



## Vertical distribution of microbial communities in soils contaminated by chromium and perfluoroalkyl substances



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### HIGHLIGHTS

- The rise of Cr and PFHxS level increase microbial abundance and richness.
- Cr determines soil microbial community structure.
- Remarkable vertical changes of microbial communities occurs in Cr- and PFASs- contaminated soils.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Both *Bacteria* and *Archaea* are important players in soil biogeochemical cycles. Both chromium (Cr) and perfluoroalkyl substances (PFASs) are widely present in soil environment. However, the depth-related distribution of microbial community in soils contaminated by Cr or/and PFASs remains unknown. Hence, the present study applied quantitative PCR assay and Illumina MiSeq sequencing to investigate the vertical variations of archaeal and bacterial communities in soils (0.5–12.5 m depth) contaminated by chrome plating waste and the potential effects of Cr and PFASs.

Both bacterial and archaeal communities displayed the remarkable depth-related changes of abundance ( $2.16 \times 10^7$ – $5.05 \times 10^9$  and  $4.95 \times 10^5$ – $2.56 \times 10^8$  16S rRNA gene copies per gram dry soil respectively for *Bacteria* and *Archaea*), diversity (bacterial and archaeal Shannon diversity indices of 5.06–6.34 and 2.91–4.61, respectively) and structure. However, at each soil depth, bacterial community had higher abundance, richness and diversity than archaeal community. Soil bacterial communities were mainly composed of *Proteobacteria*, *Chloroflexi*, *Actinobacteria* and *Firmicutes*, and archaeal communities were dominated by *Thaumarchaeota* and unclassified *Archaea*. Moreover, microbial abundance and richness increased with increasing perfluorohexane sulfonate (PFHxS) content. Microbial abundance was correlated to total Cr, and archaeal richness was correlated to total Cr and Cr(IV). In addition, total Cr might be a key determinant of soil microbial community structure.

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## 1. Introduction

Soil microorganisms play crucial roles in driving biogeochemical cycles and decomposing organic matter (Akerblom et al., 2007; Chen et al., 2016), but these microbial processes can be disturbed by metal contamination (Akerblom et al., 2007). Chromium (Cr) is widely present in soil environment due to its wide application in many industrial processes, such as electroplating, corrosion control and leather-tanning processes. Cr contamination can have adverse effects on soil bacterial communities by affecting structure (Akerblom et al., 2007; dos Santos et al., 2012; Gong et al., 2002; Z.G. He et al. 2016; Sheik et al., 2012), diversity (Z.G. He et al. 2016; Sheik et al., 2012) and abundance (Sheik et al., 2012). However, although bacterial communities in Cr-contaminated soils and their structure change with sampling location have been well documented (Desai et al., 2009; Z.G. He et al. 2016; Sheik et al., 2012), much less attention has been paid to the vertical change (with soil depth) of bacterial community structure in Cr-contaminated environment (Kamaludeen et al., 2003). To date, the influence of metal on soil archaeal community remains unclear. Two previous studies suggested that soil archaeal community structure could be altered by zinc (Zn) and copper (Cu) contamination (Macdonald et al., 2011; Sullivan et al., 2013), while Ge and Zhang (2011) indicated no significant influence of Cu pollution on soil archaeal community structure. Moreover, direct information on the effect of Cr pollution on archaeal community is still not available.

Perfluoroalkyl substances (PFASs) have found extensive industrial and household applications as surfactants and surface protectors, which leads to their ubiquity in the environment (Meng et al., 2015; Wang et al., 2013). Metal plating, especially chrome plating, is an important emission source of PFASs (Wang et al., 2013). Chrome plating industry uses chrome acid as the raw material, and also uses fluorinated surfactants (e.g. PFOS) as the chrome mist suppressant. Chromium (VI) and perfluoroalkyl substances can be released into the surrounding environment. Hence, it could be assumed that microbial community in soil contaminated by chrome plating industry might be affected by PFASs as well as Cr pollution. However, so far, little is known about the influence of PFASs on microbial community in natural environment, although the considerable influence of either perfluorooctanoic acid (PFOA) or 6:2 fluorotelomer alcohol (6:2 FTOH) was observed on sediment bacterial community structure and diversity (Sun et al., 2016; Zhang et al., 2017). In addition, the distribution of microbial community in soil contaminated by chrome plating industry remains unclear. Therefore, the main objective of the current study was to explore the vertical changes of archaeal and bacterial communities in soils contaminated by chrome plating waste and the potential effects of Cr and PFASs.

## 2. Materials and methods

### 2.1. Sampling and chemical analysis

Soil samples in triplicate were collected from fourteen different depths (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.5, 7.5 and 12.5 m) at a chrome plating waste-contaminated site, referred as samples Z1–Z14, respectively. Samples were stored in a cooler with ice packs and then immediately transferred to the laboratory after collection. To determine chemical properties, these soil samples were freeze-dried, homogenized and sieved through 0.25-mm stainless steel mesh to remove stones and other coarse materials. Soil organic carbon (OC) content was measured using the potassium dichromate oxidation spectrophotometric method. Total Cr and water soluble Cr(VI) in soils were determined according to the literature (Liao et al., 2011). To measure PFASs in soil, 0.5–2.0 g sample was spiked with 25 ng RS (reference standard) containing 13C3-PFHxS and 13C4-PFOS, extracted by 6 mL of methanol, ultrasonicated at 30 °C for 20 min, and then centrifuged at 3500 rpm for 5 min. The supernatant was collected in a precleaned 500 mL PP bottle. These procedures were repeated three

times. In order to enrich the analyte and avoid the potential matrix interferences, the extracted solution was diluted with ultrapure water and then loaded to SPE cartridges. PWAX cartridges (6 mL, 150 mg, Agela Technologies, China) were first conditioned with 4 mL of ammonium hydroxide (0.5% NH<sub>4</sub>OH in methanol), 4 mL of methanol and 4 mL of ultrapure water at a rate of 2 drops per second. Before loading, the pH of extracted solution was adjust to pH = 4–5 by using acetic acid solution. Samples were introduced to the cartridges at a flow rate of 5–10 mL/min. After loading, the cartridge was washed with 4 mL of 25 mM sodium acetate and then dried with vacuum. Subsequently, the targets were eluted with 3 mL of methanol and 3 mL of ammonium hydroxide (0.5% NH<sub>4</sub>OH in methanol). The resulting extracts were reduced using a gentle stream of nitrogen, diluted to 0.5 mL with ultrapure water and filtered by a 0.22- $\mu$ m nylon filter prior to analysis. The IS (internal standard) containing 13C8-PFOS was added at the amount of 25 ng for each sample before instrumental analysis. Extracts were analyzed using a high performance liquid chromatography-mass spectrometry (LC-MS/MS). Target compounds were separated on an Waters Xbridge BEH C18 column (3.5  $\mu$ m  $\times$  3.0 mm  $\times$  150 mm, Waters, USA) using an UltiMate 3000 HPLC (Dionex by Thermo Fisher Scientific Inc., MA). Detection was achieved using an API 3200 triple quadrupole mass spectrometer (AB SCIEX, ON, Canada). The injection volume was 10  $\mu$ L for each sample. The column unit was held at 30 °C and the flow rate was 0.3 mL/min. Initial mobile phase (40% methanol in 10 mM ammonium acetate) lasted for 1 min. A gradient ramp followed over 6 min to 100% methanol, which lasted for 3.5 min, followed by equilibrium at 40% methanol for 2.5 min. The mass spectrometer was operated in negative electrospray ionization mode with multiple reaction monitoring (MRM). The ionization was set at an ion spray voltage of –4.5 kV and at a temperature of 450 °C, using nitrogen for drying. The flows of curtain gas, collision gas, ion source gas 1 and ion source gas 2 were set at 20, 5, 30 and 60 psi, respectively. Sample concentrations were quantified based on an internal standard calibration curve. The correlation coefficient of standard curves was larger than 0.99. Procedural blank was analyzed using the same pretreatment method of soil samples. Most PFASs in blank sample were not detected, except for PFOS and PFOA. The result of all samples has been corrected using the blank sample and checked without significant laboratory interferences. The deviation of duplicate samples was <10%, illustrating a satisfactory reproducibility. The recovery rate was 70–130%. The mainly detected PFASs in soil samples were perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and chlorinated polyfluorinated ether sulfonate (F-53B, C<sub>8</sub>ClF<sub>16</sub>O<sub>4</sub>SK). The levels of PFASs, OC, water soluble Cr (VI) and total Cr in soils were listed in Table 1. The levels of OC, water soluble Cr(VI) and total Cr in soils were 0.28–2.09%, 17.16–76.28 mg/kg and 40–4400 mg/kg, respectively.

**Table 1**  
Chemical features of soil samples.

Sample	Depth (m)	PFBS ( $\mu$ g/kg)	PFHxS ( $\mu$ g/kg)	PFOS ( $\mu$ g/kg)	F53B ( $\mu$ g/kg)	OC (%)	Total Cr (mg/kg)	Cr(VI) (mg/kg)
Z1	0.5	0.22	0	46.9	9.4	0.51	75	17.16
Z2	1	0.3	0	45.9	7.9	0.38	99	76.28
Z3	1.5	0.27	0.08	32.6	5.5	0.42	1500	32.85
Z4	2	0.01	0.08	17.6	0.81	1.53	4400	50.24
Z5	2.5	0.39	0.15	47.7	8.4	1.58	3500	31.55
Z6	3	0	0	0.4	0	0.79	186	32.12
Z7	3.5	0.03	0.09	8.5	0.32	0.7	140	24.46
Z8	4	0.06	0	11.5	0.41	0.98	307	42.4
Z9	4.5	0.03	0.05	6.7	0.76	0.42	55	32.07
Z10	5	0.41	0	53.8	6.7	0.28	55	28.1
Z11	5.5	0.12	0.05	3.1	0.15	2.09	40	32.15
Z12	6.5	0.02	0.01	1.73	0.28	0.5	95	24.34
Z13	7.5	0.14	0.08	53.1	7.4	0.51	75	26.53
Z14	12.5	0.04	0.02	21.4	0.15	1.6	59	23.32

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