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ORIGINAL ARTICLE

Vertical profiles of microbial communities in perfluoroalkyl substance-contaminated soils

Yixiang Bao[1] · Bingxin Li[2] · Shuguang Xie[2] · Jun Huang[1]

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Abstract

Poly- and perfluoroalkyl compounds (PFASs) are ubiquitous in the environment, but their influences on microbial community remain poorly known. The present study investigated the depth-related changes of archaeal and bacterial communities in PFAS-contaminated soils. The abundance and structure of microbial community were characterized using quantitative PCR and high-throughput sequencing, respectively. Microbial abundance changed considerably with soil depth. The richness and diversity of both bacterial and archaeal communities increased with soil depth. At each depth, bacterial community was more abundant and had higher richness and diversity than archaeal community. The structure of either bacterial or archaeal community displayed distinct vertical variations. Moreover, a higher content of perfluorooctane sulfonate (PFOS) could have a negative impact on bacterial richness and diversity. The rise of soil organic carbon content could increase bacterial abundance but lower the richness and diversity of both bacterial and archaeal communities. In addition, *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, and *Acidobacteria* were the major bacterial groups, while *Thaumarchaeota*, *Euryarchaeota*, and unclassified *Archaea* dominated in soil archaeal communities. PFASs could influence soil microbial community.

Keywords Emerging pollutants · Microbial community · Soil depth · Organic carbon

Introduction

Due to their high chemical and thermal stability, poly- and perfluoroalkyl compounds (PFASs) have yielded wide application in commercial products. They are widely distributed in various aquatic and soil environments (Gottschall et al. 2017; Munoz et al. 2015, 2016; Rankin et al. 2016; Shan et al. 2014; Wang et al. 2016a). The ubiquity of PFASs in the environment has raised increasing concerns, due to their environmental persistency, bioaccumulation in food chain, and toxicity to invertebrates, animals, and plants (Shan et al. 2014; Sun et al. 2016). Both soil and aquatic microbial communities can be shaped by a variety of environmental factors (Li et al. 2017a; Chen et al. 2016; Ni et al. 2016), yet information on the effect of PFASs on microbial community in natural environment is still limited. Two previous studies documented that the diversity and composition of sediment bacterial community could be influenced by perfluorooctanoic acid (PFOA) (Sun et al. 2016) and 6:2 fluorotelomer alcohol (6:2 FTOH) (Zhang et al. 2017). Li et al. (2017b) pointed out that the abundance and richness of both bacterial and archaeal communities in soil were correlated with perfluorohexane sulfonate (PFHxS) content, and the impact of PFASs on microbial community might be related to the type of PFASs.

Although the distinct change of bacterial community with soil depth has been well-documented (Douterelo et al. 2010; Kim et al. 2016; Liu et al. 2015; Ma et al. 2013; Sagova-Mareckova et al. 2016; Wang et al. 2014, 2016b, 2017), the vertical change of soil archaeal community remains poorly known. In addition, there is still a paucity of knowledge on the vertical changes of soil microbial community in PFAS-contaminated site. Therefore, the objective of the present study was to investigate the vertical changes of archaeal and bacterial communities in PFAS-contaminated soils. The

✉ Shuguang Xie
xiesg@pku.edu.cn

✉ Jun Huang
huangjun@tsinghua.edu.cn

[1] School of Environment, Beijing Key Laboratory for Emerging Organic Contaminants Control (BKLEOC), State Key Joint Laboratory of Environment Simulation and Pollution Control (SKLESPC), Tsinghua University, Beijing 100084, China

[2] State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China

possible relationships between soil microbial communities and the mainly detected PFASs were also explored.

Materials and methods

Soil chemical properties

Soil samples in triplicate at five depths (0.2, 1, 5, 10, and 30 m) were collected through well drilling at a site (116° 23′ 0.16″ E, 39° 29′ 59.12″ N) with a long exposure (more than 10 years) to heavy PFAS pollution in Beijing (China). These soil samples were kept in iceboxes and transported back to the laboratory in 2 h after collection. The soils at these five depths were characterized as clay, sandy clay, sandy silt, fine sand, and coarse sand, respectively. The concentrations of soil PFASs were extracted and analyzed according to our previous study (Li et al. 2017b). In this study, the detected PFASs mainly included perfluorooctane sulfonate (PFOS) (7.7–1167 μg/kg), chlorinated polyfluorinated ether sulfonate (F-53B, $C_8ClF_{16}O_4SK$) (0–10.8 μg/kg), PFHxS (0.26–13.1 μg/kg), and perfluorobutane sulfonate (PFBS) (0.05–0.31 μg/kg) (Table 1). Soil organic carbon (OC) was determined using the potassium dichromate oxidation spectrophotometric method. OC content in soils ranged between 0.47 and 1.48%.

Molecular analyses

Genomic DNA of each soil sample (0.5 g) was extracted using PowerSoil DNA extraction kit (Mo Bio Laboratories, USA). Soil DNA quality and quantity were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA). In this study, primer sets 341F (5′-CCTACGGGAGGCAGCAG-3′)/534R (5′-ATTACCGCGGCTGCTGGCA-3′) (Jung et al. 2011) and Arch344F (5′-GYGCAGCAGGCGCGA-3′)/Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Casamayor et al. 2002) respectively were used for quantitative PCR (q-PCR) assay of bacterial and archaeal abundance, following the amplification conditions described in the literature (Liu et al. 2016). q-PCR assay was performed for each replicate soil DNA sample.

For Illumina MiSeq high-throughput sequencing of soil bacterial and archaeal communities, genomic DNA was amplified using the primer pairs 515F (5′-GTGCCAGCMGCCGCGG-3′)/907R (5′-CCGTCAATTCMTTTRAGTTT-3′) and Arch519F (5′-CAGCCGCCGCGGTAA-3′)/Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′), respectively (He et al. 2016). The amplicons from replicate soil DNA samples were pooled in the same amounts to perform sequencing using the Illumina® HiSeq 2000 system. The obtained raw bacterial and archaeal reads were deposited in NCBI short-read archive with accession numbers SRP091028 and SRP091024, respectively. Raw paired-end reads were merged using FLASH.

Quality filtering of reads was conducted with Qiime (Caporaso et al. 2010) and chimeric sequences were detected and deleted using UCHIME (Edgar et al. 2011). Operational taxonomic units (OTUs) were assigned by UPARSE (Edgar 2013) based on 97% sequence similarity. The OTUs only with one sequence (singleton) were removed for further analysis. Alpha-diversity metrics (Chao1 richness and Shannon index) were calculated using UPARSE (Edgar 2013). Representative OTU sequences were taxonomically classified using the Silva database (Quast et al. 2013). Unweighted UniFrac was calculated to identify the difference in microbial community composition among samples and then hierarchical clustering analysis was performed based on unweighted pair group method with arithmetic mean (UPGMA) using the *R* software (version i386, 3.3.0).

Statistical analysis

One-way analysis of variance (ANOVA) was used to test for significant difference ($p < 0.05$) in quantitative PCR assays. Spearman rank correlation analysis using the software SPSS 20.0 was applied to explore the links of soil chemical parameters with the abundance, richness, and diversity of microbial community. In addition, using the software CANOCO 4.5, redundancy analysis (RDA) with Monte Carlo tests was performed to identify the correlations of microbial community composition with soil chemical properties. The number of sequence in each major microbial OTU (with a threshold of 50 sequences) was assigned as species input, and soil chemical property was put as environmental input (Zhang et al. 2015).

Table 1 Soil chemical characteristics

Sample	Depth (m)	PFBS (μg/kg)	PFHxS (μg/kg)	PFOS (μg/kg)	F53B (μg/kg)	OC (%)
T1	0.2	0.16	13.1	1167	7.5	1.48
T2	1	0.23	0.22	43.2	10.5	0.8
T3	5	0.25	0.17	32.7	10.8	1.36
T4	10	0.31	0.18	7.7	4	0.53
T5	30	0.05	0.26	18.3	0	0.47

Results

Microbial abundance

In the present study, the number of bacterial 16S rRNA gene in soils were 4.65×10^8–8.57×10^9 copies per gram dry soil (Fig. 1a), while archaeal 16S rRNA gene ranged from 4.22×10^7 to 5.27×10^8 copies per gram dry soil (Fig. 1b). The soils at five depths illustrated the significant difference in both bacterial and archaeal abundance ($p < 0.05$). Soil at 0.2 m depth had the highest bacterial abundance, followed by soils at 5 and 1 m depths. Soil at 5 m depth displayed the highest archaeal abundance, and soil at 1 m depth showed significantly higher archaeal abundance than soils at 0.2, 10, and 30 m depths ($p < 0.05$). In addition, at each sampling depth, *Bacteria* were more abundant than *Archaea* (8–186:1). Spearman rank correlation analysis indicated that soil OC content was positively correlated with bacterial abundance ($p < 0.01$), while each of PFBS, PFHxS, and F53B illustrated no significant correlation with either bacterial or archaeal abundance ($p > 0.05$) (Table 2).

Microbial richness and diversity

In the present study, OTU table was normalized to the identical sequencing depth (with 35,900 sequences) for the comparison of soil microbial richness and diversity. Each soil bacterial library comprised of 613–1872 OTUs, while each soil archaeal library was composed of 287–744 OTUs (Table 3). The values of bacterial and archaeal Chao1 richness estimators and Shannon diversity index were 1047–2408 and 3.2–

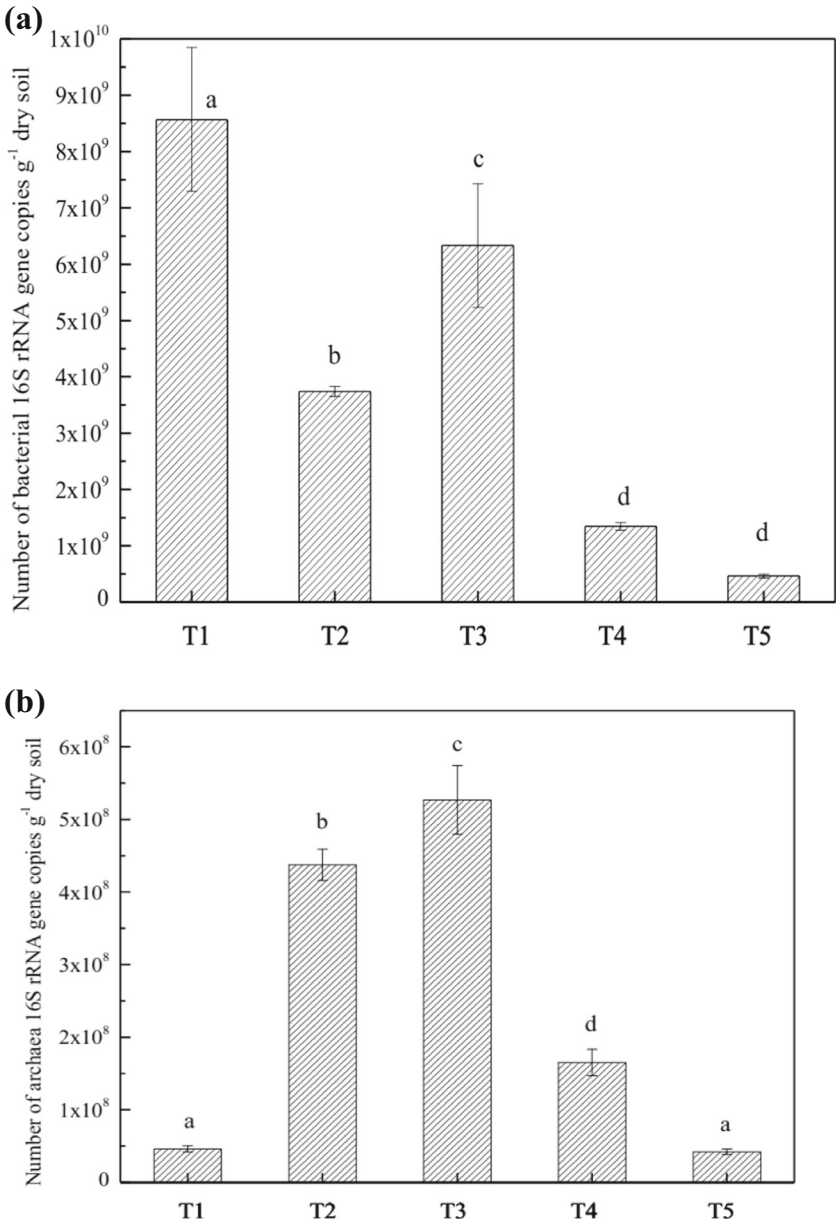

Fig. 1 The number of bacterial (**a**) and archaeal (**b**) 16S rRNA genes in soil samples. Different letters above the columns indicate the significant differences in gene abundance ($p < 0.05$)

Table 2 Spearman rank correlation analysis of soil chemical parameters with the abundance, richness, and diversity of bacterial and archaeal communities

	Abundance		Richness		Diversity	
	Bacteria	*Archaea*	*Bacteria*	*Archaea*	*Bacteria*	*Archaea*
PFBS	0.1	0.7	0	− 0.1	0	− 0.1
PFHxS	0.1	− 0.8	− 0.3	− 0.1	− 0.3	− 0.1
PFOS	0.8	0.1	− 0.9*	− 0.8	− 0.9*	− 0.8
F53B	0.4	0.8	− 0.2	− 0.4	− 0.2	− 0.4
OC	1.0**	0.4	− 0.9*	− 1.0**	− 0.9*	− 1.0**

*Correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level

6.25, and 451–836 and 3.09–3.84, respectively. For both bacterial and archaeal communities, OTUs, Chao1 richness, and Shannon diversity increased with soil depth. In addition, at each sampling depth, *Bacteria* had more OTUs and higher richness and diversity than *Archaea*. Spearman rank correlation analysis indicated that PFOS had significant negative correlations with bacterial community richness and diversity ($p < 0.05$). Soil OC content showed negative correlations with the richness and diversity of bacterial and archaeal communities ($p < 0.05$ or $p < 0.01$).

Microbial community structure

UPGMA clustering analysis illustrated that either bacterial or archaeal community in five soils could be divided into three distinct clades (Fig. 2a, b). Soil at 0.2 m depth was clearly separated from other soils. Soils at 1 and 5 m depths were clustered together, while soils at 10 and 30 m depths formed another group. The first two RDA dimensions totally represented a large amount (88.5%) of the cumulative variance of total bacterial communities (Fig. 3a). However, neither PFASs nor OC significantly contributed to the total soil bacterial assemblage–environment relationship. Moreover, the first and second RDA dimensions respectively explained 56.9 and 31.2% of variance in total soil archaeal communities (Fig. 3b). Only F53B ($F = 3.540$, $p = 0.011$, 999 Monte Carlo permutations) significantly contributed to the total soil archaeal assemblage–environment relationship.

Table 3 Soil microbial community richness and diversity

Sample	OTU		Chao1 richness		Shannon index	
	Bacteria	*Archaea*	*Bacteria*	*Archaea*	*Bacteria*	*Archaea*
T1	613	287	1047	451	3.2	3.09
T2	1548	458	1877	648	5.94	3.29
T3	1507	474	1922	683	6.06	3.32
T4	1655	562	2231	765	6.11	3.48
T5	1872	744	2408	836	6.25	3.84

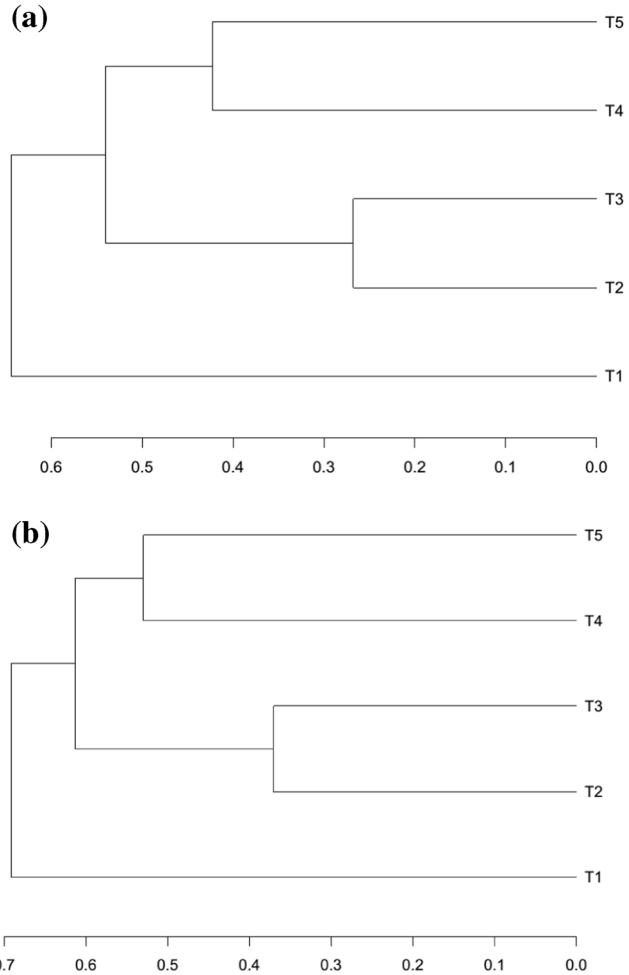

Fig. 2 UPGMA clustering of bacterial (**a**) and archaeal (**b**) assemblages based on UniFrac distance

In the current study, soil bacterial communities were mainly composed of *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, and *Acidobacteria* (Fig. 4). These organisms totally accounted for 81.3–96.8% in soil bacterial communities. *Firmicutes*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes*, and other minor bacterial groups were also detected. Moreover, the relative abundance of each major bacterial phylum illustrated a considerable vertical variation. Proteobacterial organisms (mainly comprising of *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*) predominated in soil at 0.2 m depth (accounting for 75.2%) (Fig. 5), but they became much less abundant in other four soils (17.5–23.3%). The proportions of *Proteobacteria* as well as its major classes tended to decrease with soil depth. The proportion of *Actinobacteria* organisms in soil at 10 m depth (29.7%) was higher than that in other soils (16.1–23.6%). The proportion of *Chloroflexi* organisms increased with soil depth, and they were dominant in soil at 30 m depth (41.5%). *Cyanobacteria* organisms were much abundant in soils at 1 and

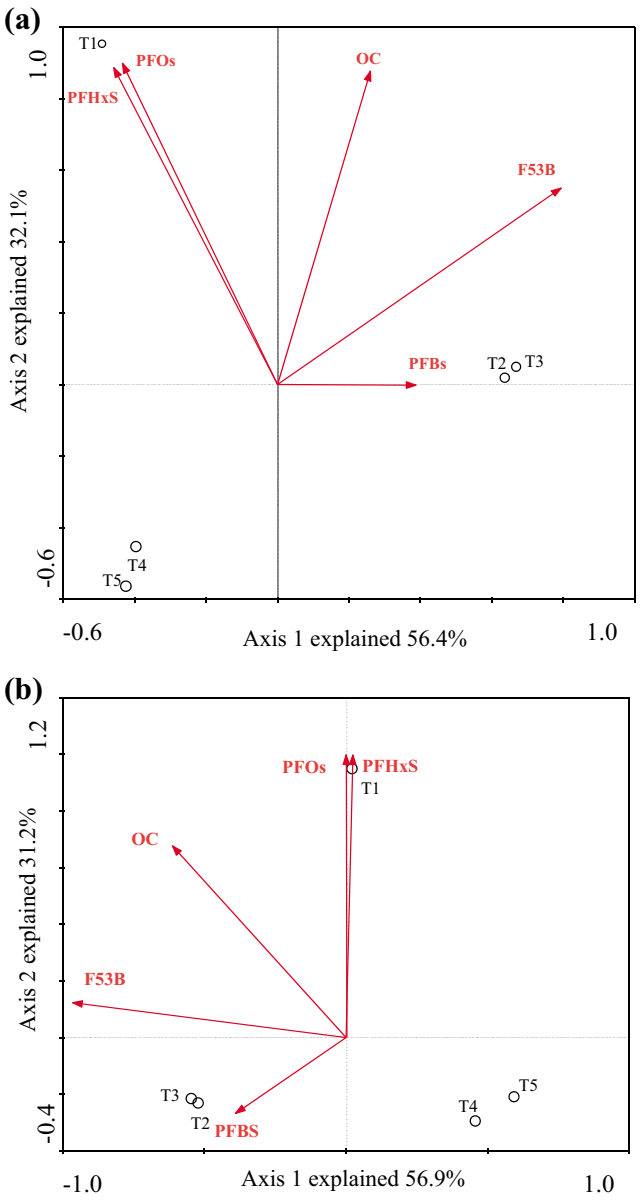

Fig. 3 RDA ordination plot for the first two principal dimensions of the relationship between bacterial (**a**) and archaeal (**b**) OTU composition and soil environmental factors

5 m depths (26.6 or 18.1%), but became a minor bacterial group in other soils (0–1.8%). In addition, *Acidobacteria* was much less abundant in soil at 0.2 m depth (0.8%) than in other soils (6.1–8.9%).

In this study, soil archaeal communities mainly included *Thaumarchaeota*, *Euryarchaeota*, and unclassified *Archaea* (Fig. 6). They comprised of 97.8–100% in soil archaeal communities. The proportion of each major archaeal groups illustrated a considerable change with soil depth. *Thaumarchaeota* organisms (mainly class *Soil_Crenarchaeotic_Group* (*SCG*)) (Fig. 7) were much less abundant in soil at 0.2 m depth (19.9%) than in other soils (42.7–66.1%). *Euryarchaeota* (mainly class *Thermoplasmata*) showed a much higher proportion in soil at 10 m depth (15.2%) than in other soils (0–4.9%).

Spearman rank correlation analysis indicated that the levels of PFBS, PFHxS, and F53B in soils were significantly correlated with the proportions of *Actinobacteria*, *Acidobacteria*, and *Alphaproteobacteria*, respectively ($p < 0.05$ or $p < 0.01$) (Table 4). The proportions of *Chloroflexi* and *Thermoplasmata* (*Euryarchaeota*) had significant correlations with PFOS and OC ($p < 0.05$). Moreover, the proportions of *Betaproteobacteria* and *Proteobacteria* were significantly correlated with the levels of PFOS and OC, respectively ($p < 0.01$).

Discussion

Vertical change of soil microbial abundance

It has been well-documented that bacterial abundance can considerably change with soil depth (Biro et al. 2014; Liu et al. 2015; Ma et al. 2013; Wang et al. 2014, 2016b). These previous studies indicated that bacterial abundance declined with soil depth. To date, little is known about the vertical change of soil bacterial abundance in PFAS-contaminated site. Only our recent study documented the remarkable change of bacterial abundance with soil depth at a chromium- and PFAS-contaminated site (Li et al. 2017b). In this study, at a site with a long exposure to heavy PFAS pollution, the highest bacterial abundance occurred in top soil (0.2 m depth). Soil bacterial abundance did not continuously decrease with soil depth, and soil at 5 m depth had higher bacterial abundance than soil at 1 m depth. This result was not in agreement with previous studies (Biro et al. 2014; Liu et al. 2015; Ma et al. 2013; Wang et al. 2014, 2016b). Although our previous study suggested that PFHxS might influence soil bacterial abundance (Li et al. 2017b), in this study, the links between bacterial abundance and PFASs were still unclear. However, the result of Spearman rank correlation analysis suggested that soil OC content might be a key determinant to bacterial abundance, which was consistent with the previous studies (Barrett et al. 2016; Ma et al. 2013). The decrease in soil carbon availability with depth might account for the depth-related decrease of bacterial abundance (Barrett et al. 2016).

Several previous studies indicated that soil depth negatively affected archaeal abundance (Barrett et al. 2016; Cao et al. 2012). Our recent study also reported the considerable change of archaeal abundance with soil depth at a chromium- and PFAS-contaminated site (Li et al. 2017b). In this study, with soil depth, archaeal abundance displayed a considerable increase followed by a considerable decline. This did not coincide with the previous studies (Barrett et al. 2016; Cao et al. 2012). In addition, our previous study suggested that PFHxS might determine soil archaeal abundance (Li et al. 2017b),

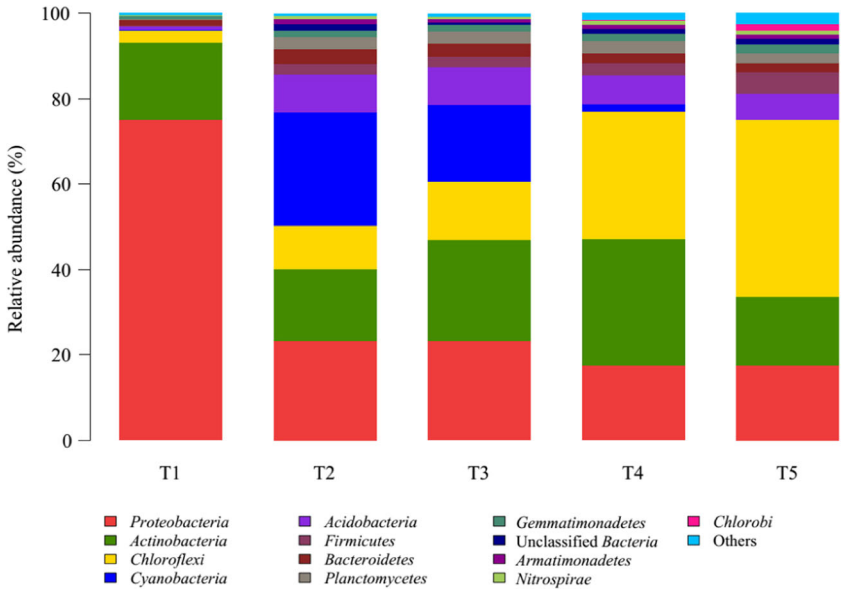

Fig. 4 Comparison of the quantitative contribution of the sequences affiliated with different bacterial phyla to the total number of bacterial sequences from soil samples. Others include the bacterial phyla with the largest relative abundance less than 1% in each sample

whereas no clear link of archaeal abundance with PFASs was not identified in the present study.

In this study, soil types were different at the different depths, which could affect DNA extraction efficiency. Soils at 0.2 and 1 m depths were characterized as clay and sandy clay, respectively. Clay could bind DNA and thus result in the lower soil microbial abundance. Hence, the number of bacterial and archaeal 16S rRNA gene copies could be underestimated. The DNA extraction efficiencies of soils at the different depths deserved further investigation.

Vertical change of soil microbial richness and diversity

Numerous previous studies indicated that bacterial diversity could decline with soil depth (Douterelo et al. 2010; Eilers et al. 2012; Wang et al. 2014, 2017). Several previous studies also reported that bacterial richness decreased with soil depth (Ma et al. 2013; Wang et al. 2017). Our previous study found that, at a chromium- and PFAS-contaminated site, soils at 0.5–4 m depths had higher richness than those at 4.5–12.5 m depths, while there was no obvious trend for the change of bacterial diversity with soil depth (Li et al. 2017b). However,

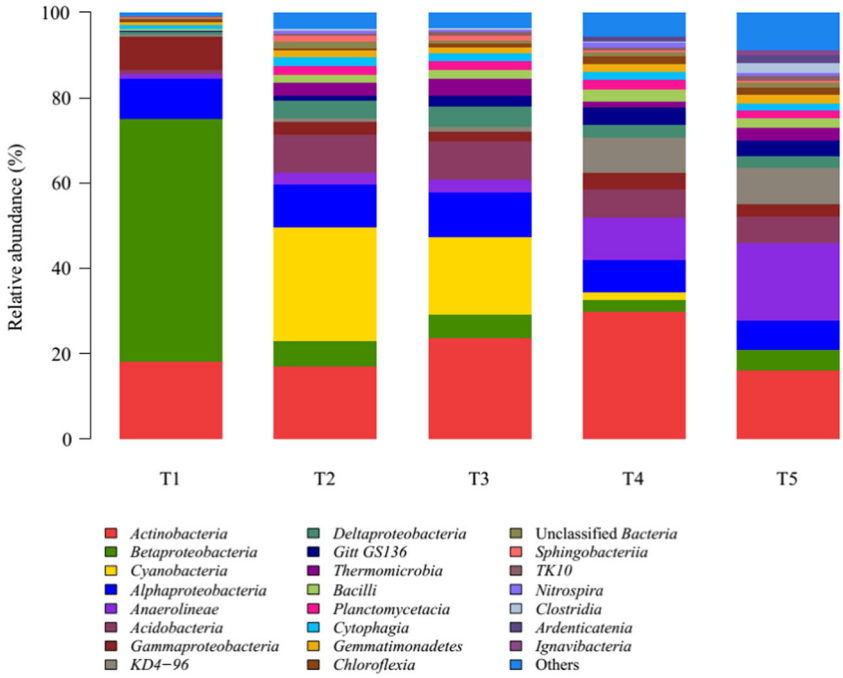

Fig. 5 Comparison of the quantitative contribution of the sequences affiliated with different bacterial classes to the total number of bacterial sequences from soil samples. Others include the bacterial classes with the largest relative abundance less than 1% in each sample

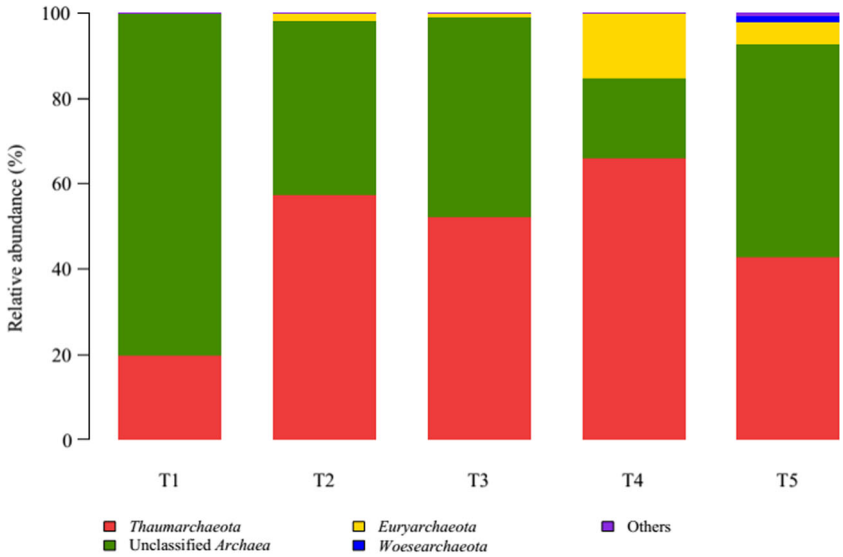

Fig. 6 Comparison of the quantitative contribution of the sequences affiliated with different archaeal phyla to the total number of archaeal sequences from soil samples. Others include the archaeal phyla with the largest relative abundance less than 1% in each sample

in this study, both bacterial richness and diversity were found to increase with soil depth at PFAS-contaminated site. This was not consistent with the results reported in previous studies (Douterelo et al. 2010; Eilers et al. 2012; Li et al. 2017b; Ma et al. 2013; Wang et al. 2014, 2017). So far, little is known about the relations between PFASs and bacterial richness and diversity. Our previous study suggested that soil bacterial richness might be positively influenced by PFHxS (Li et al. 2017b), while Sun et al. (2016) and Zhang et al. (2017) indicted that PFOA and 6:2 FTOH at higher concentration lowered sediment bacterial diversity. In this study, PFOS was found to have negative influence soil bacterial richness and diversity. To the authors' knowledge, this was the first report on the possible relation of PFOS with bacterial richness and diversity. In addition, in the present study, the result of Spearman rank correlation analysis suggested that soil OC content might also be a key determinant to bacterial richness and diversity, which was in harmony with previous studies (Ma et al. 2013; Naveed et al. 2016).

To date, the vertical changes of archaeal richness and diversity remain poorly known. Deng et al. (2015) found the decrease of archaeal richness with soil depth. Soils at 0.5–4 m depth displayed higher richness than those at 4.5–12.5 m depth at a chromium- and PFAS-contaminated site, while no trend was detected in the change of archaeal diversity with soil depth (Li et al. 2017b). In this study, both archaeal richness and diversity were found to increase with soil depth at PFAS-contaminated site. PFHxS was found to be a possible factor influencing soil archaeal richness (Li et al. 2017b), whereas the clear correlations of archaeal richness and diversity with

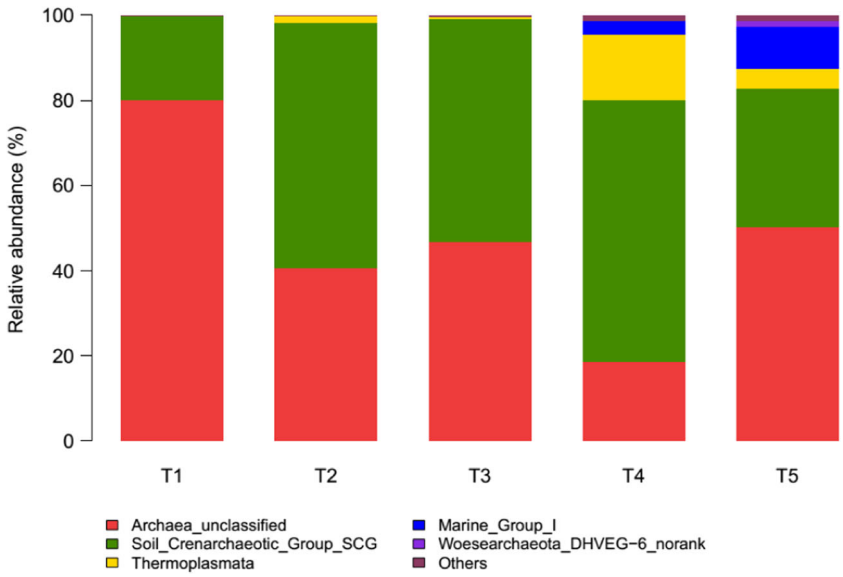

Fig. 7 Comparison of the quantitative contribution of the sequences affiliated with different archaeal classes to the total number of archaeal sequences from soil samples. Others include the archaeal classes with the largest relative abundance less than 1% in each sample

Table 4 Spearman rank correlation analysis of soil chemical parameters with the major bacterial and archaeal phyla and classes

	PFBS	PFHxS	PFOS	F53B	OC
Acidobacteria	0.6	−0.9*	−0.2	0.8	0
Actinobacteria	0.9*	−0.6	−0.3	−0.4	0.3
Cyanobacteria	0.5	−0.7	−0.1	0.6	−0.1
Chloroflexi	0	−0.3	−0.9*	−0.2	−0.9*
Proteobacteria	0.1	0.1	0.8	0.4	1.0**
Alphaproteobacteria	0.4	−0.5	0.5	1.0**	0.7
Betaproteobacteria	−0.4	0.5	1.0**	0.2	0.8
Gammaproteobacteria	−0.2	0.7	0.1	−0.8	0.1
Thaumarchaeota	0.8	−0.7	−0.6	−0.4	−0.4
Euryarchaeota	0.3	−0.3	−0.9*	−0.4	−0.9*
SCG	0.8	−0.7	−0.6	−0.4	−0.4
Thermoplasmata	0.3	−0.3	−0.9*	−0.4	−0.9*

*Correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level

PFASs were not identified in this current study. So far, the links of archaeal richness and diversity with soil OC remains unclear. A recent study suggested that soil OC played an important role in determining archaeal diversity (Dominguez et al. 2017). In this study, the result of Spearman rank correlation analysis further sustained that soil OC might be a key driver for both richness and diversity of archaeal community.

Vertical change of soil microbial structure

A number of previous studies have revealed the distinct variation of bacterial community structure with soil depth (Hu et al. 2015; Kim et al. 2016; Ma et al. 2013; Sagova-Mareckova et al. 2016; Watanabe et al. 2010). In this study, the results of both UPGMA clustering analysis and phylogenetic analysis further provided the evidence for the vertical change of soil bacterial community structure at PFAS-contaminated site. The proportion of proteobacterial organisms tended to decrease with soil depth. Our recent study also revealed the depth-related change of total bacterial community structure in chromium- and PFAS-contaminated soils (Li et al. 2017b). Sun et al. (2016) suggested that PFOA might play an important role in shaping river sediment bacterial community. Moreover, 6:2 FTOH could also considerably affect total sediment bacterial community structure (Zhang et al. 2017). In this study, although the results of RDA indicated that PFASs had a clear link with the total soil bacterial community structure, the result of Spearman rank correlation analysis suggested that the proportions of *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* were regulated by the levels of PFHxS, PFBS, and PFOS, respectively. The proportions of *Alphaproteobacteria* and *Betaproteobacteria* were governed by the levels of F53B and PFOS, respectively.

It remains unclear that whether or not archaeal community structure changes with soil depth. A few previous studies reported the distinct variation of archaeal community structure with soil depth (Eilers et al. 2012; Lee et al. 2015; Watanabe et al. 2010), while Kaurin et al. (2015) indicated that the composition of archaeal community was not affected by soil depth. In this study, the results of both UPGMA clustering analysis and phylogenetic analysis showed a considerable vertical shift in soil archaeal community. The shift in soil archaeal community with soil depth was also reported in chromium- and PFAS-contaminated soils (Li et al. 2017b). Moreover, the result of RDA indicated that F53B might play an important role in shaping total archaeal community structure. The result of Spearman rank correlation analysis further indicated that the proportion of *Thermoplasmata* (*Euryarchaeota*) was closely correlated with PFOS. To the authors' knowledge, this was the first report on the possible influence of PFASs on archaeal community structure.

In conclusion, the abundance, richness, diversity, and structure of both bacterial and archaeal communities illustrated considerable changes among different soil depths. PFASs could influence both bacterial and archaeal communities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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