

First evidence of aerobic microbial degradation of HFPO-DA in soils adjacent to fluorochemical plant: Degradation pathways, and potential functional genes and hosts



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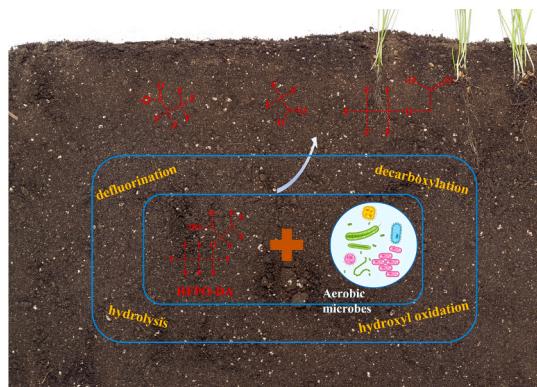
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HIGHLIGHTS

- Microbial degradation was first reported for HFPO-DA in aerobic soils.
- 3 degradation products were screened via nontarget analysis in UPLC-Q Exactive-HRMS.
- Defluorination, hydroxyl oxidation and C-C cleavage were main degradation pathways.
- 19 potential functional genes were associated with microbial degradation of HFPO-DA.
- *Gordonia*, *Megasphaera*, and *Streptomyces* were potential degradation genera of HFPO-DA.

GRAPHICAL ABSTRACT



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ABSTRACT

Hexafluoropropylene oxide (HFPO) oligomers used as the novel alternative of perfluorooctanoic acid have gained global concern due to their widespread presence and potential toxic effects. However, the microbial degradation behaviors of HFPO oligomers remain unknown. This study provided first evidence of microbial degradation of an important HFPO oligomer, HFPO-dimer acid (HFPO-DA), in aerobic soils affected by sewage of fluorochemical plant. Our findings disclosed that $51.6 \pm 3.0\%$ of initial HFPO-DA was degraded by indigenous microbes during the 150 days. A total of 3 degradation products were screened via nontarget analysis due to multiple degradation pathways, including defluorination, hydroxyl oxidation, hydrolysis and C-C bond cleavage. 2-trifluoromethyl-2-(difluoromethoxy) acetic acid was first found as the transformation product of HFPO-DA. Additionally, 19 potential functional genes associated with transmembrane transport, xenobiotic biodegradation and energy metabolism played important roles in HFPO-DA degradation. Genus *Gordonia*, *Megasphaera*, and *Streptomyces*, as hosts of potential functional genes, were found as the potential keystone taxa in HFPO-DA degradation. The findings of this study break the traditional cognition that HFPO-DA was recalcitrant to

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microbial degradation, and have important implications toward bioremediation of contaminated sites and implementation of the Action Plan on Controlling New Pollutants in China.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a class of man-made fluorinated compounds with the excellent stability and surface properties, and thus have been mass-produced and widely used in various industrial, agricultural and commercial fields since the 1950s [1]. Extensive concerns over their ubiquity, high persistence and detrimental effects have led to the phase-out of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by the Environmental Protection Agency of United States. Meanwhile, the production and usage of long-chain perfluoroalkyl carboxylic acids (PFCAs, C ≥ 8) and perfluoroalkyl sulfonic acids (PFSAs, C ≥ 7) have also been strictly regulated in the global market [2]. However, the massive global market demand for PFASs drives the manufacturers to produce alternatives and novel fluorinated compounds to compensate these restricted legacy PFASs. Hexafluoropropylene oxide (HFPO) oligomers, with the ether bond in carbon chain, have been reported as the important processing aids in synthesis of fluorinated products to replace PFOA, and shown the increasing global consumptions [3]. HFPO-dimer acid (HFPO-DA) and trimer acid (HFPO-TA) are the two major HFPO oligomers, with the wide production and application in the global market [4].

Owing to no chemical bonds with products, HFPO oligomers can be released into the environment during the production, usage and dismantling processes of HFPO-containing products. Subsequently, HFPO oligomers were widely detected in various environmental matrices, which caused the emerging environmental concerns [5,6]. Soil is regarded as one of the major reservoirs of HFPO oligomers, and can also become the HFPO sources for the soil-growing plants [6,7]. For instance, the high concentrations of HFPO-DA were detected in the soils adjacent to a fluorochemical industrial park in China, with the levels up to dozens of $\mu\text{g}/\text{kg}$, indicating the severe contamination of HFPO-DA around the pollutant sources [7]. Recently, Wang et al. reported the HFPO occurrences in the soils from 31 provinces across China, and HFPO-DA was detected in 70.2 % of soils, indicating the widespread HFPO-contamination in soils [8]. In addition, HFPO oligomers were disclosed to accumulate in crops via root uptake from the soils, which might transfer to human bodies along the food chain [6]. Although HFPO oligomers are initially anticipated to be the environment-friend replacement of PFOA, the increasing studies indicated that the persistence, bioaccumulation and toxicity of HFPO oligomers were similar to those of PFOA [9]. Hence, it is imperative to investigate the environmental behaviors and fates of HFPO oligomers in the soils, which is essential for the implementation plan of Controlling New pollutants in China.

To date, there are limited studies on transformations of HFPO oligomers, despite of the increasing shares of HFPO oligomers on the global market. HFPO-DA was reported to be completely degraded via the reductive processes in the UV/sulfite system during the 120 min, and the short-chain pentafluoropropionic acid (PFPrA) and trifluoroacetic acid (TFA) were the terminal transformation products [10]. Meanwhile, HFPO-DA can also be oxidized to generate CO_2 , PFPrA, and TFA by the advanced oxidation processes of activated persulfate in the aqueous solution, which was triggered via electron transfer reaction of SO_4^{\bullet} radicals [11]. In addition, the decarboxylation reaction of HFPO-DA was also observed in the DMSO/ H_2O system in the low temperature of 40°C, and further underwent hydroxylation and hydrolysis to form short chain PFCAs [12]. However, the limited studies of microbial degradation showed the refractoriness of HFPO oligomers. Recently, Tang et al. reported the anaerobic microbial degradation of HFPO-DA in the soils, and 5.45 % of HFPO-DA was degraded during the 10 months [13]. Jin et al. reported that HFPO-DA could not be degraded in the aerobic sludge

during the incubation of 15 days [14]. The controversial results between anaerobic and aerobic microbial degradations are mainly derived from the short period of aerobic incubation, and the fact of aerobic microbial degradation may be concealed for HFPO-DA. Thus, there are considerable knowledge gaps associated with the aerobic microbial degradation of HFPO oligomers in the environmental, which hamper the bioremediation of HFPO oligomers in the contaminated sites.

Herein, we comprehensively explored the aerobic microbial degradation of HFPO-DA by the indigenous microbes in the soils during the long-period incubation of 150 days. HFPO-DA, the widespread congener detected in the soils, was selected as the target. The aims of this study were to (1) evaluate the degradation potential of HFPO-DA by the indigenous aerobic microbes in the soils; (2) screen the unknown degradation products via nontarget identification, and disclose the potential degradation pathways; (3) characterize the dynamic changes of microbial community structures in response to HFPO-DA; (4) explore the responsible functional genes and microorganisms to enhance the understanding of HFPO-DA degradation. The findings of this study provided insights into the microbial degradation processes of HFPO-DA in the aerobic soils, and informed the development of HFPO-DA bioremediation strategies.

2. Materials and methods

2.1. Soil microcosm setup and incubation experiment

The soil samples were collected from the agricultural lands adjacent to a large fluorochemical industrial park (Fuxin, Liaoning Province, China) in July 2023 (Table S1), in which the fluoropolymer manufactures are produced by using HFPO-DA as processing aids. The soil microcosm was constructed via the addition of 10 g of homogenized soils in a 150 mL Quickfit conical flask. Prior to degradation experiment, soil in the flask was initially incubated under the aerobic condition for 14 days to active the indigenous microorganisms. The chemicals and reagents were given in details in Text S1. Four treatments were included in the microcosms: (1) the live spiked treatments (activated soils spiked with HFPO-DA), (2) sterilized control treatment (sterilized soils spiked with HFPO-DA), (3) matrix control treatments (activated soils without HFPO-DA addition), and (4) methanol control treatments (activated soils with methanol similar to amount of live spiked treatments). In the live spiked treatment, 10 μL of HFPO-DA stock solution in methanol (800 $\mu\text{g}/\text{mL}$) was added into the soil to construct the HFPO-DA spiked treatment, and the initially spiked concentration of HFPO-DA was detected to 0.74 $\mu\text{g}/\text{g}$. Subsequently, the soils were thoroughly shaken to obtain the homogenous mixture with HFPO-DA, and put into the fume cupboard to evaporate methanol. In order to verify homogeneous mixture of HFPO-DA in the spiked soils, 2 g of soil were randomly collected from the 10 g of spiked soils, and the sample collection was repeated for three times. The concentration of HFPO-DA in the collected soils showed good consistency with the relative differences $\leq 5 \%$, suggesting the homogenization of HFPO-DA in the spiked soils. To monitor the abiotic influence, the sterilized control treatments were prepared and incubated with the same ways as described above, except that the soils were autoclaved at 121°C for 3 h and repeated for three times, and then added with HgCl_2 and NaN_3 to inhibit the activities of microorganisms during the incubation time. The matrix control treatments were conducted to monitor background PFASs and potential transformations of background PFASs. In the methanol control treatments, the amount of methanol (10 μL) similar to the live spiked treatment was added into the soils to evaluate the influence of methanol on microbial community of soils. It was reported that the effect of methanol

on microbial organisms could be neglected during the aerobic microbial degradation of PFASs in the soils [15]. All of the flasks were wrapped by aluminum foils and incubated in dark environment at 25°C, during which the prepared soils maintained the 60 % of water hold capacity.

2.2. Sample preparation for analysis of HFPO-DA and its Products

The sample extraction and purification procedures of HFPO-DA from the soils were based on the method reported in our previous study [16], with a minor modification. Briefly, the freeze-dried and homogenized soils were extracted by the NH₄OH-methanol solution (0.1 %, w:w), and purified by the Oasis Wax column (Waters, USA), with the details in Text S2.

The quantitative analysis of HFPO-DA was performed by the ultra-performance liquid chromatography coupled with triple-quadrupole mass spectrometry (UPLC-MS/MS, TSQ Quantis, Thermo Fisher Scientific, Germany). A Hypersil GOLD C18 column (1.7 μm, 2.1 mm×100 mm, Thermo Fisher Scientific) was used for separation of analytes, and the mobile phases were methanol (A) and water containing 5 mM ammonium acetate (B). Details on the instrumental parameters were given in Table S2 and S3. The nontarget screening of HFPO-DA degradation products was conducted on an ultrahigh-performance liquid chromatography coupled with a high-resolution Q Exactive mass spectrometer (UPLC-Q Exactive-HRMS, Thermo Fisher Scientific, Germany), operating in both negative and positive electrospray ionization modes. Degradation products were separated by using a Waters Atlantis T3 column (3.0 μm, 100 mm × 3.0 mm). Details on the nontarget analysis were provided in Table S3. Compound Discoverer 3.3 (CD 3.3, Thermo Fisher Scientific) was applied to process the raw data to obtain feature-based data, and the workflow for the identification of potential degradation products was given in details in Text S3. TFA and PFPrA were quantified by UPLC-MS/MS (TSQ Quantis, Thermo Fisher Scientific, Germany), with instrument parameters and MS² of parent and product ions in Table S2 and Table S4. In addition, the measurement of F⁻ in the soils were provided in details in Text S4, and the quality assurance and quality control were given in details in Text S5.

2.3. Extraction of genome DNA and metagenomic sequencing

To gain insights into the potential functional genes and microorganisms responsible for HFPO-DA degradation in the aerobic soils, the soil samples at day 0, 60, 90 and 150 were collected from the non-sterilized treatments for metagenomic analysis. Details on extraction of genome DNA and metagenomic sequencing were provided in Text S6.

3. Results and discussion

3.1. Aerobic microbial degradation kinetic of HFPO-DA

During the period of 150-day incubation, there were no significant changes in HFPO-DA concentrations in the sterilized controls, with the residual percentages fluctuating at 98–117 % (Fig. 1a, blue line). However, approximate 51.6 ± 3.0 % of the initial addition dose of HFPO-DA was degraded after 150 days in the live soils with the indigenous microorganisms (Fig. 1a, red line). This result implicated that the loss of HFPO-DA in the soils was attributed to the microbial activities, and HFPO-DA could be degraded by the aerobic microorganisms. In addition, it was interesting that there were no variations of HFPO-DA concentrations during the first 30 days in live soil, which was also observed for microbial degradation of HFPO-DA in aerobic sludge during the incubation period of 15 days [14]. It was documented that when microbial communities were confronted with environmental stress, their growth entered a lag phase, during which the microorganisms regulated the expressions of genes and enzymes to adapt the changed environment [17]. Thus, the lag degradation of HFPO-DA at first 30 days might derived from the adaption of indigenously aerobic microorganisms to

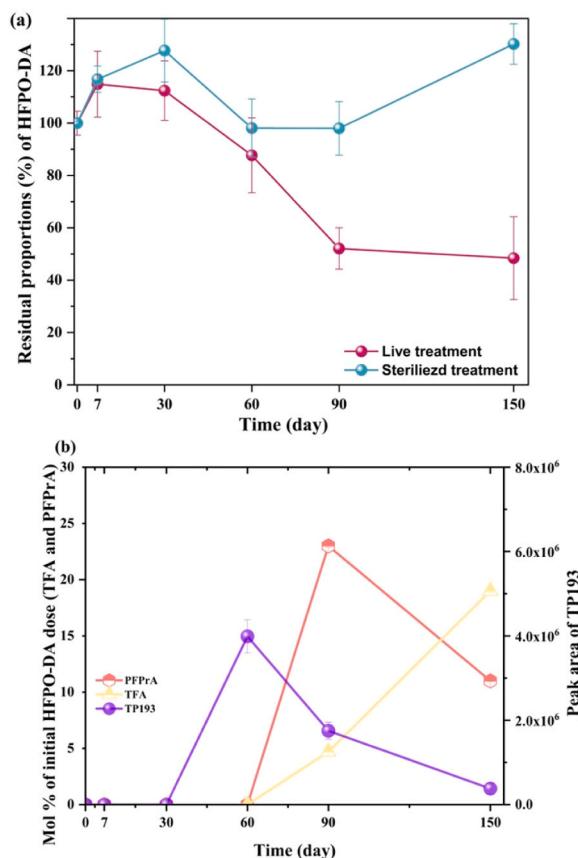


Fig. 1. Temporal changes in residual proportions of HFPO-DA (a) and molar ratio/peak area of transformation products (PFPrA, TFA and TP193) (b) during the incubation of 150 days in aerobic soil.

HFPO-DA stress in the soils. After that, HFPO-DA showed the rapid degradation, and decreased to 48.4 ± 1.6 % at day 150 (Fig. 1a, red line). These results were striking, and broke the traditional perception that HFPO-DA was hardly degraded by the aerobic microbes in the field environment.

In order to deepen understanding the degradation, the aerobic microbial degradation of HFPO-DA followed pseudo-first-order kinetic ($r^2 = 0.95$), and the degradation rate was calculated as $-0.0052 \pm 0.0005 \text{ day}^{-1}$ (Fig. S1). The half-life ($t_{1/2}$) of HFPO-DA were determined to be 134.8 ± 18.7 days in the aerobic soils. In order to get insight into persistence of HFPO-DA in the environment, the microbial degradation of HFPO-DA was compared to PFOA (predecessor of HFPO-DA) and other emerging alternatives of legacy PFASs. This degradation rate of HFPO-DA was much slower than those of other emerging PFCAs, such as 3-(trifluoromethoxy) propanoic acid ($t_{1/2} < 2$ days), 2-(2,2,2-trifluoroethoxy) acetic acid ($t_{1/2} < 4$ days) and 2-(perfluoroethoxy) acetic acid ($t_{1/2} < 20$ days) [14], and also slower than other emerging PFASs, such as 6:2 fluorotelomer sulfonate ($t_{1/2} < 30$ days) [18] and 8:2 fluorotelomer alcohol ($t_{1/2} < 4$ days) [19], but comparable to that reported for PFOA (50 % decrease for 100 days) [20]. These results indicated that HFPO-DA was very refractory to dissipation in the environment, and may be not the beneficial substitution of PFOA in the perspective of persistence.

3.2. Aerobic microbial degradation products and pathways of HFPO-DA

Three transformation products of HFPO-DA were identified though nontargeted screening using UPLC - Q Exactive HRMS (Table 1). These products were not detected in the background soils (Table S5) and matrix controls (Fig. S3), which eliminated the interference of

Table 1

Identified degradation products of HFPO-DA by UPLC-Q Exactive-HRMS.

Acronym	Formula	<i>m/z</i>		Error(ppm)	Confidence level
		Theoretical	Observed		
HFPO-DA	C ₆ F ₁₁ O ₃ H	328.96773	328.96770	0.09	1a
PFPrA	C ₅ F ₉ O ₂ H	162.98163	162.98193	-1.55	1a
TFA	C ₂ F ₃ O ₂ H	112.98397	112.98429	-2.83	1a
TP193 (2-trifluoromethyl-2-(difluoromethoxy) acetic acid)	C ₄ F ₅ O ₃ H ₃	192.99229	192.99361	-6.83	3a

background soils and HFPO-DA stock solution on the validation of transformation products. Meanwhile, the three transformation products were also not detected in the sterilized soils (Fig. S2), which further indicated the microbial degradation of HFPO-DA in the aerobic soils. For structural confirmation, the chromatograms and MS² spectra of HFPO-DA and TP193 were given in details in Fig. S5 and S6. MS² spectra of HFPO-DA were used to avoid false positive for confirmation of degradation products. In addition, two of transformation products (PFPrA and TFA) were verified by the authentic standards. Overall, the reactions of defluorination, decarboxylation, hydroxyl oxidation, hydrolysis and C-C bond cleavage were involved into the aerobic microbial degradation of HFPO-DA in soils, and thus the tentative degradation pathways were speculated in Fig. 2.

Defluorination was recognized as one of the important degradation pathways of PFASs during the microbial degradation [21,22]. In the present study, the predicated molecular structure of TP193 was CHF₂OCHCF₃COOH (2-trifluoromethyl-2-(difluoromethoxy) acetic acid) (Fig. S6), which was generated from HFPO-DA transformation via the elimination of fluoroalkyl (-CF₃ and -CF₂) and defluorination (Fig. 2). TP193 was firstly detected at day 30, and its peak area showed the first increasing and then decreasing trend with the peak at day 60, suggesting the further transformation of TP 193 (Fig. 1b). Meanwhile, compared with the sterilized soil, the gradual increase in the concentrations of F⁻ in live soil along with the incubation time also verified the occurrence of defluorination, and the defluorination degree was 15.9 ± 1.8 % (Fig. S4). This detected defluorinated product broke the traditional view that defluorination hardly occurred for the microbial degradation of HFPO-DA. It was previously reported that *Acidimicrobium* sp. strain A6 could transform PFOA via releasing fluoride ions [20]. As the alternative of PFOA, HFPO-DA might share the similar defluorinated pathways. As for C-C bond cleavage for HFPO-DA, this similar degradation pathway was also observed for other fluorinated compounds [23, 24], which might be attributed to the preferential utilization of

short-chain compounds as carbon source by microorganisms. Subsequently, TP193 may undergo a multi-step oxidation to form an alcohol intermediate product and TFA (Fig. 2). It was documented that the alcohol products occurred the spontaneous defluorination during the aerobic microbial degradation of other PFECA, such as 2-(trifluoromethoxy) acetic acid (C₃H₃F₃O₃) and 2-(perfluoroethoxy) acetic acid (C₄H₃F₅O₃) [14], and were finally oxidated into carboxylic acid. TFA and PFPrA were assigned as ultrashort-chain PFCAs (C ≤ 3), which was verified and quantified by the authentic standards in the present study (Fig. 1b). As shown in Fig. 2, TFA was generated from TP 193 via the multi-step oxidation. HFPO-DA might undergo the cleavage of ether bond, and generate PFPrA via hydrolysis. It is documented that PFPrA could be transformed to generate TFA via the C-C bond cleavage [10]. The decrease of PFPrA from day 90 also indicated the further transformation of PFPrA into other products (Fig. 1b). Thus, TFA might be derived from the further transformation of TP193 and/or C-C bond cleavage of PFPrA during the aerobic microbial degradation of HFPO-DA. Correspondingly, the first detection of TFA lagged behind TP 193 and PFPrA, and then TFA showed the continuous increasing (Fig. 1b). Numerous studies reported that TFA was end degradation products of many PFASs, and its concentrations were orders of magnitude higher than those of other PFAS [25–27]. In addition, the reproduction and liver toxicities to organisms were also reported for TFA [27, 28]. Recently, H. Arp et al. called for “The Global Threat from the Irreversible Accumulation of Trifluoroacetic Acid (TFA)” [27]. At day 150, the total molar ratio of PFPrA and TFA to initial HFPO-DA dose was 30.4 % (Fig. 1b). Coupled with the degradation degree of 51.6 % for HFPO-DA after the 150 days of incubation (Fig. 1a), the molar ratio of TP193 to initial HFPO-DA dose was calculated to be about 21.2 % under the condition of no other products except for these three degradation products. Thus, we speculated that the maximum contribution potential of TP193 to HFPO-DA loss might be 41.1 % in the soils after the 150 days of incubation, which should be further verified by the chemical synthesis

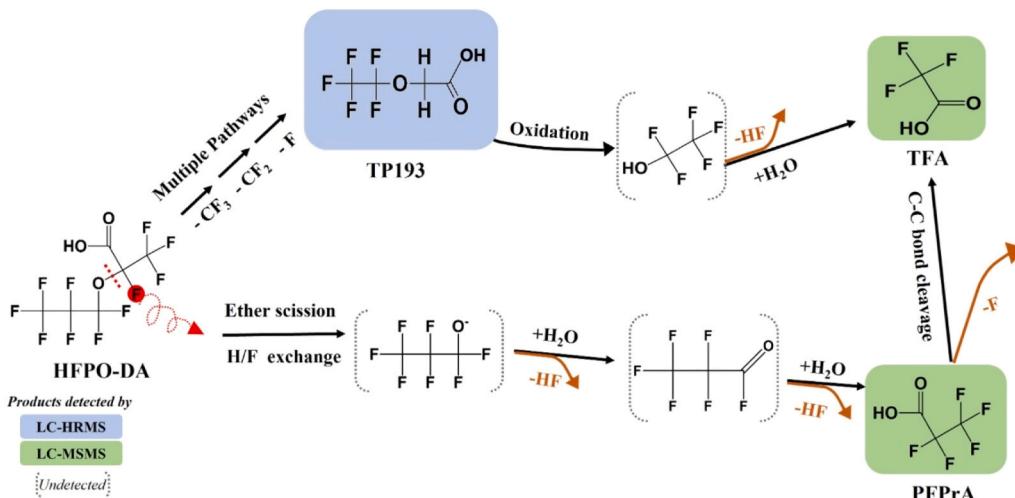


Fig. 2. Proposed microbial degradation pathways of HFPO-DA in aerobic soils. Chemicals in the blue and green boxes are detected products in the present study, and were quantified by the semi-quantitative and quantitative analyses, respectively. Chemicals in the dotted brackets were potential intermediates.

of TP193 in the future studies.

3.3. Microbial community compositions

In order to explore the influence of HFPO-DA on microbial communities, the relative abundances and compositions of microorganisms were investigated in the soils (Fig. 3). During the 150-day exposure period, the microbial community compositions in the methanol control treatments showed no detectable discrepancies compared to the matrix control treatments, indicating that the influence of methanol on microbial community could be neglected. After exposure to HFPO-DA, at the phylum level, *Actinomycetota* was the predominant phylum, and its abundances significantly increased 1.1 times during the incubation of 150 days ($p < 0.05$, Fig. S7), indicating that *Actinomycetota* could maintain self-growth under the stress of HFPO-DA and its associated degradation products (Fig. 3a). This result was consistent with the previous reports that *Actinomycetota* was widely detected in the soils contaminated with PFASs [29]. Meanwhile, some species from *Actinomycetota* were documented as the degraders of PFASs, such as *Rhodococcus jostii* RHA1, *Gordonia* sp. NB4-1Y, *Dietzia aurantiaca* J3 as the effective degraders of 6:2 FTSA [30]. Recently, *Actinomycetota* was also found to be associated with the microbial degradation of 6:2 FTSA in the aerobic sludge [18]. In addition, *Acidimicrobium* sp. strain A6, belonging to *Actinomycetota*, was reported to effectively degrade PFOA [20]. These consistence results further implied that *Actinomycetota* might play the important role in the microbial degradation of HFPO-DA in the aerobic soils. Another phylum, *Bacillota*, *Euryarchaeota* and *Cyanobacteria* were also observed with the increase in the abundances in the soils spiked with HFPO-DA during the incubation (Fig. 3a). *Bacillota* exhibited a significant enrichment after exposure to HFPO-DA at day 60 ($p < 0.05$, Fig. S7), which was observed during the aerobic biotransformation of 6:2 fluorotelomer sulfonate in soils from two aqueous film-forming foam (AFFF)-impacted sites [29]. *Euryarchaeota* was known to be tolerant to high-dose 8:2 FTOH exposure in the soils, and also participated in the microbial degradation of 8:2 FTOH [19]. In addition, a recent study found that *Cyanobacteria* had evolved several detoxification mechanisms

to degrade organic pollutants via a series of enzymes, such as monooxygenase, dioxygenase, hydroxylase, carboxylase and decarboxylase [31]. This phylum may involving the initial biodegradation of HFPO-DA, due to the enrichment at day 60. Overall, coupled with the previous studies, *Actinomycetota*, *Bacillota*, *Euryarchaeota* and *Cyanobacteria* were the important phyla highly associated with aerobic microbial degradation of HFPO-DA in the soils.

At genus level, *Nocardioides*, belonging to phylum *Actinomycetota*, was the predominant genus, and its abundance significantly increased 15.3 % from day 90 to day 150 ($p < 0.05$, Fig. S8). A previous study reported that *Nocardioides* exhibited the significant capacity to degrade polycyclic aromatic hydrocarbons (PAHs) to form diols during the aerobic microbial degradation [32]. In addition, the abundances of *Streptomyces* and *Microbacterium*, belonging to phylum *Actinomycetota*, increased 4 % and 17.5 % in the soils spiked with HFPO-DA (Fig. S8), respectively. The previous study reported *Streptomyces* could catalyze naphthalene into benzoic acid, and *Microbacterium* could be involved in oxidation degradation of naphthalene and di-2-ethylhexyl phthalate to split benzene ring catalyzed by dioxygenase and monooxygenase [33, 34]. Meanwhile, *Microbacterium* was capable of transforming phthalic acid via the successive decarboxylation and hydroxylation [33]. It is worthy to note that, despite the low increase in the abundance (1.15 %) of *Rhodococcus* (belonging to phylum *Actinomycetota*), this genus was verified to degrade 6:2 FTS and 6:2 FTOH in the soils [35, 36]. These results provided the key information on potential role of genus *Nocardioides*, *Streptomyces*, *Microbacterium* and *Rhodococcus* in the aerobic microbial degradation of HFPO-DA, and further studies are warranted to verify the degradation capacities of these genera via the isolation from the soils.

3.4. Identification of potential functional genes and hosts

Potential Functional Genes. Metagenomics analysis was used to identify the potential functional genes associated with aerobic microbial degradation of HFPO-DA. Monooxygenase, dehalogenase, dehydrogenases and hydrolases were reported to be directly involved in the microbial degradation of PFASs, such as splitting of ether bond, defluorination, elimination of fluoroalkyl and cleavage of C-C bonds [14, 37]. Meanwhile, the transmembrane transport, energy metabolism, and signal transduction had also been demonstrated to play the important roles on the degradation of halogenated compounds [37–39]. In order to comprehensively screen the potential functional genes, a total of 19 genes associated with transmembrane transport, xenobiotics degradation and energy metabolism were analyzed for the microbial degradation of HFPO-DA in aerobic soils (Fig. 4a).

Transmembrane transport of compounds into the cells was an important step prior to their biodegradation with the related intracellular enzymes within the microorganisms [40]. Thus, the genes regulating the transmembrane transport of compounds was investigated in the soils during the microbial degradation of HFPO-DA. An enrichment in the genes associated with ABC-2 type transport system ATP-binding protein (ABC-2. A, K01990) and ABC-2 type transport system permease protein (ABC-2. P, K01992) were observed during the incubation (Fig. 4a). It was documented that organic pollutants could be transported across membranes into microbial cells via the ABC transporters, and then degraded by the intracellular enzymes [38]. Overall, these results indicated that K01990 and K01992 were also the important genes regulating the transmembrane transport for the aerobic microbial degradation of HFPO-DA in the soils.

Oxidoreduction was widely reported as the key reaction step for the aerobic microbial degradation of emerging PFASs, such as fluoroalkylether substances, fluorotelomer sulfonic acid, sulfonamide amine oxide, and sulfonamide alkylbetaine [14, 18]. As shown in Fig. 4a, the genes *pmoA* (K10944), *pmoB* (K10945) and *pmoC* (K10946) encoding monooxygenase were significantly upregulated ($p < 0.05$) after the exposure to HFPO-DA in the soils. Genes *CYP199A2* (K22553) encoding

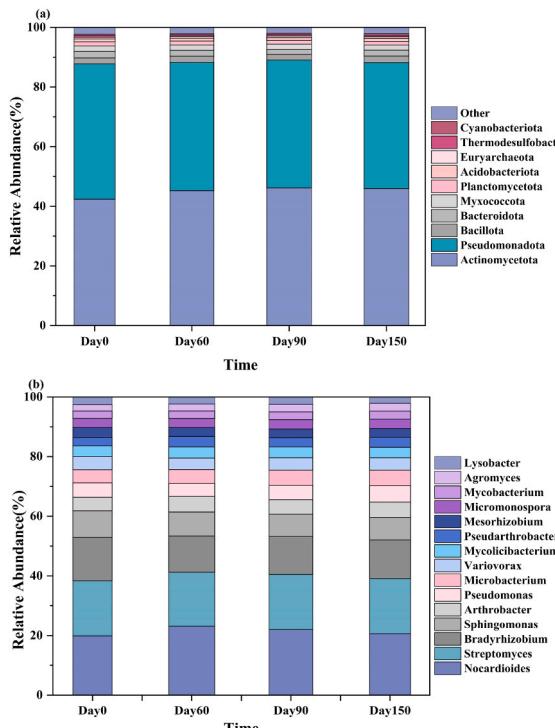


Fig. 3. Microbial community compositions and relative abundances at phylum (a) and genus level (b) in soil after exposure to HFPO-DA.

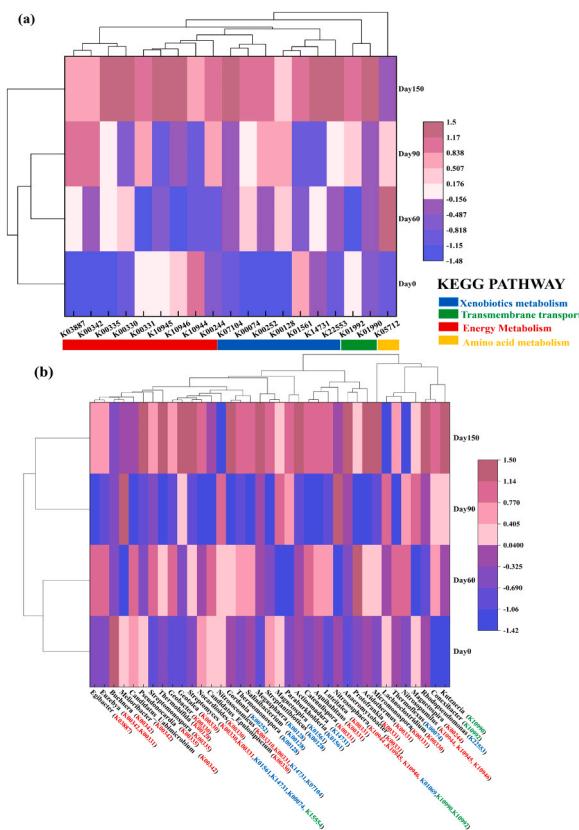


Fig. 4. Heatmap profiles showing relative abundances of potential functional genes (a) and related hosts at genus level (b) in the soils during HFPO-DA degradation.

4-methoxybenzoate monooxygenase showed the significant increase ($p < 0.05$) after exposure to HFPO-DA in the soils (Fig. 4a). It was documented that 4-methoxybenzoate monooxygenase participated in the O-demethylation of benzoate during microbial degradation [41]. Meanwhile, monooxygenase was reported to be involved into the ether bond splitting of fluoroalkylether substances (ether PFASs) during the microbial degradation [14]. Coupled with the products from the oxidation and cleavage of ether bond (Fig. 2), *pmoA*, *pmoB*, *pmoC* and *CYP199A2* were regarded as the potential functional genes encoding monooxygenase in HFPO-DA biotransformation. Gene *catE* (K07104) encoding catechol 2,3-dioxygenase also showed the enrichment after the exposure to HFPO-DA in the soils (Fig. 4a). Gene *catE* (K07104) was reported to be involved in the oxidization of alcohol into formate and dienolate during the biodegradation of aromatic compounds (Fig. S9) [42], which may corresponded to the formation of TFA/PFPrA (Fig. 2). Thus, it is speculated that *catE* was also the potential functional genes for microbial degradation of HFPO-DA. Dehydrogenase was documented as one kind of key enzymes participating in the redox degradation of PFASs [37]. As shown in Fig. 4a, these genes of *paaH*(K00074), *gcdH* (K00252), *ALDH* (K00128) and *frdA* (K00244) encoding dehydrogenase were significant upregulated ($p < 0.05$) after exposure to HFPO-DA in the soils. *paaH* (K00074) encoding 3-hydroxybutyryl-CoA dehydrogenase was reported to participate in the oxidation of hydroxy acid to dicarboxylic acids during the microbial degradation of benzoic acid [43]. *ALDH* (K00128) encoding aldehyde dehydrogenase (NAD^+) could effectively oxidize aldehydes to carboxylic acids, and had been shown to degrade halogenated pollutants, such as chlorophenols, and fipronil [44,45]. Thus, *ALDH* (K00128) was an important gene associated with the formation of short-chain PFASs (TFA and PFPrA) from HFPO-DA during the aerobic microbial degradation. Glutaryl-CoA, an electron-transfer flavoprotein 2,3-oxidoreductase, was encoded by *gcdH* (K00252), and

could promote the decarboxylation and release CO_2 . This enzyme might involve into the decarboxylation to form intermediate (Fig. 2). In addition, the genes encoding dehalogenase were also predicated for the defluorination of HFPO-DA (Fig. 4a). Based on the previous studies, *dehH* (K01561) encoding haloacetate dehalogenase, 2-HAD (K01560) encoding for 2-haloacid dehalogenase, and *dhaA* (K01563) encoding haloalkane dehalogenase could participate in the defluorination and metabolic alkane degradation [46]. Recently, *dehH* (K01561), 2-HAD (K01560) and *dhaA* (K01563) were identified as the functional genes for the defluorination of fluorotelomer sulfonic acids and fluorotelomer alcohols [46]. Accompanied by the enrichment of fluoride ion (Fig. S4) and generation of defluorinated products (Fig. 2), the high abundances of these dehalogenation genes should result into the defluorination of HFPO-DA during the microbial degradation (Fig. 4a). Enzymes involved in the hydrolysis and hydroxylation were also explored for the microbial degradation of HFPO-DA. Specifically, *chnC* (K14731) encoding epsilon-lactone hydrolase and *mphA* (K05712) encoding 3-(3-hydroxy-phenyl) propionate hydroxylase were significantly upregulated ($p < 0.05$) during the microbial degradation of HFPO-DA (Fig. 4a). These results indicated that the potential roles of genes *chnC* and *mphA* in the hydrolyzation and hydroxylation during the microbial degradation of HFPO-DA in aerobic soils, which need the further verifications in the future studies.

Energy is essential for the microbial degradation of organic pollutants, which is mainly provided by the energy metabolism in the microorganisms. A series of genes associated with energy metabolism, including *AtpE* (K03887), *nuoM* (K00342), *nuoF* (K00335), *nuoA* (K00330) and *nuoB* (K00331), were all upregulated during the microbial degradation of HFPO-DA (Fig. 4a). Based on the KEGG enrichment analysis, these genes were mainly assigned to the oxidative phosphorylation pathway (Fig. S10), which could release large amounts of ATP as the energy source for microbial degradation of HFPO-DA. Recently, it was found that natural microorganisms could enhance the degradation of fluorides via regulating phosphatases for energy generation, which reduced the toxicity to cells [40]. Thus, *AtpE*, *nuoM*, *nuoF*, and *nuoA* were inferred as the important functional genes, which was associated with the energy supply to drive the microbial degradation of HFPO-DA.

Host of Potential Functional Gene. The hosts responsible for the microbial degradation of HFPO-DA were investigated by linking the functional genes discussed above to the microorganisms. At the genus level, a total of 37 hosts harboring these functional genes associated with HFPO-DA degradation were screened (Fig. 4b). Among them, 21 functional genes hosts were identified in phylum *Actinomycetota*, which was consistent with the microbial community analysis, further verifying the important role of *Actinomycetota* in the microbial degradation of HFPO-DA. The rest hosts belonged to phylum *Bacillota*, *Myxococcota*, *Planctomycetota*, *Acidobacteriota* and *Thermodesulfobacteriota*, and *Pseudomonadota*.

In order to get insights into the microbial degradation of HFPO-DA, the hosts with increase in the abundances were analyzed in the following discussions. For phylum *Actinomycetota*, genus *Streptomyces* harbored the energy metabolism genes (K00330 and K00331), haloacetate dehalogenase gene (K01561), epsilon-lactone hydrolase (K14731), 3-hydroxybutyryl-CoA dehydrogenase gene (K00074), and sulfonate transport system permease protein gene (K15554), and its abundance increased by 1.08 times after exposure to HFPO-DA for 60 days and 1.12 times for 150 days (Fig. 4b). This genus had been reported to degrade organochlorinated pesticides [47,48]. Meanwhile, K01561 could initiate the defluorination, and genus *Streptomyces* might participate in the defluorination of HFPO-DA. The genus *Gordonia* was predicted to possess the electron-transfer flavoprotein 2,3-oxidoreductase gene (K00252), which was reported to be associated with the oxidation of 6:2 FTSA in the aerobic sludge [18]. This result implied that genus *Gordonia* was the potential oxidative degrader of HFPO-DA. For phylum *Myxococcota*, genus *Anaeromyxobacter* carried the energy metabolism gene (K00331). Genus *Anaeromyxobacter* were reported to involve in the

reductive dehalogenation of 2-chlorophenol, and energy supply was important for the reductive dehalogenation [49]. In phylum *Thermodesulfobacteriota*, genus *Geotalea* also possessed the energy metabolism gene (K00330), which was previously reported to catalyze the methylation of benzene [50]. Genus *Megasphaera* (phylum *Bacillota*) and *Thermoflexus* (phylum *Chloroflexota*) were the hosts of aldehyde dehydrogenase gene (K00128) and 3-hydroxybutyryl-CoA dehydrogenase gene (K00074), respectively, which both initiated the oxidation. Although no studies have reported their roles in xenobiotic degradation, the functional genes revealed that genus *Megasphaera* and *Thermoflexus* might participate in the oxidation of HFPO-DA, which need further studies to verify. Overall, these results provided the key information that these potential hosts were possible contributors of the microbial degradation of HFPO-DA in the aerobic soils.

3.5. Microbial co-occurrence network

In order to deepen understanding the interactions among microorganisms for microbial degradation of HFPO-DA, the microbial co-occurrence network was constructed in the soils (Fig. 5). The constructed network contained 136 nodes and 3621 edges, and each node and edge represented one genus and the strong relationship (Spearman correlation coefficient $R > 0.7$), respectively. As shown in Fig. 5a, the nodes were distributed in 10 phyla, during which *Pseudomonadota* (50.0 %) and *Actinomycetota* (40.4 %) were the major phyla, and followed by *Bacillota* (2.9 %) and *Myxococcota* (2.2 %). The high abundances and connectivity between *Pseudomonadota* and *Actinomycetota* suggested their potential interaction for HFPO-DA degradation in the soil microcosm.

Overall, all nodes in the constructed co-occurrence network were categorized into 4 modules (Fig. 5b). Module 1 was the largest module

with 53 nodes, and showed the high microbial diversity with the covering 7 phyla. Genus *Dacylosporangium*, *Agromyces* and *Gordonia* within phylum *Actinomycetota*, and genus *Rhodococcus* and *Rhodoplanes* within phylum *Pseudomonadota* behaved as the key modular hubs of Module 1. Genus *Dacylosporangium*, *Agromyces*, *Gordonia* were reported to be responsible for the microbial degradation of PAHs [51–53]. Genus *Rhodococcus* was documented as the potential degrader of PFASs [35], and *Rhodoplanes* was widely detected in the PFAS contaminated soils [37]. Members in the genus *Rhodoplanes* were also reported to be associated with biodegradation of PAHs and polybrominated diphenyl ethers (PBDEs) [54,55]. In addition, genus *Nitrosocosmus* within phylum *Thaumarchaeota* also acted as the module hub in Module 1, which was the well-known ammonia-oxidizing archaeon involved in nitrogen metabolism in the soils [55]. In the previous studies, micro-organisms associated with nitrogen metabolism were found to play important roles in the assistance with members which could degrade organic pollutants [56].

In Module 2, genus *Diaphorobacter*, *Anaeromyxabacter*, *Luteitalea* and *Nocardioides* acted as module hubs. Genus *Diaphorobacter* has been characterized as degrader of PAHs, and phenanthrene under the aerobic conditions [57,58]. Little information is available on the roles of genus *Anaeromyxabacter*, *Luteitalea* and *Nocardioides* in the degradation of pollutants, which were warranted further studies. In Module 3, genus *Streptomyces*, *Micromonospora* and *Bacillus* behaved as the modular hubs. Genus *Streptomyces* and *Micromonospora* were identified as the hosts of potential functional genes associated with microbial degradation of HFPO-DA in the above discussions. Genus *Bacillus* was found to degrade PAHs and polychlorinated biphenyl [59,60]. These results indicated that microbial degradation of HFPO-DA could be co-metabolic via the cooperation of different microorganisms. Genus *Rhizobium* was observed as the potential keystone taxa in Module 4. Recently, the strain *Rhizobium* JF-3 was reported to be capable of degrading 4-fluoroaniline by monooxygenase [61]. Thus, genus *Rhizobium* might also involve in the microbial degradation of HFPO-DA, which need further studies.

Overall, the constructed co-occurrence network provided a new sight on microbial degradation of HFPO-DA biodegradation, indicating that the degradation of HFPO-DA required the interactions of multiple microorganisms. It should be noted that many degraders of aromatic compounds and halogen compounds were also involved into the degradation of HFPO-DA, and thus the co-metabolism might occur between transformation of HFPO-DA and other pollutants. Further investigations are warranted to target the specific functions of these potential keystone taxa in the microbial degradation of HFPO-DA.

4. Conclusions

This study broke the traditional cognition that HFPO-DA was resistant to microbial degradation, and provided the first systematic characterization of microbial degradation of HFPO-DA in the aerobic soils. During the long-period incubation of 150 days, about $51.6 \pm 3.0\%$ of HFPO-DA was degraded by the indigenous aerobic microorganisms in soils. In total, 3 transformation products were identified based on the nontarget screening analysis, including a novel product (2-trifluoromethyl-2-(difluoromethoxy) acetic acid), which enriched our understanding on the microbial degradation behaviors of HFPO-DA. In addition, TFA was detected as the terminal product, which caused the increasing concerns around the world due to its ubiquitous detection and high persistence. Based on the transformation products, the multiple degradation pathways were involved in the microbial degradation of HFPO-DA, including defluorination, decarboxylation, hydroxyl oxidation, hydrolysis and C-C bond cleavage. In addition, 19 potential functional genes and 37 functional gene hosts were screened for their contributions to HFPO-DA degradation. Meanwhile, genera *Gordonia*, *Megasphaera*, and *Streptomyces* were found as the potential keystone taxa in microbial degradation of HFPO-DA. Overall, the results of this study have the important implications for the development of bioremediation

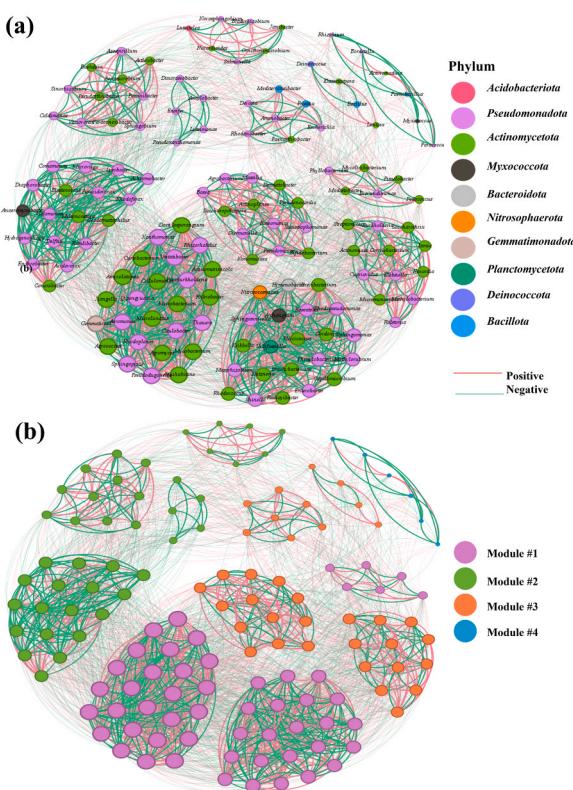


Fig. 5. Microbial co-occurrence networks for phylum (a) and module (b) in the soils after the exposure to HFPO-DA. Edge: each edge represents a strong correlation (Spearman coefficient > 0.7), with red for positive correlation and green for negative correlation, and thickness for degree of correlation. Node: each node represents a genus.

strategies and regulations associated with HFPO-DA contaminated sites, which promote the implementation of the Action Plan on Controlling New Pollutants in China.

It should be noted that the potential functional genes found in this study should be verified in the microbial degradation of HFPO-DA via the gene knockout in the future studies. Meanwhile, further studies are warrant to confirm the degradation capacities of the hosts of potential functional for HFPO-DA via the isolation of hosts from the soils. Additionally, the enzymatic studies are needed to explore the key enzymes associated with the HFPO-DA degradation in the microorganisms. It is imperative to resolve these knowledge gaps to inform policies toward the management and remediation of emerging PFASs in the world.

Environmental implication

This study provided first evidence that HFPO-DA, a typical novel alternative of PFOA, could be degradation by aerobic microbes. Degradation pathways, and potential functional genes and hosts were revealed for HFPO-DA, which was meaningful for bioremediation of contaminated sites.

CRediT authorship contribution statement

Guangzhi Rong: Visualization, Validation, Software. **Haixia Wang:** Visualization, Resources, Methodology. **Yu Liu:** Writing – original draft, Supervision, Funding acquisition. **Guoguang Wang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Ziao Xing:** Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Shuaihao Liu:** Visualization, Validation, Software, Resources, Formal analysis. **Hongyi Xue:** Visualization, Software, Resources, Investigation. **Yana Wang:** Visualization, Validation, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.139880](https://doi.org/10.1016/j.jhazmat.2025.139880).

Data availability

Data will be made available on request.

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