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Adaptive Variations of Sediment Microbial Communities and Indication of Fecal-Associated Bacteria to Nutrients in a Regulated Urban River

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Abstract: Anthropogenic activities strongly influence river habitat conditions and surrounding landscape patterns. A major challenge is to understand how these changes impact microbial community composition and structure. Here, a comprehensive analysis combining physicochemical characteristics in sediment with sequencing targeting the V4 region of the 16S rRNA gene was conducted to test the hypothesis that diverse habitat conditions induce dissimilarity of microbial community composition and structure in a regulated urban river. The results suggested that observed species richness and Shannon–Wiener diversity had a decreasing variation along the land use intensified gradient, while beta diversity also revealed significant separation of microbial community structure between headwaters and urban reaches. Total nitrogen (TN), total phosphorus (TP), oxidation–reduction potential (ORP) and total organic carbon (TOC) in sediment were the dominant factors in structuring bacterial and archaeal community assemblages. Further analysis in dominant fecal-associated bacteria indicated that elevated nutrient concentrations may significantly ($p < 0.05$) increase the relative abundance of *Clostridium* and *Acinetobacter* in sediment. The findings highlight the pivotal roles of alpha diversity and fecal-associated bacteria in understanding the dynamics of microbial communities in a regulated urban river ecosystem.

Keywords: adaptive variations; dissimilarity; microbial community; headwater; urban reach; fecal-associated bacteria

1. Introduction

It is well known that microbial communities play the key roles in global biogeochemical cycles, especially that of nitrogen, phosphorous and organic matter in aquatic and terrestrial ecosystems [1–3]. An understanding of the biogeographical patterns of microbial communities in complex freshwater ecosystems is essential to developing and predicting ecosystem responses to environmental changes [4–6]. However, the high dynamics and diversity of microbial communities in complex fluvial habitats at different temporal and spatial scales remain major challenges [7,8].

Current efforts have revealed that the biogeography patterns of microbial communities in complex freshwater ecosystems were different within their habitat conditions, which can be deciphered by their origins in upstream freshwater and surrounding terrestrial environments [8–10]. The temporal and spatial variation of water, sediment, channel morphology and land use have strongly changed a range

of ecosystem processes [6,11,12]. Land use, especially in urban and agricultural areas, appeared to induce greater changes of river environmental factors in water and sediment than forest and grassland. Nevertheless, the differential contributions of spatially-distributed controlling factors to affect microbial community structure and function are still poorly understood [3,7]. Previous studies have shown that pH [13,14], transparency [15], temperature [16], nutrients [17,18], water residence time [19] or other stressors [20] directly or indirectly altered the microbial community composition, while no consensus has been formed for the diverse drivers in the different river ecosystems. Besides, in the complex fluvial network, main streams may have similar dominant sub-communities with their tributaries, while the distinct dominant taxa were also probably identified between main streams and tributaries due to the distance-related dispersal limitation [6], perhaps which were mainly recruited from the terrestrial environment [9].

In addition, there is a growing concern over the threat posed by fecal-associated bacteria to human health in urban reaches [21,22]. The fecal-associated bacteria may originate from multiple sources including upstream agricultural animal feces, sewage released from sewer overflows and even wastewater treatment plants (WWTPs) [23,24]. A handful of studies have used denaturing gel gradient electrophoresis (DGGE), automated ribosomal intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphism (T-RFLP) and next-generation sequencing (NGS) to analyze the sources, distribution and community composition of fecal-association bacteria [3,23,25–28]. However, the impact of nutrient concentration on fecal-association bacteria community composition is less clear, although the nutrient enrichment in rivers may dramatically affect the persistence patterns of fecal-associated bacteria [29]. Therefore, understanding the relationships between nutrient concentration and fecal-associated bacteria community could enhance our knowledge of the dynamics of microbial communities in relation to rapid changes of environmental stress.

The Qingshui River, originating in the Liupan Mountains, flows through Guyuan City, to the north, and then into the Yellow River, which is located in the central Loess Plateau of northwestern China. Upstream agricultural pollution and urban wastewater discharge has resulted in deterioration of the Qingshui River. This area has a continental monsoon climate characterized by an annual mean temperature of 6.6 °C and a mean precipitation of 450 mm [30]. The annual evaporation ranges from 1250 to 2000 mm. Due to extreme shortage of water resources, Guyuan City was selected to be one of the second batch of pilot cities for Sponge City construction, which was designed by the Chinese government in 2016 [31]. The Sponge City concept is similar to low impact developments (LID) in United States, sustainable urban drainage systems (SuDs) in United Kingdom and low impact developments urban design (LIDUD) in New Zealand [32]. An ecological dredging project was completed in the urban reach of the Qingshui River between 2016 and 2017. It is one of the directly effective measures to restore the river ecosystem by removing severely contaminated river sediments, and further improving the river habitat conditions in an ecological way. To the best of our knowledge, the microbial community in the Qingshui River has not yet been investigated.

Herein, the present study hypothesized that diverse habitat conditions affected by landscape patterns would induce dissimilarity of microbial community composition and structure, and that ecological dredging would narrow the dissimilarity of different river reaches. The specific objectives were to (i) characterize the spatial variations of alpha diversity and beta diversity in different river reaches; (ii) identify the environmental factors in sediment that explain variations in bacterial and archaeal community structure; and (iii) analyze the relationships between nutrients and fecal-associated bacteria. The findings can provide insights for ecological restoration in urban rivers and Sponge City construction.

2. Materials and Methods

2.1. Sample Collection and Environmental Variables

During October 18–20, 2018, 31 sites distributed throughout seven different reaches (R1–R7) of the Qingshui River were sampled for sediment (Figure 1). According to the landscape pattern, R1 (15A–17A), R3 (6A–8A), R4 (9A–12A) and R7 (13A, 14A, 18A) were considered to be agricultural reaches, while R2 (1A–5A) was primarily located in urban areas. R5 (25A–30A) and R6 (19A–24A, 31A) were mainly surrounded by grassland, cropland and forest. Surface sediments were collected using a Peterson grab sampler. Each sample was obtained by mixing sediments randomly collected (3 times) at each sampling site. The sediment samples for molecular biological analysis were immediately refrigerated for transport to the laboratory and stored at −80 °C until DNA extraction.

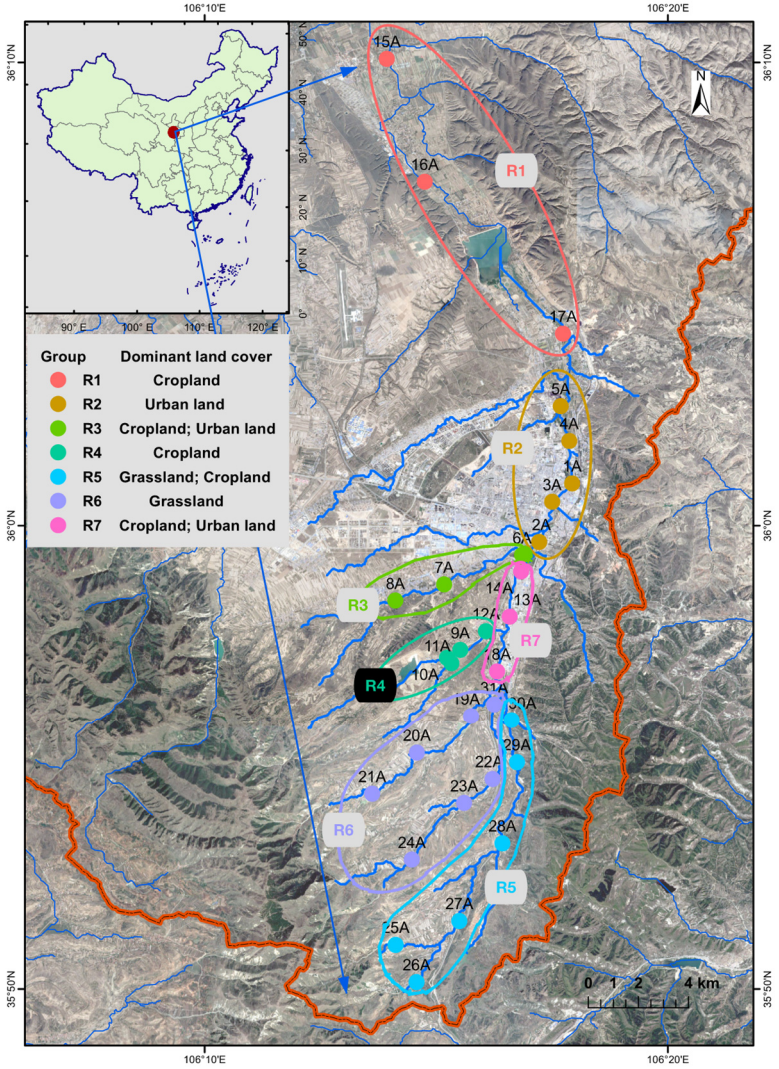


Figure 1. Locations of sampling sites in the Qingshui River.

The pH, total nitrogen (TN), total phosphorus (TP), oxidation–reduction potential (ORP), nitrate nitrogen (NO_3-N), chemical oxygen demand (COD_{Mn}), total organic carbon (TOC), Ca, Mg, Al, Fe, K, Na and Mn for sediment chemistry were measured at the Pony Testing International Group (Pony, Beijing, China) following the standard methods described by the American Public Health Association.

2.2. Extraction of DNA and Sequencing of 16S rRNA Amplicon

Sediment DNA (0.5 g) was extracted from of each sediment sample using a PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA) following the manufacturer's protocol. The V4 region of the bacteria 16S ribosomal RNA gene was amplified by a polymerase chain reaction using forward primer 515F and reverse primer 806R [33]. This primer set was designed to be universal for nearly all archaeal and bacterial taxa [34]. PCR reactions (an initial denaturation at 94 °C for 3 min, 30 cycles at 94 °C for 45 s, annealing at 50 °C for 60 s, extension at 72 °C for 90 s, followed by a final extension step at 72 °C for 10 min) were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). Further Illumina sequencing was done using the Illumina Hiseq 2500 platform at the Beijing Genomics Institute (BGI, Wuhan, China).

2.3. Bioinformatics Analysis

Paired-end sequence reads were assembled and quality filtered using FLASH v1.2.11 [35]. Low-quality sequences containing ambiguous 'N', quality score less than 20 over a 30 bp sliding window, low complexity with 10 consecutive same base and length shorter than 75% of their original length were discarded. Operational taxonomic units (OTUs) at 97% similarity were used to cluster using UPARSE v v7.0.1090, and chimeric sequences were identified and removed using UCHIME v4.2.40 [36]. The representative sequences for each OTU were obtained by searching against the Greengene v201305 database at the 60% confidence and then were chosen for classification using Ribosomal Database Project (RDP) classifier v2.2 [37]. All sequences were deposited into the NCBI Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/Traces/sra/) database (Accession No. PRJNA598417). To examine the effect of ecological dredging and the potential risk posed by the microbial communities in the present study area, fecal-associated bacteria were screened and analyzed at the genus level by comparing the previous findings.

2.4. Diversity and Statistical Analysis

Microbial alpha diversity indices including the observed species richness, Chao1 richness, ACE richness, Shannon–Wiener diversity and Simpson diversity in Mothur (v1.31.2) [38] were calculated. One-way ANOVA followed by post hoc LSD test was employed to display the difference of microbial community composition in different samples. Variation in beta diversity was visualized using principal coordinate analysis (PCoA) on the basis of Bray–Curtis distances in R package vegan. Permutational multivariate analysis of variance test (PERMANOVA, n = 999) [39] subsequently examined the statistical significance of differences among a prior defined sampling reaches (Figure 1).

To further investigate the taxonomic distribution and differentially dominant clades between river reaches, the linear discriminant analysis (LDA) effect size (LEfSe) was employed to compare the abundance of microbial compositions and identify differentially abundant microbial taxa at each taxonomic level [40]. Only those taxa that obtained a log LDA score > 2 were considered. Kruskal–Wallis test with false discovery rate (FDR) correction was used to detect features with significant differential abundance at $p < 0.05$ [40].

The effects of environmental variables on bacterial community structure were analyzed using canonical correspondence analysis (CCA) or redundancy analysis (RDA) with the Monte Carlo permutation test (permutations = 999, $p < 0.05$) in the R package vegan. The function "envfit" was performed with 999 permutations to select the significant variables ($p < 0.05$). The environmental variables and taxa abundances were log-transformed prior to ordination analysis. Ordinary least squares (OLS) regression was used to explore the relationship between nutrients and the relative abundance of dominant fecal bacteria in SYSTAT (v13.2).

3. Results

3.1. Microbial Alpha Diversity in Qingshui River

A total of 1,435,589 high-quality paired-end sequences in 31 sediment samples after pre-filtration of ambiguous sequences and the sequence range from 40,129 to 57,698 with the average sequences of 46,309 were obtained by high-throughput sequencing. A total of 10,593 OTUs were assigned to bacteria (10,435), archaea (157) and unclassified (1), respectively. The rarefaction curves based on observed OTUs, chao1 and ace illustrated that the produced sequences were enough to represent the microbial communities in 31 sediment samples. Rarefaction curves revealed that most samples reached the saturation stage (Supplementary Materials Figure S1), combining the relatively high Good's coverage ranging from 96.48% to 99.89% for sediment samples indicated the major bacterial and archaeal communities were correctly covered by current microbial profiles (Figure 2). Three indices of alpha diversity including observed OTUs, Chao1 and ACE were calculated to represent species richness, while Shannon–Wiener and Simpson were calculated for species diversity. The medium values of observed species, Chao1 and ACE in R5, R6 and R7 were higher than those in R1, R3 and R4 (Figure 2 and Supplementary Materials Table S1), and they had the highest Shannon–Wiener and the lowest Simpson diversity than other reaches in mean value (Figure 2 and Supplementary Materials Table S1). A one-way ANOVA analysis with post hoc LSD test revealed that the significant differences of alpha diversity in Shannon–Wiener diversity index ($p = 0.009$) and Simpson diversity index ($p = 0.003$) were found among different river reaches (Supplementary Materials Table S1). Specifically, observed species richness and Shannon–Wiener diversity were decreased from headwaters to lower reaches (Figure 2). Besides, there was a significant difference ($p < 0.05$) in observed OTUs, Chao1, Shannon–Wiener diversity and Simpson diversity between R1 and R7, as well as R4 and R7. R2 was also different from R6 in Shannon–Wiener and Simpson diversity ($p < 0.05$) (Figure 2).

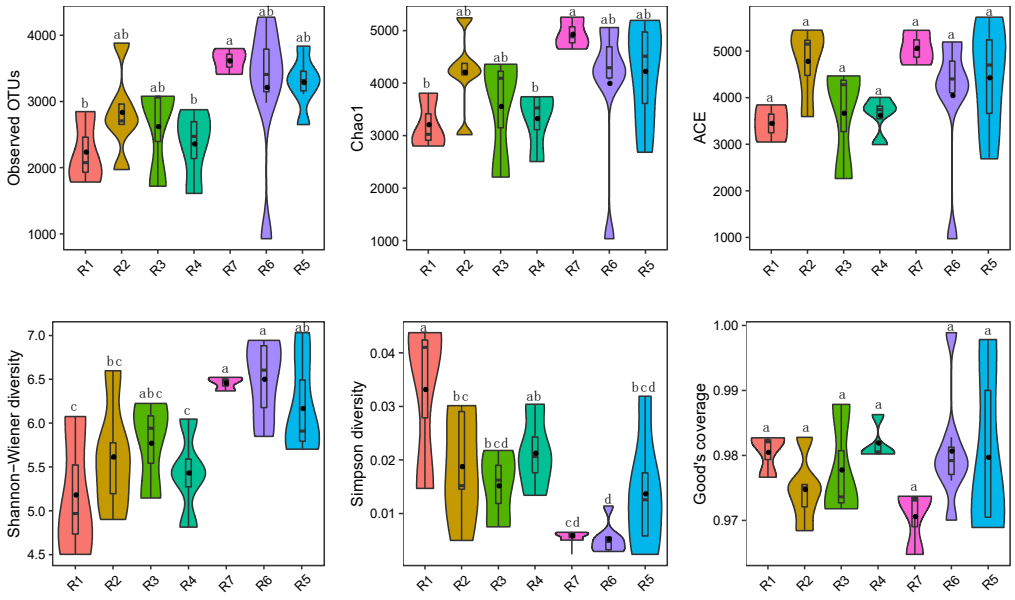


Figure 2. Comparison of alpha diversity among different river reaches. Statistical significances are indicated by letters above the boxes. Boxes with the same letters (like a and a, a and ab) represent no significant difference ($p > 0.05$), while with different letters (like a and b, a and bc) differ significantly ($p < 0.05$) between paired reaches.

3.2. Spatial Variations of Microbial Composition and Beta Diversity

Sequences from the Proteobacteria (44.2%) phyla with the highest average relative abundance dominated most samples, followed by Verrucomicrobia (14.4%), Bacteroidetes (9.6%), Cyanobacteria (5.6%), Planctomycetes (5.3%), Acidobacteria (5.2%) and Actinobacteria (3.3%) (Figure 3). In the urban

reach R2, the relative abundance of Verrucomicrobia was mostly higher than headwater reaches R6 and R5. Cyanobacteria and Bacteroidetes dominated in sampling site 27A and 6A, respectively, while the relative abundance of Proteobacteria was the highest in sampling site 5A and 11A. Furthermore, the relative abundance of Firmicutes and Euryarchaeota were right behind Bacteroidetes in sampling site 6A (Figure 3). Additionally, different sites also showed the distinct microbial composition at the genus and order levels (Supplementary Materials Figure S2).

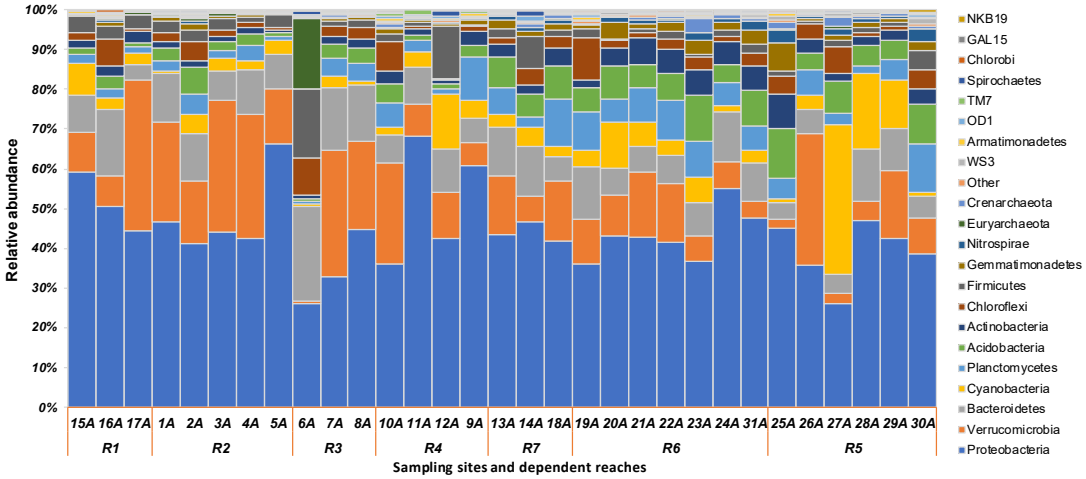


Figure 3. Taxonomic composition distribution of each sample at the phylum level. Only phyla with relative abundance > 0.10% are shown.

The PCoA plot of Bray–Curtis distance revealed distinct beta diversity separation of the microbial community structure on R1–R7, which was confirmed by PERMANOVA ($R^2 = 0.239$, $p = 0.044 < 0.05$) (Figure 4). The significant difference of beta diversity between urban reach R2 and upstream reaches (R5 and R6) was obvious ($p < 0.001$), while R3 and R4 as adjacent reaches were not different than the R2 reach (Figure 4). Moreover, the distinct patterns of the microbial community along the PCoA1 gradient presented the spatial succession from headwaters to lower reaches.

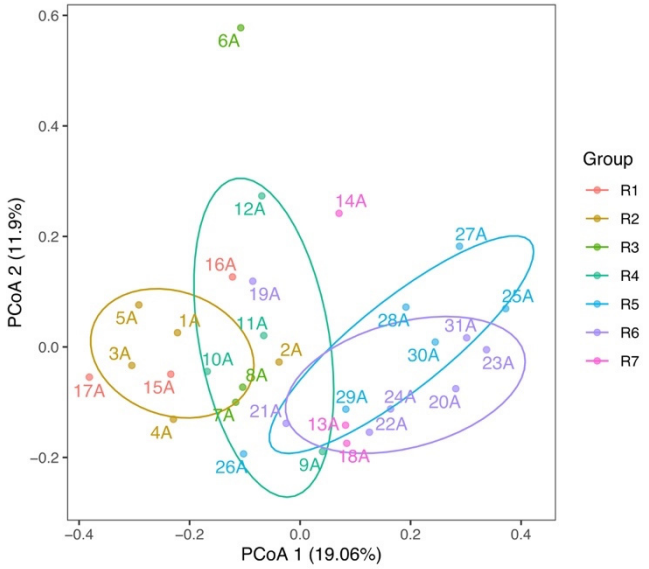


Figure 4. Principal coordinate analysis (PCoA) showing microbial composition differences among different reaches. Only ellipse with points in each reach greater than 3 are shown.

LEfSe analysis based on the Kruskal–Wallis test was applied to identify the key phylotypes responsible for the difference among river reaches. The results revealed that phylum Planctomycetes (p = 0.038) in R6, phylum Crenarchaeota (p = 0.030) and its class Thaumarchaeota (p = 0.027) in R5 and class Methanomicrobia (p = 0.014) in R3 were most abundant, contributing to the significant differences among all the groups (Figure 5 and Supplementary Materials Figure S3). Besides, order Methanomicrobiales (p = 0.014) in R3 and order Hydrogenophilales (p = 0.024) in R1 were the important taxa within their groups. However, after false discovery rate (FDR) correction of p-values, no significant differential abundance among all groups ($p > 0.05$) was found (Figure 5).

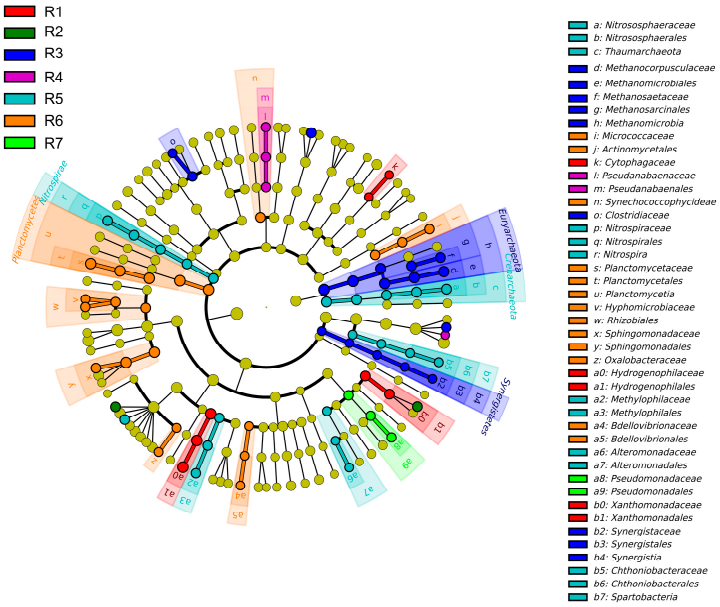


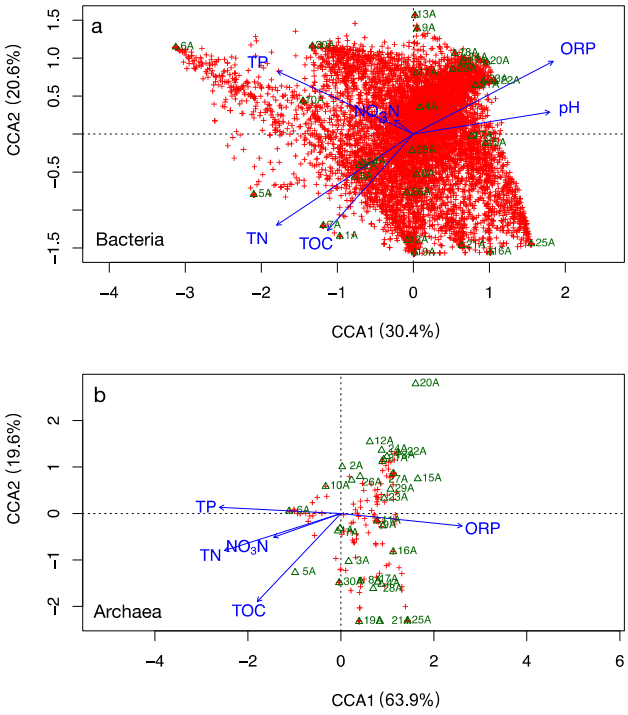
Figure 5. Taxonomic cladogram obtained from LEfSe analysis of 16S sequences. In the LEfSe tree, different colors indicate different groups. Each small circle with different colors represent a taxon, and the diameter of the small circle is proportional to the relative abundance. The yellow circles indicate non-significant differences in abundance among groups.

3.3. Environmental Influences on the Microbial Community

Canonical correspondence analysis of microbial communities indicated distinct correlations with sediment variables. COD_{Mn}, Ca, Mg, Al, Fe, K, Na and Mn were removed due to collinearity after the completion of the function "envfit". Six variables explained 24.2% of the total variation in bacterial community assemblage with the Monte Carlo permutation test (F = 1.277, p = 0.021), revealing that TN (R^2 = 0.585, p = 0.001), ORP (R^2 = 0.536, p = 0.001), TP (R^2 = 0.505, p = 0.001), pH (R^2 = 0.411, p = 0.001) and TOC (R^2 = 0.368, p = 0.005) in sediment were the significant factors in structuring bacterial community assemblages (Figure 6a). CCA also showed that five variables explained 32.6% of the total variation in archaeal community assemblage, which, combined with the Monte Carlo permutation test (F = 2.422, p = 0.001), proved that TN (R^2 = 0.761, p = 0.001), ORP (R^2 = 0.759, p = 0.002), TP (R^2 = 0.749, p = 0.008) and TOC (R^2 = 0.604, p = 0.046) in sediment dramatically affected the archaeal community (Figure 6b). It was apparent that TN, TP, ORP and TOC were altogether responsible for shaping microbial community assemblage in the Qingshui River.

According to the previous findings [24,27,41], at the genus level, 15 genera of fecal-associated bacteria were identified as follows: *Bifidobacterium, Bacteroides, Parabacteroides, Prevotella, Streptococcus, Clostridium, Roseburia, Ruminococcus, Faecalibacterium, Veillonella, Sutterella, Burkholderia, Acinetobacter, Pseudomonas* and *Akkermansia*. Specifically, *Clostridium, Acinetobacter* and *Pseudomonas* were the dominant fecal-associated bacteria with a relative abundance of 10.24%, 7.72% and 7.65% in all of the sampling sites, respectively (Figure 7). The relative abundance of *Acinetobacter* in R2 was notably higher

than in other river reaches, while the relative abundance of *Pseudomonas* in R1 and R4, and *Clostridium* in R6 was substantially lower than in others. The relative abundance of *Clostridium* in site 6A and *Pseudomonas* in site 24A was the highest in all river reaches (Figure 7).



Variables	R^2	*p*-value
Bacteria		
pH	0.411	0.001
TOC	0.368	0.005
ORP	0.536	0.001
TN	0.585	0.001
TP	0.505	0.001
NO_3N	0.013	0.772
Archaea		
TOC	0.604	0.044
ORP	0.759	0.001
TN	0.761	0.002
TP	0.749	0.008
NO_3N	0.233	0.322

Figure 6. Canonical correspondence analysis of microbial community (bacteria (**a**) and archaea (**b**)) and environmental variables in sediment. The red cross represents OTUs, while green triangle represents sampling sites.

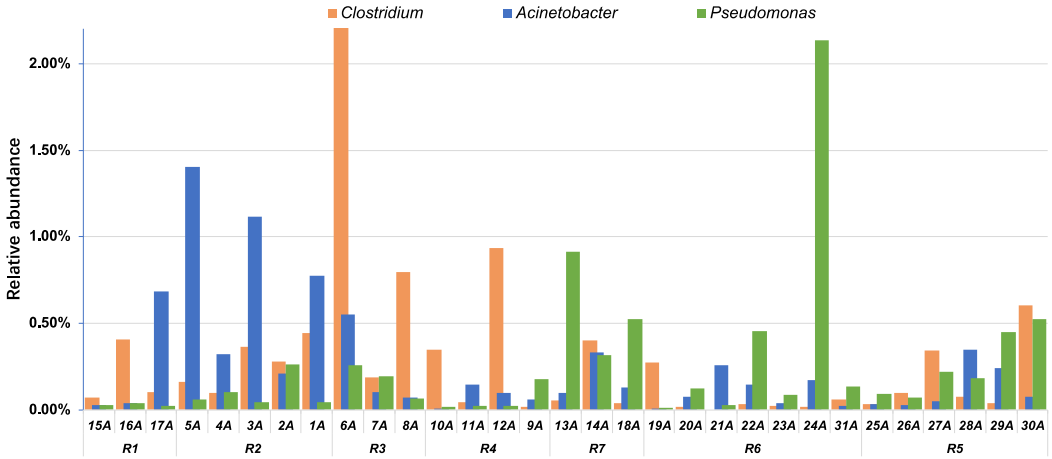


Figure 7. Distribution and composition of dominant fecal bacteria at the genus level.

Given the above, for the dominant fecal-associated bacteria, the relationships between their relative abundance and nutrient level in sediment were further analyzed using OLS regression, which demonstrated that the relative abundance of *Clostridium* and *Acinetobacter* significantly increased with increasing TN ($R^2 = 0.199$, $p = 0.012$; $R^2 = 0.328$, $p = 0.001$, respectively) and TP ($R^2 = 0.227$, $p = 0.007$; $R^2 = 0.195$, $p = 0.013$, respectively) concentration in sediment (Figure 8). However, the difference was that the relative abundance of *Pseudomonas* was not associated with TN ($R^2 = 0.091$, $p = 0.099$) and TP ($R^2 = 0.021$, $p = 0.442$) (Figure 8).

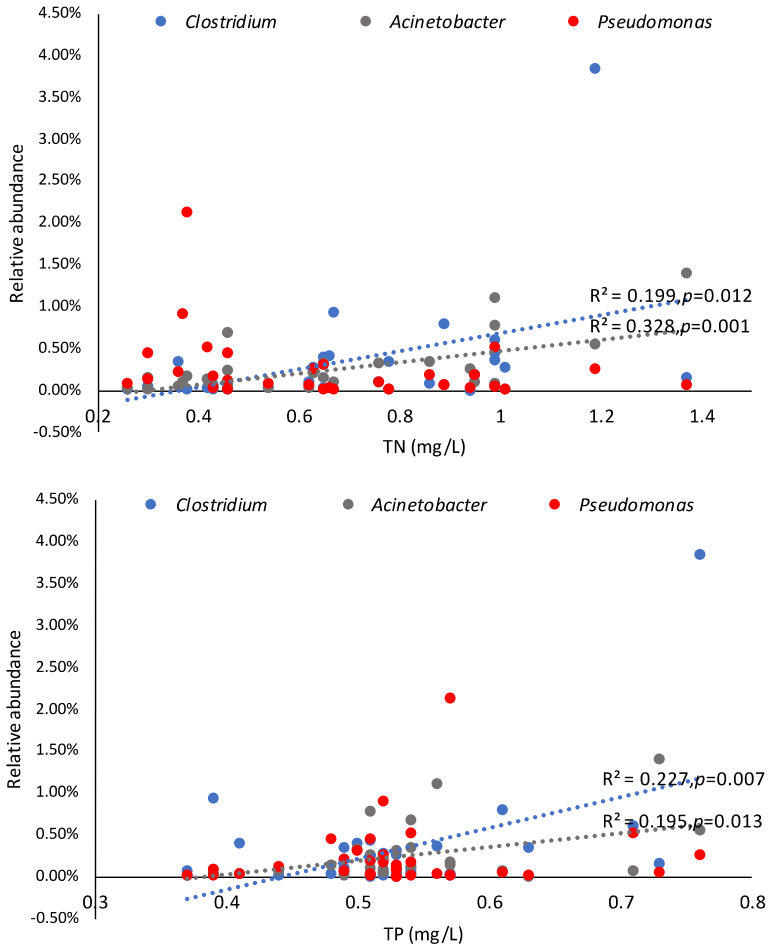


Figure 8. Relationships between nutrients and the relative abundance of dominant fecal bacteria. Significant ordinary least squares (OLS) regression lines were fit to the data. The blue and grey dash lines represent *Clostridium* and *Acinetobacter*, respectively. Statistical significances are indicated by *p*-values.

4. Discussion

4.1. Adaptive Patterns and Their Driving Factors in Microbial Communities

In the present study, the variations of microbial community structure among the different river reaches revealed a significantly dissimilar pattern of alpha and beta diversity of microbial communities. To be specific, there were more species and higher Shannon–Wiener diversity observed in upstream reaches (R5, R6 and R7) than the other river reaches (Figure 2). Instead, Simpson diversity index was a decreasing pattern. Obviously, different surrounding land use types resulted in differences in microbial community structure, which was that urbanization and agriculture decreased the species richness and Shannon–Wiener diversity. This was consistent with previous findings [3,8]. However, unlike these results, Savio et al. [42] revealed that bacterial community richness gradually increased from the headwaters to the lower reaches. In addition, although R7 was considered to be influenced by surrounding impervious surfaces and cropland, the community composition was more likely recruited from R5 and R6 due to the fast water flow, and there were no significant differences in environmental variables between R5, R6 and R7 (Supplementary Materials Figure S4). Similar to alpha diversity, our results also suggested that there were adaptive and distinct patterns of beta diversity distributed in different river reaches in regards to the significant difference of anthropogenic disturbance on the landscape (Figures 1 and 4), which has been confirmed in previous studies [3,27,43–46].

Further analysis suggested the spatial alteration of the bacterial and archaeal community structure in sediment responding to TN, TP, ORP and TOC in the present study. However, the most prevailing ecological drivers seemed to be the pH in bacteria of the Santa Ana River [44]. Fortunato et al. [47] demonstrated that hydrological and climatic conditions may be some of the primary factors structuring the composition and dynamics of microbial communities in running water systems. Hosen et al. [3] showed that bacterial communities in forested river sediments were correlated with TP, while TN was associated with communities in urbanized river sediments. Taken all together, the results of the present study and previous research [25,44–48] implied that there is currently no agreement to explain the relationship between key drivers and microbial diversity. Compared with current efforts to decipher variations of microbial community structures in lakes and oceans, stream microbial communities still received less attention [49,50]. Therefore, further research is required to address the interactive effect of excessive nutrients and other critical physicochemical variables on shaping microbial community structure in lotic ecosystems.

4.2. Implications for the Watershed Management to Sponge City Construction and Indication of Fecal-Associated Bacteria

According to our results, the microbial community composition in the Qingshui River was generally dominated by Proteobacteria (44.2%), Verrucomicrobia (14.4%) and Bacteroidetes (9.6%) at the phylum level, which were usually observed in freshwater ecosystems and highlighted in the biogeochemical processes [6,13,25,51]. As a whole, there was no distinct difference of taxonomic composition at the phylum level except for sampling site 6A and 27A in the Qingshui River (Figure 3). LEfSe analysis confirmed that no remarkable biomarkers were observed in urban reach R2, which showed no significant differences with other river reaches (Figure 5), since ecological dredging was implemented only in urban reach R2 in 2016 and 2017. However, the contrasting composition of archaeal communities in sediment was observed. Specifically, methanogens in the order Methanomicrobiales, class Methanomicrobia, belonging to Euryarchaeota showed prominence in river reach R3 (Figure 5 and Supplementary Materials Figure S3), which may be responsible for anaerobic oxidation of methane [52]. The sampling site 6A in steam reach R3 played an important role in the variations of archaeal communities (Figure 5) on account of degraded habitat conditions with the lowest ORP (133 mV), the highest TP (0.76 g/kg) and the second highest TN (1.19 g/kg) concentration among all the sampling sites (Supplementary Materials Figure S4). Thus, we propose effective ecological restoration measures in relation to the sediment to improve the habitat conditions in R3.

In addition, domestic sewage and industrial wastewater is still probably dispersed into the river with urban runoff after ecological dredging, although the sewage outlets directly discharging sewage into the Qingshui River were blocked during the process of Sponge City construction. Previous reports used high-throughput sequencing of microbial community composition and advanced statistical approaches to identify pollution sources in aquatic environments [26,53–56]. All these studies pointed out that fecal-associated bacteria deriving from sewage and wastewater influent strongly altered microbial community composition and structure [23,24,27]. Both Clostridiales and Bacteroidales bacterial groups with high abundance in human and other animal feces were used to identify sources of fecal contamination in the environmental samples [23,54,57]. Besides, Lactobacillales and Ruminococcaceae, as dominant bacteria in feces, have also been documented in WWTPs [26,28]. In this study, profound changes in the composition of fecal-associated bacteria were uncovered in sediment of the Qingshui River (Figure 7). Only Bacteroidales, Clostridiales and Lactobacillales with higher relative abundance were detected and enriched in the urban and agricultural reaches, as compared to the headwaters (Supplementary Materials Figure S5). At the genus level, we found a potential shift from a *Pseudomonas*-dominated community in the headwaters to a *Clostridium*-dominated community in the agricultural reaches, and to an *Acinetobacter*-dominated community in the urban reaches (Figures 1 and 6). The shifting pattern indicated an ecological succession of microbial communities

along the land use intensified gradient. Despite the ecological dredging project having been finished in 2016–2017, sewage, wastewater influent and agricultural fertilization may have led to nutrient enrichment in sediment in the past two years (Supplementary Materials Figure S4). It has been suggested that TN and TP in sediment were more important than other environmental variables in structuring microbial communities. Nevertheless, a handful of studies have provided insights to explore the variations of fecal-associated bacteria in conditions with elevated nutrient levels [24]. Here, there was evidence of the significantly positive relationships between two dominant genera (*Clostridium* and *Acinetobacter*) of fecal-associated bacteria and nutrient concentrations (Figure 8), and the trends may extend the persistence period of fecal pollution and possible growth, further increasing public health risks [25,29,44,58]. The relative abundance of *Pseudomonas* presented distinctively poor correlations with nutrients in this study (Figure 8). This was probably because the highest abundance of *Pseudomonas* from animal feces in sampling sites 24A disturbed their relationships (Figures 7 and 8), where the surrounding grassland was good for grazing cattle or sheep (Figure 1).

5. Conclusions

The study showed that evident differences of alpha diversity including observed species richness, Shannon–Wiener diversity and Simpson diversity were observed in sediment microbial communities between downstream and headwaters, which implied a decline in urban and cropland reaches. PCoA analysis based on Bray–Curtis distance also showed adaptive and distinct patterns of beta diversity, which were shaped by the key environmental factors like TN, TP, ORP and TOC, responding to the significant gradient of anthropogenic disturbance on the landscape. Although ecological dredging has been implemented to restore the river habitat conditions, non-point source pollution from urban and cropland areas should be explicitly considered. Moreover, the considerable variability in community composition of fecal-associated bacteria was caused by elevated nutrient concentrations, and the findings demonstrated that fecal-associated bacteria may be an alternative indicator of non-point source pollution to provide implications for Sponge City construction and management.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/5/1344/s1, Figure S1: Rarefaction curves for 31 samples, Figure S2: Taxonomic composition distribution of each sample at the genus and order level, Figure S3: LDA scores as calculated by LEfSe of taxa differentially abundant among different river reaches, Figure S4: Physicochemical characteristics and statistical comparison of key environmental variables in sediment of the Qingshui River, Figure S5: Distribution and relative abundance of three dominant orders in fecal-associated bacteria, Table S1: The statistical description of alpha diversity among groups.

Author Contributions: Conceptualization, X.C., M.H. and J.P.; Data curation, Y.W.; Formal analysis, X.C.; Funding acquisition, J.P.; Investigation, X.C., Y.W., Y.X. and G.D.; Methodology, X.C. and Y.W.; Resources, M.H.; Visualization, J.P.; Writing—original draft, X.C.; Writing—review and editing, X.C. and J.P. All authors have read and agreed to the published version of the manuscript.

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