 3 by J3, while J2 and J3 were included in cluster 4. The clusters were recognized to have hypereutrophic, moderate eutrophic, light eutrophic, and high heavy metal pollution risk. The deterioration of water quality in this basin mainly resulted from domestic sewage and industrial wastewater [49,50], which was consistent with the results of the present study. Moreover, hierarchical cluster analysis showed that the pollution types of the two creeks were significantly different. Previous studies indicated that the nutrients and heavy metals might alter microbiological processes (e.g., decomposition of organic matter) and lead to changes in the microbial density and composition [51,52].

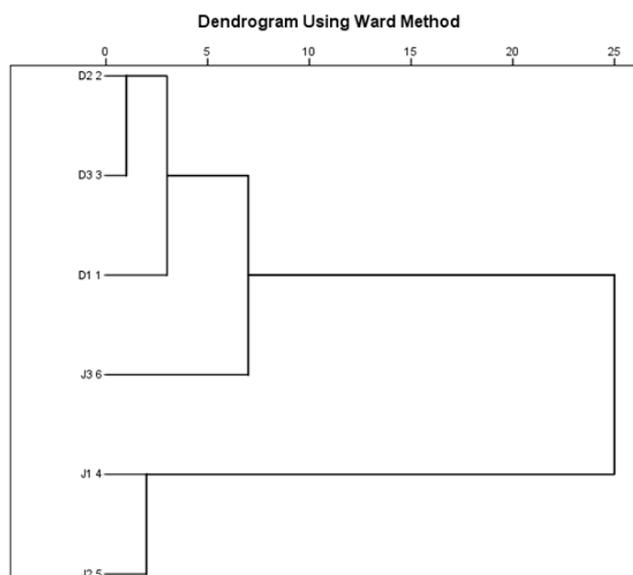


Figure 8. Cluster dendrogram showing the grouping of sampling sites based on RI, TSI, and concentrations of SO_4^{2-} , Cl^- , NH_4^+ -N, and NO_3^- -N.

As shown in Table 6, the wastewater entering the creek in Duangou Village was mainly sewage, and the primary pollutants were nitrogen and phosphorus. The wastewater discharging into the creek in Jixian Village was mainly from a chemical fiber factory and iron foundry. The concentration of nitrogen and phosphorus in the water in the industry creek was lower than that of the sewage creek. As shown in Table 2, the concentration of heavy metals (Cr, Cu, As, Cd and Pb) in the sediment of the industry creek was higher than that of the sewage creek. The microbial community of the sewage creek was more abundant than that of the industry creek, and the abundance of the microbial diversity increased with increased distance of the sampling sites from the pollutant source. It is interesting that the order of bacterial diversity in both creeks was $D3 \approx D2 > D1$, but $D1 > D2 \approx D3$ for archaea, which indicated that water quality was the main factor for determining the microbial community of both creeks.

As shown in Figures 3 and 4, the microbial community composition of the two creeks between D2 and J3, as well as that of D3 and J2 were classified into the same cluster, which was inconsistent with the result of the hierarchical cluster analysis (Figure 8). This may indicate that the heat map reflected the classification of OTUs of the microbial communities, but could not accurately reflect the effects of environmental factors on the microbial structure and relationship between microbial abundance and environment factors. Moreover, the physiochemical properties of sediment could have had some impact on the microbial community structure as indicated in the previous studies [53].

According to RDA, the environmental factors in the first two axes explained 60.3 and 23.8% of the total variance in bacterial composition, as well as 59.8% and 30.2% of that of the archaeal composition in the sediment of the two creeks. However, only SO_4^{2-} ($p = 0.009$, $F = 3.34$, 999 Monte Carlo permutations) significantly contributed to the bacterial assemblage-environment relationship, while no other factors passed the Monte Carlo significance test. These findings indicate that the archaeal population was less influenced by environmental factors.

Among bacteria, *Proteobacteria* was the main phylum in the sediment at all six sites in both creeks, which was consistent with the results of previous studies of freshwater systems [10,22,54], including Taihu Lake and the Yangtze River estuary [55,56]. As shown in Table S1, *Betaproteobacteria* was dominant in the sediment of the sewage creek, and the abundance of *Betaproteobacteria* at the site D1 was higher than at sites D2 and D3. *Betaproteobacteria* was positively correlated with nitrogen nutrients and TSI, which was consistent with the results of previous studies that found that the abundance of *Betaproteobacteria* was correlated with nutrient concentrations and cellulose decomposition [57,58]. *Epsilonproteobacteria* and *Deltaproteobacteria* were dominant in the sediment at the site (J1), which was close to the industrial effluents' source, where the concentrations of heavy metals and sulfate ions were higher than at other sites. Previous studies indicated that *Epsilonproteobacteria* was abundant in the animals' digestive tracts and near undersea hydrothermal vents, and that it exhibited good resistance to toxicity [59,60] and the ability to degrade some toxic substances [61]. However, *Epsilonproteobacteria* has rarely been detected in the sediments in the Taihu Basin [62]. Previous studies indicated that *Deltaproteobacteria* was ubiquitous in the environment, and closely related to the sulfur cycle between the water and sediment [63]. According to the RDA, *Epsilonproteobacteria* and *Deltaproteobacteria* were positively correlated with SO_4^{2-} concentration and RI. Previous studies showed that some *Epsilonproteobacteria* have the ability to oxidize sulfur and sulfide into sulfate, as well as the ability to degrade some toxic substances [64,65]. Additionally, *Deltaproteobacteria* were shown to be closely related to sulfate reduction in sediment [66]. Further analysis found that the main genera belonging to *Epsilonproteobacteria* at J1 were *Sulfurovum*, *Sulfurimonas*, *Sulfurospirillum*, and *Sulfuricurvum*, which was similar to the results of previous studies of a submarine hydrothermal spring [67], marine surface sediment [68], and pond sediments [69]. Moreover, some researchers found that these bacterial genera could utilize elemental sulfur, sulfite, arsenate, or nitrate as electron acceptors [70]. The main bacterial genera of *Deltaproteobacteria* at J1 were *Desulforhabdus amnigena*, *Desulfobacterium*, and *Desulfobacteraceae*, which have been shown to use acetate as a substrate, as well as to strongly degrade organic pollutants [71]. *Flavobacteria* belonging to *Bacteroidetes* was more abundant at sites

near the pollution source than at other sites in the same creek, and was positively correlated with Cl^- , TSI, and nutrients (Figure 9a). These findings were consistent with the results reported by Abell et al. [72]. Previous studies indicated that *Flavobacteria* was dominant in a coking wastewater treatment system [73], alkaline environment system [74], and a highly saline environment [75]. *Chloroflexia*, which was found to be ubiquitous in Taihu Lake [76], was only detected in the sediment of the sewage creek. *Chloroflexia* was positively correlated with Cl^- , TSI, TOC, and nutrients, but negatively correlated with RI (Figure 9a). Previous studies indicated that *Chloroflexia* is a photosynthetic bacteria that cannot live without light [77]. The Secchi-depth of the industry creek was low (mean (SD) = 0.24 m), indicating that light does not reach the bottom; therefore, the environment was unfavorable for the survival of *Chloroflexi*. Previous studies indicated that *Chloroflexia* was suited to live under brackish, nutrient-rich conditions [78]. *Nitrospira*, which was found to be more abundant at sites far away from the pollution source (D2, D3, J2, and J3), has often been found in freshwater ecosystems [79,80], sewage treatment systems [81], and anoxic environments as nitrite oxidizers [82]. Based on the results of RDA (Figure 9a), *Nitrospira* was positively correlated with $\text{NH}_4^+\text{-N}$, NO_3^- , Cl^- -N, TOC, and TSI, but negatively correlated with RI. Previous studies indicated that heavy metals might lead to a decline in the population of *Nitrospira* [83,84], but an increase in the population of *Nitrospira* with a low concentration of NH_4^+ (10 mg/L) in water [85].

Fewer phyla of archaea than bacteria were found in all samples of both creeks. Moreover, the effects of water quality of different pollution sources on archaeal diversity were not as obvious as those on bacterial diversity. Most archaeal sequences from the sediment of both creeks were affiliated with *Euryarchaeota*, which was consistent with the results reported by Liu et al. [86]. *Halobacteria* belonging to the phylum *Euryarchaeota* was dominant at all sites of both creeks, and showed no significant positive correlation with the environmental factors. However, previous studies indicated that *Halobacteria* exists in highly saline aquatic environments [87,88]. The second most abundant archaea in the sediment of the two creeks belonged to *Thaumarchaeota*. These previous studies indicated that *Thaumarchaeota* have been linked to methanogenic activities and ammonia oxidation in the environment [89,90]. The relative abundance of *Methanobacteria* was slightly higher in the sediment of the industry creek than that of the sewage creek. RDA analysis showed that *Methanobacteria* was positively correlated with TSI, $\text{NH}_4^+\text{-N}$, NO_3^- , Cl^- -N, TOC, Cl^- , and SO_4^{2-} , but not with RI. Previous studies have shown that *Methanobacteria* was often found in sewage treatment facilities and some freshwater environments [91,92], and exhibited good resistance to toxicity [93].

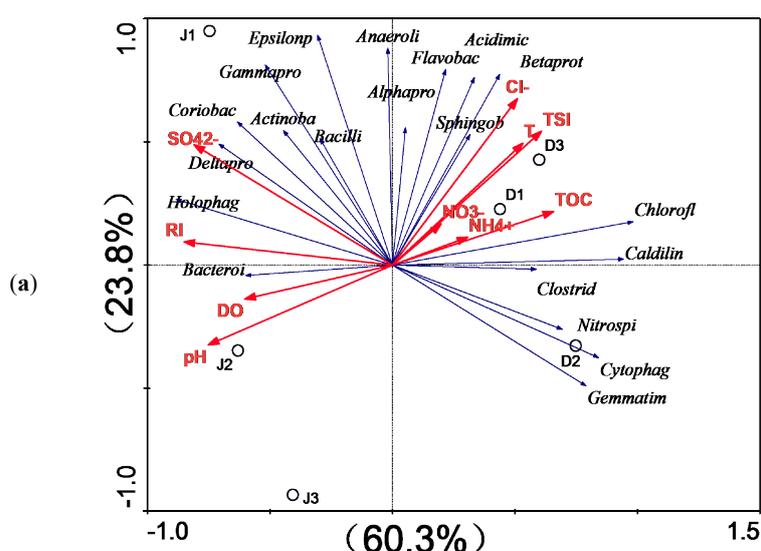


Figure 9. Cont.

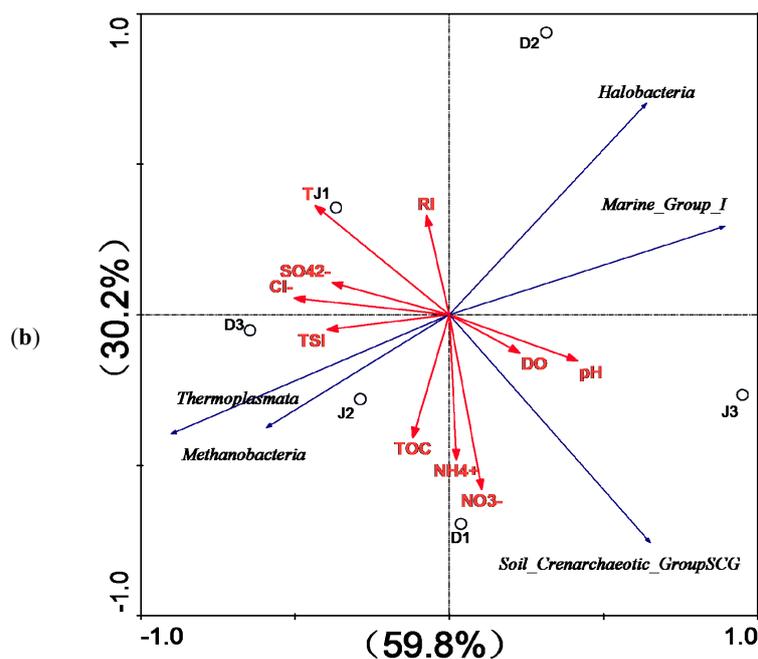


Figure 9. RDA for the first two principal dimensions of the relationship between (a) bacterial and (b) archaeal OTUs and environmental factors.

5. Conclusions

The analysis of the diversity of bacteria and archaea in creeks influenced by sewage and industrial waste revealed that domestic and industrial pollution had different effects on the diversity and microbial abundance of the creeks. The influence of domestic and industrial pollution on the diversity and microbial abundance was dependent on the distances between the sampling sites and their pollution sources. A higher diversity and similarity among the sites was observed for bacteria in the sewage impacted creek than for that in the industrial waste impacted creek. Additionally, the water quality and diversity and abundance of both bacteria increased with the distance from the pollutant source in both creeks. The diversity and abundance of bacteria were more heavily influenced by the distance between the sampling sites and the pollutant source than those of archaea; therefore, the bacterial diversity and abundance could be a better index of ecological changes in creeks affected by domestic and industrial pollution than the archaeal diversity and abundance.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/9/6/373/s1.

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Ethical Statement: In this paper, we guarantee that the information we provide above has not been published or reviewed elsewhere, and that we did not violate ethical standards. There is no plagiarism in this study.

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