Long-Term Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on Soil Microbial Community

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Abstract

The extensive application of perfluoroalkyl and polyfluoroalkyl substances (PFASs) causes their frequent detection in various environments. Nevertheless, the effects of PFASs exposure on environmental microorganisms still remain unknown. In current work, two typical PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are selected to investigate their long-term effects on soil microbes. Microbial community structure and diversity were investigated by high-throughput sequencing and multiple statistical methods. Under 90-days of exposure, PFAS treatments increased the alpha-diversity of soil microbial communities with PFOS treatment, followed by PFOA treatment. The long-term exposure of PFASs substantially changed the compositions of soil microbial communities. The most abundant phylum Proteobacteria decreased from 82.9% (without amended PFASs) to 62.1% (with PFOA treatment) and 77.8% (with PFOS treatment). As a comparison, the relative abundance of Bacteroidetes, Chloroflexi, Acidobacteria, and Ignavibacteriae increased in the PFOA or PFOS groups. Comparative co-occurrence networks were constructed to investigate the biotic interactions in the two treatments. It was found that most taxonomy nodes in the PFOA and PFOS networks were associated with the genus *Hydrogenophaga* and *Pseudoxanthomonas*, respectively. The LEfSe analysis identified a set of core taxonomies (e.g., *Azospirillum*, *Methyloversatilis*, *Ancylobacter*, *Hydrogenophaga*, and *Methylomonas*) in the soil microbial communities and suggested their different preferences to PFAS exposures. Functional gene prediction suggested that the microbial metabolism processes, such as nucleotide transport and metabolism, cell motility, carbohydrate transport and metabolism, energy production and conversion, and secondary metabolites biosynthesis transport and catabolism, might be significantly inhibited under PFAS exposure, which may further affect soil ecological services.

1 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are oleophobic and hydrophobic compounds with high surfactant properties and thermal stability. To date, a variety of PFASs have been commercially produced. Meanwhile, PFASs have been widely used in various industrial products, such as textiles, paints, lubricants, and fire extinguishing agents [1, 2]. It is defined that PFASs are aliphatic substances containing one or more C atoms on which all of the H substituents present in the nonfluorinated analogues from which they are notionally derived have been replaced by F atoms in such a way that PFASs contain the perfluoroalkyl moiety $C_nF_{2n+1}-$ [3]. Among them, two perfluoroalkyl acids, perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) and perfluorooctane sulfonic acid (PFOS, $C_8F_{17}SO_3H$), are the most reported categories [4] that have been reported to threat human health and ecosystems. [5, 6].

Due to the intensive utilization and emissions, PFASs have been frequently detected in various environmental samples, as well as bio-samples. It should be noted that the PFASs are harmful and hard to degrade, which leads to an increasing level in the ecosystems. The mass usage of PFASs inevitably leads to their release into environments and serious pollution problems (Liu and Mejia Avendano, 2013). The first global estimation of PFASs was performed for PFOS in wildlife by Giesy and Kannan [7]. Latter studies frequently reported PFAS pollution in numerous samples, such as rivers, the atmosphere,

wastewater, municipal sewage sludge, organisms, as well as soils [8–10]. In east China, the total content of PFASs was estimated over 700 ng/g in soil samples (Li et al., 2010). Concerns regarding the potential toxicological and environmental effects of PFASs have result in a phase-out of related products and elimination by 2015. Recently, several large research programs have been launched in an aim to elucidate the environmental origin, fate, and impact of PFASs [3, 11].

Soil represents one of the most important sinks of PFASs by receiving surface runoff, atmospheric deposition, and point-source contamination. The accumulation of PFASs in soils has raised health concerns as they might enter the food chain through the soil-plant-humans pathway (Ruan et al., 2015). Recent studies on the effects of PFASs in soil have primarily focused on soil microbes [12]. Because soil bacteria are significant components in soil ecosystems, their activity and composition are critical for maintaining soil ecological services [13–15]. Moreover, soil microbial communities reflect changes of soil properties thus indicate the health and quality of soils. It is well known that the high-energy C-F bonds in PFASs leads to they are highly resistant to biodegradation [16]. Toxicity of PFASs to bacteria has been also reported recently, including DNA damage, oxidative damage, membrane damage, and general cell lesions [17, 18]. These findings suggested that the accumulation of PFASs may alter soil microbial communities and thus restrict soil functions. Therefore, it is of great importance to evaluate the impacts of typical PFASs on soil ecosystems, especially the dynamics of soil microbial communities and functions.

In the current work, two typical PFASs, i.e., PFOA and PFOS, are selected to investigate their long-term effects on the dynamics of the soil microbial community. With the long-term exposure of PFAS, the changes in soil microbial diversity and community composition are characterized using high-throughput sequencing and a co-occurrence network analysis. This study provides a better understanding of the potential for PFAS distributions in terrestrial habitats and their impact on the dynamics of soil microbes.

2 Methods

2.1 Soil collection

Surface soil samples (0–10 cm depth) were collected from a farm in Guizhou province, China (25° 07' N, 107° 42' E). Soil samples were then homogenized and kept at 4°C during transport. The air-dried soils were sieved through 2.0 mm mesh in the laboratory and divided into three parts. One part is for geochemical analysis, one is for molecular analysis of indigenous community and the other part is for incubation experiment.

2.2 Chemicals and reagents

All the analytical grade chemicals used in the experiments, such as PFOA (CAS# 335-67-1) and PFOS (CAS# 1763-23-1), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution (100 mg/L) of PFOA or PFOS was dissolved in grade methanol (> 99.8%). The composition of mineral salt

medium (MSM) contains: $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (10.55 g /L), KH_2PO_4 (1.5 g /L), NH_4Cl (0.3 g /L), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1 g /L), vitamin solution (1 mL /L) of, and trace elements solution (1 mL /L).

2.3 Microcosms experiment design

To evaluate the long-term impacts of PFOA or PFOS on soil microbial community, microcosms experiments were incubated in sterilized 250-mL glass serum bottles containing 20 g soil, 200 mL of MSM (pH = 7.0). The treatment groups added 500 μL of PFOA or PFOS stock solution (final concentration = 0.25 mg/L). The control groups (Ct) only added 500 μL methanol as comparisons. Triplicate microcosms were prepared for each experimental condition. All microcosms were incubated under aerobic conditions in the dark room (25°C). 500 μL of PFOA or PFOS or methanol stock solutions (as described above) were spiked into each group every week and continuously cultured for 90 days (a total of 6-times spiking). During the long-term exposure experiments, supernatant and soil samples in each group were collected every two weeks for downstream analysis.

2.4 Illumina high-throughput sequencing

Soil samples under the long-term exposure of PFOA or PFOS were collected on day 60 and day 90 for the analysis of microbial community by Illumina high-throughput sequencing. Total DNA was extracted from 0.25 g of soil samples using Fast DNA SPIN kit (MP Biomdecals, CA, USA) according to the manufacturer's protocol. Genomic DNA was qualified by gel electrophoresis and quantified by Qubit (Invotrogen 3.0 Fluorometer), respectively [19, 20]. The V3-V4 region of the bacterial 16S rRNA gene was amplified using the recommend primer pair (338F/806R) [21]. The purified PCR products were sent for Illumina Miseq PE-250 sequencing [22].

2.5 Bioinformatic analysis and statistical method

The raw sequencing reads were merged, trimmed, and filtered by USEARCH (v 10.0). Clean sequences were dereplicated using '–derep_fulllength' with the 'min-unique-size' of 20. The non-redundant sequence was denoised by '–uniose3' to obtain the unique amplicon sequence variants (ASV). The taxonomy of each represent ASV was classified by GreenGene database (v 13.5). Functional genes were predicated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) with the default parameters [23]. Gene annotations were based on the Clusters of Orthologous Genes (COG) database [24].

Alpha-diversity of microbial community was determined by '–alpha_div' function. Beta-diversity of microbial community was calculated by '–beta_div' function to generate the Bray-Curtis-based distance matrix [25]. Analysis for the relative abundance, principal coordinates analysis (PCoA) was performed by MicrobiomeAnalyst platform [26]. The statistical difference was calculated by the Statistical Analyses of Metagenomic Profiles (STAMP) toolkit [27]. Analysis of COG pathway enrichment were performed with the OmicShare tools [28]. Co-occurrence network analysis was performed by R package 'ggcorrplot' [29]. The strong and significant Spearman's correlations ($|R| > 0.6$, $P < 0.05$) was visualized on the Gephi (v. 0.92) platform [30–32].

3 Results

3.1 Effects of PFASs on soil microbial community diversity

The long-term exposure of PFASs increased the alpha-diversity of soil microbial communities within two PFAS treatments compared to the Ct (without amended PFASs, Fig. 1). For example, the averaged observed species number in the Ct group was 1390, and the value in the PFOS and PFOA groups were 1765 and 1694, respectively. The Chao 1 index, the Shannon index, and the Simpson index showed a similar trend, where PFOS > PFOA > Ct. For the measurement of beta-diversity of soil microbial communities, the PCoA results showed that the samples in Ct group were separated from those with the PFOA or PFOS treatments (Fig. 2). It is suggested that the long-term exposure of PFASs significantly changed the compositions of the soil microbial communities.

3.2 Response of soil microbial community to PFASs

The composition and abundance of the soil microbial communities were characterized based on 16S rRNA gene amplicon sequencing. The number of effective counts from the different soil samples varied in the range of 34,568 to 50,470. All of the quality-controlled counts were affiliated into 4044 ASVs and annotated as 19 phyla, 34 classes, 63 orders, 133 families, and 316 genera. It was found that long-term treatment with PFOA and PFOS changed the major compositions of the soil microbial communities. After 90 days of treatment, the most abundant phylum, Proteobacteria, decreased from 82.9% (Ct) to 62.1% (PFOA) and 77.8% (PFOS) (Fig. 3A). The relative abundance of the second most abundant phylum, Bacteroidetes, gradually increased from 12.6% (60 d) to 27.6% (90 d) with PFOA treatment, which were much higher than that of the Ct group (6.2% at 60 d and 10.9% at 90 d, respectively). The high relative abundance of Bacteroidetes was also observed with PFOS treatment on 60 d with a value of 15.6%; however, they dramatically decreased to 7.4% on 90 d. Additionally, the enrichment of Chloroflexi and Acidobacteria were observed both with the PFOA and PFOS treatments. The high relative abundance of Ignavibacteriae was only observed in the PFOS group after 90 d of treatment (2.2%) when compared to PFOA (0.2%) or Ct (0.1%).

The changes in the relative abundance of the major orders further confirmed the effects of PFASs on soil microbial communities (Fig. 3B). The long-term treatment significantly increased the abundance of *Rhodocyclales* in the PFOA group (9.6–39.5%) and PFOS group (6.7–24.0%) as compared to the Ct group (2.2–5.1%). In contrast, the most abundant order, *Rhodospirillales*, in the Ct group (52.8–71.7%) became the minor abundant groups with both the PFOA (6.9–13.4%) and PFOS (6.8–19.4%) treatments. In addition, the relative abundances of *Burkholderiales*, *Rhizobiales*, *Bacteroidales*, and *Methylococcales* were also detected to be much higher with both PFOA and PFOS treatments as compared to the Ct group.

3.3 Effects of PFASs on soil microbial interactions

To elucidate the soil microbial interactions under the long-term exposure of PFASs, two co-occurrence networks, i.e., “PFOA network” and “PFOS network,” were constructed to compare the correlations within taxonomies (Fig. 4). It was found that most taxonomic nodes in the PFOA and PFOS networks could be

assigned to the phylum Proteobacteria (70.2% and 76.4%), followed by Bacteroidetes (13.1% and 7.6%) and Acidobacteria (4.5% and 4.4%). More importantly, a set of intensively connected nodes was identified in the two networks. For example, in the PFOA network, the ASVs associated with the genus *Hydrogenophaga*, *Azospirillum*, *Methyloversatilis*, and *Methylobacter* were found to be highly connected with the other ASVs. However, in the PFOS network, the ASVs associated with the genus *Pseudoxanthomonas*, as well as *Methyloversatilis*, *Hydrogenophaga*, and *Azospirillum*, were identified as the most connected members.

In addition, the topological parameters of the two networks were also calculated (Table 1). A total of 246 and 226 nodes were obtained from the PFOA and PFOS networks, generating 10,473 and 11,358 pair-wise correlations, respectively. The modularity of the two networks were 0.45 and 0.43, respectively. The PFOS network had a higher average clustering coefficient than the PFOA network (0.64 vs. 0.03). The average path length in the PFOS network was lower than in the PFOA network (2.08 vs. 3.36). Comparisons of topological parameters suggested that the PFOS treatment increased the intensive connection of the network than in the PFOA treatment.

Table 1
Topological features of the PFOA network and PFOS network.

Topological features	PFOA network	PFOS network
Modularity	0.457	0.431
Average Degree	5.967	7.38
Average Clustering Coefficient	0.034	0.643
Average Path Length	3.368	2.087
Nodes	246	226
Positive links	8343	9237
Negative links	2130	2121
All links	10473	11358

3.4 Effects of PFASs on the core taxonomy

The LEfSe analysis was performed to identify the core taxonomies in the soil microbial communities treated with the PFOA or PFOS treatments (Fig. 5). The identification of the core taxonomies can be used as biomarkers to distinguish the different influences of PFOA and PFOS. The genera *Azospirillum*, *Methyloversatilis*, *Ancylobacter*, *Hydrogenophaga*, and *Methylobacter* were detected as the top five core taxonomies (i.e., biomarkers) according to the LDA score (default > 2.0). In addition, *Pseudoxanthomonas*, *Geobacter*, *Holophaga*, *Acetobacterium*, as well as *Anaerocella*, were also potential core taxonomies in current study. Moreover, the relative abundance of these core taxonomies showed their different preferences to PFAS treatments. For example, the PFOA treatment significantly enriched the

abundances of *Methyloversatilis*, *Ancylobacter*, *Methylothermobacter*, *Geobacter*, *Sulfurisoma*, and *Anaerocella*, but reduced the abundances of *Azospirillum*, *Hydrogenophaga*, *Pseudoxanthomonas*, and *Acetobacterium*. In a comparison of the PFOS treatment, the abundances of *Hydrogenophaga*, *Methylobacter*, *Prosthecomicrobium*, *Pseudoxanthomonas*, *Holophaga*, and *Brevundimonas* were significantly increased, whereas the abundance of *Dokdonella* decreased. Collectively, the long-term exposure of PFOA or PFOS both reduced the abundance of *Azospirillum*, *Dokdonella*, and *Acetobacterium*.

3.5 Response of soil microbial functions to PFASs

The functional genes of soil microbe were compared according to the PICRUSt predication (Fig. 6). The abundances of the functional genes with the PFAS treatments were significantly different from the Ct group. The PFAS treatments increased the abundances of several functional genes, such as the signal transduction mechanisms, the chromatin structure and dynamics, and RNA processing and modification. In contrast, the majority of functional categories in the PFOA or PFOS groups showed a lower abundance than the Ct group, such as carbohydrate transport and metabolism, nucleotide transport and metabolism, cell motility, defense mechanisms, energy production and conversion, secondary metabolites biosynthesis transport and catabolism, and cell wall membrane envelope biogenesis. The results suggested that a wide range of microbial metabolism processes might be inhibited by PFASs.

4 Discussion

4.1 PFASs increased the soil microbial richness and diversity and richness

This study examined the effects of the long-term treatment of PFOA or PFOS, which resulted in increased diversity and richness of soil microbes (Fig. 1). In previous studies, Li, Bao, Xu, Xie and Huang [33] observed that soil microbial richness were facilitated as the concentrations of PFASs increased. Qiao, Xie, Zhang, Liu, Xie, Huang and Yu [34] also indicated that the typical PFASs, such as PFOS, increased the observed species numbers in soils. Cai, Chen, Yuan, Wang, Chen and Zhou [12] reported an increased diversity of microbial communities in PFOA or PFOS amended soils up to 3000 mg/kg higher than in un-amended soils. The richness and diversity of soil microbes correlated with the soil ecological functions, such as the mineralization of carbon and nitrogen and the storage of total carbon, nitrogen, and phosphorus [35]. These correlations suggest that changes in the soil microbial diversity caused by PFASs could cause disturbances in soil functions. Besides, the microbial diversity and richness are inherent properties of the soil microbe to adapt to various environmental disturbances, however, few studies investigated the effects of PFASs on soil microbes from this viewpoint. Moreover, it was observed in the current study that exposure to PFOS (eight carbon chain structures, C8) caused a larger effect on the microbial community richness or diversity than PFOA (seven carbon chain structures, C7) did, indicating that the extents of these effects varied among different PFAS species. Cai, Chen, Yuan, Wang, Chen and

Zhou [12] suggested that the species of PFAS was a critical factor to govern microbial community structures that led to changes in diversity and richness.

4.2 PFASs changed the soil microbial community composition

The PCoA results supported that the PFASs significantly altered the soil microbial community composition as indicated by the formation of three distinct clusters associated with their corresponding treatments (Fig. 2). The exposure of PFOA or PFOS enriched the abundances of the phyla Bacteroidetes, Chloroflexi, Acidobacteria, and Ignavibacteriae (Fig. 3). Many orders were also increased with PFOA or PFOS treatment, such as *Rhodocyclales*, *Burkholderiales*, *Rhizobiales*, *Bacteroidales*, and *Methylococcales*. Enrichment of these taxonomies suggested a soil microbial response to PFASs, such as preference, tolerance, or degradation. Similarly, other studies have suggested that PFASs have close correlations with these soil bacteria. Bao, Li, Xie and Huang [36] reported that in soils contaminated by high-level of PFASs over ten years, the soil microbial communities were primarily dominated by the phyla Chloroflexi. Cai, Chen, Yuan, Wang, Chen and Zhou [12] investigated the effects of PFASs on soil microbial communities and observed that Acidobacteria, Actinobacteria, and Chloroflexi were the primary bacterial phyla. Among them, Acidobacteria is a kind of versatile heterotrophic bacteria which can tolerate various extreme environments [37, 38]. Due to the increase of Acidobacteria in this work, it is suggested that they are tolerant to the presence of toxic PFASs.

In contrast, the inhibition of PFAS exposures on soil microbial communities was also observed (Fig. 3). Among them, the relative abundance of the order *Rhodospirillales* (within the phylum Proteobacteria) was largely reduced in both the PFOA and PFOS groups, which might have been attributed to the toxicity of the PFAS treatment. The PICRUSt results supported that the majority of the microbial metabolic pathways were inhibited in the PFOA and PFOS groups (Fig. 6), such as cell wall membrane envelope biogenesis and energy production and conversion. Qiao, Xie, Zhang, Liu, Xie, Huang and Yu [34] suggested that PFASs would restrict microbial growth and gene expression, such as disrupting immune function and cellular structure. Moreover, the potential biological damage of PFOS include the breaks in the DNA strand, genotoxic effects in cells, and alteration of the permeability of cell membranes [39–41]. Notably, few researches have reported the effects of PFASs on the cell membrane. PFOS might increase the membrane fluidity of fish leukocytes in a dose-dependent way, due to their relatively non-specific detergent-like effects on the membrane [42]. Liu, Zhang, Yang, Zhu and Lin [17] reported that the exposure to high-levels of PFOA and PFOS might change cell surface hydrophobicity. As a result, the hydrophobic parts of PFOA and PFOS would bond to lipid bilayer of the cell membrane thus increase the membrane fluidity, thereby causing cell inactivation and mortality or disrupting the membrane structure. This evidence might explain the significant reduction in the above-mentioned soil microbes.

4.3 Response of core taxonomies to PFASs

A set of core taxonomies was identified using the co-occurrence network analysis, such as *Hydrogenophaga*, *Azospirillum*, *Methyloversatilis*, *Ancylobacter*, *Methylobacter*, and *Pseudoxanthomonas*

(Fig. 4). The identification of core taxonomies indicated their important roles for community structure and integrity [43]. The LEfSe analysis further confirmed these genera could be used as biomarkers to indicate the different influences due to PFOA or PFOS exposure (Fig. 5). For example, the abundance of *Hydrogenophaga* was enriched under PFOS exposure. *Hydrogenophaga* is known as an aerobic dechlorinating bacterium [44] that hydrogen can be used as electron donor for its autotrophic growth [45]. *Hydrogenophaga* strains have been isolated from textile wastewater treatment plants, PAH-contaminated river sediments, and haloaromatic-contaminated soils, confirming they are able to degrade various organic pollutants, such as 4-aminobenzenesulfonate, 4-chlorobenzoate, and high molecular weight-polycyclic aromatic hydrocarbons [46–48]. In addition, the abundance of *Methyloversatilis* and *Ancylobacter* increased under PFOA exposure. *Methyloversatilis* and *Ancylobacter* have been characterized as methylotrophic groups that are obligate methyl utilizers [49, 50]. The aerobic utilization of dichloromethane by *Ancylobacter* has been confirmed in contaminated soil [50]. *Methyloversatilis* has also been identified as a contributor of benazolin-ethyl biodegradation in activated sludge [51]. The enrichment of *Methyloversatilis* and *Ancylobacter* suggested their potential capabilities to degrade PFOA. Additionally, the abundance of *Azospirillum* was largely inhibited by PFOA and PFOS. *Azospirillum* is a well-known rhizobacteria of plant growth promoting (PGP). *Azospirillum* can colonize plenty of plant species and promote their growth, development, and productivity [52].

4.4 PFASs may affect soil function

The prediction of functional genes suggested that PFOA or PFOS exposure may exert adverse effects on a wide range of soil functions (Fig. 6). For example, the abundance of genes involved in secondary metabolites biosynthesis transport and catabolism were inhibited within the PFOA and PFOS treatments. Secondary metabolite biosynthesis has been considered as an important function that soil microbes utilize to regulate community dynamics [53]. Sun, Xu, Dong, Li, Tao, Kong, Zhang, Qiu, Wang and Sun [54] demonstrated that secondary metabolite production might affect soil microbial interactions and affect soil functions. In fact, secondary metabolites include a group of different organic compounds, which do not directly participate in the growth or reproduction of the organism, but play a variety of functions in interactions with other organisms in soil microbial communities or plants [55]. Therefore, the current study suggested that soil ecological services would be harmed under PFOA or PFOS long-term exposure due to the inhibition of soil microbial functions. Previous researches have also suggested similar conclusions and have explained the potential damage from PFASs to soil functions, such as enzyme activities. Zhang, Lin, Yang and Zhang [56] observed the activities of soil phosphatase and catalase that decreased with increasing PFOA concentration. Qiao, Xie, Zhang, Liu, Xie, Huang and Yu [34] reported that PFOS exposure (10 µg/g) reduced soil sucrase by 30% and damaged the soil ammonia oxidation process. Ke, Chen, Hu, Tong, Huang and Xie [57] observed that soil nitrifying activity was potentially restricted by PFASs in a dose-dependent way.

5 Conclusions

The long-term effects of two typical PFASs, i.e., PFOA and PFOS, on soil microbes were investigated over 90-days of exposure. The alpha-diversity of the soil microbial communities significantly increased with PFOS treatment, followed by PFOA treatment. The compositions of the soil microbial communities, such as Proteobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, and Ignavibacteriae, substantially changed with PFOS or PFOA treatment. The genus *Hydrogenophaga* and *Pseudoxanthomonas* were identified as two most connected nodes in the PFOA and PFOS co-occurrence network, respectively. A number of core taxonomies, such as *Azospirillum*, *Methyloversatilis*, *Ancylobacter*, *Hydrogenophaga*, and *Methylomonas*, showed their different preferences to PFASs exposure. The functional gene prediction suggested that the long-term exposure of PFASs may exert adverse effects on a wide range of soil functions by inhibiting various microbial metabolism processes.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The datasets generated during the current study are available in the NCBI database under the project number PRJNA694469.

Authors' contributions

Conceptualization: Weimin Sun, Rui Xu; **Methodology:** Rui Xu, Wan Tao, Xiaoxu Sun; **Formal analysis and investigation:** Hanzhi Lin; **Writing - original draft preparation:** Wan Tao, Duanyi Huang; **Writing - review and editing:** Rui Xu, Pin Gao, Zhaohui Yang; **Funding acquisition:** Rui Xu, Weimin Sun; **Supervision:** Weimin Sun.

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Figures

Figure 1

Measurements of the alpha-diversity of the soil microbial communities under PFOA or PFOS treatment over 90 days. The box chart indicates the measurements of (A) observed species number, (B) the Chao1 index, (C) the Shannon index, and (D) the Simpson index.

Figure 2

The PCoA showed the Bray-Curtis distance-based beta-diversity of the soil microbial communities under the PFOA or PFOS treatment over 90 days.

Figure 3

Exposure of PFOA and PFOS over 90 days changed the compositions of the soil microbial community. The bar charts showed (A) the major phyla and (B) the major orders.

Figure 4

The co-occurrence networks of the soil taxonomies under the (A) PFOA and (B) PFOS treatments over 90 days. The color of each node (OTU) is marked using the assignment at the phylum level. Each node was labelled using the assignment at the genus level. The node size was proportional to its connection number (mean degree). The links represent the significant and strong Spearman's correlations with the $|R|>0.6$ and $P<0.05$.

Figure 5

Identification of the core genus according to the LefSe analysis. The LDA score indicates the importance of each genus. The relative abundance of each genus in the different treatment groups is highlighted by the red (high), blue (low), and white (medium) squares.

Figure 6

Comparisons of the functional gene abundances in the different treatment groups using the COG database.