Bacterial community compositions in sediment polluted by perfluoroalkyl acids (PFAAs) using Illumina high-throughput sequencing

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Received: 30 June 2015 / Accepted: 4 January 2016 / Published online: 16 January 2016
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Abstract The characterization of bacterial community compositions and the change in perfluoroalkyl acids (PFAAs) along a natural river distribution system were explored in the present study. Illumina high-throughput sequencing was used to explore bacterial community diversity and structure in sediment polluted by PFAAs from the Xiaoqing River, the area with concentrated fluorochemical facilities in China. The concentration of PFAAs was in the range of 8.44–465.60 ng/g dry weight (dw) in sediment. Perfluorooctanoic acid (PFOA) was the dominant PFAA in all samples, which accounted for 94.2 % of total PFAAs. High-level PFOA could lead to an obvious increase in relative abundance of *Proteobacteria*, ϵ -*Proteobacteria*, *Thiobacillus*, and *Sulfurimonas* and the decrease in relative abundance of other bacteria. Redundancy analysis revealed that PFOA played an important role in the formation of bacterial community, and PFOA at higher concentration could reduce the diversity of bacterial community. When the concentration of PFOA was below 100 ng/g dw in sediment, no significant effect on microbial community structure was observed. *Thiobacillus* and

Sulfurimonas were positively correlated with the concentration of PFOA, suggesting that both genera were resistant to PFOA contamination.

Keywords PFOA · Bacterial community structure · Illumina high-throughput sequencing · Redundancy analysis · *Thiobacillus* · *Sulfurimona*

Introduction

Perfluoroalkyl acids (PFAAs), mostly known as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), have unique physicochemical properties, such as high surface activity, thermal inertness, resistance to acidic and alkaline conditions, and amphiphilicity. These properties contribute their popularity for the application as surfactants, fire-fighting foams, pesticides, shampoos, oil-resistant coating for carpets, car polishes, and food packaging materials for more than 60 years (Higgins et al. 2005; Paul et al. 2009). Perfluorooctanoic acid (PFOA) is one of the most widely detected PFCAs in various environmental media (river water, oceans, sediment, and soil) and tissues of wild animals and human (Ahrens et al. 2010; Dai et al. 2006; Higgins et al. 2005; Moody et al. 2002; Wang et al. 2013b). The application of PFOA is regulated in some developed countries such as USA and Canada, and PFOA manufacturing has been gradually phased out in these countries over the past few years. Even though their use is regulated, PFOA is still ubiquitous in the environment due to the huge demand for various products and industrial processes in some developing countries, especially in China (Wang et al. 2009; Wang et al. 2014c).

The widespread occurrence of PFOA has also caused increasing concern about its potential risk to organisms (Rayne and Forest 2009). The different toxic effects of PFOA towards

Responsible editor: Robert Duran

Electronic supplementary material The online version of this article (doi:10.1007/s11356-016-6055-0) contains supplementary material, which is available to authorized users.

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human and animals have been explored, such as reproductive toxicity, developmental toxicity, carcinogenicity, and immunotoxicity in experimental animals (Lau et al. 2007; Olsen et al. 2009). Additionally, according to previous studies, PFOA could inhibit the growth and development of macrophytes (Hanson et al. 2005) and affect the survival of several invertebrates (Hazelton et al. 2012; Kawamoto et al. 2010). However, these toxicity studies of PFOA mainly focused on animals and plants, while the information on microorganisms is limited.

Bacterial communities are ubiquitous and have a crucial role in the biogeochemical cycling processes. Similarly, bacteria are the most diverse and abundant group in soil organisms (Acosta-Martinez et al. 2008). So it is essential to understand the association between bacterial community compositions and PFAA-polluted ecosystem. Environmental biodegradability of PFAAs by bacteria has been explored in previous studies, which focuses on microbial degradation pathways, defluorination potential, and novel degradation intermediates and products (Liu and Avendano 2013), but few studies were conducted to evaluate the toxicity of PFAAs to bacterial community. Meanwhile, various methods have been used to investigate bacterial compositions in sediment, including denaturing gradient gel electrophoresis (DGGE), cloning library of 16S rRNA genes, and fluorescence in situ hybridization (FISH) as well as terminal restriction fragment length polymorphism (T-RFLP). These conventional molecular biological methods lack sufficient sequences to capture systematic and comprehensive information on various microbial communities (Lu et al. 2012). With the development of the second-generation sequencing, recently, Illumina high-throughput sequencing technology has confirmed as a highly efficient tool for identifying the entire profile of microbial communities, which can characterize over 10^7 reads in a single running through a paired-end sequencing approach (Matochko et al. 2012). This method has been widely used to analyze microbial compositions in various environmental samples, such as wastewater treatment plant (Ma et al. 2015), drinking water (Huang et al. 2014), and soil (Bartram et al. 2011). However, few studies have been conducted to analyze microbial communities in high PFOA-loading area by high-throughput sequencing.

In the present study, we selected the Xiaoqing River in Shandong Province of China as an area with fluorochemical industry for the investigation, and high PFOA loads were quantified (Wang et al. 2014b; Zhu et al. 2014). A total of 10 samples of surface sediment along the river were collected and sequenced using Illumina high-throughput platform. The objectives were: (1) to monitor the pollution status of PFAAs in sediment, (2) to uncover the bacterial community diversity and structure, and (3) to explore the relationship between bacterial community and environmental variables, especially PFOA contamination.

Materials and methods

Site description and sample collection

Xiaoqing River, with the length of 240 km and watershed area of 11,000 km², is located in Shandong Province of China. In April 2014, 10 samples of surface sediment were collected from 10 sampling sites (XQ1–XQ10). The location and physicochemical properties for each site are shown in Fig. 1 and Table 1. It is worth noting that a major fluoropolymer manufacturer was identified at the upstream site XQ5 (Wang et al. 2014b). Sediment samples were harvested from four corners and the center point of each site with the area of 2 m × 2 m using a sediment sampler (Peterson dredge, China). Surface sediment samples (0–15 cm) were carefully collected into 500 mL polypropylene (PP) bottles and mixed to produce one composite sample for each site. The sediment samples were stored in ice box and transferred to the laboratory immediately. One part was stored at −80 °C before DNA extraction, the other part was dried in a FreeZone 2.5 Liter Benchtop Freeze Dry System (LABCONCO, Kansas City, MO), and ground to pass a 2-mm mesh sieve before further analysis.

Determination of PFAAs

Sample extraction and analysis

Sediment samples were extracted according to a previously published method with minor optimizations and modifications (Naile et al. 2010; Wang et al. 2013b; Zhu et al. 2014). A total of 12 PFAAs, including 9 PFCAs (perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA)), and 3 PFSAAs (potassium perfluorobutanesulfonate (PFBS), sodium perfluorohexanesulfonate (PFHxS), potassium perfluorooctanesulfonate (PFOS)) were analyzed (Wang et al. 2014b). Briefly, 2-g sediment was placed in a 50-mL PP centrifuge tube and spiked with mass-labeled internal standards (5 ng). Then, the sediment samples were digested with 2 mL of 100 mM NaOH in acetonitrile (ACN) and subjected to sonication for 30 min. The mixture was shaken at 250 rpm for 30 min after adding 20 mL of acetonitrile. Subsequently, 0.1 mL of 2 mol/L hydrochloric acid was added and the mixture was centrifuged at 3000 rpm for 15 min. The supernatant was transferred to another 50 mL centrifuge tube. The above steps were repeated once except that 10 mL acetonitrile was added instead of 20 mL. Finally, the supernatant was pooled and evaporated to 1 mL under a gentle flow of nitrogen with high purity (99.999 %, Haidian District, BJ). The extracts were further loaded onto pre-cleaned ENVI-Carb tubes and

Fig. 1 Sediment sampling sites along the Xiaoqing River, Shandong Province of China

purified with OASIS-WAX cartridges. After loading, cartridges were washed with 4 mL of 25 mM ammonium acetate and air-dried. Then, 4 mL of methanol and 4 mL of 0.1 % NH_4OH in methanol were added to cartridges to wash the target analytes. The sample volume was reduced to 1 mL under high purity nitrogen and filtered through 0.2 mm nylon filter, and then it was transferred into a 1.5-mL PP snap top brown glass vial with polyethylene (PE) septa for further analysis. The instrumental analysis was implemented via Agilent 1290 high performance liquid chromatography system equipped with an Agilent 6460 Triple Quadrupole LC/MS

System (Agilent Technologies, Palo Alto, CA) that was operated in a negative electrospray ionization (ESI) mode. The HPLC and ESI-MS instrument conditions were listed in Table S1.

Quality assurance and quality control

During collection and extraction, samples were kept away from any fluorinated materials to avoid contamination. Besides, transport blanks, field blanks, procedure blanks, and solvent blanks were conducted with every sample set.

Table 1 Physicochemical properties of each sampling point

	XQ1	XQ2	XQ3	XQ4	XQ5	XQ6	XQ7	XQ8	XQ9	XQ10
Temperature (°C)	21.9	21.8	21.3	23.9	23.8	24.3	22.3	22.5	19.9	19.9
DO (mg/L)	6.8	8.8	8.8	10.3	7.7	7.5	12.9	9.4	9.8	10.5
Conductivity (mS/cm)	1.5	1.4	1.8	6.8	6.5	2.7	3.3	2.8	7.0	28.8
Salinity (‰)	0.8	0.7	1.0	3.8	3.6	1.4	1.8	1.5	4.3	19.9
pH	7.7	7.8	7.6	7.7	7.9	7.9	7.8	8.0	8.2	8.0
TOC (g/kg)	20.8	19.4	13.7	20.3	14.5	21.5	20.6	16.2	17.8	7.3
TN	1.4	0.9	1.4	0.8	0.4	1.4	0.8	1.3	0.7	0.3
TP	1.5	1.0	1.2	1.38	0.5	1.4	0.9	0.7	0.51	0.5
Moisture (%)	38.4	36.0	42.6	30.3	43.3	47.1	39.4	30.4	28	26.9
Clay (%)	12.7	13.0	13.7	17.1	11.2	15.2	11.3	8.6	15	10.7
Powder (%)	42.2	43.5	45.0	38.1	40.1	36.9	50.9	21.6	28.8	34.4
Sand (%)	45.1	43.5	41.3	44.8	48.7	48	37.7	69.7	56.2	54.9

Nine-point internal standard curves with native standards ranging from 0.01 to 100 ng/mL and internal standards of 5 ng/mL were prepared for the quantification of individual PFAA with determination coefficients (r^2) for all target analytes over 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were defined as the peak of analytes to yield a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. Matrix spiked recovery rates ranged from 75 to 117 %. No detectable PFAA was observed exceeding LOQ in all fields, transport, and solvent blanks. Detailed QA/QC measurements of PFAAs in sediment samples were listed in Table S2.

Microbial analysis

DNA extraction, PCR amplification, and sequencing

DNA was extracted from sediment samples with the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA) following the manufacturer's protocols. The concentrations of extracted DNA were determined through Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA). Additionally, the extracted DNA was evaluated by 1.5 % agarose gel electrophoresis and stored at $-20\text{ }^{\circ}\text{C}$ prior to further analysis.

Taxonomic profiling of the sediment microbial community was performed using Illumina® MiSeq sequencing combinatorial sequence-tagged PCR products. The 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers with different barcodes were used to amplify bacteria specific V4 hyper variable region of the 16S rRNA gene (Bates et al. 2011). PCR amplification was conducted in a reaction system (30 μL) containing 15 μL Phusion® High-Fidelity PCR Master Mix with GC buffer (New England Biolabs, USA), 0.2 μM of forward and reverse primers, and 10 μL of template DNA. PCR program included initial denaturation at $98\text{ }^{\circ}\text{C}$ for 1 min, followed by 30 cycles of $98\text{ }^{\circ}\text{C}$ for 10 s, annealing at $50\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 60 s, with a final elongation at $72\text{ }^{\circ}\text{C}$ for 5 min. The PCR products were pooled and purified using Gene JET Gel Extraction Kit (Thermo Scientific). Finally, the DNA library was constructed and run on an Illumina MiSeq platform and 250 bp/300 bp paired-end reads were generated.

Sequence and microbial community analysis

Paired-end reads were merged by using Flash (Magoc and Salzberg 2011), and the low quality sequences were removed (http://qiime.org/scripts/split_libraries_fastq.html). Chimera was detected by UCHIME, and the resultant high quality sequences were grouped into operational taxonomic units, at a 97 % sequence similarity threshold, using the UPARSE program (Edgar 2013) in the QIIME (V1.6.0, <http://qiime.org/index.html>) software package.

The taxonomic assignment was performed with the Ribosomal Database Project (RDP) classifier with minimum confidence of 80 %. We subsampled the DNA sequences, so for comparative analysis, the samples contained the same number of sequences. We also rarified the OTU table to compute community richness estimators of Chao1 and ACE, community diversity estimators of Shannon and Simpson and the Good's coverage, and Rarefaction curves were generated based on these three metrics. In order to describe the similarity and difference among 10 samples and detect the relationship among samples, environmental factors and bacterial richness/diversity revealed by sequencing of 16S rRNA. Redundancy analysis (RDA) (Gao et al. 2014), a direct gradient analysis, was constructed based on unweighted UniFrac metric with CANOCO5.0.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics V21 (SPSS Inc. Quarry Bay, HK). Before the analysis of PFAAs, concentrations less than LOQs were set to one-half of the LOQs, and those less than LODs were assigned as values of LODs/ $\sqrt{2}$ (Bao et al. 2011). Spearman correlation coefficients (Puth et al. 2015) were calculated to assess the association among environmental factors.

Results and discussion

PFAAs in sediment samples from Xiaoqing River

The concentrations of PFAAs in sediment samples from Xiaoqing River are shown in Fig. 2. The total concentration of PFAAs in surface sediment ranged from 8.44 to 465.60 ng/g dry weight (dw) with a mean of 109.47 ng/g dw. PFOA was the dominant PFAAs and ranged from 3.86 to 456.20 ng/g dw with a mean concentration of 103.14 ng/g dw and with the contribution rate of 94.2 % to total PFAAs, which was followed by PFOS (0.40 to 5.16 ng/g dw, 1.49 ng/g dw on average, 1.35 %) and short chain PFCAs, including PFBA (0.04 to 14.45 ng/g dw, 1.98 ng/g dw on average, 1.8 %), PFHxA (0.02 to 2.98 ng/g dw, 0.78 ng/g dw on average, 0.71 %), PFHpA (0.02 to 1.23 ng/g dw, 0.64 ng/g dw on average, 0.59 %), and PFPeA (0.04 to 1.06 ng/g dw, 0.39 ng/g dw on average, 0.36 %). For concentrations of the other long chain PFCAs and other short chain PFASs, the total contribution was less than 1 %. The highest concentrations of total PFAA (465.60 ng/g dw) and PFOA (456.20 ng/g dw) were observed in sample XQ5 (Fig. 2). These results suggested that the dominant PFAA in sediment was PFOA, which is similar to the previous report (Wang et al. 2014b).

Fig. 2 Concentrations of PFAAs in sediment samples from Xiaoqing River

Sediment samples from Xiaoqing River contained extremely high concentration of PFOA (a maximum of 456.20 ng/g dw) when compared with other riverine and coastal sediment studies in both China and other countries, such as North Bohai coastal rivers (n.d.–0.54 ng/g dw) (Wang et al. 2011), South Bohai coastal rivers (0.01–1.05 ng/g dw) (Zhu et al. 2014), Zhujiang River in Guangzhou, China (0.09–0.28 ng/g dw) (Bao et al. 2010), Western coast of Korea (<0.20–2.40 ng/g dw) (Naile et al. 2013), Kyoto area River, Japan (1.31–3.92 ng/g dw) (Senthilkumar et al. 2007), Ganges River of India (<0.50–14.09 ng/g dw) (Corsolini et al. 2012), Roter Main River, Germany (0.02–0.07 ng/g dw) (Becker et al. 2008), and San Francisco Bay, USA (nd–0.40 ng/g dw) (Higgins et al. 2005). High level of PFOA in sediment especially at sample XQ5 implied local point source in this area, and the manufacturing facility had been proven in the upstream of XQ5 (Wang et al. 2014b), which produced an annual capacity of 50,000 tons of TFE, 37,000 tons of PTFE, 10,000 tons of hexafluoropropylene (HFP), and more than 200,000 tons of different types of fluorinated refrigerants by the end of 2012.

Bacterial community compositions

Illumina high-throughput sequencing was conducted to explore bacterial community diversity and structure of Xiaoqing River. After trimming the barcodes, adapters, and primers and filtering the low quality sequences, totally 612,615 reads for 10 samples were obtained. The chimeras in the sequencing reads were further removed. Totally, 35,135–114,367 effective sequences for these samples were obtained (Table 2).

Richness and diversity of microbial community

Biodiversity of 10 sediment samples was investigated based on the analysis of OTUs, Chao1, ACE, Good's coverage, Shannon and Simpson at cutoff level of 3 % (Table 2). Results showed that OTU numbers of these samples ranged from 2319 to 4878 based on the effective sequences, and XQ6 had the richest diversity (4878 OTUs), whereas XQ5 displayed the lowest richness (2319 OTUs). The patterns of Chao1 and ACE were accordant with the estimation of OTUs, and XQ5 had lower community richness while XQ6 had higher community richness. According to two diversity indices (Shannon and Simpson), sample XQ5 had lower community diversity (6.76 and 0.0711) compared to the other samples with indices of 8.58–9.89 and 0.003–0.0185, respectively (Table 2). The diversity and richness of sediment samples from Xiaoqing River were similar to intertidal sediment samples from Laizhou Bay of Bohai Sea (Wang et al. 2013a; Wang et al. 2014a). Numerous studies showed that the ecosystem of the Bohai Sea in its coastal environment was heavily polluted and was one of the most degraded areas in China (Ma et al. 2001; Yu and Mao 2002; Zhang et al. 2009). The ecosystem of Xiaoqing River has suffered from serious damage and the contaminants mainly come from fluorine chemical industry (Hong et al. 2010; Ongley et al. 2010). Other pollutants have not been reported from the Xiaoqing River; therefore, the lower bacterial diversity in the sediment from the Xiaoqing River is possibly more similar to that in the highly polluted sediment from the Laizhou Bay of the Bohai sea due to both sites being highly contaminated which in the case of the Xiaoqing River is due to PFAAs.

Table 2 The richness and diversity of bacterial community

Sample ID	Number of sequences	OTU	Chao 1	ACE	Good's coverage	Shannon	Simpson
XQ1	101,031	4468	3433.5	5731.7	96.9 %	9.53	0.0059
XQ2	45,160	3174	2957.6	5087.9	94.2 %	9.37	0.0054
XQ3	36,135	2629	2608.9	4673.6	90.3 %	8.53	0.0185
XQ4	36,388	2977	2999.7	5963.2	93.3 %	9.05	0.0075
XQ5	35,181	2319	2508.7	4258.2	95.1 %	6.76	0.0711
XQ6	114,367	4878	3809.8	5798.9	97.4 %	9.44	0.0095
XQ7	80,290	4344	3646.5	5199.8	96.3 %	9.65	0.0042
XQ8	19,117	2697	2858.2	5621.1	89.8 %	9.89	0.0030
XQ9	36,846	3445	3549.2	5706.4	92.8 %	9.60	0.0067
XQ10	91,437	3975	3279.5	5143.3	97.1 %	8.83	0.0095

Chao 1 - community richness. A higher number represents more richness

ACE - community richness. A higher number represents more richness

Coverage - sampling depth

Shannon - community diversity. A higher number represents more diversity

Simpson - community diversity. A higher number represents less diversity

Abundance profiles for phylum and classes

The phylogenetic classification of bacterial 16S rRNA sequences from 10 sediment samples is shown in Fig. 3 based on phylum and in Table S3 based on classes. Figure 3 showed that the most abundant and largest phylum was *Proteobacteria* with 59.42 % of the sequences on average (minimum 50.4 % in XQ1 and maximum 85.1 % in XQ5). Previous studies have shown that *Proteobacteria* is the prominent phylum in sediment from a drinking water reservoir (Roeske et al. 2012) and intertidal zones of Bohai Bay (Wang et al. 2013a), industrial wastewaters from steel

industrial (Ma et al. 2015), pharmaceutical, petroleum refinery and sewage (Ibarbalz et al. 2013), soils of livestock-cotton production (Acosta-Martinez et al. 2010), and CO₂ gas vent (Saenz de Miera et al. 2014). Moreover, the abundance of *Proteobacteria* was higher when environmental conditions were aggravated, such as an increasing abundance of *Proteobacteria* in drinking water after chlorine disinfection (Huang et al. 2014) and river sediment after huge nitrate injection (Chen et al. 2013). Consistent with previous studies, *Proteobacteria* occupied an obviously higher percentage in sample XQ5 (85.1 %) with serious pollution of PFOA than that in other samples with a range from 50.4 to 68.6 %. The

Fig. 3 Bacterial community compositions at phylum level in each sample

following major phyla were *Bacteroidetes* (2.5–18.7 %), *Gemmatimonadetes* (1.9–11.1 %), *Acidobacteria* (2.0–12.0 %), *Verrucomicrobia* (2.3–8.0 %), and *Chloroflexi* (0.8–5.2 %).

At the class level, distinctly higher relative abundances of sequences affiliated with the classes of *Flavobacteria* and α -*Proteobacteria* were observed (15.8 and 13.9 %) in sample XQ10 (Table S3). A large number of studies have shown that *Flavobacteria* is abundant in the sea (Abell and Bowman 2005; Zamora et al. 2013), that is consistent with the position of XQ10 located in the estuary (Fig. 1).

Among all freshwater sediment samples, there was considerable consistency between bacterial communities except at site XQ5. For all freshwater sediment samples except XQ5, β -*Proteobacteria* was the most prevalent class with relative abundance in samples ranging from 17.1 to 31.9 % (Table S3). The second and third prominent classes in all freshwater sediment but XQ5 were γ -*Proteobacteria* and δ -*Proteobacteria* with relative abundance ranging from 15.7 to 24.5 % and 6.0 to 10.9 %. Except for three subdivisions of *Proteobacteria*, other major classes contained *Gemmatimonadetes-1* (2.6 to 8.2 %), α -*Proteobacteria* (1.9 to 5.8 %), *Sphingobacteria* (1.4 to 3.3 %), and *Pedospirae* (1.1 to 4.1 %). As for XQ5, β -*Proteobacteria* (39.1 %) was still the largest class, while the second and third prominent classes were ε -*Proteobacteria* (23.4 %) and γ -*Proteobacteria* (16.4 %). ε -*Proteobacteria*, as the second major class in sample XQ5, was obviously higher than that in other freshwater sediment samples with a range from 0.6 to 1.7 %. This is different from the results of microbial community in freshwater sediments (Roeske et al. 2012; Wang et al. 2012), which showed that β -, δ -, and γ -*Proteobacteria* were the major abundant types of *Proteobacteria*. Other major classes in sample XQ5 contained δ -*Proteobacteria* (3.7 %), α -*Proteobacteria* (2.2 %), *Verrucomicrobiae* (1.4 %), and *Gemmatimonadetes-1* (1.3 %). These findings suggest that elevated PFAAs in sediment cause a shift in the bacterial community towards ε -*Proteobacteria*.

Identification of the core genera

A total of 455 genera were identified in 10 sediment samples. Only 94 genera were shared by all samples, whereas these genera represented 92.5 % of the total identified sequences. Percentage of the most abundant genus in each sample is shown in Table 3. In sample XQ10, the dominant genera were *Lutimonas*, *Marinobacter*, *Aequorivita*, *Luteolibacter*, and *Shewanella* with 4.8, 3.2, 2.1, 1.6, and 1.1 %, respectively, which were all reported as marine bacteria. *Lutimonas*, as the core genus, was known to be an important member of the bacterial community in many aquatic environments and has

a special role in using high molecular mass dissolved organic matter (Kirchman 2002). *Thiobacillus* (22.9 %), *Sulfurimonas* (21.9 %), and *Dok59* (1.6 %) were the dominant genus and represented half of the sequences at XQ5 (Table 3), while the percentage of genera was very low (almost all <0.6 %). In other freshwater sediment samples, the dominant genera were similar, mainly including *Thiobacillus* (1.5–6.8 %), *Dok59* (1.2–4.4 %), and *Candidatus solibacter* (0.8–1.3 %). Although *Thiobacillus* was the dominant genus in all freshwater sediment samples, it was more prevalent in the sediment at XQ5 (Table 3), while it was only up to 1.5–6.8 % of the community at the other sites.

Thiobacillus is a genus found in water, air, soil (Vidyalakshmi et al. 2009), sewage pipelines (Roberts et al. 2002), and even degraded concrete structure (Okabe et al. 2007). Most species of *Thiobacillus* use ammonium salt as nitrogen source (Allegretti et al. 2006; Nikolov et al. 2002; Yousefi et al. 2014), and some of them are capable of denitrification that can reduce the content of nitrogen in the soil nutrition (Torrentó et al. 2010). Based on the reported functional role of *Thiobacillus* in soil, the lower total nitrogen recorded at XQ5 may be potentially due to metabolic activity of this genus. The higher prevalence of *Thiobacillus* and *Sulfurimonas* at XQ5 suggests that the growth of *Thiobacillus* and *Sulfurimonas* may be promoted under certain PFOA concentrations, or the two bacteria may have resistance to high concentration of PFOA, which needs to be further explored.

Relationship between environmental factors and community structure

After Spearman correlation analysis among environmental factors (Table S4), and RDA screening between environmental factors and bacterial community, three environmental factors (TOC, salinity, and PFOA) were displayed as the important factors for affecting bacterial community structure. Then, RDA analysis (Gao et al. 2013) was conducted to explore the possible correlation between bacterial community structure and major environmental variables. Three environmental variables were all significant according to the permutation test analysis ($p < 0.05$). The length of the arrows in the RDA plot corresponds with the strength of the correlation between variables and community structure.

As shown in Fig. 4, the first and second canonical axes represented 47.2 and 24.4 % of variation, respectively. RDA analysis at the genus level showed that the bacterial communities in 10 sediment samples were clustered into 3 distinct groups including group I (XQ5), group II (XQ10), and group III (sample points except XQ5 and XQ10). XQ5 and XQ10 were separated from others, suggesting the clear distinct structures of microbial community among three groups. Group I (XQ5), with the highest concentrations of PFOA (456.2 ng/g

Table 3 Percentage of the most abundant genus in each sample (%)

	XQ1	XQ2	XQ3	XQ4	XQ5	XQ6	XQ7	XQ8	XQ9	XQ10
<i>Thiobacillus</i>	1.53	2.22	6.84	4.52	22.91	4.40	2.27	2.41	6.49	1.07
<i>Sulfurimonas</i>	0.42	0.61	0.88	1.00	21.10	0.43	0.34	0.40	0.75	0.28
<i>Dok59</i>	2.30	2.36	3.39	1.89	1.58	4.44	1.97	1.14	1.72	0.72
<i>Lutimonas</i>	0.04	0.17	0.09	0.13	0.11	0.30	0.36	0.26	0.18	4.81
<i>Marinobacter</i>	0.16	0.24	0.16	0.16	0.14	0.46	0.51	0.38	0.42	3.16
<i>Aequorivita</i>	0.04	0.08	0.07	0.03	0.05	0.14	0.17	0.14	0.07	2.13
<i>Rubrivivax</i>	0.22	0.32	0.23	0.38	0.34	0.42	2.00	0.44	0.49	0.26
<i>Luteolibacter</i>	0.40	1.72	0.86	0.20	0.15	0.69	0.65	0.42	0.31	1.58
<i>Candidatus Solibacter</i>	1.04	0.83	0.87	0.77	0.31	0.85	0.79	1.40	1.00	0.33
<i>Sulfuritalea</i>	0.78	0.96	1.41	1.10	0.56	0.73	0.43	0.29	0.49	0.19

dw) (Fig. 2), had the lowest diversity, with a Shannon index of 6.76 and a Simpson index of 0.071. At the site of XQ5, PFOA was the most important environmental factor affecting the bacterial community structure based on RDA analysis. Group II (XQ10) was located in the estuary of Xiaoqing River, while other samples were from freshwater, so its bacterial community was mainly affected by salinity. Other sites clustered together forming group III, the community structure at sites in group III was mainly effected by TOC and there was no significant effect by PFOA based on RDA analysis. These findings suggest that PFOA concentrations below 100 ng/g do

not result in the impact on the microbial community structure. The grouping pattern indicated that PFOA played an important role in community formation, and high concentration of PFOA could reduce the diversity of bacterial community. When the concentration of PFOA was below 100 ng/g, no significant effect on microbial community structure was observed.

Triplot analysis suggested that the dominant genera of *Sulfurimonas* and *Thiobacillus* confined to XQ5 were positively correlated with the concentration of PFOA, while the genera of *C. solibacter*, *Sulfuritalea*, *Dok59*, and *Luteolibacter* had negative correlation with PFOA concentration. It assumed that the species of *Thiobacillus* and *Sulfurimonas* might be the PFOA contamination indicators or resistant strain in a certain concentration range of PFOA, which needs to be further studied. *Lutimonas*, *Marinobacter*, and *Aequorivita* with high correlation with XQ10 play key roles in marine environment. In the present study, only a single sampling point was investigated. It could be speculated that the bacterial community in sediment also shifted seasonally, and further investigations are needed to reveal the relationship between temporal dynamics of the community and concentrations of PFAAs.

Conclusion

According to our current knowledge, the relationship between bacterial community compositions in sediment and PFOA pollution evaluated by Illumina high-throughput sequencing and high concentration of PFOA (456.20 ng/g dw) can reduce the diversity of bacterial community. While the concentration of PFOA is below 100 ng/g, no significant effect on microbial community structure is observed. *Proteobacteria* is the dominant phylum in all samples, while the abundance of *Proteobacteria* (85.1 %) in sample XQ5 is significantly higher when compared with other 9 samples with the abundance of class of ϵ -*Proteobacteria* (23.4 %), genera of *Thiobacillus*

Fig. 4 Redundancy analysis triplot to explore the correlation between sediment properties and bacterial community based on genus level structure

(22.9 %), and *Sulfurimonas* (21.1 %). *Thiobacillus* and *Sulfurimonas* are positively correlated with the concentration of PFOA, so it has the potential to be the resistant strain for PFOA contamination. The profound understanding of bacterial communities in Xiaoqing River will be helpful to understand the risk of PFAAs to ecosystem.

Acknowledgments This study was supported by the National Natural Science Foundation of China (nos. 41571478 and 41171394), the Key Research Program of the Chinese Academy of Sciences (no. KZZD-EW-TZ-12), and the National Fundamental Field Study Program (no. 2013FY111110).

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