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

Rui Xu1,2 · Wan Tao1,2 · Hanzhi Lin1,2 · Duanyi Huang1,2,3 · Pingzhou Su1,2 · Pin Gao1,2 · Xiaoxu Sun1,2 · Zhaohui Yang3 · Weimin Sun1,2,4,5

Abstract
The extensive application of perfluoroalkyl and polyfluoroalkyl substances (PFASs) causes their frequent detection in various environments. In this work, two typical PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are selected to investigate their effects on soil microorganisms. Microbial community structure and microbe–microbe relationships were investigated by high-throughput sequencing and co-occurrence network analysis. Under 90 days of exposure, the alpha-diversity of soil microbial communities was increased with the PFOS treatment, followed by the PFOA treatment. The exposure of PFASs substantially changed the compositions of soil microbial communities, leading to the enrichment of more PFASs-tolerant bacteria, such as Proteobacteria, Burkholderiales, and Rhodocyclales. Comparative co-occurrence networks were constructed to investigate the microbe–microbe interactions under different PFASs treatments. The majority of nodes in the PFOA and PFOS networks were associated with the genus Azospirillum and Hydrogenophaga, respectively. The LEfSe analysis further identified a set of biomarkers in the soil microbial communities, such as Azospirillum, Methyl-oversatilis, Hydrogenophaga, Pseudoxanthomonas, and Fusibacter. The relative abundances of these biomarkers were also changed by different PFASs treatments. 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 inhibited under PFAS exposure, which may further affect soil ecological services.

Keywords Perfluoroalkyl and polyfluoroalkyl substances (PFASs) · Perfluorooctanoic acid (PFOA) · Perfluorooctane sulfonic acid (PFOS) · Soil microbial community · High-throughput sequencing · Co-occurrence network

Introduction
Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are highly polar and stable organic compounds that contain multiple carbon–fluorine (C-F) bonds [1]. A variety of PFASs have been widely used in various industrial products, such as

1 National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-Environmental Pollution Control and Management, Institute of Eco-Environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou 510650, China
2 Guangdong-Hong Kong-Macao Joint Laboratory for Environmental Pollution and Control, Guangzhou
3 College of Environmental Science and Engineering, Hunan University, Changsha 410082, People’s Republic of China
4 School of Environment, Henan Normal University, Xinxiang, China
5 Key Laboratory of Yellow River and Huai River Water Environment and Pollution Control, Ministry of Education, 808 Tianyuan Road, Guangzhou, Guangdong, China
textiles, paints, lubricants, and fire extinguishing agents [2, 3]. Among them, two specific PFASs compounds, perfluorooctanoic acid (PFOA, C₇F₁₅COOH) and perfluorooctane sulfonic acid (PFOS, C₈F₁₇SO₃H), are the most reported categories [4, 5]. PFASs are considered to be both hydrophobic and lipophobic tendencies, which result in ready accumulation within lipids (fats) and proteins [6, 7]. A number of PFASs-related health issues, such as kidney [8], ulcerative colitis [9], high cholesterol [10], and cancers [11], have also been reported. In addition, PFASs are believed to act as endocrine disruptors among PFASs-exposed individuals [6].

It should be noted that most of PFASs have high chemical stability, high thermal resistance, and high resistance to biodegradation [1]. As a result of continued production and utilization, PFASs have been frequently detected in the environment, including drinking water [12], rivers [13], groundwater [14], wastewater [15], household dust [16], and soils [17]. Soil represents one of the most important sinks of PFASs by receiving surface runoff, atmospheric deposition, and point-source contamination [18]. In east China, the total content of PFASs was estimated over 700 μg/kg in soil samples [19]. The accumulation of PFASs in soils has raised health concerns as they might enter the food chain through the soil–plant-humans pathway [20]. Recent studies on the effects of PFASs in soil have primarily focused on soil microorganisms [21] because their activity and composition have critical roles in maintaining soil ecological services or indicating the soil properties [22–24].

Researches have indicated that the continued exposure to PFASs resulted in a significant shift in soil microbial community structure, leading to the enrichment of more PFASs-tolerant bacteria, such as Proteobacteria, Acidobacteria, and Bacteroidetes [25–27]. Additionally, the toxicity of PFASs to bacteria has been also reported recently, including DNA damage, oxidative damage, membrane damage, general cell lesions, and restrained microbial growth [28–30].

Although these findings suggested that the accumulation of PFASs may alter soil microbial communities and thus restrict soil functions. However, the effects of PFASs on the co-occurrence patterns of soil microorganisms are still unknown. Co-occurrence patterns are ubiquitous and particularly important in understanding the structure of complex microbial communities [31], which may help to decipher the potential inter-taxa correlations, and revealing niche spaces shared by community members [32–34]. This information would comprise a systems-level understanding of community structure and function [35]. Recent studies have investigated co-occurrence patterns between microorganisms in complex environments using network analysis-based approaches [36–38]. Network analysis has demonstrated power for exploring the features of co-occurrence patterns and identifying key taxa in different soils [39, 40]. Here, we advance this research by providing a further understanding of the soil microbial co-occurrence patterns under the exposure of PFASs.

In the current work, two typical PFASs, PFOA and PFOS, are selected to investigate their effects on the dynamics of the soil microbial community. With the exposure of PFASs, the changes of microbial community structures and microbial interactions are characterized using the high-throughput sequencing and co-occurrence network analysis. This study provides a better understanding of the response of soil microorganisms to the exposure of PFASs.

**Materials and Methods**

**Soil Collection**

Surface soil samples (0–10 cm depth) were collected from a typical rice paddy in Guizhou province, China (25° 07′ N, 107° 42′ E). These samples can be used to study the response of indigenous bacteria in uncontaminated soil to new PFASs pollutions. 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 soil geochemical analysis, one is for molecular analysis of soil microbial community and the other part is for incubation experiment. The measurements of geochemical parameters showed that soil samples had a pH of 5.58 ± 0.42, nitrate contents of 18.4 ± 1.7 mg/kg, sulfate contents of 95.7 ± 6.5 mg/kg, and total organic carbon (TOC) of 0.7%.

**Chemical Reagents and Analysis Methods**

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, 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: Na₂HPO₄·12H₂O (10.55 g /L), KH₂PO₄ (1.5 g /L), NH₄Cl (0.3 g /L), MgCl₂·6H₂O (0.1 g /L), vitamin solution (1 mL /L) [41], and trace elements solution (1 mL /L, SL-10, DSMZ GmbH, 2010). For the measurements of geochemical parameters, pH was measured by a pH meter (HQ30d, Hach, USA). The contents of nitrate and sulfate were measured using an ion chromatography ( Dionex ICS-40, USA) [23]. The contents of TOC were measured by an elemental analyzer (Hanau, Germany) using the fully digested soil samples (5% HCl, v/v).
Microcosm Experiment Design

To evaluate the impacts of PFASs on soil microbial community, a total of three groups of microcosm experiment were conducted in sterilized 250-mL glass serum bottles [42]. Initially, all bottles contain 20 g soil and 200 mL of MSM (pH = 7.0). Five hundred microliters of PFOA or PFOS stock solution was added to two groups to make a final concentration of 0.25 mg/L. Five hundred microliters of methanol was added to the control group (denoted as “Ct,” hereafter) as a comparison. The test concentration of this study refers to the PFASs concentrations of the contaminated site reported in the literature [43]. Microcosms were prepared in triplicates for each experimental condition. All microcosms were incubated under aerobic conditions by purging the compressed air in the darkroom (25 °C). Five hundred microliters of PFOA or PFOS or methanol stock solutions (as described above) were spiked into each group every 15 days and continuously cultured for 90 days (a total of 6-times spiking).

Measurement of Microbial Community Structure and Biomass

Soil samples were collected in triplicates at three time points (day 30, day 60, and day 90) from each treatment (PFOA, PFOS, and Ct). The inoculums soils (day 0) were also collected in triplicates. A total of 30 soil samples were obtained for the analysis of microbial community by Illumina high-throughput sequencing. Initially, the collected samples were freeze-dried. Total DNA was extracted from 0.25 g of freeze-dried soil samples using Fast DNA SPIN kit (MP Biomedicals, CA, USA) according to the manufacturer’s protocol [44]. Genomic DNA was qualified by gel electrophoresis and quantified by Qubit (Invitrogen 3.0 Fluorometer), respectively [45, 46]. The V3-V4 region of the bacterial 16S rRNA gene was amplified using the recommend primer pair (338F/806R) [47]. The PCR-amplification cycles followed an initial denaturation at 98 °C for 60 s, with 30 cycles of denaturation at 98 °C for 10 s, 50 °C annealing for 30 s and 72 °C for 30-s extension. A final extension at 72 °C for 5 min was included before holding at 4 °C. The purified PCR products were sent for Illumina Miseq PE-250 sequencing [48, 49].

For the measurement of microbial biomass, the quantitative PCR (qPCR) was performed to amplify the bacterial 16S rRNA genes using the primer pair 338F/518R. A 20 μL reaction solution included 10 μL SYBR premix, 0.2 μL DNA template, and 0.4 μL of each primer. The two-step amplifications were performed using a CFX96TM Real-Time Detection System (Bio-RAD, CA, USA) and consisted of an initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 55 °C for 30 s [50]. In addition, the amplicons of 16S rRNA gene were cloned into plasmids using the TA cloning kit (Invitrogen, CA, USA). The plasmid DNA was then serially diluted to generate the qPCR standards. PCR grade water was used as the negative control.

Bioinformatic Analysis and Statistical Method

The raw sequencing reads were merged, trimmed, and filtered using USEARCH (v 10.0). Clean sequences were dereplicated using “--derep_fulllength” with the “min-uniqueness” of 20. The non-redundant sequence was denoised by “--unioise3” to obtain the unique amplicon sequence variants (ASV). The taxonomy of each representing ASV was classified by GreenGene database (v 13.5). Functional genes were predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) with the default parameters [51]. Gene annotations were based on the Clusters of Orthologous Genes (COG) database [37].

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 [52]. Analysis for the relative abundance, principal coordinates analysis (PCoA), and linear discriminant analysis (LDA) effect size (LEfSe) was performed by MicrobiomeAnalyst platform [53, 54]. The statistical difference was calculated by the Statistical Analyses of Metagenomic Profiles (STAMP) toolkit [55]. Analysis of COG pathway enrichment was performed with the OmicShare tools [56]. Co-occurrence network analysis was performed by R package “ggcorrplot” [57]. The strong and significant Spearman’s correlations (|R|>0.8, P < 0.01) was visualized on the Gephi (v. 0.92) platform [58–60]. All the replicate samples of either PFOA treatments (n = 9) or PFOS treatments (n = 9) were grouped to construct the PFOA or PFOS network, respectively.

Results

Effects of PFASs on Soil pH and Microbial Biomass

Soil pH were monitored during the 90-days exposure of PFASs, but no significant difference was observed among the Ct treatment, PFOA treatment, and PFOS treatment at each time point (Fig. 1A). In addition, the microbial growth under different PFAS treatments was evaluated by targeting the bacterial 16S rRNA gene numbers using qPCR (Fig. 1B). At first 30 days, the gene abundance showed no significant difference in all treatments. From day 60 to 90, the PFOA treatment and PFOS treatment significantly decreased bacterial 16S rRNA gene abundance as compared to the Ct treatment (P < 0.05).

© Springer
Effects of PFASs on Soil Microbial Community Diversity

At the first 30 days, the alpha-diversity of soil microbial communities was reduced by the exposure of PFOA and PFOS (Fig. 2). However, with the prolongation of exposure time from 60 to 90 days, the diversity indexes increased significantly in two PFASs treatments compared to the Ct. At days 30, 60, and 90, the averaged Chao 1 index and Shannon 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 under the exposure of PFOA or PFOS (Fig. 3). It is suggested that the exposure of PFASs significantly changed the diversity and composition of soil microbial communities.

Response of Soil Microbial Community to PFASs

The composition and abundance of the soil microbial communities were characterized using the 16S rRNA gene amplicon sequencing. The phylum Proteobacteria was dominated in all samples (67–96%), followed by Bacteroidetes (2–22%), Firmicutes (1–11%), and Acidobacteria (1–3%). Specifically, the PFOA and PFOS treatments changed the major compositions of the soil microbial communities (Fig. 4A). At the first 30-day exposure of PFASs, Proteobacteria increased to 96% in the PFOS treatment while it was only 67% in the PFOA treatment. After 90 days exposure of PFASs, the relative abundances of
Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on Soil Microbial Communities

Proteobacteria were both higher in the PFOA treatment (80%) and the PFOS treatment (88%), as compared to the Ct (74%) treatment. Besides, at day 30, the relative abundance of Bacteroidetes increased to 22% in the PFOA treatment while it was only 2% in the PFOS treatment. However, after 90 days of exposure, the relative abundances of Bacteroidetes were both lower in the PFOA treatment (12%) and the PFOS treatment (6%), as compared to the Ct (19%).

The changes in the relative abundances of the major orders further revealed the effects of PFASs on soil microbial communities (Fig. 4B). As compared to the Ct treatment, the relative abundances of the order Rhodospirillales were always lower in PFOA or PFOS treatments at day 30 (Ct: 46%, PFOA: 31%, PFOS: 3%), day 60 (Ct: 56%, PFOA: 6%, PFOS: 6%), and day 90 (Ct: 31%, PFOA: 17%, PFOS: 9%). On the contrary, the relative abundances of Burkholderiales in the PFOS treatment were always higher than those in the Ct treatment from day 30 to 90. In addition, at the first 30 days of PFASs exposure, the relative abundance of the order Rhodocyclales was reduced in the PFOA treatment (7%) as compared to the Ct treatment (17%). While its abundance largely increased in the PFOA treatment (55%, day 60) and the PFOS treatment (33%, day 90).

**Effects of PFASs on Soil Microbial Interactions**

To elucidate the soil microbial interactions under the exposure of PFASs, two co-occurrence networks, “PFOA network” and “PFOS network,” were constructed to compare the potential interactions between microbial taxa (Fig. 5). The topological parameters of the two networks were calculated (data not shown). A total of 250 and 223 nodes were obtained from the PFOA and PFOS networks, generating 5202 (3079 positive links + 2123 negative links) and 5216 (3114 positive links + 2102 negative links) pair-wise correlations, respectively. The modularity of the two networks was 0.40 (PFOA) and 0.37 (PFOS), respectively. The average degree the two networks was 31 (PFOA) and 34 (PFOS), respectively. The clustering coefficients of the two networks were 0.05 (PFOA) and 0.42 (PFOS), respectively. The average path length in the PFOA network (3.7) was longer than that in the PFOS network (2.3). Comparisons of topological parameters collectively suggested that the PFOS treatment increased the intensive connection of the network than in the PFOA treatment.

In addition, the majority of nodes in the PFOA networks could be assigned to the phylum Proteobacteria (182/250), followed by Firmicutes (26/250) and Bacteroidetes (24/250). In the PFOS network, the majority of nodes were still found to be Proteobacteria (182/223), but Bacteroidetes-associated nodes became the second phylum (17/223), followed by Firmicutes (14/223). More importantly, a set of nodes with high connection degrees were identified. These nodes were associated with different ASVs in the two networks. In the PFOA network, the nodes associated with the genus Azospirillum (29/250), Methyloversatilis (22/250), and Hydrogenophaga (16/250) were found to be highly connected with the other ASVs. However, in the PFOS network, the most connected nodes became the genus Hydrogenophaga (30/223), followed by Methyloversatilis (17/223) and Azospirillum (9/223).

**Identification of Biomarkers Under Different PFAS Treatments**

The LEfSe model is an algorithm for high-dimensional biomarker discovery that identifies taxonomic features characterizing the differences among three treatment conditions (Ct, PFOA, and PFOS). The identification of the biomarkers (estimating by the LDA score) can be used to distinguish the different influences of PFASs (Fig. 6A). The genera Azospirillum, Methyloversatilis, Hydrogenophaga,
**Pseudoxanthomonas**, and **Fusibacter** were detected as the top five biomarkers according to the LDA score (default > 2.0). In addition, **Dokdonella**, **Geobacter**, **Thermomonas**, **Methylotenera**, **Hyphomicrobium**, **Bdellovibrio**, **Anaeromyxobacter**, and **Desulfovibrio** were also potential biomarkers. Moreover, the relative abundances of these biomarkers have been largely influenced by different PFASs treatments (Fig. 6B). The exposure of PFOA or PFOS both reduced the abundance of **Azospirillum**, **Dokdonella**, **Acetobacterium**, and **Pandoraea**, because these genera showed
Fig. 5 The co-occurrence networks of the soil microbiomes under the PFOA treatments (A) (n = 9) and PFOS (n = 9) treatments (B). The color of the most connected node (top 3) is marked 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| > 0.8 and P < 0.05.

Fig. 6 A Identification of the biomarkers from Ct treatments (red), PFOA treatments (green), and PFOS (blue) treatments using the LEfSe analysis. The LDA score indicates the effect size of each differential biomarker. B The relative abundances (evaluated by log 10) of each biomarker in the three treatment groups are visualized using the bar-chart figure.
higher relative abundances in the Ct group. In contrast, the PFOA treatment enriched the abundances of *Fusibacter*, *Geobacter*, *Thermomonas*, and *Methylotenera* while the PFOS treatment increased the abundances of *Methyloversatilis*, *Hydrogenophaga*, *Pseudoxanthomonas*, and *Desulfovibrio*.

**Response of Soil Microbial Functions to PFASs**

The functional genes of soil microorganisms were predicted using the PICRUSt model (Fig. 7). After 60 days of exposure, the abundances of the functional genes in PFOA and PFOS treatments were different from the Ct group. It is found that the majority of functional categories in the PFOA or PFOS groups showed a lower abundance than the Ct group, such as nucleotide transport and metabolism [F], energy production and conversion [C], defense mechanisms [V], transcription [K], secondary metabolites biosynthesis transport and catabolism [Q], carbohydrate transport and metabolism [G], RNA processing and modification [A], extracellular structures [W], cell motility [N], and cell wall membrane envelope biogenesis [M]. The results suggested that a wide range of microbial metabolism processes might be inhibited by PFASs.

**Discussion**

**PFASs Restricted the Microbial Growth but Increased the Diversity of Soil Microorganisms**

This study examined the effects of the PFOA or PFOS treatments on soil microorganisms, which resulted in a reduction of soil microbial growth (Fig. 1B). A published report documented that PFASs decreased bacterial 16S rRNA gene abundance in soil [61]. Likewise, Yu et al. [30] found that PFOA restrained the microbial growth in the activated sludge, but these negative impacts could be recovered after long-term adaptation (> 290 days). Previous research reported the toxicity of PFOA to *Escherichia coli*, which led to growth inhibition of *E. coli* by membrane disruption, oxidative stress, and DNA damage [28]. These observations are consistent with our results, which indicate that the negative impacts of PFASs on microbial growth might widely exist in many mixed microbial consortia.
In addition, previous studies indicated that PFAS pollution is able to reduce microbial diversity in soil [27], sediment [62], and sludge [30]. However, this study observed that the diversity and richness of soil microorganisms were increased under the exposure of PFOA or PFOS (Fig. 2). Similarly, Li et al. [63] observed that soil microbial richness was facilitated as the concentrations of PFASs increased. Qiao et al. [26] also indicated that the typical PFASs, such as PFOA, increased the observed species numbers in soils. Cai et al. [21] reported an increased diversity of microbial communities in PFOA or PFOS amended soils up to 3000 mg/kg higher than those in un-amended soils. Although the impacts of PFASs on microbial diversity remain in conflict, the stability of soil microbial structure and activity depends on microbial diversity in theory [64]. A higher microbial diversity may promote ecosystem functioning [65]. Thus, the diversity and richness of soil microorganisms are important indicators to reflect the various environmental disturbances. The diversity and richness of soil microorganisms correlated with the soil ecological functions, such as the mineralization of carbon and nitrogen and the storage of total carbon, nitrogen, and phosphorus [66]. These correlations suggest that changes in the soil microbial diversity caused by PFASs might lead to disturbances of soil functions. Further studies are still needed to evaluate the impacts of PFASs on soil microbial diversity. In addition, this study observed that the exposure to PFOS (eight carbon chain structures, C8) caused a greater effect on the microbial community richness and diversity than PFOA (seven carbon chain structures, C7) did, indicating that the extents of these effects varied among different PFASs species. Cai et al. [21] suggested that the species of PFASs was a critical factor to govern microbial community structures that led to changes in diversity and richness.

**PFASs Changed the Soil Microbial Community Composition**

The PCoA results supported that different PFAS treatments significantly altered the composition of soil microbial communities (Fig. 3). Several bacteria groups, such as Proteobacteria, were enriched under the exposure of PFASs (Fig. 4). Enrichment of these taxonomies suggested a soil microbial response to PFASs, such as preference, tolerance, or degradation. Researches have suggested that the continued exposure to PFASs resulted in the enrichment of more PFAS-tolerant bacteria, such as Proteobacteria, Acidobacteria, and Bacteroidetes [25–27]. It should be noted that the biodegradation of PFAS is poorly understood and limited literature reported the slow or incomplete biodegradation of PFASs under natural condition [1], because PFASs contain strong carbon–fluorine bonds (C-F), which is difficult to destroy. Therefore, the degradation of PFASs may not occur in our case.

In contrast to the enrichment, the reduction of soil microbial abundances under the exposure of PFASs was also observed (Fig. 4). 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 PFASs. The decrease of function microbe Rhodospirillales with an increase of PFOA concentration was also observed in aerobic granular sludge systems [67]. In addition, the PICRUSt results further supported that the majority of the microbial metabolic pathways were inhibited in the PFOA and PFOS groups (Fig. 7), such as cell wall membrane envelope biogenesis and energy production and conversion. Qiao et al. [26] 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 includes the breaks in the DNA strand, genotoxic effects in cells, and alteration of the permeability of cell membranes [68–70]. In addition, 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 [71]. Liu et al. [28] 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 microorganisms.

**Response of Core Taxonomies to PFASs**

The highly connected taxa can be statistically identified as core taxonomies in a microbial co-occurrence network by calculating their network properties, such as high mean degree (i.e., connection) [72]. According to this criterion, a set of highly connected taxa was identified as core taxonomies using the co-occurrence network analysis, such as *Azospirillum, Hydrogenophaga*, and *Methyloversatilis* (Fig. 5). The identification of core taxonomies indicated their important roles for community structure and integrity [72]. The LEfSe analysis further confirmed these genera could be used as biomarkers to indicate the differences from the PFOA or PFOS exposure (Fig. 6A). The abundance of *Azospirillum* was largely inhibited by PFOA and PFOS. *Azospirillum* is a well-known rhizobacteria of plant growth promotion. *Azospirillum* can colonize plenty of plant species and promote their growth, development, and productivity [73]. The reduction of *Azospirillum* suggested that PFASs pollutions might have negative impacts on plant’s growth.
In contrast to the decrease of *Azospirillum*, the abundance of *Hydrogenophaga* was enriched under PFOS exposure. The enrichment of *Hydrogenophaga* is probably due to their abilities to resist organic pollutants because many *Hydrogenophaga* strains were isolated from the environments with high concentrations of organic pollutants, such as the textile wastewater treatment plants, polycyclic aromatic hydrocarbon (PAH)-contaminated river sediments, and haloaromatic-contaminated soils [74–76]. In addition, the abundance of *Methyloversatilis* also increased under PFOA exposure. *Methyloversatilis* has been characterized as methylotrophic groups that are obligate methyl utilizers [77, 78]. *Methyloversatilis* has been identified as a contributor of benazolin-ethyl biodegradation in activated sludge [79]. The enrichment of *Methyloversatilis* suggested their potential capabilities to degrade PFOA. The characterization of these PFAS-tolerant bacteria would be useful to optimally select the soil microorganisms that are capable of degrading these compounds.

**PFASs May Affect Soil Microbial Function**

The negative effects of toxic PFASs on soil organisms have been frequently reported [80, 81]. In these studies, DNA damage, inhibition of organism growth, and even mortality occurred with the increase of exposure concentrations of PFASs in soil. In this work, the prediction of functional genes suggested that PFOA or PFOS exposure may also have inhibition effects on a wide range of soil microbial functions (Fig. 7). For example, the abundance of genes involved in secondary metabolite biosynthesis transport was inhibited under the exposure of PFASs. Secondary metabolite biosynthesis has been considered an important function that soil microorganisms utilize to regulate community dynamics [82]. Sun et al. [38] demonstrated that secondary metabolite production might affect soil microbial interactions and affect soil functions. 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 [83]. Therefore, the current study suggested that soil ecological services would be harmed under the exposure of PFOA or PFOS 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 et al. [84] observed the activities of soil phosphatase and catalase that decreased with increasing PFOA concentration. Qiao et al. [26] reported that PFOS exposure (10 μg/g) reduced soil sucrase by 30% and damaged the soil ammonia oxidation process. Ke et al. [61] observed that soil nitrifying activity was potentially restricted by PFASs in a dose-dependent way. In the future, more robust pieces of evidence, such as metagenomics, are required to uncover the effects of PFASs on the genomic potentials of soil microorganisms.

**Conclusions**

The effects of two typical PFASs, PFOA and PFOS, on soil microorganisms 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, Burkholderiales, Rhodocyclales, and Rhodospirillales, were substantially changed by the PFOS or PFOA treatments. The genus *Azospirillum* and *Hydrogenophaga* were identified as the two most connected nodes in the PFOA and PFOS co-occurrence network, respectively. A number of biomarkers, such as *Azospirillum*, *Methyloversatilis*, *Hydrogenophaga*, *Pseudoxanthomonas*, and *Fusibacter*, showed their different preferences to different PFASs exposure. The functional gene prediction suggested that the exposure of PFASs may exert adverse effects on a wide range of soil microbial functions by inhibiting various metabolic processes.

**Acknowledgements** We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

**Author Contribution** 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.

**Funding** This work was supported by the National Natural Science Foundation of China (Grant Nos. 42007357 and 41771301), the China Postdoctoral Science Foundation (Grant Nos. 2020T131027 and 2019M662825), the Science and Technology Planning Project of Guangzhou (Grant No. 202002030271), Guangdong Basic and Applied Basic Research Foundation (Grant No. 2019A1515110351), GDAS’ Project of Science and Technology Development (Grant Nos. 2020GDASYL-20200103086, 2020GDASYL-20200102015, 2020GDASYL-20200102014, and 2019GDASYL-0301002), Guangdong Foundation for Program of Science and Technology Research (Grant No. 2019B121205006), and Guangdong Introducing Innovative and Entrepreneurial Talents (Grant No. 2017GC010570).

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

**Declarations**

**Conflict of Interest** The authors declare no competing interests.
References

1. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, De Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SP (2011) Perfluorooctyl and polyfluorooctyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag 7:513–541
2. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, De Voogt P, Jensen AA, Kannan K, Mabury SA, van Leeuwen SPJLeea, management (2011) Perfluorooalkyl and polyfluorooalkyl substances in the environment: terminology, classification, and origins. 7:513–541
3. Lindstrom AB, Strynar MJ, Libelo EL (2011) Polyfluorinated compounds: past, present, and future. Environ Sci Technol 45:7954–7961
4. Rankin K, Mabury SA, Jenkins TM, Washington JW (2016) A North American and global survey of perfluorooalkyl substances in surface soils: Distribution patterns and mode of occurrence. Chemosphere 161:333–341
5. Seo S-H, Son M-H, Choi S-D, Lee D-H, Chang Y-S (2018) Influence of exposure to perfluorooalkyl substances (PFASs) on the Korean general population: 10-year trend and health effects. Environ Int 113:149–161
6. Mora AM, Oken E, Rifas-Shiman SL, Webster TF, Gillman MW, Calafat AM, Ye X, Sagiv SK (2017) Prenatal exposure to perfluoroalkyl substances and adiposity in early and mid-childhood. Environ Health Perspect 125:467–473
7. Shahsavari E, Rouch D, Khudur LS, Thomas D, Aburto-Medina A, Ball AS (2021) Challenges and current status of the biological treatment of PFAS-contaminated soils. Front Bioeng Biotechnol 8
8. Blake BE, Pinney SM, Hines EP, Fenton SE, Ferguson KK (2018) Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. Environ Pollut 242:894–904
9. Steenland K, Kugathasan S, Barr DB (2018) PFOA and ulcerative colitis. Environ Res 165:317–321
10. Gruber JM, Alexander C, Laumbach RJ, Black K, Strickland PO, Georgopoulos PG, Marshall EG, Shendell DG, Alderson D, Mi Z (2019) Per and polyfluorooalkyl substances (PFAS) blood levels after contamination of a community water supply and comparison with 2013–2014 NHANES. J Eposure Sci Environ Epidemiol 29:172–182
11. Steenland K, Winquist A (2020) PFAS and cancer: a scoping review of the epidemiologic evidence. Environ Res 110690
12. Thompson J, Eaglesham G, Mueller J (2011) Concentrations of PFOS, PFOA and other perfluorinated alkyl acids in Australian drinking water. Chemosphere 83:1320–1325
13. Shi Y, Vestergren R, Xu L, Song X, Niu X, Zhang C, Cai Y (2015) Characterizing direct emissions of perfluorooalkyl substances from ongoing fluoropolymer production sources: A spatial trend study of Xiaoting River, China. Environ Pollut 206:104–112
14. von der Trenck KT, Konietzka R, Biegel-Engler A, Brodsky J, Hädicke A, Quadflieg A, Stocker R, Stahl T (2018) Significance thresholds for the assessment of contaminated groundwater: perfluorinated and polyfluorinated chemicals. Environ Sci Eur 30:19
15. Eriksson U, Haglund P, Kärman A (2017) Contribution of precursor compounds to the release of per- and polyfluorooalkyl substances (PFASs) from waste water treatment plants (WWTPs). J Environ Sci 61:80–90
16. Eriksson U, Kärman A (2015) World-wide indoor exposure to perfluorooalkyl phosphate esters (PAPs) and other PFASs in household dust. Environ Sci Technol 49:14503–14511
17. Washington JW, Rankin K, Libelo EL, Lynch DG, Cyterski M (2019) Determining global background soil PFAS loads and the fluoroelomer-based polymer degradation rates that can account for these loads. Sci Total Environ 651:2444–2449
18. Brusseau ML, Anderson RH, Guo B (2020) PFAS concentrations in soils: background levels versus contaminated sites. Science of The Total Environment 740:140017
19. Li F, Zhang C, Qu Y, Chen J, Chen L, Liu Y, Zhou Q (2010) Quantitative characterization of short- and long-chain perfluorinated acids in solid matrices in Shanghai, China. Sci Total Environ 408:617–623
20. Ruan T, Lin Y, Wang T, Liu R, Jiang G (2015) Identification of novel polyfluorinated ether sulfonates as PFOS alternatives in municipal sewage sludge in China. Environ Sci Technol 49:6519–6527
21. Cai Y, Chen H, Yuan R, Wang F, Chen Z, Zhou B (2020) Metagenomic analysis of soil microbial community under PFOA and PFOS stress. Environ Res 188:109838
22. Li B, Xu R, Sun X, Han F, Xiao E, Chen L, Qiu L, Sun W (2021) Microbiome–environment interactions in antimony-contaminated rice paddies and the correlation of core microbiome with arsenic and antimony contamination. Chemosphere 263:128227
23. XU R, Sun X, Han F, Li B, Xiao E, Xiao T, Yang Z, Sun W (2020) Impacts of antimony and arsenic co-contamination on the river sedimentary microbial community in an antimony-contaminated river. Sci Total Environ 713:136451
24. Sun X, Kong T, Häggblom MM, Kolton M, Li F, Dong Y, Huang Y, Li B, Sun W (2020) Chemolithoautotrophic diazotrophy dominates the nitrogen fixation process in mine tailings. Environ Sci Technol 54:6082–6093
25. Yu X, Nishimura F, Hidaka T (2018) Impact of long-term perfluorooctanoic acid (PFOA) exposure on activated sludge process. Water Air Soil Pollut 229:1–12
26. Qiao W, Xie Z, Zhang Y, Liu X, Xie S, Huang J, Yu L (2018) Perfluorooalkyl substances (PFASs) influence the structure and function of soil bacterial community: Greenhouse experiment. Sci Total Environ 642:1118–1126
27. Bao Y, Li B, Xie S, Huang J (2018) Vertical profiles of microbial communities in perfluorooalkyl substance-contaminated soils. Ann Microbiol 68:399–408
28. Liu G, Zhang S, Yang K, Zhu L, Lin D (2016) Toxicity of perfluorooctane sulfonate and perfluorooctanoic acid to Escherichia coli: membrane disruption, oxidative stress, and DNA damage induced cell inactivation and/or death. Environ Pollut 214:806–815
29. Nobels I, Dardenne F, De Coen W, Blust R (2010) Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying chain length. Toxicol In Vitro 24:1768–1774
30. Yu X, Nishimura F, Hidaka T (2018) Impact of Long-Term Perfluorooctanoic Acid (PFOA) Exposure on activated sludge process. Water Air Soil Pollut 229:134
31. Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J 6:343–351
32. Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538
33. Zengler K, Zaramela LS (2018) The social network of microorganisms — how auxotrophies shape complex communities. Nat Rev Microbiol 16:383–390
34. Coyte KZ, Schluter J, Foster KR (2015) The ecology of the microbiome: Networks, competition, and stability. Science 350:663
Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on Soil…

69. Choi SK, Kim JH, Park JK, Lee KM, Kim E, Jeon WB (2013) Cytotoxicity and inhibition of intercellular interaction in N2a neurospheroids by perfluorooctanoic acid and perfluorooctanesulfonic acid. Food Chem Toxicol 60:520–529
70. Qian Y, Ducatman A, Ward R, Leonard S, Bukowski V, Lan Guo N, Shi X, Vallyathan V, Castranova V (2010) Perfluoroctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. J Toxicol Environ Health 73:819–836
71. Hu W, Jones PD, DeCoe W, King L, Fraker P, Newsted J, Giesy JP (2003) Alterations in cell membrane properties caused by perfluorinated compounds. Comp Biochem Physiol C Toxicol Pharmacol 135:77–88
72. Banerjee S, Schlaeppi K, Mga VDH (2018) Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol 16:1
73. Cassán F, Diaz-Zorita M (2016) Azospirillum sp. in current agriculture: From the laboratory to the field. Soil Biol Biochem 103:117–130
74. Gan HM, Shahir S, Ibrahim Z, Yahya A (2011) Biodegradation of 4-aminobenzenesulfonate by Ralstonia sp. PBA and Hydrogenophaga sp. PBC isolated from textile wastewater treatment plant. Chemosphere 82:507–513
75. Xu C, Zang X, Hang X, Liu X, Yang H, Liu X, Jiang J (2017) Degradation of three monochlorobenzoate isomers by different bacteria isolated from contaminated soil. Int Biodeterior Biodegradation 120:192–202
76. Yan Z, Zhang Y, Wu H, Yang M, Zhang H, Hao Z, Jiang H (2017) Isolation and characterization of a bacterial strain Hydrogenophaga sp. PYR1 for anaerobic pyrene and benzo [a] pyrene biodegradation. RSC Adv 7:46690–46698
77. Kalyuzhnaya MG, De Marco P, Bowerman S, Pacheco CC, Lara JC, Lidstrom ME, Chistoserdova L (2006) Methyloversatilis universalis gen. nov., sp. nov., a novel taxon within the Betaproteobacteria represented by three methylotrophic isolates. Int J Syst Evol Microbiol 56:2517–2522
78. Firsova J, Dronina N, Lang E, Spröer C, Vuilleumier S, Totsenko Y (2009) Ancylobacter dichloromethanicus sp. nov. – a new aerobic facultatively methylotrophic bacterium utilizing dichloromethane. Syst Appl Microbiol 32:227–232
79. Cai T, Qian L, Cai S, Chen L (2011) Biodegradation of benazolin-ethyl by strain Methyloversatilis sp. cd-1 isolated from activated sludge. Curr Microbiol 62:570–577
80. Navarro I, de la Torre A, Sanz P, Porcel MÁ, Pro J, Carbonell G, MdlÁ M (2017) Uptake of perfluoroalkyl substances and halogenated flame retardants by crop plants grown in biosolids-amended soils. Environ Res 152:199–206
81. Rich CD, Blaine AC, Hundal L, Higgins CP (2015) Bioaccumulation of perfluoroalkyl acids by earthworms (Eisenia fetida) exposed to contaminated soils. Environ Sci Technol 49:881–888
82. Riley MA, Wertz JE (2002) Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56:117–137
83. Tyc O, Song C, Dickschat JS, Vos M, Garbeva P (2017) The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends Microbiol 25:280–292
84. Zhang W, Lin K-F, Yang S-S, Zhang M (2013) Enzyme activities in perfluorooctanoic acid (PFOA)-polluted soils. Pedosphere 23:120–127