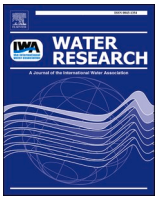
Water Research 249 (2024) 120941

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



Aerobic biotransformation of 6:2 fluorotelomer sulfonate in soils from two aqueous film-forming foam (AFFF)-impacted sites

Peng-Fei Yan [a,*], Sheng Dong [a], Katherine E. Manz [b], Matthew J. Woodcock [b], Chen Liu [b], Melissa P. Mezzari [c], Linda M. Abriola [b], Kurt D. Pennell [b], Natalie L. Cápiro [a,*]

[a] Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA
[b] School of Engineering, Brown University, Providence, RI, USA
[c] Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA

ARTICLE INFO

Keywords:
6:2 fluorotelomer sulfonate
FTS
AFFF
PFAS
Microbial community
Biotransformation

ABSTRACT

Although 6:2 fluorotelomer sulfonate (6:2 FTS) is a common ingredient in aqueous film-forming foam (AFFF) formulations, its environmental fate at AFFF-impacted sites remains poorly understood. This study investigated the biotransformation of 6:2 FTS in microcosms prepared with soils collected from two AFFF-impacted sites; the former Loring Air Force Base (AFB) and Robins AFB. The half-life of 6:2 FTS in Loring soil was 43.3 days; while >60 mol% of initially spiked 6:2 FTS remained in Robins soil microcosms after a 224-day incubation. Differences in initial sulfate concentrations and the depletion of sulfate over the incubation likely contributed to the different 6:2 FTS biotransformation rates between the two soils. At day 224, stable transformation products, i.e., C_4—C_7 perfluoroalkyl carboxylates, were formed with combined molar yields of 13.8 mol% and 1.2 mol% in Loring and Robins soils, respectively. Based on all detected transformation products, the biotransformation pathways of 6:2 FTS in the two soils were proposed. Microbial community analysis suggests that Desulfobacterota microorganisms may promote 6:2 FTS biotransformation via more efficient desulfonation. In addition, species from the genus *Sphingomonas,* which exhibited higher tolerance to elevated concentrations of 6:2 FTS and its biotransformation products, are likely to have contributed to 6:2 FTS biotransformation. This study demonstrates the potential role of biotransformation processes on the fate of 6:2 FTS at AFFF-impacted sites and highlights the need to characterize site biogeochemical properties for improved assessment of 6:2 FTS biotransformation behavior.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have been used in a wide variety of industrial and consumer products owing to their unique properties (e.g., water- and oil-repellency, surface tension reduction) (Buck et al., 2011). However, concerns over the impacts that these compounds may pose to environmental and human health are growing (Grandjean and Clapp, 2015). Aqueous film-forming foams (AFFFs), which were commonly used to extinguish hydrocarbon-based fuel fires at airports and military bases (Moody and Field, 2000), were identified as a major source of PFAS in the environment (Baduel et al., 2015; Houtz et al., 2013). 6:2 fluorotelomer sulfonate (6:2 FTS) has been identified as a component of AFFF formulations (Backe et al., 2013; Herzke et al., 2012; Schultz et al., 2004). Moreover, 6:2 FTS is a major transformation product of other PFAS compounds in AFFFs, such as 6:2 fluorotelomer thioether amido sulfonates (6:2 FtTAoS) and 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB) (Harding-Marjanovic et al., 2015; Moe et al., 2012). As a consequence, 6:2 FTS has been widely detected at AFFF-impacted sites and in the nearby terrestrial and aquatic ecosystems (Backe et al., 2013; Houtz et al., 2013), with concentrations up to 14,600 μg/L and 2,101 μg/kg reported in water and soil, respectively (Kärrman et al., 2011; Schultz et al., 2004). Furthermore, the concentration of 6:2 FTS is anticipated to increase at AFFF-impacted sites, consistent with the shift to global production of shorter-chain length fluorotelomer-based AFFFs containing 6:2 FTS or its precursors (Herzke et al., 2012; Moe et al., 2012).

Biotransformation of 6:2 FTS has been reported by pure bacterial strains (e.g., *Gordonia* sp. strain NB4-1Y, *Rhodococcus jostii* RHA1, and *Dietzia aurantiaca* J3) (Méndez et al., 2022; Van Hamme et al., 2013; Yang et al., 2022b), and various mixed microbial cultures from activated

* Corresponding authors at: Department of Biological and Environmental Engineering, Cornell University, 111 Wing Dr., Ithaca, NY 14853, USA.
E-mail addresses: py222@cornell.edu (P.-F. Yan), natalie.capiro@cornell.edu (N.L. Cápiro).

https://doi.org/10.1016/j.watres.2023.120941
Received 28 July 2023; Received in revised form 14 November 2023; Accepted 28 November 2023
Available online 29 November 2023

sludge (Wang et al., 2011), river sediment (Zhang et al., 2016), wetland slurry (Yin et al., 2019), landfill leachate (Hamid et al., 2020), and soils (Chen et al., 2019; Yang et al., 2022a). However, large discrepancies in the 6:2 FTS biotransformation rate and product formation were documented across those studies. For example, the half-life of 6:2 FTS was less than 5 days in the river sediment, but approximately 86 days in landfill leachate sediment and 2 years in activated sludge (Hamid et al., 2020; Wang et al., 2011; Zhang et al., 2016). Under sulfur-limiting conditions, rapid biotransformation of 6:2 FTS (>99 mol% depletion after 7 days) was achieved by pure bacterial cultures, while no biotransformation was observed in the presence of other sulfur sources (e.g., sulfate) (Méndez et al., 2022; Yang et al., 2022b). Although polyfluorinated compounds such as 5:3 fluorotelomer carboxylic acid (5:3 acid) and short chain perfluoroalkyl carboxylates, including perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA), were commonly reported as biotransformation products of 6:2 FTS, the detection frequencies and molar yields of those products varied among prior studies (Hamid et al., 2020; Wang et al., 2011; Yang et al., 2022b; Zhang et al., 2016). These findings suggest that the biotransformation of 6:2 FTS is likely dependent on the environmental matrix. Thus, more research is needed to improve our understanding of 6:2 FTS biotransformation in various environments, and to explore the role of different microbial communities involved in the biotransformation. Bridging these knowledge gaps will provide valuable insights into the assessment of 6:2 FTS fate and behavior in the environment.

Despite the widespread occurrence and anticipated increased concentration at AFFF-impacted sites, the environmental fate of 6:2 FTS at those sites remains poorly understood. Harding-Marjanovic et al. (2015) demonstrated that a native AFFF-impacted soil microbial community biotransformed 6:2 FtTAoS to 6:2 FTS, which was further transformed to products including 5:3 acid, PFPeA and PFHxA. However, the rate and pathway of 6:2 FTS biotransformation, as well as the role of the microbial community in the biotransformation, were not elucidated. Therefore, the present study aims to (a) examine 6:2 FTS biotransformation (rates, transformation products, and pathways) under conditions representative of AFFF-impacted sites using native microbial communities, and (b) explore soil microbial community dynamics during the 6:2 FTS biotransformation. To this end, surface soils collected from two U.S. military bases were used for separate 6:2 FTS microcosm studies. Biotransformation rates and product formation in the two sets of microcosms were examined over a 224-day incubation period. Additionally, the composition shift of two soil microbial communities during 6:2 FTS biotransformation was evaluated, and microbial taxa that potentially played an important role in the biotransformation were identified.

2. Materials and methods

2.1. Chemicals and materials

The chemical names, acronyms, molecular structures, and suppliers of PFAS targeted in liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis are listed in the Supplementary Materials (SM), Table S1. The LC–MS grade water and methanol (>99.9 %) were obtained from Honeywell Burdick & Jackson (Honeywell, Charlotte, NC). All other chemicals were reagent grade or higher. AFFF-impacted soils collected from two military sites, the former Loring Air Force Base (Aroostook County, ME) and Robins Air Force Base (Houston County, GA) were used in this study (referred to hereafter as Loring soil and Robins soil). Detailed information on soil collection, and physical and chemical properties (pH, moisture content, organic matter content, cation exchange capacity (CEC), particle size distribution) are provided in SM-section S1 and Table S2.

2.2. Microcosm set-up and sampling

Two sets of microcosms were constructed in 60-mL Wheaton serum bottles with either Loring or Robins soil. Each set consisted of live treatments, abiotic controls, and positive controls (composition described in Table S3). In each bottle, 3 g (dry weight) of Loring or Robins soil and 30 mL of synthetic groundwater (SM-section S1) were added. For live treatments, each bottle was dosed with approximately 1,700 μg/L of 6:2 FTS prepared in diethylene glycol butyl ether (DGBE), which is a primary organic solvent in AFFF formulations and has also been demonstrated as an electron donor and carbon source in prior microcosm studies (Dong et al., 2023; Yan et al., 2022). The initial concentration of DGBE added into the microcosms was approximately 5 mM. Abiotic controls were prepared similarly to live treatments, except with the addition of 1 g/L NaN_3 to inhibit microbial growth (1 g/L was determined to be effective in preliminary experiments; data not shown). Abiotic controls were used to evaluate potential abiotic transformation of 6:2 FTS and/or background PFAS originally present in Loring and Robins soils. In positive control bottles, only DGBE (no 6:2 FTS) was added to monitor background PFAS and potential PFAS biotransformation in Loring and Robins soils. Throughout the experiments, DGBE was amended into the live treatment and positive control microcosms as needed to support the microbial growth (Fig. S1). All bottles were crimp-sealed with rubber septa and aluminum caps (Chemglass; Vineland, NJ). One C_{18} cartridge (Maxi-Clean™, Alltech, Deerfield, IL) pre-conditioned with methanol, was connected to an 18 G × 1" needle that was pushed through the septum into the headspace of each bottle. The cartridge ensured sufficient aeration, and captured potential volatile transformation products (Zhao et al., 2013). All bottles (98 bottles in total; each soil set comprised of 21 bottles for live treatment, 14 bottles for abiotic control, and 14 bottles for positive control) were continuously agitated at 150 rpm on an orbital shaker (Innova 2350, New Brunswick Scientific) at room temperature until sampling.

On days 0, 7, 28, 56, 98, 154, and 224, triplicate bottles from live treatments, and duplicate bottles from abiotic and positive controls were destructively sampled. The headspace of each bottle was initially purged through the C_{18} cartridge using an aquarium air pump (Imagitarium, Petco Brand). The cartridges were then eluted with 5 mL methanol for subsequent PFAS analysis described below. To ensure the efficiency of C_{18} cartridges during the long-term incubation (i.e., 224 days), new C_{18} cartridges were installed to replace the old ones on the remaining bottles on days 28, 56, 98, 126, 154, and 189. The cartridge eluents from each bottle were combined to account for the total volatile PFAS captured over the course of the experiment. After headspace sampling, supernatant (0.5-mL) was collected from each bottle and immediately added into methanol (9.5-mL) to minimize potential loss of volatile PFAS. The diluted sample was then filtered through a 0.22-μm nylon filter (Corning Inc., Corning, NY). Each bottle was then shaken vigorously, and well-mixed slurry (1-mL) was withdrawn for the measurements of dissolved organic carbon (DOC) and sulfate (SM-section S1). All remaining slurry was transferred into a 50-mL centrifuge tube, along with the rubber septum. The bottle was rinsed with 5 mL ultrapure water and the rinse water was decanted into the 50-mL tube. The tube was then centrifuged at 4,000 rpm for 20 min, and the supernatant was discarded. The soil pellet was resuspended with 30 mL methanol and vortexed for 30 min, followed by 30 min sonication (operating frequency of 35 kHz) at 60 °C. The methanol extract from soil and septum were collected after centrifugation, and further filtered through a 0.22-μm nylon filter. The C_{18} cartridge eluents, and the filtrates of methanol-diluted aqueous samples and methanol extracts of soil and septum were stored at −20 °C prior to PFAS analysis.

2.3. PFAS analysis

PFAS analysis was performed using a Waters ACQUITY ultra-performance liquid chromatography system coupled with a Waters Xevo TQ-S Micro triple quadrupole mass spectrometer (UPLC-MS/MS) (Waters Corporation, Milford, MA). Samples from each of the three phases (i.e., headspace, aqueous, and solid phases) were analyzed by LC–MS/MS separately. Target PFAS analytes are listed in Table S1. Perfluoroalkyl acids (PFAAs) were analyzed following established methods (McCord et al., 2018), while polyfluorinated compounds were analyzed using a method (Szostek et al., 2006) with no ammonium acetate addition to mobile phases. A different method was used for polyfluorinated compounds because fluorotelomer alcohols (e.g., 6:2 FTOH) that are potential transformation products of 6:2 FTS could form adducts with ammonium acetate under negative electrospray ionization. In both methods, analyte separation was achieved using a Waters ACQUITY UPLC BEH C18 Column (130 Å, 1.7 μm, 2.1 mm X 50 mm). Details on the solvent gradient and instrumental parameters used in each targeted method are provided in Tables S4-S7. The detection limits of target PFAS are provided in Table S8. Information on the procedures taken to minimize matrix effects on PFAS analysis, and the steps taken to verify target PFAS recovery throughout the experimental procedure are provided in SM-Section S1.

2.4. Microbial community analysis

To gain insight into the role of native microbial communities in 6:2 FTS biotransformation in AFFF-impacted soils, duplicate microcosm samples at two sampling points (day 0 and 224) were collected from Loring and Robins live treatments and positive controls for microbial community analysis. Since the quantity of DNA extracted from Robins soil microcosms on day 0 was too low to be amplified, day 7 samples from the positive control (i.e., biostimulation with DGBE) were used as the initial samples. Amplification and sequencing of Loring and Robins soil DNA samples were performed at the Alkek Center for Metagenomics and Microbiome Research at Baylor College of Medicine, following the procedures detailed in our recent study (Dong et al., 2023).

3. Results and discussion

3.1. Experimental system

The consumption of DGBE was monitored throughout the experiments conducted in Loring and Robins soil bioactive microcosms (i.e., live treatments and positive controls), which were amended 4–5 times with 10 mM DGBE (Fig. S1). No change in DOC concentration was detected in abiotic controls (Fig. S1). These results indicated that aeration was sufficient to support DGBE biodegradation by the aerobic microorganisms in both soils. In addition, no differences in the consumption rates of DGBE were observed between live treatments and positive controls (Fig. S1), suggesting that the addition of 6:2 FTS did not negatively impact substrate consumption by the native microbial community.

Background levels of 6:2 FTS and potential transformation products (Wang et al., 2011; Zhang et al., 2016) in the Loring and Robins soils were determined from the day 0 positive control samples (Table S9). Additional discussion on the occurrence of background PFAS in the two AFFF-impacted soils is provided in SM-section S2. The total mass of 6:2 FTS initially spiked into the live treatments and abiotic controls was approximately 180-fold and 22-fold greater than the background 6:2 FTS (this term refers to the total mass of 6:2 FTS originally present) in the Loring and Robins soils, respectively (Tables S10 and S11). Such spiked amounts allowed for the identification and quantification of 6:2 FTS transformation products. As shown in Fig. 1, 102.9 ± 5.5 mol% and 95.9 ± 6.6 mol% of the initially spiked 6:2 FTS remained in the Loring and Robins abiotic controls, respectively, with no significant changes ($p > 0.05$) over the 224-day incubation period. This finding demonstrates the integrity of the experimental system and the efficiency of extraction methods used in this study, and that 6:2 FTS was stable under the abiotic experimental conditions.

3.2. Biotransformation of 6:2 FTS in Loring and Robins soil microcosms

3.2.1. Biotransformation rates

In the Loring soil microcosm live treatments, rapid 6:2 FTS biotransformation was observed with only 0.2 mol% of the initially spiked 6:2 FTS remaining after 154 days (Fig. 1A). The half-life was determined to be 43.3 days by fitting 7 data points using a first-order kinetic model ($R^2 = 0.98$). Biotransformation of background 6:2 FTS was also observed in positive controls, where the total mass of 6:2 FTS decreased from 0.8 ± 0.2 nmol at day 0 to 0.0 ± 0.0 nmol at day 28 (Table S10). In contrast, much slower 6:2 FTS biotransformation was observed in Robins soil, with 63.7 ± 6.0 mol% of initially spiked 6:2 FTS remaining in live treatments after the 224-day incubation (Fig. 1B). Similarly, background 6:2 FTS in positive controls was biotransformed slowly, with 7.0 ± 2.0 nmol remaining at day 98 (8.3 ± 0.3 nmol at day 0, Table S11). The measurement of background 6:2 FTS biotransformation in the Loring and Robins soils suggests that a lack of electron

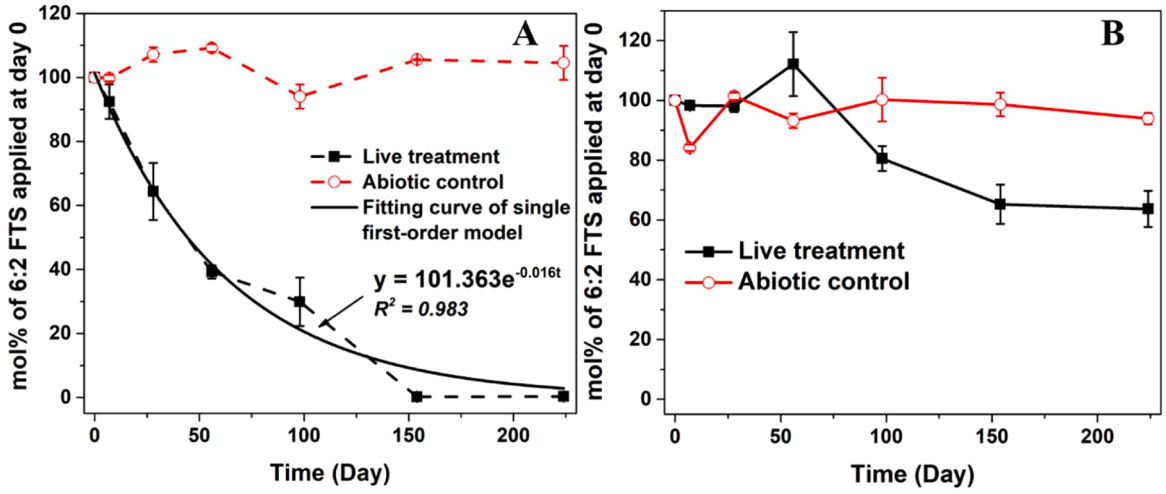


Fig. 1. Changes in molar ratios of residual 6:2 FTS during aerobic biotransformation in Loring (Panel A) and Robins (Panel B) soil microcosms. Error bars represent the standard error of triplicate live treatments and duplicate abiotic controls.

donor, carbon source, and/or nutrients limited natural attenuation of 6:2 FTS at the field sites.

3.2.2. *Biotransformation products quantified by LC–MS/MS analysis*

Four polyfluorinated compounds, including 6:2 fluorotelomer saturated carboxylic acid (6:2 FTCA), 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUA), 5:2 secondary fluorotelomer alcohol (5:2 sFTOH) and 5:3 acid, and four perfluorinated compounds (perfluorobutanoic acid-PFBA, PFPeA, PFHxA, and perfluoroheptanoic acid-PFHpA), were identified as biotransformation products of 6:2 FTS in the Loring soil using LC-MS/MS analysis. Significant increases ($p < 0.05$) in the mass of these compounds were detected in live treatments compared to abiotic and positive controls (Table S10). Formation of 6:2 FTUA and 5:2 sFTOH in positive controls confirmed the occurrence of background 6:2 FTS biotransformation in the Loring soil (Table S10). An initial intermediate of 6:2 FTS biotransformation reported previously (Zhang et al., 2016), 6:2 FTOH, was below the limit of detection (LOD), suggesting that it was rapidly converted to downstream products. This finding is consistent with observations in activated sludge (Wang et al., 2011) and river sediment (Zhang et al., 2016), where 6:2 FTOH was absent or detected at low levels (<2.5 mol%). Similarly, low levels of 6:2 FTCA (<0.5 mol%) were detected over the 224-day incubation likely due to its rapid biotransformation to 6:2 FTUA, which peaked at day 28 (3.7 ± 0.5 mol%) and decreased to less than 0.5 mol% by day 224 (Fig. 2A). As a major intermediate product reported previously (Zhang et al., 2016), 5:2 sFTOH concentrations increased throughout the incubation period, reaching a peak molar yield of 13.3 ± 5.1 mol% by day 224 (Fig. 2A). The majority of the 5:2 sFTOH was measured in the headspace of the microcosm bottles. As an example, >75 % of 5:2 sFTOH was in the headspace between day 28 and day 224, and thus, a majority of the 5:2 sFTOH was likely unavailable for further biotranformation by the Loring soil microbial community. Another major transformation product of 6:2 FTS (Wang et al., 2011; Zhang et al., 2016), 5:3 acid, reached its peak molar yield at 8.9 ± 1.2 mol% by day 154 then decreased to 0.2 ± 0.1 mol% at day 224 (Fig. 2A). The biotransformation of 5:3 acid was reported previously in activated sludge via "one--carbon removal pathways", leading to the formation of two major products (4:3 acid and PFPeA) and two minor products (3:3 acid and PFBA) (Wang et al., 2012). In the present study, the substantial decrease in 5:3 acid and increases in PFPeA and PFBA between days 154 and 224 indicated that reactions in "one-carbon removal pathways" likely occurred in the later stage of incubation (Fig. 2A). However, 4:3 acid could not be quantified due to the absence of an authentic standard, while the formation of 3:3 acid was not found to be significant ($p > 0.05$) in live treatments (Table S10). Neither 4:3 acid nor 3:3 acid was identified in subsequent non-targeted PFAS analysis (SM-section S1), suggesting that both compounds were not formed or formed with an extremely low molar yield that cannot be distinguished from the background levels. PFBA, PFPeA, and PFHxA were the major stable transformation products in Loring soil with molar yields of 1.3 ± 0.5 mol%, 7.0 ± 2.7 mol%, and 5.3 ± 0.7 mol% by day 224, respectively (Fig. 2B). Low amounts of PFHpA (0.2 ± 0.1 mol%) were also formed. The detection of PFPeA and PFHxA as the most abundant terminal products is consistent with previous studies of 6:2 FTS biotransformation in

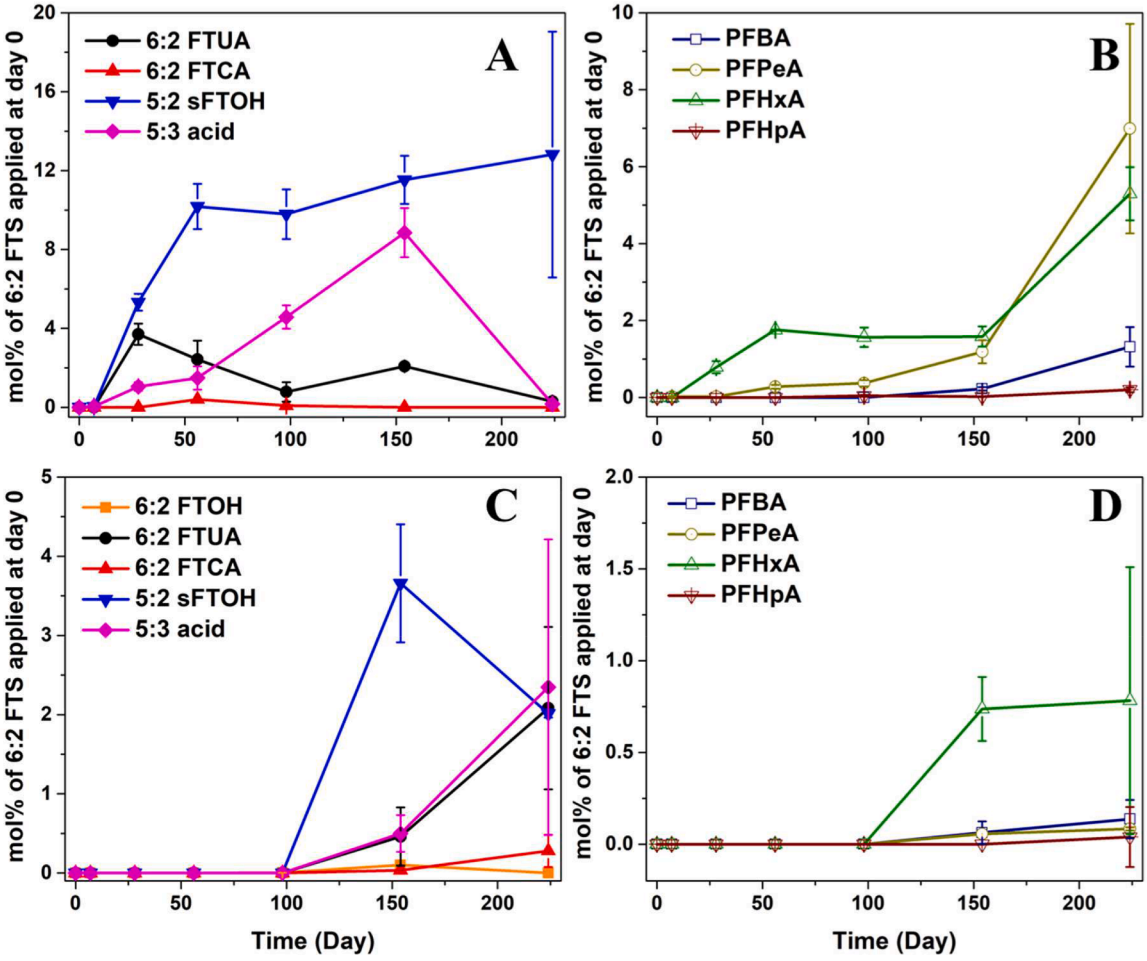


Fig. 2. Changes in concentrations of poly- (Panels A and C) and perfluorinated (Panels B and D) biotransformation products during 6:2 FTS aerobic biotransformation in Loring (Panels A and B) and Robins (Panels C and D) soil microcosms. Error bars represent the standard error of triplicate live treatments. Note that different y-axis scales were used in Panels A–D.

activated sludge (Wang et al., 2011), sediment (Zhang et al., 2016), wetland slurry (Yin et al., 2019), and landfill leachate (Hamid et al., 2020).

Fig. 2C and D show the molar yields of 6:2 FTS biotransformation products in the live treatments of Robins soil microcosms. Similar to the Loring soil, 6:2 FTUA, 6:2 FTCA, and 5:2 sFTOH were detected in positive controls but not in abiotic controls, indicating that biotransformation of background 6:2 FTS occurred in Robins soil (Table S11). In live treatments, trace amounts (<0.01 mol%) of 6:2 FTOH were detected at day 98, which increased to 0.1 ± 0.0 mol% by day 154, while other transformation products were below the LODs until day 154 (Fig. 2C and D). At day 224, 6:2 FTS biotransformation in Robins soil yielded 6:2 FTUA (2.1 ± 1.0 mol%), 6:2 FTCA (0.3 ± 0.2 mol%), 5:2 sFTOH (2.0 ± 0.1 mol%), 5:3 acid (2.3 ± 1.9 mol%), PFBA (0.3 ± 0.1 mol%), PFPeA (0.1 ± 0.0 mol%), and PFHxA (0.8 ± 0.7 mol%). Taken together, the total molar yield of all quantified transformation products in the Robins soil microcosms by day 224 was ca. 8.0 mol%, in contrast to that of ca. 27.1 mol% in the Loring soil microcosms. These results illustrate that 6:2 FTS was biotransformed in the Robins soil at a much slower rate and to a lesser extent than in the Loring soil.

3.2.3. *Comparison of 6:2 FTS biotransformation in AFFF-impacted soils to other environmental matrices and pure cultures*

The 6:2 FTS transformation rates and product formation in the Loring and Robins soils were compared to other environmental matrices and pure cultures reported previously (Table 1). Rapid biotransformation of 6:2 FTS was generally achieved in studies utilizing pure bacterial cultures (*Gordonia* sp. strain NB4-1Y, *Rhodococcus jostii* RHA1, and *Dietzia aurantiaca* J3), with a substantial decrease (i.e., 44–100 mol%) in 6:2 FTS during short incubation periods (<10 days) (Table 1). In contrast, relatively slow 6:2 FTS biotransformation was observed in various environmental matrices including activated sludge, wetland slurry, landfill leachate, and AFFF-impacted soils, with the exception of river sediment where a half-life of <5 days was reported (Table 1). 6:2 FTS biotransformation in Robins soil (63.7 mol% remaining after 224 days) was comparable to that in activated sludge (63.7 mol% remaining after 90 days) and constructed wetlands (91.1 mol% remaining after 142 days), but was lower than that observed in landfill leachate (50 mol% remaining after 90 days; half-life = 86 days) (Table 1). The biotransformation in the Loring soil (half-life = 43.3 days), however, was approximately 2-fold faster than that observed in landfill leachate (Table 1). The presence of more easily assimilated sulfur sources (e.g., sulfate) was reported to impact the 6:2 FTS biotransformation rate by inhibiting the initial step of biotransformation, i.e., enzymatic desulfonation (Key et al., 1998). For example, rapid 6:2 FTS biotransformation was achieved by pure microbial cultures under sulfur-limiting conditions (Méndez et al., 2022; Shaw et al., 2019; Yang et al., 2022b), while the presence of sulfate (10 mM) suppressed the desulfonation process (Yang et al., 2022b). The sulfate present in activated sludge (0.21–0.52 mM) (Wang et al., 2011), landfill leachate (0.32 mM) (Hamid et al., 2020), and wetland slurry (8 mM) (Yin et al., 2019) was also assumed by researchers to cause the observed slow 6:2 FTS biotransformation. In the present study, sulfate concentrations in the Loring and Robins soil microcosms were monitored throughout incubation (Fig. S2). The initial concentration was 3–5 times higher in Robins soil than Loring soil (0.10 ± 0.00 mM and 0.02 ± 0.01 mM, respectively). Sulfate in bioactive Loring soil microcosms was rapidly and completely consumed by day 7, whereas sulfate was not nearly depleted (<0.01 mM) until day 98 in Robins soil (Fig. S2). The time point of the initiation of 6:2 FTS biotransformation (e.g., formation of biotransformation products) appeared to coincide with the time point of sulfate depletion in both microcosms (Figs. 1, 2, and S2; day 7 and day 98 for Loring and Robins microcosms, respectively), indicating that 6:2 FTS biotransformation was favored by the microbial consortium in both soils once sulfur sources were depleted. Therefore, differences in the initial sulfate concentrations and its depletion over the incubation period were likely critical factors resulting in the differing 6:2 FTS biotransformation rates observed between the Loring and Robins soil microcosms. These findings indicate that availability of alternate sulfur sources in the environment could inhibit the desulfonation of 6:2 FTS, making it a limiting step for 6:2 FTS biotransformation.

In addition to biotransformation rates, variations in 6:2 FTS transformation products and yields are observed for different environmental matrices and pure cultures (Table 1). The biotransformation of 6:2 FTS by pure bacterial cultures yielded minimal or negligible amounts of perfluoroalkyl carboxylic acids (PFCAs), whereas the biotransformation by microbial communities in environmental media consistently resulted in PFCAs formation. Although the molar yields of PFCAs varied among the experimental systems, PFPeA and PFHxA were the most abundant (Table 1), and PFBA and PFHpA were not consistently detected as 6:2 FTS biotransformation products. Small amounts of PFHpA were only detected in the studies using river sediment (Zhang et al., 2016), pure strain *Rhodococcus jostii* RHA (Yang et al., 2022b), and in the present study. Polyfluorinated products, including 6:2 FTOH, 6:2 FTCA, 6:2

Table 1
Comparison of 6:2 FTS biotransformation rates and product formation in AFFF-impacted soils to other environmental matrices and pure cultures.

Environmental matrix/ pure culture	Duration (days)	Biotransformation rate (half-life, if applicable)	Biotransformation products at the end of experiment (yield in mol%, if determined)	Reference
Pseudomonas sp. strain D2	1	Not available	Not available	Key et al. (1998)
Gordonia sp. strain NB4-1Y	5	56 mol% remaining at day 5	6:2 FTCA, 6:2 FTUA, 5:3 acid, 5:3U acid	Van Hamme et al. (2013)
Gordonia sp. strain NB4-1Y	7	0.1 mol% remaining at day 7	6:2 FTOH (4.1), 6:2 FTCA (4.3), 6:2 FTUA (13.7), 5:2 sFTOH (9.0), 5:2 ketone (43.9), 5:3 acid (0.4), PFBA (<0.1), PFPeA(<0.1), PFHxA(0.6)	Shaw et al. (2019)
Rhodococcus jostii RHA1	6	<1 mol% remaining at day 6	6:2 FTCA, 6:2 FTUA, α-OH-5:3 acid, PFHpA	Yang et al. (2022b)
Dietzia aurantiaca J3	7	0 mol% remaining at day 6	6:2 FTCA, 6:2 FTUA, 5:3 acid, PFPeA, PFHxA	Méndez et al. (2022)
Activated sludge	90	63.7 mol% remaining at day 90	5:2 ketone & 5:2 sFTOH (3.4), 5:3 acid (0.1), PFBA (0.1), PFPeA (1.5), PFHxA (1.1)	Wang et al. (2011)
River sediment	90	1.9 mol% remaining at day 90 (<5 days)	6:2 FTOH (<2.5), 6:2 FTCA (12), 5:2 sFTOH (<8), 5:2 ketone (<8), 5:3 acid (16), PFPeA(21), PFHxA(20), PFHpA (0.6)	Zhang et al. (2016)
Wetland slurry	142	91.1 mol% remaining at day 142	5:3 acid (2.7), PFPeA (6.1), PFHxA (2.1)	Yin et al. (2019)
Landfill leachate and sediment	90	~50 mol% remaining at day 90 (86 days)	5:2 sFTOH (0.6), 5:3 acid (0.2), PFBA (0.6), PFPeA (5.6), PFHxA (3.1)	Hamid et al. (2020)
Loring soil	224	0.2 mol% remaining at day 224 (43.3 days)	6:2 FTUA (0.3), 5:2 sFTOH (12.8), 5:3 acid (0.2), PFBA (1.3), PFPeA (7.0), PFHxA (5.3), PFHpA (0.2)	This study
Robins soil	224	63.7 mol% remaining at day 224	6:2 FTUA (2.1), 6:2 FTCA (0.3), 5:2 sFTOH (2.0), 5:3 acid (2.3), PFBA (0.3), PFPeA (0.1), PFHxA (0.8)	This study

FTUA, 5:2 sFTOH, 5:2 ketone and 5:3 acid, were commonly reported during 6:2 FTS biotransformation. However, the detection frequency and molar yield of each product varied across these studies (Table 1). Taken together, these results suggest that 6:2 FTS biotransformation in different environments and/or by different microbial communities is likely to undergo different pathways.

3.2.4. *Biotransformation pathways of 6:2 FTS in AFFF-impacted soils*

In the Loring and Robins soil microcosm live treatments, total mass recovery of 6:2 FTS and transformation products generally decreased with incubation time. For Loring soil, mass recovery decreased from 92.5 ± 5.4 mol% at day 7 to 55.8 ± 4.8 mol% at day 56, then further to 25.7 ± 3.2 mol% at day 154, and for Robins soil, recovery decreased from 98.3 ± 1.1 mol% at day 7 to 71.1 ± 8.1 mol% at day 154 (Fig. S3). However, a similar decrease was not observed in the abiotic controls (Fig. S3). The relatively lower mass recovery observed in the live treatments of Loring soil compared with Robins soil can, thus, be attributed to an increased extent of 6:2 FTS biotransformation and the associated formation of unknown transformation products. Non-targeted LC—HRMS analysis was then performed on the samples from each treatment of the Loring and Robins microcosms (SM-section S1), with an attempt to identify unknown 6:2 FTS biotransformation product. Unfortunately, no potential product was putatively identified by LC—HRMS analysis.

Based on the transformation products detected by targeted LC-MS/MS analysis (Fig. 2), the biotransformation pathways of 6:2 FTS in the Loring and Robins soil microcosms are proposed in Fig. 3. The detection of 5:2 sFTOH, 5:3 acid, and PFCAs indicated that pathways reported

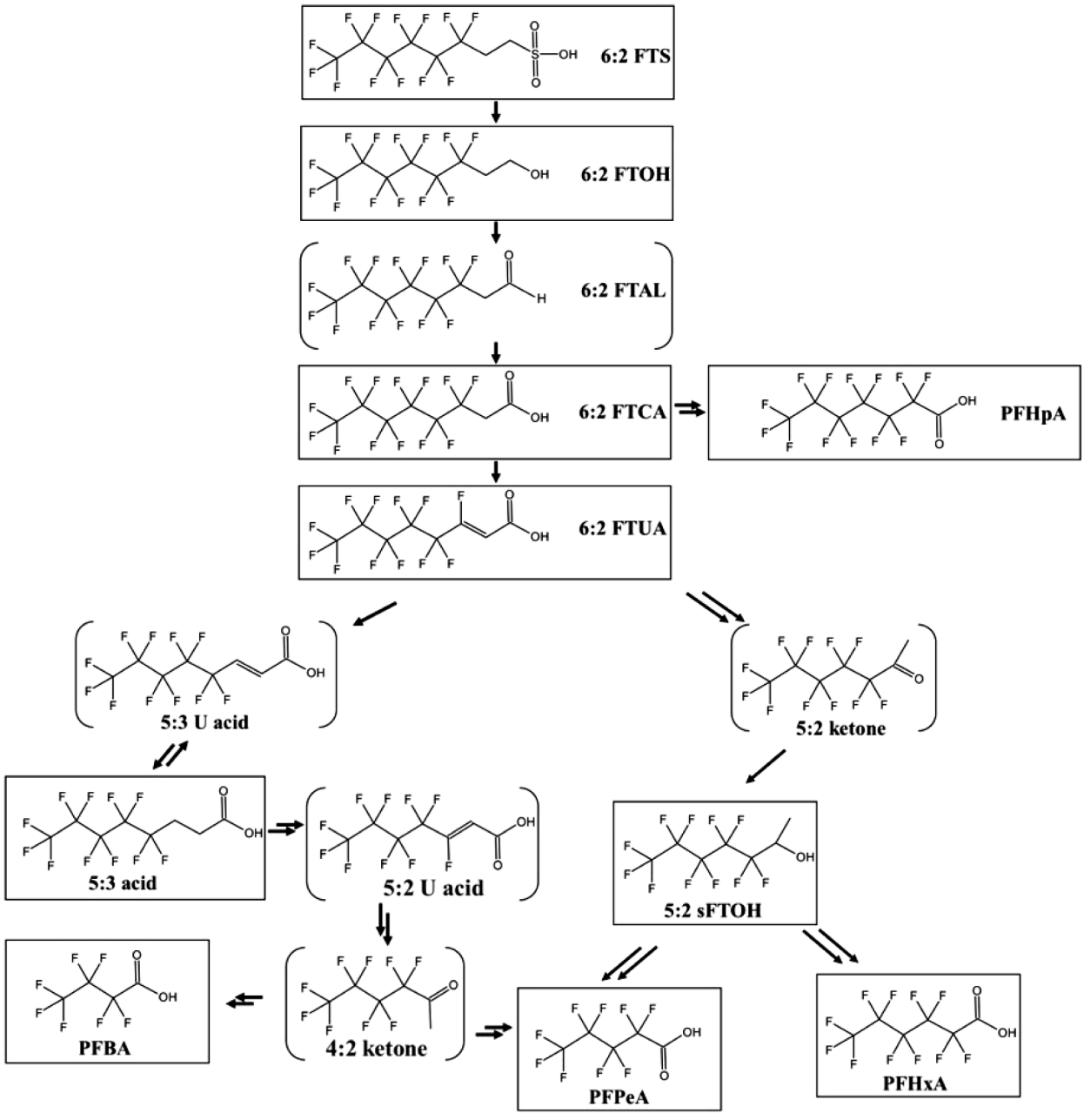


Fig. 3. Proposed aerobic biotransformation pathways of 6:2 FTS in AFFF-impacted soils. Compounds in the rectangular boxes were detected by LC–MS/MS analysis. Compounds in the brackets are proposed transformation products and were not detected in this study. The solid arrows represent the biotransformation steps expected to occur based on the present and previous studies (Shaw et al., 2019; Wang et al., 2011; Zhang et al., 2016). The double arrows represent multiple enzymatic steps involved.

previously in activated sludge (Wang et al., 2011), river sediment (Zhang et al., 2016), and landfill leachate (Hamid et al., 2020), also occurred during 6:2 FTS biotransformation by native microbial communities in AFFF-impacted soils. The biotransformation initiated by the microbial desulfonation of 6:2 FTS catalyzed by monooxygenases (Van Hamme et al., 2013; Yang et al., 2022b), resulted in the formation of 6:2 FTOH. Then, 6:2 FTOH was oxidized to 6:2 fluorotelomer aldehyde (6:2 FTAL) by an alcohol dehydrogenase, which was further oxidized to 6:2 FTCA catalyzed by an aldehyde dehydrogenase. Through a dehydrohalogenation reaction, 6:2 FTCA was converted to 6:2 FTUA involving elimination of a hydrogen fluoride (HF). Similar to those previously reported (Liu et al., 2010b, 2010a; Yang et al., 2022b), the biotransformation of 6:2 FTOH to 6:2 FTAL, to 6:2 FTCA, and then to 6:2 FTUA was likely a rapid process, so that accumulation of 6:2 FTCA (<0.5 mol%) was minimal (Fig. 2). The low level of PFHpA formation (Fig. 2, Tables S10 and S11) observed in this study suggests that α-oxidation of 6:2 FTCA might be a minor pathway during 6:2 FTS biotransformation. Although microbial α-oxidation of fluorotelomer compounds (e.g., 6:2 FTOH, 8:2 FTOH) was not previously observed in studies conducted with bacterial cultures, activated sludge or pristine soils (Liu et al., 2010b, 2010a, 2007; Wang et al., 2011, 2009), the detection of PFHpA in recent 6:2 FTS and 6:2 polyfluoroalkyl phosphates (PAPs) biotransformation studies supports the possibility that microbial α-oxidation of 6:2 FTS to PFHpA occurred (Lee et al., 2010; Yang et al., 2022b; Zhang et al., 2016). A key branching point for further biotransformation was 6:2 FTUA, after which the pathways diverged. Through multiple enzymatic steps (e.g., defluorination and decarboxylation) (Wang et al., 2011), 6:2 FTUA was metabolized to 5:2 ketone. Then, the ketone was converted to 5:2 sFTOH, which is the precursor to PFPeA and PFHxA via multiple unknown reactions involving the removal of fluorine and carbon atoms (Liu et al., 2010b; Wang et al., 2011). The other transformation pathway for 6:2 FTUA led to the formation of 5:3 U acid, possibly via reductive defluorination (Hamid et al., 2020). Recently, Yu et al. (2020) demonstrated the microbial reductive defluorination of unsaturated PFAS by a mixed microbial culture. Further, 5:3 U acid was converted to 5:3 acid through a reduction step facilitated by nicotinamide-adenine dinucleotide phosphate (NADPH), and 5:3 acid could then enter into the "one-carbon removal pathways", leading to the formation of 5:2 U acid and 4:2 ketone, which are the precursors to PFBA and PFPeA (Wang et al., 2012).

3.3. Microbial community dynamics during aerobic biotransformation of 6:2 FTS

3.3.1. Diversity and richness of microbial community

The mapped reads of 16S rRNA gene amplicons from Loring and Robins soil microcosms varied in the range of 1,878 to 18,059 (Table S12). To compare the diversity and richness of microbial communities, the number of reads in all samples was rarefied to an identical sequencing depth (1,878). The quality-filtered reads were clustered into operational taxonomic units (OTUs) ranging from 25 to 554 at a 97 % similarity level. Alpha diversity, defined as the mean diversity of species in a community (Whittaker, 1972), was estimated using Shannon and Simpson indices. Species richness, the number of species or OTUs in a community, was estimated using Chao1 index. The original Robins soil has lower Shannon, Simpson and Chao 1 indices than those of Loring soil (Table S12), indicating Robins soil has both lower microbial richness and diversity than Loring soil. Although both Robins and Loring soils were heavily contaminated by PFAS (Table S9), the lower richness and diversity of Robins soil could be associated with its milieu conditions; for example, the lack of sufficient carbon sources for microbial growth at the site (lower organic carbon content in Robins soil (0.6 %) than Loring soil (3.5 %)). A significant difference ($p < 0.05$) in Chao1, Shannon, or Simpson indices was not found between day 224 samples from the live treatment (~1,700 μg/L 6:2 FTS spiked) and the positive control (no 6:2 FTS spiked) in both Loring and Robins soil microcosms (Table S12), suggesting that the presences of spiked 6:2 FTS and its biotransformation products did not significantly change the microbial diversity in this study. Although the results are not in agreement with previous studies that found microbial diversity (in river sediment and soil) was reduced by exposure to PFCAs or 6:2 FTS biotransformation products, the concentrations of PFAS detected or applied in those studies were much higher (e.g., 15 mg/L) than that in the present study (Qiao et al., 2018; Zhang et al., 2017). Principle coordinate analysis (PCoA) was also applied as a measurement of beta diversity to visualize microbial community alteration among the different treatments in Loring and Robins microcosms. The minimal impact of spiked 6:2 FTS on overall diversity was further supported by the clear grouping of day 224 samples from the live treatment and positive controls (Fig. S4). In addition, the apparent separation of Loring and Robins soil samples revealed the distinct differences between microbial community compositions in two soils (Fig. S4).

3.3.2. Microbial community composition in Loring and Robins soil microcosms

The microbial community composition in Loring and Robins soils was further analyzed on various taxonomic levels. A total of 36 classified phyla in the domain Bacteria and 5 phyla in the domain Archaea were shared. Fig. 4 shows Proteobacteria was the most dominant phylum in Loring and Robins soils (relative abundance >35 %). The results corroborate with previous studies that identified Proteobacteria as the predominant phylum in surface soils and river sediments that were heavily contaminated by PFAS (Bao et al., 2018; Li et al., 2017). Additionally, prior studies observed obvious increases (12.5–65.7 %) in Proteobacteria in wetland slurry and fresh water following exposure to PFAS (Yin et al., 2019; Zhang et al., 2020). These results suggest that Proteobacteria is likely more tolerant of PFAS than other phyla. In this study, two classes of Proteobacteria, Alpha- and Gamma-proteobacteria, were observed in Loring and Robins soils; these microorganisms have been reported to degrade various hydrocarbon compounds (Sutton et al., 2013), as well as PFAS (e.g., FTOHs, 6:2 FTS) (Key et al., 1998; Kim et al., 2014; Lewis et al., 2016). Another phylum, Actinobacteriota was also observed in all of Loring and Robins soil samples (Fig. 4). Some genera from Actinobacteriota are known as PFAS degraders; for example, *Mycobacterium* is known to degrade 6:2 FTOH (Kim et al., 2014), and *Gordonia, Rhodococcus*, and *Dietzia* have been shown to transform 6:2 FTS (Méndez et al., 2022; Shaw et al., 2019; Yang et al., 2022b). In the current study, *Rhodococcus* was present in Loring and Robins soils (Fig. 5). Taken together with prior work, *Rhodococcus* and/or other strains belonging to the Proteobacteria and Actinobacteriota phyla are likely tolerant to 6:2 FTS and associated biotransformation products; some of these strains may also play a role in the 6:2 FTS biotransformation observed in both soils.

Desulfobacterota was present in Loring soil at a relative abundance of 1.7–6.5 % whereas it was absent in Robins soil (Fig. 4). Many genera in Desulfobacterota have been reported to desulfonate the organosulfonates (Cook et al., 1998). Since desulfonation is a critical and often a rate-limiting step in 6:2 FTS biotransformation (Hamid et al., 2020; Wang et al., 2011), the distinct difference in the abundance of Desulfobacterota could have contributed to the observed differences in biotransformation rates of 6:2 FTS in Loring and Robins soils. In addition, higher abundances of Bacteroidota (12.7 ± 3.4% vs. 0.9 ± 1.3 %), Zixibacteria (3.0 ± 2.5% vs. 0 %), Cyanobacteria (2.1 ± 1.5% vs. 0 %), Patescibacteria (1.6 ± 0.8% vs. 0 %), as well as Archaea (1.2 ± 0.5% vs. 0 %), in Loring soil than Robins soil may partially account for the observed differences in 6:2 FTS biotransformation (Fig. 4). Bacteroidota and Archaea were shown to be associated with diesel- and PFAS-contaminated soils (Sutton et al., 2013; Zhang et al., 2017). Cyanobacteria species were reported to degrade aromatic hydrocarbons and xenobiotics using various enzymes (Touliabah et al., 2022). Patescibacteria was widely detected at sites with organic and metal pollutants (Tripathi et al., 2022), and was linked to the remediation of aromatic

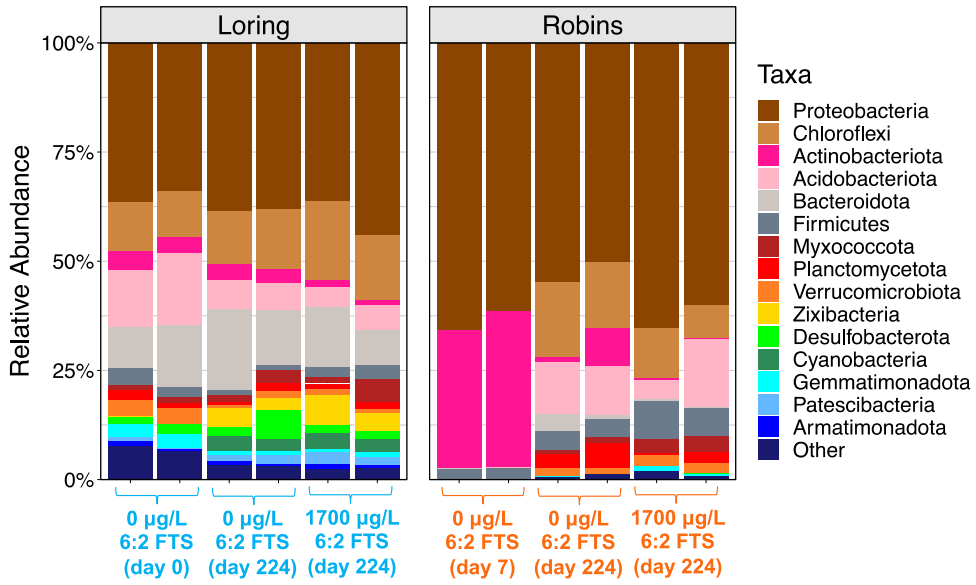


Fig. 4. The relative abundance of microbial community composition at the phylum level in Loring (left) and Robins (right) soil microcosms.

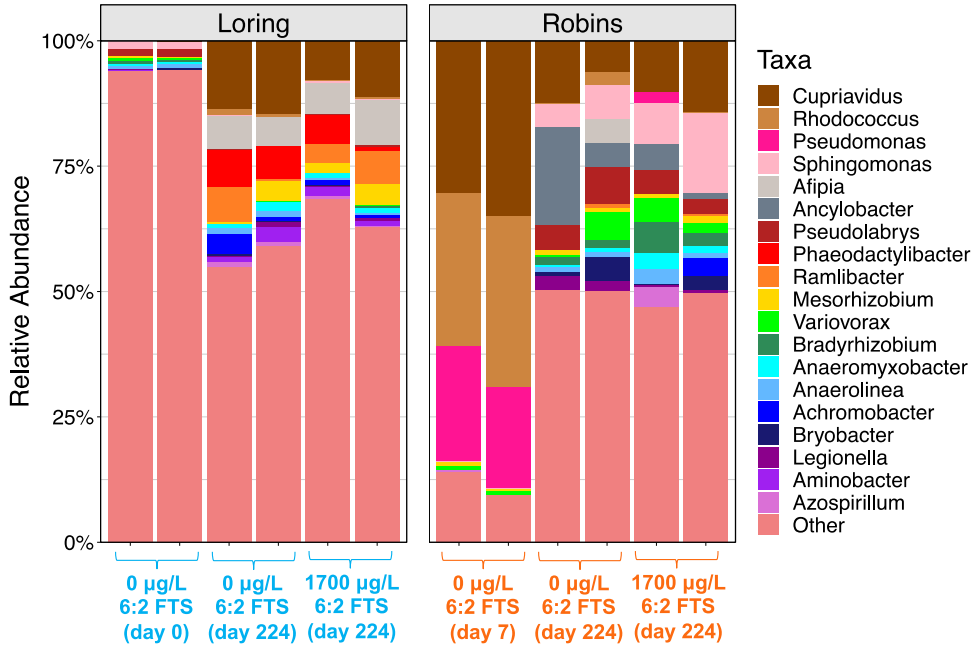


Fig. 5. The relative abundance of microbial community composition at the genus level in Loring (left) and Robins (right) soil microcosms.

hydrocarbons and heavy metals (Cao et al., 2022). Zixibacteria was recently proposed as a new bacterial phylum, and the representative organism in the phylum was reported to be metabolically versatile (Castelle et al., 2013).

The impacts of 6:2 FTS and its biotransformation products on microbial community composition were also investigated by comparing the day 224 samples from live treatments (~1,700 µg/L 6:2 FTS spiked) and from positive controls (no 6:2 FTS spiked). At the phylum level, the increases in Myxococcota and Firmicutes were found in both Loring and Robins soils after being exposed to 6:2 FTS (Fig. 4). Specifically, the relative abundance of Myxococcota was 2.2 ± 0.7 % and 1.1 ± 0.4 % in non-spiked Loring and Robins soils, respectively; while it increased to 3.5 ± 1.9 % and 3.5 ± 0.3 % in the 6:2 FTS spiked soils, respectively. Likewise, Firmicutes increased from 1.3 ± 0.1 % and 4.3 ± 0.2 % in non-spiked soils, to 2.7 ± 0.5 % and 7.5 ± 1.1 % in the 6:2 FTS spiked Loring and Robins soils, respectively. These results suggest that Myxococcota and Firmicutes were relatively more tolerant to 6:2 FTS and/or its transformation products. Both phyla were found capable of biotransformation of chlorinated solvents (Krzmarzick et al., 2014; Sanford et al., 2002), and Firmicutes was a dominant phylum in the PFAS-contaminated soils (Li et al., 2017; Qiao et al., 2018). At the genus level, a total of 320 genera were shared, and the Welch *t*-test with Benjamini-Hochberg false discovery rate (FDR) for multiple test corrections was applied to compare the relative abundance of each genus in live treatments and positive controls. It was found that *Sphingomonas* was the only genus that increased significantly (adjusted $p < 0.05$) in live treatments compared to positive controls in both the Loring and Robins soil microcosms. In Loring soil, the relative abundance of *Sphingomonas* was 0.3 ± 0.0 % in live treatments compared to positive controls (0.2 ± 0.0 %); in Robins soil, the relative abundance was 12.0

± 3.8 % in live treatments compared to positive controls (5.7 ± 1.1 %) (Fig. 5). These results suggest that the genus of *Sphingomonas* likely has higher tolerance to the elevated concentrations of 6:2 FTS and its biotransformation products. Moreover, some species in *Sphingomonas* may play a role in 6:2 FTS biotransformation. Species in *Sphingomonas* are present in various natural environments, and widely known to degrade many kinds of environmental pollutants (e.g., polycyclic aromatic hydrocarbons, chlorinated pesticides) (Bhatt et al., 2020; Nagata et al., 1999; Premnath et al., 2021). Recent studies reported that the relative abundance of *Sphingomonas* increased under PFAS-spiked (e.g., PFOS, 6:2 FTS) conditions (Qiao et al., 2018; Yin et al., 2019).

4. Conclusions

This study investigated the aerobic biotransformation of 6:2 FTS in two different AFFF-impacted soils. Different biotransformation rates were observed for the two soils; a half-life of 43.3 days was calculated for the Loring soil microcosms, while >60 mol% of initially spiked 6:2 FTS remained in the Robins soil microcosms after a 224-day incubation. Differences in initial sulfate concentrations and its depletion rate over the incubation were likely associated with the different biotransformation rates between the two soils. Previously documented 6:2 FTS biotransformation products, including 6:2 FTCA, 6:2 FTUA, 5:2 sFTOH, 5:3 acid, and C_4—C_7 PFCAs, were identified and quantified by LC–MS/MS. After 224 days incubation, C_4—C_7 PFCAs were formed with combined molar yields of 13.8 mol% and 1.2 mol% in Loring and Robins soils, respectively. Based on these identified products, the aerobic biotransformation pathways of 6:2 FTS in AFFF-impacted soils were proposed.

This study also explored the microbial community composition and dynamics during the 6:2 FTS biotransformation in the Loring and Robins soil microcosms. *Rhodococcus* and/or other strains belonging to the Proteobacteria and Actinobacteriota, which were found to be present in both soils, could play a role in the 6:2 FTS biotransformation. Moreover, microorganisms from phylum Desulfobacterota, which has a relative abundance of 1.7–6.5 % in Loring soil but was absent in Robins soil, likely promoted 6:2 FTS biotransformation via more efficient desulfonation. Additionally, species in the genus *Sphingomonas,* which exhibited higher tolerance to the elevated concentrations of 6:2 FTS and its biotransformation products, are likely to have contributed to 6:2 FTS biotransformation. These findings provide insights into the role of native soil microbial communities in the biotransformation of fluorotelomer compounds, which can be valuable for future screening and isolation of microorganisms that could biodegrade fluorotelomers and/or other PFAS.

Overall, this study advances our understanding of the biotransformation rates and reaction pathways of 6:2 FTS, and the associated microbial mechanisms in AFFF-impacted soils. The different biotransformation rates observed in the two AFFF-impacted soils also highlight that evaluations of site geochemical and biological attributes are critical for achieving a better understanding of 6:2 FTS transformation in the field. The findings in this study could also serve as a reference for predicting the environmental fate of 6:2 FTS that can be used in the development of improved future conceptual site models.

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.

Data availability

Data will be made available on request.

Acknowledgments

This study was supported by the Strategic Environmental Research and Development Program (SERDP) under contract W912HQ-18-C-0014 for Project ER18-1149, "Development and Laboratory Validation of Mathematical Modeling Tools for Prediction of PFAS Transformation, Transport, and Retention in AFFF Source Areas". We acknowledge Naji Akladiss at Maine Department of Environmental Protection for providing access to collect Loring soil, and the Air Force Civil Engineer Center and Geosyntec Consultants for the assistance in Robins soil collection. Peng-Fei Yan and Sheng Dong also acknowledge the financial support from the China Scholarship Council, and Auburn University Graduate School and Office of International Programs. Katherine Manz was funded by the NIEHS Training in Environmental Pathology T32 program (grant #T32 ES007272). The high-resolution mass spectrometer used to identify reaction byproducts was partially funded by NSF Major Research Instrumentation (MRI) award CBET-1919870.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.120941.

References

Backe, W.J., Day, T.C., Field, J.A., 2013. Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. Environ. Sci. Technol. 47, 5226–5234. https://doi.org/10.1021/es3034999.

Baduel, C., Paxman, C.J., Mueller, J.F., 2015. Perfluoroalkyl substances in a firefighting training ground (FTG), distribution and potential future release. J. Hazard. Mater. 296, 46–53. https://doi.org/10.1016/j.jhazmat.2015.03.007.

Bao, Y., Li, B., Xie, S., Huang, J., 2018. Vertical profiles of microbial communities in perfluoroalkyl substance-contaminated soils. Ann. Microbiol. 68, 399–408. https://doi.org/10.1007/s13213-018-1346-y.

Bhatt, P., Huang, Y., Rene, E.R., Kumar, A.J., Chen, S., 2020. Mechanism of allethrin biodegradation by a newly isolated Sphingomonas trueperi strain CW3 from wastewater sludge. Bioresour. Technol. 305, 123074 https://doi.org/10.1016/j.biortech.2020.123074.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541. https://doi.org/10.1002/ieam.258.

Cao, X., Cui, X., Xie, M., Zhao, R., Xu, L., Ni, S., Cui, Z., 2022. Amendments and bioaugmentation enhanced phytoremediation and micro-ecology for PAHs and heavy metals co-contaminated soils. J. Hazard. Mater. 426, 128096 https://doi.org/10.1016/j.jhazmat.2021.128096.

Castelle, C.J., Hug, L.A., Wrighton, K.C., Thomas, B.C., Williams, K.H., Wu, D., Tringe, S. G., Singer, S.W., Eisen, J.A., Banfield, J.F., 2013. Extraordinary phylogenetic diversity and metabolic versatility in aquifer sediment. Nat. Commun. 4, 2120. https://doi.org/10.1038/ncomms3120.

Chen, H., Zhao, L., Wang, N., Yao, Y., Sun, H., 2019. Aerobic biotransformation of 6: 2 fluorotelomer sulfonic acid in soil. CSB 64, 3441–3448. https://doi.org/10.1360/TB-2019-0126.

Cook, A.M., Laue, H., Junker, F., 1998. Microbial desulfonation. FEMS Microbiol. Rev. 22, 399–419. https://doi.org/10.1111/j.1574-6976.1998.tb00378.x.

Dong, S., Yan, P.-F., Liu, C., Manz, K.E., Mezzari, M.P., Abriola, L.M., Pennell, K.D., Cápiro, N.L., 2023. Assessing aerobic biotransformation of 8:2 fluorotelomer alcohol in aqueous film-forming foam (AFFF)-impacted soils: pathways and microbial community dynamics. J. Hazard. Mater. 446, 130629 https://doi.org/10.1016/j.jhazmat.2022.130629.

Grandjean, P., Clapp, R., 2015. Perfluorinated Alkyl Substances: emerging Insights Into Health Risks. New Solut 25, 147–163. https://doi.org/10.1177/1048291115590506.

Hamid, H., Li, L.Y., Grace, J.R., 2020. Aerobic biotransformation of fluorotelomer compounds in landfill leachate-sediment. Sci. Total Environ. 713, 136547 https://doi.org/10.1016/j.scitotenv.2020.136547.

Harding-Marjanovic, K.C., Houtz, E.F., Yi, S., Field, J.A., Sedlak, D.L., Alvarez-Cohen, L., 2015. Aerobic biotransformation of fluorotelomer thioether amido sulfonate (Lodyne) in AFFF-amended microcosms. Environ. Sci. Technol. 49, 7666–7674. https://doi.org/10.1021/acs.est.5b01219.

Herzke, D., Olsson, E., Posner, S., 2012. Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway – A pilot study. Chemosphere 88, 980–987. https://doi.org/10.1016/j.chemosphere.2012.03.035.

Houtz, E.F., Higgins, C.P., Field, J.A., Sedlak, D.L., 2013. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. Environ. Sci. Technol. 47, 8187–8195. https://doi.org/10.1021/es4018877.

Kärrman, A., Elgh-Dalgren, K., Lafossas, C., Møskeland, T., Kärrman, A., Elgh-Dalgren, K., Lafossas, C., Møskeland, T., 2011. Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination. Environ. Chem. 8, 372–380. https://doi.org/10.1071/EN10145.

Key, B.D., Howell, R.D., Criddle, C.S., 1998. Defluorination of organofluorine sulfur Compounds by *Pseudomonas* Sp. Strain D2. Environ. Sci. Technol. 32, 2283–2287. https://doi.org/10.1021/es9800129.

Kim, M.H., Wang, N., Chu, K.H., 2014. 6:2 Fluorotelomer alcohol (6:2 FTOH) biodegradation by multiple microbial species under different physiological conditions. Appl. Microbiol. Biotechnol. 98, 1831–1840. https://doi.org/10.1007/s00253-013-5131-3.

Krzmarzick, M.J., Miller, H.R., Yan, T., Novak, P.J., 2014. Novel firmicutes group implicated in the dechlorination of two chlorinated xanthones, analogues of natural organochlorines. Appl. Environ. Microbiol. 80, 1210–1218. https://doi.org/10.1128/AEM.03472-13.

Lee, H., D'eon, J., Mabury, S.A., 2010. Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. Environ. Sci. Technol. 44, 3305–3310. https://doi.org/10.1021/es9028183.

Lewis, M., Kim, M.-H., Wang, N., Chu, K.-H., 2016. Engineering artificial communities for enhanced FTOH degradation. Sci. Total Environ. 572, 935–942. https://doi.org/10.1016/j.scitotenv.2016.07.223.

Li, B., Bao, Y., Xu, Y., Xie, S., Huang, J., 2017. Vertical distribution of microbial communities in soils contaminated by chromium and perfluoroalkyl substances. Sci. Total Environ. 599–600, 156–164. https://doi.org/10.1016/j.scitotenv.2017.04.241.

Liu, J., Lee, L.S., Nies, L.F., Nakatsu, C.H., Turco, R.F., 2007. Biotransformation of 8:2 fluorotelomer alcohol in soil and by soil bacteria isolates. Environ. Sci. Technol. 41, 8024–8030. https://doi.org/10.1021/es0708722.

Liu, J., Wang, N., Buck, R.C., Wolstenholme, B.W., Folsom, P.W., Sulecki, L.M., Bellin, C. A., 2010a. Aerobic biodegradation of [14C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system. Chemosphere 80, 716–723. https://doi.org/10.1016/j.chemosphere.2010.05.027.

Liu, J., Wang, N., Szostek, B., Buck, R.C., Panciroli, P.K., Folsom, P.W., Sulecki, L.M., Bellin, C.A., 2010b. 6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. Chemosphere 78, 437–444. https://doi.org/10.1016/j.chemosphere.2009.10.044.

McCord, J., Newton, S., Strynar, M., 2018. Validation of quantitative measurements and semi-quantitative estimates of emerging perfluoroethercarboxylic acids (PFECAs) and hexfluoroprolyene oxide acids (HFPOAs). J. Chromatogr. A 1551, 52–58. https://doi.org/10.1016/j.chroma.2018.03.047.

Méndez, V., Holland, S., Bhardwaj, S., McDonald, J., Khan, S., O'Carroll, D., Pickford, R., Richards, S., O'Farrell, C., Coleman, N., Lee, M., Manefield, M.J., 2022. Aerobic biotransformation of 6:2 fluorotelomer sulfonate by Dietzia aurantiaca J3 under sulfur-limiting conditions. Sci. Total Environ. 829, 154587 https://doi.org/10.1016/j.scitotenv.2022.154587.

Moe, M.K., Huber, S., Svenson, J., Hagenaars, A., Pabon, M., Trümper, M., Berger, U., Knapen, D., Herzke, D., 2012. The structure of the fire fighting foam surfactant Forafac®1157 and its biological and photolytic transformation products. Chemosphere 89, 869–875. https://doi.org/10.1016/j.chemosphere.2012.05.012.

Moody, C.A., Field, J.A., 2000. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. Environ. Sci. Technol. 34, 3864–3870. https://doi.org/10.1021/es991359u.

Nagata, Y., Miyauchi, K., Takagi, M., 1999. Complete analysis of genes and enzymes for γ-hexachlorocyclohexane degradation in Sphingomonas paucimobilis UT26. J. Ind. Microbiol. Biotechnol. 23, 380–390. https://doi.org/10.1038/sj.jim.2900736.

Premnath, N., Mohanrasu, K., Guru Raj Rao, R., Dinesh, G.H., Prakash, G.S., Ananthi, V., Ponnuchamy, K., Muthusamy, G., Arun, A., 2021. A crucial review on polycyclic aromatic Hydrocarbons - Environmental occurrence and strategies for microbial degradation. Chemosphere 280, 130608. https://doi.org/10.1016/j.chemosphere.2021.130608.

Qiao, W., Xie, Z., Zhang, Y., Liu, X., Xie, S., Huang, J., Yu, L., 2018. Perfluoroalkyl substances (PFASs) influence the structure and function of soil bacterial community: greenhouse experiment. Sci. Total Environ. 642, 1118–1126. https://doi.org/10.1016/j.scitotenv.2018.06.113.

Sanford, R.A., Cole, J.R., Tiedje, J.M., 2002. Characterization and Description of Anaeromyxobacter dehalogenans gen. nov., sp. nov., an aryl-halorespiring facultative anaerobic myxobacterium. Appl. Environ. Microbiol. 68, 893–900. https://doi.org/10.1128/AEM.68.2.893-900.2002.

Schultz, M.M., Barofsky, D.F., Field, J.A., 2004. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. Environ. Sci. Technol. 38, 1828–1835. https://doi.org/10.1021/es035031j.

Shaw, D.M.J., Munoz, G., Bottos, E.M., Duy, S.V., Sauvé, S., Liu, J., Van Hamme, J.D., 2019. Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by Gordonia sp. strain NB4-1Y under sulfur-limiting conditions. Sci. Total Environ. 647, 690–698. https://doi.org/10.1016/j.scitotenv.2018.08.012.

Sutton, N.B., Maphosa, F., Morillo, J.A., Abu Al-Soud, W., Langenhoff, A.A.M., Grotenhuis, T., Rijnaarts, H.H.M., Smidt, H., 2013. Impact of long-term diesel contamination on soil microbial community structure. Appl. Environ. Microbiol. 79, 619–630. https://doi.org/10.1128/AEM.02747-12.

Szostek, B., Prickett, K.B., Buck, R.C., 2006. Determination of fluorotelomer alcohols by liquid chromatography/tandem mass spectrometry in water. Rapid Commun. Mass Spectrom. 20, 2837–2844. https://doi.org/10.1002/rcm.2667.

Touliabah, H.E.-S., El-Sheekh, M.M., Ismail, M.M., El-Kassas, H., 2022. A review of microalgae- and cyanobacteria-based biodegradation of organic pollutants. Molecules 27, 1141. https://doi.org/10.3390/molecules27031141.

Tripathi, S., Purchase, D., Al-Rashed, S., Chandra, R., 2022. Microbial community dynamics and their relationships with organic and metal pollutants of sugarcane molasses-based distillery wastewater sludge. Environ. Pollut. 292, 118267 https://doi.org/10.1016/j.envpol.2021.118267.

Van Hamme, J.D., Bottos, E.M., Bilbey, N.J., Brewer, S.E., 2013. Genomic and proteomic characterization of Gordonia sp. NB4-1Y in relation to 6 : 2 fluorotelomer sulfonate biodegradation. Microbiology 159, 1618–1628. https://doi.org/10.1099/mic.0.068932-0.

Wang, N., Buck, R.C., Szostek, B., Sulecki, L.M., Wolstenholme, B.W., 2012. 5:3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel "one-carbon removal pathways. Chemosphere 87, 527–534. https://doi.org/10.1016/j.chemosphere.2011.12.056.

Wang, N., Liu, J., Buck, R.C., Korzeniowski, S.H., Wolstenholme, B.W., Folsom, P.W., Sulecki, L.M., 2011. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. Chemosphere 82, 853–858. https://doi.org/10.1016/j.chemosphere.2010.11.003.

Wang, N., Szostek, B., Buck, R.C., Folsom, P.W., Sulecki, L.M., Gannon, J.T., 2009. 8-2 Fluorotelomer alcohol aerobic soil biodegradation: pathways, metabolites, and metabolite yields. Chemosphere 75, 1089–1096. https://doi.org/10.1016/j.chemosphere.2009.01.033.

Whittaker, R.H., 1972. Evolution and measurement of species diversity. Taxon 21, 213–251. https://doi.org/10.2307/1218190.

Yan, P.-F., Dong, S., Manz, K.E., Liu, C., Woodcock, M.J., Mezzari, M.P., Abriola, L.M., Pennell, K.D., Cápiro, N.L., 2022. Biotransformation of 8:2 fluorotelomer alcohol in soil from aqueous film-forming foams (AFFFs)-impacted sites under nitrate-, sulfate-, and iron-reducing conditions. Environ. Sci. Technol. 56, 13728–13739. https://doi.org/10.1021/acs.est.2c03669.

Yang, S.-H., Shan, L., Chu, K.-H., 2022a. Fate and transformation of 6:2 fluorotelomer sulfonic acid affected by plant, nutrient, bioaugmentation, and soil microbiome interactions. Environ. Sci. Technol. 56, 10721–10731. https://doi.org/10.1021/acs.est.2c01867.

Yang, S.-H., Shi, Y., Strynar, M., Chu, K.-H., 2022b. Desulfonation and defluorination of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) by Rhodococcus jostii RHA1: carbon and sulfur sources, enzymes, and pathways. J. Hazard. Mater. 423, 127052 https://doi.org/10.1016/j.jhazmat.2021.127052.

Yin, T., Tran, N.H., Huiting, C., He, Y., Gin, K.Y.-H., 2019. Biotransformation of polyfluoroalkyl substances by microbial consortia from constructed wetlands under aerobic and anoxic conditions. Chemosphere 233, 101–109. https://doi.org/10.1016/j.chemosphere.2019.05.227.

Yu, Y., Zhang, K., Li, Z., Ren, C., Chen, J., Lin, Y.-H., Liu, J., Men, Y., 2020. Microbial cleavage of C–F bonds in two C $_6$ Per- and polyfluorinated compounds via reductive defluorination. Environ. Sci. Technol. 54, 14393–14402. https://doi.org/10.1021/acs.est.0c04483.

Zhang, D., Zhang, W., Liang, Y., 2020. Bacterial community in a freshwater pond responding to the presence of perfluorooctanoic acid (PFOA). Environ. Technol. 41, 3646–3656. https://doi.org/10.1080/09593330.2019.1616828.

Zhang, S., Lu, X., Wang, N., Buck, R.C., 2016. Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. Chemosphere 154, 224–230. https://doi.org/10.1016/j.chemosphere.2016.03.062.

Zhang, S., Merino, N., Wang, N., Ruan, T., Lu, X., 2017. Impact of 6:2 fluorotelomer alcohol aerobic biotransformation on a sediment microbial community. Sci. Total Environ. 575, 1361–1368. https://doi.org/10.1016/j.scitotenv.2016.09.214.

Zhao, L., Folsom, P.W., Wolstenholme, B.W., Sun, H., Wang, N., Buck, R.C., 2013. 6:2 Fluorotelomer alcohol biotransformation in an aerobic river sediment system. Chemosphere 90, 203–209. https://doi.org/10.1016/j.chemosphere.2012.06.035.