Occurrence and Environmental Fate of Per- and Polyfluoroalkyl Substances at Aqueous Film-Forming Foams Impacted Sites

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Abstract

Over the years, people have recognized that widely used per- and polyfluoroalkyl substances (PFAS) in industry and consumer products often appear as anthropogenic pollutants in the environment and biota. PFAS-containing aqueous film-forming forms (AFFFs) used for controlling class B fires constitute significant sources of PFAS pollution. The persistent, bioaccumulative and toxic properties of select PFAS call for a better understanding of the large chemical class in terms of their environmental behaviours and impact. In addition to anionic perfluoroalkyl acids (PFAAs), zwitterionic and cationic PFAS have been recognized as important constituents in AFFFs and at AFFF-impacted sites. However, assessing the environmental impact of AFFF deployment is challenging due to the large variety of PFAS species and complex chemistry. In particular, information on the appearance and abundance of zwitterionic and cationic PFAS is sparse, and the environmental behaviours of such compounds remain poorly understood. This thesis intends to fill such knowledge gaps.

First, the PFAS profiles at four Canadian airports were characterized using a range of advanced analytical tools, which was the first study of its kind in Canada. Results showed that these airports were commonly impacted by more than one AFFF chemistry, while distinct PFAS profiles and loads indicate the influence of AFFF use history. In source zone areas, zwitterions and cations made a high contribution (34.5-85.5%) in surface soils but a low contribution (<20%) in groundwaters. The PFAS in source zone soils had limited horizontal transfer, while the vertical migration down soil columns occurred even in locations of low permeability. In the background soils where AFFF impact was insignificant, unidentified precursors made up high percentages, probably resulting from atmospheric deposition. The study provides improved methodology, new knowledge and a priority list of PFAS to support future PFAS monitoring and remediation efforts.

Next, the biotransformation potential of zwitterionic polyfluoroalkyl compounds made via the historical electrochemical fluorination (ECF) process was investigated in aerobic soils. Two compounds with betaine head groups and two with tertiary amine groups were examined for the first time. The perfluoroalkyl sulfonamide betaine and tertiary amine were confirmed to be the precursors to perfluorooctane sulfonate (PFOS), while the amide betaine and tertiary amine were precursors to perfluorooctane carboxylate (PFOA). Comparing their transformation kinetics with

four other previously reported ECF-zwitterions and cations indicates the great influence of structure, especially the nitrogen head groups, on the chemical persistence. Specifically, i) the ECF precursors with sulfonamide group have higher microbial stability than those with an amide group; ii) the ECF precursors containing quaternary ammonium or betaine groups have high stability in soils with DT_{50} in years or decades, while those with tertiary amine or amine oxide groups were less stable with DT_{50} of weeks or months. For the first time, this study establishes a preliminary structure-degradability relationship for ECF precursors.

Finally, the biotransformation potential of novel fluorotelomer betaine (FTB) compounds was investigated. AFFFs containing these compounds are permitted to use as of today. Two short-chain FTBs and a commercial AFFF primarily containing n:3 and n:1:2 FTBs (n = 5, 7, 9, 11, and 13) were explored for the first time in aerobic soils. Results showed that 5:3 and 5:1:2 FTBs exhibited high persistence with negligible production of short-chain polyfluoroalkyl acids and PFAAs. In contrast, the commercial AFFF was slowly biotransformed, resulting in low yields of short and long-chain PFAAs (0.023-0.252 mol% by day 120), including PFOA and longer-chain PFAAs that have been banned. High-resolution mass spectrometry (HRMS) did not reveal any other biotransformation products. The high stability of FTBs and FTB-containing AFFF highlights the importance of considering these rarely monitored PFAS in monitoring, risk assessment, and remediation activities at AFFF-impacted sites.

This research emphasized the presence of diverse PFAS compounds at AFFF-impacted sites and revealed different environmental behaviors of zwitterionic and cationic PFASs contained in historical and current AFFF formulations. The improved understanding contributes to the knowledge base for assessing and managing such contaminated sites.

Résumé

Ces dernières années, il a été établi que les substances per- et polyfluoroalkyles (PFAS), largement utilisées dans l'industrie et les produits de consommation, étaient également devenues des polluants anthropiques de l'environnement et du biote. Les mousses à formation de pellicule aqueuse (AFFF) utilisées pour contrôler les feux de classe B constituent des sources importantes de pollution par les PFAS. Les propriétés persistantes, bioaccumulatives et toxiques de certains PFAS nécessitent une meilleure compréhension de cette grande classe chimique en termes de devenir et d'impacts environnementaux. Outre les acides perfluoroalkyles anioniques (PFAAs), les PFAS zwitterioniques et cationiques ont été reconnus comme des constituants importants des formulations d'AFFF; leur présence a également été documentée au niveau de certains sites impactés. L'évaluation de l'impact environnemental du déploiement des AFFF est cependant difficile en raison de la grande variété de PFAS et de leur complexité chimique. En particulier, les informations sur l'occurrence et l'abondance des PFAS zwitterioniques et cationiques sont rares, et les comportements environnementaux de ces composés restent mal compris. Cette thèse vise à combler ces lacunes dans les connaissances.

Dans un premier temps, les profils des PFAS ont été caractérisés dans quatre aéroports canadiens à l'aide d'une gamme d'outils analytiques avancés. Ceci constitue la première étude du genre au Canada. Les résultats ont montré que ces aéroports étaient communément affectés par des AFFF de chimie diverse; les profils et quantités des PFAS retrouvés sur ces sites retracent l'historique d'utilisation des AFFF. Dans la zone source de la contamination, les zwitterions et les cations ont représenté une abondance élevée (34,5-85,5 %) dans les sols de surface, mais une modeste contribution (<20 %) dans les eaux souterraines. Les PFAS dans les sols de la zone source avaient un transfert horizontal limité; en revanche, la migration verticale vers le bas a été observée même dans les endroits de faible perméabilité. Dans les sols plus éloignés de la zone source où l'impact des AFFF était réduit ou négligeable, les précurseurs non identifiés représentaient des pourcentages élevés, résultant probablement du dépôt atmosphérique. L'étude fournit une méthodologie améliorée, de nouvelles connaissances et une liste prioritaire de PFAS pour soutenir les futurs efforts de surveillance et de remédiation des PFAS.

Dans un deuxième temps, le potentiel de biotransformation de PFAS zwitterioniques issus de la voie de synthèse historique de fluoration électrochimique (ECF) a été étudié dans des sols aérobies. Deux composés avec des groupes fonctionnels bétaïne et deux avec des groupes amine tertiaire ont été examinés pour la première fois. Les sulfonamides bétaïne et amine ont été confirmées comme étant les précurseurs du sulfonate de perfluorooctane (PFOS), tandis que les amides bétaïne et amine tertiaire étaient les précurseurs de l'acide perfluorooctanoïque (PFOA). La comparaison de leur cinétique de transformation avec quatre autres zwitterions et cations de type ECF indique la grande influence de la structure, notamment des groupes de tête azotés, sur leur persistance. Plus précisément, i) les précurseurs ECF avec un groupe sulfonamide ont une plus grande stabilité microbienne que ceux avec un groupe amide ; ii) les précurseurs ECF contenant des groupes ammonium quaternaire ou bétaïne ont une grande stabilité dans les sols avec un DT50 de plusieurs années ou décennies, tandis que ceux avec des groupes amine tertiaire ou amine oxyde étaient moins stables avec un DT50 de quelques semaines ou mois. Pour la première fois, cette étude établit une relation préliminaire structure-dégradabilité pour les précurseurs de type ECF.

Enfin, le potentiel de biotransformation de nouveaux composés de type fluorotélomère bétaïne (FTB) a été étudié. L'utilisation des AFFF contenant ces composés n'est pas interdite à l'heure actuelle. Deux FTB à chaîne courte et une formulation technique d'AFFF contenant principalement des FTB n:3 et n:1:2 (n = 5, 7, 9, 11 et 13) ont été étudiés pour la première fois dans des sols aérobies. Les résultats ont montré que les FTB 5:3 et 5:1:2 présentaient une persistance élevée avec une production négligeable d'acides polyfluoroalkyliques à chaîne courte et de PFAAs. L'AFFF commerciale a été lentement biotransformée, avec de faibles rendements en PFAAs à chaîne courte et longue (0,023-0,252 % molaire au jour 120), y compris le PFOA et les PFAAs à chaîne longue. La spectrométrie de masse à haute résolution (HRMS) n'a pas révélé d'autres produits de biotransformation. La grande stabilité des FTB souligne l'importance de prendre en compte ces PFAS rarement surveillés dans les activités de caractérisation environnementale, d'évaluation des risques et de dépollution des sites touchés par les AFFF.

Ces travaux de recherche ont mis en évidence la présence de divers PFAS sur les sites impactés par les AFFF. Différents comportements environnementaux des PFAS zwitterioniques et cationiques contenus dans les formulations AFFF historiques et actuelles ont été révélés. Cette

meilleure compréhension contribue aux connaissances scientifiques pour l'évaluation et la gestion de ces sites contaminés.

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PFAS	Perfluoroalkyl and polyfluoroalkyl substance
PFAA	Perfluoroalkyl acid
PFCA	Perfluoroalkyl carboxylate
PFSA	Perfluoroalkyl sulfonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
FTB	Fluorotelomer betaine
FTAB or FTSA-PrB	Fluorotelomer sulfonamide betaine
FTAA	Fluorotelomer sulfonamide amine
FTSA or FTS	Fluorotelomer sulfonate
FTCA	Fluorotelomer carboxylic acid
FTUCA	Fluorotelomer unsaturated carboxylic acid
FTOH	Fluorotelomer alcohol
AFFF	Aqueous film forming foam
ECF	Electrochemicalfluorination
FT	Fluorotelomer
HRMS	High resolution mass spectrometry
LC-MS	Liquid chromatography coupled to mass spectrometry
IS	Internal Standard
LOD	Limit of detection
LOQ	Limit of quantification
MRM	Multiple Reaction Monitoring
PIGE	proton-induced gamma-ray emission
HRMS	high-resolution mass spectrometry
UHPLC-HRMS	ultra-high-performance liquid chromatography coupled to high-resolution orbitrap mass spectrometry
TOP assay	Total oxidizable precursor assay
TOF-CIC	Total organofluorine-combustion ion chromatography
EOF	Extractable organic fluorine

Preface

This thesis is presented in a manuscript-based format. A general introduction and literature review are presented in Chapters 1 and 2. Chapters 3 to 5 comprise the manuscripts that have been published or are in preparation for submission. In the final chapter, a summary of the thesis and future research directions are presented. The Ph.D. candidate and author of this thesis, Min Liu, held primary responsibility for performing the literature review, designing and performing experiments, analyzing and interpreting experimental data, and preparing the manuscripts for publication and the doctoral dissertation. Below is a detailed description of the efforts of contributing authors to each manuscript.

Chapter 3. Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Per- and Polyfluoroalkyl Substances in Contaminated Soil and Groundwater at Airports: A Canadian Case Study. *Environ. Sci. Technol.* **2022,** *56,* (2), 885–895.

Liu, M. Designed the study, conducted the experimental procedures, analyzed the results, and wrote the manuscript.

Munoz, G. Contributed to the soil analysis method development, statistical interpretation of results and revised the manuscript.

Vo Duy, S. Assisted with checking the instrument performance and qualitative chemical analysis.

Sauvé, S. Supervised the research, assisted with the interpretation of results, and revised the manuscript.

Liu, J. Acquired the research funding, defined research scope and objectives, supervised the design of experiments, contributed to the interpretation of results, and revised the manuscript.

Chapter 4. Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Stability of Nitrogen-Containing Polyfluoroalkyl Substances in Aerobic Soils. *Environ. Sci. Technol.* **2021,** *55,* (8), 4698-4708.

Liu, M. Designed the study, conducted the experimental procedures, analyzed the results, and wrote the manuscript.

Munoz, G. Contributed to the statistical interpretation of results and revised the manuscript.

Vo Duy, S. Assisted with the qualitative chemical analysis.

Sauvé, S. Supervised the research, assisted with results interpretation, and revised the manuscript.

Liu, J. Acquired the research funding, defined research scope and objectives, supervised the design of experiments, contributed to the interpretation of results, and revised the manuscript.

Chapter 5. Liu, M.; Munoz, G.; Hermiston, J.; Vo Duy, S.; Zhang, J., Wang, D.; Bottos, E., Van Hamme, J.; Lee, L. S.; Sauvé, S.; Liu, J., High persistence of novel polyfluoroalkyl betaines in aerobic soils. In preparation for submission to *Environ. Sci. Technol.*

Liu, M. Designed the study, conducted the experimental procedures, analyzed the results, and wrote the manuscript.

Munoz, G. Contributed to the optimization of the PFAS analysis method, interpretation of the experimental results and revised the manuscript.

Hermiston, J. Assisted with the soil sample collection and microbial community analysis.

Vo Duy, S. Assisted with the development and optimization of analysis methods for transformation products.

Zhang, J. Assisted with the soil sample preparation.

Wang, D. Assisted with the microbial community result interpretation.

Bottos, E. Contributed to the analysis of soil microbial community, interpretation of the experimental results and revised the manuscript.

Van Hamme, J. Contributed to the analysis of soil microbial community, interpretation of the experimental results and revised the manuscript.

Lee, L. S. Assisted with the soil sample collection.

Sauvé, S. Supervised the research, assisted with the interpretation of results, and revised the manuscript.

Liu, J. Acquired the research funding, defined research scope and objectives, supervised the design of experiments, contributed to the interpretation of results, and revised the manuscript.

Chapter 1. Introduction

1.1 Background

Per- and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals whose structural hydrogens are fully or mostly replaced by fluorines. They have been manufactured and widely used in a myriad of industrial, commercial, and domestic products for almost four decades,[1, 2] before the first report of global contamination by PFASs was published in 1999.[3] The recent report on the global commercial uses of PFASs, published in 2018 by the OECD, estimated that approximately 4800 PFASs have been produced since the 1950s.[4] In 2019, the U.S. EPA assembled a master list of 6330 PFAS that combines information from several existing lists.[5] These studies, however, only included the information from the public domain, and therefore the actual variety of PFASs may be even greater. Among many applications, aqueous film-forming foams (AFFFs) represent a critical usage of PFASs as fire extinguishing agents of hydrocarbon fuels in civil, military aviation, and oil industries since the 1970s. Owing to the lack of regulations and awareness of their toxic effect, PFASs were historically discharged into the environment at different stages of their life cycle. As a result, PFASs such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were globally distributed in the environment,[6-11] wildlife,[12-15] and humans.[16-18]

Due to their persistence, bioaccumulation, and toxicity,[12] PFOS and perfluorooctane sulfonyl fluoride (POSF)-based compounds were phased out of production in 2000-2002 in North America.[19] PFOA and related chemicals were also regulated in the PFOA stewardship program toward the elimination of emissions and products by 2015.[20] Short-chain PFASs have been introduced as substitutes that are not bioaccumulative,[21] but these new PFASs remain highly persistent and mobile in the environment.[22] Fluorine-free firefighting foams have also been introduced as alternatives to AFFFs, but whether they can replace all PFAS-based AFFFs is not certain, suggesting AFFFs might be in use for the foreseeable futures.[23] The PFAS profiles at impacted sites can change significantly due to weathering and natural attenuation. To date, the Canadian federal government has identified over 22,000 contaminated or suspected contaminated

sites across Canada. For the sites where either historical or ongoing fire-equipment testing activities result in high PFAS levels, they have been recognized to be a high priority for action. Detailed site characterization of such AFFF-contaminated sites is necessary to allow sound decisions to be made before effective management or remedial efforts are implemented.

The information on AFFF components is proprietary. However, the increasing availability of PFAS chemical standards and high-resolution mass spectrometry during recent years enabled the discovery of some critical AFFF components. Aside from perfluoroalkyl carboxylic acids (PFCAs) and sulfonates (PFSAs), various cationic, zwitterionic, and nonionic PFAS were recently identified as components of AFFFs.[24-27] The newly identified PFASs include N- and/or S-containing functional groups such as amine, sulfonamide, amine oxide, quaternary ammonium, and betaines, among others. The high diversity of PFAS molecular structures associated with AFFF formulations makes it difficult to completely understand the nature of AFFFs. Besides, many unidentified PFAS components in AFFFs and PFAS transformation products in the environment turn up as "dark matter" that escapes our grasp.[26, 28] As the existing chemical standards and analytical methods cannot fully resolve such "dark matter", underestimation of the PFASs in AFFF-impacted sites is believed to be widespread. Hence, determining the identity of unknown PFASs and the total PFAS level is necessary to fully characterize AFFF-contaminated sites.

Surrogate parameter methods may provide a solution for determining total PFAS and/or total organofluorine in AFFF formulations and AFFF-impacted sites. One surrogate parameter method is the total oxidizable precursor (TOP) assay,[26, 29] which measures the total PFCAs after a sample is subject to an oxidation reaction to allow non-fluorinated functionalities to convert to carboxyl groups.[30, 31] The TOP assay has been validated using anionic and neutral precursors as model compounds, but very few studies integrated those cationic and zwitterionic AFFF-derived PFASs.[31] In addition, this TOP assay was validated for aqueous samples, while its suitability for cationic and zwitterionic PFASs in other environmental matrixes, including soil and aquifer solids,

remains unclear. Therefore, an optimized TOP method that works for various PFAS structures in different environmental matrixes is urgently needed for an accurate estimation of the extent of PFAS contamination.

Biotransformation processes greatly influence the environmental fate of AFFF-derived PFASs. A large fraction of PFASs present in AFFFs and environmental samples impacted by them are *polyfluoroalkyl* substances that can undergo abiotic or biotic transformations and therefore collectively are termed "precursors".[28] Known AFFF-derived precursors include electrochemical fluorination (ECF)-based precursors such as perfluorooctane sulfonamide quaternary ammonium salt (PFOSAmS),[32] and fluorotelomer (FT)-based precursors such as thioamidosulfonate (6:2 FTSAS),[29, 33] sulfonamide amine (6:2 FTAA) and sulfonamide alkyl betaine (6:2 FTAB).[34, 35] Without complete mineralization,[36] their biotransformation can yield *perfluoroalkyl* acids (PFAAs) as final products, as well as a great variety of other *polyfluoroalkyl* intermediates, some of which may show slow degradation kinetics and high persistence.[29, 33] The environmental fate of the precursors and their degradation intermediates remain poorly understood and need further characterization efforts of their chemical degradation pathways. Additionally, omitting such infrequently monitored PFAS could seriously underestimate the total PFAS burden at AFFF-impacted sites.[28]

Overall, there is a great knowledge gap on aspects spanning from the identity and concentration of PFASs present in the environment to their potential transformation processes in environmental systems, which impedes the proper assessment and management of AFFF-impacted sites. Therefore, a series of investigations, including the PFAS composition profiles of characteristic airports and biotransformation of different PFAS precursors, were performed for better understanding and ultimately predicting the behaviour and fate of PFASs contained in AFFFs at impacted sites.

1.2 Research Objectives and Hypothesis

The overarching goal of this research project is to produce a better understanding of the extent of AFFF impacts at impacted sites and the fate of PFAS precursors in soil ─ an important reservoir for PFAS.[37-39] This would provide more elements for proper assessment, management, and remediation of AFFF-impacted sites. The specific objectives and hypothesis are detailed below:

Objective 1. Characterize the PFAS at representative Canadian airports impacted by firefighting activities due to the historical use of AFFFs, delineate the profiles of AFFF-derived precursors and their potential transformation products in soil and groundwater, and examine the horizontal transfer and vertical transport of PFAS. The **hypothesis** is that the various impacted airports can exhibit different PFAS concentrations and profiles because of different AFFFs released, site-specific geochemical conditions, and different climates.

Objective 2. Investigate the biotransformation potential of ECF-based precursors with different N-containing groups, which constitute important components of historical AFFFs, in aerobic soil; and establish a preliminary structure-degradability relationship. The **hypotheses** are *i*) Zwitterionic ECF-based betaine and tertiary amine PFASs can biotransform to PFSA/PFCA as a result of microbial activity, *ii*) Intermediate biotransformation products are generated along with PFSA/PFCA, and *iii*) These N-containing PFAS precursors with different terminal functional groups can exhibit different microbial stability in the soil environment.

Objective 3. Investigate the biotransformation potential of novel FT-based polyfluoroalkyl betaines, which represent important components of current-in-use AFFFs, in aerobic soils. The **hypotheses** are *i*) These novel zwitterionic betaines with n:3 and n:1:2 polyfluoroalkyl chains can biotransform to PFCAs or H-substituted PFCAs as a result of microbial activity, and *ii*) These novel betaines with unique structures can exhibit different biotransformation potential compared with the conventional n:2 fluorotelomer-based and ECF-based betaines.

1.3 Thesis Organization

Chapter 1 of this thesis provides an overview of the thesis organization and objectives to be addressed in subsequent chapters.

Chapter 2 provides a detailed literature review of PFAS chemistry and production, concerns over PFAS, the regulatory status of PFAS, PFAS in AFFFs and at AFFF-impacted sites, PFAS characterization methods at AFFF-impacted sites, and the aerobic, anaerobic and anoxic biotransformation of AFFF-derived precursors.

Chapter 3 addresses Objective 1 and surveys the status of PFAS contamination in soil and groundwater at Canadian airports, with the concentrations of both PFAS with known identity and unknown precursors disclosed. The PFAS profile differences between the source zone and background area were elucidated. In addition, the potential *in-situ* transformation pathways of both ECF and FT-based precursors occurring in the soil and groundwater environment were proposed, and the transport of PFAS from surface to deep layer soil was revealed. This chapter has been published as:

Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Per- and Polyfluoroalkyl Substances in Contaminated Soil and Groundwater at Airports: A Canadian Case Study. *Environ. Sci. Technol.* **2022,** *56*, (2), 885–895.

Chapter 4 addresses objective 2. It investigated the biotransformation potential in aerobic soils of four ECF polyfluoroalkyl substances (two betaines and two tertiary amines) that are used in historical AFFF formulations. It compares the biotransformation pathways and kinetics of these precursors to four other known ECF precursors in aerobic soils. The microbial stability of these eight ECF-derived compounds allows for establishing a preliminary structure-degradability relationship for ECF precursors. Chapter 4 has been published as:

Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Stability of Nitrogen-Containing Polyfluoroalkyl Substances in Aerobic Soils. *Environ. Sci. Technol.* **2021,** *55*, (8), 4698-4708.

Chapter 5 addresses objective 3 and explores the biotransformation potential of two short-chain novel fluorotelomer betaines that are used in current AFFF formulations in aerobic soils. It also investigates the biotransformation potential and/or persistence of a commercial AFFF primarily containing such novel fluorotelomer betaines with different carbon chain lengths. This chapter is in preparation for submission to *Environ. Sci. Technol*.

Liu, M.; Munoz, G.; Hermiston, J.; Vo Duy, S.; Zhang, J., Wang, D.; Bottos, E., Van Hamme, J.; Lee, L. S.; Sauvé, S.; Liu, J., High persistence of novel polyfluoroalkyl betaines in aerobic soils. In preparation for submission to *Environ. Sci. Technol.*

Chapter 6 summarizes the thesis, its general findings, and directions for future work.

1.4 Original Contributions to New Knowledge

This thesis addresses the environmental occurrence and fate of AFFF-derived PFASs in soil. The specific contributions to knowledge are highlighted below.

The PFAS concentration profiles in soil and groundwater at four airports in Canada were investigated, which represent the first comprehensive characterization of PFAS pollution at civilian airports in North America. The PFAS profile of the sites impacted by fluorotelomer-based AFFF was newly disclosed. In addition, it was informed for the first time that a new class of fluorotelomers that bear n:3 and n:1:2 polyfluoroalkyl chains make up a large proportion of total PFAS in selected sites, and many fluorotelomers (e.g., n:3, n:1:2, n:2 fluorotelomers) were highly persistent in soils. Finally, the discovery of a high percentage of unidentified PFAS in background soils was emphasized by using an improved TOP assay. This work provides a critical dataset to support developing new priority analyte lists and integrating TOP assay into the current PFAS analysis workflow to allow comprehensive PFAS monitoring.

The biotransformation potential and persistence in aerobic surface soils of four ECF zwitterionic PFAS (two betaines and two tertiary amines) used in historical AFFFs were examined

for the first time. Their environmental fate was established for the first time as precursors to PFOS or PFOA. In addition, the common transformation pathways among various ECF-based precursors were revealed, providing the knowledge to predict the fate of other ECF-based precursors. Furthermore, a preliminary structure-degradability relationship was established for the first time by comparing the microbial stability of several ECF-based precursors. This work will enhance our ability to predict the persistence of other AFFF-derived precursors and guide PFAS prioritization for related studies, e.g., environmental monitoring and risk assessment.

The biotransformation potential and persistence of both short-chain 5:3 and 5:1:2 fluoroalkyl betaines (FTB) used in current AFFF and a commercial AFFF primarily containing n:3 and n:1:2 FTB (n = 5, 7, 9, 11 and 13) in aerobic soils was investigated for the first time. The high persistence of the 5:3 and 5:1:2 FTB and the AFFF predominant with novel FTBs in aerobic soils was revealed for the first time. The FTB-containing AFFF was newly discovered to contribute to both short-chain and long-chain PFCA production. This study contributes to understanding the environmental fate of zwitterionic PFASs and provides insights for future environmental monitoring, risk assessment, and remediation activities at AFFF-impacted sites.

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Chapter 2. Literature Review

2.1 Introduction

2.1.1 PFAS chemistry and production

Per- and polyfluoroalkyl substances (PFASs), unified by the moiety-C_nF_{2n+1}-, are synthetic polymeric or nonpolymeric chemicals having a carbon backbone, in which the hydrogen atoms are fully or partially substituted with the fluorine atoms.[1] Due to the strength of multiple C-F bonds,[2] PFAS show high thermal and chemical stability.[3] The high electronegativity of fluorine also imparts simultaneous hydrophobicity and lipophobicity to some PFAS structures. These properties endow PFAS with applications in diverse industrial, commercial and domestic products, such as nonstick surfaces, performance plastics, paints, fabric, and paper coatings, cosmetics, aqueous film-forming foam (AFFFs), etc.[4, 5]

Before 2010, most of the PFAS used in commerce were manufactured through two processes: electrochemical fluorination (ECF) or telomerization.[1] In the ECF process, a hydrocarbon analog of perfluorooctanoyl fluoride (POSF) is subject to electrolysis in anhydrous HF to produce perfluorooctane sulfonyl fluoride (POSF) or perfluorooctanoyl fluoride, which is further derivatized to produce sulfonamide or amide-based precursor substances (structures shown in Figure 2.1a). In the telomerization process, a two-step polymerization reaction of perfluoroalkyl iodide results in fluorotelomer iodides, which are raw material intermediates used to produce fluorotelomer (FT)-based PFAS (structures shown in Figure 2.1b).[1] The former process produced both branched and linear isomers of PFAS that hold fully fluorinated carbon chains with homologs of varying $-CF_2-$ units, while the latter produces only linear isomers of PFAS, whose even-numbered perfluoroalkyl carbon chain is connected to a polar functional group via an ethyl spacer $-C_2H_4-$.[6] Both chemistries have been utilized in AFFF formulations for several decades. However, since the phase-out of PFOS and related eight-carbon ECF derivatives in 2000-2002 in North America,[7] FT-derived compounds, which neither contain nor break down into PFOS,[8] have been preferentially used in current AFFF formulations.[9] Notably, FT-based PFAS contain about 30-60% less fluorine than ECF-based ones.[8] In addition, ECF-based chemistry has also shifted from C8

perfluoroalkyl chains to C4 chains to reduce the bioaccumulation potential of these chemicals; even before the 3M phase-out, other chain lengths other than C8 were also present in products despite at a low abundance.

In the past few years, another family of FT chemicals, which are characterized by n:1:2 fluoroalkyl chains with n being an odd number (n = 5, 7, 9, 11, 13, and 15) have also emerged mainly as fluorosurfactants in AFFF formulations. They often contain low levels of compounds carrying n:3 fluoroalkyl chains as impurities. The synthesis route for this new family is not publicly known. Their structural resemblance to n:2 FTs suggests they likely share similar environmental fates and effects, but few data are available to confirm this hypothesis.

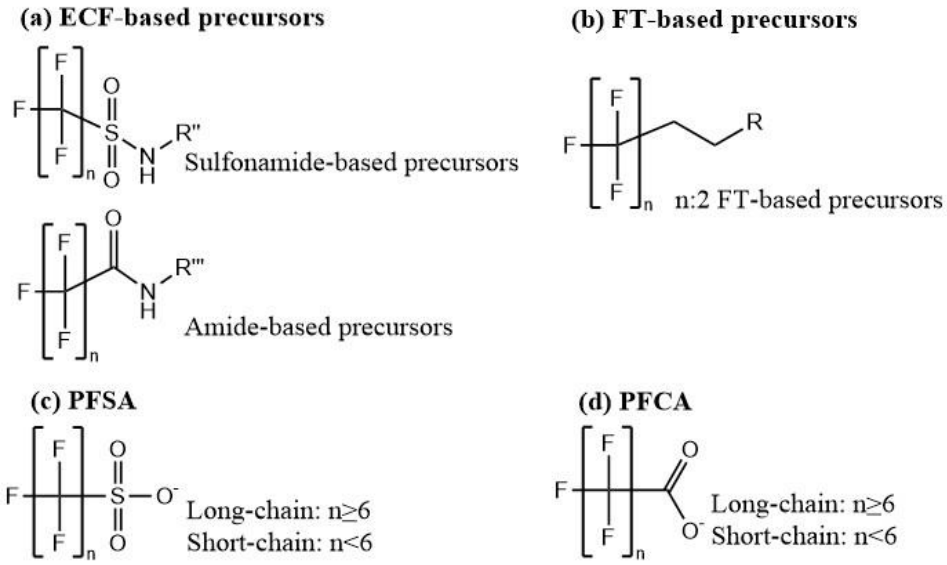

Figure 2.1 Structures of ECF-based precursors (including sulfonamide and amide-based), n:2 FT-based precursors, PFSA and PFCA.

2.1.2 Concerns over PFAS and PFAS regulations

The global distribution of perfluoroalkyl acids (PFAAs), including PFSAs and PFCAs (structures shown in Figure 2.1 c~d), in the environment, wildlife and humans is well documented.[10-13] PFOS and PFOA with eight carbons are the two most widely detected PFAS.[10,

[14, 15] This drives extensive research on their environmental behaviours and fate,[16, 17] and toxicological impacts on biota and humans.[4, 18, 19] Long-chain PFAAs show great persistency and long-range transport propensity in the environment, have a higher bioaccumulation potential and exhibit a much longer elimination half-life in exposed animals and humans than their short-chain homologs.[20] Toxicity and epidemiological studies show that PFOS and PFOA can cause developmental, endocrine, liver, immune and other effects in animals, while human exposure is associated with negative effects on the immune, endocrine, metabolic, and reproductive systems (including fertility and pregnancy outcomes), and increased risk for cancer.[4, 19, 21, 22] These aspects led to the ban or restriction in the use of PFOS, PFOA, the longer-chain PFAAs and related precursors since 2000. Specifically, 3M, a major fluorochemical manufacturer, ceased its PFOS production in North America in 2000-2002. PFOS and related substances were listed under Annex B (restriction of production and use) of the Stockholm Convention in 2009. PFOA, ammonium perfluorooctanoate (APFO), and C_{11}–C_{14} PFCAs were listed in the *Candidate List of Substances of Very High Concern* under the European chemicals regulation REACH in 2012-2013.[23] Many government agencies throughout the world also proposed provisional advisory health guidelines or screening values for most PFSAs and PFCAs in drinking water,[24-27] such as USEPA health advisory level of 70 ng/L for PFOS and PFOA, individual or combined.[25]

Alternatively, major global manufacturers have been replacing the long-chain PFAS with short-chain or other fluorinated chemicals (e.g., ether-based PFAS, etc.).[9] Experimental studies demonstrate that short-chain n:2 FT-based precursors (e.g., 6:2 FTSAS, 6:2 FTAB, 6:2 FTAA, etc) can be biotransformed into short-chain intermediates (e.g., 6:2 FTSA and 6:2 FTCA, etc), which ultimately degrade into short-chain PFAAs as stable products.[28-30] Compared with PFOS/PFOA, 6:2 FTSA and 6:2 FTCA exhibited weak or moderate hepatotoxicity,[31] while 6:2 FTSA showed greater toxic effects on cell viability.[32] Therefore, these short-chain alternatives may still represent great concerns as environmental toxicants. Additionally, the short-chain PFAAs showed extreme persistence similar to long-chain PFAAs and even had a higher potential for long-range transport than the long-chain homologs due to their low adsorption potential and increased mobility in the

water environment.[33] Short-chain PFAAs can also be enriched in the edible parts of plants,[33] though the information on the toxicity and long-term health effects of these compounds (e.g., reproductive effects for PFBS, etc.) are still very limited. [33, 34]

2.1.3 PFAS in AFFFs and at AFFF-impacted sites

AFFFs have been used for fighting hydrocarbon-fuel fires for decades.[35] They function by lowering the surface tension at the air-AFFF interface through fluorosurfactants and by cutting off oxygen via the foam blankets formed from surfactants.[36] In recent years, the discovery of dozens of PFAS in AFFFs showed the complexity of formulations.[35, 37, 38] Anionic PFSAs, mostly containing C_8 and C_6 perfluoroalkyl chains, and trace levels of PFCAs were in historically manufactured ECF-based AFFFs.[35, 37, 39] Besides, a large array of zwitterionic or cationic perfluoroalkyl sulfonamide-based PFASs, such as amino carboxylates (PFSaAmA, C_3-C_8),[35] betaines (PFASB, C_3-C_6),[38] quaternary ammonium compounds (PFASAmS, C_3-C_8),[40] amines (PFASAm, C_3-C_8),[38] and amine oxides (PFASNO, C_6-C_9),[38] are also present. In contrast, FT-based PFASs tend to dominate in more recent AFFFs, though their uses can be dated before the 1980s. FT-based AFFFs also contain anionic, cationic, zwitterionic, and even nonionic surfactants, with perfluoroalkyl chain lengths ranging from 3 to 15. [35, 37-39] The representative compounds identified in these AFFFs include fluorotelomer thioether amidosulfonates (FTSAS), fluorotelomer sulfonamidoalkyl betaines (FTAB), fluorotelomer betaines (FTB) and fluorotelomer thiohydroxyammonium (FTSHA). [35, 37-39, 41]

The occurrence of ECF- and FT-based PFAS and their transformation products [e.g., perfluoroalkyl sulfonamide (FASA), n:2 fluorotelomer sulfonates (n:2 FTSAs, n = 6, 8)] at AFFF-impacted sites illustrates the contribution of historical or ongoing use of AFFFs.[37, 41-43] Notably, PFAS concentrations at contaminated sites (soils, groundwater, etc.) are often orders of magnitude greater than those at the background sites.[44, 45] The PFAS profiles of AFFF formulations (ECF-based and FT-based) and environmental samples (such as groundwater) impacted by those AFFFs are often quite distinct.[37] For instance, FTSAs often make up a large portion of the total PFAS

burden at AFFF-impacted sites,[39, 46] but they are not major components of the AFFF formulations.[37, 38] Additionally, some PFAS may preferentially partition after release, leading to changes to the initial PFAS profile in the parent foam. For example, in one study, only a single component of AFFF formulations, perfluorohexane sulfonamide amine (PFHxSAm), was detected in soils and aquifer solids, while other PFASs initially present in formulations were undetectable in the impacted environment.[39] In another study, a change in the FTB profile was observed in earthworms exposed to soil amended with an Ansul firefighting foam; long-chain FTB were predominately concentrated in the worms due to high bioaccumulation potential.[47] Besides, the potential abiotic and biotic transformation of many polyfluoroalkyl compounds (so-called precursors) in the environment to PFCAs, PFSAs, FTSAs, and/or other intermediates may also contribute to the varied PFAS profiles.[28-30] Therefore, in addition to the proprietary nature of AFFF components, the complexity in environmental behaviours and fate related to AFFF-derived precursors makes it challenging to predict PFAS profiles at impacted sites based solely on AFFF use history.

When combining the PFAS soil and groundwater concentration data of many samples throughout the world, Brusseau et al. found the soil PFAS concentrations at contaminated sites were generally orders of magnitude higher than groundwater concentrations,[45] indicating the importance of soil as a PFAS sink. However, compared with the widely-studied PFAS distribution in atmospheric and aquatic environments,[48, 49] the data on soil PFAS contamination are very limited.[50] The understanding of PFAS contamination in soils is greatly needed.

2.2 Methods for PFAS Characterization at AFFF-impacted Sites

Most studies have focused on quantitative analysis of a suite of PFASs with available standards. As the number of authentic PFAS standards is far fewer than the types of PFAS used in commerce, such target analysis would only reveal a fraction of PFAS present in AFFFs or AFFF-impacted environmental samples, such as PFAAs, FASAs, FTSAs, etc.[51] Past few years have seen a vast improvement in the qualitative analysis of PFASs using advanced mass spectrometry. [52, 53]

The suspect screening method, when chemical standards are not available, has been used to identify many novel PFASs, including anionic, cationic, and zwitterionic compounds, as reviewed by Xiao [54].[39, 42, 43] However, suspect screening is limited to the classes of compounds (or some individual substances) whose chemical formula and structures are known or expected before the analysis is performed.[55] Non-target screening does not limit the number and origin of potential analytes,[56] using methods such as mass defect filtering,[57] in source fragmentation flagging scan,[58, 59]and TOF-MS^E high-resolution parent ion search (HRPIS).[60] The use of these methods further discovered various classes of novel anionic, zwitterionic, and cationic PFAS.[35, 38 61] Therefore, target analysis in combination with non-target and suspect screening is essential for a comprehensive characterization of PFAS, as well as for the evaluation of possible transformation products.

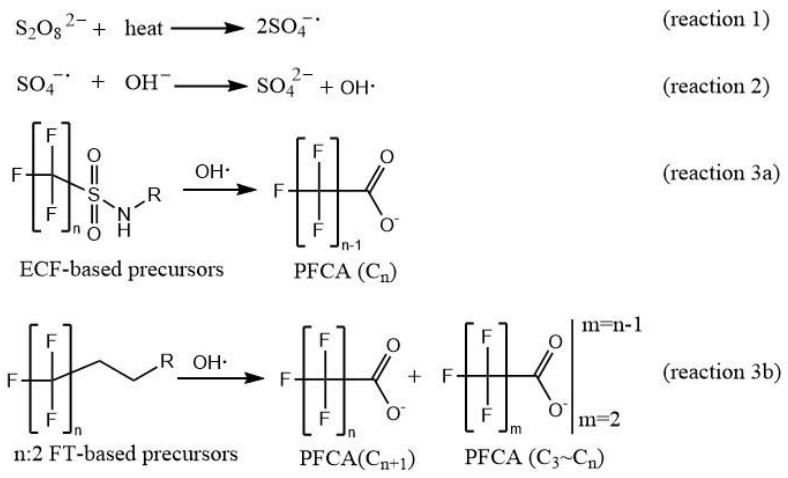

Figure 2.2 The reactions involved in the TOP assay for both ECF-based and n:2 FT-based precursors. [62, 63]

To better understand the extent of PFAS contamination without resorting to a detailed analysis of each PFAS, researchers have developed nonspecific methods, such as the TOP assay, to reveal those PFAS that cannot be easily identified or quantified. The technique converts polyfluoroalkyl precursors (including unquantifiable and unidentified ones) into PFCAs through

reactions with hydroxyl radicals. Persulfate salt is the preferred oxidant for the assay, and its thermolysis (85 °C and pH>12) produces radicals that can partially break down the precursors without completely mineralizing them (the reactions shown in Figure 2.2). [62, 63] Since PFCAs and PFSAs typically remain intact under the condition, differences between the concentrations of PFCAs before and after oxidation can be considered as contributions from the *polyfluoroalkyl* precursors. The TOP method has been widely employed for diverse environmental samples and even consumer products.[39, 62-64] However, some limitations in the established TOP assay method came to be recognized. For instance, less than 75% of the conversion rates for C_6 and short-chain fluorotelomer compounds would underestimate the actual concentration of precursors in environmental samples because ultra-short-chain products are often not captured by existing analytical approaches.[39] The validity of the method for novel PFASs in nonaqueous matrices (such as soil, aquifer solids, etc.) remains unconfirmed; matrix interferences could reduce the fraction of hydroxyl radicals that is available to react with PFAS. Since the TOP assay can provide quantitative estimates of precursors in the environment that otherwise cannot be obtained,[28] a validated TOP method applicable to solid matrices is greatly needed for applications where unknown PFAS cannot be quantified otherwise.

2.3 Environmental Fate of AFFF-derived Precursors

Previous studies found that a significant fraction of PFAS present in AFFFs and environmental samples impacted by them are *polyfluoroalkyl* substances that can undergo abiotic or biotic transformations and therefore collectively termed "precursors".[39, 63] Compared with the fully fluorinated PFAAs, nonfluorinated functional groups in PFAS precursors may enable them to interact with soils differently or be more susceptible to microbial attack, thus exhibiting distinct behaviors and fate in the environment.

2.3.1 Aerobic biotransformation

Biotransformation greatly influences the environmental fate of polyfluoroalkyl substances.[65] To date, a few studies have investigated the aerobic biotransformation of AFFF-derived precursors

in the environment, most of which focused on FT-based surfactants (shown in Table 2.1). For instance, Weiner et al. and Harding-Marjanovic et al. found that 6:2 FTSAS could be readily biotransformed into FTSAs and PFCAs in wastewater treatment sewage sludge (WWTP) and soil, [28, 29] illuminating another source of persistent PFCAs in AFFF-impacted environment. More recently, D'Agostino & Mabury reported the biotransformation of 6:2 FTAA and 6:2 FTAB in aerobic WWTP, with the generation of polyfluoroalkyl acids and PFCAs.[30] The higher yields of each product (0.38~6.9% versus <LOQ~0.9%) and all products (12~16% versus 3~6%) from 6:2 FTAA than from 6:2 FTAB demonstrated its higher biotransformation potential. Li et al. found that the slow biotransformation and environmental persistence of 6:2 FTAB in oil-impacted soils could not even result in a detectable increase of PFCAs,[66] while Shaw et al. demonstrated fast and near-complete biotransformation of 6:2 FTAB by a pure microbial culture under sulfur-limiting conditions, with the formation of 5:2 fluorotelomer ketone (5:2 ketone) as the major PFAS.[67] These findings show that the biotransformation potential and PFAA yields of novel zwitterionic betaine PFASs vary greatly depending on microbes present in a particular system and other factors.

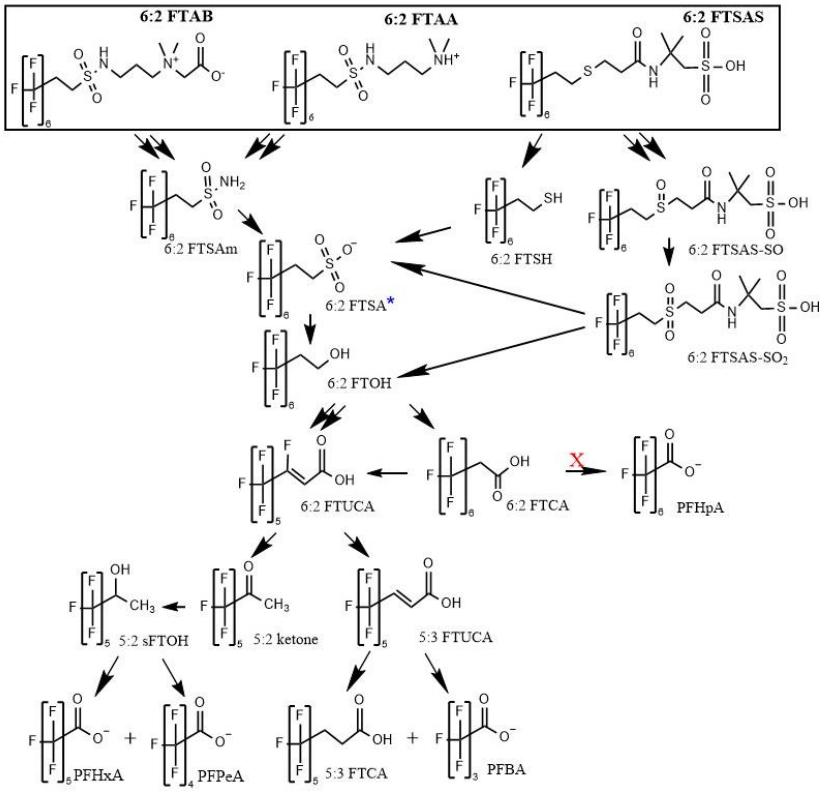

Figure 2.3 The aerobic biotransformation pathways of three n:2 FT-based precursors derived from AFFFs, adapted from literature.[28-30] The red "X" represents the absence of this pathway during the biotransformation of 6:2 FTSAS in aerobic soil and 6:2 FTAA and 6:2 FTAB in activated sludge,[28, 30] while this pathway occurred during the biotransformation of 6:2 FTSAS in aerobic WWTP sludge.[29] The blue star (*)with 6:2 FTSA indicates that it accumulated only in sterile WWTP sludge.

The aerobic biotransformation pathways for three AFFF-derived FT-based precursors are shown in Figure 2.3. The aerobic biotransformation of 6:2 FTSAS in WWTP sludge involved two pathways: Pathway I resulted in the formation of 6:2 FTSH (thiol) via S-dealkylation, which was further oxidized to 6:2 FTSA, while the pathway II resulted in the formation of 6:2 FTSAS-SO (sulfoxide) followed by 6:2 FTSAS-SO_2 (sulfone) via S-oxygenation. [29] Both 6:2 FTSA and 6:2 FTSAS-SO_2 were further transformed into a common intermediate 6:2 FTOH, which then followed similar pathways as previously reported.[68-71] Specifically, 6:2 FTOH was oxidized to 6:2

FTCA, which could degrade to a stable product PFHpA via α-oxidation, [72, 73] or 6:2 FTUCA, [68, 74] whose further reactions split into two pathways (PFCA pathway n:3 FTCA pathway).[70] The PFCA pathway resulted in the formation of 5:2 ketone followed by 5:2s FTOH, with the latter further forming PFPeA and PFHxA, while the n:3 FTCA pathway resulted in the formation of 5:3 FTCA.[29] The 4-5 times higher yield of 5:3 FTCA than the sum of PFPeA and PFHxA indicates the dominance of the n:3 FTCA pathway during this process. The preferential formation of polyfluoroalkyl acids (e.g., 5:3 FTCA) during the biotransformation of 6:2 FTOH was also observed in fungus cultures.[75, 76] In addition, previous studies reported the generation of relatively minor short-chain PFCA (e.g., PFBA) from 6:2 FTOH in soil, pure and mixed culture, etc.,[68, 71] and the formation of even shorter-chain polyfluoroalkyl acid (4:3 FTCA, 3:3 FTCA, etc.) from 5:3 FTCA in activated sludge.[77] However, not all the pathways would be observed for a given system; 6:2 FTSAS biotransformation in WWTP sludge showed no formation of PFBA and n:3 FTCA (n = 3, 4).[29]

During the biotransformation of 6:2 FTSAS in aerobic soil, the formation of 6:2 FTSAS-SO, 6:2 FTSAS-SO_2, followed by 6:2 FTSA, and then 6:2 FTUCA, 5:3 FTCA, PFHxA, PFPeA, and PFBA were similar as observed in WWTP sludge,[28] but with no PFHpA production. The yields of both PFCA and polyfluoroalkyl acids from 6:2 FTSAS in aerobic soil were much lower than in WWTP sludge (Table 2.1), which might be due to the microbial desulfonation of 6:2 FTSA as the rate-limiting step. Previous studies reported the varied transformation kinetics of 6:2 FTSA in different matrixes, from a half-life of 2 years in activated sludge to < 5 d in aerobic sediment,[74, 78] which is possibly influenced by different microorganisms, distinct microbial enzymes encoded for desulfonation and defluorination,[79] absorptions to organic material,[74] etc.

As opposed to the 6:2 FTSAS biotransformation in aerobic soil, 6:2 FTSA was not the major biotransformation product of both 6:2 FTAA and 6:2 FTAB in aerobic WWTP sludge; instead, the 6:2 fluorotelomer sulfonamide (6:2 FTSAm) was the most abundant one.[30] The 6:2 FTSAm intermediate was slowly biotransformed into 6:2 FTOH,[30] which then followed the pathways as

reported for 6:2 FTOH in soil, mixed bacterial culture, pure culture, etc., producing 5:3 FTCA, PFHxA, PFPeA, and PFBA. [68, 70, 71] Notably, 6:2 FTSA was formed in sterile soil only, indicating its formation via abiotic mechanisms.

There were two studies on the biotransformation potential of cationic or zwitterionic ECF-based PFASs in aerobic soil (shown in Table 2.1), [80, 81] one for perfluoroalkyl quaternary ammonium compounds and the other for perfluoroalkyl amine oxides. The amido-based compound (PFOAAmS) was degraded with a DT_{50} of 142 days and generated PFOA at a yield of 30 mol% after 180 d incubation, while the sulfonamide-based compound (PFOSAmS) was biotransformed into PFOS (0.3 mol% by day 180) at a much slower rate (DT_{50} >>180d). These results demonstrate that the extent to which these perfluoroalkyl ammonium salts (PFOSAmS and PFOAAmS) might form PFAAs in soil microcosms could vary greatly with the functionality attached to the perfluoroalkyl chain. In contrast, amido and sulfonamide-based amine oxides (PFOANO and PFOSNO) could rapidly degrade (DT_{50}: 3~15d) in aerobic soils, with the former producing PFOA at a yield of 15~21 mol% while the latter forming PFOS at a yield of ~2 mol %. [81] The distinct transformation kinetics between the compounds containing quaternary ammonium and the amine oxides could be related to the differences in their chemical structures (e.g., different hydrophilic functional groups), soil microorganisms, sorption potential influenced by soil properties, etc.

To date, the transformation of cationic and zwitterionic PFASs remains poorly understood. Perfluoroalkane sulfonamide-based surfactants identified in AFFFs were likely candidates for biotransformation to PFSAs due to their structural similarity with PFOSAmS and PFOSNO.[38] Likewise, many identified FT-based PFASs (such as FTBs, FTSHAs, etc.) were likely to be PFCA precursors based on hydrogenated carbons next to fluorinated carbons in their structures [38]. Further investigation of the transformation patterns of such AFFF-derived novel PFASs in the natural environment and their contribution, if any, to the secondary formation of PFSAs and PFCAs in these systems is warranted.

Table 2.1 Summary of the studies on the aerobic transformation of AFFF-derived precursors and/or related transformation products.

Precursor	Types of microbes or microcosms	Incubation conditions	Incubation duration	Estimated half-life ($t_{1/2}$)	Transformation product yields	Reference
6:2 FTSAS	Aerobic WWTP sludge	Polypropylene bottles with a purge-and-trap system	42 d	NA	6:2 FTSH (0.25%), 6:2 FTOH (6.2%), 6:2 FTSA (0.1%), 6:2 FTUCA (0.3%), 6:2 FTCA (2.9%), 5:3 FTCA (17.4%), PFPeA (3.2%), PFHxA (0.7%), PFHpA (0.4%)	29
6:2 FTSAS	Aerobic soil slurry	Shaken closed glass bottle at 30℃	60 d	NA.	6:2 FTSA (8 %), 6:2 FTUCA (0.18%), 5:3 FTCA (0.5%), PFHxA (0.72 %), PFPeA (0.6 %), PFBA (0.15 %)	28
6:2 FTAA	Aerobic WWTP sludge	Closed polypropylene bottles	109 d	NA	6:2 FTSAm (6.9 %), 6:2 FTOH (1.37 %), 5:3 FTCA (4.01%), PFHxA (0.76%), PFPeA (0.95%), PFBA (0.38%)	30
6:2 FTAB	Aerobic WWTP sludge	Closed polypropylene bottles	109 d	NA	6:2 FTSAm (0.9 %), 6:2 FTOH (0.75 %), 5:3 FTCA (0.76%), PFHxA (0.34%), PFPeA (0.23%), PFBA (ND)	30
6:2 FTAB	*Gordonia* sp. strain NB4-1Y	Shaken closed serum bottles at 30℃	7 d	NA	6:2 FTOH (2.42 %),6:2 FTCA (7.47 %), 5:2 FT ketone (18.6 %), 5:2 sFTOH (1.25 %), 5:3 FTCA (0.413 %), 4:3 FTCA (0.018 %), PFHxA (0.026 %), PFPeA (0.001 %), PFBA (0.018 %)	67
6:2 FTAB	Aerobic oily soil	Semi-closed glass bottles	60 d	31 d	PFCA: ND	66
6:2 FTSA	Aerobic WWTP mixed liquor sludge	Glass bottles with purging and closed glass bottles	77 d (purge), 148 d (closed)	NA	Purge system: 6:2 FTOH (7%) Closed system: 6:2 FTCA (10%), 6:2 FTUCA (1.4%), PFHxA (1.7%)	82
6:2 FTSA	Aerobic diluted activated sludge	Shaken closed vessels	90 d	2 years	∑5:2 ketone, 5:2 sFTOH (3.4%), 5:3 FTCA (0.12 %), PFHxA (1.1%), PFPeA (1.5%), PFBA (0.14%)	74
6:2 FTSA	Aerobic river sediment	Shaken closed glass serum bottles at 20 °C under the dark	90 d	< 5 d	6:2 FTCA (12 %), 6:2 FTUCA (< 1 %), 5:3 FTUCA (< 1 %), ∑5:2 sFTOH, 5:2 ketone (<8 %), 5:3 FTCA (16 %), 4:3 FTCA (< 1 %), PFHpA (0.55 %), PFHxA (20 %), PFPeA (21 %)	78
6:2 FTSA	Aerobic sediment	Shaken closed glass bottles at 20 °C under the dark	12 d	NA	DI water microcosm: ∑PFHxA, PFPeA, PFBA (~14 %). Leachate added microcosms: ∑PFHxA, PFPeA, PFBA (10~13 %).	83
6:2 FTSA	Aerobic wetland slurry	Shaken glass serum bottles at 30 °C in the dark.	142 d	NA	5:3 FTCA (2.7 %), PFHxA (2.1 %), PFPeA (6.1 %)	84
6:2 FTSA	*Pseudomonas sp. strain D2*	Shaken closed glass vials at 30 °C	50 d	NA	Six volatiles transformation products (unknown identity)	85
6:2 FTSA	*Gordonia* sp. strain NB4-1Y	Shaken closed serum bottles at 30℃	7 d	NA	6:2 FTOH (4.14%), 5:2 FT ketone (43.9 %),5:2 sFTOH (8.97 %), 5:3 FTCA (0.35%), PFHxA (0.55 %), PFPeA (0.1 %)	67
6:2 FTSA	*Gordonia* sp. strain NB4-1Y	Shaken closed amber bottles at 30 °C	5 d	NA	6:2 FTUCA (3%), 6:2 FTCA (2.5%), 5:3 FTCA (NA), 5:3 FTUCA (NA), PFHxA (ND), PFBA (ND)	86
6:2 FTSA	*Rhodococcus jostii RHA1*	Shaken closed glass vials at 30 °C under sulfur-free condition	6 d	NA	Unquantified 6:2 FTUCA, α-OH 5:3 FTCA, PFHpA	87
6:2 FTSA	*Dietzia aurantiaca J3*	Erlenmeyer flasks	7 d	NA	Unquantified 6:2 FTUCA, 6:2 FTCA, 5:3 FTCA, PFHxA, PFPeA	88
6:2 FTOH	Aerobic river sediment	Shaken closed glass serum bottles	100 d	1.8 d	6:2 FTCA (0.2%), 6:2 FTUCA (ND), 5:3 FTCA (22.4 %), 4:3 FTCA (2.7 %), PFHxA (8.4 %), PFPeA (10.4 %), PFBA (1.5 %)	89
6:2 FTOH	Aerobic sediment	Shaken closed glass serum bottles at 20-25 °C	28 d	< 3 d	6:2 FTUCA (0~0.1%)5:2 sFTOH (28~73%), 5:3 FTCA (9.6~23.2 %), PFHxA (11~26%), PFPeA (2.0~5.3%), PFBA (0.5~2.9 %)	90
6:2 FTOH	Aerobic activated sludge	Closed glass serum bottles	56 d	NA	6:2 FTUCA (<1.0 %), 6:2 FTCA(ND), 5:3 FTCA (14%), PFHxA (11%), PFPeA (4.4 %), PFBA (<0.5 %)	91

6:2 FTOH	Aerobic mixed bacterial culture	Shaken closed vessels at 20-25 °C	90 d	<2 d	6:2 FTUCA (25 %), 6:2 FTCA (5.7 %), 5:3 FTCA (5.5 %), PFHxA (5.1%), PFPeA (<0.5%), PFBA (<0.5%)	68
6:2 FTOH	Aerobic microbial culture	Shaken closed glass serum bottles	32 d	NA	6:2 FTUCA (9.9%), 6:2 FTCA (27%), 5:3 FTCA (12.5%), PFHxA (2%), PFPeA (1.6 %), PFBA (1.7%), TFA (2.3%)	92
6:2 FTOH	Aerobic soil	Shaken closed vessels at 20-25 °C	180 d	<2 d	6:2 FTUCA (ND), 6:2 FTCA (ND), 5:3 FTCA (15%), PFHxA (8.1%), PFPeA (30%), PFBA (1.8%)	68
6:2 FTOH	Aerobic soil	Flow-through system	84 d	<2 d	PFHxA (4.5%), PFPeA (4.2%), PFBA (0.8%)	69
6:2 FTOH	*Pseudomonas oleovorans*, *Pseudomonas butanovora*	Shaken closed glass vials in a dark room at 30 °C	28 d	NA	*P. oleovorans*: 6:2 FTUCA (7.26%), 6:2 FTCA (0.23%), 5:3 FTCA (5.7%), PFHxA (2.8%), PFPeA (ND), PFBA (0.44%) *P. butanovora*: 6:2 FTUCA (43.5%), 6:2 FTCA (33.4%), 5:3 FTCA (ND), PFHxA (2.9%), PFPeA (ND), PFBA (ND).	70
6:2 FTOH	*Mycobacterium vaccae JOB5*, *Pseudomonas fluorescens DSM8341*	Shaken closed glass bottles in a dark room at 30 °C	28 d	NA	*M. vaccae:* 6:2 FTUCA (32%), 6:2 FTCA (13%), 5:3 FTCA (3.4 %), 5:3 FTUCA (1.1 %), PFHxA (0.89 %), PFPeA (0.26 %), PFBA (0.37 %) *P. fluorescens*: 6:2 FTUCA (16 %), 6:2 FTCA (38%), 5:2 ketone (21 %), 5:2 sFTOH (27 %), 5:3 FTCA (4.8 %), 5:3 FTUCA (0.25 %), PFHxA (1.6 %), PFPeA (0.57 %), PFBA (ND)	71
6:2 FTOH	White-rot fungus *Phanerochaete Chrysosporium*	Shaken closed serum bottles	28 d	NA	6:2 FTCA (0.42%), 6:2 FTUCA (1.61%), 5:3 FTCA (32 %), PFHxA (4.2 %), PFPeA (1.5%), PFBA (0.15 %)	75
6:2 FTOH	Two fungal strains and six fungal isolates	Closed serum bottles	28~30 d	NA	∑6:2 FTCA, 6:2 FTUCA (<1~30.1%), ∑5:2 ketone, 5:2 sFTOH (ND~4.5%), ∑5:3FTCA,4:3 FTCA (<1~51.4 %), ∑PFHxA, PFPeA, PFBA (<1~ 6.7%)	76
5:3 FTCA	*Pseudomonas oleovorans*, *Pseudomonas fluorescens*	Shaken closed glass bottles in a dark room at 30 °C	90 d	NA	*P. Fluorescens* (sodium fluoroacetate and yeast extract): 4:3 FTCA (<1.54%), PFPeA (0.36 %). *P. oleovorans:* ND	71
5:3 FTCA	Aerobic soil	Shaken closed vessels at 20-25 °C	60 d	NA	4:3 FTCA (2.3%)	68
5:3 FTCA	Diluted domestic WWTP activated sludge	Shaken glass serum bottles at 20-25 °C	90 d	NA	4:3 FTCA (14.2 %), 3:3 FTCA (0.9 %), PFPeA (5.9 %), PFBA (0.8 %)	93
PFOAAmS, PFOSAmS	Aerobic soil	Closed amber serum bottles	180 d	PFOAAmS: 142 d, PFOSAmS:>>180 d	PFOAAmS: PFOA (30 %) PFOSAmS: PFOS (0.03 %)	94
PFOANO, PFOSNO	Aerobic soil	Semi-closed glass bottles	90 d	PFOANO :3~7 d, PFOSNO: ~15 d.	PFOANO: PFOA (15−21 %) PFOSNO: PFOS (2 %)	81

2.3.2 Anaerobic and anoxic biotransformation

There are fewer studies on the anaerobic and anoxic biotransformation of AFFF-derived precursors (shown in Table 2.2) than on aerobic conditions. Yi et al. investigated the biotransformation of 6:2 FTSAS under sulfate-reducing conditions in microcosms inoculated with pristine or AFFF-impacted solids.[95] Results showed that the 6:2 FTSAS was biotransformed

primarily to a stable polyfluoroalkyl compound, 6:2 fluorotelomer thioether propionate (6:2 FtTP, 30-36%),[95] which could not be further biodegraded into PFCA, FTSA and FTCAs as observed for aerobic transformation,[28, 29] indicating the stability of the thioether group under sulfate-reducing conditions. 6:2 FTSA, the common intermediate from aerobic biotransformation of AFFF-derived fluorotelomer precursors,[28, 29] was found to be resistant to biodegradation in both anaerobic sediment and anaerobic digester sludge, with no PFCA production over the >100 d incubation.[78, 96] In an anoxic wetland slurry, 6:2 FTSA slowly biodegraded into 0.7 mol% 5:3 FTCA only over 116-d incubation, in sharp contrast with a much higher yield of 5:3 FTCA (2.7 mol%), PFPeA and PFHxA (2.1~6.1 mol% by day 142) in the aerobic wetland slurry. [78, 84] Despite the slow anoxic biotransformation of 6:2 FTSA, these findings were significant since they demonstrated the capability of anoxic microorganisms at partially breaking down the perfluoroalkyl chains to produce smaller molecules in the environment where oxic and anoxic biological activities can co-occur or alternate. In addition, 6:2 FTOH, the common intermediate from the aerobic biotransformation of AFFF-derived FT precursors (e.g., 6:2 FTSAS, 6:2 FTAA, 6:2 FTAB),[29, 30] was transformed primarily into 6:2 FTCA and/or 5:3 FTCA in anaerobic digester sludge under methanogenic conditions. [97, 98]

Table 2.2 A summary of studies on the anaerobic and anoxic transformation of AFFF-derived precursors and related transformation intermediates.

Precursors	Types of microbes or microcosms	Incubation conditions	Incubation duration	Estimated half-life	Transformation product yields (%)	References
6:2 FTSAS	Microcosms inoculated with pristine or AFFF-impacted solid under sulfate-reducing conditions	Closed glass serum bottles purged with N_2/CO_2	300 d	NA	Pristine solids: 6:2 FtTP (36%), ∑6:2 FtTPlA, 6:2 FtTPlAA, 6:2 FtTPoP (<0.1%), 6:2 FTSA (ND), FTCA(ND), PFCA(ND) AFFF-impacted solids: 6:2 FtTP(30%), ∑6:2 FtTPlA, 6:2 FtTPlAA, 6:2 FtTPoP (<0.1%), 6:2 FTSA (ND), FTCA(ND), PFCA (ND)	95
6:2 FtTP	Microcosms inoculated with pristine or AFFF-impacted solids under sulfate-reducing conditions	Closed glass serum bottles purged with N_2/CO_2	150 d	NA	ND	95
6:2 FTSA	Anaerobic river sediment	Sealed plastic bottles inside an	100 d	NA	ND	78

Precursors	Types of microbes or microcosms	Incubation conditions	Incubation duration	Estimated half-life	Transformation product yields (%)	References
		anaerobic chamber				
6:2 FTSA	Anaerobically digested sewage at 30°C	Closed serum flasks flushed with N_2/CO_2 gas	110~162 weeks	NA	ND	[99]
6:2 FTSA	Anoxic wetland slurry	Closed glass serum bottles	116 d	NA	5:3 FTCA (0.7 %), PFHxA (ND), PFPeA (ND)	[84]
6:2 FTSA	Anaerobic digester sludge	Laboratory-scale anaerobic digester	8 weeks	NA	ND	[96]
6:2 FTOH	Anaerobic river sediment	Closed glass serum bottle at 20 °C under the dark	100 d	NA	6:2 FTCA (60 %), 5:3 FTCA (12 %), PFHxA (0.6%)	[78]
6:2 FTOH	Anaerobic WWTP digester sludge under methanogenic condition	Closed bottles at 29 °C inside anaerobic chamber	90 d~176 d	30 d	6:2 FTCA (32~44%), 6:2 FTUCA (1.8 ~8.0%), 5:2 sFTOH (0.6~2.5%), 5:3 FTCA (18~23%), PFHxA (0.2~0.4%)	[97]
6:2 FTOH	Anaerobic municipal digester sludge under methanogenic condition	Anaerobic glove box	94 d	NA	6:2 FTCA (11 %), 6:2 FTUCA (ND)	[98]
6:2 FTUCA	Anaerobic WWTP digester sludge under methanogenic condition	Closed bottles at 29 °C inside anaerobic chamber	56 d	NA	3-fluoro 5:3 FTCA (0~53%), 5:3 FTCA (94%)	[97]
5:3 FTUCA	Anaerobic WWTP digester sludge under methanogenic condition	Closed bottles at 29 °C inside anaerobic chamber	56 d	<3 h	5:3 FTCA (95%), α−OH 5:3 FTCA (ND)	[97]
5:3 FTCA	Anaerobic WWTP digester sludge under methanogenic condition	Closed bottles at 29 °C inside anaerobic chamber	56 d	NA	ND	[97]
α-OH 5:3 FTCA	Anaerobic WWTP digester sludge under methanogenic condition	Closed bottles at 29 °C inside anaerobic chamber	90 d	NA	5:2 FTCA (0.4%), 4:3 FTCA (17%), PFPeA (0.9%)	[97]

Overall, the results for anaerobic biotransformation of the above-mentioned FT precursors demonstrate that these compounds did not represent a major source of PFCA detected in anaerobic environmental matrices. Since researchers mainly focus on the anaerobic/anoxic biotransformation of n:2 fluorotelomers, the anoxic and anaerobic biotransformation of both ECF precursors (e.g. TAmPr-FHxSA, TAmPr-N-MeFBSA, etc) and n:3 and n:1:2 fluorotelomers (n:3, n:1:2 FTBs), which commonly showed up at historical or current AFFF contaminated sites, [42, 44, 100] remains unclear and warrants further research.

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Chapter 3. Per- and Polyfluoroalkyl Substances in Contaminated Soil and Groundwater at Airports: a Canadian Case Study

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Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Per- and Polyfluoroalkyl Substances in Contaminated Soil and Groundwater at Airports: A Canadian Case Study. *Environ. Sci. Technol.* **2022,** *56*, (2), 885–895.

Preface

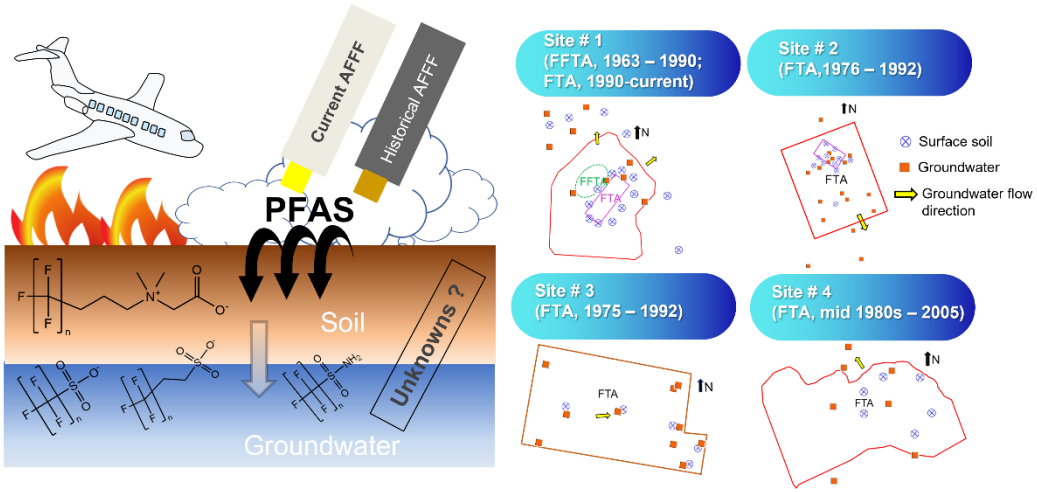
Utilizing PFAS-containing AFFFs for firefighting training was a common practice in many countries for decades. Due to the lack of knowledge of the environmental impact of such substances, AFFFs were released with limited or no treatment, resulting in contamination of directly impacted soil and groundwater, as well as adjacent areas indirectly impacted. Site assessments of AFFF-impacted areas reported in the USA and Europe have shown that a wide range of PFAS would persist in impacted areas decades after AFFF release. However, such data are not yet available for Canada, which has similar firefighting training practices. What is different are the types of AFFFs utilized because of product availability and types of applications. In addition, due to varying geochemical conditions, climate, land utilization and past remediation history, PFAS in impacted sites are expected to exhibit large spatial variability. Therefore it necessities a comprehensive characterization of PFAS in AFFF-impacted sites in Canada to support risk assessment and future remediation efforts.

Abstract

The occurrence of 93 classes of per- and polyfluoroalkyl substances (PFAS) was investigated at aqueous film-forming foam (AFFF)-impacted sites of four Canadian airports. Surface/subsurface soils and groundwater samples were characterized using high-resolution mass spectrometry (HRMS) and an improved total oxidizable precursor (TOP) assay. PFAS profiles, loads, and spatial trends were highly site-specific, influenced by AFFF use history, variations in sorption, transport and in-situ transformation potential of PFAS, and site remediation history. All sites have been impacted by more than one AFFF chemistry, with the active firefighter training area (FTA) exhibiting greater PFAS variety and total PFAS burden than decommissioned sites. Zwitterionic and cationic compounds composed a large percentage (34.5-85.5%) of the total PFAS mass in most surface soil samples in the source zone but a relatively low percentage (<20%) in groundwater samples. Background soils surrounding the source zone contained predominantly unidentified precursors attributed to atmospheric deposition, while in AFFF-impacted soils, precursors originating from AFFFs can be largely captured by HRMS using available suspect lists. Horizontal transfer of PFAS in surface soils was limited, but vertical migration down the soil column occurred even in locations of low permeability. The study provides a critical dataset to support developing new priority analyte lists and integrating TOP assay for comprehensive PFAS monitoring at AFFF-impacted sites.

Keywords: Aqueous film-forming foam (AFFF), Zwitterionic PFAS, Airports, Soil, Groundwater, Site characterization, Suspect screening, Total oxidizable precursor (TOP assay)

Graphical Abstract



3.1 Introduction

Aqueous film-forming foams (AFFFs) represent a critical usage of per- and polyfluoroalkyl substances (PFASs) as fire extinguishing agents for hydrocarbon fuel fires at airports, military bases, firefighter training areas, oil industries, and other installations.[1] Over decades, releases of AFFFs and the lack of proper containment and treatment measures have caused severe PFAS contamination of soil, surface water and groundwater.[2] Toxicological and epidemiological studies on a subset of perfluoroalkyl sulfonates (PFSAs) and carboxylates (PFCAs) have driven the changes in regulatory actions, environmental policy, and industrial activities surrounding PFASs. With the phase-out of perfluorooctane sulfonate (PFOS) and carboxylate (PFOA), as well as their precursor substances, PFAS-based formulations also evolved. PFAS manufacturing has shifted to (or continued to use) non-regulated alternatives that are less bioaccumulative, such as those with shorter perfluoroalkyl moieties, fewer perfluorinated carbons, or polyether linkages.[3-5] Investigations of AFFF-impacted sites present the great challenge of comprehensively characterizing a large variety of chemical species, reflecting the varying formulations a site has been exposed to over time and assessing how PFAS naturally transport and attenuate.

In 2004, ~ 45% of the total AFFF inventory in the US was electrochemical fluorination (ECF) based, while about two-thirds of the AFFFs that met military specifications (MilSpec) were of ECF chemistry.[6] The past fifteen years have seen a shift to fluorotelomer (FT)-based AFFFs away from the ECF-based formulations due to the PFOS phase-out. Nevertheless, recent site assessments of AFFF-impacted sites in the US revealed the dominance of ECF-based chemistry in soil and groundwater due to the decadal use of MilSpec AFFFs at those military sites.[5, 7] Some US sites also showed characteristic fluorotelomers, primarily n:2 fluorotelomer sulfonates (FTSAs).[8, 9] Other studies have reported PFAS contamination at airports (both military and civilian) and firefighting training areas (FTA) in European countries (e.g., The Netherlands[10], Sweden[11], France[12], and Norway[13]) with frequent detection of PFSAs, PFCAs and FTSAs. Dauchy et al. recently reported the overwhelming contribution of fluorotelomers in runoff water and wastewater drained from a large firefighter training area in France, and also deep seepage of PFAS into subsurface soil and groundwater.[12] Several fluorotelomer classes (e.g., 6:2 fluorotelomer sulfonamidopropyl betaine) and their related by-products were identified in their study.[12] We have also detected dozens of fluorotelomers and their partial transformation products in soils after the

AFFF emergency response to a major terrestrial oil spill and fire at Lac-Mégantic (QC, Canada).[14] Although FT-based AFFFs have been widely used and released, detailed site investigations of impacted soil and groundwater on these PFAS have been infrequently reported. In Canada, PFAS contamination at places with a history of AFFF usage is recognized (Auditor General's response to Petition 332, Canada).[15] Based on publically available resources, Milley et al. estimated that 152 out of 2071 airports/heliports likely have PFAS contamination linked to FTAs and/or accidents where fires occurred, and another 268 sites are possibly impacted by PFAS linked to storage of petroleum products and presence of AFFF systems at sites.[16] The data mining approach narrowed down the number of places for further investigation, but PFAS monitoring data remain lacking for most FTA sites.

High-resolution mass spectrometry (HRMS) allowed identifying cationic and zwitterionic PFAS as major AFFF components and in AFFF-impacted environments.[5, 9, 17-19] Many of these polyfluoroalkyl compounds are likely precursors to PFSAs and PFCAs because non-fluorinated functionalities can be susceptible to environmental transformation processes. Adamson et al. reported that in a former US FTA, 52% of the total PFAS mass was associated with polyfluoroalkyl precursors, and zwitterionic and cationic species (primarily ECF-based) represented 83% of the total precursor mass, even more than 20 years after the last AFFF deployment. [20] Strong retention and/or slow transformation of those precursors on source zone soils created a slow but sustained long-term PFAS flux to adjacent surface water and groundwater.[20] We surmise that zwitterionic and cationic fluorotelomer surfactants may also exhibit strong retention by surface soils, but few field monitoring data are available to verify the hypothesis.

Ongoing analytical refinement has contributed to discovering the strong association of zwitterionic and cationic PFAS with surface soils. However, the methods previously developed for perfluoroalkyl acids are not necessarily transferable to zwitterionic and cationic PFAS. For instance, a widely used soil extraction method could not effectively recover PFAS zwitterions and cations in soils, especially for soils with high organic matter and clay content.[21-23] The total oxidizable precursor (TOP) assay can capture unidentified precursor substances by quantifying the increase in PFCA concentrations after thermal-activated alkaline persulfate oxidation reactions where precursors are converted to PFCAs.[24 9] The approach can account for those PFAS without analytical standards or overlooked by UHPLC-HRMS analysis, but the unextracted PFAS fraction

would remain unidentified regardless of the analytical approach.[25] Therefore, we suspect that underestimating PFAS contamination of AFFF sites is widespread, as many studies did not perform detailed recovery tests. The TOP assay was recently validated using a more expansive suite of precursors for aqueous samples than the original method,[26] but few studies have applied it to nonaqueous environmental matrices.[9, 26-28]

Therefore, the project was initiated to provide a thorough characterization of PFAS at AFFF-impacted sites using up-to-date tools and methodology. Soil and groundwater samples were collected from FTA sites within four airports in Central and Eastern Canada. Target and suspect screening high-resolution mass spectrometry (HRMS) informed the concentration of individual PFAS, while the improved TOP assay procedures (new exhaustive soil extraction method, small volume reaction,[26] and modification of post-oxidation procedure[26]) revealed the contributions of unidentifiable PFAS, not only in AFFF-impacted areas but also the background soils. The complementary techniques and the inclusion of a wide breadth of PFAS allowed improved delineation. At the same time, the data revealed the persistence of fluorotelomer compounds (e.g., n:3 and n:1:2 FTB, and 6:2 fluorotelomers) in impacted sites. This first comprehensive characterization of PFAS pollution at civilian airports in North America provides critical information and methodology to support future PFAS monitoring, mitigation, and remediation efforts in Canada and other countries.

3.2 Materials and Methods

3.2.1 Chemicals and reagents

The sources of PFAS analytical standards included Wellington Laboratories (Guelph, ON, Canada), DuPont (Wilmington, DE, USA), and SynQuest Laboratories (Alachua, FL, USA). Six cationic and zwitterionic PFAS standards were custom-synthesized at the Beijing Surfactant Institute (Beijing, China).[29, 30] All isotope-labeled internal standards were obtained from Wellington Laboratories. Further details are provided in the Appendix (Table A.1 and A.2).

3.2.2 Study sites and sample collection

The four FTA sites (Figure A.1) are within four airports located in central and eastern Canada, representing four climate regions—Northeastern Forest, Great Lakes, St. Lawrence, and Atlantic

Canada. The sites have had varying periods of active fire-training activities, as illustrated in Figure A.1e, with Site #1 being the only in-activity firefighter training area (FTA, pink line). Site #1 (Figure A.1a) was built near a decommissioned former FTA area (green line, decommissioned in 1990), has a double-liner system in the ground, and has been in operation since 1990. Site #2 (Figure A.1b) was operational between 1976 and 1992 and is located within a larger area later redeveloped as a commercial airport park. Site #3 (Figure A.1c) was operational from 1975 to 1992; after decommissioning, around 17,000 m^3 of soil were excavated and moved to a nearby location for bioremediation of BTEX contamination, and clean soil was backfilled. Site #4 (Figure A.1d) was operational from the mid-1980s to 2005, and remediation was performed afterward; approximately 19,000 metric tons of soil were removed to remediate contamination by petroleum hydrocarbons, and clean soils were backfilled.

Field sampling was conducted by engineering firms contracted by federal and provincial transportation authorities between September 2016 and February 2017. The field soil samples at Site #1 covered the upgradient, the vicinity, and the downgradient of FTAs, while other sites only included the FTA vicinity areas. Soils were collected into pre-cleaned high-density polyethylene (HDPE) containers. Forty-five surface soil samples (0.15-0.3 m below ground surface (bgs) for all sites, listed in Table A.3a, n = 23, 11, 5, and 6 from Site #1, #2, #3, and #4, respectively) and thirty-seven subsurface soil samples (0.4-8.8 m bgs for Site #3) were collected. For site #1, samples SS01-SS03 outside the FTA might be impacted by the soil removed from the FFTA area during decommissioning. Samples collected from Site #2, #3 and #4 were original soils at the FTA areas, not impacted by site development or remediation activities.

Groundwater samples at Site #1, #2, and #4 covered the upgradient, the vicinity, and downgradient of the FTAs, while Site #3 included only the FTA vicinity area. Groundwater was collected by purging the monitoring wells and using dedicated ¼" HDPE tubing and peristaltic pumps directly into pre-cleaned HDPE bottles. Sixty-two groundwater samples (listed in Table A.3b, n = 14, 21, 18, and 9 from Site #1, #2, #3, and #4, respectively) were collected. All samples were shipped on ice to our labs. Further details can be found in the Appendix (Table A.3 and Figure A.1).

3.2.3 Sample preparation for UHPLC-HRMS analysis

Soils samples were stored at -20 °C upon reception, as were soil extracts in between preparation steps. Sample preparation started with soil air-drying, homogenization with a ceramic mortar and pestle, sieving via a 2-mm sieve, and freeze-drying for 24 h. As soil samples were freeze-dried before solvent extraction, PFAS concentrations included the fractions bound to soils and associated with the porewater. Losses from freeze-drying the soils were investigated via spiking experiments; suitable freeze-drying recoveries were obtained (91.2-115%, shown in Figure A.2), suggesting minimal losses from this sample pre-treatment step. Freeze-dried soils were extracted using the previously validated procedures that utilize sequential extractions with methanol and ammonium acetate (**Method I**) to effectively recover anionic, zwitterionic and cationic PFAS.[22] Groundwater samples were stored at 4°C once received, and the preparation was via previously validated procedures (dilution with organic cosolvent).[26] Detailed preparation methods are given in the Appendix.

3.2.4 Sample preparation for the TOP assay

Method I used for preparing soil samples for direct UHPLC-HRMS analysis has demonstrated satisfactory performance for recovering PFAS of various classes (detailed in Table A.4 and A.5), but ammonium acetate would be carried forward into the TOP assay affecting oxidation yields. Therefore, we applied **Method II** for soil extraction before the TOP assay after performing a complete validation of soil extraction efficiency (see Table A.6). We also verified the oxidation yields of eight representative precursors spiked into clean soils (see Figure A.3, A.4), including 6:2 FTSA, 8:2 FTSA, 6:2 fluorotelomer sulfonamidopropyl betaine (6:2 FTAB, also referred as 6:2 FTSA-PrB), 5:3 fluorotelomer betaine (5:3 FTB), 5:1:2 FTB, perfluorohexane sulfonamide (FHxSA), perfluorohexane sulfonamido amine (AmPr-FHxSA) and quaternary ammonium (TAmPr-FHxSA). The validation procedures are detailed in Appendix Briefly, **Method II** involved extraction by methanol/400mM NaOH (2 cycles) and methanol/400mM HCl (one cycle), followed by ENVI-Carb cartridge cleanup and N_2 evaporation. The dried soil extracts were then subjected to oxidation by hot alkaline persulfate for 6 h (60 mM potassium persulfate, 125 mM NaOH, 85°C). After oxidation, samples were cooled down to ambient temperature, neutralized with concentrated HCl, and quenched with methanol. The terminal PFCA products were quantified using the LC-MS method described in *Instrumental Analysis*.

The TOP assay for groundwater samples was conducted using previously published procedures.[26] Water sample was aliquoted after centrifugation and directly subjected to oxidation by hot alkaline persulfate for 6 h under the same conditions described above. Then the aqueous samples underwent the same post-oxidation treatment procedures as those for soil samples before instrumental analysis.

3.2.5 Instrumental analysis

Samples were analyzed via ultra-high-performance liquid chromatography coupled to high-resolution Q-Exactive Orbitrap mass spectrometry (UHPLC-HRMS, Thermo Fisher Scientific, Waltham, MA, USA). A total of 93 PFAS classes (430 individual PFAS) (shown in Table A.7) were quantified or semi-quantified in the field samples. The HRMS was operated in full scan MS mode (mass scan range: 150-1000 m/z) with a resolution setting of 70,000 FWHM at *m/z* 200. Targeted MS/MS (t-MS^2) under both positive and negative ionization modes (normalized collision energy, NCE = 20−70%) was used for the structure elucidation of semi-quantitatively identified PFAS, for which the confidence levels were assigned as per Schymanski et al.[31] An internal calibration curve (1/x weighted) was used to quantify 53 target analytes (structure shown in Figure A.5), using the available authentic standards and internal standards (Table A.8). Soil samples showed relatively low matrix effects (Table A.7), allowing the use of solvent-based calibration.

Suspect-screening was conducted for those PFAS previously reported in AFFF formulations,[5, 17-19] AFFF-impacted sites[5, 14, 29] or from industrial sources,[32, 33] but for which no authentic standards are available. The concentrations of suspect PFAS were estimated from the calibration curve of an assigned reference calibrant assuming equimolar response, as performed in our previous studies.[14, 29] The similarity to the reference calibrant was used to assign semi-quantification confidence levels for the suspect PFAS (Table A.9). Further details on instrument operation and calibration method/performance can be found in SI text and Table A.10. Quality assurance/quality control measures implemented throughout the analytical process are also summarized in the Appendix.

3.2.6 Data analysis

The molar concentrations of *total precursors* were estimated as the differences of PFCA molar concentrations after and before the persulfate oxidation. The molar concentration of *unknown precursors* was estimated as the difference between the TOP assay results and direct

UHPLC-HRMS analysis encompassing both target and suspect analytes. Origin(Pro) Version 2020b (OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis. A Shapiro–Wilk test was first used to test the normal distribution of the PFAS concentration in the source zone of each FTA site, and a Kruskal-Wallis ANOVA test was then performed to test the overall significant different results. Mann-Whitney pairwise comparisons were run as a post hoc test.

3.3 Results and Discussion

3.3.1 PFAS occurrence and levels in soil and groundwater

Summed PFAS. Of the 45 surface soil samples collected from the four sites, all presented detections of at least one PFAS, including the samples from the upgradient or downgradient areas. In the source zone areas (or the vicinity of FTA), up to 47 quantitative and 181 semi-quantitative PFAS (belonging to 66 distinct PFAS classes) were detected in impacted surface soils, with the maximum summed PFAS concentration (ΣPFAS) of 9200 µg/kg detected at Site #1 (Figure A.6). The first three sites displayed median ΣPFAS levels (2670, 1340, 1480 µg/kg dw, at Site #1, #2, and #3, respectively) orders of magnitude higher than Site #4 (53 µg/kg dw). Statistical analysis confirmed that ΣPFAS in soils of the first three sites was significantly higher (p-value = 6.9×10^{-4}, 5.5×10^{-4} and 4.1×10^{-2}, respectively) than Site #4.

Of the 70 groundwater samples, 66 showed detectable levels of at least one PFAS, with up to 38 quantitative and 139 semi-quantitative PFAS (belonging to 58 distinct PFAS classes) identified. The highest ∑PFAS was also detected in the source zone of Site #1, about 10800 µg/L (Figure A.6). Site #1 again showed a median concentration of ΣPFAS (1590 µg/L) orders of magnitude higher than the other three sites (29.2~90.0 µg/L). Statistical analysis revealed that the groundwater ΣPFAS level at Site #1 was significantly higher (*p*-value = 6.5×10^{-4}, 8.6×10^{-4} and 4.0×10^{-3}, respectively) than those at the other three sites, while the ΣPFAS of the latter three sites were not significantly different (*p*-value > 0.05). Overall, based on the soil and groundwater PFAS concentrations data, Site #1 ranked as the most impacted site, attributed to its long history of AFFF use and continued AFFF input. The highest levels seen at Site #1 exceeded those of a historic US AFFF site, which recorded maximum concentrations of 3810 µg/kg dw for surface soils and 5180 µg/L for groundwater.[7]

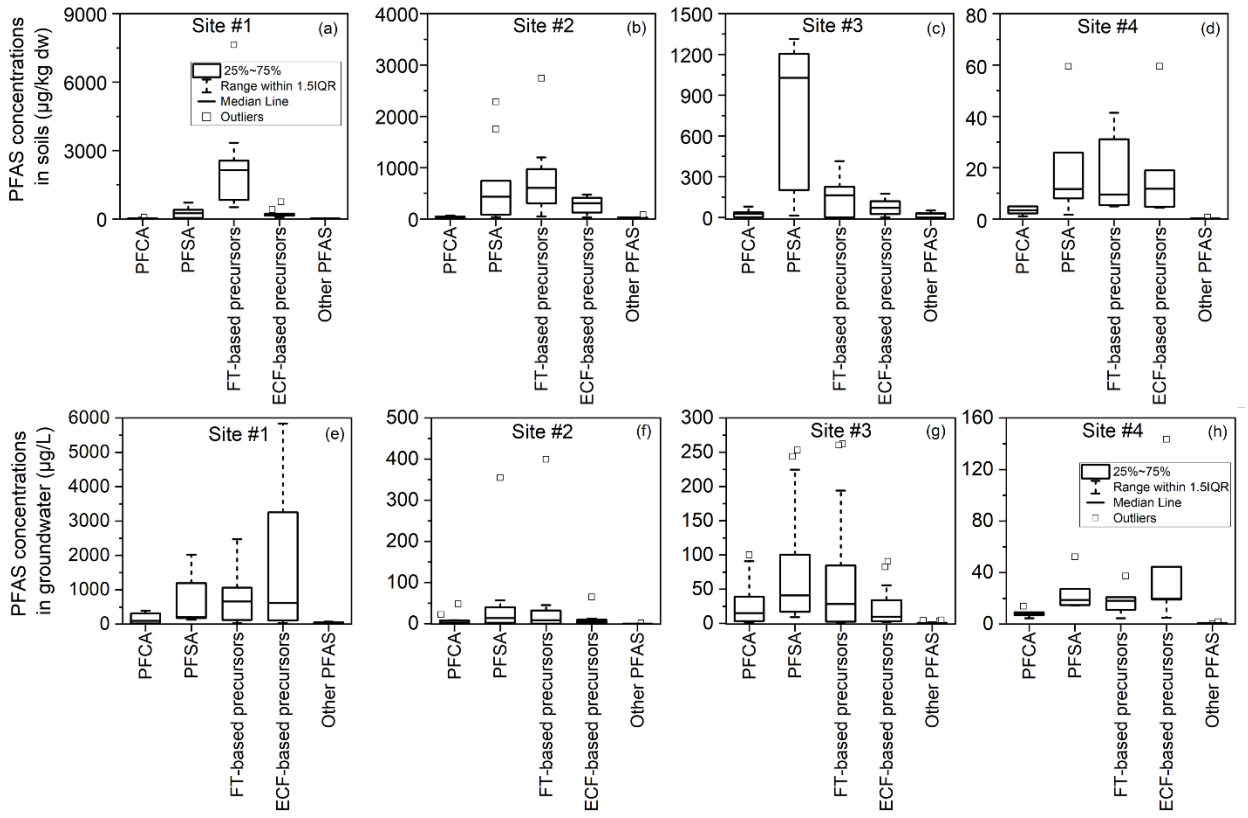


Figure 3.1 The box plots of the concentrations of each PFAS class in surface soils (0–0.5 m) (a-d) and groundwater (e-h) in the vicinity area at four Canadian FTA sites, the concentrations included quantitative and semi-quantitative values determined under both ESI- and ESI+ modes of UHPLC-HRMS.

Prevalence of PFAS superclasses. All targeted and suspect PFAS detections were grouped into five PFAS superclasses:[7] perfluorinated carboxylates (PFCAs), perfluorinated sulfonates (PFSAs), FT-derived compounds, ECF-derived sulfonamides and amides, and other PFAS. Superclasses 3 and 4 together constitute the known precursors. The "other PFAS" included cyclic and unsaturated PFAAs, substituted PFAA derivatives (e.g., Cl-PFAAs, O-PFAAs, H-PFAAs), perfluoroalkyl sulfinates, and other classes that did not fit the descriptions of the first four superclasses. As illustrated in Figure 3.1**,** the relative dominance of each superclass varied from site to site without displaying similar patterns. The relative predominance of each superclass in groundwater samples did not always correlate with soil samples for a given site. Site #1 was

predominated by fluorotelomers, whose median concentration (2140 µg/kg dw) was one order of magnitude higher than PFSAs or ECF-sulfonamides (211~251 µg/kg dw), while the groundwater showed higher levels of both fluorotelomers and ECF-sulfonamides (median: 614~660 µg/L) than PFSAs (median: 200 µg/L). In surface soils at Site #2, the median levels ranked as fluorotelomers (606 µg/kg dw) > ECF-sulfonamides (435 µg/kg dw) > PFSAs (310 µg/kg dw); in contrast, PFSAs (median: 13.9 µg/L) and fluorotelomers (median: 8.61 µg/L) were relatively more abundant in groundwater. At Site #3, both the surface soils and groundwater samples exhibited a dominance of PFSAs, while at Site #4, soils and groundwater comprised roughly comparable levels of ECF-sulfonamides and PFSAs, followed by fluorotelomers. Overall, all four sites indicated the historical use of the AFFFs of both fluorotelomer and ECF chemistry.

Previous soil surveys found a preponderance of PFOA over other PFCAs in AFFF-impacted soils;[34-37] however, we found the short-chain PFCA analogs (e.g., PFHxA and PFPeA) were more abundant than PFOA at the source zones. For instance, PFHxA was detected at relatively high abundance in surface soils (median: 0.489~11.5 µg/kg dw) and groundwater (median: 2.50~182 µg/L) at the four sites. As reported, ECF-based AFFFs manufactured before 1988 were enriched with PFCAs,[38] yet those manufactured around 1988-2001 only contained trace level PFCAs. FT-based AFFFs contained trace levels of PFCAs,[18] and equally important, the biotransformation of 6:2 fluorotelomers (6:2 FTSAS, 6:2 FTAA, and 6:2 FTAB, the dominant PFAS in several current-use AFFFs[17-19]) could also result in the generation of short-chain PFCAs (PFHxA, PFPeA, and PFBA).[25, 39, 40] Therefore, the presence of PFCAs at these sites can be attributed to the 3M AFFFs made before 1988 and/or *in-situ* biotransformation of fluorotelomers. However, the transformation appears quite limited, as further discussed below. Across all sites, "other PFAS" were present at relatively low concentrations, possibly due to their presence as minor components in AFFFs. The detection of a cyclic perfluoroalkyl sulfonate (class #73), similar to observations for Beijing international airport,[41] could be related to aircraft operations rather than firefighter training.

Major individual PFAS. As illustrated in Figures S7 and S8, PFOS was detected as one of the most abundant compounds in soil (median: 238~754 µg/kg dw) and groundwater (median: 12.2~171 µg/L) at the source zones of all the sites. The finding was consistent with previous reports of PFOS at high abundance in AFFF-impacted soils[2, 9] and groundwater [8, 12, 42] from similar sites in other regions. PFHxS was generally detected at lower levels than PFOS in both surface soils

(median: 1.60~99.8 µg/kg dw) and groundwater (median: 1.40~80.7 µg/L), which also agrees with the lower abundance of PFHxS than PFOS in ECF-based AFFFs.[18] As PFHxS could also reflect biotransformation of C6 precursors, PFHxS:PFOS concentration ratios may be used to indicate the extent of *in-situ* biotransformation. Previous studies reported increasing PFHxS:PFOS ratios in soil and groundwater along the groundwater flow path;[7] we also observed elevated PFHxS/PFOS ratios when moving downgradient of the FTA for soil (Site #1) and groundwater (Site #1 and #2) (Table A.11). This may be related to the differential transport of PFHxS and PFOS and the *in-situ* transformation of C6 ECF-derived sulfonamides.

The ECF-derived sulfonamides in surface soils at the four sites included both AFFF-derived ECF precursors and their transformation products (e.g., FASA) (Figure A.7, A.9, compounds' full names in Table A.7). The profiles of AFFF-derived ECF precursors were site-specific. At Site #1, N-CMAmP-FBSAP and N-TAmP-FHxSA (median concentration: 26.5 and 14.2 µg/kg, respectively) were among the most abundant; Site #2 primarily comprised N-TAmP-FHxSA and N-HOEAmP-FHxSA, whose concentrations (median: 90.4~115.7 µg/kg) were 5 to 900 fold higher than those at the other three FTA sites; Site #3 mainly contained N-HOEAmP-FHxSA (median: 16.2 µg/kg); while Site #4 was mainly composed of AmPr-FHxSA, N-TAmP-FOSA and N-CMAmP-FHxSA (median: 1.56~3.08 µg/kg). These ECF-sulfonamides were previously identified in 3M AFFF,[5] and this is the first time N-HOEAmP-FHxSA was observed in AFFF-impacted soils. Based on the knowledge of model C8 ECF precursors,[43] *in-situ* transformation and interconversion between C6 ECF precursors are likely (shown in Figure A.10c,d). Specifically, N-CMAmP-FHxSA or AmPr-FHxSAP may break down to AmPr-FHxSA, which is also an AFFF component.[17, 19] These three precursors with six perfluorinated carbons can further break down to FHxSA (median: 0.526~25.7 µg/kg at the four sites), a precursor to PFHxS. In groundwater, FHxSA (median: 2.51~182 µg/L) was the major ECF-derived sulfonamide found at the four sites, while other analogs with shorter perfluoroalkyl chains (including FPrSA, FBSA, and FPeSA) were also measured at high concentrations (median: 51.0~130 µg/L) at Site #1 (Figure A.7, A.9).

The abundance of individual fluorotelomers was also site-specific, as shown in Figures S7 and S11. The fluorotelomers in the surface soils of Site #1 included those with n:2 polyfluoroalkyl chains such as 6:2 FTAB, 6:2 FTSA, 8:2 FTSA, 6:2 fluorotelomer thiohydroxyammonium-sulfoxide (6:2 FTSHA-SO) and a demethylated analog of 6:2 fluorotelomer sulfonamide amine

(6:2 demethyl-FTA), but also n:1:2 and n:3 FTBs (n = 5, 7, 9,11 and 13). Site #2, #3 and #4 comprised mainly n:2 fluorotelomers, with 6:2 FTSA and/or 8:2 FTSA detected at high levels. 6:2 demethyl-FTA and 6:2 FTSHA-SO were also among the abundant ones at Site #2, while 8:2 demethyl-FTA was a major contributor at Site #4. 6:2 FTAB has been recognized as a major component of several brands of AFFFs (e.g., National Foams, Angus Fire and Fire Service Plus).[13, 18, 19] Previous studies have frequently reported 6:2 FTAB in AFFF-impacted soils,[13, 14] sediment,[29, 44], sludges,[45] surface water,[46] and fish.[29] However, n:3 FTB and n:1:2 FTB (n = 5, 7, 9, 11, 13, 15), which were detected in several AFFF brands (e.g., Buckeye[17, 18], Ansul and other AFFFs[12, 19]), started to show up in the environment only in very recent years, to the best of our knowledge. Previously we detected these compounds in earthworms from another Canadian airport that is not part of this survey[47] and the AFFF-impacted soils after the Lac-Mégantic derailment accident,[14] suggesting their current use in Canada.

Again, *in-situ* transformations (Figure A.10) that are likely to progress very slowly (as judged by the low PFCA levels) can partly explain the relative abundance of some precursors observed at surface soils of Site #1. For example, 6:2 FTSHA is an AFFF component, but its oxidation product 6:2 FTSHA-SO appeared at levels >30 times higher in sample SS-01. Similarly, 6:2 FTA, an AFFF component, appeared at least five times lower than its demethylated product (6:2 demethyl-FTA). Besides, 6:2 FTSA, as a common transformation intermediate from many possible precursors, appeared at high levels in select samples. The high abundance of 6:2 FTSHA-SO, 6:2 demethyl-FTA and 6:2 FTSA suggested their further degradation could be the rate-limiting step in the long pathways to forming PFCAs. In addition, we could not identify possible (bio)transformation products of n:3 FTB and n:1:2 FTB. Their very high abundance suggests very slow (bio)transformation in the field or probably lack thereof. A few anionic precursors that were detected in groundwater at high abundance at Site #1 were noticeably missing in surface soils (Figure A.7, A.11), such as 6:2 fluorotelomermercaptoalkylamido sulfonate (6:2 FTSAS) and its sulfoxide/sulfone oxidation products (Figure A.10). The lack of a positive charge (as in the quaternary ammonium group) in their structure may explain their low retention by soils.

We ranked the 15 most abundant PFAS detected in surface soils and groundwater for each site. As shown in Figure A.7, each site comprised precursor substances that have not been routinely monitored nor covered by the analyte lists of various published standard methods.[48] For surface

soils at Site #1, only three compounds have been included in the published standard methods,[48] while most of the other PFAS (largely zwitterionic or cationic) are not routinely monitored. The significance of such non-anionic compounds is further elaborated below.

3.3.2 Contribution of zwitterionic and cationic PFAS

Most surface soils (10/10 at site #1, 6/11 at site #2, 4/6 at site #4) in the source zone of Site #1, #2, and #4 were predominated by known precursors (>50% of ΣPFAS mass), with zwitterionic/cationic precursors (i.e., ESI (+) PFAS) making up a high mass fraction (38.4~92.7% of the total known precursors, or 34.5~85.5% of ΣPFAS) (Figure 3.2). The ESI (+) precursors at Site #1 were largely FT-based ones, while those at Site #2 and #4 have roughly equal contributions of both chemistries or are slightly predominated by ECF-based ones. The surface soils at Site #3 contained high percentages of PFSA but low percentages of known precursors (12.0~25.9% of ΣPFAS); in particular, ESI(+) precursors made smaller percentages (6.7~8.5% in ∑PFAS), comparable to the downgradient locations at Site #1. Despite overall low ΣPFAS at Site #4, ESI(+) precursors still made up a significant mass fraction.

Comparatively, in groundwater, known ESI (+) precursors represented more limited fractions of ΣPFAS. The mass percentage was largely less than 20%, except for Site #2, where some samples with relatively high contributions (>20%) were found in the source zone and at downgradient locations. Interestingly, the ECF-based ESI(+) precursors were detected at a higher mass fraction (median:1.4~8.1% of ΣPFAS) than FT-based ones (median:0~1.9% of ΣPFAS) in groundwater at source zone areas across all sites, contrasting the patterns in soils.

Several major zwitterions/cations of either fluorotelomer or ECF chemistry (e.g., FTBs, 6:2 FTAB, N-TAmP-FHxSA, N-HOEAmP-FHxSA) found in surface soils were measured at trace or non-detectable levels in corresponding groundwater samples (see Figure A.7), confirming the strong soil retention of zwitterions/cations.[7] Previously, Mejia-Avendaño et al. reported that the zwitterionic 6:2 FTAB exhibited higher sorption than anionic 6:2 FTSA over a wide range of aqueous concentrations (10-1000 nM).[49] Nguyen et al. also found that cationic N-TAmP-FHxSA and zwitterionic AmPr-FHxSA had soil sorption coefficients (K_d) 1-2 orders of magnitude higher than those of anionic compounds (e.g., C4-C8 PFCA, C5-C7 PFSA), indicating their higher affinity for soils than anionic precursors.[50] Although sorption behaviors of n:3 and n:1:2

fluorotelomer betaines have yet to be investigated, the current field monitoring data suggest that they may behave similarly as 6:2 FTAB and strongly sorb to soils. The quaternary ammonium at the polar head group allows the compounds to engage electrostatic interactions with negatively charged soil components. The current findings are thus consistent with previous investigations, and our data further revealed that soils serve as important sinks for ESI(+) precursors in source zone areas, and act as a long-term PFAS source to groundwater and adjacent surface w ater.[51, 52]

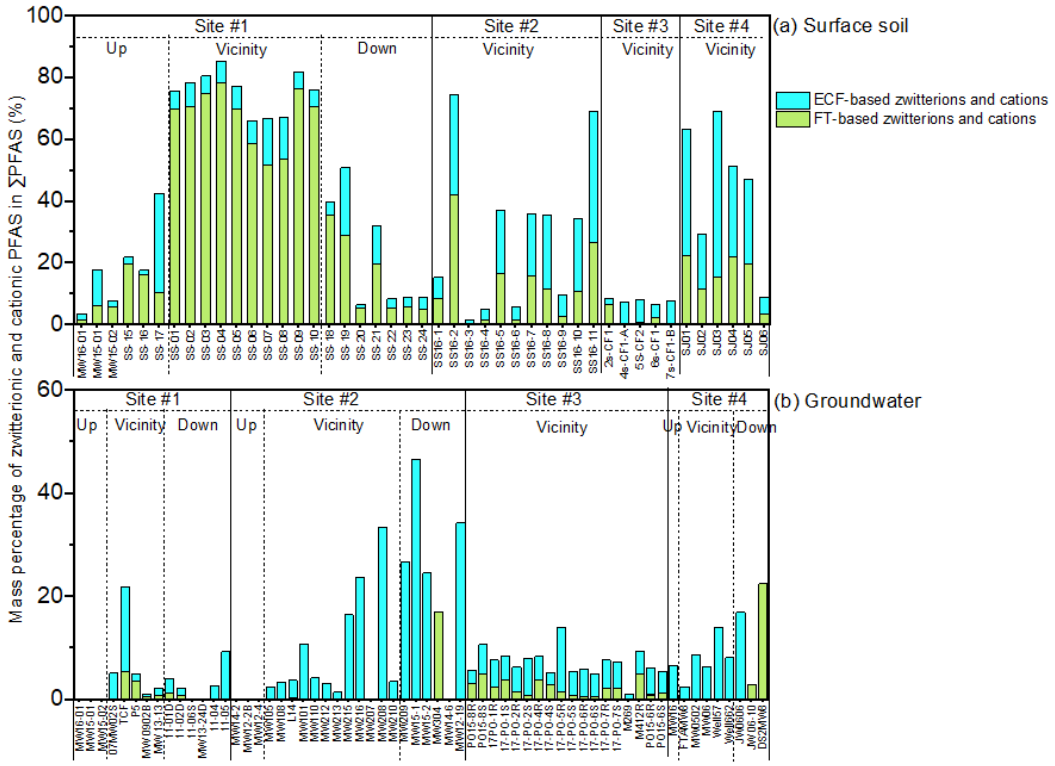


Figure 3.2 Contribution (in mass percentage) of FT-based and ECF-based zwitterionic and cationic PFAS to the summed PFAS concentration in surface soil (a) and groundwater (b) samples.

3.2.3 Spatial trends of PFAS in soil and groundwater

At Site #1, surface soil sampling was conducted inside and outside the existing FTA boundary to improve PFAS delineation. Figure A.6a confirmed the general trend that summed PFAS concentrations declined with the radial distance away from the active FTA boundary (or the AFFF source zone). The soil PFAS background (ΣPFAS: 2.42~9.91 µg/kg dw) at upgradient locations fell in the lower range of reported soil background (<0.001~237 µg/kg dw) from >1400 sampling

locations around the world.[2] The source zone contained ΣPFAS (median: 2670 µg/kg dw) orders-of-magnitude greater than the upgradient area (median: 4.65 µg/kg dw). The two immediate downgradient soil samples (SS18 and SS19) 10 ~ 20 m away from the FTA boundary still showed high levels of ΣPFAS (median:1430 µg/kg dw), while the far downgradient area (28-74 m away) showed levels (median: 4.92 µg/kg dw) comparable to the upgradient area. Locations most distant from the source zone (SS-23 and SS-24) displayed not only low ΣPFAS, but also minor contributions by the known precursors. As AFFF overspray or wind drift might have occurred, the source zone may be expanded to include some areas outside of the existing FTA boundary but is mainly limited to ~20 m to the northeast.

In groundwater samples of Site #1 (Figure A.6b), the low PFAS background (median: 0.215 µg/L) at the upgradient contrasted with the high concentrations (median: 1890 µg/L) within the FTA boundary, corresponding to the trend observed for surface soils. The six groundwater samples located 34-95 m downgradient from the active FTA area boundary still had high PFAS levels (median: 282 µg/L), with 6:2 FTSA, PFHxS, PFHxA and perfluorobutane sulfonamide (FBSA) dominating the profile. The mobility of these short-chain anionic PFAS along the hydraulic gradient is consistent with previous reports.[53, 54] As no samples beyond 100-m downgradient were examined, we cannot evaluate further downgradient transport.

3.2.4 Vertical distribution of PFAS in soils

Figure 3.3 illustrates the vertical soil profiles at several locations of Site #3, where PFAS were detectable at all depths down to 8.8 m (Location 5S). Nickerson et al. detected PFAS at ~15 m bgs in groundwater and soil, while Dauchy et al. also reported the deep seepage of PFAS to similar depths for firefighter training sites.[23, 55] Nickerson et al. also described increasing total PFAS concentrations with soil depth,[7] while the trend was less evident in the present study. Location 4S showed that PFAS were mostly restricted to the shallow soils (<0.6 m), with detectable but much lower levels at deeper layers; silty clay and clay layers with low hydraulic conductivity likely prevented the downward migration of PFAS. In contrast, subsurface soils within ~ 2.1 m for location 6S, ~0.9 m for location 7S and at ~4.0 m for location 5S, showed summed PFAS within the same magnitude as the surface soils (Figure 3.3). These three locations are within close range, but ΣPFAS in surface soils varied by two orders of magnitude (20.7~1621 µg/kg), while the deeper horizons also showed location-specific vertical profiles. The silty clay

and clay layers in these locations did not prevent PFAS transport, consistent with findings by Dauchy et al., who reported that clay layers did not stop PFAS deep seepage.[55] This was probably due to preferential flow pathways generated by soil heterogeneities resulting from either destruction of air-water interfaces (greater water saturation) or reduced air-water interfacial area (due to the presence of coarse grain media).[56]

PFOS as the most predominant compound in surface soils (Figure A.12) moved to deep soils (4.0, 2.1, and 7.0 m bgs at locations 5S, 6S, and 7S, respectively), while the PFHxS, 6:2 FTSA, and PFHxA reached a depth of 2.1 m at location 6S. Zwitterions such as 6:2 demethyl-FTA and N-HOEAmP-FHxSA were detectable at 2.1 m (Location 6S), but not at deeper depths, similar to observations by Nickerson et al.[7] ∑PFAS of 22.4~52.1 µg/kg was observed at a depth of 6.4~8.8 m bgs at Location 2S, 5S and 6S, higher than the levels seen for background surface soils. Figures 3 and S12 provide evidence that significant retention of PFAS occurs in unsaturated zones and capillary fringes.

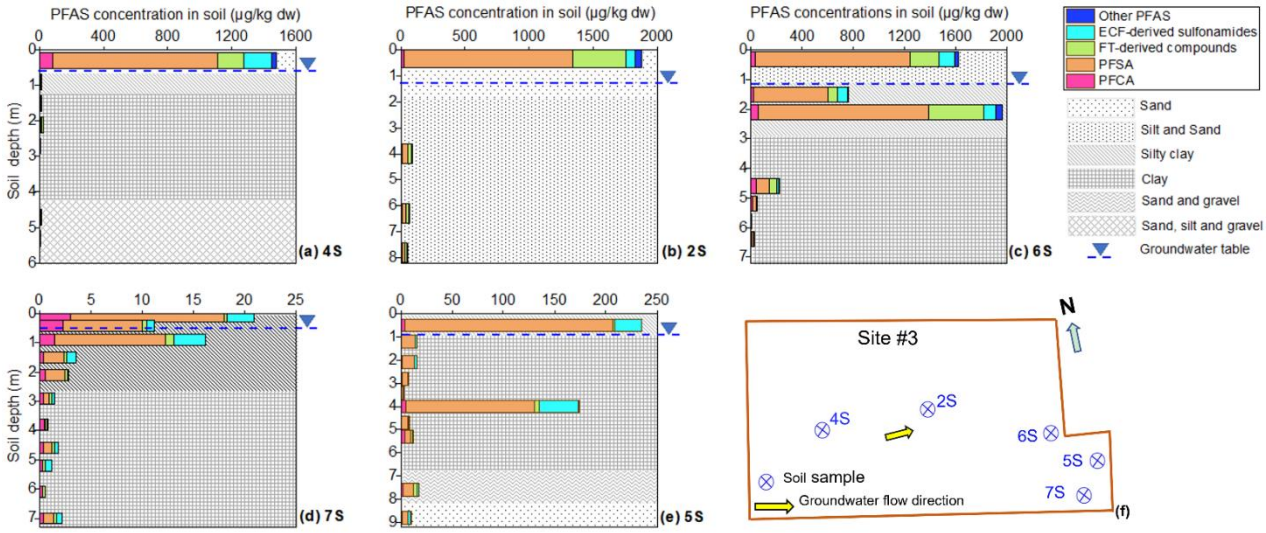


Figure 3.3 The vertical concentration profiles of five superclasses of PFAS in soils at Site #3 at five sampling locations (a) 4S, (b)2S, (c) 6S, (d)7S, (e) 5S. The five sample locations are shown in the scheme map (f).

3.2.5 Total and unknown precursors in soil and groundwater

Implementation of the TOP assay to soil requires exhaustive extraction of PFAS of various polarities, some of which form strong interactions with soil. **Method II** achieved satisfactory spike recoveries for 53 target PFAS (70-99%), except for FOSAA (62%) in one soil type (Table A.6). Oxidation yields of 8 selected precursors were also verified on three soils with different textures and organic matter content (Table A.4). Figure A.4 indicates that six model precursors, including 6:2 FTAB, 6:2 FTSA, 8:2 FTSA, FHxSA, AmPr-FHxSA, and N-TAmP-FHxSA, demonstrated acceptable or excellent oxidation yields. The fluorotelomers were converted to PFCAs (C_3 to C_{n+1}), with the dominance of C_{n-1}, $C_{n-2,}$ and C_n PFCAs. The C6 ECF precursors were converted to C_6 PFCA (PFHxA) as the primary product and C_5 PFCA (PFPeA) as the minor product. For the first time, the persulfate oxidation conversion yields of 5:3 FTB and 5:1:2 FTB were investigated. The total PFCA yields from 5:3 FTB and 5:1:2 FTB in soils, however, only reached 43%~57% and 7.2%~40%, respectively, and were lower than the yields in ultra-pure water (81% for 5:3 FTB and 40% for 5:1:2 FTB). Other potential oxidation products were screened using HRMS, but no fluorinated products were identified. We speculate that some products might not be captured by the current RPLC (C18) chromatographic methods or instrument, such as ultra-short chain PFCAs (e.g., trifluoroacetate) or H/F exchanged PFAS.

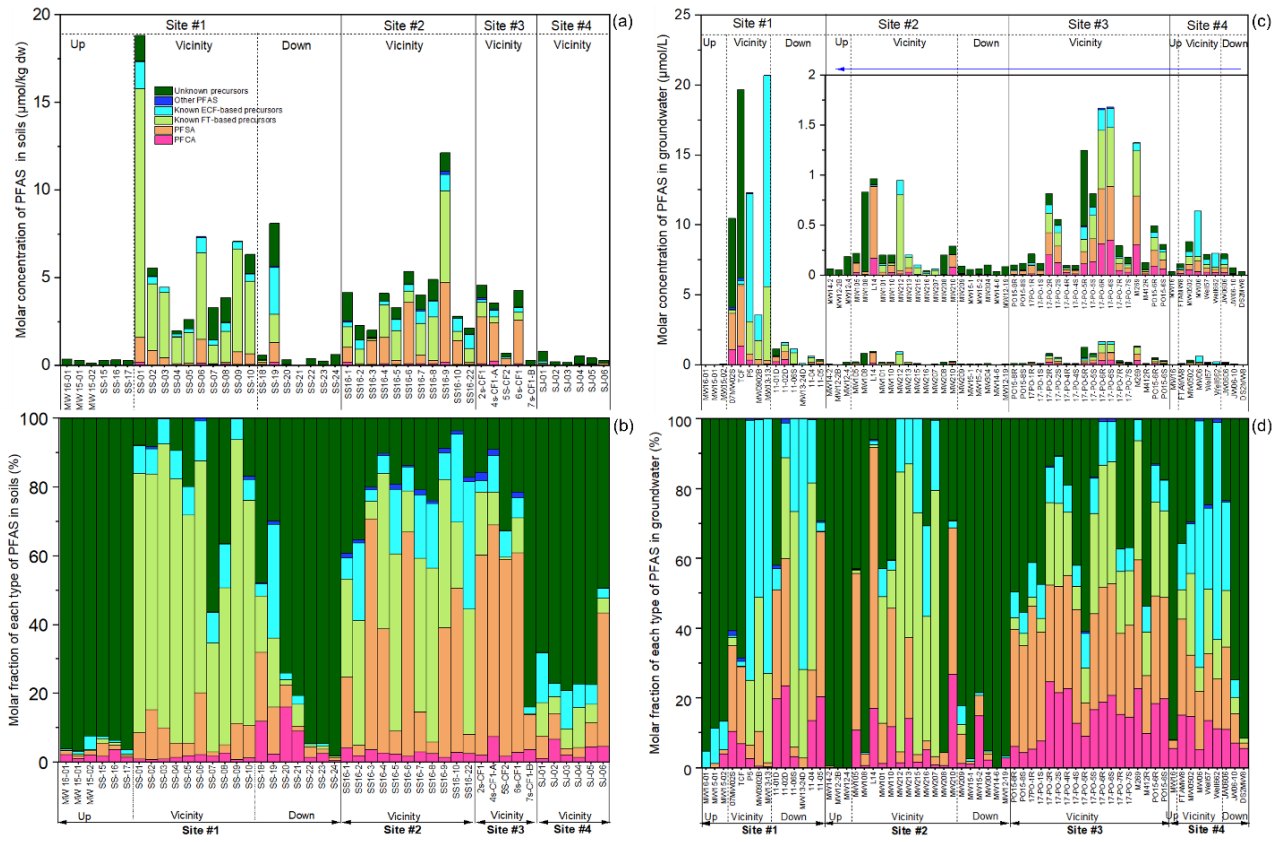


Figure 3.4 The molar concentrations (a, c) and molar distribution (b, d) of each identified PFAS class and unknown precursors in surface soil (a,b) and groundwater samples (c, d). The concentrations for all classes included quantitative and semi-quantitative values in both ESI- and ESI+ modes of UHPLC-HRMS; the unknown precursors (in dark green) were estimated as the moles only identified via the TOP assay.

The TOP assay revealed relatively high concentrations of total precursors in both surface soil (0.03~17.2 μmol/kg dw) and groundwater (0.02~13.8 μmol/L) at the four sites (Figure A.13), with the concentration at the source zone areas much higher than those at the upgradient and downgradient areas at both Site #1 (e.g., median: 3.8 vs 0.3~0.4 μmol/kg in surface soil while 1.76 vs 0.05~0.09 μmol/L in groundwater) and #4. Precursors with distinct chain lengths probably existed at the source zone, and the upgradient/downgradient area, as indicated by the distinct chain lengths of PFCA forming as major oxidation products from soil samples after TOP (e.g., for soils from Site #1, C4-C8 PFCAs formed in the source zone while C7-C8 PFCAs in the upgradient and

downgradient). However, a few samples (e.g., 3 of 45 soils) showed higher concentrations of known precursors (target and suspect-screening) than total precursors estimated by the TOP assay (Figure A.13). Underestimation of PFAS concentration via the TOP assay can be attributed to the incomplete conversion of some precursors, the production of ultra-short-chain PFCA not retained by the current chromatographic approach,[57] or specific soil constituents or co-contaminants impeding effective oxidation.

Despite some limitations, the TOP assay revealed unknown PFAS that were not always captured by HRMS analysis (Figure 3.4). The contribution of unknown precursors to the total PFAS was low (median: 9.4%) in the source zone area but increased in the background area: 96.2% (median) for the upgradient and 94.2% (median) for the downgradient. A sensitivity analysis (assuming different molar yields from TOP) results (shown in Figure A.14) indicate that the variability of yields of TOP could not explain the large fraction of unknowns for the background areas. We attributed these differences to distinct PFAS sources. All locations receive PFAS through atmospheric deposition. One prevalent theory proposed that a major source to PFAS soil background was volatile PFAS such as fluorinated alcohols and amide, as well as fluorotelomer polymers.[58, 59] The TOP assay products in our study suggested background soil contained a large percentage of ECF and/or FT-based PFAS with C8 perfluoroalkyl chains.[60] Based on precedent literature, the ECF- (e.g., MeFOSE, EtFOSE) and FT-based alcohols (e.g., 8:2 FTOH) could explain part of the PFAS soil background.[61, 62] However, the abundant PFAS that can be largely identified via LC-HRMS using available suspect lists in the source zones indicates the primary AFFFs source, dwarfing the contribution of the untargeted volatile PFAS from diffuse atmospheric sources.

Compared with the active site #1 (median: 8.8%), unknown precursors contributed more to the soil total PFAS at the other three historical sites (median: 19.0~71.3%). The highest contribution of unknown precursors was at site #4 (median: 71.3%), consistent with the above conjecture—the predominance of unknown precursors from atmospheric deposition is only evident when the PFAS associated with AFFFs are at low levels.

3.4 Environmental Implications

The study characterized multiple AFFF-impacted sites at four Canadian airports through complementary uses of LC-HRMS and TOP assay. PFAS profiles, loads, and spatial trends were highly site-specific, influenced by AFFF use history, variations in sorption, transport and *in-situ* transformation potential of PFAS, and site remediation history. All four sites commonly had elevated levels of PFSAs and 6:2 fluorotelomers above the background levels, demonstrating the historical use of both ECF-based and fluorotelomer AFFFs in all airports. Despite different geographical locations and AFFF use history, the Canadian sites shared some common characteristics with an AFFF site in the United States[7]: 1) ESI(+) PFAS can make up a large percentage of the total PFAS burden in the soil, more so than in groundwater; 2) PFAS can seep into the deep subsurface even for locations with low subsurface permeability; 3) PFAS can transport out of source zones to downgradient locations in the subsurface.

Zwitterionic n:3 and n:1:2 FTB (n = 5, 7, 9, 11 and 13) were exclusively measured in the active FTA area at Site #1, indicating the AFFFs permitted to use nowadays rely on this new type of fluorotelomer chemistry. Their extremely high concentrations, as well as the existence of long-chain analogues (e.g., n >7) are concerning. Toxicity and environmental fates of n:3 FTB and n:1:2 FTB remain largely unexplored. Their low oxidative conversion yields via TOP assay suggest their transformation, if it occurs, might deviate from the recognized pathways for n:2 fluorotelomers.[63, 64] The PFAS prevalently detected in the present study and other surveys [7] could be used to develop a draft priority PFAS list (Table A.12 for both soil and groundwater) for streamlined monitoring efforts at FTA sites. Many of the most abundant PFAS found in the study are not routinely monitored, while some commonly targeted PFAS are less relevant to AFFF sites. Despite the increasing availability of HRMS, the TOP assay proves to be a valuable tool to estimate total PFAS and unknown precursors in both AFFF-impacted areas and background soils. The assay revealed that the PFAS soil background might have been largely underestimated in previous studies, which primarily focused on individual PFAAs and a limited number of precursors. Determining the identity of unknown precursors remains a challenge and probably requires analytical tools complementary to LC-HRMS.

Lack of information on AFFF types, quantities applied, and the timing of applications, among others, poses challenges for investigating the in-situ transformation pathways of precursors and evaluating the fundamental transport, fate, or behavior of PFAS. Previous studies reported the

remedial activities could alter the subsurface PFAA and precursor distribution at AFFF-impacted sites.[27] However, the impact of distinct remediation efforts, largely aiming at non-PFAS cocontaminants, on the fate of zwitterionic/cationic precursors deserves future research. Besides, modeling PFAS transport and comparison with field monitoring data should be performed in future studies.

3.5 References

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Chapter 4. Stability of Nitrogen-Containing Polyfluoroalkyl Substances in Aerobic Soils

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Liu, M.; Munoz, G.; Vo Duy, S.; Sauvé, S.; Liu, J., Stability of Nitrogen-Containing Polyfluoroalkyl Substances in Aerobic Soils. *Environ. Sci. Technol.* **2021,** *55,* (8), 4698-4708.

Preface

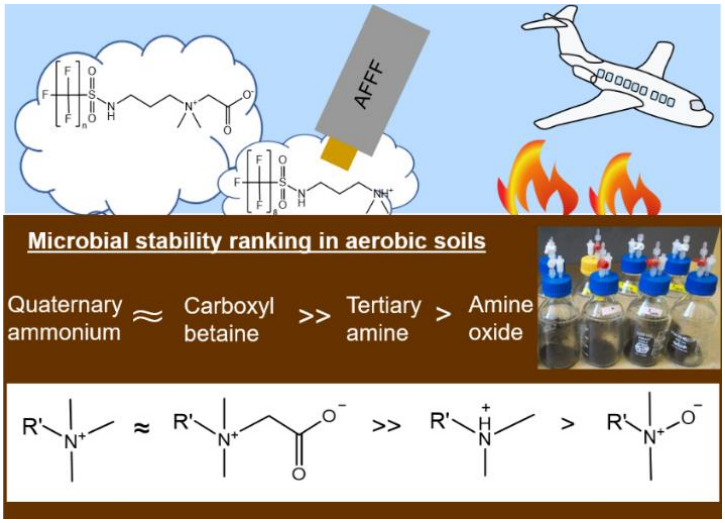
In Chapter 3, the characterization of PFAS concentration profiles at the source zone soils and groundwater from FTA sites within four Canadian airports revealed the high contributions of zwitterionic and cationic compounds to total PFAS loads in most surface soils, suggesting that soil represents an important sink of these compounds. At the sites with the historical use of AFFFs, the abundance of ECF-based zwitterions and cations (e.g., TAmP-FHxSA, AmPr-FHxSA, N-TAmP-FOSA and N-CMAmP-FHxSA, etc) in surface soils indicates their high retention and/or slow transformation in the soil environment. However, these assumptions have not been verified in experimental studies. In addition, we noted that these ECF-based zwitterions and cations exhibited structural differences in nonfluorinated functional groups, especially N-containing headgroups (e.g., sulfonamide, amide, quaternary ammonium, tertiary amine, and betaine, etc.). The influence of these functional groups on their microbial stability in the soil environment remains unclear. Therefore, this chapter focused on investigating the abiotic and biotic transformation potential of ECF-based betaines and amines in aerobic soils and establishing the structural-degradability relationship of ECF-based precursors.

Abstract

Zwitterionic per- and polyfluoroalkyl substances (PFASs) used in aqueous film-forming foams (AFFFs) could face diverse environmental fates once released at military bases, airports, fire-training areas, and accidental release sites. Here, we studied for the first time the transformation potential of four electrochemical fluorination (ECF)-based PFAS zwitterions (two carboxyl betaines and two tertiary amines) in aerobic soils. The two perfluoroalkyl sulfonamide derivatives were precursors to perfluorooctanesulfonate (PFOS), while the amide derivatives were precursors to perfluorooctane carboxylate (PFOA). These zwitterions and four other previously reported zwitterions or cations were compared for their transformation pathways and kinetics. Structural differences, especially the nitrogen head groups, largely influenced the persistence of these compounds in aerobic soils. The perfluoroalkyl sulfonamide-based compounds showed higher microbial stability than the corresponding perfluoroalkyl amide-based ones. Their stability in aerobic soils is ranked based on the magnitude of DT_{50} (time for 50% of substance to disappear): quaternary ammonium ≈ carboxyl betaine ≫ tertiary amine > amine oxide. The PFASs containing quaternary ammonium or betaine groups showed high stability in soils, with the longest DT_{50} likely to be years or decades, while those with tertiary amine or amine oxide groups showed DT_{50} of weeks or months. These eight ECF-based precursors provide insights into the degradation pathways and persistence in surface soils of other perfluoroalkyl cations and zwitterions present in AFFFs.

Keywords: PFOSB; PFOAB; PFOSAm; biotransformation potential; stability; structure-degradability relationship

Graphical Abstract



4.1 Introduction

Per- and polyfluoroalkyl substances (PFASs) have been used in a large variety of industrial, commercial, and domestic products, including aqueous film-forming films (AFFFs) for extinguishing hydrocarbon fuel fires.[1] Release of the water-based AFFFs without prevention measures or remediation strategies resulted in severe contamination with hydrocarbon-based surfactants, solvents, and PFAS at military bases, airports, fire-training areas, and accidental release sites. The prevalence of PFASs in surface waters,[2] groundwater,[3,4] soils,[4,5] sediments,[6] and biota[6] was thus documented. In addition to the commonly investigated perfluoroalkyl acids (PFAAs), a large variety of cationic, zwitterionic, and other anionic polyfluoroalkyl substances were detected in such environments. Great variations were noted between PFASs compositions in AFFF formulations and those in the environment,[4,7] indicating that abiotic or biotic transformation, sorption or other environmental processes (e.g., transfer, photodegradation, abiotic oxidation, natural reduction) occur to these PFASs after release into the environment. Understanding their environmental behavior and fate, especially for numerous zwitterionic and cationic polyfluoroalkyl substances that have been recently identified,[8] can provide crucial knowledge to allow proper site assessment and design of effective mitigation and remediation measures.

In the AFFF formulations manufactured before 2002, electrochemical fluorination (ECF)-based PFASs, including perfluoroalkyl sulfonates (PFSAs) and their precursor substances, represent significant components.[9,10] These precursors are known to be predominantly fluorinated sulfonamide derivatives ($F(CF_2)_n$─$S(O)_2NH$─).[9] For instance, perfluoroalkyl sulfonamide betaines (PFASB, C_3-C_6), perfluoroalkyl sulfonamide quaternary ammonium compounds (PFASAmS, C_3-C_8), and their synthesis intermediates perfluoroalkyl sulfonamide amines (PFASAm, C_3-C_8), among many other structures, were documented in patents[11] and identified in 3M AFFFs.[10,12] What is less known is that the ECF process also produced fluorinated amide derivatives ($F(CF_2)_n$─$C(O)NH$─) for various uses; for instance, perfluoroalkyl amido betaines (PFAAB) (C_6-C_{14}) were used in fire suppressants and commercial fluorinated surfactants.[10,13] Amide derivatives are potential precursors to perfluoroalkyl carboxylates (PFCAs), including perfluorooctane carboxylate (PFOA).[14] Since perfluorooctane sulfonate (PFOS) and their derivatives were phased out of production in 2000-2002 in North America due to their persistent, bioaccumulative, mobile and toxic (PBMT) properties, their concentrations in humans have been in decline,[15] suggesting

that direct exposure to PFOS contributed to a large percentage of human body burden. However, for AFFF-impacted sites, debates are ongoing regarding the role of precursors in contributing to PFAA burdens and how much effort should be placed on precursors in terms of chemical analysis, risk assessment, and remediation activities.

To date, a limited number of studies reported that AFFF-related precursors would undergo partial degradations under aerobic conditions in laboratory studies. Anionic 6:2 fluorotelomer thioether amido sulfonate (6:2 FTTh-PrAd-DiMeEtS, also previously referred as 6:2 FTSAS),[16,17] zwitterionic 6:2 fluorotelomer sulfonamide alkylamine (6:2 FTSAPr-DiMeAn, or 6:2 FTAA) and 6:2 fluorotelomer sulfonamide alkyl betaine (6:2 FTSA-PrB, or 6:2 FTAB)[18,19] were found to be biotransformed into PFCAs by mixed culture derived from activated sludge,[16,18,19] or soil microcosms.[17] We have recently investigated the biotransformation potential of several AFFF-related ECF-based compounds in aerobic soil microcosms. Perfluorooctane amido quaternary ammonium salt (PFOAAmS) degraded with an estimated half-life of 142 d and generated PFOA at a yield of 30 mol% by day 180, while perfluorooctane sulfonamido quaternary ammonium salt (PFOSAmS) produced PFOS at a yield of 0.3 mol% without noticeable changes in PFOSAmS concentrations (half-life >>180 d).[14] The presence of a quaternary ammonium group ($R{-}N^{+}(CH_3)_2{-}R'$), associated with strong sorption to solids and biocidal nature, seemed to contribute to the persistence of the compounds but still cannot prevent the nonfluorinated segment from breaking down. In contrast, two other PFAS with a terminal amine oxide group ($R{-}N^{+}{-}(CH_3)_2O^{-}$), perfluorooctane amido amine oxide (PFOANO) and perfluorooctane sulfonamido amine oxide (PFOSNO), showed much lower stability with DT_{50} (time for 50% of substance to disappear) < 15 days and significant production of PFOS and PFOA (yields of 2 mol% and 15~21 mol%, respectively, by day 90), in comparison to the corresponding quaternary ammonium salts.[20] It is intriguing that PFASs of similar molecular weights, with a minor difference in N-containing groups, i.e., $R{-}N^{+}(CH_3)_2{-}R'$ versus $R{-}(CH_3)_2N^{+}{-}O^{-}$ groups, would result in such drastic changes in environmental persistence.

In the past, we observed in aerobic soils a roughly linear correlation of DT_{50} with the molecular weight of nine fluorotelomers and two perfluoroalkyl sulfonamide compounds, suggesting high molecular weight increased microbial recalcitrance.[21] In light of the recent findings, the stability of some newly-identified ECF-based precursors does not seem to correlate

with their molecular weights; rather, their structures have a substantial influence. There is a paucity of information on how structures of PFAA precursors influence the biotransformation potential and kinetics. To fill the knowledge gap on the structure-degradability relationship of precursors, we believe a reasonable starting point is to examine those compounds with N-containing groups, which are very common in the structures of newly identified AFFF components.[12] The new knowledge to be acquired will enhance our ability to predict the persistence of AFFF-derived precursors and also guide PFAS prioritization for other related studies.

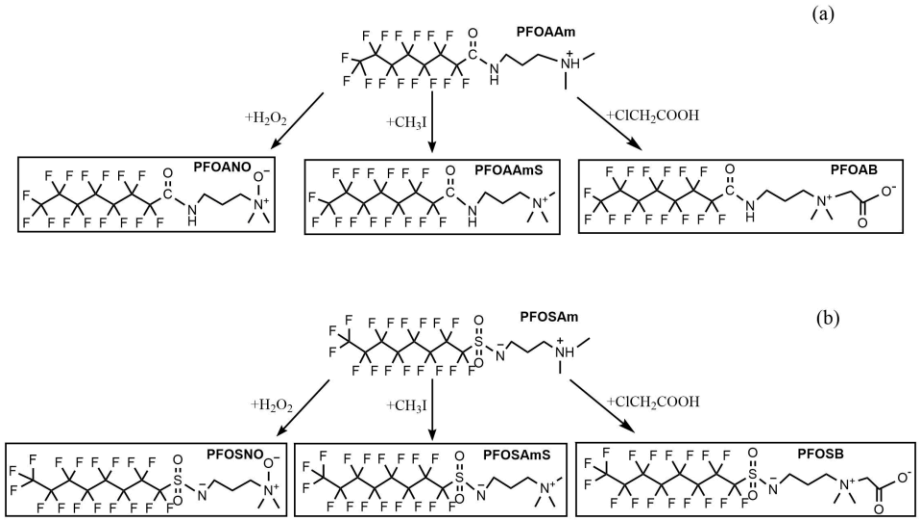


Figure 4.1 The custom synthesis routes of amine oxides (PFOANO and PFOSNO), quaternary ammoniums (PFOAAmS and PFOSAmS), and betaines (PFOAB and PFOSB) from tertiary amines (PFOAAm and PFOSAm). Amine oxides were synthesized via H_2O_2 oxidation, while the quaternary ammoniums and betaines were synthesized via Menshutkin reaction with an alkyl halide and a halogen carboxylic acid, respectively.

In the present study, we first applied modified OECD biodegradability tests to evaluate the biotransformation potential of four ECF-synthesized polyfluoroalkyl compounds, including perfluorooctane sulfonamido betaine (PFOSB), perfluorooctane amido betaine (PFOAB), 3-dimethyl amino perfluorooctanesulfonamide (PFOSAm), and 3-dimethyl amino perfluorooctaneamide (PFOAAm). They are suspected PFOS or PFOA precursors and contain either betaine or amine in the polar head groups. PFOSAm and PFOAAm were the custom

synthesis intermediates, from which the other fluorosurfactants (e.g., PFOSB, PFOAB) were created (Figure 4.1), but these synthesis materials frequently appear in AFFFs probably as impurities.[9,10] The transformation pathways and kinetics of the precursors were compared to four other ECF-based precursors (including PFOSAmS, PFOAAmS, PFOSNO and PFOANO, Figure 4.1) in aerobic soils, which were studied in recent years.[14,20] The acronyms have not yet been unified, and other alternatives in the literature are provided in the Appendix (Table B.1). The experimental evidence on microbial stability of these eight ECF-derived compounds allows establishing a preliminary structure-degradability relationship for ECF-based PFOS/PFOA precursors.

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Standards of PFOAB [CAS No. 90179-39-8, $F(CF_2)_7CONH(CH_2)_3N^+(CH_3)_2CH_2COOH$], PFOSB [CAS No. 75046-16-1, $F(CF_2)_8SO_2NH(CH_2)_3N^+(CH_3)_2CH_2COOH$] and PFOSAm [CAS No. 13417-01-1, $F(CF_2)_8SO_2NH(CH_2)_3N(CH_3)_2$] were custom-synthesized at the Beijing Surfactant Institute (Beijing, China) as per the synthesis processes summarized in the Appendix. All three materials received still contained some impurities: PFOSB contained PFOSAm and PFOS as impurities, PFOSAm contained PFOS, and PFOAB contained PFOAAm and PFOA impurities (Table B.2). Since PFOS/PFOA as impurities would prevent reliable quantification of PFOS/PFOA as biotransformation products, purification using solid-phase extraction and fractionation (SPE, detailed in the Appendix) was performed, resulting in nondetectable levels of PFOS or PFOA in purified PFOSB, PFOAB, and PFOSAm methanolic solutions. The purity of purified PFOSB, PFOAB, and PFOSAm solutions used for the present study was 89.6%, 98.3% and 100%, respectively. PFOSAm (10.4 mol %) or PFOAAm (1.7 mol%) could not be removed from prospective PFOSB and PFOAB solutions, respectively, and therefore were introduced simultaneously with the parent PFOSB/PFOAB compounds into soil microcosms. Custom-synthesized pure PFOAAm could not be obtained, but the transformation potential of PFOAAm could still be indirectly evaluated when it was present in PFOAB as an impurity. Further details on chemicals and materials, as well as purification procedures, are included in the Appendix.

4.2.2 Test soil and soil microcosm setup

Two soils (abbreviated as M and P soil, respectively) were selected based on "OECD Guideline 304A–Inherent Biodegradability in Soil" to have properties similar to Spodosol and Alfisol,[22] and have been used previously for the aerobic transformation study of PFOSNO and PFOANO.[20] The M soil was collected from McGill's McDonald campus in Sainte-Anne-de-Bellevue, Canada, in September 2018, whereas P soil from an urban forest area next to Rue de Gaspé, Verdun, Canada in October 2018. Both soils were collected at the top 20 cm layer, sieved via a 2 mm sieve immediately after collection, and stored at 4 °C, and used within three months. The soil properties were shown in Table B.3. The same semi-dynamic setup as in previous studies was employed for soil incubations ─ 500-mL glass bottles fitted with an airtight cap and a vent with an SPE C18 cartridge (Maxi-Clean, Canadian Life Science) for passive aeration and capturing volatiles.[21,23] Three treatments were prepared for each soil: (1) live soils spiked with purified parent compounds (in methanol); (2) sterile soils spiked with the same concentration of purified parent compounds; and (3) live (matrix) soil spiked with the same volume of methanol. The addition of a solvent carrier such as methanol is necessary to ensure even mixing into the soil, although it may temporarily impact soil biogeochemistry (eg. microbial community) and probably mildly on PFAS biotransformation kinetics. It has little impact on research outcome as long as the prevailing redox condition is not altered. Soil for treatment (2) was rendered sterile by autoclaving and addition of antibiotics as described in Mejia-Avendaño et al.[14] The sterility of treatment (2) was verified at the end of incubation by subjecting soils (1 g dw) from both the live and sterile treatments to an ATP assay (bioluminescence assay), using the Deposit and Surface Analysis test kit (DSA-25C) from LuminUltra (New Brunswick, Canada). PFOSB and PFOAB (prepared in methanol) were spiked together into live/sterile soils at an initial concentration of 1.8 μg g^{-1} for each. After spiking, soils were mixed with a sterile spatula to achieve homogeneous soil distribution. Another set of bottles, including the same three treatments for each soil as mentioned above, were used for the PFOSAm transformation study. The initial concentration of PFOSAm in soils was 2.0 μg g^{-1}.

Full details on the soils and soil microcosm setup can be found in the Appendix. The soil moisture content was measured gravimetrically in the live matrix control vessels throughout the study, and constant moisture content was maintained (Figure B.1).

4.2.3 Sample preparation

PFOSB and PFOAB were incubated in soil microcosms for 150 d, PFOSAm for 90 d. The incubation was carried out at ~22 °C in the dark. At each sampling time point, the bottle headspace was first purged through the SPE C18 cartridge using filter-sterilized air,[23] and then the cartridge was removed and eluted with 5 mL acetonitrile to extract volatile compounds. The soils were homogenized using a spatula before the bottle cap was removed to allow soil sampling. Roughly 2 g (dry weight, dw) of soil were taken out from each bottle and weighed in precleaned 15-mL polypropylene tubes. Each sample was then processed as per the procedure described in Text B.5, which includes solvent extraction, nitrogen evaporation, and SPE fractionation.

4.2.4. Instrumental analysis

Soil extracts and headspace extracts were analyzed by ultra-high-performance liquid chromatography coupled to high-resolution Orbitrap mass spectrometry (UHPLC-HRMS) as described in our recent studies.[5,14,24,25] Quantitative analysis was performed for the PFASs for which authentic standards were available (listed in Table B.6), while qualitative analysis was performed for suspected transformation products (listed in Table B.14 and S15), which were anticipated with the EAWAG's biodegradation/biocatalysis predictive function,[26] and enviPath (https://envipath.org/). PFOAAm was semi-quantified using PFOSAm as the reference standard. Soil extracts at different time points were analyzed under t-MS^2 (targeted MS/MS) positive and negative ionization modes (normalized collision energy, NCE=20−70%) for structure elucidation of qualitatively identified products, for which the confidence levels were assigned as per Schymanski et al.[27] Full details on UHPLC-HRMS operating conditions (Table B.5) and unknown elucidation are available in the Appendix B. Detection and quantification limits (iLOD, iLOQ, mLOD, mLOQ) of quantifiable PFAS analytes are provided in Table B.7). No polar volatile PFAS as listed in Table B.15 (b) were detected in headspace extracts by the current HRMS methodology. The possible production of nonpolar volatile transformation products requires GC-HRMS for detection; production of such volatile products was highly unlikely.

4.2.5 Quality assurance and quality control

All setups were prepared and processed in triplicate, and analytical results were reported as the average when applicable, with acceptable standard deviations (≤ 20%) between triplicates.

Except for soil incubation vessels, plastic tubes and vials were always used to minimize adsorption to solid surfaces.[28] The possibility of in-source fragmentation of PFOSB/PFOAB/PFOSAm during HRMS analysis to release PFOS or PFOA was verified and ruled out. Procedural blanks were included in each extraction batch, and injection blanks were run during each analysis sequence; both showed no PFAS detection.

The whole method recovery was determined as per Matuszewski et al.[29] All quantitative PFASs showed acceptable or suitable absolute recovery (70% ~ 120%) in both types of soils (Table B.8), supporting the efficiency of the extraction (MeOH/CH_3COONH_4) and SPE fractionation methods. Besides, the low absolute and effective matrix effects (Table B.9) in both soils indicate the negligible influence of soil matrix on the instrument responses. Determination coefficients (R^2) of calibration curves are provided in SI. Calibration verification standards were also run every 7-10 samples along each LC-MS batch sequence, with suitable accuracy and precision (Table B.10).

4.2.6 Determination of biotransformation kinetics.

The R-based software Kinetic Graphic User Interface (KinGUII) v2.1 (2015) was used to determine the DT_{50} values.[14,30] Four kinetic models, including Single First-Order (SFO), Double First-Order in Parallel (DFOP), Hockey Stick (SH) and First Order Multi-Compartments (FOMC) were fitted to soil degradation data of PFOAB, PFOSB, and PFOSAm.[14] Further information on these models was described in SI. The SFO model fitted the best for PFOAB transformation data with the smallest $\chi 2$ error (detailed in Table B.12), while the DFOP model fitted the best for both PFOSB and PFOSAm transformation data. In the SFO model, the DT_{50} value is also referred to as the half-life.

4.3 Results and Discussion

4.3.1 Transformation of PFOAB and coexistent PFOAAm in soils

The faster decline of PFOAB in live M soil than in the sterile treatment (Figure 4.2a), concurrent with the significant production of PFOA (Figure 4.2c), confirmed PFOAB to be a precursor to PFOA. PFOA was formed at a yield of 32.6 mol% by day 150 in live M soil. In contrast, PFOAB showed a very similar minor decline in both live and sterile P soils (Figure 4.2d), but the significant PFOA production (a yield of 6.1 mol% by day 150) was only observed in the

live P soil (Figure 4.2f). The vast differences in PFOA yields between these two soils may be associated with different soil biogeochemical properties (e.g., microbial community, pH, mineralogy, etc.). At the end of the incubation, the PFOA levels in both live soils were 22 ~ 45 times higher than those in the corresponding sterile soils, indicating PFOA formation mainly via biotic processes. Note PFOA had not reached a plateau when the incubation ended and was expected to continue to increase if the incubation would continue. The biotransformation rate constants of PFOAB in live M and P soil were determined to be 2.6×10^{-3} d^{-1} and 1.1×10^{-3} d^{-1}, respectively, which corresponded to DT_{50} of 266 d for M soil and 630 d for P soil. It is worth mentioning that the PFOA may be formed from PFOAB/PFOAAm through abiotic hydrolysis or other abiotic oxidation processes (shown in SI Figure B.5) in sterile soils.

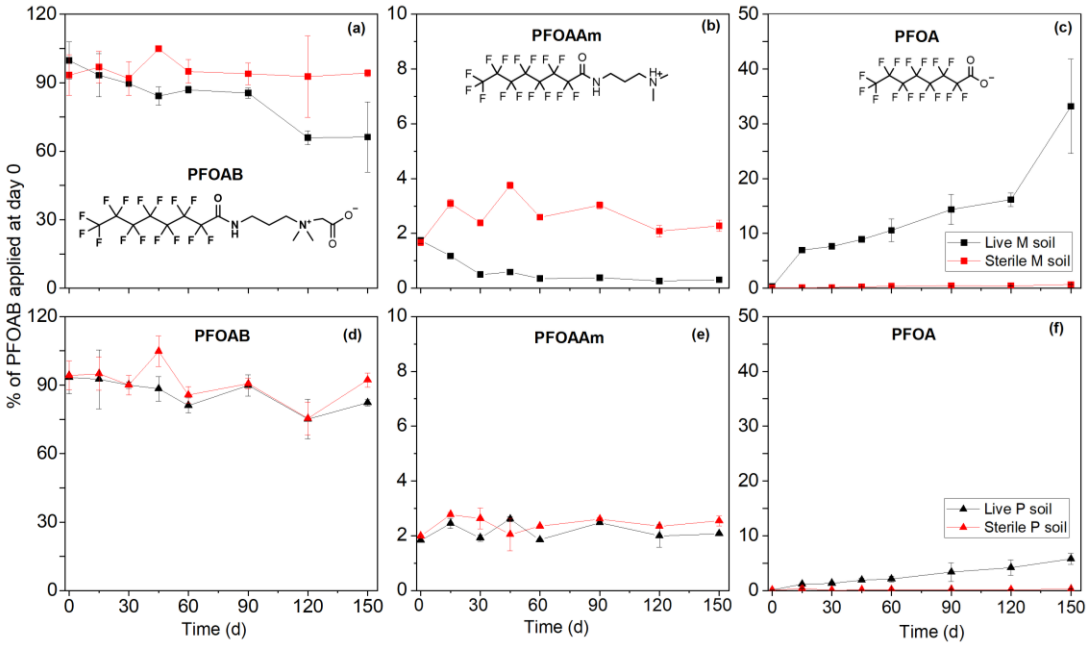


Figure 4.2 Concentration profiles of PFOAB and its transformation products (PFOAAm and PFOA) in aerobic live soils and sterile controls; (a), (b), (c) are for soil M, and (d), (e), (f) for soil P.

PFOAAm material was not available to the study but was examined indirectly for its transformation kinetics using the KinGUII package. The transformation of PFOAB also produces PFOAAm as an intermediate product, as discussed later. PFOAAm decreased continuously in live

M soil from 1.7 mol% at day 0 to 0.31 mol% at day 150 (Figure 4.2b), while it fluctuated between 1.8 and 2.6 mol% in live P soil (Figure 4.2e). Kinetics calculation via KinGUII estimated the biotransformation rate constant of PFOAAm to be 4.9×10^{-2} d^{-1} in live M soil, corresponding to a DT_{50} of 14.2 d. In addition, the KinGUII simulation results suggested that the PFOAAm impurity contributed to little of the total PFOA produced, likely due to the relatively small quantity (Table B.12). If PFOAAm was separately incubated, its production to PFOA would be quantifiable. The total PFOA yield from PFOAB was 32.6 mol% by day 150 in live M soil, comparable to those of PFOAAmS (30 mol% by day 180) or PFOANO (15 ~ 21 mol% by day 90 in two soils).[14,20]

In sterilized M and P soil, PFOAAm showed no clear trend over time, fluctuating between 1.7-3.8 mol% (Figure 4.2b). The soil sterilization via repeated autoclaving, reinforced with the addition of three antibiotics, was previously found effective in nearly eliminating biotic transformation.[14,23,31] The soil ATP assay performed on the last incubation day also indicated a low ATP level or residual biomass C in sterilized soils (Table B.11). We believe the degradation mechanisms in these two sterile soils were dominantly abiotic, but the possibility of weak microbial activities cannot be entirely ruled out.

Aside from PFOA and PFOAAm, the chromatograms and spectra recorded by UHPLC-MS for soil extracts revealed the formation of four other transformation products of PFOAB (Table B.14). Different dynamics for these transformation products were observed in two soils. For instance, compounds #3 (amido primary amine) and #4 (amido propionate) were produced only in the live M soil and probably biotically, while compounds #1 (PFOAAm), #2 (amido secondary amine), and #5 (hydroxylated PFOAB) experienced noticeable changes in the sterilized M soil, likely through abiotic mechanisms (Figure B.6). Note that in P soil, compound #3 (amido primary amine) was not detected, while compound #4 (amido propionate) was produced in both live and sterile soil. Given the variations observed in two different soils, we can only conclude that for certain reactions, strict division of biotic *vs.* abiotic reactions cannot be made.

The same transformation products, except for compound #5 (hydroxylated PFOAB), were also identified as the transformation products of cationic PFOAAmS or PFOANO.[14,20] In fact, we find that PFOAB (Figure B.5), PFOAAmS and PFOANO share essentially the same transformation pathways. A major route is to first produce PFOAAm; PFOAAm then undergoes N-demethylation, N-dealkylation, and oxidation to form secondary amine (# 2), primary amine

(#3), amide propionate (#4) and finally PFOA. Common to all three parent compounds, PFOA might also be formed directly from a one-step hydrolysis reaction. PFOAB may undergo a hydroxylation reaction to form hydroxyl-substituted betaine (#5), biotically or abiotically, while no equivalent was found for PFOAAmS or PFOANO. Based on the quantitatively targeted products alone, the molar balance over time for PFOAB (plus PFOAAm impurity) ranged between 81~113% (Figure B.4), suggesting that other qualitative products were minor contributors.

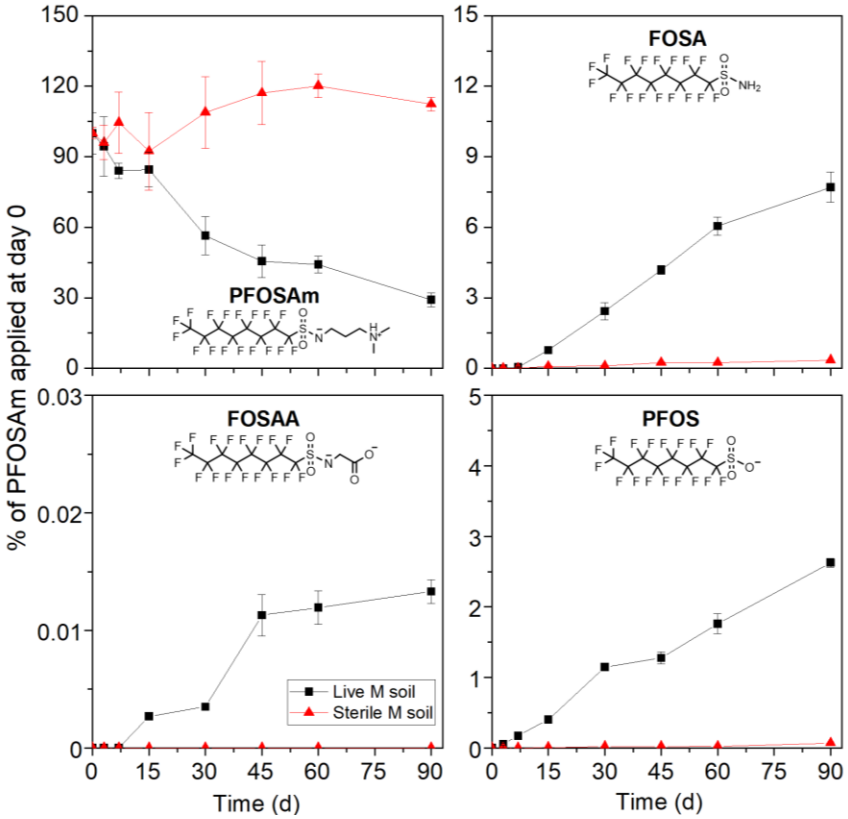


Figure 4.3 Concentration profiles of PFOSAm and its transformation products (including FOSA, FOSAA, and PFOS) in live and sterile M soil.

4.3.2 Transformation of PFOSAm in soils

PFOSAm can be used to synthesize other fluorosurfactants with examples shown in Figure 4.1 and is also a predicted common transformation intermediate of those fluorosurfactants. The 90-day incubation (Figure 4.3) verifies that PFOSAm in live M soil is readily transformable with the decline of PFOSAm concentration. FOSAA and FOSA, which were often formed from other

ECF-based eight-carbon precursors,[14, 20, 23] were also important transformation products of PFOSAm. The PFOS yield in live M soil was 2.7 mol% by day 90. The DFOP model estimated a DT_{50} of 47.5 d for PFOSAm in live M soil, while no DT_{50} could be determined for live P soil due to an unnoticeable change of PFOSAm (Figure B.2). The PFOS yield (0.06 mol% by day 90) in the live P soil was nonetheless above the soil background level, suggesting PFOSAm was biotically transformed despite slow kinetics.

In sterile M and P soils (Figure 4.3 and Figure B.2), the slight increase in FOSA and PFOS concentrations over time evidenced some active but insignificant mechanisms. The rather low amount of total ATP in the sterile soils (Table B.11) suggested that weak microbial activities were responsible for the production of PFOS, as no study reported PFOS being an abiotic product in aerobic soil studies. [14,20,23,32]

4.3.3 Transformation of PFOSB and coexistent PFOSAm in soils

In live M soil (Figure 4.4), a general decline of PFOSB was concurrent with significant production of FOSA, FOSAA and PFOS, and their concentrations were in contrast with those in sterile M soil. The coexistent PFOSAm declined faster in the live M soil than the sterile M soil, decreasing by 8.3 and 3.9 mol%, respectively, by day 150. Assuming the coexistent PFOSAm exhibited the same kinetics as it was degrading alone (Figure 4.3), we estimated the product yields attributable to the initial amine impurity and PFOSB using a molar balance comparison approach.[33] As shown in Table B.13, the FOSA, FOSAA, and PFOS yields from PFOSB biotransformation by day 90 were determined to be 0.52, 0.064, and 1.5 mol%, respectively (see Table B.12), and from the amine impurity, 0.80%, 0.001% and 0.27%, respectively. The PFOS yield from PFOSB biotransformation was 4.6 times higher than that formed from the amine impurity, supporting that PFOSB is a precursor to PFOS. The DT_{50} of PFOSB in live M soil was estimated to be 675 d. As shown in Table B.12 (b), the varied DT_{50} of PFOSAm when introduced as PFOSB mixture compared with the PFOSAm alone (15.7 d versus 47.5 d) may be due to the different initial concentrations of amine that influenced the biotransformation kinetics, or different preferential transformation pathways of PFOSAm in the presence of other PFASs.

In P soils (Figure B.3), PFOSB and PFOSAm remained essentially unchanged either in live or sterile soil over the time course of the experiment. FOSA and FOSAA yields were higher in the

sterile soil than the live soil, whereas PFOS showed higher yields in the live soil, which may be due to the more favorable transformation of FOSA and FOSAA to PFOS under biotic conditions.

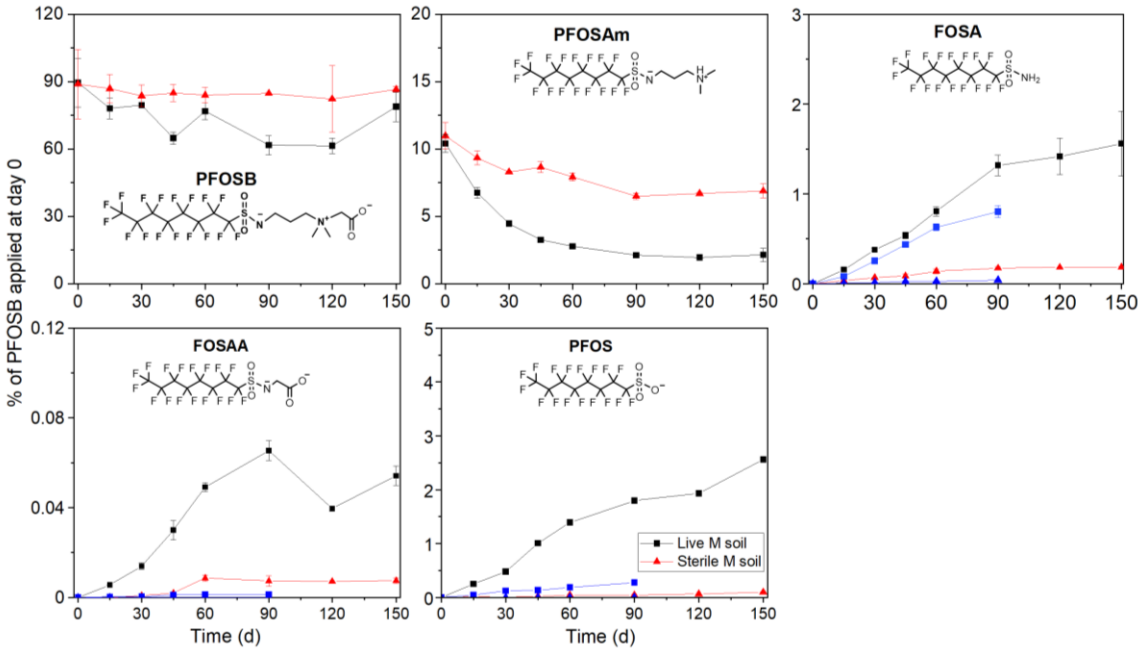


Figure 4.4 Concentration profiles of PFOSB plus the PFOSAm impurity and their transformation products (including FOSA, FOSAA, and PFOS) in live and sterile M soils. The blue symbol lines indicate the FOSA/FOSAA/PFOS formed from the PFOSAm impurity from day 0 to day 90.

Using HRMS, six additional transformation products (#6 through #11) were identified at different confidence levels (Table B.14) in PFOSB-spiked live and sterile soil extracts; #6 through #9 were also observed in the separate PFOSAm experiments. The biotic and abiotic transformation of PFOSB is proposed to proceed via three different initial pathways, as illustrated in Figure 4.5. Analogous to PFOAB, in Pathway I, PFOSB may first form the tertiary amine (PFOSAm), and then PFOSAm goes through similar steps as observed in the PFOSAmS or PFOSNO biotransformation studies,[14,20] forming #6 (sulfonamido secondary amine), #7 (sulfonamido primary amine), #8 (sulfonamido propionate). Compound #8 demethylated to form FOSAA then underwent deacetylation (or decarboxylation followed by N-demethylation) to form FOSA, which generated perfluorooctane sulfinate (PFOSI, #9) via deamination.[34] Up to this point, this pathway had been shown to be predominant and similar to those of PFOSAmS and PFOSNO; however,

PFOSI was not discovered in these previous studies. PFOSI is terminally degraded into PFOS mainly via microbially-mediated sulfur oxidation mechanism.[34]

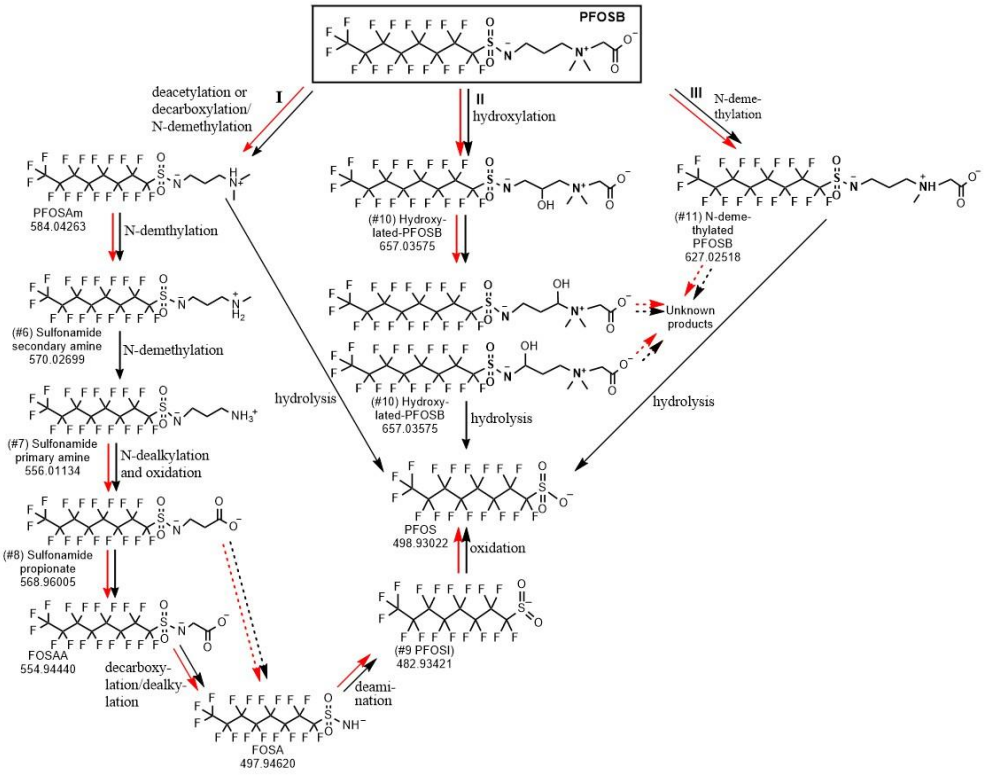


Figure 4.5 Proposed abiotic (red arrow line) and biotic (black arrow line) transformation way of PFOSB in aerobic soils (M soil as an example). All PFAS structures shown above are the speciation under M soil pH condition (pH 7.2). The dashed arrow line represents hypothetical multiple-step pathways.

Based on the quantitative products alone, the molar balance over time for PFOSB (plus PFOSAm impurity) (Figure B.4) ranged between 67~103%, suggesting that other qualitative products, as well as other unidentifiable products, make up a less significant portion of the total mass, especially for soil P with lower transformation rates. KinGUII results indicate that direct formation of PFOS from PFOSB via a one-step hydrolysis reaction, previously reported for PFOSB and PFOSNO,[14,20] did not occur while this direct hydrolysis reaction did occur for coexistent PFOSAm during the biotransformation process (Table B.12). In pathway II, PFOSB is converted to hydroxyl-substituted betaines (#10), with three possible positions of hydroxylation

on the aliphatic carbons. PFOSB may also form demethylated betaine (#11) in pathway III. These hydroxylated or N-demethylated compounds may biotically form PFOS via direct hydrolysis or further abiotically and biotically transform into other unidentified products. The slow PFOS production and the slightly increasing trend for #10 and #11 indicates that the three pathways may be minor for PFOSB, while pathways II and III do not seem to be functional for PFOSAmS and PFOSNO.[14,20]

4.3.4 Environmental stability of structurally related PFAS

As discussed above, some transformation products with long perfluoroalkyl chain (C=8 for PFOSB and PFOSAm, C=7 for PFOAB) were confirmed; however, no other potential transformation products with shorter perfluoroalkyl chain (C≤7 for PFOSB and PFOSAm, C≤6 for PFOAB, as listed in Table B.15a) were detected in soil extracts during the incubation of PFOSB, PFOSAm and PFOAB. This supported that the biotransformation of ECF-based betaines and amines is limited to the nonfluorinated moieties in aerobic soils, in agreement with those observed for other ECF-based PFASs, including EtFOSE, EtFOSA, PFOAAmS, PFOSAmS, PFOSNO and PFOANO.[14,20,23,32] As shown in Table 4.1, hydrophilic head groups in the structures of the eight ECF-based precursors strongly influence their biodegradability.

PFOSAmS (with a quaternary ammonium group) and PFOSB (with a betaine group) show high microbial stability in aerobic soils,[14] although they can still degrade to form PFOS at very slow rates. PFOSAm (with an amine group) is less resistant to transformation in live soils than these two. Previously, a higher biotransformation potential and PFCA yield were reported for 6:2 FTAA (with an amine group) than 6:2 FTAB (with a betaine group) in WWTP sludge.[18] Furthermore, the sulfonamido amine oxide (PFOSNO) showed even lower stability in soils than the above three.[20] The DT_{50} of PFOSNO (15~24 d) was among the shortest and was comparable to that of EtFOSA (13.9 d)[23] and slightly larger than that of EtFOSE (5.2 d) in aerobic soils. Overall, the stability of sulfonamide-based precursors followed the order based on the magnitude of DT_{50}: PFOSAmS ≈ PFOSB >> PFOSAm > PFOSNO. Similarly, amido-based precursors listed in Table 4.1 have a similar trend in terms of microbial stability, as ranked based on the type of hydrophilic head groups: PFOAAmS ≈ PFOAB >> PFOAAm > PFOANO.

The sulfonamide-based PFOSAm, PFOSB, PFOSAmS, and PFOSNO also showed higher microbial stability compared with corresponding amide-based PFOAAm, PFOAB, PFOAAmS, and PFOANO (Table 4.1). The higher hydrophobicity of sulfonamide-based compounds results from the longer perfluoroalkyl chain (eight-carbon) than amide-based compounds (seven-carbon), as well as the larger sulfonyl group. Both features increased the sorption of the sulfonamides onto soils and reduced their bioavailability.[14,35] In addition, C-N fission of the amide group might occur more easily than the S-N fission of the sulfonamide group.[36.] The lower PFAA yields of PFOSB compared with PFOAB (Table 4.1) in aerobic soils are also in agreement with a previous study where PFOSB was resistant to conventional water chlorination whereas PFOAB was converted to PFOA.[37]

Table 4.1 PFAA yields and DT_{50} of N-containing precursors in aerobic soils.

Type	Compound	Incubation time	PFOS/PFOA yield	DT_{50}	Test system	Reference
PFOS precursors	PFOSAmS	180 d	0.3%	>> 180 d	Closed system with intermittent oxygenation	[14]
	PFOSB	150 d	0.07 ~ 1.5%[a]	675 d	Semi-dynamic system	This study
	PFOSAm	90 d	0.06 ~ 2.7%	47.5 d	Semi-dynamic system	This study
	PFOSNO	90 d	5 ~ 10%	15 ~ 24 d	Semi-dynamic system	[20]
PFOA precursors	PFOAAmS	180 d	30%	142 d	Closed system with intermittent oxygenation	[14]
	PFOAB	150 d	5.8 ~ 32.6%[b]	266 ~ 630 d	Semi-dynamic system	This study
	PFOAAm	180 d	n/a [b]	14 d	Semi-dynamic system	This study
	PFOANO	60 d	15 ~ 20%	7 ~ 10 d	Semi-dynamic system	[20]

[a] The PFOS yield from PFOSB is for day 90 by deducting the amount predicted to be formed from the PFOSAm impurity.

[b] The PFOA yield from PFOAAm in live M soil is 0% according to KinGUII simulation results, while the yield in live P soil is not available.

PFOSAm is an intermediate in the preparation of many fluorosurfactants with a sulfonamide functional group. It was obtained by reacting perfluorooctane sulfonyl fluoride ($C_8F_{17}SO_2F$) with N, N-dimethyl-1,3-propanediamine. The low stability of PFOSAm in aerobic soils may be explained by the two oxidizable nitrogens with a lone pair of electrons on this tertiary amine. PFOSAmS as a quaternary ammonium compound is produced through the reaction of PFOSAm with an alkyl halide (CH_3I)–a Menshutkin reaction (as illustrated in Figure 4.1). A carboxyl betaine

(e.g., PFOSB) is produced through the reaction of PFOSAm with $ClCH_2COONa$,[38] also a Menshutkin reaction. Quaternization reactions create quaternary ammonium cations (e.g., PFOSAmS and PFOSB) that are unreactive towards even strong electrophiles, oxidants and acids, and also most nucleophiles. The lack of one oxidizable nitrogen with a lone pair of electrons on the betaine or quaternary ammonium group possibly resulted in the greater chemical and environmental stability of quaternary ammonium and carboxyl betaine compared with a tertiary amine.[2] In contrast, PFOSNO, an amine oxide, is produced by reacting PFOSAm with hydrogen peroxide–an oxidation reaction.[39] Though the amine oxide group imparts similar polarity as a quaternary ammonium group to a surfactant molecule, it is also known to be reactive. Such chemical reactivity may contribute to the low environmental stability of PFOSNO in aerobic soils.[20] The above discussion applies equally to the amide-based precursors in terms of the structure-degradability relationship.

The microbial stability ranking also shows that the formation of a precursor to the tertiary amines (PFOSAm or PFOAAm) can be a rate-limiting step, as in the case of quaternary ammonium and betaine compounds. Additional rate-limiting steps can be found in the downstream reactions to the eventual formation of PFOS, such as through FOSA. Previously, we predicted the half-life of FOSA in aerobic soil could be >700 d.[31] The DT_{50} predicted using PFOSB data also showed high persistence (>1000 day) for FOSA in M soil. The data might explain the frequent detection of FOSA in AFFF-impacted soils[4,40], aquifer solids[4], sediments,[30-42]surface water[40,41] and groundwater[40]. FHxSA was prevalently found in AFFF-impacted environments.[2,4,43] As FOSA is confirmed to be produced from PFOSB or PFOSAm with eight perfluorinated carbons, it is reasonable to surmise that FHxSA can be formed from perfluorohexane sulfonamide betaine (PFHxSB) and amine (PFHxSAm) (C_6 analogs of PFOSB and PFOSAm), important components of some ECF-based AFFFs.[9,12] FOSAA was produced at lower yields than FOSA during the biotransformation of PFOSB/PFOSAm in both live soils. It appeared that the formation of FOSAA was a minor pathway, similar to what was observed for the biotransformation of EtFOSA in aerobic soil,[23] or FOSAA might be quickly converted into FOSA as occurred in activated sludge[32]. Similarly, FHxSAA has been a less frequent PFAS in impacted sites than FHxSA.[25] This would help prioritize the PFAS analytes in future environmental monitoring efforts and remediation work.

4.4 Environmental Implications

Through a series of experimental studies conducted in aerobic surface soils, we have shown that just as nitrogen functional groups play a significant role in directing and controlling organic reactions, they are equally crucial in determining the environmental stability of polyfluoroalkyl substances. Despite variability in DT_{50} and PFAA yields associated with distinct types of hydrophilic head groups in fluorosurfactants, these polyfluoroalkyl substances have all been confirmed to be precursors to PFOS or PFOA in the laboratory, suggesting that they could be sources to PFAAs at historical AFFF contamination sites. The precursors with relatively labile groups such as amines and amine oxides may quickly degrade to below detection while producing PFAAs and other polyfluoroalkyl products in the early days of AFFF release into the environment. For instance, PFASs with amine oxide groups have been infrequently detected in AFFF-impacted sites.[24] Their detection in 2 out of 11 AFFF foams[10] might be due to their only presence in foams from one or two manufacturers or infrequent usage as AFFF components, but their low environmental stability might also be a deciding factor. It is noted that these 11 AFFF formulations might have been tested based on sample availability and thus may not be necessarily representative of all AFFFs. However, PFAS cations or zwitterions containing quaternary ammonium groups (e.g., PFOSB and PFOSAmS) can be quite persistent in the field even years after AFFF releases. If they do transform to produce PFOS, the rates might be diminutive in natural field conditions.

The detection of many similar cationic and zwitterionic structures in aged environmental samples has further provided the field evidence of the persistence of such PFASs, [12,43] consistent with our laboratory findings. Barzen-Hanson et al. found among the new classes of PFASs only detected in groundwater impacted by AFFF dated decades ago,[12], 11 out of 13 classes were ECF-based sulfonamide derivatives, while 8 out of the 11 classes contained quaternary ammonium groups. In the study by Nickerson et al.,[43] PFAS cations and zwitterions (with quaternary ammonium groups) were measured to be up to 97% of the total PFAS mass found in soil cores and the prevalence of ECF-derivatives was also observed. Aside from the low propensity to biotransform, the retention of such compounds in soils is also due to strong sorption to soils owing to ionic interactions between positively charged quaternary ammonium and negatively charged soil particles.[44] Such compounds were largely missed out until very recently with the use of high-resolution mass spectrometry, positive electrospray ionization technique, and optimized extraction methods.[25,43]

We also noticed that among 40 classes of new PFASs found in AFFFs and impacted water samples, 23 classes are derivatives of PFOSAmS with more complex hydrophilic groups.[12] The substitutes on the sulfonamide nitrogen and quaternary ammonium can contain multiple carbons and/or additional hydroxyl, carboxyl or sulfonyl groups, creating bulky hydrophilic head groups. Within each class, the perfluoroalkyl chain typically varies from 2 to 8. Given the high persistence of PFOSAmS observed in the laboratory study,[14] we surmise that those eight-carbon derivatives reported by Barzen-Hanson et al.[12] pose even higher environmental persistence than PFOSAmS (DT_{50} >> 180 d) and can persist for years or decades. The shorter-chain derivatives (e.g., precursors to perfluorohexane sulfonate or perfluorobutane sulfonate) might be more prone to environmental degradation than the eight carbon equivalents. Still, no data are yet available regarding the chain-length dependent kinetics for ECF compounds. Future experiments or computation tools might be necessary to generate such knowledge. Previously, fluorotelomer alcohols as precursors to PFCAs showed chain-length dependent transformation kinetics in soils and activated sludge.[45,46]

Amide derivatives such as PFOAB, PFOAAmS and PFOANO are part of ECF chemistry, and as we found out, are precursors to PFOA.[14,20] Recent monitoring studies suggest fewer types of amides than sulfonamides,[12] but as sources to PFOA, ECF-based amides have not often been targeted. We also detected branched amide isomers as well as branched PFOA (unpublished data), but it is beyond the scope of the current study to explore isomer-specific transformation potential or kinetics. Should the understanding of such a phenomenon become essential, for instance, for environmental forensics or source tracking, the methodology developed in the current and past studies would prove useful.

The study also revealed the challenges of differentiating abiotic from biotic reactions solely based on the differences of chemical species observed between a sterilized soil microcosm and a non-sterilized one. The aerobic soil also cannot represent other types of natural environments where abiotic reactions (e.g., radical based) could be significant. For instance, hydroxyl radical (•OH) may be produced by photochemical reactions of dissolved organic matter (DOM) in soils.[47] The dark formation of •OH may also occur when reduced DOM and Fe(II) produced by anaerobic microbial respiration[48,49] come into contact with O_2 at oxic-anoxic boundaries,[50] such as in contaminated source zones or sediments. As previously reported for other amide/sulfonamide-based precursors,[51,52] •OH could oxidatively degrade PFOSAm, PFOSB or PFOAB, probably

attacking the hydrophilic head groups. Future studies may focus on the abiotic transformation of these N-containing PFASs. The effect of ECF-based precursors and their transformation products on the bacterial/fungal/archaeal community changes, the microorganisms responsible for precursor biotransformation, and the enzymes or functional genes involved warrant further research. These steps are crucial for a deep understanding of the degradation mechanisms and will help predict microbial community changes in response to PFAS and identifying robust microbial strains capable of degrading polyfluoroalkyl substances.

4.5 References

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Chapter 5. High Persistence of Novel Polyfluoroalkyl Betaines in Aerobic Soils

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Liu, M.; Munoz, G.; Hermiston, J.; Vo Duy, S.; Zhang, J., Wang, D.; Bottos, E., Van Hamme, J.; Lee, L. S.; Sauvé, S.; Liu, J., High persistence of novel polyfluoroalkyl betaines in aerobic soils. In preparation for submission to *Environ. Sci. Technol.*

Preface

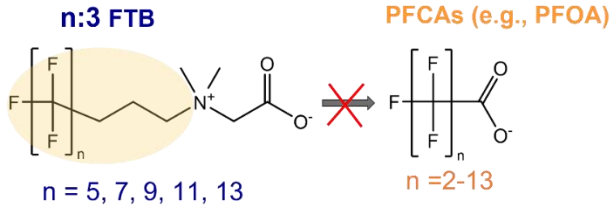
In Chapter 3, the PFAS concentration profiles at the source zone soils from FTA sites within four Canadian airports revealed high concentrations of novel FT-based betaines at the active site with the current use of AFFF, in contrast with the abundance of ECF-based zwitterions and cations at the areas with the historical use of AFFFs. Chapter 4 demonstrated the slow transformation of two ECF-based betaines in aerobic soils, resulting in low yields of PFCA (for amide betaine) or PFSA (for sulfonamide betaine). In contrast, no study has explored the biotransformation of these novel FT betaines manufactured via different chemistry, and whether and to what extent they would contribute to the PFCA remains unclear. In a real scenario, the novel FT betaines are released from the current-in-use AFFFs as a mixture solution. Therefore, this chapter focused on examining the transformation of novel FT betaines and an AFFF containing these betaines in aerobic soils, which is expected to deepen our understanding of the structure-degradability relationship for FT-based precursors.

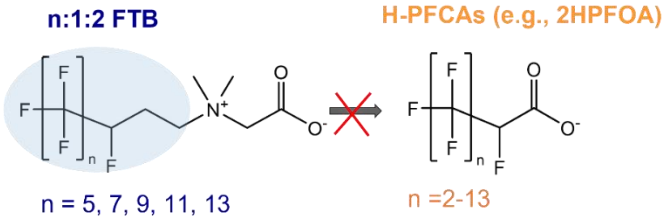
Abstract

Fluorotelomer betaines (FTBs) with n:3 and n:1:2 polyfluoroalkyl chains are major components of some contemporary aqueous film-forming foams (AFFFs) and have been frequently detected in AFFF-impacted sites. Although they are permitted post-PFOS/PFOA phaseout, their environmental fate and impact are largely unexplored. In this study, we investigated the biotransformation of 5:3 and 5:1:2 FTBs and a commercial AFFF containing n:3 and n:1:2 FTB (n = 5, 7, 9, 11, and 13) in aerobic soil microcosms. Results showed that the biotransformation of 5:3 and 5:1:2 FTBs occurred slowly in aerobic soil microcosms with little or no production of predicted transformation products after 120 d. Specifically, 5:3 FTB did not degrade to n:3 polyfluoroalkyl acids (n = 2~5) or perfluoroalkyl carboxylic acids (C_3~C_6 PFCA), and 5:1:2 FTB did not produce short-chain hydrogen-substituted polyfluoroalkyl acids (n:2 H-FTCA, n = 2~5) or hydrogen-substituted PFCA (2H-PFCA, C_3~C_7). The incubation of a commercial Ansulite AFFF in four soils with different soil properties and microbial communities resulted in the production of 0.023~0.25 mol% PFCAs by day 120. The products are hypothesized to be transformation products of n:2 fluorotelomers, which were only minor AFFF components, rather than from the breakdown of n:3 and n:1:2 FTBs. We postulate that FTB resistance to biotransformation is partly due to the stable quaternary ammonium group. These findings highlight that current structure-biodegradability relationship models cannot explain the persistence of these widely detected emerging polyfluoroalkyl compounds.

Keywords: n:3 fluorotelomer betaine, n:1:2 fluorotelomer betaine, perfluoroalkyl carboxylic acids, aqueous film-forming foams, persistence, soil microcosm

Graphical Abstract





5.1 Introduction

The hydrophobic and lipophobic nature, as well as the chemical and thermal stability of the perfluoroalkyl chain, endows per- and polyfluoroalkyl substances (PFASs) with broad applications in industrial, commercial, and domestic products,[1] including aqueous film-forming foams (AFFFs). However, decades of AFFF use has resulted in severe PFAS contamination of surface waters, groundwater, soils, sediments, and biota at military bases, airports, and firefighting training areas.[1-2] The PFAS identified in AFFFs and impacted sites includes the commonly investigated perfluoroalkyl acids (PFAAs) and an array of cationic, zwitterionic, and anionic polyfluoroalkyl substances (referred to as "precursors") with varied perfluoroalkyl chain lengths and hydrophilic functional groups.[3, 4] Notably, AFFF-impacted environmental samples have shown different PFAS patterns compared with AFFFs,[3, 4] as sorption, microbial transformation, abiotic oxidation and reduction, and photodegradation alter chemical structures. Understanding the environmental behavior and fate of AFFF-derived precursors can provide crucial knowledge for risk assessment, site management, and remediation efforts.

Historically manufactured AFFFs contained either dominantly perfluorooctane sulfonate (PFOS) and its sulfonamido derivatives, or 6:2 and 8:2 fluorotelomer (FT) compounds. Such formulations were either direct sources of long-chain PFAAs (e.g., PFOS, PFOA), which are categorized as persistent, bioaccumulative, and toxic, or contained precursor substances that may degrade to PFAAs. Therefore, the uses and production of such AFFFs have been phased out or restricted in the past two decades in North America.[5] AFFF products currently permitted tend to contain largely C_4 or C_6 perfluoroalkyl chains because smaller PFAS molecules generally have lower bioaccumulation potentials.[6]

In recent years, zwitterionic fluorotelomer betaines (FTBs) characterized by n:3 and n:1:2 polyfluoroalkyl chains have been increasingly identified in currently used AFFFs including those under the brand names Buckeye and Ansul.[4, 7-9] These n:3 and n:1:2 FTBs are dominated by C_5

perfluoroalkyl chains, but longer chain lengths are also present (n =7, 9, 11, 13 and 15). Their manufacturing process is not publicly known, but they may be synthesized through hydrogenation of unsaturated polyfluoroalkylamines.[10, 11] Not surprisingly, these new betaines have also started to appear in the environment (e.g., surface water,[12] soils,[13, 14] and sediments,[2], etc.) and biota (earthworm,[15] and fish[2]). Notably, n:1:2 and n:3 FTBs have been detected at high concentrations in surface soils but at low to nondetectable levels in groundwater at source zone areas impacted by AFFF,[14] which may indicate their high retention and/or slow transformation in soil environments. Recently, Munoz et al. reported the moderate bioaccumulation potential of n:3 and n:1:2 FTBs in earthworms, especially for long-chain homologs with $C \geq 9$.[15] Both the wide environmental occurrence and bioaccumulative nature of n:3 and n:1:2 FTBs has spurred interest about their environmental fate and behavior, especially in soils that serve as an important sink of these compounds.[13, 14]

Results from multiple laboratory studies have shown aerobic biotransformation of AFFF-derived precursors, suggesting degradation of FTBs would occur under similar conditions. Eight electrochemical fluorination (ECF)-based precursors with quaternary ammonium, betaine, tertiary amine, or amine oxide terminal functional groups were transformed at varying rates in aerobic soils, producing PFOS or PFOA at different yields (0.06-32.6 mol%).[16] Such transformation involved the breakdown of nonfluorinated chains, while the perfluoroalkyl chains remained intact with no defluorination. In contrast, 6:2 FT-derived precursors, such as 6:2 fluorotelomer thioether amido sulfonate (6:2 FTSAS),[17, 18] 6:2 fluorotelomer sulfonamide alkylamine (6:2 FTAA),[19] and 6:2 fluorotelomer sulfonamide alkyl betaine (6:2 FTSA-PrB, or 6:2 FTAB)[19] undergo partial breakdown of both nonfluorinated chains and perfluoroalkyl chains, often accompanied by defluorination. In activated sludge[19], soil microcosms[17], and pure bacterial cultures,[20] major transformation products were detected including fluorotelomer polyfluoroalkyl acids (e.g., 5:3 FTCA) and a series of short-chain PFCAs (e.g. C_4~C_6 PFCAs). Compared with n:2 FTs (manufactured by many fluorochemical producers, including Chemours) and ECF-derived

precursors (manufactured by 3M), n:3 and n:1:2 FTBs (manufactured by Dynax corporation) have distinct fluorinated carbon chains. However, it remains unexplored how the presence of the singly fluorinated carbon linkage in n:1:2 FTBs or the odd number of hydrocarbon moieties in n:3 FTBs may affect biodegradability. There have been reports that short-chain PFCAs were detected at some recent AFFF-impacted sites,[13] while long-chain PFCAs have been accumulating in the tissues of arctic animals long after their phase-out.[21] Some commercial AFFF formulations (e.g., Ansulite® AFFF) contain both short-chain and a high percentage of long-chain FTB analogs, but whether the recent use of such AFFFs could contribute to the environmental presence of short- and long-chain PFCAs via biotransformation is not known. Therefore, understanding the fate of these novel PFASs in aerobic soils and their links to PFAA burdens in the environment is necessary.

Based on the literature, we hypothesized that FTBs can be biotransformed in ways similar to n:2 FTs to release PFCAs when the carbons in the polar functional groups are metabolized by soil microorganisms. To test the hypothesis, we incubated 5:3 and 5:1:2 FTBs and a commercial AFFF, which contains n:3 and n:1:2 FTBs (n = 5, 7, 9, 11, and 13) as major fluorosurfactant components, in four aerobic soils for up to 120 days, and investigated parent compounds and their potential transformation using high-resolution mass spectrometry (HRMS). The experimental evidence on the microbial stability of the novel FTBs, or the lack thereof, provides much-needed knowledge to allow for proper assessment and management of those sites that still receive AFFFs. This work provides insights into the structure-degradability relationship for FT-based precursors.

5.2 Materials and Methods

5.2.1 Chemicals and reagents

Standards of 5:3 FTB [CAS No. 171184-14-8, $F(CF_2)_5(CH_2)_3N^+(CH_3)_2CH_2COOH$] and 5:1:2 FTB [CAS No. 171184-02-4, $F(CF_2)_5CFH(CH_2)_2N^+(CH_3)_2CH_2COOH$] with purity >98% were provided by Wellington Laboratories (Guelph, ON, Canada). A known impurity in 5:1:2 FTB is 5:3 FTB, estimated at 0.3 mol%. The commercial AFFF formulation (Ansulite®) was purchased

in Canada. Details on other chemicals and materials are provided in Text C.1 in Appendix C.

5.2.2 Soil microcosm setup

Four soils (abbreviated as CA-M, CA-L, US-F, and US-G soil), collected in Canada and USA (the collection locations and soil properties listed in Table C.1), were selected for the biotransformation study based on "OECD Guideline 304A–Inherent Biodegradability in Soil".[22] The soils were sieved using a 2 mm sieve immediately after collection, stored at 4 °C, and used within 3 months. Fifty-two quantitative PFAS were found at <LOQ~0.90 ng/g (Table C.2), comparable to other non-contaminated soils.[23]

The same closed test vessels as those in the previous studies were employed for soil incubations.[24, 25] Amber serum bottles (50 mL) were fitted with crimp-sealed natural rubber stoppers and a vent created with an SPE C18 cartridge (Maxi-Clean, Canadian Life Science) for passive aeration and capturing volatiles.[21, 23] Incubations were performed for the single 5:3 FTB, the single 5:1:2 FTB, a mix of 5:3 FTB and 5:1:2 FTB, and the Anusite AFFF concentrate. Three treatments were prepared for each chemical or mixture: (1) live soils spiked with FTB(s) or Ansulite AFFF methanolic solutions; (2) sterile soils spiked with the same levels of PFAS as in treatment (1); (3) live (matrix) soil spiked with the same volume of methanol only. As reported, using a solvent carrier (such as methanol) during spiking is necessary for evenly dispersing PFAS and has little impact on biotransformation outcomes.[16] Soils used in treatment (2) were rendered sterile by repeated autoclaving and amending with three antibiotics (chloramphenicol, kanamycin, and cycloheximide) at an approximate concentration of 100 mg/(kg of soil).[16, 24, 26, 27] Soil moisture content was adjusted to 70% of maximum water holding capacity before chemical spiking. Then soils were homogenized by manual mixing with a sterile spatula. For treatments (1) and (2), the initial PFAS concentration was ~0.8 μg g^{-1} dry-weight (dw) of 5:3 or 5:1:2 FTB in the single betaine experiment and the two-FTB mixture experiments (achieved by spiking 48 μl of 500 ppm 5:3 FTB or 5:1:2 FTB or mixture of 5:3 and 5:1:2 FTB into 30 g dw soils); while the initial

concentrations of PFAS derived from the AFFF are listed in Table C.3 (48 ul of 6.95 times-diluted Ansulite AFFF methanolic solution spiked into 30 g dw soils). All test vessels were incubated for up to 120 d at ~22 °C in the dark. Relatively constant moisture content in the live soil controls was maintained throughout the incubation, as illustrated in Figure C.1.

5.2.3 Sampling and sample preparation

Aliquots of soils were aseptically removed from the incubation vessels on Day 0, 7, 15, 30, 45, 60, 90, and 120 for the single FTB experiments, Day 0 and 120 for the two-FTB mixture experiment, and Day 0, 15, 30, 60, and 120 for the AFFF experiment. At each sampling time point, the bottle headspace was first purged through the SPE C18 cartridge using filter-sterilized air,[27] and then the cartridge was removed and eluted with 5 mL acetonitrile to extract volatile compounds. The soil was homogenized using a sterile spatula before soil sampling. Roughly 1.0 g (dry weight, dw) of soil was taken from each bottle for chemical analysis following the procedures described in Text C.2. An additional 0.25 g was removed and stored at -80 °C for microbial community analysis. The headspace extracts were stored at -20 °C in the freezer for chemical analysis.

5.2.4 Instrument analysis

The soil and headspace extracts were analyzed using ultra-high-performance liquid chromatography coupled to a high-resolution Orbitrap mass spectrometer (UHPLC-HRMS) as described in our recent studies.[2, 16, 24] The samples were first analyzed in full-scan mode (details in Text C.3). EAWAG's Biocatalysis/Biodegradation Database (BBD)[28] and previous literature[25, 29] were referred to predict possible transformation products for n:3 and n:1:2 FTBs (n = 5, 7, 9, 11 and 13), and other n:2 fluorotelomers derived from the AFFF (e.g., n:2 FTS, n = 8, 10, and n:2 FTB, n = 6 and 10). Target analysis enabled the quantification of the parent compounds (e.g., 5:3 FTB, 5:1:2 FTB, 8:2 FTSA) and predicted metabolites with available authentic standards (listed in Table C.4). Nontarget analysis (procedures shown in Text C.3, and the workflow diagram in Figure C.2) and suspect screening enabled the qualitative analysis of other transformation products

without available standards (Table C.5), including but not limited to the predicted ones. Select samples were also analyzed using t-MS^2 (targeted MS/MS, detailed in Text C.3) positive and negative ionization modes (normalized collision energy, NCE = 20−50%) for the structure elucidation of qualitatively identified products, for which the confidence levels were assigned as per Schymanski et al.[30] Different UHPLC-HRMS operating conditions (Text C.4, Table C.6 and Figure C.3) were tested for the optimal analysis of the quantitative PFAS, especially volatile ones. Full details on the optimized instrument conditions are provided in Table C.7. Detection and quantification limits (iLOD, iLOQ, mLOD, mLOQ) of quantitative PFAS are provided in Table C.8. The current LC-HRMS methodology can detect volatile PFAS (listed in Table C.4 and C.5) in headspace extracts, but no PFAS were detected during the incubation of single FTB, two-FTB mixture or Ansulite AFFF, possibly due to the high detection limits of some volatiles (e.g., 5:2 sFTOH, shown in Table C.8). GC-HRMS might be a more effective tool to identify possible volatile transformation products that deserve future efforts.

5.2.5 Soil microbial community analysis

Genomic DNA was isolated from soils for 16S rRNA gene amplicon sequencing to examine the differences in bacterial community composition between the four soils. DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Then the V4 hypervariable region of bacterial 16S rRNA genes was amplified by polymerase chain reaction (PCR) for two rounds. DNA extracts and PCR products were quantified using a Quant-iT dsDNA HS Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts, USA). Second-round PCR amplicons were pooled to equimolar amounts based on qPCR quantification with an Ion Library Quantitation Kit (ThermoFisher Scientific). Sequencing libraries were prepared using an Ion 520 and Ion 530 Kit-Chef on an Ion Chef system and subsequently sequenced on an Ion S5 XL using 400 bp chemistry (ThermoFisher Scientific).

Further details on PCR amplification and sequencing data processing method were provided in Text C.5.

5.2.6 Quality assurance and quality control

Triplicate samples were prepared for all experiments, and analytical results were reported as the average when applicable. To minimize adsorption to solid surfaces,[31] we used plastic tubes and vials whenever possible, except for the soil incubation vessels, which were glass. Procedural blanks were included in each extraction batch, and injection blanks were analyzed during each analysis sequence; no PFAS were detected in the blanks. The whole method recovery (Text C.6) was determined as per Matuszewski et al.[32] All quantitative PFASs but 5:2 ketone, and qualitative PFAS derived from Ansulite AFFF showed acceptable or suitable absolute recovery (70% ~ 130%) in four types of soils (Figure C.4), supporting the efficiency of the sample preparation method. Besides, the low absolute and effective matrix effects (Text C.7 and Figure C.5) in soils indicate the negligible influence of soil matrix on the instrument responses. The quality of calibration is provided in Tables C.8 and C.9.

5.3 Results and Discussion

5.3.1 Persistence of 5:3 FTB in aerobic soil

During the 120-d incubation, 5:3 FTB concentrations showed little change over time in both live and sterile CA-M soil, staying between 82.4~99.6 mol% and 98.3~117 mol% of the Day 0 concentration, respectively (Figure 5.1). We initially predicted that 5:3 fluorotelomer carboxylic acid (5:3 FTCA) would be formed from 5:3 FTB degradation when the quaternized nitrogen and the carboxylic group were metabolized by microorganisms or through other processes. However, 5:3 FTCA was only sporadically detected at low levels (0.01 mol%) on Days 30 and 60 in the live CA-M soil, but not any other treatment. 5:3 FTCA was first discovered as a biotransformation product of 6:2 fluorotelomer alcohol (6:2 FTOH),[25, 33, 34] without any other known origins. During

6:2 FTOH biotransformation, 5:3 FTCA showed strong binding with soil or sediment,[25, 33] but could be effectively recovered by a strong base (e.g., NaOH) in acetonitrile.[25] The present study showed that MeOH/CH_3COONH_4 extraction could also effectively recover 5:3 FTCA from soils (see Figure C.4), thereby excluding the possibility of 5:3 FTCA loss due to strong binding with soil. In addition, 5:3 FTCA has been observed to degrade to 4:3 FTCA with a low molar yield (2.3 mol%) in aerobic soils,[25] and in activated sludge with additional PFCA products (e.g., PFPeA and PFBA).[35] However, in the present experiment, no 4:3 FTCA was detected in CA-M soil. The PFPeA and PFHxA concentrations in live soils showed no evident trends over time, albeit at slightly higher or comparable levels compared with the sterile and live soil matrix controls; PFBA and PFPrA remain undetectable or sporadically detected at trace levels (0.001~0.004 mol%) in both live and sterile soils. These findings preclude the identification of these short-chain PFCAs as abiotic or biotransformation products. We also performed nontarget analysis and suspect screening to identify other possible biotransformation products of 5:3 FTB. Only one compound, tentatively identified as 5:3 fluorotelomer methyl amine (5:3 FT-MeAn or 5:3 demethyl-FTA) was detected in live soils with relatively small peak areas with an increasing trend followed by a decreasing trend over time (Figure C.6), confirming it as a biotransformation product. When doing a retrospective analysis, low levels of n:3 FT-MeAn (n=5, 7, 9 and 11) (1~2 orders of magnitube lower than n:3 FTB for the absolute peak areas) were found to be present in the AFFF-impacted soil samples containing relatively high concentrations of n:3 FTBs in the vicinity of a fire-fighting site (site #1) close to a Canadian airport, which may be formed from the slow biotransformation of n:3 FTBs. No other qualitative transformation products of 5:3 FTB, including those predicted by the EAWAG BBD/PPS, were detected.

We also detected several other PFASs, but they are improbable products of 5:3 FTB. These PFAS mainly originate from the soil as ambient anthropogenic background but could also come from other materials used in the experiments. First, since 5:3 FTB contains five fluorinated carbons, PFASs with six or more fluorinated carbons, such as C_7~C_{12} PFCAs, C_6, C_8, and C_{10} PFSA, are

improbable transformation products. Figure 5.1 shows similar levels and fluctuation patterns of these compounds in the live and sterile CA-M soil, as well as the soil matrix control, suggesting their origins in the ambient soil background. Second, due to the lack of sulfonamide or sulfonate group in 5:3 FTB, 5:3 FTB would not be biotransformed into any sulfonamide or sulfonamide derivative. Surprisingly, perfluorobutane sulfonamide (FBSA) and perfluorobutane sulfonate (PFBS) continually increased over time in the live CA-M soil only, rising by 0.02 mol% and 0.12 mol%, respectively, by Day 120. FBSA and PFBS were most likely produced from C_4 ECF-based precursors rather than 5:3 FTB, analogous to the generation of perfluoroctane sulfonamide (FOSA) and PFOS from biotransformation of C_8 precursors, as reported in the literature.[24, 26, 27, 36] In contrast, the sporadic or lack of formation of FBSA and PFBS in the live M soil matrix control suggested that such precursors were not present in the ambient soil background but were likely introduced during the incubation experiments. Previous studies reported the detection of PFBS and PFBS-based compounds, such as N-methyl-perfluorobutane sulfonamide (MeFBSA) and FBSA in air or dust, [37-39] but we could not detect any such precursors in any of the materials used in the present study. Although the source of FBSA and PFBS remain unidentified, their increasing trends over time in the 5:3 FTB-spiked live CA-M soil, but not in the sterile and live soil matrix control, confirmed the microbial activity in the former soil. The production of FBSA and PFBS unlikely from 5:3 FTB would not impact our conclusion on the 5:3 FTB biotransformation or the lack of it.

Satisfactory mass balances were recorded for 5:3 FTB, 101~120 mol%, and 82.8~100 mol% in live and sterile CA-M soil, respectively (Figure C.7a), which confirmed the integrity of the test vessels, and the effectiveness and suitability of the extraction methods employed. Therefore, we conclude that 5:3 FTB was not readily biodegradable in CA-M soil under the current test conditions.

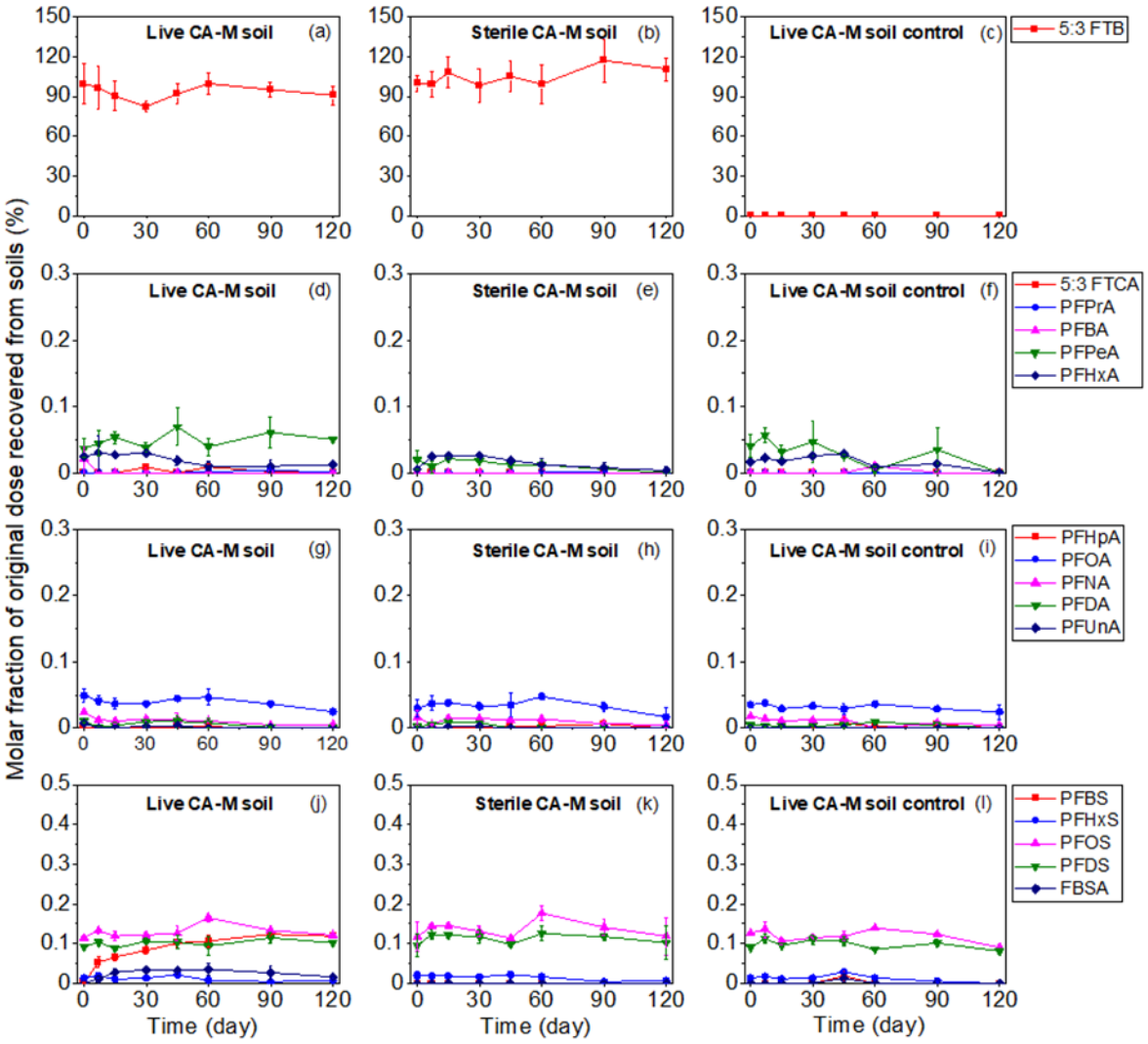


Figure 5.1 Concentration profiles of parent 5:3 FTB (a-c) and its potential transformation products (5:3 FTCA, PFPrA, PFBA, PFPeA PFHxA) (d-f) in aerobic live CA-M soil, sterile CA-M soil and live CA-M soil matrix control. Concentration profiles of other PFAS, which were present as backgrounds and not linked to 5:3 FTB biotransformation, included perfluoroalkyl carboxylates (PFHpA~PFDoA) (g~i), perfluoroalkyl sulfonates (PFBS, PFHxS, PFOS, PFDS) and FBSA (j~l).

5.3.2 Persistence of 5:1:2 FTB in aerobic soil

The 120-d soil incubation with 5:1:2 FTB (and the coexistent 5:3 FTB impurity) showed similar results as those of 5:3 FTB described in section 5.3.1, so we conclude that the 5:1:2 FTB

is also not readily biodegradable in the CA-M soil. The evidence that supports the conclusion is as follows. First, as shown in Figure 5.2, 5:1:2 FTB showed a negligible decline after 120 d with concentrations in the range of 91.7~115 mol% of Day 0, concurrent with insignificant production of PFAS that might be attributed to 5:1:2 FTB or 5:3 FTB degradation. Despite slightly higher or comparable levels compared with the sterile and live matrix controls, PFPeA and PFHxA concentrations in live soils showed no clear trends over time except a high PFPeA concentration stood out at a time point (day 90) possibly due to recovery variations or analytical errors. PFPrA, PFBA, and other short-chain polyfluoroalkyl acids (n:3 FTCA, n = 2~5) were undetectable or sporadically detected at trace levels (0.01~0.02 mol%) throughout the incubation period. Nontarget analysis and suspect screening methods did not reveal the presence of any other possible transformation products. For instance, hydrogen-substituted polyfluoroalkyl acid 5:1:2 FTCA ($F(CF_2)_5CHFCH_2COOH$) and 2H-PFHpA ($F(CF_2)_5CHFCOOH$) were predicted but were not detected. Second, other detectable PFAS, which are not associated with 5:1:2 FTB biotransformation, only reflected the PFAS initially present in the soil or those that were unintentionally introduced during the incubation experiments. Examples are C_7-C_{12} PFCAs and even-chained PFSA (PFHxS, PFOS, and PFDS). Again, FBSA and PFBS were observed in the 5:1:2 FTB-spiked live CA-M soil only, reaching 0.02 mol% and 0.14 mol%, respectively, by Day 120, while their origins remain unresolved. Lastly, the molar balance for 5:1:2 FTB ranged between 92.3~115.8% in live CA-M soil and 93.6~121.1% in sterile CA-M soil (Figure C.7b), suggesting that other products, even if present, were minor contributors to the 5:1:2 FTB biotransformation.

When 5:3 and 5:1:2 FTB were added to soil microcosms together, either 5:3 FTB or 5:1:2 FTB showed negligible degradation (Figures C.7c and C.8, Text C.8), with minimal production of PFPeA and PFHxA in live soils (0.010~0.028 mol% by day 120). As such, we were unable to explore the potential of differential metabolism of these two compounds.

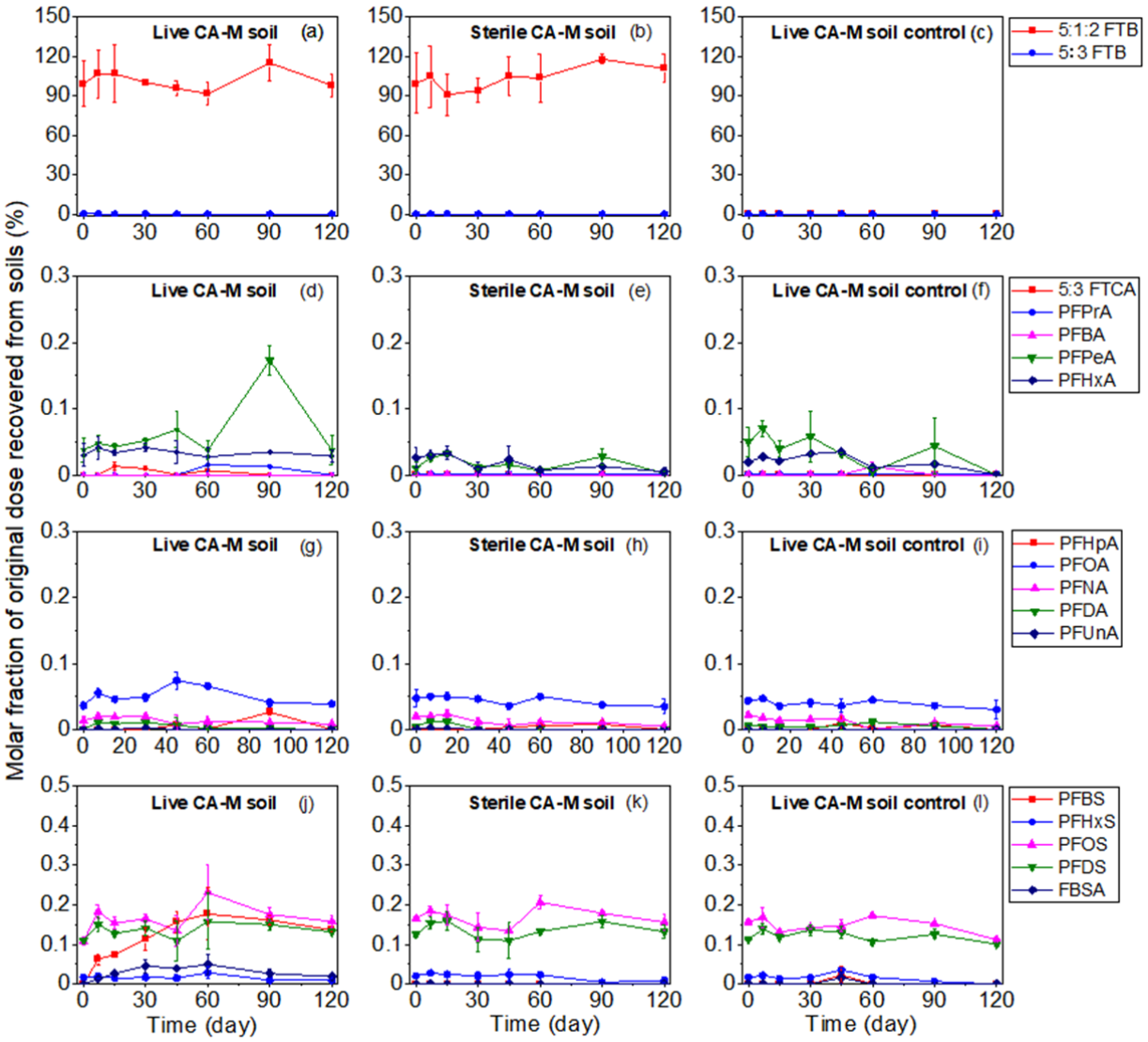


Figure 5.2 Concentration profiles of parent 5:1:2 FTB and coexistent 5:3 FTB impurity (a-c), and the potential transformation products (5:3 FTCA, PFPrA, PFBA, PFPeA PFHxA) of 5:3 FTB impurity (d-f) in aerobic live CA-M soil, sterile CA-M soil, and live CA-M soil matrix control. Concentration profiles of other PFAS, which were present as background and not linked to 5:3 FTB biotransformation, including perfluoroalkyl carboxylates (PFHpA~PFDoA) (g~i), perfluoroalkyl sulfonates (PFBS, PFHxS, PFOS, PFDS) and FBSA (j~l).

5.3.3 PFAS in the Ansulite AFFF

The composition of the Ansuilite AFFF was characterized using target, nontarget (CF_2 scale mass defect plots shown in Figure C.9), and suspect screening analyses, and allowed for the identification of nine classes of PFAS (structures in Figure C.10; t-MS^2 mass spectra and other details of qualitative PFAS in Figure C.11 and Table C.10) in the Ansulite AFFF, with a summed PFAS concentration of 1.03×10^4 ppm. FTBs with n:1:2 and n:3 polyfluoroalkyl chains stand out as the most abundant classes at 7.86×10^3 and 1.92×10^3 ppm, respectively, followed by n:1:3 FTB and n:4 FTB (177~ 193 ppm) (Figure C.12a). Individually, the species detected at >200 ppm included 5:1:2, 7:1:2, and 9:1:2 FTBs, followed by 5:3, 7:3 and 9:3 FTBs, and then by 11:1:2 FTB, together accounting for 93.9% of the summed PFAS (Figure C.12b). Other polyfluoroalkyl substances detected at low levels (11.8~90.9 ppm) included 8:2 FTSA, n:4 FTB (n = 4, 6, 8, 10), n:2 FTB (n = 6, 10), and n:1:3 FTB (n = 4, 6, 8), all together contributing to 6.0% of the summed PFAS concentration. In addition, we also detected other minor polyfluorinated substances, including n:2 FTS (n = 6, 10) and 10:1:3 FTB, which only accounted for 0.07% of the summed PFAS. This is the first time that n:1:3 FTBs were discovered in current-in-use AFFFs.

5.3.4 Aerobic transformation of the Ansulite AFFF

The Ansulite AFFF was added to the four soils collected from different geographical locations to examine variations in biotransformation outcomes. Although the four soils possess varying physical and chemical properties (Table C.1), as well as different microbial communities (see section 5.3.5), time profiles and trends of the AFFF-derived PFAS showed similar general patterns with minor differences. In CA-L soil (collected from Lac Du Bois Grasslands, British Columbia, Canada), the AFFF's major components, including n:3 FTB (n = 5, 7, 9, 11) and n:1:2 FTB (n =5, 7, 9), showed little change over the 120-d incubation, with the live and sterile soils showing similar profiles (Figure 5.3). PFAS concentrations in the other three soils exhibited the same general trends

(Figure C.13), illustrating the persistence of the n:3 and n:1:2 FTB compounds with varying chain lengths, not only for those with n = 5 as discussed in sections 5.3.1 and 5.3.2.

In addition to the predominant n:3 and n:1:2 fluorotelomers, the Ansulite AFFF also contain other fluorotelomers with distinct polyfluoroalkyl chains, such as n:2, n:4, and n:1:3 FTs (Figures C.10, C.14 and C.15), and these polyfluoroalkyl components were labeled as "minor" and "trace-level" PFAS due to their relatively low abundance; each accounting for 0.09~1.3 mol% and 0~0.21 mol%, respectively, of the summed PFAS. These minor and trace-level components generally showed similar high persistence as the major PFAS components, with their concentrations remaining essentially unchanged after 120 days in both live and sterile soils (Figures C.14 and C.15). An exception was 8:2 FTS in the live US-G soil, whose concentration significantly declined over time (from 1.5 mol% on Day 0 to 0.90 mol% on Day 120) (Figures C.14 and C.15). This indicates that 8:2 FTS may be degraded and/or defluorinated, forming polyfluoroalkyl acids (e.g., 7:3 FTCA) and a series of PFCA (e.g., C_6~C_9 PFCA), as observed for 6:2 FTS in aerobic river sediment,[40] and wetland slurry.[41] The persistence of 8:2 FTS in the other three live soils may be due to microbial desulfonation as the rate-limiting step, as observed for 6:2 FTS in activated sludge.[42]

Since the long-chain n:3 and n:1:2 FTBs with ≥7 fluorinated carbons were simultaneously introduced into the soil along with the short-chain 5:3 and 5:1:2 FTBs, a wide range of PFCAs (C_3~C_{14}) and n:3 FTCA (n = 2~15) were monitored for their potential production. Figure 5.3 demonstrates more rapid increases of PFPeA, PFHxA, PFHpA, PFOA, and PFNA concentrations in the live CA-L soil (increase by 0.13, 0.063, 0.054, 0.15, and 0.015 mol%, respectively, by day 120) than the sterile control (increase by 0.024, 0.010, 0.002, 0.008 and 0.003 mol%, by day 120), confirming their formation from the biotransformation of the Ansulite AFFF. Both 5:3 and 7:3 FTCAs increased in concentration (0.061 and 0.015 mol% by day 120, respectively) in the sterile CA-L soil, while their concentrations remain undetectable in the live CA-L soil, which may be

consumed by biotransformation to further downstream products.. Previous studies found that 7:3 FTCA was slowly converted to PFHpA in activated sludge,[35] but was not degradable in aerobic soil.[43] In our previously published work, we tested the efficacy of the soil sterilization method using the same CA-M soil used in the present study; repeated autoclaving and the amendment of three antibiotics can significantly reduce soil ATP levels, but not to zero, suggesting weak microbial activities were still possible.[16] Nevertheless, it is uncertain if any abiotic mechanisms or microbial activities were responsible for the appearance of 5:3 or 7:3 FTCAs in sterile soils. Other PFCAs and polyfluoroalkyl acids remained at background or undetectable levels in both live and sterile CA-L soil, eliminating the possibility of their formation from the Ansulite AFFF. Overall, the negligible loss of parent polyfluoroalkyl compounds concurrent with the low yields of PFCAs (0.40 mol% in total) demonstrates the high persistence of the FTB-containing AFFF in one aerobic soil.

Similar to what was observed in CA-L soil, we observed faster increases in C_5~C_9 PFCA concentrations in the other three live soils than in their corresponding sterile soils (Figure C.13). Specifically, PFPeA, PFHxA, PFHpA, PFOA, and PFNA were produced at a yield of 0.028~0.032, 0.018~0.038, 0.004~0.027, 0.016~0.05, and 0.005~0.019 mol%, respectively, by day 120, in the three live soils, while at a yield of 0~0.01 mol% in the three sterile soils(Figure C.13). At the end of incubation, the total PFCA yields in the four live soils ranked as CA-L soil (0.41 mol%) > US-G soil (0.39 mol%) > US-F soil (0.18 mol%) > CA-M soil (0.073 mol%). The separate incubation with 5:3 and 5:1:2 FTB showed limited degradation to PFCAs, so we expected their longer-chained analogs to be no less persistent. Surveying the literature on the limited number of studies on polyfluoroalkyl precursors, we hypothesize that the most likely precursors of C_5~C_9 PFCA are n:2 fluorotelomers in the Ansulite AFFF, such as 8:2 FTS. Previously, 6:2 FTS was found to be degraded into C_4~C_7 PFCAs in pure bacterial cultures, aerobic sediment and surface soil with a wide range in half-life (<5 d ~ 2 years).[40, 42] Despite the lack of studies on biotransformation of 8:2 FTS, its similar structure to 6:2 FTS suggests that it would be transformed to 8:2 FTOH via

microbial desulfonation, with further biodegradation of 8:2 FTOH into PFCAs (e.g., PFHxA, PFOA), as previously observed for 8:2 FTOH in mixed bacterial culture, activated sludge.[44-46]

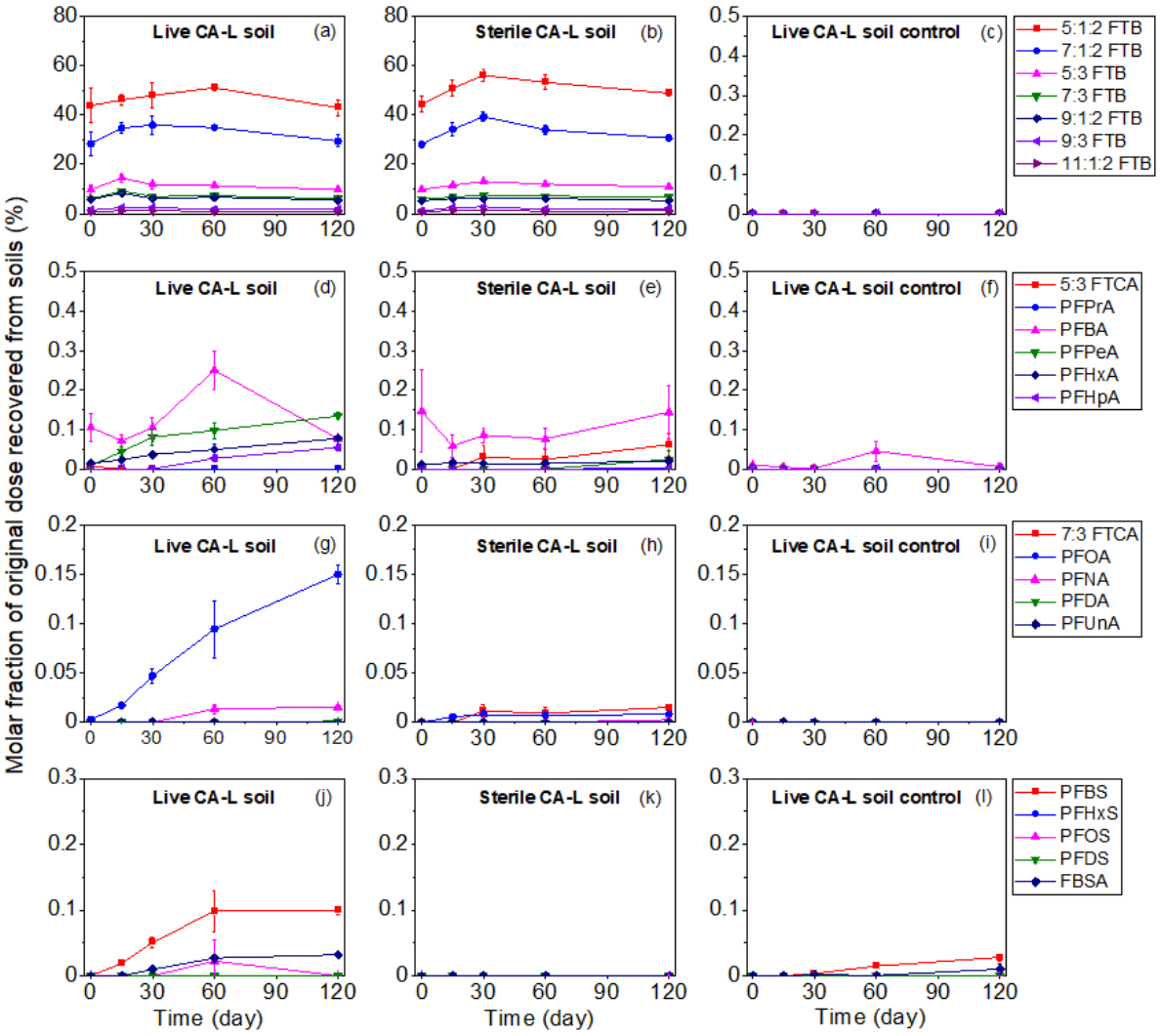


Figure 5.3 Incubation of an Ansulite AFFF in CA-L soil over 120 d; concentration profiles in live, sterile, and live soil matrix controls are shown for the major PFAS (a-c) contained in the Ansulite AFFF, and their potential transformation products, including C3 ~ C11 PFCA and n:3 FTCAs (d-i), as well as those of other detectable ECF-based PFAS that were not derived from the AFFF (j-l).

The satisfactory mass balance for PFAS precursors predominant in the Ansulite AFFF and their quantitative transformation products in the four live and sterile soils (97.0~128 mol% and 84.0~129 mol%, respectively) (Figure C.8d) indicates the minor contribution of the qualitative transformation products. Consistent with what was observed in the two-betaine mixture biotransformation experiment, no n:2 H-FTCA, 2H-PFCA, or other qualitative transformation products were identified in the four soils by nontarget analysis and suspect-screening methods.

The Ansulite AFFF does not contain any sulfonamide-derived compounds; again, the production of FBSA and PFBS (a yield of 0.01~0.03 mol% and 0.08~0.13 mol%, respectively, by day 120) was observed in the four live soils (Figure 5.3 and S13). Different from 5:3 and 5:1:2 FTBs, the production of PFBS was also observed in all soil controls for which no AFFF was spiked, though at levels lower than the live soils. This indicates the likely presence of C_4 ECF-based precursors in all four soils as ambient soil background or the precursors of unknown sources were introduced during the experiments. The presence and increasing trends of FBSA and PFBS in four live soils suggest the ubiquitous nature of these PFAS.

5.3.5 Microbial community analysis

We extracted DNA from all the live soil samples over the incubation period to analyze the impact of PFAS treatments on microbial community composition over time. All four soil microcosms were predominantly composed of Proteobacteria, Actinobacteria, and Acidobacteria, which is consistent with phyla-level compositions across diverse soils.[47] The phylum-level compositions were largely consistent across treatments for the duration of the experiment (Figure C.16).

We generated multidimensional scaling (MDS) plots based on Bray-Curtis dissimilarity between samples at the OTU level (Figures 4 and S17) to analyze trends in beta diversity. Despite similar phylum-level community compositions, the MDS plots showed that the four soils had distinct microbial communities at the OTU level. No clear trends with different PFAS treatments

(Figure 5.4) or over time (Figure C.17) were observed. Both the phylum-level and OTU-level analyses indicate that the microbial communities were little impacted by the dosed PFAS and their carrier solvents over the incubation period, regardless of the soil type.

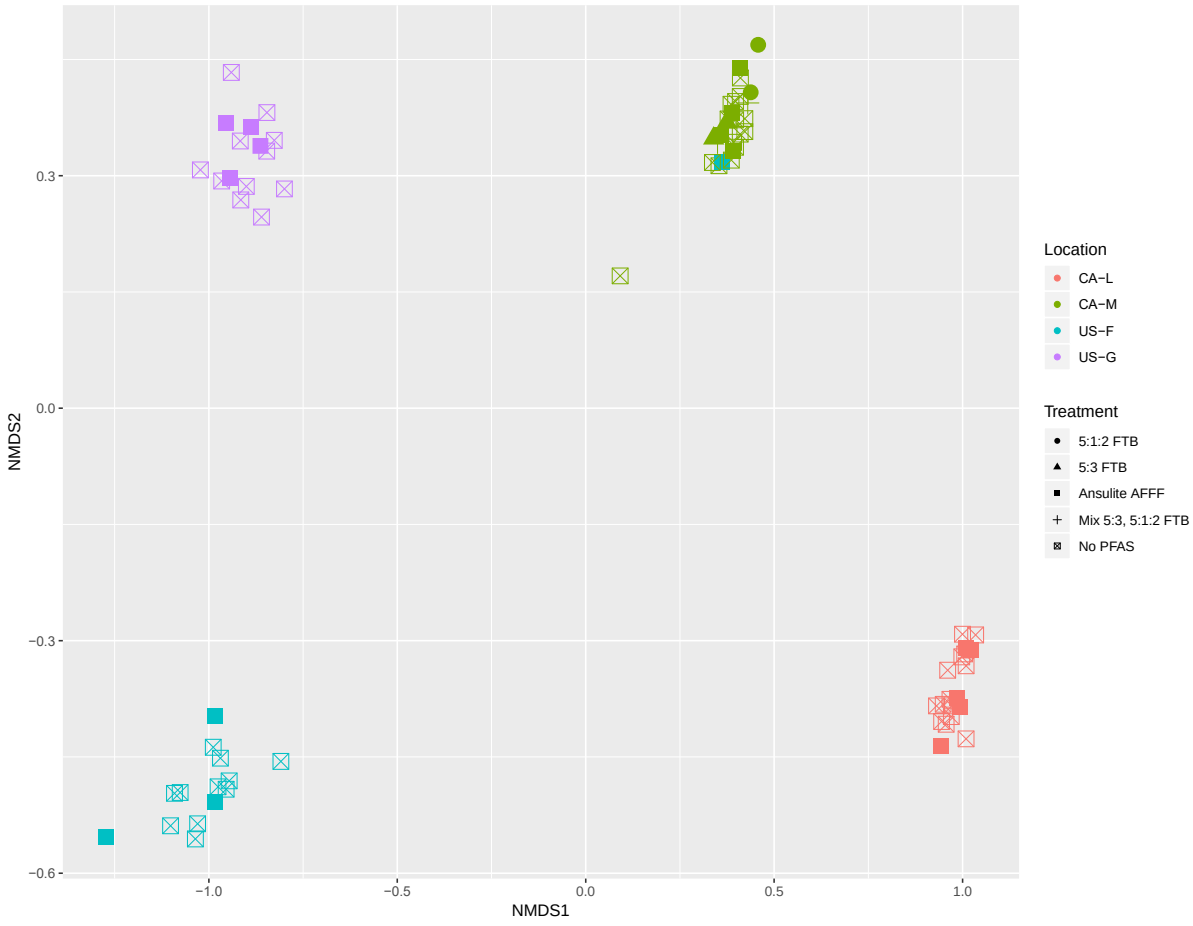


Figure 5.4 MDS plot representing Bray-Curtis dissimilarity in community composition between samples at the OTU level. Samples are coloured by location, and different shapes represent different treatments.

5.4 Environmental Implications

The information on the environmental fate of the current-in-use AFFFs is sparse. For the first time, we demonstrate that the major components of common AFFFs, such as n:3 and n:1:2 FTBs, are highly persistent and, at best, can only produce trace amounts of polyfluoroalkyl acids (e.g.,

5:3 and 7:3 FTCAs) and PFCAs in aerobic soils. The findings are at odds with the long-held preconception that fluorotelomers with hydrocarbon polar groups would readily biodegrade to form perfluoroalkyl acids. Despite the presence of three hydrocarbon atoms or two hydrocarbon atoms connected to a singly fluorinated carbon on n:3 or n:1:2 FTBs, they both showed high resistance to biodegradation, with aerobic soils of different origins, properties, and distinct microbial communities having very similar biotransformation outcomes with only minor differences. These results suggest the odd number of hydrocarbon moieties or the singly fluorinated carbon linkage could not increase the susceptibility of n:3 and n:1:2 FTBs to biotransformation. The persistence of n:3 and n:1:2 FTBs indicates that the AFFFs containing these compounds would constitute a long-term PFAS source once released into the soil environment, highlighting the importance of bringing these precursors that are not routinely measured into focus for monitoring, risk assessment, and remediation activities at many AFFF-impacted sites.

Despite the negligible contribution of the AFFF-derived FTBs to polyfluoroalkyl acids and PFCAs, the minor components (e.g., 8:2 FTS) contained in these FT-based AFFFs can potentially serve as an indirect source of both short- (C_5-C_7) and long-chain PFCAs (C_8-C_9) in the environment. This deserves great attention since the short-chain PFCAs can readily migrate into groundwater and further into surface water, or even drinking water, owing to their persistence, low sorption potential, and high mobility,[48] while the long-chain PFCAs can persist in soil, and accumulate in plants and animals. Whether the continuously accumulating PFOA and other long-chain PFCAs in the tissues of arctic animals,[21] long after their phase-out, is related to the current use of such FTB-containing AFFFs, needs further investigation.

The causes for the persistence of n:3 and n:1:2 FTBs remain unknown, but several possibilities can be eliminated: (1) FTBs are not known to be biocidal and inhibit microbial activities; thus, the persistence is not expected to be due to their biocidal nature; (2) It is unlikely

that the high sorption potential of n:3 and n:1:2 FTBs (n = 5, 7) onto soil reduced their bioavailability, restricting their biodegradation because their LogK_d (solid-water distribution coefficient) or LogK_{oc} (organic carbon-water partition coefficient) values are not expected to be high based on previous literature. Specifically, 7:1:2 and 7:3 FTBs showed comparable LogK_{oc} values (3.0 and 3.3, respectively) to 6:2 FTSA-PrB (2.7) in water-sediment,[12] while the LogK_d value of 6:2 FTSA-PrB in soils is comparable to that of 6:2 FTS and PFHxS, slightly lower than PFOA, [49] but significantly lower than PFOS and other longer-chain PFAAs. The K_d/K_{oc} values for short-chain 5:3 and 5:1:2 FTBs may be even lower considering their short perfluoroalkyl chains.[12] In addition to the hydrophobic interactions from the fluorinated chain,[12] the electrostatic interactions of the quaternary ammonium at the polar head group with negatively charged soil components may influence the sorption of FTBs, similar to those reported for 6:2 FTSA-PrB.[51]

The persistence of FTBs may be due to the structural quaternary ammonium group, as reported for ECF-based PFOSB and PFOAB in aerobic soils.[16] One possible synthesis route for n:3 FTB and n:1:2 FTB is through hydrogenation of unsaturated polyfluoroalkylamines,[10, 11] followed by the reaction of saturated fluoroalkylamines with a halocarboxylic acid (X-CH_2COOH, X is preferably Cl or Br),[10, 11] a Menshutkin reaction. This quaternization reaction creates quaternary ammonium cations that are unreactive toward even strong electrophiles, oxidants, acids, and most nucleophiles. However, we previously reported that both PFOSB and PFOAB could biotransform to produce detectable levels of PFOS (from PFOSB) or PFOA (from PFOA),[16] while the transformation of PFOAB to PFOA was quite significant in the same CA-M soil. In the case of 5:3 and 5:1:2 FTBs, it is quite puzzling that a shorter perfluoroalkyl chain and extra hydrocarbons did not result in higher transformation potential.

Further studies on the sorption behaviors, the vertical transport in the vadose zone, and leaching from soil into the aqueous environment of n:3 and n:1:2 FTB are warranted. The toxicity and adverse health effects of PFAAs have been well studied,[50-52] the toxic nature of a legacy AFFF

mixture and/or AFFF-related transformation products (e.g., 6:2 FTCA, 6:2 FTS) were also reported; [53-55] while the toxicological data on both the FTBs and the current-in-use AFFFs predominant with FTBs are lacking and warrant further research.

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Chapter 6. Summary, Conclusions, and Future Work

6.1 Summary and Conclusions

This thesis aimed to elucidate the environmental fate of AFFF-derived from PFAS by thoroughly characterizing PFAS at AFFF-impacted sites and investigating the biotransformation potential of ECF-based zwitterions (used in historical AFFFs) and FT-based zwitterions (used in current AFFFs) in aerobic soils.

In chapter 3, the status of PFAS contamination at four Canadian airports was evaluated. All four airports have been impacted by more than one AFFF chemistry (ECF and FT chemistry), as indicated by the detection of PFSA, PFCA, ECF-based precursors, FT-based precursors, and other PFAS in both soil and groundwater. However, these four sites displayed distinct PFAS profiles and loads, with the active site exhibiting greater PFAS variety and total PFAS burden than decommissioned sites, indicating the influence of AFFF use history on the PFAS concentration profiles. In addition, the PFAS profile differences between soil and groundwater in the source zone area were noted. (1) Zwitterionic and cationic PFAS composed a large percentage (34.5-85.5%) of the total PFAS mass in most surface soil samples but a relatively low percentage (<20%) in groundwater samples; (2) Many zwitterionic and cationic fluorotelomers, including n:3 FTBs, n:1:2 FTBs and 6:2 fluorotelomers (e.g. 6:2 FTAB, 6:2 FTSHA-SO, etc.), were abundant in soil but low or nondetectable in groundwater at the active site, while anionic precursors (e.g., 6:2 FTSAS, sulfoxide/sulfone oxidation products, etc.) were exclusively detected at high abundance in groundwater. The spatial trends of PFAS in soil and groundwater were also different. Specifically, the PFAS in the source zone soil underwent limited horizontal transfer and seldomly reached the background area surrounding the source zone, but the vertical migration of PFAS down the soil column occurred even in locations of low permeability; PFAS in source zone groundwater could be transferred to the downgradient area, but the exact extent was unclear because of the limited number of samples. These differences may be due to variations in sorption, transport, and in-situ transformation potential of PFAS, geochemical and hydrologic conditions,[1], etc.

Furthermore, a high percentage of unidentified precursors possibly resulting from atmospheric deposition was noted in background soils around the source zone, and the identity of those precursors needs to be determined. This first comprehensive characterization of PFAS pollution at civilian airports in North America provides critical information and methodology to support future PFAS monitoring, mitigation, and remediation efforts in Canada and other countries.

In chapter 4, the biotransformation potential and persistence in aerobic soils of ECF-based betaines and tertiary amines used in historical AFFF formulations were investigated and compared with those with quaternary ammonium salts and amine oxides, biotransformation of which were previously known. Results demonstrated that the amide betaine and tertiary amine were precursors to PFOA, while the perfluoroalkyl sulfonamide betaine and tertiary amine were precursors to PFOS. Specifically, PFOAB degraded with an estimated half-life of ≥ 266 d and generated PFOA at a yield of 5.8~32.6 mol% by day 150, PFOAAm was biotransformed into PFOA with a half-life of ≥ 14 d, while PFOSB slowly degraded with a half-life ≥ 675 d and produced PFOS at a yield of 0.07~1.5mol%, PFOSAm was biotransformed into PFOS (0.06~2.7% by day 90) with a half-life ≥ 47 d. Therefore, these four ECF zwitterions displayed varied transformation kinetics in aerobic soils. The comparison of their transformation kinetics with four other previously reported zwitterions or cations indicates the great influence of structure, especially the nitrogen head groups, on the persistence of these ECF-based precursors in aerobic soil. The ECF precursors with the sulfonamide group linked with the perfluoroalkyl chain showed higher microbial stability than those with the amide group. In addition, the ECF precursors containing quaternary ammonium or betaine groups showed high stability in soils, with the longest DT_{50} likely to be years or decades, while those with tertiary amine or amine oxide groups were less stable, with a shorter DT_{50} of weeks or months. Furthermore, the transformation pathways commonly shared by the amide-based or sulfonamide-based precursors were proposed based on the transformation product profiles.

These eight ECF-based precursors provide insights into the degradation pathways and persistence in surface soils of other perfluoroalkyl cations and zwitterions present in AFFFs.

In chapter 5, the biotransformation potential and persistence of short-chain 5:3 and 5:1:2 FTBs and a commercial AFFF primarily containing n:3 and n:1:2 FTBs (n = 5, 7, 9, 11, and 13) in aerobic soils was explored. Results demonstrated the high persistence of 5:3 and 5:1:2 FTBs and their little contribution to short-chain PFCAs in aerobic soils, as indicated by the negligible change of the parent betaine(s) concentration concurrent with the low yields of short-chain 5:3 FTCA, PFPeA, and PFHxA. In contrast, the slow biotransformation of the Ansulite AFFF that contains both short-chain and long-chain n:3 and n:1:2 FTBs resulted in low production of both short- and long-chain PFCAs, including PFOA and the longer-chain PFCAs (PFNA, PFDA, and PFUnA) that have been restricted in use due to their adverse environmental and health effects. In addition, the high persistence of FTBs and FTB-containing AFFF highlights the importance of integrating novel betaine precursors, which are not routinely measured, into monitoring, risk assessment, and remediation activities of AFFF-impacted sites.

6.2 Future Work

The complementary uses of LC-HRMS and TOP assay enabled the comprehensive characterization of PFAS in AFFF-impacted soil and water samples. However, there remains some difficulty in accurately evaluating the extent of AFFF impact due to the limited chemical standard availability. The study implemented an approximate quantification for PFAS without available native standards by comparing them to standards with similar structures. However, this could lead to the under- or overestimation of PFAS concentrations, as suggested by higher concentrations determined using LC-HRMS than those estimated through the TOP assay.[2] In this study, the TOP assay was improved (e.g., a new exhaustive soil extraction method, a small reaction solution, and modifications of post-oxidation procedures) to achieve satisfactory oxidation of selected

precursors in soils, thus enabling relatively accurate estimation of the unknown precursors and total PFAS in soils. However, this TOP method still has limitations, such as interferences from co-extracted soil matrix components, the resistance of some known (e.g., ether-based PFAS) or unknown PFAS to oxidation,[3] and the inability to capture some ultra-short-chain PFCAs or other products by LC-HRMS.[4] Therefore, more work is warranted on improving the TOP assay method to overcome such limitations. Recently, the TOP assay was modified by fully oxidizing small amounts of the solid samples (e.g., hens' feed and eggs) instead of oxidizing their extracts in order to overcome potential losses during extraction and avoid incomplete oxidation presumably due to high matrix load in the extracts, which proved to be a powerful tool to assess the total burden of PFAS.[5] However, the method availability for complex matrixes (e.g., soil, aquifer solids, etc) warrant further study. In addition, alternative approaches that capture all organofluorine, including combustion ion chromatography (CIC), could be applied. CIC was used to determine the extractable organic fluorine (EOF) content of water,[6] animal blood,[7] human serum, and placenta samples.[8] Previous studies also reported the application of particle-induced gamma-ray emission (PIGE) to quantify total fluorine concentrations in soil samples.[9] However, these analytical tools cannot distinguish organic fluorine from fluoride, and only proper sample preparation steps can remove or reduce the level of fluoride. The applicability of these two methods to complex solid matrixes (e.g., soils with high organic matter or with hydrocarbon co-contaminants, biosolids, etc.) deserves future research.

The investigation of the biotransformation potential of several ECF-based zwitterions in aerobic soils revealed a preliminary structure-degradability relationship for ECF-based precursors with varying terminal functional groups, including quaternary ammonium, tertiary amine, amine oxide, and carboxyl betaine. In addition to these N-containing functional groups with positive charges, the historical and current AFFFs contain a great variety of precursors with negatively charged functional groups (e.g., sulfonate, hydroxyl, carboxyl, a combination of two or more functional groups, etc.).[10] How these functional groups influence the sorption, migration, and

degradation potential of AFFF-derived precursors remains unknown. In addition, other PFAS zwitterions and cations with more complex head groups are present at high levels in AFFF-impacted sites; [10-13] further studies on these compounds could be conducted.

The FTB biotransformation experiment demonstrated the high persistence of n:3 and n:1:2 FTBs in aerobic soils. However, their environmental behaviours, including sorption, transport, leaching, and migration, remain largely unexplored. In the field, PFAS always appear as a mixture rather than a single component, and other non-fluorinated compounds such as hydrocarbon surfactants and chlorinated solvents also coexist with PFAS. Therefore, the influence of co-contaminants on the behaviour and fate of AFFF-derived precursors must be considered. Moreover, little is known about the toxicity of PFAS mixtures or AFFFs. A recent study characterized the toxicity of a legacy AFFF mixture predominant with PFOS in zebrafish, with developmental, morphological, and liver effects identified for the first time.[14] Although legacy AFFF has been banned, the toxicity of current-in-use AFFFs such as those containing n:2 fluorotelomers and novel n:3 and n:1:2 FTBs is unknown and needs further study research.

6.3 References

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Appendix A. Supplemental information for Chapter 3

Text A.1 Details on Chemicals and materials

HPLC-water, HPLC-water containing 0.1% formic acid, methanol, and acetonitrile were of LC-MS grade and were obtained from Fisher Scientific (Whitby, ON, Canada). Ammonium acetate (purity ≥ 98%), sodium hydroxide (pellets, purity ≥ 97%), ammonium hydroxide (25–30% in water), and formic acid (reagent grade, purity ≥ 95%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (35–38% in water), and glacial acetic acid were obtained from Fisher Scientific (Whitby, ON, Canada). Nitrogen (N_2) (purity 99.998%) was from MEGS InB. (St-Laurent, QC, Canada). Superclean ENVI-Carb cartridges (250 mg/6 mL) were obtained from Supelco (Bellefonte, PA, USA).

The sources of 53 PFAS with available native standards are shown in Table A.1. The native PFASs obtained from Wellington Labs, InB. (Whitby, ON, Canada) had chemical purities >98%. These standards were either acquired at 2 μg mL^{-1} (as compound or salt) as mixtures or separately at 50 μg mL^{-1} (as compound or salt) as individual compounds.

Isotope-labeled internal standards (IS) (see Table A.2) were all obtained from Wellington Labs, InB. (Whitby, ON, Canada). Perfluorooctane amidoakyl ammonium salt (PFOAAmS) was custom synthesized at Beijing Surfactant Institute (Peking, China) and was used as an internal standard for positive mode native analytes. $^{13}C_4$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, $^{13}C_2$-PFDoA, $^{13}C_2$-PFTeDA, $^{13}C_2$-PFHxDA, $^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, $^{13}C_8$-PFOS, $^{13}C_8$-FOSA, d_3-MeFOSA, d_5-EtFOSA, d_3-MeFOSAA, $^{13}C_2$-6:2-FtS, $^{13}C_2$-8:2-FtS, $^{13}C_2$-6:2-FTUA, $^{13}C_2$-8:2-FTUA, and PFOAAmS were the internal standards used in the present study. The association between the native analyte and internal standard is provided in Table A.8.

Text A.2 Field soil and groundwater sample preparation without persulfate oxidation

Soil. The method modified from previous studies was applied for the field soil samples.[1] Specifically, 1 g (dw) of soil for each sample was weighed in 15-mL polypropylene (PP) tubes (previously cleaned with MeOH). Then 5 µg/kg dw of surrogate internal standard solution mixture (100 µL, 50 ng mL^{-1}) was spiked into each soil sample. After a wait time of ~60 min, soil samples were submitted to three sequential solvent extraction cycles. In each cycle, the soil was extracted with 4 mL of 100 mM of ammonium acetate (AmmoAce) in methanol, vortexed for 0.5 min, ultrasonicated for 10 min, and subjected to centrifugation (5000 rpm, 5 min). The supernatant was transferred into a new 15-mL PP tube.

The combined extract (~12 mL) was then subjected to cleanup. The extract was transferred onto an ENVI-Carb graphite cartridge (250 mg/6 mL, pre-cleaned with 4 mL of MeOH), and the eluate was directly recovered in a new 15-mL PP tube. The tubes containing soil extracts were also rinsed with 0.5 mL of MeOH and passed through the cartridges. The cartridges were rinsed with 1 mL of MeOH in the end. The resulting extract was concentrated using a gentle stream of N_2 and mild heating (40°C) and finally adjusted to a volume of 2 mL. Following brief vortexing (0.25 min), a 150-µL aliquot of sample was introduced into a 250-µL polypropylene HPLC vial, along with 50 µL of a 20 ng mL^{-1} injection internal standard solution mixture (MPFAC-C-IS from Wellington Labs). Following brief vortexing (0.1 min), the extracts were submitted to UHPLC-HRMS analysis. For those PFAS present at very high concentrations, new sample preparation was performed if necessary to fall within linear working range.

Groundwater. Groundwater samples were prepared as follows. After gently inverting the bottles for homogenization (but avoiding foaming), a 40-mL aliquot of sample from the original collection bottle was collected from ~10 cm below the air-water interface, introduced in a 50-mL PP tube, and submitted to centrifugation (5 min; 6000 rpm). A 41.7-µL aliquot of groundwater was then transferred to a polypropylene tube, to which 28.3 µL of HPLC-water, 140 µL of MeOH, and 140 µL of a 6.25 ng mL^{-1} internal standard solution (prepared in MeOH) were added. The sample was briefly vortexed and a 200-µL aliquot of sample was transferred to a 250-µL polypropylene HPLC vial. The final extract composition was 80:20 MeOH:water (v/v). Note that internal calibration curves were built accordingly (80:20 MeOH:HPLC-water v/v) with the same IS concentration. The dilution factor of 8.4× was considered to derive the actual concentration.

Text A.3 Procedures for spike recovery and matrix effect assessment

The procedures for the determination of whole-method spike recovery and instrumental matrix effect assessment were adapted from previous studies. [2, 3]

Validation of the method spike recovery

Three soils were obtained locally from areas without known PFAS point sources (Table A.2), and were used to evaluate the spike recovery of the final retained method (extraction using 100 mM CH_3COONH_4 in MeOH, Envicarb cartridge cleanup, and evaporation). The soil samples (n = 3 per soil matrix) were spiked with 10 µg/kg dw of native standards (i.e., 100 µL of a mixture containing the 53 certified PFAS at 100 ng mL^{-1} in MeOH) and then processed using the procedures as described above. These samples were referred to as "spiked before" (SB). In parallel, for each soil type, six non-spiked soil samples were added with 200 µL of MeOH and processed as per the same procedure. At the end of the preparation procedure, three of the latter samples were spiked with 10 ng/g of native standards (referred to as "spiked after" [SA] samples), while the other three were left unspiked (referred to as "non-spiked" [NS] samples). The three sets of samples (i.e. SB, SA, and NS) were then spiked with internal standards, briefly vortexed, and analyzed by HPLC-MS. The spike recovery was determined as per the following equation:

$$Recovery\ (\%) = 100 * \frac{SB-NS}{SA-NS} \qquad \textbf{(Equation 1)}$$

where 'SB' is the native analyte to internal standard area ratio observed in a sample spiked before extraction with native analytes, 'SA' is the native analyte to internal standard area ratio observed in a sample spiked at the end of the analytical procedure with native analytes, and 'NS' is the native analyte to internal standard area ratio of the non-spiked sample.

The recovery test for soil sample preparation gave satisfactory recovery for the 53 quantitative PFAS with available standards (in the range of 60%-140%), validating the method efficiency. However, trifluoroacetic acid (TFA) and perfluorooctadecanoic acid (PFOcDA) were not quantified due to instrument limitations.

Assessment of the instrumental matrix effect

The same three soils were used to examine potential matrix effects at the UHPLC-MS analysis stage. For each soil type, the matrix effect at the instrumental analysis stage was evaluated by

comparing aliquots of soil extract spiked post-preparation (extraction, cleanup, and concentration) to that in a matrix-free (solvent-based) reference.

Two types of matrix effects were investigated. The absolute matrix effect (Equation 2) is determined based on the native analyte absolute area, while the effective matrix effect refers to that evaluated based on the native analyte to internal standard area ratio (Equation 3):

$$Absolute\ matrix\ effect\ (\%) = 100 * \left(\frac{M-NS}{S} - 1\right) \qquad \text{(Equation 2)}$$

where 'M' is the native analyte absolute area in the spiked soil matrix, 'NS' is the native analyte absolute area in the non-spiked soil matrix, and 'S' is the native analyte absolute area in the spiked matrix-free reference.

$$Effective\ matrix\ effect\ (\%) = 100 * \left(\frac{m-ns}{s} - 1\right) \qquad \text{(Equation 3)}$$

where 'm' is the native analyte to internal standard area ratio in the spiked soil matrix, 'ns' is the native analyte to internal standard area ratio in the non-spiked soil matrix, and 's' is the native analyte to internal standard area ratio in the spiked matrix-free reference.

Text A.4 Method validation of total oxidizable precursor (TOP) assay for soils

The same three soils (Table A.2) were also used for TOP method validation, with all treatments in triplicate for each type of soil. The soil sample preparation method before TOP was modified from Nickerson et al.[4] In detail,1 g dw of soil was weighed into a pre-cleaned PP tube and then spiked with 67 µl of an individual precursor solution (6:2 FTSA, 8:2 FTSA, 6:2 FTAB, FHxSA, PFHxSAm or PFHxSAmS methanolic solution at a concentration of 17.91 ppm). After 1-h stabilization, the soils were extracted with a basic solvent (MeOH with 0.4 M NaOH) for two cycles, followed by an acidic solvent (MeOH with 0.4 M HCl) for one cycle. Each extraction cycle consisted of high-speed vortexing for 30 s, ultrasonication for 10 min, and centrifugation at 6000 rpm for 5 min. The supernatants of the first two cycles were combined (4 ml), while the supernatant from the third cycle (2ml) was separately collected. Then the basic extracts (4 ml) and acid extracts (2 ml) were sequentially passed through Envi-Carb graphite cartridges (6 mL/ 250 mg) for cleanup and stored in separate PP tubes. The two fractions of polished extracts were combined (10 ml), neutralized with HCl (adjust pH to 7), and adjusted to a final volume of 11 mL. After centrifugation (5000 rpm, 5 min), an aliquot (1 ml) of the supernatant was transferred to a 15-mL HDPE tube and evaporated to dryness at 45 ºC.

The TOP assay procedure was modified from Houtz and Sedlak's.[5] Specifically, 3.6 mL of water was added into the tube with dried soil extract, sonicated for 20 min, then 1968 µL of potassium persulfate at 175 mM in HPLC-water was added (final concentration of 60 mM), followed by the addition of 172.2 µL of 5 M NaOH (a final concentration of 150 mM). After mixing, the tubes with the aqueous solution were placed into a heated water bath (85 ºC) for 6 hours. At the end of the reaction, the tubes were removed from the water bath and a wait time was applied to let the samples cool down to room temperature. Subsequently, 30 µL of HCl 6M and 300 µL of methanol were sequentially added, and the capped tubes were inverted for mixing. After that, 70 uL of TOP sample, 140 uL of internal standard solution at 6.25 ppb (in MeOH), and 140 uL of methanol were added into a separate vial (2 mL). After brief vortexing and centrifugation (to separate out the salt precipitate), a 180-µL aliquot of the supernatant was transferred to an injection vial for instrument analysis.

The tubes with the three soil matrixes without PFAS spike were processed in parallel to the spiked soils for subtracting the initial PFCAs extracted/generated from the background soil matrix.
The TOP assay was also performed on the precursors in HPLC water; reference tubes were spiked with an equivalent amount of precursor and subjected to the TOP procedure. The PFCA oxidation yields in soil and ultrapure water matrixes were thus compared.

TOP assay performance – verification of volatile loss and sorption loss

The potential PFCA loss from the TOP procedure (including adsorption to vials, tubes, and volatilization) was checked to ensure the accuracy of the TOP assay result. Figure A.2 illustrates the PFAA recovery in three types of soils during the whole TOP procedure. Both PFCA (C3-C9) and PFSA (C4, C6, C7, and C8) showed acceptable recovery, ranging from 60.2% to 127.8%, during the soil sample preparation and TOP procedure. This indicates a minimal influence of adsorption to vials, tubes, and volatilization on PFAA losses during TOP. Given that the PFCA background levels from soil preparation and TOP procedures (procedural blank) and the PFCA background levels in soil matrixes could influence the determination of the PFCA yields, procedural blanks and soil matrix blanks (non-spiked soils) were submitted to the preparation and TOP procedure at the same time as the soil matrixes spiked with precursors. The procedural blanks

showed nondetectable levels of each PFCA, while the PFCA background levels in soil matrixes were deducted when calculating the PFCA molar yields resulting from the spiked precursors.

TOP assay performance – verification of oxidation yields

Implementation of the TOP assay to soil requires efficient extraction of PFAS of various polarities, some of which form strong interactions with soil, preceding sample cleanup and persulfate oxidation. **Method II** achieved satisfactory spike recoveries for 53 target PFAS (70-99%), except for FOSAA (62%) in one soil (Table A.6). The oxidation yields of 8 selected precursors (6:2 FTSA, 8:2 FTSA, 6:2 FTAB, 5:3 FTB, 5:1:2 FTB, FHxSA, PFHxSAm, and PFHxSAmS) were verified on N_2-dried extracts of three types of soils with different textures and organic matter content (See the soil properties and PFAS background in Table A.2). Figure A3 showed that n:2 fluorotelomers (6:2 FTAB, 6:2 FTSA, 8:2 FTSA) were completely consumed in both soils and ultra-pure water (as matrix-free control) during the TOP procedure (conversion ratio of 95.2-100%), resulting in the production of PFCAs (chain length ranging from C_3 to C_{n+1}), with the dominance of C_{n-1}, $C_{n-2,}$ and C_n PFCA. The total PFCA yields from these 6:2 FTAB, 6:2 FTSA, and 8:2 FTSA in the tested soils fell between 63.3-92.3 mol%, 74.2-98.1 mol%, 66.0-84.9 mol%, respectively.

The ECF-based C_6 precursors (FHxSA, PFHxSAm, and PFHxSAmS) achieved total PFCA yields of 68.1-69.8 mol%, 73.6-84.5 mol%, and 70.8-91.0 mol%, respectively, in the three soils (Figure A3). C_6 PFCA (PFHxA) was the major product and C_5 PFCA (PFPeA) was the minor product, which agrees with the production of C_8 PFOA (major product) and C_7 PFHpA (minor product) from ECF-based C_8 precursors observed by Martin et al. in ultra-pure water.[6] Though slightly biased-low, the PFCA yields in soils were consistent with those in HPLC-grade water tested in parallel, indicating acceptable oxidation efficiency. The low oxidative yield of FHxSA may be due to limited stability in the TOP aqueous medium or partial losses during the evaporation step. The PFCA yields were generally lowest for soil 2N, which contained more than 10% organic matter that may compete for hydroxyl radicals to render incomplete PFAS oxidation.[7] Given the satisfactory PFCA yields from six selected precursors (except 5:3 FTB and 5:1:2 FTB), the validated TOP assay procedures were applied to the 45 soil samples for estimating unidentified precursors and total PFAS equivalent.

Text A.5 TOP assay procedures for field soil and groundwater samples

Soil samples. The field soil samples were first extracted (MeOH with 0.4 M NaOH for 2 cycles followed by MeOH with 0.4 M HCl for 1 cycle), separately cleaned up on Envi-Carb cartridges, combined, and then the solvent extracts were evaporated to dryness. The dried soil extracts were subjected to a TOP procedure modified from Houtz and Sedlak's,[5] as described in the section "Method validation of total oxidizable precursor (TOP) assay for soils".

Groundwater samples. For groundwater samples, the TOP procedures described by Martin et al. were used.[6] In detail, a 1200-µL groundwater sample aliquot was added to a 2-mL centrifuge tube after centrifugation (5 min; 6000 rpm). Following the addition of 656 µL of 175 mM potassium persulfate and 57.4 µL of 5 M NaOH, the centrifuge tubes were placed in a water bath at 85 °C for 6 h. The samples were then removed from the bath and left to cool down to ambient temperature. The TOP medium was brought to pH ~8 with hydrochloric acid and amended with 100 µL of MeOH. After briefly vortexing the samples, a 70-µL aliquot of oxidized sample was added to a polypropylene tube, along with 140 µL of MeOH and 140 µL of a 6.25 ng/mL internal standard solution (prepared in MeOH). The sample was briefly vortexed, centrifuged (3 min; 6000 rpm), and a 200µL aliquot of sample was transferred to a 250-µL polypropylene HPLC vial. Internal calibration curves (i.e., solvent-based: 80:20 MeOH: HPLC-water v/v) were used for quantification purposes after verifying the lack of matrix effects in the presence of the methanol diluted TOP medium.[6] The procedure derived the same dilution factor of 8.4× as that of the samples analyzed without persulfate oxidation.

Text A.6 Instrumental analysis parameters and method performance

Details on UHPLC-MS operating parameters. The Dionex Ultimate 3000 LC was controlled via the Chromeleon 7.2 Software (Thermo Fisher Scientific, Waltham, MA, USA). A Thermo Hypersil Gold C18 column (100 mm x 2.1 mm; 1.9 µm particle size) thermostated at 40°C was used for analyte separation. A trap column (Thermo Hypercarb, 20 mm x 2.1 mm; 7 µm particle size) was positioned immediately after the aqueous and organic LC mobile phases mixing point but before the injector. The aqueous mobile phase (A) consisted of 0.1% HCOOH in HPLC-water

(v/v) and the organic mobile phase (B) of 0.1% HCOOH in acetonitrile (v/v). The injection volume was 10 μL or 15 μL (for TOP assays).

Chromatographic gradient elution conditions were as follows: gradual increase of B channel from 10 to 72.5% (0–7 min), and then from 72.5 to 100% (7–8.5 min). The 0:100 A: B ratio was held for 4 minutes (8.5–12.5 min), then returned to the 90:10 initial set up (12.5–12.6 min), kept constant for 2 minutes for re-equilibration (12.6–14.5 min). Before each injection, the injection needle and injection port were rinsed sequentially with i) an equal volumetric mixture of acetonitrile/methanol/isopropanol and ii) HPLC-water containing 0.1% HCOOH.

Analyte detection was performed using a Q-Exactive Orbitrap mass spectrometer controlled by the Xcalibur 4.0 software (Thermo Fisher Scientific, Waltham, MA, USA) in full scan mode and with t-MS^2 mode, with positive and negative heated electrospray ionization (fast polarity switching mode).[3, 8] Orbitrap parameters were set as follows: AGC target (maximum capacity in C-trap) was set at 3 x 10^6, maximum injection time at 50 ms, and resolution at 70,000 FWHM at *m/z* 200. The mass scan range was set at *m/z* 150–1000 (Full Scan MS mode).

Text A.7 Quality assurance/quality control

Replicate field/trip water blanks were performed (two for each FTA site), and consisted of DI water poured on-site in pre-cleaned HDPE bottles during the sampling campaigns. The blank samples were shipped together with the other field samples and all samples were processed together at the analytical facilities. Method (laboratory) blanks were also performed for both water and soil samples. The method blanks and field/trip blanks presented nondetectable levels of PFAS. Upon the characterization of low to moderate matrix effects (<±25%, listed in Table A.5), solvent-based calibration curves were used for both soil and groundwater samples. Analytes were quantified using inverse-weighted internal regression lines with determination coefficients (R^2) ranging from 0.9906 to 0.9999 and suitable accuracy (70.6-130.5%). After running the calibration curve, continued calibration verification standards (quality control CCV samples) were inserted every 10-15 samples during the LC-MS batch sequence. The mean accuracy of CCV standards (n = 5) ranged between 80-119% (Table A.10), within the 70-130% acceptance criterion set by EPA

methods.[9] As an additional control of precision, field duplicates for two soil samples and method triplicates for four groundwater samples were performed.

Text A.8 Rationales for associating quantification confidence levels

Quantification confidence levels associated with each of the detected PFASs in the samples from the field survey are shown in Table A.9. To attain the highest quantification confidence level, a certified native standard and a matching IS, or at least closely related ones, were used if available.[8] Identification confidence levels were assigned adapted from Schymanski's classification.[10]

Table A.1 Ion formula, theoretical and observed m/z, mass error, retention time (RT) and commercial sources of 53 native PFASs with available standards.

Analyte	Name	Ion Formula	Theoretical m/z	Observed m/z	Error (ppm)	RT (min)	Sources of standards
PFPrA	Perfluoropropionoic acid	$[C_3F_5O_2]^-$	162.98185	162.98169	-1.0	0.89	
PFBA	Perfluorobutanoic acid	$[C_4F_7O_2]^-$	212.97947	212.97906	-1.9	2.21	
PFPeA	Perfluoropentanoic acid	$[C_5F_9O_2]^-$	262.97669	262.97644	-1.0	3.33	
PFHxA	Perfluorohexanoic acid	$[C_6F_{11}O_2]^-$	312.97335	312.97336	0.0	4.15	
PFHpA	Perfluoroheptanoic acid	$[C_7F_{13}O_2]^-$	362.97013	362.97055	1.2	4.82	
PFOA	Perfluorooctanoic acid	$[C_8F_{15}O_2]^-$	412.96714	412.96701	-0.3	5.40	
PFNA	Perfluorononanoic acid	$[C_9F_{17}O_2]^-$	462.96414	462.96408	-0.1	5.96	
PFDA	Perfluorodecanoic acid	$[C_{10}F_{19}O_2]^-$	512.96066	512.96063	-0.1	6.50	
PFUnA	Perfluoroundecanoic acid	$[C_{11}F_{21}O_2]^-$	562.95865	562.95782	-1.5	7.00	
PFDoA	Perfluorododecanoic acid	$[C_{12}F_{23}O_2]^-$	612.95461	612.95477	0.3	7.52	PFAC-MXC from Wellington Laboratories (Guelph, ON, Canada)
PFTrDA	Perfluorotridecanoic acid	$[C_{13}F_{25}O_2]^-$	662.95041	662.95209	2.5	7.99	
PFTeDA	Perfluorotetradecanoic acid	$[C_{14}F_{27}O_2]^-$	712.94808	712.94928	1.7	8.45	
PFHxDA	Perfluorohexadecanoic acid	$[C_{16}F_{31}O_2]^-$	812.94292	812.94379	1.1	9.26	
PFOcDA	Perfluorooctadecanoic acid	$[C_{18}F_{35}O_2]$-	912.93394	912.93701	3.4	10.06	
PFPrS	Perfluoropropane sulfonate	$[C_3F_7SO_3]^-$	248.94564	248.94719	6.2	3.19	
PFBS	Perfluorobutane sulfonate	$[C_4F_9SO_3]^-$	298.94326	298.94351	0.8	4.15	
PFPeS	Perfluorohexane sulfonate	$[C_5F_{11}SO_3]^-$	348.93925	348.94052	3.6	4.90	
PFHxS	Perfluorohexane sulfonate	$[C_6F_{13}SO_3]^-$	398.93712	398.93719	0.2	5.52	
PFHpS	Perfluoroheptane sulfonate	$[C_7F_{15}SO_3]^-$	448.93286	448.93408	2.7	6.08	
PFOS	Perfluorooctane sulfonate	$[C_8F_{17}SO_3]^-$	498.93126	498.93008	-2.4	6.60	
PFNS	Perfluorononane sulfonate	$[C_9F_{19}SO_3]^-$	548.92647	548.92798	2.8	7.12	
PFDS	Perfluorodecane sulfonate	$[C_{10}F_{21}SO_3]^-$	598.92487	598.92474	-0.2	7.60	
PFDoS	Perfluorododecane sulfonate	$[C_{12}F_{25}SO_3]^-$	698.91689	698.91937	3.5	8.47	
PFECHS	Perfluoro-4-ethylcyclohexane	$[C_8F_{15}SO_3]^-$	460.93286	460.93585	6.5	6.02	
FBSA	Perfluorobutane sulfonamide	$[C_4F_9SO_2NH]^-$	297.95843	297.95938	3.2	5.21	
FHxSA	Perfluorohexane sulfonamide	$[C_6F_{13}SO_2NH]^-$	397.95204	397.95282	2.0	6.56	
FOSA	Perfluorooctane sulfonamide	$[C_8F_{17}SO_2NH]^-$	497.94631	497.94745	2.3	7.58	Wellington Laboratories (Guelph, ON, Canada)
MeFOSA	N-methyl-perfluorooctane	$[C_9F_{17}SO_2NH_3]^-$	511.96130	511.96326	3.8	8.18	
EtFOSA	N-ethyl-perfluorooctane	$[C_{10}F_{17}SO_2NH_5]^-$	525.97695	525.97882	3.6	8.41	
FOSAA	Perfluorooctane	$[C_{10}F_{17}SO_4NH_3]^-$	555.95113	555.95331	3.9	7.48	
MeFOSAA	N-methyl-perfluorooctane	$[C_{11}F_{17}SO_4NH_5]^-$	569.96678	569.96893	3.8	7.51	
EtFOSAA	N-ethyl-perfluorooctane	$[C_{12}F_{17}SO_4NH_7]^-$	583.98243	583.98511	4.6	8.20	
3:3 FTCA	3:3 fluorotelomer carboxylate	$[C_6F_7H_4O_2]^-$	241.00995	241.01096	4.2	4.94	
4:3 FTCA	4:3 fluorotelomer carboxylate	$[C_7F_9H_4O_2]^-$	291.00676	291.00836	5.5	5.69	DuPont USA
5:3 FTCA	5:3 fluorotelomer carboxylate	$[C_8F_{11}H_4O_2]^-$	341.00356	341.00516	4.7	6.31	
7:3 FTCA	7:3 fluorotelomer carboxylate	$[C_{10}F_{15}H_4O_2]^-$	440.99717	440.99921	4.6	7.39	
4:2 FTSA	4:2 fluorotelomer sulfonate	$[C_6F_9H_4SO_2]^-$	326.97374	326.97528	4.7	3.86	
6:2 FTSA	6:2 fluorotelomer sulfonate	$[C_8F_{13}H_4SO_2]^-$	426.96866	426.96902	0.8	5.11	
8:2 FTSA	8:2 fluorotelomer sulfonate	$[C_{10}F_{17}H_4SO_2]^-$	526.96097	526.96289	3.6	6.17	
10:2 FTSA	10:2 fluorotelomer sulfonate	$[C_{12}F_{21}H_4SO_3]^-$	626.95458	626.95715	4.1	7.18	Wellington Laboratories (Guelph, ON, Canada)
6:2 FTUA	6:2 fluorotelomer unsaturated	$[C_8F_{12}H_2O_2]^-$	356.97849	356.98026	5.0	6.46	
8:2 FTUA	8:2 fluorotelomer unsaturated	$[C_{10}F_{16}H_2O_2]^-$	456.97210	456.97412	4.4	7.54	
10:2 FTUA	10:2 fluorotelomer unsaturated	$[C_{12}F_{20}H_2O_2]^-$	556.96571	556.96808	4.3	8.39	
6:2 FTAB	6:2 fluorotelomer	$[C_{15}F_{13}H_{20}N_2SO_4]^+$	571.09362	571.09387	0.4	5.37	
5:3 FTB	5:3 fluorotelomer betaine	$[C_{12}F_{11}H_{15}NO_2]^+$	414.09271	414.09247	-0.6	4.47	
5:1:2 FTB	5:1:2 fluorotelomer betaine	$[C_{12}F_{12}H_{14}NO_2]^+$	432.08329	432.08324	-0.1	4.58	
PFHxSAm(AmPr-FHxSA)	Perfluorohexane sulfonamidoalkyl amine	$[C_{11}H_{14}F_{13}N_2O_2S]^+$	485.05684	485.05692	0.2	5.28	Beijing Surfactant

PFOSAm(AmPr-FOSA)	Perfluorooctane sulfonamidoalkyl amine	$[C_{13}H_{14}F_{17}N_2O_2S]^+$	585.04990	585.05054	1.1	6.32	Institute (Peking, China).
PFHxSAmS(N-TAmP-FHxSA)	Perfluorohexane sulfonamidoalkyl ammonium	$[C_{12}H_{16}F_{13}N_2O_2S]^+$	499.07249	499.07251	0.0	5.34	
PFOSAmS(N-TAmP-FOSA)	Perfluorooctane sulfonamidoalkyl ammonium	$[C_{14}H_{16}F_{17}N_2O_2S]^+$	599.06555	599.06610	0.9	6.38	
PFOSNO(N-OxAmP-FOSA)	Perfluorooctane sulfonamidoalkyl amine oxide	$[C_{13}H_{14}F_{17}N_2O_3S]^+$	601.04482	601.04510	0.5	6.36	
PFOANO(N-OxAmP-FOAd)	Perfluorooctane amidoalkyl amine oxide	$[C_{13}H_{14}F_{15}N_2O_2]^+$	515.08103	515.08197	1.8	5.49	
PFOSB(N-CMAmP-FOSA)	Perfluorooctane sulfonamidoalkyl betaine	$[C_{15}H_{16}F_{17}N_2O_4S]^+$	643.05538	643.05591	0.8	6.32	
PFOAB(N-CMAmP-FOAd)	Perfluorooctane amidoalkyl betaine	$[C_{15}H_{16}F_{15}N_2O_3]^+$	557.09159	557.09229	1.3	5.39	

Table A.2 The acronym, full name, theoretical and observed m/z, RT and commercial sources of isotope-labeled IS. (a) Surrogate IS., (b) Injection IS.

(a)

Acronym	Acronym	Full Name	M^+, $[M+H]^+$ or $[M-H]^-$	Theoretical m/z	Observed m/z	Mass erro	RT, min	Ionization mode	Commercial Sources
$^{13}C_4$-PFBA	MPFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	$[^{13}C_4F_7O_2]^-$	216.99177	216.99271	4.3	2.21	ESI–	
$^{13}C_5$-PFPeA	M5PFPeA	Perfluoro-n-[$^{13}C_5$]pentanoic acid	$[^{13}C_5F_9O_2]^-$	267.99345	267.99338	-0.3	3.33	ESI–	
$^{13}C_5$-PFHxA	M5PFHxA	Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$[^{13}C_5CF_{11}0_2]^-$	317.99046	317.99026	-0.6	4.15	ESI–	
$^{13}C_4$-PFHpA	M4PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$[^{13}C_4C_3F_{13}0_2]^-$	366.98249	366.98407	4.3	4.82	ESI–	
$^{13}C_8$-PFOA	M8PFOA	Perfluoro-n-[$^{13}C_8$]octanoic acid	$[^{13}C_8F_{15}O_2]^-$	420.99272	420.99429	3.7	5.40	ESI–	
$^{13}C_9$-PFNA	M9PFNA	Perfluoro-n-[$^{13}C_9$]nonanoic acid	$[^{13}C_9F_{17}O_2]^-$	471.99288	471.99435	3.1	5.96	ESI–	
$^{13}C_6$-PFDA	M6PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$[^{13}C_6C_4F_{19}O_2]^-$	518.97962	518.98120	3.0	6.50	ESI–	
$^{13}C_7$-PFUnA	M7PFUdA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$[^{13}C_7C_4F_{21}O_2]^-$	569.97978	569.98175	3.5	7.00	ESI–	
$^{13}C_2$-PFDoA	MPFDoA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$[^{13}C_2C_{10}F_{23}O_2]^-$	614.95981	614.96191	3.4	7.52	ESI–	
$^{13}C_2$-PFTeDA	M2PFTeDA	Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic	$[^{13}C_2C_{12}F_{27}O_2]^-$	714.95342	714.95636	4.1	8.45	ESI–	Wellington Laboratories (Guelph, ON, Canada)
$^{13}C_3$-PFBS	M3PFBS	Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	$[^{13}C_3C_1F_9SO_3]^-$	301.95251	301.95352	3.3	4.15	ESI–	
$^{13}C_3$-PFHxS	M3PFHxS	Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	$[^{13}C_3C_3F_{13}SO_3]^-$	401.94612	401.94727	2.9	5.52	ESI–	
$^{13}C_8$-PFOS	M8PFOS	Perfluoro-1-[$^{13}C_8$]octanesulfonate	$[^{13}C_8F_{17}SO_3]^-$	506.95641	506.95837	3.9	6.60	ESI–	
$^{13}C_2$-6:2 FtS	M2-6:2 FTSA	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]octanesulfonate	$[^{13}C_2C_8F_{16}HO_2]^-$	428.97537	428.97568	0.7	5.11	ESI–	
$^{13}C_2$-8:2 FtS	M2-8:2FTSA	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]decanesulfonate	$[C_{10}F_{17}D_5NSO_2]^-$	528.96898	528.96960	1.2	6.17	ESI–	
$^{13}C_2$-6:2 FTUA	M6:2 FTUA	2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoic acid	$[^{13}C_2C_8F_{17}H_4SO_3]^-$	358.98520	358.98685	4.6	6.46	ESI–	
$^{13}C_2$-8:2 FTUA	M8:2 FTUA	2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	$[^{13}C_2C_6F_{12}HO_2]^-$	458.97881	458.98083	4.4	7.54	ESI–	
$^{13}C_8$-FOSA	M8FOSA-I	Perfluoro-1-[$^{13}C_8$]octane	$[^{13}C_8F_{17}NHSO_2]^-$	505.97249	505.97430	3.6	7.58	ESI–	
d_3-N-MeFOSA	d-N-MeFOSA-M	N-methyl-d_3-perfluoro-1-	$[C_9F_{17}NSO_2D_3]^-$	514.98013	514.98187	3.4	8.18	ESI–	
d_5-N-EtFOSA	d-N-EtFOSA-M	N-ethyl-d5-perfluoro-1-	$[C_{10}F_{17}NSO_2D_5]^-$	531.00830	531.01001	3.2	8.41	ESI–	
d_3-N-MeFOSAA	d3-N-MeFOSA	N-methyl-d_3-perfluoro-1-	$[C_{11}F_{17}NSO_4D_3H_2]^-$	572.98561	572.98798	4.1	7.94	ESI–	
d_5-N-EtFOSAA	d5-N-EtFOSAA	N-ethyl-d5-perfluoro-1-	$[C_{12}F_{17}NSO_4D_5H_2]^-$	589.01437	589.01599	2.8	8.20	ESI–	
PFOAAmS(N-TAmP-FOAd)	PFOAAmS	Perfluorooctane amidoalkyl ammonium	$[C_{14}H_{16}F_{15}N_2O]^+$	513.10176	513.10254	1.5	5.38	ESI+	Beijing Surfactant Institute (Peking,

(b)

Acronym	Acronym	Full Name	M^+, $[M+H]^+$ or $[M-H]^-$	Theoretical m/z	Observed m/z	Mass error, ppm	RT, min	Ionization mode	Commercial Sources
$^{13}C_3$-PFBA	M3PFBA	Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic	$[^{13}C_3CF_7O_2]^-$	215.98926	215.98917	-0.4	2.21	ESI–	Wellington Laboratories (Guelph, ON, Canada)
$^{13}C_2$-PFOA	M2PFOA	Perfluoro-n-[1,2-13C2]octanoic	$[^{13}C_2C_6F_{15}O_2]^-$	414.97314	414.97385	1.7	5.40	ESI–	
$^{13}C_2$-PFDA	MPFDA	Perfluoro-n-[1,2-13C2]decanoic	$[^{13}C_2C_8F_{19}O_2]^-$	514.96675	514.96729	1.0	6.50	ESI–	
$^{13}C_4$-PFOS	MPFOS	Sodium perfluoro-1-	$[^{13}C_4C_4F_{17}SO_3]^-$	502.94364	502.94379	0.3	6.60	ESI–	

Table A.3 Detailed information about field soil and groundwater samples.

(a) Soil sample list.

Sample Name	Location	Site Name	Collection Time
MW16-01 MW15-01 MW15-02 SS-15* SS-16 SS-17	Upgradient	Site #1, Ontario, Canada	Sept-Oct. 2016
SS-01 SS-02* SS-03 SS-04 SS-05 SS-06 SS-07 SS-08 SS-09 SS-10	Vicinity of FTA/FFTA		
SS-18 SS-19 SS-20 SS-21 SS-22 SS-23 SS-24	Downgradient		
SS16-1 SS16-2 SS16-3 SS16-4	Vicinity of FTA area	Site #2, Ontario, Canada	Nov. 2016
SS16-5 SS16-6 SS16-7 SS16-8 SS16-9 SS16-10 SS16-22	Vicinity of FTA area	Site #2, Ontario, Canada	Nov. 2016
2s-CF1 4s-CF1-A 5s-CF2 6s-CF1 7s-CF1-B	Vicinity of FTA area	Site #3, Quebec, Canada	Feb. 2017
SJ-01 SJ-02 SJ-03 SJ-04 SJ-05 SJ-06	Vicinity of FTA area	Site #4, Newfoundland, Canada	Sep. 2016

Note: The red star (*) represents the collection, preparation and analysis of a duplicate of the field soil sample for PFAS. For duplicate soil samples, the standard deviations for the concentration of each PFAS were lower than 20%, therefore, the average value for each PFAS concentration was considered as the individual PFAS concentration.

(b) Groundwater sample list.

Sample Name	The depths to groundwater table (m bgs)	Location	Site Name	Collection Time
MW16-01	2.58	Upgradient	Site #1, Ontario, Canada	Sept-Oct. 2016
MW15-01	2.54			
MW15-02	3.18			
07MW02S	3.37	Vicinity of FTA/FFTA area		
TCF	3.23			
P5	3.29			
MW 0902B	2.88			
MW13-13	3.20			
11-01D	8.68	Downgradient		
11-02D	8.89			
11-06S	1.88			
MW13-24D	3.63			
11-04	3.55			
11-05	4.51			
MW14-2	2.38	Upgradient	Site #2, Ontario, Canada	Sept-Oct. 2016
MW12-2B	2.06			
MW 12-4	1.76			
MW105	1.90	Vicinity of FTA area		
MW108	1.84			
L14	1.76			
MW101	2.28			
MW110	2.29			
MW212	1.12			
MW213	1.18			
MW215	2.07			
MW216	1.30			
MW207	1.68			
MW208	1.13			
MW210	1.42			
MW209	1.36	Downgradient		
MW15-1	NA*			
MW15-2	NA*			
MW304	1.67			
MW14-6	1.39			
MW12-19	0.78			
PO 15 8R	1.71	Vicinity of FTA area	Site #3, Quebec, Canada	Sept-Oct. 2016
PO 15 8S	1.65			
17 PO 1R	0.48			
17 PO 1S	0.53			
17 PO 2R	1.24			
17 PO 2S	1.28			
17 PO 4R	0.62			
17 PO 4S	0.65			
17 PO 5R	0.86			
17 PO 5S	0.83			
17 PO 6R	1.16			
17 PO 6S	1.13			
17 PO 7R	0.58			
17 PO 7S	0.59			
M269	0.36			
M412R	0.44			
PO 15 6R	0.54			
PO 15 6S	0.49			
MW16	NA*	Upgradient	Site #4, Newfoundland, Canada	Sept-Oct. 2016
FTA MW8	NA*	Vicinity of FTA area		
MW 0502	NA*			
MW06	NA*			
Well 57	NA*			
Well 662	NA*			
JW0606	NA*	Downgradient		
JW 06-10	NA*			
DS2MW8	NA*			

Note: The data for depths to groundwater table at site #1 was from 2019 data, while those for site #2 was from 2014.NA means that the groundwater table levels of monitoring wells at site #4 were not available in engineering reports.

Table A.4 The property and PFAS background levels of soils used for spike recovery test, matrix effect assessment, and TOP method validation purposes.

(a) Soil property.

Name	Type	Sampling location	% sand	% silt	% clay	Textural class	% OM	pH
Soil #1R	Background	Chaudière watershed, QC, Canada	59.2	32.2	8.6	Sandy loam	3.1	5
Soil #2N	Background	Nuns' Island, QC, Canada	51.2	36.2	12.6	Loam	12.6	4.5
Soil #3F	Background	Parc Elgar, QC, Canada	47.2	40.0	12.8	Loam	4.0	5.2

(b) The PFAS background levels. Each soil matrix was prepared and analyzed in triplicate using the same extraction (MeOH with 100 mM ammonium acetate) and cleanup method described for field soil samples.

PFAS analyte	**Soil from Riverine Chaudière (1R soil, ng/d dw)**	**Soil from Nun Island (2N soil, µg/kg dw)**	**Soil from Parc Elgar (3F soil, µg/kg dw)**
PFPrA	ND	ND	0.80±0.10
PFBA	0.20±0.08	1.43±0.33	0.71±0.22
PFPeA	0.26±0.01	0.29±0.01	0.26±0.05
PFHxA	0.11±0.06	0.37±0.02	0.27±0.01
PFHpA	0.17±0.01	0.31±0.06	0.18±0.01
PFOA	0.16±0.01	0.43±0.00	0.38±0.04
PFNA	0.14±0.03	0.15±0.02	0.15±0.01
PFDA	0.08±0.02	0.05±0.07	0.04±0.06
PFUnA	0.11±0.01	0.06±0.00	ND
PFDoA	ND	ND	ND
PFTrA	ND	ND	ND
PFTeDA	ND	ND	ND
PFHxDA	0.07±0.04	0.06±0.04	0.05±0.01
PFPrS	ND	ND	ND
PFBS	0.07±0.01	0.05±0.00	0.13±0.04
PFPeS	ND.	ND.	ND.
PFHxS	0.01±0.02	ND	0.05±0.00
PFHpS	ND	ND	ND
PFOS	0.50±0.12	0.79±0.03	1.01±0.04
PFNS	ND	ND	ND
PFDS	0.05±0.01	0.99±0.08	0.02±0.00
PFDoS	ND	ND	ND
3:3 FTCA	ND	ND	ND
4:3 FTCA	ND	ND	ND
5:3 FTCA	ND	ND	ND
7:3 FTCA	ND	ND	ND
6:2 FTUA	ND	ND	ND
8:2 FTUA	ND	ND	ND
10:2 FTUA	ND	ND	ND
42 FTSA	ND	ND	ND
6:2FTSA	0.13±0.03	ND	ND
8:2 FTSA	0.02±0.03	ND	ND
10:2 FTSA	ND	ND	ND
6:2 FTAB	ND	ND	ND
5:3 FTB	ND	ND	ND
5:1:2 FTB	ND	ND	ND

FBSA	ND	ND	ND
FHXSA	ND	ND	ND
FOSA	ND	ND	ND
FOSAA	ND	ND	ND
MeFOSAA	2.05±0.23	1.86±0.10	2.14±0.03
EtFOSA	ND	ND	ND
MeFOSA	ND	ND	ND
EtFOSAA	ND	ND	ND
PFHxSAm(AmPr-FHxSA)	ND	ND	ND
PFHxSAmS(N-TAmP-FHxSA)	ND	ND	ND
PFOAB(N-CMAmP-FOAd)	ND	ND	ND
PFOANO(N-OxAmP-FOAd)	ND	ND	ND
PFOSAm(AmPr-FOSA)	ND	ND	ND
PFOSB(N-CMAmP-FOSA)	ND	ND	ND
PFOSNO(N-OxAmP-FOSA)	ND	ND	ND
PFOSAmS(N-TAmP-FOSA)	ND	ND	0.06±0.00
PFECHS	ND	ND	ND

Table A.5 The spike recovery (mean ± SE, %) and matrix effects (mean ± SE, %) of 53 quantitative PFAS analytes in three types of soil matrixes.

(a) Spike recovery of 53 quantitative PFAS analytes in three types of soil matrixes. These soils were extracted by MeOH with the 100mM AA method.

PFAS analyte	**1R soil (%)**	**2N soil(%)**	**3F soil (%)**
PFPrA	87 ± 14	96 ± 7	85 ± 15
PFBA	81 ± 5	95 ± 17	92 ± 9
PFPeA	81 ± 9	90 ± 10	90 ± 7
PFHxA	81 ± 9	88 ± 9	90 ± 7
PFHpA	79 ± 8	87 ± 10	89 ± 6
PFOA	80 ± 7	89 ± 10	87 ± 6
PFNA	79 ± 8	91 ± 9	87 ± 6
PFDA	80 ± 8	87 ± 10	86 ± 7
PFUnA	80 ± 8	90 ± 10	88 ± 6
PFDoA	78 ± 6	89 ± 10	88 ± 8
PFTrDA	79 ± 6	90 ± 7	85 ± 6
PFTeDA	80 ± 8	88 ± 10	86 ± 7
PFHxDA	68 ± 5	68 ± 10	67 ± 12
PFPrS	78 ± 7	89 ± 10	87 ± 7
PFBS	81 ± 9	93 ± 10	88 ± 6
PFPeS	78 ± 7	89 ± 10	87 ± 7
PFHxS	79 ± 8	90 ± 10	87 ± 7
PFHpS	78 ± 8	87 ± 9	89 ± 7
PFOS	80 ± 7	91 ± 10	88 ± 7
PFNS	80 ± 8	87 ± 8	88 ± 8
PFDS	79 ± 8	89 ± 8	87 ± 8
PFDoS	78 ± 7	85 ± 9	88 ± 10
4:2 FTSA	83 ± 6	94 ± 11	87 ± 7
6:2 FTSA	81 ± 7	92 ± 11	83 ± 6
8:2FTSA	83 ± 4	90 ± 11	87 ± 6
10:2 FTSA	81 ± 8	88 ± 8	87 ± 7
3:3 FTCA	73 ± 7	85 ± 12	80 ± 7
4:3 FTCA	76 ± 9	85 ± 7	83 ± 4
5:3 FTCA	79 ± 10	87 ± 6	79 ± 8
7:3 FTCA	77 ± 10	89 ± 4	79 ± 8
6:2 FTUA	80 ± 7	89 ± 10	86 ± 8
8:2 FTUA	81 ± 8	91 ± 9	85 ± 7
10:2 FTUA	80 ± 8	86 ± 11	87 ± 8
6:2 FTAB	73 ± 5	70 ± 13	56 ± 5

5:3 FTB	93±10	86±15	102±5
5:1:2 FTB	100±8	88±11	104±2
FBSA	78 ± 9	86 ± 9	82 ± 7
FHxSA	77 ± 8	86 ± 11	86 ± 6
FOSA	77 ± 8	86 ± 10	86 ± 8
FOSAA	70 ± 7	78 ± 8	62 ± 5
MeFOSAA	75 ± 11	93 ± 12	81 ± 8
EtFOSAA	75 ± 10	94 ± 15	80 ± 5
MeFOSA	63 ± 6	67 ± 7	79 ± 9
EtFOSA	62 ± 5	68 ± 8	79 ± 9
PFOAB	78 ± 5	75 ± 5	68 ± 4
PFOSB	72 ± 5	75 ± 11	68 ± 6
PFOANO	81 ± 6	99 ± 14	82 ± 7
PFOSNO	75 ± 6	91 ± 13	90 ± 9
PFOSAm	73 ± 6	91 ± 15	86 ± 5
PFHxSAm	80 ± 4	97 ± 13	84 ± 5
PFOSAmS	73 ± 5	87 ± 15	74 ± 4
PFHxSAmS	82 ± 5	88 ± 10	71 ± 5
PFECHS	78 ± 8	89 ± 11	88 ± 7

(b) Matrix effects (mean ± SE, %) of 53 quantitative PFAS in soils when a solvent-based calibration curve was used.

PFAS analyte	**Matrix effects in different soil matrixes (Mean ± SE, %)**		
	1R soil	2N soil	3F soil
PFBA	16 ± 2	1 ± 13	9 ± 6
PFPeA	8 ± 3	-5 ± 11	6 ± 3
PFHxA	9 ± 3	-3 ± 13	9 ± 5
PFHpA	16 ± 2	1 ± 16	14 ± 5
PFOA	11 ± 2	-1 ± 14	14 ± 4
PFNA	9 ± 3	-4 ± 12	11 ± 4
PFDA	7 ± 2	-2 ± 13	10 ± 5
PFUnA	6 ± 3	-6 ± 13	8 ± 3
PFDoA	8 ± 3	-5 ± 11	8 ± 3
PFTrDA	6 ± 2	-9 ± 10	7 ± 4
PFTeDA	8 ± 3	-7 ± 10	7 ± 2
PFHxDA	7 ± 2	-5 ± 12	8 ± 4
PFPrS	12 ± 2	3 ± 12	12 ± 1
PFBS	9 ± 7	-5 ± 14	11 ± 7
PFPeS	11 ± 1	-3 ± 14	12 ± 6
PFHxS	9 ± 1	-5 ± 12	10 ± 4
PFHpS	8 ± 1	-4 ± 13	8 ± 5
PFOS	14 ± 4	6 ± 17	21 ± 4
PFNS	10 ± 2	-1 ± 12	11 ± 3
PFDS	10 ± 3	-1 ± 12	12 ± 4
PFDoS	10 ± 3	1 ± 10	12 ± 3
4:2 FTSA	10 ± 7	-5 ± 13	11 ± 7
6:2 FTSA	26 ± 5	10 ± 13	22 ± 20
8:2 FTSA	9 ± 3	-4 ± 12	8 ± 5
10:2 FTSA	9 ± 4	-2 ± 12	9 ± 4
3:3 FTCA	-1 ± 2	-7 ± 12	6 ± 3
4:3 FTCA	7 ± 6	-6 ± 14	7 ± 9
5:3 FTCA	4 ± 7	-7 ± 12	8 ± 8
7:3 FTCA	4 ± 7	-8 ± 12	6 ± 5
6:2 FTUA	10 ± 3	-2 ± 12	10 ± 2
8:2 FTUA	10 ± 2	-4 ± 12	11 ± 4
10:2 FTUA	12 ± 3	1 ± 15	12 ± 2
6:2 FTAB	16 ± 4	1 ± 9	21 ± 14
5:3 FTB	-22±5	-21±3	-24±7
5:1:2 FTB	-34±4	-19±3	-19±5

PFOAB	10 ± 1	-2 ± 13	11 ± 3
PFOANO	10 ± 1	-6 ± 11	13 ± 1
PFHxSAm	-8 ± 12	9 ± 2	-13 ± 5
PFHxSAmS	-14 ± 10	11 ± 4	-9 ± 6
PFECHS	7 ± 0	-5 ± 13	8 ± 4
PFOSAM	22 ± 7	1 ± 14	11 ± 2
PFOSB	19 ± 5	1 ± 19	12 ± 2
PFOSNO	17 ± 5	0 ± 12	10 ± 2
PFOSAmS	23 ± 8	0 ± 15	14 ± 1
FBSA	11 ± 9	-8 ± 11	11 ± 7
FHxSA	11 ± 6	-5 ± 12	9 ± 5
FOSA	10 ± 3	-3 ± 12	11 ± 4
FOSAA	14 ± 1	-2 ± 11	10 ± 2
MeFOSAA	8 ± 2	-12 ± 18	7 ± 2
MeFOSA	7 ± 5	-5 ± 9	10 ± 3
EtFOSA	9 ± 3	-3 ± 13	9 ± 5
EtFOSAA	3 ± 4	-16 ± 8	4 ± 5

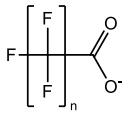
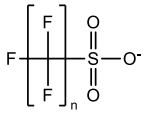
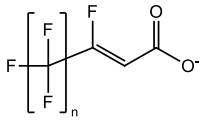
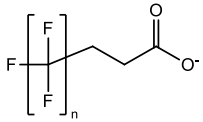
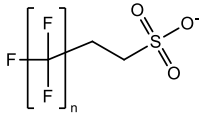
Table A.6 The spike recoveries (mean±SE, %) of 53 quantitative PFAS in three types of soils with the new exhaustive extraction method.

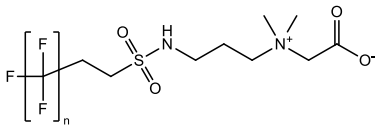
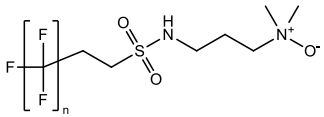
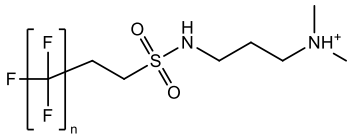
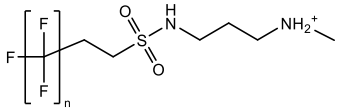
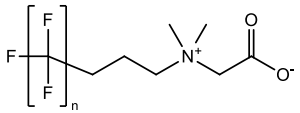
These soils were extracted using the comprehensive method (extraction by methanol with 400 mM NaOH for two cycles followed by methanol with 400 mM HCl for one cycle). Absolute recoveries lower than 60% are highlighted in red font. The low recoveries of FTUCAs are probably due to a reaction with methanol in the presence of a base to form methoxy-substituted unsaturated telomer acids.[12]

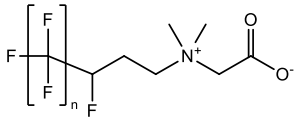
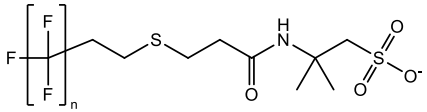
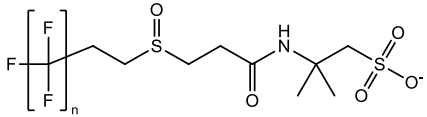
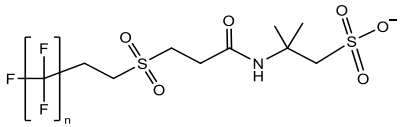
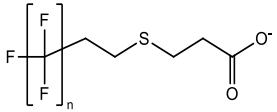
PFAS analyte	1R soil	2N soil	3F soil
PFPrA	104±32	95±23	112±5
PFBA	98±5	101±4	122±21
PFPeA	99±1	95±4	111±5
PFHxA	91±8	93±5	117±2
PFHpA	106±6	92±5	112±4
PFOA	116±8	92±5	116±2
PFNA	103±7	93±2	112±1
PFDA	106±4	92±2	122±1
PFUnA	100±5	98±4	115±2
PFDoA	110±5	93±3	113±1
PFTrDA	100±9	95±2	111±2
PFTeDA	107±6	94±2	113±1
PFHxDA	100±4	86±2	103±1
PFPrS	106±9	84±2	111±3
PFBS	103±7	91±1	116±1
PFPeS	108±7	93±0	115±2
PFHxS	103±9	87±2	111±1
PFHpS	102±7	93±2	112±3
PFOS	100±4	88±7	121±3
PFNS	105±12	93±1	114±3
PFDS	104±5	92±2	109±0
PFDoS	101±6	90±5	112±4
PFECHS	103±9	85±2	112±0
42 FTSA	107±11	120±0	113±16
62 FTSA	112±15	125±5	122±9
82 FTSA	106±8	120±9	129±12
10:2 FTSA	104±9	127±9	115±8

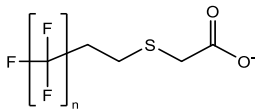
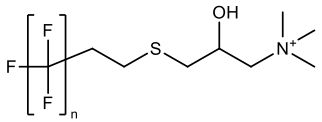
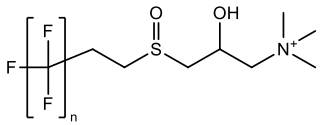
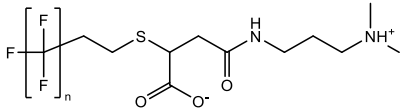
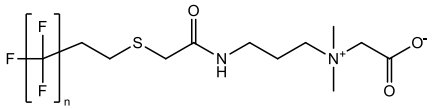
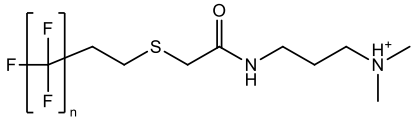
3:3 FTCA	110±18	86±4	127±9
4:3 FTCA	107±1	41±2	116±6
5:3 FTCA	96±6	108±7	117±0
7:3 FTCA	109±9	100±8	110±8
6:2 FTUA	24±3	76±1	39±1
8:2 FTUA	27±1	40±3	41±2
10:2 FTUA	30±5	63±4	44±2
6:2 FTAB	99±6	84±5	103±3
5:3 FTB	98±8	90±5	110±7
5:1:2 FTB	77±1	98±0	91±12
FBSA	110±7	102±2	117±1
FHxSA	107±4	107±1	120±5
FOSA	107±8	105±1	114±1
FOSAA	53±6	40±6	55±5
MeFOSA	102±18	76±12	125±0
EtFOSA	98±36	104±3	124±11
MeFOSAA	132±8	82±22	144±11
EtFOSAA	20±28	76±18	89±20
PFHxSAm	108±7	95±19	102±7
PFOSAm	109±6	66±11	107±3
PFHxSAmS	108±10	95±11	108±4
PFOSAmS	119±8	75±10	102±0
PFOAB	94±14	89±32	92±10
PFOSB	115±11	79±34	104±0
PFOANO	101±10	73±3	96±3
PFOSNO	107±10	71±5	96±2

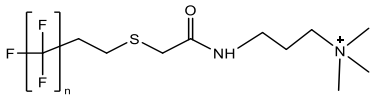
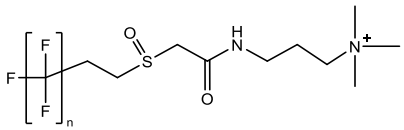
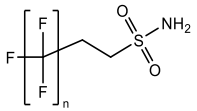
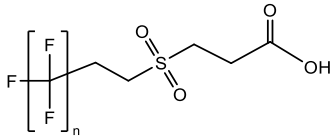
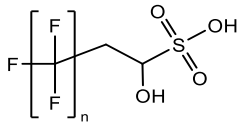
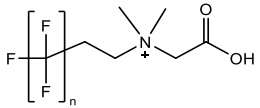
Table A.7 PFAS analyte list (target and suspect-screening) for field soil and groundwater samples.

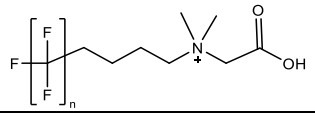
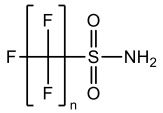
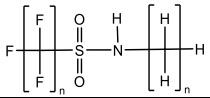
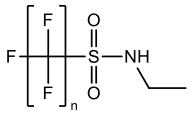
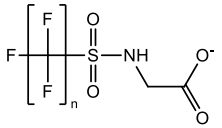
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
PFCA				
1	PFCA 	2-13,15	[11-13]	Perfluoroalkyl carboxylic acid
PFSA				
2	PFSA 	2-10	[11-14]	Perfluoroalkyl sulfonic acid
Fluorotelomer (FT)-derived compounds				
3	n:2 FTUA 	6, 8, 10	[12]	n:2 fluorotelomer unsaturated acid
4	n:3 FTCA 	3-11	[12]	n:3 fluorotelomer carboxylic acid
5	n:2 FTSA 	4, 6, 8, 10, 12, 14	[7, 11, 15]	n:2 fluorotelomer sulfonic acid

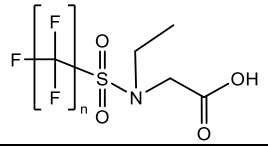
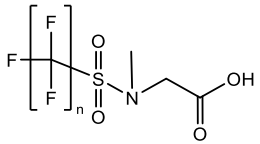
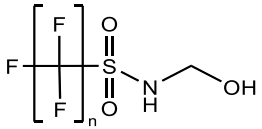
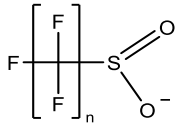
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
6	n:2 FTAB 	4, 6, 8, 10, 12, 14,16	7, 11, 14, 16	n:2 fluorotelomer sulfonamide betaine
7	n:2 FTNO 	6, 8, 10	8, 17	n:2 fluorotelomer sulfonamide amine oxide
8	n:2 FTA (or FTAA, or FtSaAM, or M4) 	4, 6, 8, 10, 12	3, 7, 11, 14, 16	n:2 fluorotelomer sulfonamide amine
9	n:2 demethyl-FTA 	6, 8, 10, 12	3	n:2 demethyl-fluorotelomer sulfonamide amine
10	n:3 FTB 	5, 7, 9, 11, 13, 15	7, 11, 14, 16	n:3 fluorotelomer betaine
11	n:1:2 FTB	5, 7, 9, 11, 13, 15	7, 11, 14, 16	n:1:2 fluorotelomer betaine

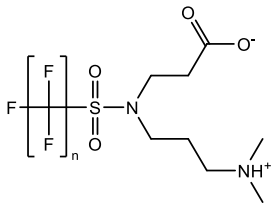
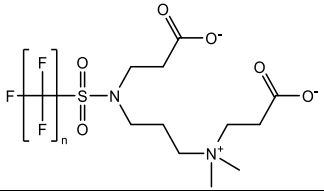
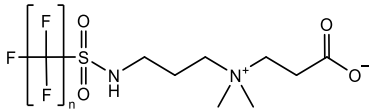
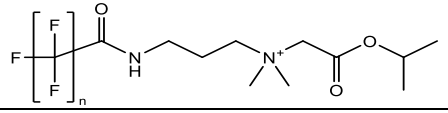
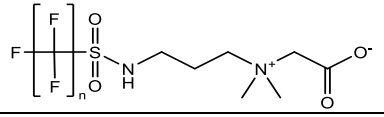
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
12	n:2 FTSAS 	2, 4, 6, 8, 10, 12, 14	7, 11, 14-16, 18	n:2 fluorotelomermercaptoalkylamido sulfonate
13	n:2 FTSAS-SO (or n:2 FTSAS-sulfoxide) 	4, 6, 8, 10, 12	14-16, 19	n:2 fluorotelomermercaptoalkylamido sulfonate-sulfoxide
14	n:2 FTSAS-SO_2 (or n:2 FASO2PA-MePS) 	4, 6, 8, 10, 12	20	n:2 fluorotelomer mercaptoalkylamido sulfonate sulfone or n:2 tridecafluoroalkyl sulfonyl (SO2) propanoamido-methylpropylsulfonate
15	n:2:2 FTSC (or n:2 FTS-C_2H_4-COOH) 	4, 6, 8, 10, 12, 14	3, 12, 16	n:2:2 fluorotelomer thioether propanoate (n:2:2 fluorotelomer mercaptoalkyl carboxylate)
16	n:2:1 FTSC (or n:2 FTS-CH_2-COOH)	4, 6, 8,10, 12, 14	3, 12, 16	n:2:1 fluorotelomer thioether ethanoate (n:2:1 fluorotelomer mercaptoalkyl carboxylate)

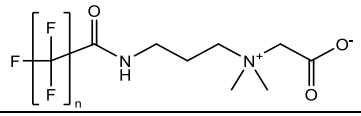
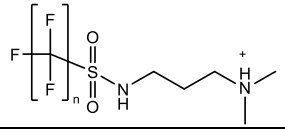
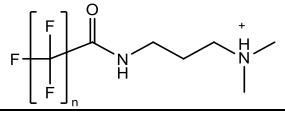
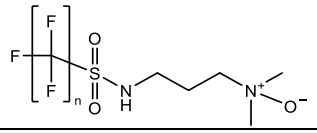
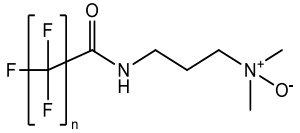
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
17	n:2 FTSHA 	4, 6, 8, 10, 12	3, 7, 11, 14, 16	n:2 fluorotelomer thiohydroxyammonium
18	n:2 FTSHA-SO (or n:2 FTSHA-sulfoxide) 	4, 6, 8, 10	12, 16	n:2 fluorotelomer thiohydroxyammonium-sulfoxide
19	n:2 FTSAAC 	6, 8, 10	3, 14	n:2 fluorotelomer thioether amino carboxylic acids (n:2 fluorotelomer thioalkylamido amine carboxylate)
20	n:2 FTSAB 	4, 6, 8, 10, 12, 14	7, 11, 14, 16	n:2 fluorotelomer sulfonamide betaines
21	n:2 FTSAA 	4, 6, 8, 10, 12, 14	16	n:2 fluorotelomer thio alkylamine

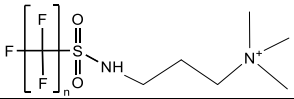
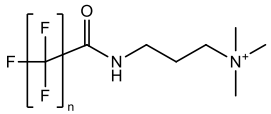
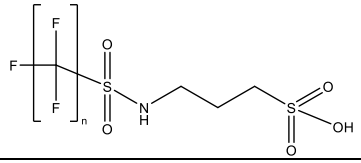
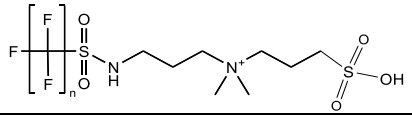
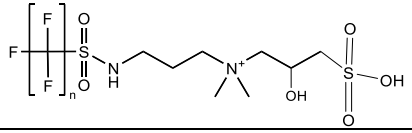
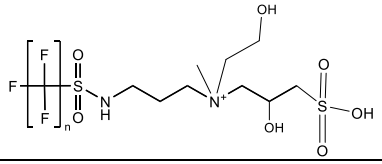
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
22	n:2 FTSAAmS or n:2 FTStrA 	2, 4, 6, 8, 10, 12, 14	16	n:2 fluorotelomer thio alkylamido ammonium or n:2 fluorotelomer thio trialkylamine
23	n:2 FTStrA-SO (or n:2 FTSoAAmS) 	2, 4, 6, 8, 10, 12, 14	16	n:2 fluorotelomer sulfinyl alkylamido ammonium or n:2 fluorotelomer thio trialkylamine sulfoxide
24	n:2 FTSAm (or n:2 FASA) 	6, 8, 10, 12, 14	3, 21	n:2 fluorotelomer sulfonamide
25	n:2 FTSO$_2$PA 	4, 6, 8	22	n:2 fluorotelomersulfonyl(O$_2$) propanoic acid
26	1HO-n:2 FTS 	4-8	22	1-hydroxy(HO)-n:2 fluorotelomer Sulfonate
27	CMAmEt-FA (or n:2 FTB) 	6, 8,10	22	Carboxymethyldimethylammonioethyl- Perfluoroalkane

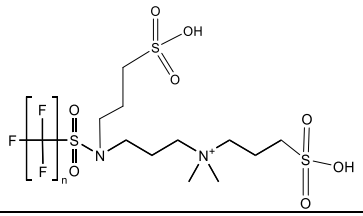
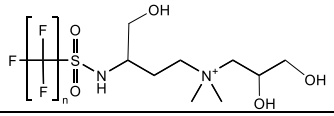
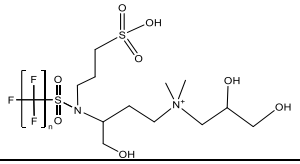
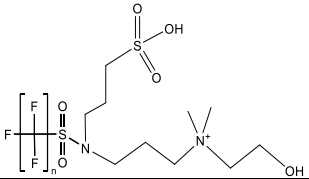
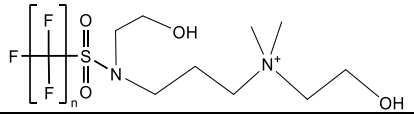
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
28	CmAmB-FA (or n:4 FTB) 	4, 6, 8	22	Carboxymethyldimethylammoniobutyl-Perfluoroalkane
ECF-derived sulfonamides and amides				
29	FASA 	3-10	12, 20, 22	Perfluoroalkane sulfonamide
30	MeFASA 	6, 8	1	N-methyl-perfluoroalkyl sulfonamide
31	EtFASA 	6, 8	12	N-ethyl-perfluoroalkyl sulfonamide
32	FASAA 	4-8	12, 22	Perfluoroalkyl sulfonamidoacetic acid
33	N-EtFASAA	2-10	22	N-ethyl perfluoroalkyl sulfonamide acetic acid

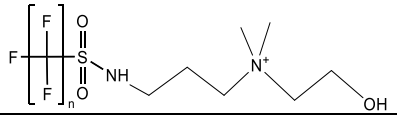
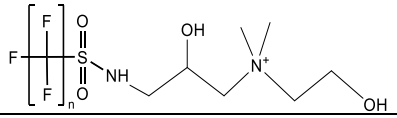
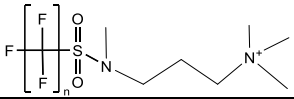
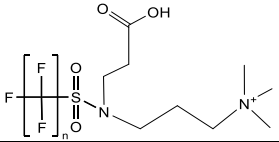
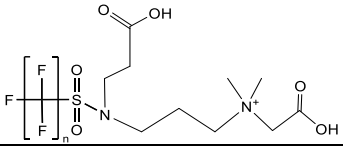
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
34	N-MeFASAA 	3-8	22	N-methyl perfluoroalkyl sulfonamide acetic acid
35	FASE ($C_n H_5 F_{2n-3} NSO_3^-$) 	4-10	13	Perfluoroalkyl sulfonamidoethanols
36	PFSiAs 	4-8	1, 13	Perfuoroalkane sulfinate
37	PFASAC (PFSaAmA or PFASAmA or FASAAA or AmPr-FASAP)	3-8	3, 7, 14, 16	Perfluoroalkyl sulfonamide amino carboxylates

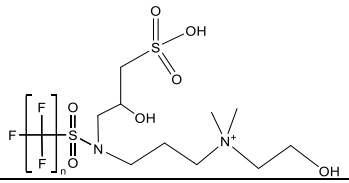
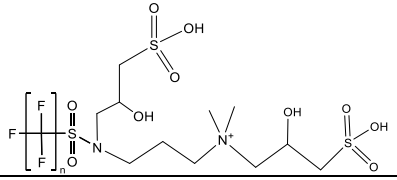
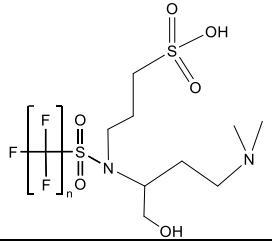
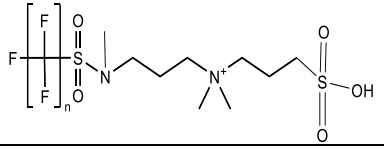
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
38	PFASBC (PFASA2C or FASADA or N-CEAmP-FASAP) 	3-8	3, 14, 16	Perfluoroalkyl amido betaine carboxylate (or Perfluoroalkyl sulfonamide amino dicarboxylic acids)
39	Isomeric side product betaine of PFASAC 	3-8	16	Isomeric side product betaine of PFSaAmA
40	PFAAiPrE (or Prop-PFAA) 	8, 10, 12	3, 11, 16	Perfluoroalkyl amido amine isopropyl acetate
41	PFASB (or FASAB, or N-CMAmP-FASA) 	3- 8	11, 12, 16, 20	Perfluoroalkylsulfonamide betaine, or N-carboxymethyldimethylammoniopropyl-perfluoroalkanesulfonamide

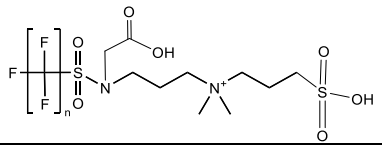
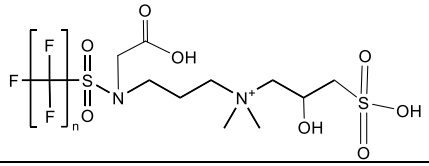
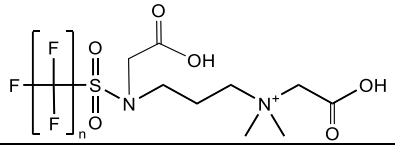
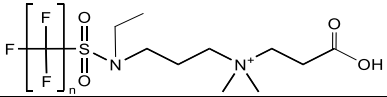
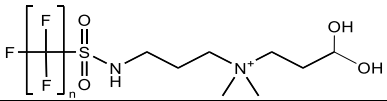
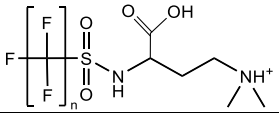
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
42	PFAAB (or N-CMAmP-FAAd) 	2-14	11, 12, 16	Perfluoroalkylamido betaine
43	PFASAm (FASAAm or AmPr-FASA) 	3-9	7, 14, 16, 20	Perfluoroalkyl sulfonamide amines
44	PFAAAm (or AmPr-FAAd) 	3-14	3, 16	Perfluoroalkyl amido amine (or N-(3-(dimethylamino)propyl)-Perfluoroalkylamide)
45	PFASNO (or N-OxAmP-FASA, OAmPr-FASA) 	4-9	16	Perfluoroalkyl sulfonamide amine oxide
46	PFAANO (or N-OxAmP-FAAd, OAmPr-FAAd) 	3-9	3	Perfluoroalkyl amido amine oxide
47	PFASAmS (or N-TAmP-FASA)	3-8	20, 23	N-trimethylammoniopropyl Perfluoroalkanesulfonamide

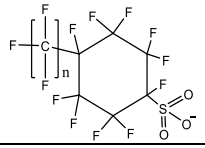
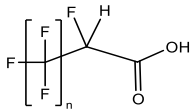
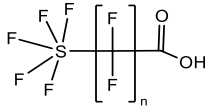
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
48	PFAAAmS (or N-TAmP-FAAd) 	3-7	12, 16	Perfluoroalkyl-amido ammonium salt
49	N-SP-FASA 	3-6	22	N-sulfopropyl Perfluoroalkanesulfonamide
50	N-SPAmP-FASA 	3-8	22	N-sulfopropyldimethylammonio propyl perfluoroalkanesulfonamide
51	N-SHOPAmP-FASA 	3-6	22	N-sulfohydroxypropyl(hop)dimethyl ammonio propyl perfluoroalkanesulfonamide
52	N-SPHOEAmP-FASA 	4-6	22	N-sulfohydroxypropyl(hop)dimethyl ammonio propyl perfluoroalkanesulfonamide

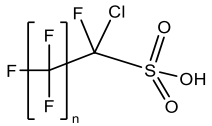
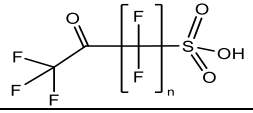
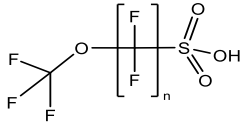
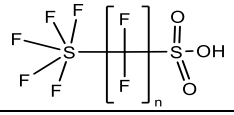
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
53	N-SPAmP-FASAPS 	3-8	22	N-sulfopropyldimethylammoniopropyl Perfluoroalkane sulfonamidopropylsulfonate
54	N-diHOPAmHOB-FASA 	3-6	22	N-dihydroxy(ho)propyldimethylammonio hydroxybutyl(hob)-perfluoroalkanesulfonamide
55	N-diHOPAmHOB-FASAPS 	2-6	22	N-dihydroxy(ho)propyldimethylammonio hydroxybutyl(hob)-perfluoroalkanesulfonamidopropyl sulfonate
56	N-HOEAmP-FASAPS 	2-8	22	N-hydroxyethyl(hoe)dimethylammonio propyl perfluoroalkanesulfonamido propylsulfonate
57	N-HOEAmP-FASE 	2-8	22	N-hydroxyethyl(hoe)dimethylammonio propyl perfluoroalkane sulfonamidoethanol

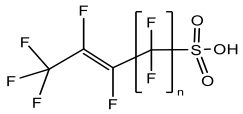
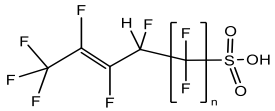
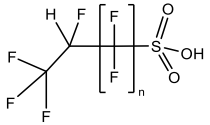
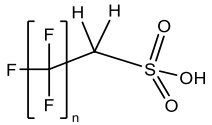
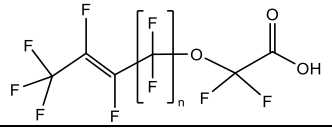
Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
58	N-HOEAmP-FASA 	2-8	22	N-hydroxyethyl(hoe)dimethylammonio propyl perfluoroalkanesulfonamide
59	N-HOEAmHOP-FASA 	4-6	22	N-hydroxyethyl(hoe)dimethylammonio hydroxypropyl(hop) perfluoroalkanesulfonamide
60	N-TAmP-N-MeFASA 	4-8	22	N-trimethylammoniopropyl n-methyl perfluoroalkanesulfonamide
61	N-TAmP-FASAP 	3-6	22	N-trimethylammoniopropyl perfluoroalkylsulfonamido propanoic acid
62	N-CMAmP-FASAP 	4-6	22	N-carboxymethyldimethylammoniopropyl-perfluoroalkylsulfonamido propanoic acid
63	N-HOEAmP-FASAHOPS	4-6	22	N-hydroxyethyl(hoe)dimethylammonio propyl perfluoroalkanesulfonamido hydroxy(ho)propyl sulfonate

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
64	N-SHOPAmP-FASAHOPS 	2-6	22	N-sulfohydroxypropyl(hop)dimethyl ammonio propyl perfluoroalkanesulfonamido hydroxy(HO)Propyl Sulfonate
65	N-AHOB-FASAPS 	5-6	22	N-dimethylaminohydroxybutyl(hob)-Perfluoroalkanesulfoamido propylsulfonate
66	N-SPAmP-MeFASA 	3-6	22	N-sulfopropyldimethylammoniopropyl methyl perfluoroalkanesulfonamide
67	N-SPAmP-FASAA	3-6	22	N-sulfopropyldimethylammoniopropyl-perfluoroalkanesulfonamido acetic acid

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
				
68	N-SHOPAmP-FASAA 	3-6	22	N-sulfohydroxypropyl(hop)dimethyl ammoniopropyl perfluoroalkanesulfonamido acetic acid
69	N-CMAmP-FASAA 	3-6	22	N-carboxymethyldimethylammoniopropyl-perfluoroalkanesulfonamido acetic acid
70	N-CEAmP-EtFASA 	5-6	22	N-carboxyethyldimethylammoniopropyl-ethyl perfluoroalkanesulfonamide
71	N-diHOBAmP-FASA 	4-6	22	N-dihydroxybutyl(dihob)dimethyl Ammoniopropyl perfluoroalkanesulfonamide
72	N-AmCP-FASA 	4-6	22	N-ammoniocarboxypropyl-perfluoroalkanesulfonamide

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
Other PFAS				
73	PFACHS 	1,2	3, 12	Perfluoroalkyl cyclohexane sulfonate
74	H-PFCA (HCnF2nCOO−) 	5-16	24, 25	Hydro substituted perfluorocarboxylates (H-PFCAs)
75	Cl-PFCA ($ClC_nF_{2n}COO^-$)	4-11	24	Chlorine substituted perfluorocarboxylates
76	PFS ($C_2F_5(C_2H_4)_nCHFOSO_3^-$)	1-6	24	Polyfluorinated sulfonates (PFSs)
77	H-PFE/As ($C_nF_{2n-2}HO^-$)	7-11	24	Unsaturated hydro substituted perfluorinated ethers/alcohols
78	Cl-PFE/As ($ClC_nF_{2n-2}O^-$)	6-10	24	Unsaturated chlorine substituted perfluorinated ethers/alcohols
79	n+1-F5S-PFAA 	6-8	22	(n+1-Pentafluoro(5)sulfide)-perfluoroalkanoic acid
80	Chlorinated PFSAs	3, 4, 5, 6, 7, 8	1, 13, 26	Chloro(Cl)-perfluoroalkanesulfonate

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
	(or Cl-PFSA) 			
81	Dichlorinated PFSAs ($C_n F_{2n-1}Cl_2 SO_3$)	3, 8	[13]	Chlorinated Perfluoroalkyl Sulphonate
82	K-PFSA (or Ketone PFSA) ($C_n F_{2n-1}SO4$) 	3-13	[13, 26]	Ketone perfluoroalyl sulfonate
83	Ether-PFSA (O-PFSA) 	3, 4, 5, 6, 7, 8, 9, 10	[1, 26]	Ether perfluoroalkane sulfonate
84	PFSA-Un $C_n F_{2n-1}SO_3^-$	8	[13]	Perfluoroalkene sulphonate
85	n-F5S-PFAS 	3-9	[22]	(n-Pentafluoro(5)sulfide)- perfluoroalkane sulfonate
86	UPFAS	1-10	[22]	Unsaturated perfluoroalkane sulfonate

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
	 Multiple isomers possible			
87	H-UPFAS  Multiple isomers possible	1-6	[22]	Hydrido-unsaturated perfluoroalkane sulfonate
88	H-PFAS  Multiple isomers possible	0-8	[22]	Hydrido-perfluoroalkane sulfonate
89	n:1 PFAS  Multiple isomers possible	5,7	[22]	n:1 perfluoroalkanesulfonate
90	O-U-PFAA 	0-4[a]	[22]	Oxa-unsaturated-perfluoroalkanoic acid

Class Number	Analyte acronym and structure	Perfluoroalkyl chain, n	Reference	Full Name
	Multiple isomers possible			
91	$C_{n+8}H_{16}O_2SN_2F_{2n+1}$	6, 8, 10	[22]	Not applicable
92	$C_{n+10}H_{20}O_7SN_2F_{2n+1}$ or $C_{n+10}H_{18}O_4SN_2F_{2n+1}$	Unknown	[22]	Not applicable
93	$C_{n+9}H_{22}O_2SN_2F_{2n+1}$	3, 4, 6	[22]	Not applicable

Table A.8 Correspondence between native PFAS analytes and isotopically labeled surrogate IS.

Analyte	Surrogate IS
PFPrA	MPFBA
PFBA	MPFBA
PFPeA	M5PFhxA
PFHxA	M5PFhxA
PFHpA	M8PFOA
PFOA	M8PFOA
PFNA	M9PFNA
PFDA	M6PFDA
PFUnA	M7PFUnA
PFDoA	MPFDoA
PFTrDA	M2PFTeDA
PFTeDA	M2PFTeDA
PFHxDA	M2PFTeDA
PFOcDA	M2PFTeDA
PFPrS	M3PFBS
PFBS	M3PFBS
PFPeS	M3PFHxS
PFHxS	M3PFHxS
PFHpS	M8PFOS
PFOS	M8PFOS
PFNS	M8PFOS
PFDS	M8PFOS
PFDoS	M8PFOS
PFECHS	M3PFHxS
FBSA	M8FOSA-I
FHxSA	M8FOSA-I
FOSA	M8FOSA-I
MeFOSA	d-N-MeFOSA-M
EtFOSA	d-N-EtFOSA-M
FOSAA	d3-N-MeFOSAA
MeFOSAA	d3-N-MeFOSAA
EtFOSAA	d3-N-MeFOSAA
3:3 FTCA	M8PFOA
4:3 FTCA	M8PFOA
5:3 FTCA	M8PFOA
7:3 FTCA	M8PFOA
4:2 FTSA	M2-6:2 FTSA
6:2 FTSA	M2-6:2 FTSA
8:2 FTSA	M2-8:2 FTSA
10:2 FTSA	M2-8:2 FTSA
6:2 FTUA	M6:2 FTUA
8:2 FTUA	M8:2 FTUA
10:2 FTUA	M8:2 FTUA
6:2 FTAB	PFOAAmS(N-TAmP-FOAd)
5:3 FTB	PFOAAmS(N-TAmP-FOAd)
5:1:2 FTB	PFOAAmS(N-TAmP-FOAd)
PFHxSAm(AmPr-FHxSA)	PFOAAmS(N-TAmP-FOAd)
PFOSAm(AmPr-FOSA)	PFOAAmS(N-TAmP-FOAd)
PFHxSAmS(N-TAmP-FHxSA)	PFOAAmS(N-TAmP-FOAd)
PFOSAmS(N-TAmP-FOSA)	PFOAAmS(N-TAmP-FOAd)
PFOSNO(N-OxAmP-FOSA)	PFOAAmS(N-TAmP-FOAd)
PFOANO(N-OxAmP-FOAd)	PFOAAmS(N-TAmP-FOAd)
PFOSB(N-CMAmP-FOSA)	PFOAAmS(N-TAmP-FOAd)
PFOAB(N-CMAmP-FOAd)	PFOAAmS(N-TAmP-FOAd)

Table A.9 The acronym, theoretical and observed m/z, mass error, retention time (RT), analysis method, and identification confidence level of suspect-screening PFAS.

No.	PFAS Class	Acronym	Theoretical m/z	Observed m/z	Mass error, ppm	RT, min	Ionization mode	Identification confidence level
1	n:2 FTSA	14:2 FTSA	826.94230	826.94552	3.89	8.93	ESI-	Level 4
2	n:3 FTCA	9:3 FTCA	540.99141	540.99225	1.55	8.29	ESI-	Level 4
3	n:2 FTSAS (or FtTAoS)	6:2 FTSAS	586.03914	586.03912	-0.03	6.08	ESI-	Level 2a
4	n:2 FTSAS-SO (or FtSOAoS or n:2 FTSAS-sulfoxide)	6:2 FTSAS-sulfoxide	602.03457	602.03363	-1.56	5.12	ESI-	Level 2a
5	n:2 FTSAS-SO_2	8:2 FTSAS-SO_2	718.02300	718.02423	1.71	6.53	ESI-	Level 2a
6	n:2:1 FTSC	4:2:1 FTSC	336.99448	336.99460	0.36	5.42	ESI-	Level 4
7	n:2 FASA	6:2 FASA	425.98313	425.98785	11.08	6.49	ESI-	Level 4
8	1HO-n:2 FTS	1HO-6:2 FTS	442.96248	442.96228	-0.45	4.94	ESI-	Level 2a
9	n:2 FTSO2PA	8:2 FTSO2PA	582.98760	582.98859	1.70	7.53	ESI-	Level 4
10	FASAs	FPeSA	347.95544	347.95627	2.39	6.00	ESI-	Level 2a
11	FASAA	FHxSAA	455.95773	455.95905	2.90	6.52	ESI-	Level 2a
12	MeFASA	MeFHxSA	411.96748	411.96936	4.56	7.30	ESI-	Level 2a
13	EtFASA	EtFHxSA	425.98313	425.98523	4.93	6.49	ESI-	Level 4
14	N-EtFASAA	N-EtFHxSAA	483.98861	483.99069	4.30	7.38	ESI-	Level 2a
15	N-MeFASAA	N-MeFBSAA	369.97914	369.97934	0.54	5.44	ESI-	Level 4
16	FASE	FOSE	441.97867	441.97894	0.61	6.53	ESI-	Level 4
17	N-SP-FASA	N-SP-FHxSA	519.95612	519.95557	-1.06	5.45	ESI-	Level 4
18	PFASi	PFHxSi	382.94135	382.94232	2.53	5.00	ESI	Level 4
19	n-F5S-PFAS	8-F5S-PFAS	606.89587	606.89728	2.32	7.66 (major), 7.45 (minor)	ESI-	Level 2a
20	UPFAS	UPFUnS	610.92401	610.92511	1.80	7.49	ESI-	Level 2a
21	H-UPFAS	H-UPFOS	442.94260	442.94568	6.95	5.86	ESI-	Level 2a
22	H-PFAS	H-PFHxS	480.93951	480.93909	-0.87	5.91	ESI-	Level 2a
23	Cl-PFAS (Cl-PFSA)	Cl-PFOS	514.90064	514.90232	3.26	6.57	ESI-	Level 2a
24	O-PFAS (ether-PFSA)	O-PFOS	514.92490	514.92572	1.59	6.84	ESI-	Level 2a
25	ketone-PFSAs	ketone-PFOS	476.92819	476.92780	-0.82	6.27	ESI-	Level 2a
26	n:2 FTAB	8:2 FTAB	671.08723	671.08759	0.54	6.33	ESI+	Level 2a
27	n:2 FTA (or FtSaAM, or M4)	6:2 FTA	513.08814	513.08813	-0.02	5.33	ESI+	Level 2a
28	n:2-demethyl-FTA	6:2 dimethyl-FTA	499.07249	499.07166	-1.66	5.39	ESI+	Level 2a
29	n:3 FTB	7:3 FTB	514.08632	514.08569	-1.23	5.64	ESI+	Level 2a
30	n:1:2 FTB	7:1:2 FTB	532.07711	532.07587	-2.33	5.71	ESI+	Level 2a
31	n:2 FTSHA (or FtTHN+)	6:2 FTSHA	496.09818	496.09723	-1.91	5.70	ESI+	Level 2a
32	n:2 FTSHA-SO (FtTHN+-SO)	6:2 FTSHA-SO	512.09234	512.09296	1.21	4.88	ESI+	Level 2a
34	n:2 FTSAA	6:2 FTSAA	523.10908	523.10156	-14.38	5.12	ESI+	Level 4
35	CMAmEt-FA	6CMAmEt-FA (or 6:2 FTB)	450.07387	450.07367	-0.44	4.76	ESI+	Level 2a
36	CMAmB-FA	6CMAmB-FA (or 6:4 FTB)	478.10496	478.10477	-0.40	5.47	ESI+	Level 2a
37	AmPr-FASAP (PFASAC, or PFnSAmA)	AmPr-FHxSAP	557.07828	557.07776	-0.93	5.37	ESI+	Level 2a

38	N-CEAmP-FASAP (PFASBC or PFASA2C)	N-CEAmP-FPeSAP	579.10250	579.10175	-1.30	6.46	ESI+	Level 2a
39	N-CMAmP-FAAd (PFAAB)	N-CMAmP-FHpAd	507.09668	507.09134	-10.53	5.45	ESI+	Level 4
40	N-CMAmP-FASA (PFASB)	N-CMAmP-FHxSA	543.06211	543.06165	-0.85	5.34 (major), 5.19 (minor)	ESI+	Level 2a
41	N-TAmP-FASA (PFASAmS)	N-TAmP-FPeSA	449.07537	449.07535	-0.04	4.87	ESI+	Level 2a
42	AmPr-FASA (PFASAm)	AmPr-FPeSA	435.05972	435.05966	-0.14	4.84 (major), 4.70 (minor)	ESI+	Level 2a
43	AmPr-FAAd (PFAAAm)	AmPr-FHxAd	399.09325	399.09314	-0.28	4.18	ESI+	Level 2a
44	N-OxAmP-FASA (PFASNO)	N-OxAmP-FHxSA	501.05155	501.05096	-1.18	5.42	ESI+	Level 2a
45	N-SPAmP-FASAA	N-SPAmP-FHxSAA	663.05075	663.05072	-0.05	5.35	ESI+	Level 4
46	N-SPAmP-FASA	N-SPAmP-FHxSA	607.06040	607.05963	-1.27	5.18	ESI+	Level 2a
47	N-SPHOEAmP-FASA	N-SPHOEAmP-FBSA	537.07756	537.07892	2.53	5.50	ESI+	Level 2a
48	N-HOEAmP-FASAPS	N-HOEAmP-FHxSAPS	651.08724	651.08661	-0.97	5.07	ESI+	Level 2a
49	N-HOEAmP-FASA	N-HOEAmP-FHxSA	529.08316	529.08234	-1.55	5.28	ESI+	Leve 2a
50	N-HOEAmP-FASE	N-HOEAmP-FHxSE	573.10968	573.10870	-1.71	5.25 (major), 5.11 (minor)	ESI+	Level 2a
51	N-HOEAmHOP-FASA	N-HOEAmHOP-FHxSA	545.07818	545.07794	-0.44	5.18 (major). 5.07 (minor)	ESI+	Level 2a
52	N-SPAmP-FASAPS	N-SPAmP-FBSAPS	629.07087	629.06647	-6.99	4.96	ESI+	Level 4
53	N-TamP-N-MeFASA	N-TamP-N-MeFHxSA	513.08835	513.08698	-2.67	5.58 (major), 5.32 (minor)	ESI+	Level 2a
54	N-TAmP-FASAP	N-TAmP-FHxSAP	571.09393	571.09344	-0.86	5.38	ESI+	Level 2a
55	N-CMAmP-FASAP	N-CMAmP-FBSAP	515.08963	515.08893	-1.36	5.66	ESI+	Level 2a
56	N-CMAmP-FASAA	N-CMAmP-FPrSAA	451.07738	451.07724	-0.31	4.76	ESI+	Level 2a
57	N-CEAmP-EtFASA	N-CEAmP-EtFHxSA	585.10937	585.10944	0.12	5.58	ESI+	Level 2a

Table A.10 Compound-specific instrumental limits of detection (iLOD), instrumental limits of quantification (iLOQ), method limits of detection (mLOD), and limits of quantification (mLOQ) and linearity performance of 53 quantitative PFAS in soil and groundwater.

(a) In soil. The accuracy and precision performance of QC samples inserted along the analytical sequence at a medium spike level (5 ng/mL)

Analyte	Linearity range	R^2	iLOD (ng/mL)	iLOQ (ng/mL)	mLOD (µg/kg dw)	mLOQ (µg/kg dw)	Accuracy (%) at 5 ng/mL n = 5, (Average ± SD)	Precision N = 5 (RSD, %)
PFPrA	0.2-25	0.9984	0.090	0.200	0.180	0.400	98±5	5.2
PFBA	0.2-25	0.9989	0.080	0.200	0.160	0.400	97±2	1.8
PFPeA	0.05-25	0.9992	0.020	0.050	0.040	0.100	96±2	1.6
PFHxA	0.025-25	0.9989	0.030	0.025	0.060	0.050	96±3	2.5
PFHpA	0.025-25	0.9994	0.007	0.025	0.014	0.050	97±1	1.1
PFOA	0.025-25	0.9985	0.009	0.025	0.018	0.050	96±2	1.6
PFNA	0.025-25	0.9992	0.010	0.025	0.020	0.050	96±1	1.0
PFDA	0.025-25	0.9991	0.010	0.025	0.020	0.050	95±1	0.6
PFUnA	0.025-25	0.9992	0.020	0.025	0.040	0.050	96±1	0.8
PFDoA	0.05-25	0.9992	0.030	0.050	0.060	0.100	97±1	1.3
PFTrDA	0.05-25	0.9986	0.030	0.050	0.060	0.100	94±3	3.2
PFTeDA	0.05-25	0.9992	0.030	0.050	0.060	0.100	98±2	1.6
PFHxDA	0.05-25	0.9970	0.030	0.050	0.060	0.100	99±4	4.2
PFPrS	0.025-25	0.9999	0.005	0.025	0.010	0.050	93±4	3.8
PFBS	0.025-25	0.9994	0.007	0.025	0.014	0.050	95±1	0.9
PFPeS	0.025-25	0.9977	0.005	0.025	0.010	0.050	98±3	2.7
PFHxS	0.025-25	0.9992	0.006	0.025	0.012	0.050	94±1	0.6
PFHpS	0.025-25	0.9967	0.006	0.025	0.012	0.050	95±4	3.7
PFOS	0.025-25	0.9990	0.020	0.025	0.040	0.050	96±1	0.8
PFNS	0.025-25	0.9992	0.007	0.025	0.014	0.050	100±3	3.3
PFDS	0.025-25	0.9991	0.005	0.025	0.010	0.050	95±3	3.1
PFDoS	0.05-25	0.9994	0.010	0.050	0.020	0.100	102±9	8.6
4:2 FTSA	0.025-25	0.9986	0.005	0.025	0.010	0.050	98±2	2.2
6:2 FTSA	0.05-25	0.9928	0.040	0.050	0.080	0.100	116±12	12.4
8:2 FTSA	0.025-25	0.9988	0.010	0.025	0.020	0.050	96±2	1.7
10:2 FTSA	0.05-25	0.9996	0.020	0.050	0.040	0.100	96±3	2.6
3:3 FTCA	0.2-25	0.9987	0.050	0.200	0.100	0.400	93±6	5.9
4:3 FTCA	0.1-25	0.9975	0.050	0.100	0.100	0.200	94±3	2.8
5:3 FTCA	0.1-25	0.9969	0.050	0.100	0.100	0.200	94±3	3.5
7:3 FTCA	0.1-25	0.9990	0.030	0.100	0.060	0.200	97±4	4.1
6:2 FTUA	0.1-25	0.9986	0.100	0.100	0.200	0.200	100±1	1.4
8:2 FTUA	0.05-25	0.9987	0.050	0.050	0.100	0.100	98±1	1.3
10:2 FTUA	0.05-25	0.9970	0.050	0.050	0.100	0.100	103±3	3.4
6:2 FTAB	0.05-25	0.9966	0.040	0.050	0.080	0.100	95±7	6.6
5:3 FTB	0.05-25	0.9992	0.040	0.050	0.080	0.100	119±12	11.6
5:1:2 FTB	0.05-25	0.9998	0.040	0.050	0.080	0.100	92±2	2.1
FBSA	0.025-10	0.9935	0.008	0.025	0.016	0.050	99±7	6.9
FHxSA	0.025-25	0.9979	0.009	0.025	0.018	0.050	101±5	4.9
FOSA	0.025-25	0.9992	0.010	0.025	0.020	0.050	97±1	0.9
MeFOSA	0.025-25	0.9922	0.010	0.025	0.020	0.050	87±5	4.7
EtFOSA	0.025-25	0.9978	0.010	0.025	0.020	0.050	99±6	5.6
FOSAA	0.05-25	0.9978	0.020	0.050	0.040	0.100	96±3	2.8
MeFOSAA	0.1-25	0.9955	0.070	0.100	0.140	0.200	113±16	16.4
EtFOSAA	0.1-25	0.9906	0.060	0.100	0.120	0.200	93±9	9.3
PFHxSAm	0.05-25	0.9991	0.010	0.050	0.020	0.100	88±7	6.7
PFOSAm	0.05-25	0.9990	0.010	0.050	0.020	0.100	90±6	6.0
PFHxSAmS	0.05-25	0.9967	0.020	0.050	0.040	0.100	82±5	4.6
PFOSAmS	0.05-25	0.9972	0.020	0.050	0.040	0.100	80±5	4.7
PFOAB	0.1-25	0.9977	0.080	0.100	0.160	0.200	94±4	4.0
PFOSB	0.2-25	0.9981	0.070	0.200	0.140	0.400	92±3	2.6
PFOANO	0.05-25	0.9988	0.030	0.050	0.060	0.100	90±8	7.8
PFOSNO	0.05-25	0.9975	0.007	0.050	0.014	0.100	90±4	3.9
PFECHS	0.025-25	0.9986	0.005	0.025	0.010	0.050	91±2	1.

(b) In groundwater. The volume of MeOH and HPLC grade-water was 80/20 (v/v) when building the matrix-free solvent calibration curve.

Analyte	Linearity range	R^2	iLOD (ng/mL)	iLOQ (ng/mL)	mLOD (ng/ml)	mLOQ (ng/ml)
PFPrA	0.05-25	0.9998	0.025	0.050	0.210	0.420
PFBA	0.05-26	0.9998	0.014	0.050	0.118	0.420
PFPeA	0.01-25	0.9998	0.004	0.010	0.034	0.084
PFHxA	0.01-25	0.9998	0.004	0.010	0.034	0.084
PFHpA	0.01-25	0.9997	0.005	0.010	0.042	0.084
PFOA	0.01-25	0.9997	0.006	0.010	0.050	0.084
PFNA	0.01-25	0.9995	0.005	0.010	0.042	0.084
PFDA	0.01-25	0.9998	0.006	0.010	0.050	0.084
PFUnA	0.025-25	0.9997	0.007	0.025	0.059	0.210
PFDoA	0.05-25	0.9992	0.010	0.050	0.084	0.420
PFTrDA	0.05-25	0.9986	0.010	0.050	0.084	0.420
PFTeDA	0.05-25	0.9992	0.010	0.050	0.084	0.420
PFHxDA	0.05-25	0.9970	0.010	0.050	0.084	0.420
PFPrS	0.1-25	0.9998	0.050	0.100	0.420	0.840
PFBS	0.01-25	0.9996	0.008	0.010	0.067	0.084
PFPeS	0.01-25	0.9997	0.005	0.010	0.042	0.084
PFHxS	0.01-25	0.9998	0.005	0.010	0.042	0.084
PFHpS	0.01-25	0.9994	0.002	0.010	0.017	0.084
PFOS	0.01-25	0.9995	0.002	0.010	0.017	0.084
PFNS	0.01-25	0.9999	0.002	0.010	0.017	0.084
PFDS	0.01-25	1.0000	0.004	0.010	0.034	0.084
PFDoS	0.01-25	0.9994	0.005	0.010	0.042	0.084
4:2 FTSA	0.025-25	0.9999	0.006	0.025	0.050	0.210
6:2 FTSA	0.025-25	0.9999	0.012	0.025	0.101	0.210
8:2 FTSA	0.025-25	0.9993	0.013	0.025	0.109	0.210
10:2 FTSA	0.05-25	0.9991	0.026	0.050	0.218	0.420
3:3 FTCA	0.1-25	0.9987	0.050	0.100	0.420	0.840
4:3 FTCA	0.1-25	0.9975	0.050	0.100	0.420	0.840
5:3 FTCA	0.1-25	0.9999	0.020	0.100	0.168	0.840
7:3 FTCA	0.1-25	0.9996	0.075	0.100	0.630	0.840
6:2 FTUA	0.25-25	0.9989	0.008	0.250	0.067	2.100
8:2 FTUA	0.05-25	0.9980	0.006	0.050	0.050	0.420
10:2 FTUA	0.025-25	0.9979	0.006	0.025	0.050	0.210
FBSA	0.01-25	0.9999	0.002	0.010	0.017	0.084
FHxSA	0.01-25	0.9999	0.002	0.010	0.017	0.084
FOSA	0.01-25	0.9998	0.005	0.010	0.042	0.084
MeFOSA	0.01-25	0.9999	0.002	0.010	0.017	0.084
EtFOSA	0.025-25	0.9998	0.008	0.025	0.067	0.210
FOSAA	0.05-25	0.9995	0.012	0.050	0.101	0.420
MeFOSAA	0.05-25	0.9999	0.025	0.050	0.210	0.420
EtFOSAA	0.05-25	0.9999	0.007	0.050	0.059	0.420
PFHxSAm	0.05-25	0.9996	0.007	0.050	0.059	0.420
PFOSAm	0.05-25	0.9996	0.007	0.050	0.059	0.420
PFHxSAmS	0.025-25	0.9995	0.014	0.025	0.118	0.210
PFOSAmS	0.025-25	0.9995	0.014	0.025	0.118	0.210
PFOAB	0.05-25	0.9977	0.080	0.050	0.672	0.420
PFOSB	0.1-25	0.9981	0.070	0.100	0.588	0.840
PFOANO	0.05-25	0.9988	0.030	0.050	0.252	0.420
PFOSNO	0.05-25	0.9975	0.007	0.050	0.059	0.420
6:2 FTAB	0.05-25	0.9966	0.040	0.050	0.336	0.420
5:3 FTB	0.05-50	0.9992	0.020	0.050	0.168	0.420
5:1:2 FTB	0.05-50	0.9998	0.020	0.050	0.168	0.420
PFECHS	0.01-25	1.0000	0.002	0.010	0.0168	0.084

Table A.11 The median PFHxS/PFOS ratio in surface soil and groundwater at different areas of four FTA sites.

Sample type	Site name	Area	PFHxS/PFOS (median)
Surface soil	Site #1	Upgradient	0.17
		Vicinity	0.01
		Downgradient	0.05
	Site #2	Vicinity	0.04
	Site #3	Vicinity	0.07
	Site #4	Vicinity	0.09
Groundwater	Site #1	Upgradient	0.14
		Vicinity	0.20
		Downgradient	1.04
	Site #2	Upgradient	NA*
		Vicinity	0.19
		Downgradient	0.81
	Site #3	Vicinity	0.35
	Site#4	Upgradient	0.33
		Vicinity	0.19
		Downgradient	0.13

*The PFOS concentrations in the upgradient groundwater samples at site #2 were nondetectable thus the PFHxS/PFOS ratios were not available for those samples.

Table A.12 A draft priority PFAS analyte list for surface soil (a) and groundwater (b).

(a) PFAS analyte priority list for surface soil

No.	PFAS Analyte
1	PFOS
2	7:1:2 FTB
3	8:2 FTSA
4	6:2 FTAB
5	5:1:2 FTB
6	6:2 FTSA
7	6:2 demethyl-FTA
8	N-TAmP-FHxSA
9	6:2 FTSHA-SO
10	N-HOEAmP-FHxSA
11	PFHxS
12	7:3 FTB
13	5:3 FTB
14	9:1:2 FTB
15	N-CMAmP-FBSAP
16	FHxSA
17	11:1:2 FTB
18	PFHpS
19	PFHxA
20	10:2 FTSA
21	6:2 FTA
22	AmPr-FHxSA
23	8:2 $FTSO_2PA$
24	PFNS
25	N-CMAmP-FHxSA

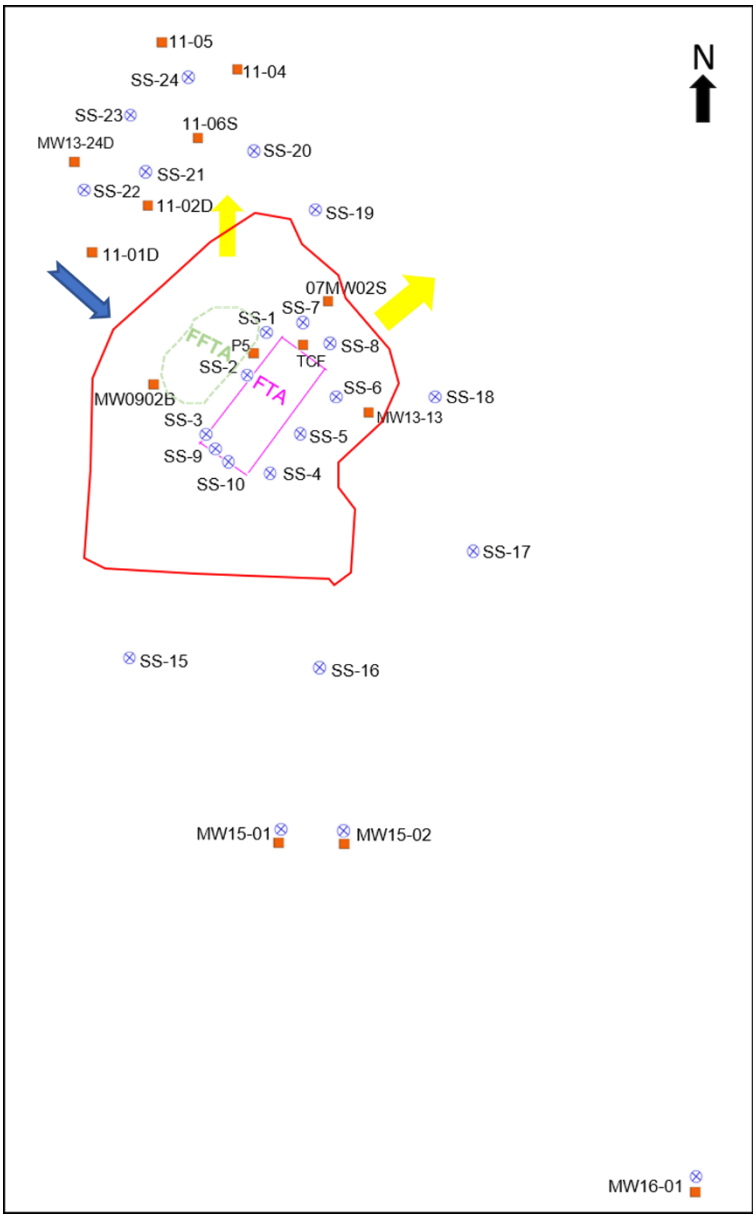
(b) PFAS analyte priority list for groundwater

No.	PFAS Analyte
1	6:2 FTSA
2	FBSA
3	FHxSA
4	PFOS
5	FPeSA
6	6:2 FTSAS-SO
7	PFHxS
8	FPrSA
9	6:2 FTSAS-SO_2
10	PFHxA
11	PFPeA
12	MeFBSAA
13	PFOA
14	PFBA
15	PFHpA
16	8:2 FTSA
17	N-CMAmP-FHxSA
18	N-SPAmP-FHxSA
19	8:2 FTSAS-SO_2
20	4:2 FTA
21	PFPeS
22	N-TamP-N-MeFBSA
23	AmPr-FHxSAP
24	N-SP-FHxSA
25	PFHpS

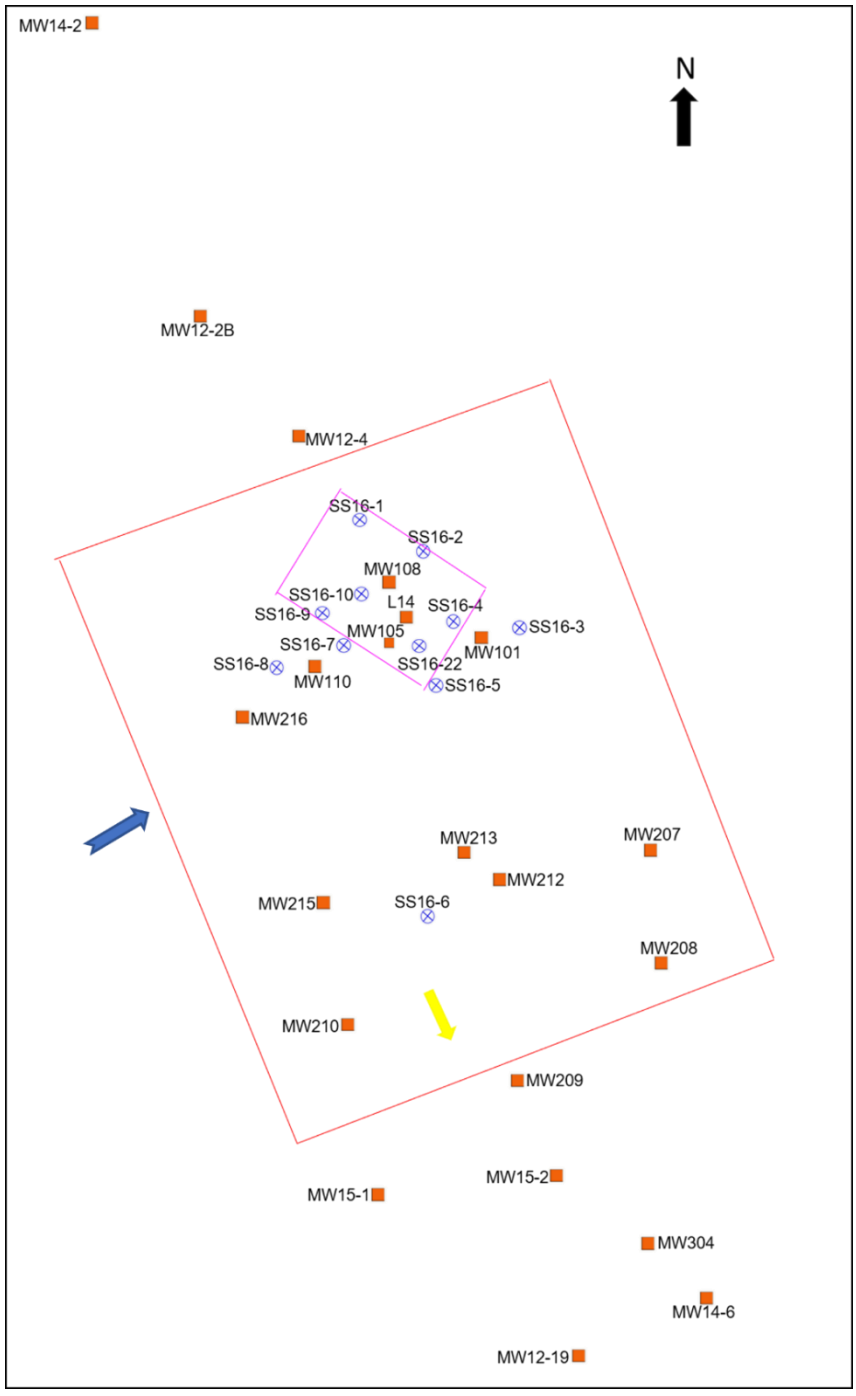
Figure A.1 Sampling locations for soil and groundwater samples from the four FTA sites, and site history.

(a) Site #1. (Green line – decommissioned FTA; pink line – active FTA)

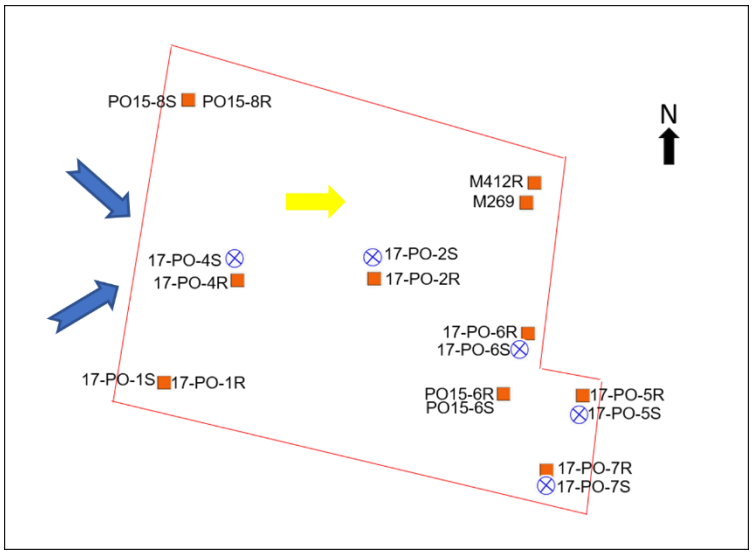
Note: ⊗ soil samples, ■ groundwater samples, ➡ Groundwater flow direction, and ➡ prevailing wind direction. The red line represents the FTA area boundary.



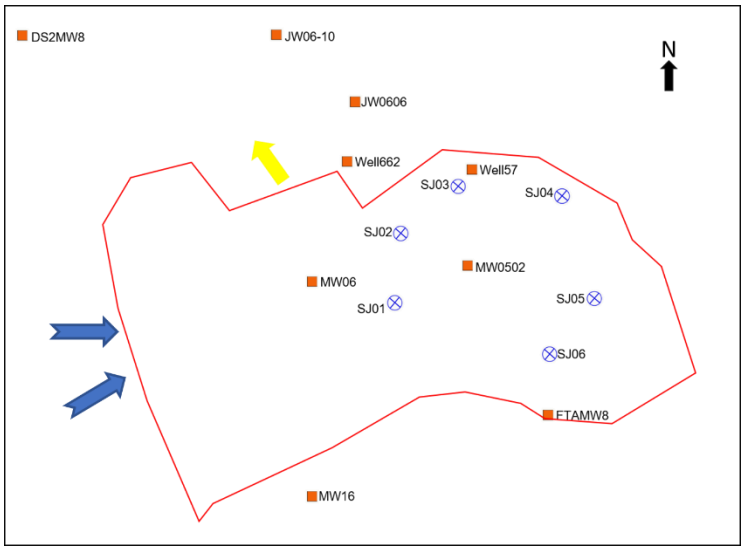
(b) Site #2 (Decommissioned FTA).



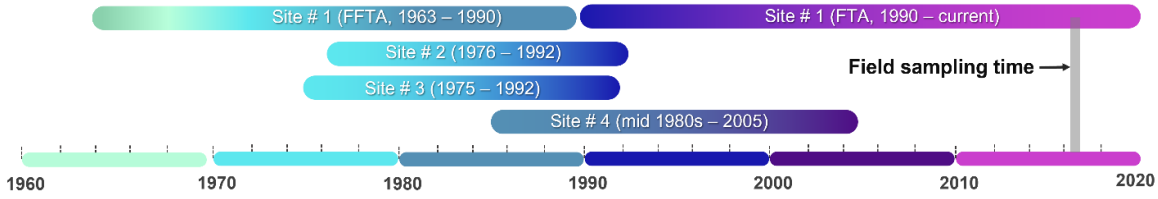
(c) Site #3 (Decommissioned FTA).



(d) Site #4 (Decommissioned FTA).



(e) The periods of active fire training for the four Canadian FTA sites: Site #1 included a former FTA area (FFTA) and an active FTA area (FTA). The field soil and groundwater samples were sampled between Sep. 2016 and FeA. 2017.



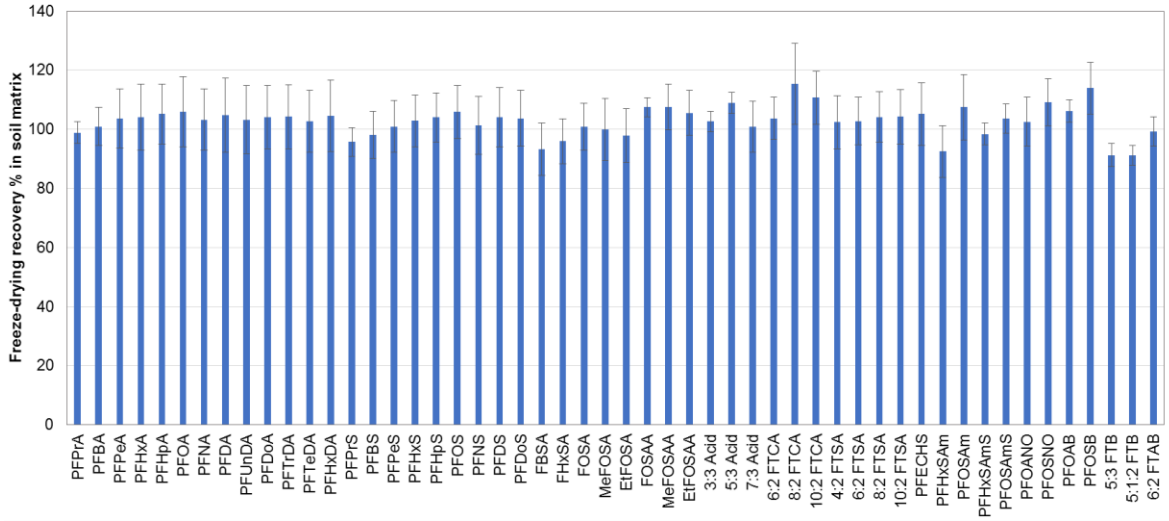


Figure A.2 Recovery of PFAS during the freeze-drying step in the soil matrix. Error bars represent standard deviations.

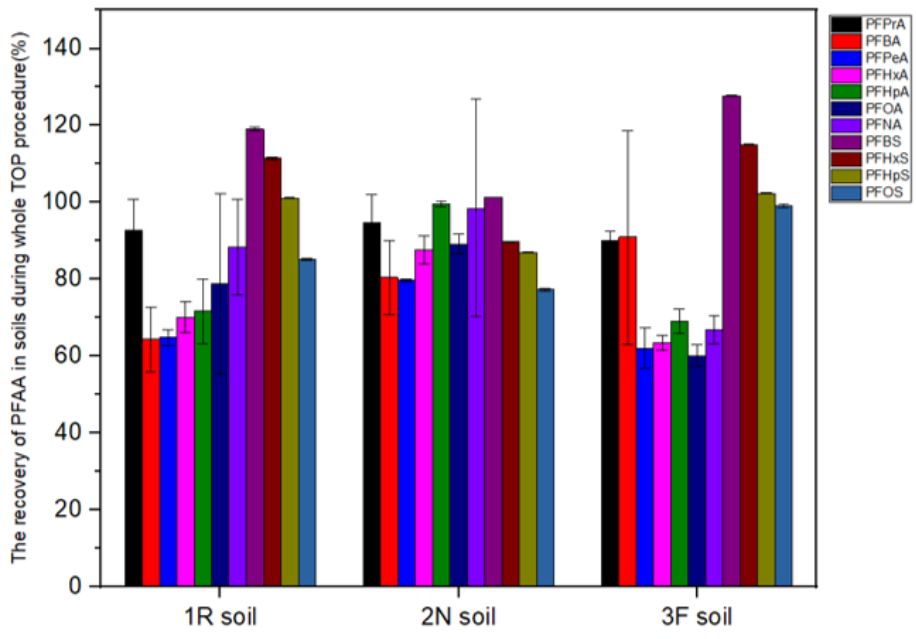


Figure A.3 The PFAA recovery in three types of soils during the whole TOP procedure.

Note: Sixty-seven microliters of 1.79 ppm of PFCA (C3-C9) and PFSA (C4, C6, C7, and C8) were spiked into 1 g-dw soil, which then underwent extraction, ENVI-Carb cleanup, nitrogen evaporation to dryness, and the TOP procedure before instrument analysis. PFAAs do not undergo degradation during the TOP assay.

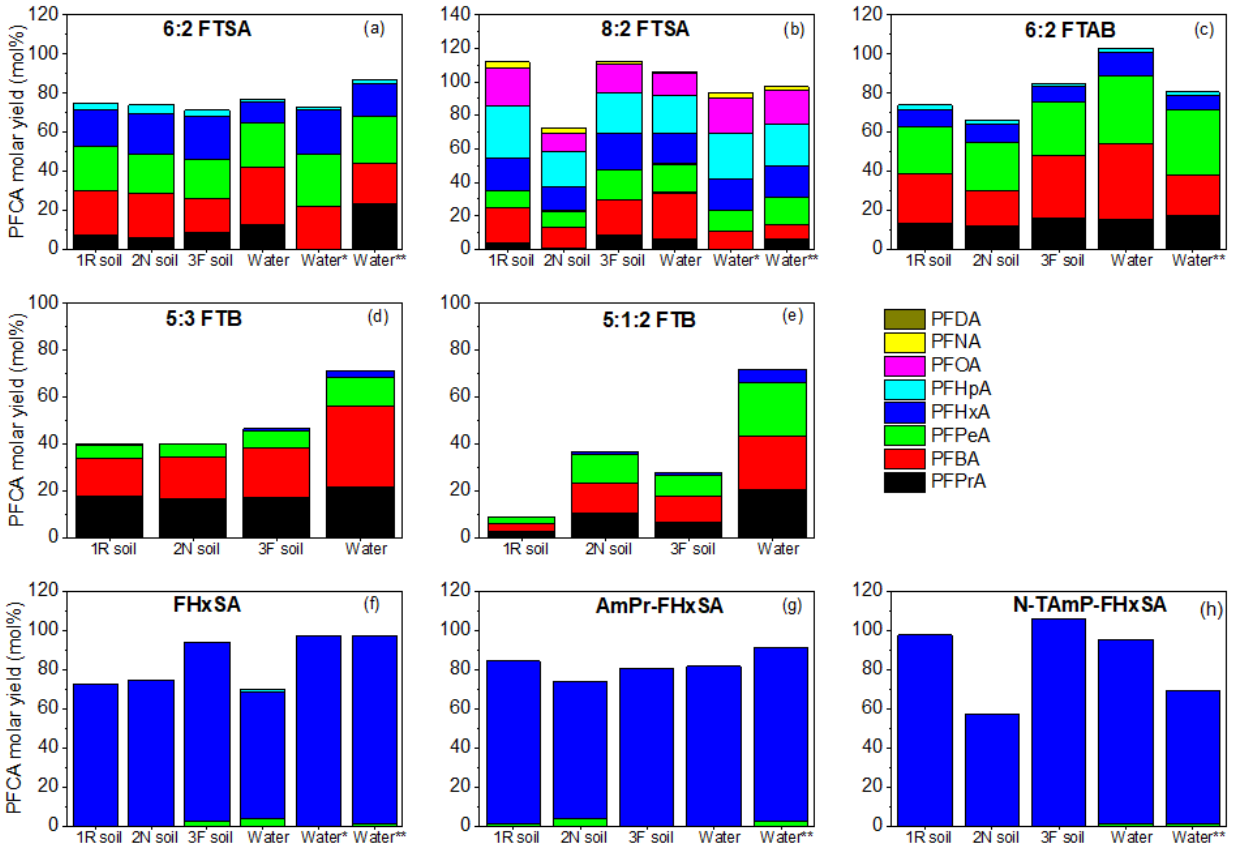


Figure A.4 The molar conversion yields of five fluorotelomer-based and three ECF-based precursors into C3-C10 PFCA post TOP assay in three types of soil matrixes (1R, 2N and 3F soil) and ultra-pure water; the precursors included 6:2 FTSA (a), 8:2 FTSA (b), 6:2 FTAB (c), 5:3 FTB (d), 5:1:2 FTB (e), FHxSA (f), PFHxSAm (g), and PFHxSAmS (h).

Note: The oxidation conditions, including oxidant concentration and reaction time, were selected from the literature [5]. Sixty-seven µl of 179 ppm stock solution of each precursor was spiked into 1 g-dw soil matrixes, which then underwent extraction, Envi-carb cleanup, nitrogen evaporation to dryness, and TOP assay procedure before instrument analysis. Three replicates were executed per treatment condition. The asterisk indicates oxidation data from Houtz et al,[5] and the double-asterisk (**) represent oxidation data obtained or estimated from Martin et al.[9]

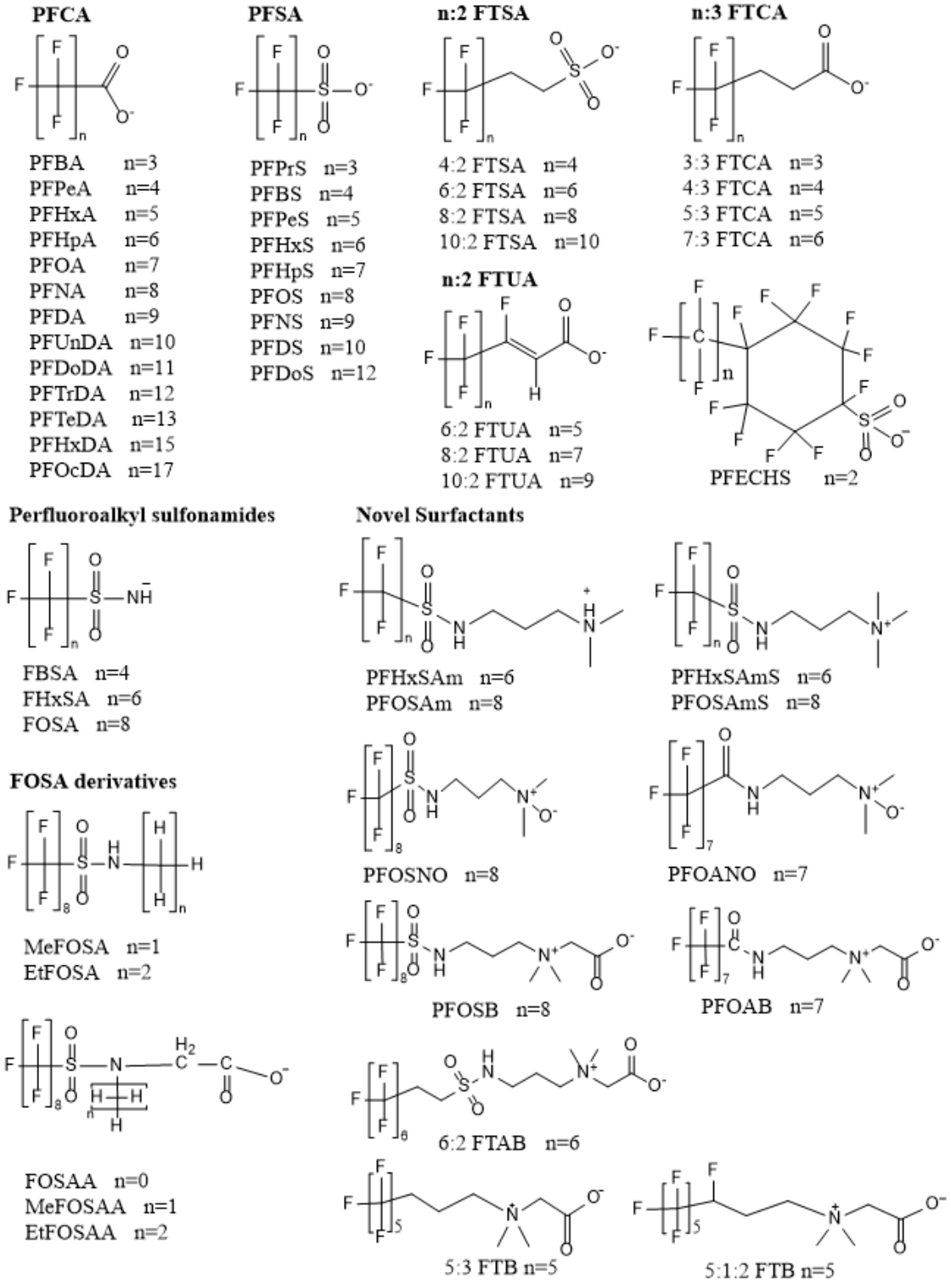


Figure A.5 The structure of 53 quantitative PFAS.

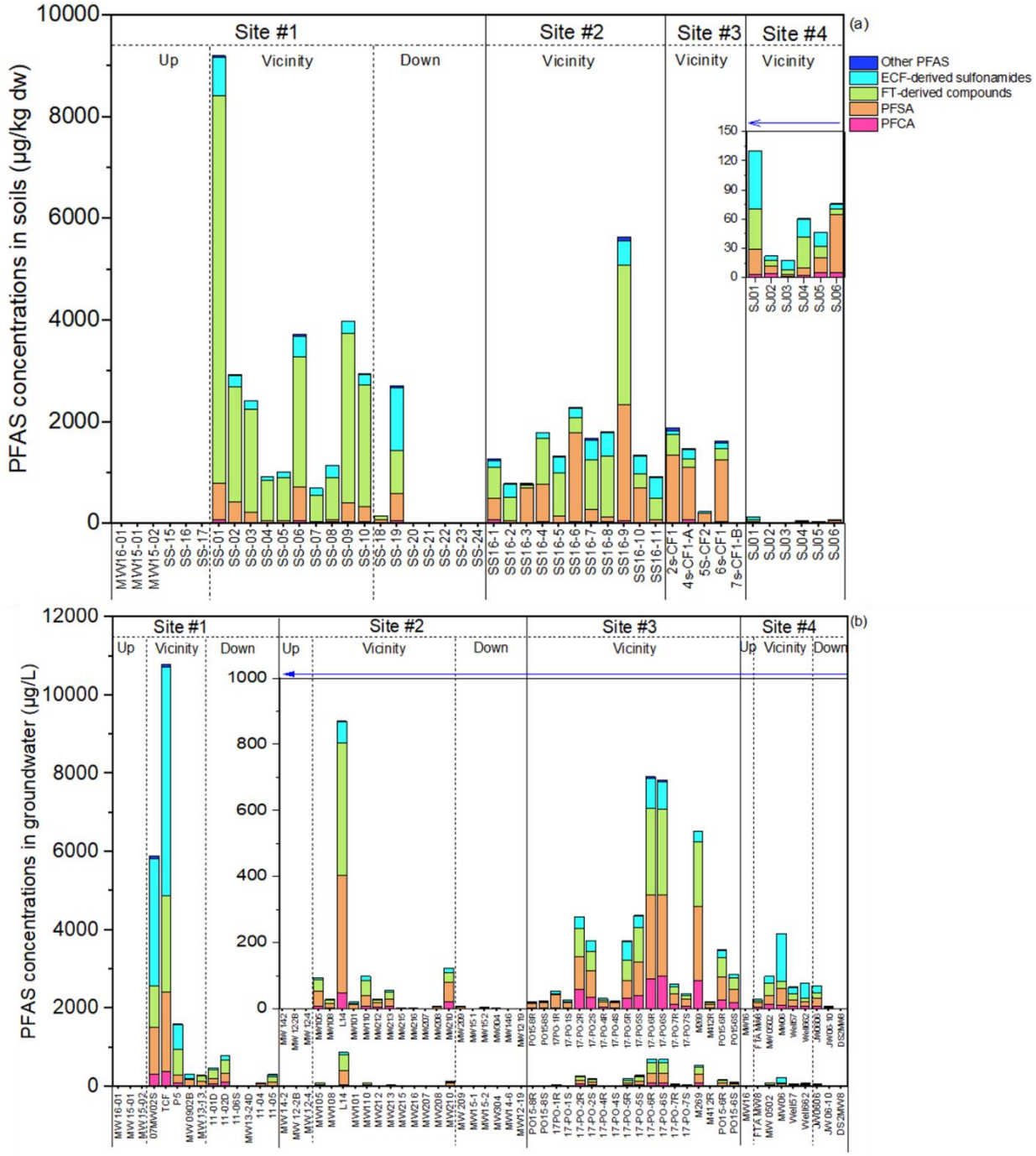


Figure A.6 The concentrations of five types of PFAS in surface soils (a) and groundwater (b) from the four Canadian FTA sites.

Note: "up" refers to the upgradient area, "vicinity" refers to the vicinity of the FTA area, while "down" refers to the downgradient area.

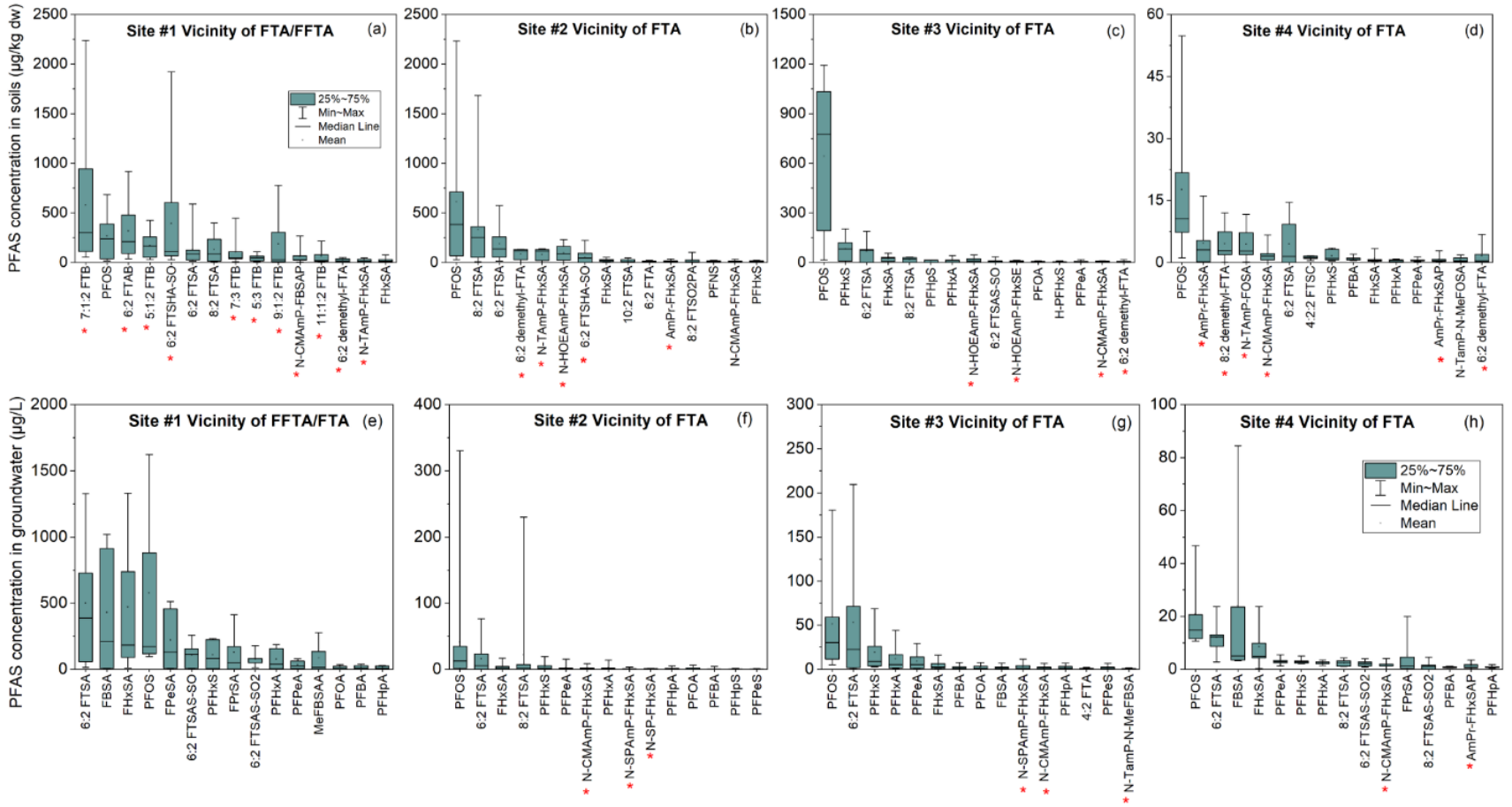


Figure A.7 The fifteen highest PFAS measured in AFFF-impacted soils (a-d) and groundwater (e-h) in the vicinity of FTA area at the four FTA sites. The zwitterionic and cationic precursors were marked with a red asterisk (*).

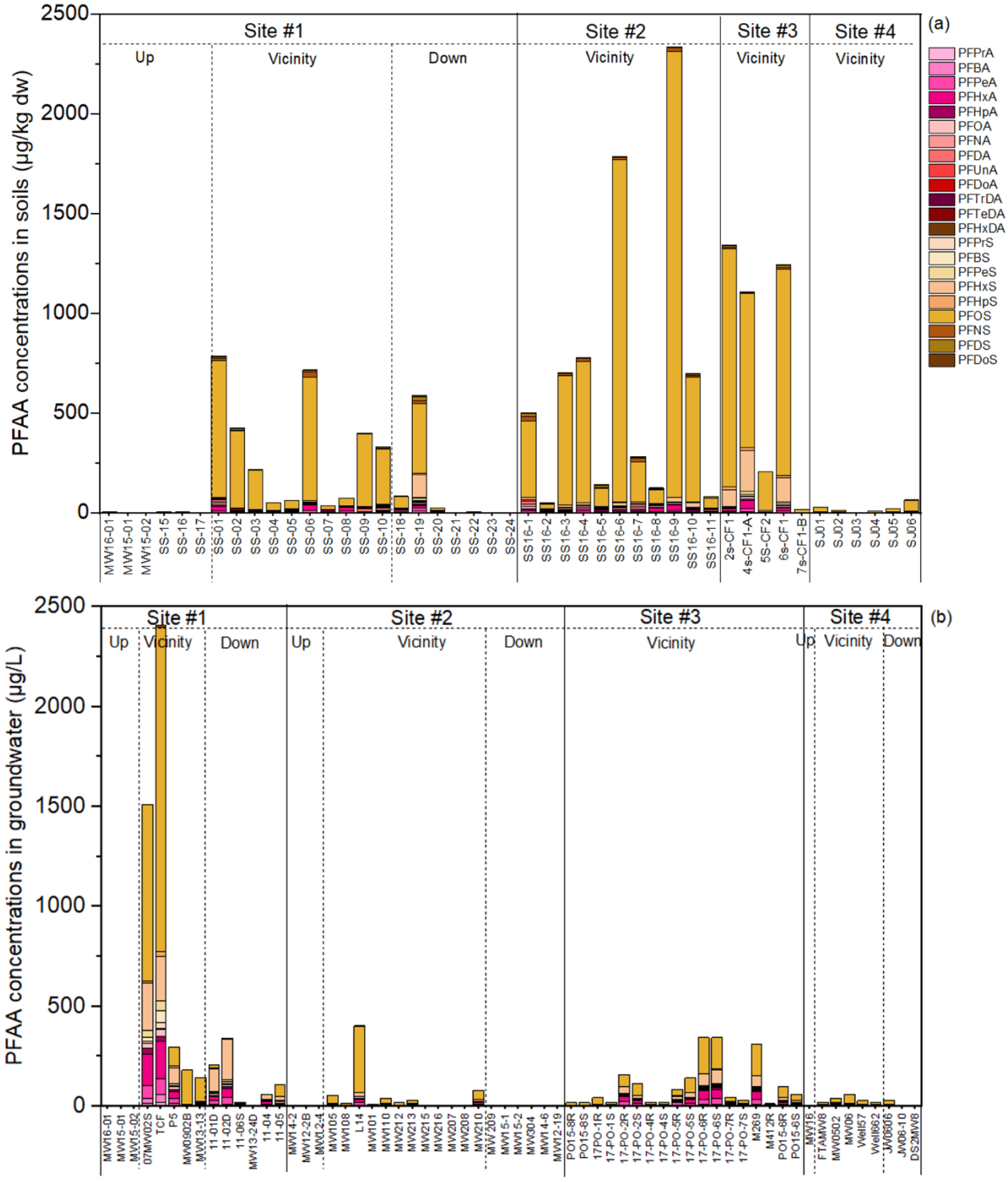


Figure A.8 The PFAA concentrations in surface soil (a) and groundwater (b) samples from the four Canadian FTA sites**.**

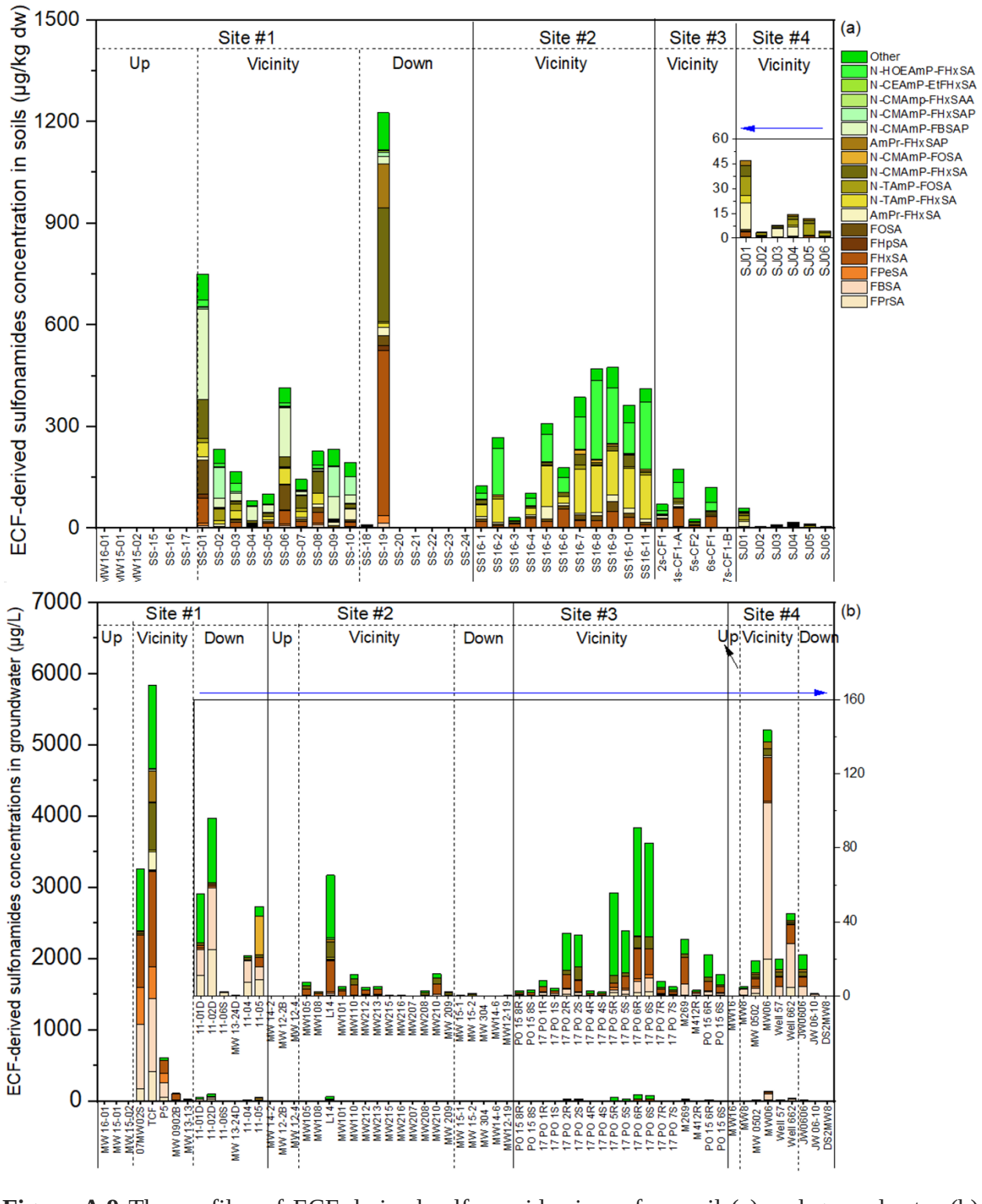
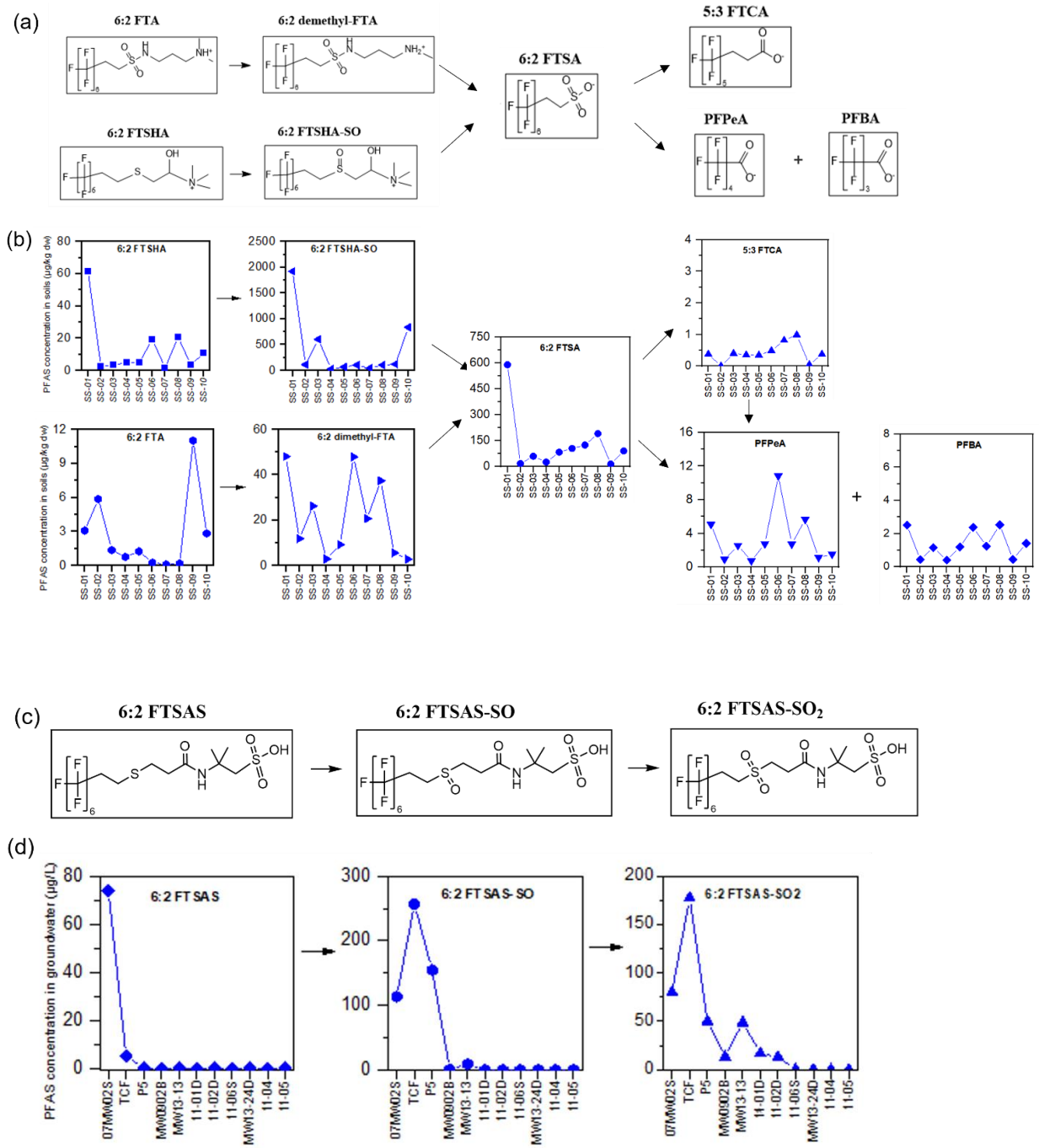


Figure A.9 The profiles of ECF-derived sulfonamides in surface soil (a) and groundwater (b) samples from the four Canadian FTA sites.


(a)
6:2 FTA
6:2 demethyl-FTA
6:2 FTSHA
6:2 FTSHA-SO
6:2 FTSA
5:3 FTCA
PFPeA
PFBA
(b)
6:2 FTSHA
6:2 FTSHA-SO
6:2 FTA
6:2 dimethyl-FTA
6:2 FTSA
5:3 FTCA
PFPeA
PFBA
PFAS concentration in soils (μg/kg dw)
(c)
6:2 FTSAS
6:2 FTSAS-SO
6:2 FTSAS-SO2
(d)
6:2 FTSAS
6:2 FTSAS-SO
6:2 FTSAS-SO2
PFAS concentration in groundwater (μg/L)
07MW02S
TCF
P5
MW0902B
MW13-13
11-01D
11-02D
11-06S
MW13-24D
11-04
11-05

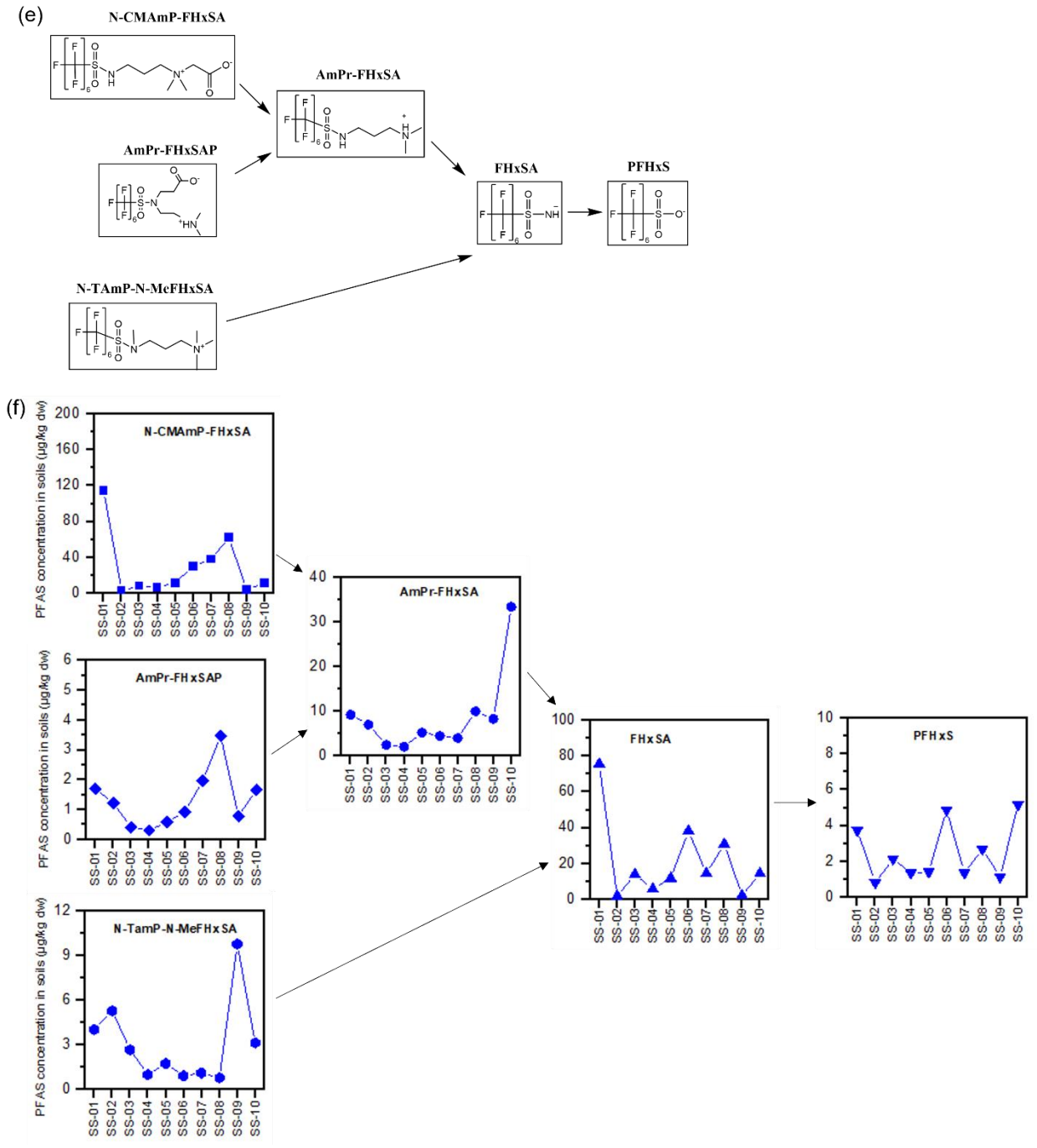


Figure A.10 Likely in-situ transformation pathways of fluorotelomer precursors in source zone soils (a) and groundwater (c) of Site #1 and the concentrations of the precursor and transformation products in soil (b) and groundwater (d) samples. Likely in-situ transformation pathways of ECF-derived sulfonamides in source zone soils of Site #1 (e) and the concentrations of the precursor and transformation products in all samples (f).

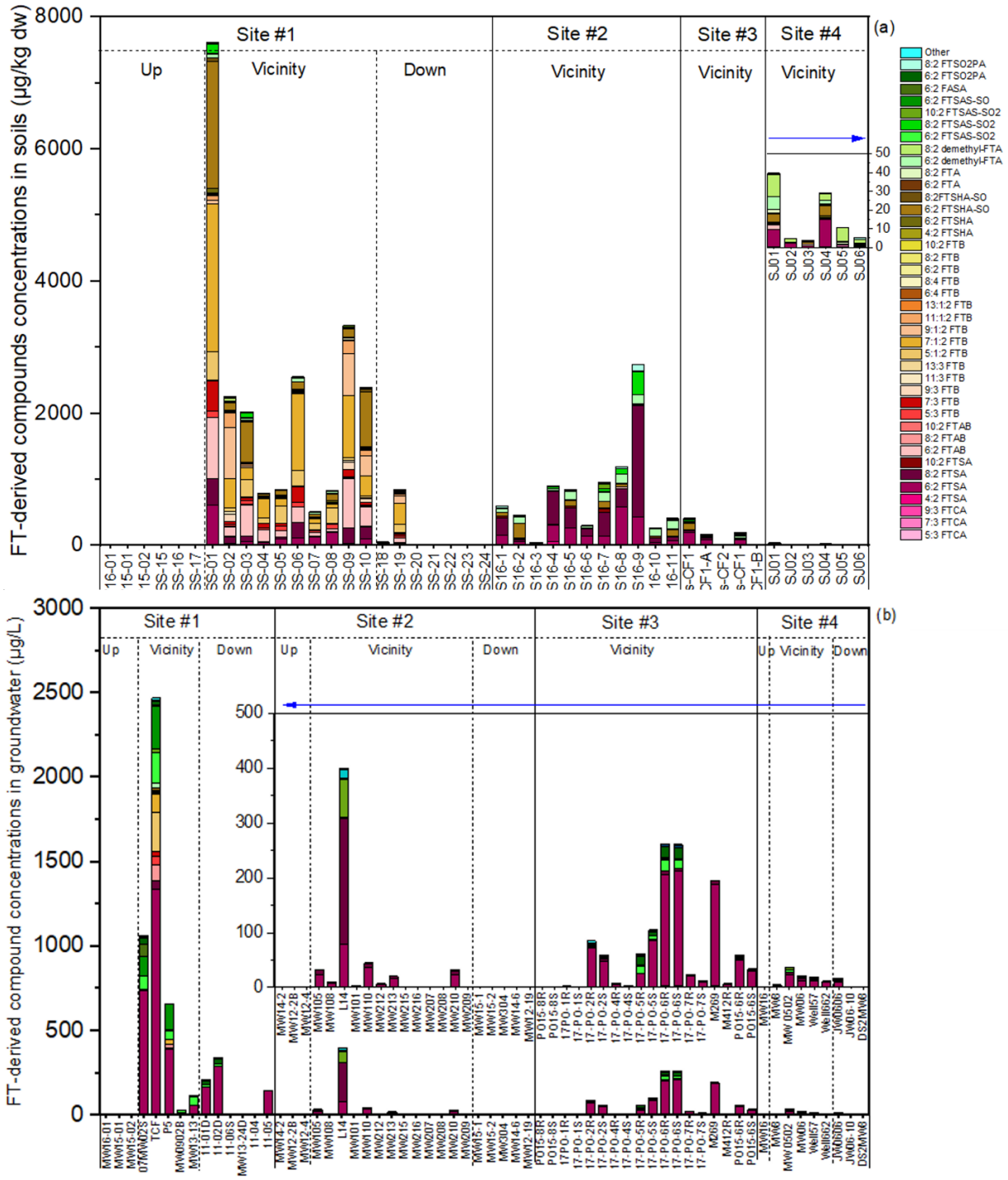


Figure A.11 The profiles of FT-derived compounds in surface soil (a) and groundwater (b) samples from the four Canadian FTA sites.

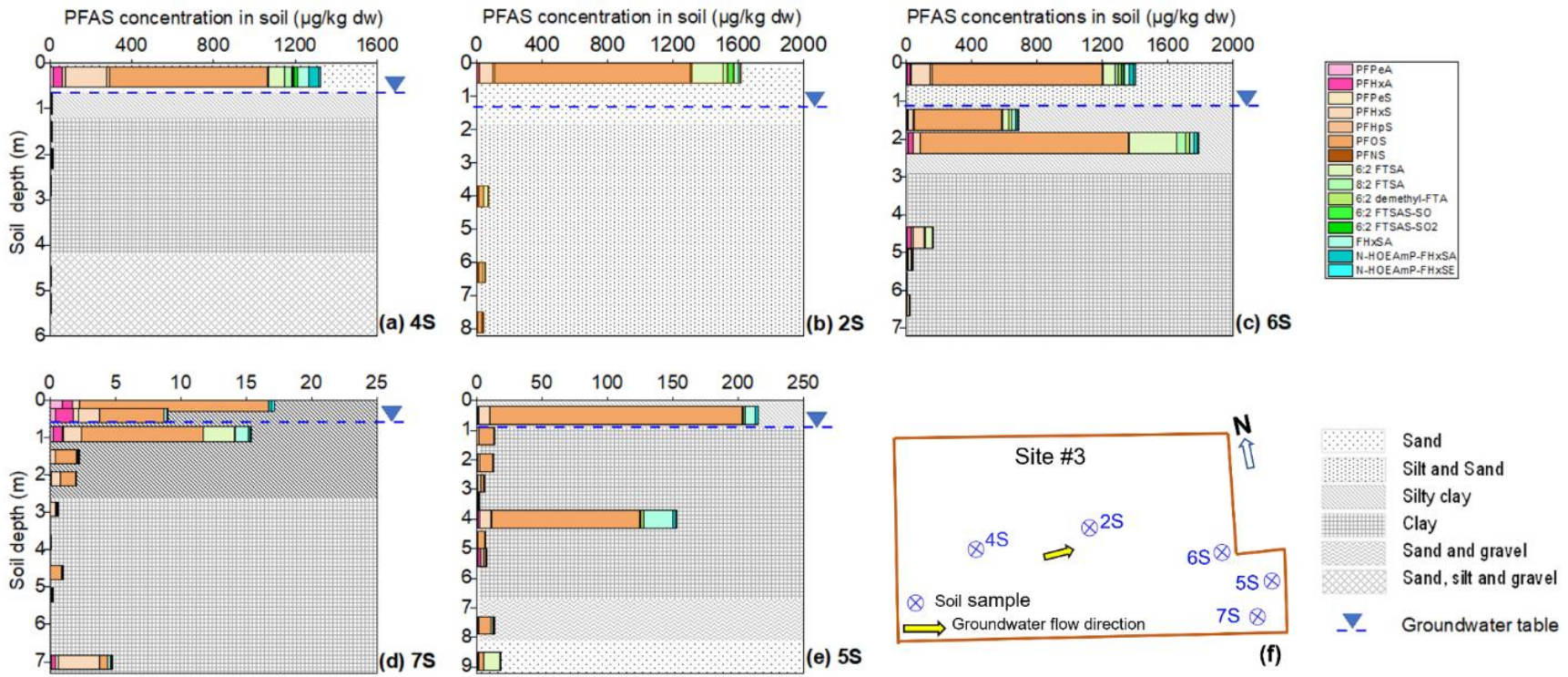


Figure A.12 The changes in the concentrations of 15 PFAS (mainly detected in surface soils) over depths at five sampling locations (a) 4S, (b)2S, (c) 6S, (d)7S, (e) 5S at site #3. The five sample locations are shown in the scheme map (f).

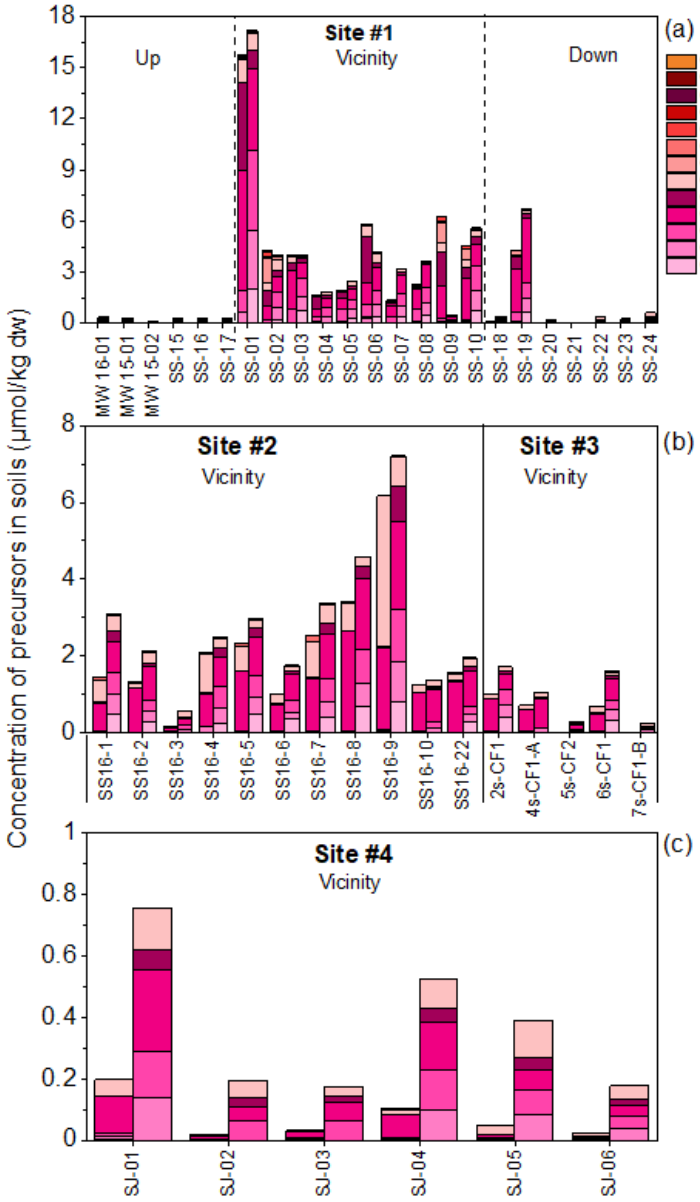
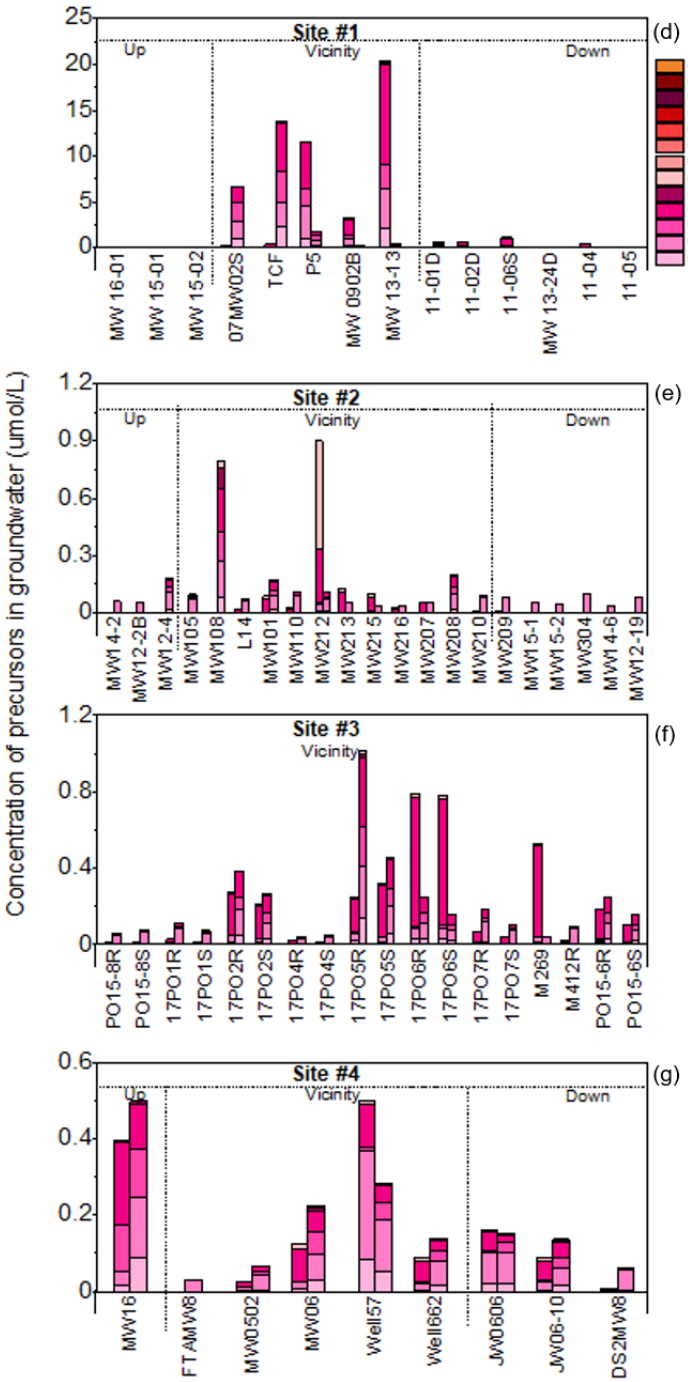


Figure A.13 The concentration of both known and total precursors in (a-c) surface soil and (d-g) groundwater samples at the four Canadian FTA sites. The left bar represents the concentration of known precursors identified via UHPLC-HRMS through the target and suspect-screening methods, while the right bar shows the concentration of total precursors determined by TOP assay. The C_3-C_{15} represented the carbon numbers of the known precursors (left bars) and the carbon number of PFCA produced from the precursors (right bars).


Concentration of precursors in groundwater (umol/L)
Site #1
(d)
Up
Vicinity
Down
C16
C14
C13
C12
C11
C10
C9
C8
C7
C6
C5
C4
C3
MW 16-01
MW 15-01
MW 15-02
07MW02S
TCF
P5
MW 0902B
MW 13-13
11-01D
11-02D
11-06S
MW 13-24D
11-04
11-05
Site #2
(e)
MW14-2
MW12-2B
MW12-4
MW105
MW108
L14
MW101
MW110
MW212
MW213
MW215
MW216
MW207
MW208
MW210
MW209
MW15-1
MW15-2
MW304
MW14-6
MW12-19
Site #3
(f)
PO15-8R
PO15-8S
17PO1R
17PO1S
17PO2R
17PO2S
17PO4R
17PO4S
17PO5R
17PO5S
17PO6R
17PO6S
17PO7R
17PO7S
M269
M412R
PO15-6R
PO15-6S
Site #4
(g)
MW16
FTAMW8
MW0502
MW06
Well57
Well662
JW0606
JW06-10
DS2MW8

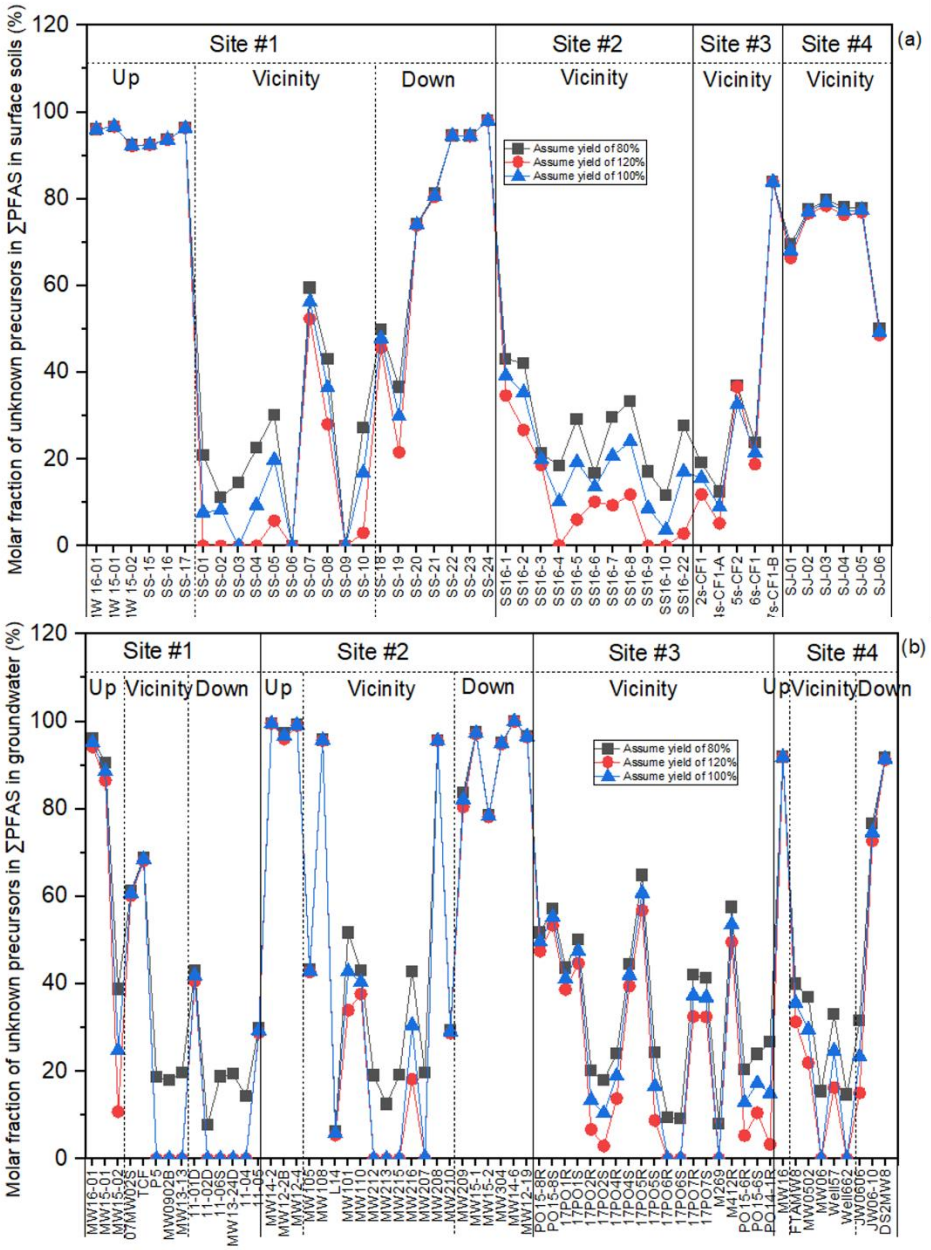


Figure A.14 **The molar fraction of unknown precursors in ∑PFAS in both surface soil (a) and groundwater (b) when assuming molar PFCA yields of 80%, 100% and 120% from TOP.**

Text A.9 References

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Appendix B. Supplemental information for Chapter 4

Text B.1 Synthesis of PFOSB, PFOAB and PFOSAm

The synthesis processes for PFOSB, PFOAB, and PFOSAm were as previously reported:[1] (1) the compounds containing a sulfonamide group start with the reaction of perfluorooctanesulfonyl fluoride [POSF, $F(CF_2)_8SO_2F$] with N,N-dimethyl-1,3-propanediamine to yield PFOSAm [$F(CF_2)_8SO_2NHC_3H_6N(CH_3)_2$] (Mejia-Avendaño, Duy et al. 2016), which is then reacted with sodium chloroacetate ($ClCH_2COONa$) to produce PFOSB; (2) analogously, the amide compound was synthesized via an identical approach with the only difference of using $F(CF_2)_7CONH(CH_2)_3N(CH_3)_2$ as the synthesis starting material.

Text B.2 Additional information on chemicals and materials

A standard mixture of PFAAs (2 μg mL^{-1} as compound or salt, >98% purity), which included PFOS (linear isomer) and PFOA (linear isomer), was obtained from Wellington Laboratories (Guelph, ON, Canada). Other PFAS standards, including N-ethyl perfluorooctane sulfonamide (EtFOSA, 50 μgmL^{-1}, >98% purity), perfluorooctane sulfonamide (FOSA, 50 μgmL^{-1}, >98% purity) and perfluorooctane sulfonamide acetate (FOSAA, 50 μgmL^{-1}, >98% purity), and isotopically labeled internal standards perfluoro-1-[13C8] octanesulfonamide (M8FOSA-I) and N-ethyl-d5-perfluoro-1-octanesulfonamide (d-N-EtFOSA-M), were also obtained from Wellington Laboratories (Guelph, ON, Canada). HPLC-grade acetonitrile (ACN) and methanol (MeOH), LC/MS-grade water and formic acid, certified sodium hydroxide (NaOH, 1 N) and hydrochloric acid (HCl, 1 N), and ACS-grade calcium chloride (CaCl2) were purchased from Fisher Scientific (Whitby, ON, Canada). Ammonium acetate (purity ≥ 98%) and anhydrous sodium acetate were acquired from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (25–30% in water) was obtained from Fisher Scientific (Whitby, ON, Canada). Nitrogen (N2) (purity 99.998%) was from MEGS InB. (St-Laurent, QC, Canada). The high-speed vortex (LP Vortex Mixer) was from Fisher Scientific (Whitby, ON, Canada).

Text B.3 Soil Microcosm Setup

Based on "OECD Guideline 304A–Inherent Biodegradability in Soil", two types of soils, including a sandy loam soil (referred as M soil) and a loam soil (referred as P soil), were selected to be used

for the microcosm experiments. M soil was collected from McGill University McDonald campus in Montreal, QC, while P soil was collected from an urban forest area next to Rue de Gaspé, Verdun, Montreal, QB. Each soil was sieved with a 2-mm sieve immediately upon collection, stored at 4°C, and used within three months. Part of the soil was rendered sterile by the autoclaving and addition of antibiotics (chloramphenicol, kanamycin, and cycloheximide) as described in Mejia-Avendaño et al.[5] The soil moisture was adjusted to a gravimetric moisture content of 20%-23%, representing ca. 80% of the soil water holding capacity.

Text B.4 Purification of parent compounds

Methods. The fractionation procedure modified from Ballesteros-Gómez et al.[2] was used to eliminate PFAA impurities (PFOS or PFOA) from PFOSB, PFOAB and PFOSAm solutions used in biodegradation experiments.

Aqueous diluted methanolic solutions of PFOSB or PFOAB or PFOSAm were loaded into Strata X-AW cartridges (200 mg/6 mL). After sample loading, the cartridges were rinsed with 25 mM sodium acetate (pH adjusted to 4 with acetic acid) and left to dry under vacuum for 1 hour. Afterwards, 4 mL of methanol and 4 mL of 0.2% NH_4OH in methanol were applied sequentially to collect Fractions A (PFOSB or PFOAB or PFOSAm) and B (PFOA or PFOS impurity), respectively. The eluents were then analyzed by high-resolution mass spectrometry (UHPLC-Orbitrap MS), along with reference solutions, i.e., PFAC-MXC standard for PFAA quantitation, and non-purified PFOSB, PFOAB and PFOSAm solutions to estimate the SPE recovery.

Results. The initial PFOSB methanolic solution contained PFOSAm (12 mol%) and PFOS (0.66 mol%) as impurities, and PFOAB contained PFOA (10 mol%) as an impurity. After SPE fractionation of the original PFOSB solution, a recovery of 80% can be achieved for PFOSB, and the level of PFOS in the purified PFOSB solution was nondetectable (lower than the detection limit or <0.006 mol%). But the coexistent synthetic intermediate PFOSAm could not be fractionated from PFOSB due to their similar physicochemical properties; thus, the purified PFOSB solution still contained 12 mol% of PFOSAm.

Similarly, the SPE fractionation can achieve a satisfactory recovery (92%) of PFOAB and resulted in as low as 0.03 mol% of PFOA impurity in the purified PFOAB solution. Therefore, the fractionation was successfully applied to eliminate PFOA and PFOS from the initial solution of PFOAB and PFOSB, respectively, even though the removal of PFOSAm from PFOSB is challenging.

The initial PFOSAm contained 1.0 mol% of PFOS as an impurity. The SPE fractionation procedure successfully removed the PFOS in the original PFOSAm methanolic solution and resulted in a satisfactory recovery of PFOSAm (94%).

Text B.5 Sample preparation

The soil samples were extracted by a previously reported method.[3] Approximately 2 grams (dw) of soil were placed in a 15 mL polypropylene centrifuge tube and submitted to three sequential solvent extraction cycles. Each cycle consisted of the addition of 4 mL of 400 mM of ammonium acetate in methanol followed by high-speed vortexing for 0.5 min, a 10 min ultrasonication step, and centrifugation (5000 rpm, 5 min). The extracts from the three cycles were combined, and if needed the volume was adjusted to 12 mL with methanol. A small aliquot (1 mL) was then collected ("Fraction O") and stored at -20°C until instrumental analysis. The remaining extract (11 mL) was submitted to SPE fractionation as follows. The extract was first concentrated to 1 mL using nitrogen evaporation (45℃). The concentrated extracts were diluted in HPLC-grade water prior to loading into previously conditioned Strata X-AW cartridges. After loading, the cartridges were rinsed with 4 mL of a solution of 25 mM sodium acetate buffer (previously adjusted to pH 4.5 with concentrated acetic acid). The cartridges were left to dry for 1 h under vacuum. The extracts were then eluted by 4 mL of methanol twice to collect fraction A (cationic and neutral fraction), after which 4 mL of 0.2% NH_4OH in methanol was applied twice to collect fraction B (anionic fraction). For fraction A, the final extract volume was adjusted to ~8 mL, while fraction B was concentrated by nitrogen evaporation (45℃) to 2 mL. Before instrumental analysis, 150 µl of each fraction (Fraction O, A and B) were separately spiked with a mix of internal standards (50 µl) for a final concentration of 2.5 ng/mL each. The levels of PFOSB and PFOAB were quantified

in Fraction O; the amount of PFOSAm, EtFOSA, FOSA and FOSAA were quantified in Fraction A, while the level of PFOS and PFOA were quantified in Fraction B.

Text B.6 High-resolution MS/MS analysis

The day 60 sample collected during PFOSB/PFOAB incubation in both live and sterile sandy loam soil was further concentrated and analyzed under t-MS^2 mode (Orbitrap Q-Exactive). Fraction A eluent was further concentrated 8 times, and Fraction B eluent was concentrated 4 times under a gentle stream of N_2 at 40℃. Compound #4 and #8 existed in Fraction B while other compounds (shown in Table 4.1 of the main text) were present in fraction A eluent. We provide in SI the full-scan MS and t-MS^2 chromatograms, as well as annotated MS/MS spectra with elucidated fragment ions and their corresponding mass accuracy.

Text B.7 Identification of suspected abiotic and biotic transformation products

The chromatograms, mass spectra, and monoisotopic intensity distribution of these compounds are shown in Figure B6. The signal intensity at several time points (day 0, 15, 30, 45, 60, 90 and 150) were also presented, showing the temporal trend of potential transformation products in both live and sterile soils.

Compounds #1(PFOAAm), #4 (perfluorooctane amide propionate), and #8 (perfluorooctane sulfonamide propionate) and #9 (perfluorooctane sulfonamide sulfinate) were identified with high confidence and assigned to the probable diagnostic structure (level 2b). Compounds #2 and #6 were assigned the tentative candidate category (level 3). The MS/MS spectrum could not be generated for compounds #3, #5, #7, #10 and #11 due to low response of the parent ion; these compounds were therefore assigned a confidence level of 4 (unequivocal molecular formula).

Text B.8 Procedures for spike recovery assessment

Prior to the start of the soil biotransformation study, the extraction solvent (methanol with 400 mM of ammonium acetate) was tested for recovering PFOSB, PFOAB and their possible transformation products from soil.

In each soil matrix, the recovery was determined based on the analyte response in soil samples spiked before extraction, divided by that in the matrix-matched extracts spiked at the end of the preparation procedure. Further details are provided below.

Spiked Before samples: About 2.0 g of soil (oven-dry weight) was weighed into a 15-mL polypropylene tube and spiked with PFOSB (or PFOAB) to give an initial concentration of about 200 ng g-1 -soil for each perfluoroalkyl betaine or spiked with other possible products (including PFOSAm, EtFOSA, FOSA, FOSAA, PFOS, PFOA) to give an initial concentration of about 10 ng g^{-1} for each. Then soil was extracted with 400 mM of ammonium acetate in methanol at 1:2 (w:v) soil:solvent ratio. The soil-solvent slurry was subject to high-speed vortexing for 0.5 min followed by a 10 min ultrasonication step and centrifugation (5000 rpm, 5 min). The supernatant was pipetted out and transferred to a polypropylene tube. The same extraction process of the soil sample was repeated twice more.

Spike After samples: Another triplicate of live soil sample was not spiked initially with PFAS but subject to the same extraction and fractionation procedure as the spiked before samples. They were spiked just before LC-MS analysis. Such samples were referred to as "Spiked After", and are used to provide a post-extraction matrix-matched reference for calculating the recovery.

Non-Spiked reference: A triplicate of live soil samples not spiked with PFAS were also extracted and fractionated using SPE in the same fashion. No native standards were added to these samples. These samples were referred to as "Non-spiked" samples.

For all three sets of samples (Spiked Before, Spiked After, and Non-Spiked samples), the internal standards (IS) were added at the end of the sample preparation process (just before LC-MS analysis).

Calculation of the recovery. The recovery of native analytes from the soil samples (i.e., live sterile soil) was determined as follows: Recovery (%) = $100\times \frac{SB-NS}{SA-NS}$.

Where "SB" is the analyte to IS response ratio of the sample spiked at the start of the preparation procedure with native analytes ("Spiked Before" samples), "SA" is the analyte to IS response ratio of the sample spiked at the end of the preparation procedure with native analytes ("Spiked After" samples), and "NS" is the analyte to IS response ratio of the reference (Non-spiked samples).[1]

Text B.9 Assessment of linearity performance and matrix effect

Linearity performance. Calibration curves were generated in the matrix-free solvent (MeOH) and also in live soil final extracts by spiking native analytes at 7 incremental calibration levels while the concentration of the internal standards was set at an intermediate level of 2.5 ng mL^{-1}. Using both the parent compound (PFOSB/PFOAB/PFOSAm) and suspected intermediate transformation products (FOSA, FOSAA, PFOS and PFOA) spiking solutions, the matrix-matched calibration levels were constructed in such a way that the soil matrix concentration would be constant between the different calibration levels. Additionally, the matrix dilution factor of the calibration curve levels was equivalent to that of the samples. Inverse-weighted (1/x) linear regressions were generated by plotting the native analyte to internal standard peak area ratio (y-axis) as a function of native analyte spiked concentration (x-axis). Linearity range, determination coefficients (R^2), and bias between calculated-back ($\hat{x}$) and expected (x) concentrations were monitored.

Absolute matrix effect. This parameter was assessed by comparing the absolute responses of isotope-labeled internal standards in each of the soil matrices to those in the matrix-free solvent reference, following the methodology previously described.[4]

Effective matrix effect. The soil matrix was subject to the previously mentioned extraction procedure. Native PFASs (PFOSB, PFOAB and their quantifiable transformation products) and isotope-labeled ISs were spiked post-extraction to create matrix-matched calibration curves. Meanwhile, a clean solvent spiked with native PFAS analytes was also used to produce a matrix-free calibration curve. The slopes of the resulting matrix-matched calibration curves (based on area ratios of analytes to internal standards) were then compared to those prepared in a clean solvent to assess the effective matrix effects at the instrumental stage.

Effective matrix effects were evaluated by comparing the solvent-based slope (S) to that of soil extracts spiked post-extraction (M) corrected by the non-spiked sample initial contribution (ref), as described previously[5]:

Effective matrix effect (%) = $100\times \left(\frac{\mathrm{M-ref}}{\mathrm{S}}-1\right)$

Results of the matrix effect assessment. There was no significant difference between the absolute signal of each internal standard in live soil matrix (or sterile soil matrix) and that in the solvent reference. Thus, the absolute matrix effect of each internal standard used for quantification was found to be low to moderate (less than $\pm$ 15%) (Table B.8 (a)). In addition, the effective matrix effect for each analyte was suitable (<5%) (Table B.8 (b)).

Text B.10 Kinetic modeling of PFOAB, PFOSB and PFOSAm biotransformation

The kinetics of PFOAB, PFOSB and PFOSAm was modeled using Kinetic Graphic User Interface (KinGUII) v2.12, a model developed based on R for environmental fate studies. Four kinetic models used in a previous biotransformation study,[5] including Single First Order (SFO), Double First-Order in Parallel (DFOP), Hockey Stick (SH) and First Order Multi Compartments (FOMC) models, were tested to fit with all individual data points. A goodness of fit test was applied with χ^2 value, and since all models passed the test, χ^2 error was set as the measurement of comparison between models. χ^2 error was defined as

$$x^2_{error} = 100 \cdot \sqrt{\frac{1}{x^2_{tab}} \sum \frac{(C-O)^2}{\bar{O}^2}}$$

Where C is the calculated value at time i, O is a single observed value at time i, $\bar{O}$ is the average of the observed values at time i, and χ^2_{tab} is the tabulated χ^2 value for the corresponding degrees of freedom at $\alpha = 0.05$. The model with the smallest χ^2 error is the best fit for the data. Table A.11 shows the comparison of the results of the four models, by comparing the value of χ^2 error, it was decided that the best fit for PFOAB transformation data in live M soil was the SFO model.

Table B.1 (a) The acronyms of PFOSB, PFOAB, PFOSAm, PFOAAm, PFOSNO and PFOANO used in previous literature. (b) Name, acronyms and formula of native and isotope labelled PFAS standards.

(a)

Name of PFAS in the present study	Acronyms also used in literature	Relevant literature
PFOSB	N-CMAmPFOSA,[6] CMeAmPr-FOSA[7]	6, 7
PFOSAm	PFOSaAm, [8] AmPr-FOSA[7]	7-9
PFOSAmS	N-TAmPFOSA,[6] TAmPr-FOSA[7]	6, 7
PFOSNO	PFOSNO,[10] N-OxAmPFOSA,[6] OAmPr-FOSA[7]	6, 7, 10
PFOAB	PFOAB,[10] BPr-FOAd[7]	7, 10
PFOAAm	PFOAAm,[10] AmPr-FOAd[7]	7, 10
PFOAAmS	PFOAAmS[5]	5
PFOANO	PFOANO[10]	7, 10

(b)

Acronym	Name	Formula
Native standards		
PFOA	Perfluorooctanoic acid	$F(CF_2)_7COOH$
PFOS	Perfluorooctane sulfonate	$F(CF_2)_8SO_3^-$
EtFOSA	N-ethyl perfluorooctane sulfonamide	$F(CF_2)_8SO_2NHCH_2CH_3$
FOSAA	Perfluorooctane sulfonamidoacetic acid	$F(CF_2)_8SO_2NHCH_2COOH$
FOSA	Perfluorooctane sulfonamide	$F(CF_2)_8SO_2NH_2$
PFOSAm	Perfluorooctane sulfonamido amine	$F(CF_2)_8SO_2NHCH_2CH_2CH_2N(CH_3)_2$
PFOSB	Perfluoroctane sulfonamido betaine	$F(CF_2)_8SO_2NH(CH_2)_3N^+(CH_3)_2CH_2COOH$
PFOAB	Perfluorooctane amido betaine	$F(CF_2)_7CONH(CH_2)_3N^+(CH_3)_2CH_2COOH$
Internal standards		
MPFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	$F(CF_2)_4(^{13}CF_2)_3{}^{13}COOH$
MPFOS	Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	$F(CF_2)_4(^{13}CF_2)_4SO_3^-$
d5-EtFOSA-M	Ethyl-d_5-perfluorooctanesulfonamide	$F(CF_2)_8SO_2NHCD_2CD_3$
M8FOSA-I	Perfluoro-1-[^{13}C8] octanesulfonamide	$F(^{13}CF_2)_8SO_2NH_2$
PFOAAmS	Perfluorooctane amido ammonium salt	$F(CF_2)_7CONHCH_2CH_2CH_2N^+(CH_3)_3$

Table B.2 Molar fraction (mol%) of certified standards determined in an individual solution of synthesized compounds before purification.

Possible impurity	Initial compound				
	PFOAB	**PFOSB**	**PFOSAm**	**PFOAAmS**	**PFOSAmS**
PFOS	n.d.	0.66	1.0	n.d.	0.03
PFOA	10.0	n.d.	n.d.	n.d.	n.d.
FOSA	n.d.	n.d.	n.d.	n.d.	n.d.
EtFOSA	n.d.	n.d.	n.d.	n.d.	n.d.
FOSAA	n.d.	n.d.	n.d.	n.d.	n.d.
EtFOSAA	n.d.	n.d.	n.d.	n.d.	n.d.
PFOSAm	n.d.	10.4	–	n.d.	n.d.

n.d: non-detected.

Based on these results, the PFOS and PFOA impurities present in PFOAB, PFOSB or PFOSAm solutions were then removed as per the fractionation procedure described in Text B.4.

Table B.3 Properties of soils used for the biotransformation study.

	M Soil	**P Soil**
Textural Class	Sandy loam	Loam
Sand percentage	64.9	47.2
Silt percentage	26.0	40.0
Clay percentage	9.1	12.8
Organic matter (%)	4.0	4.0
Bulk Density (kg/m^3)	1435	1107
pH	7.2	5.2
Cation Exchange capacity (CEC, meq/100g)	18.9	14.4
Phosphate (μg/g)	90	13
Potassium (μg/g)	158	62
Magnesium (μg/g)	142	139
Calcium (μg/g)	3260	1150
Sodium (μg/g)	31	23
Aluminium (μg/g)	465	1026
Fe(III) (μg/g)	8.7	8.6
Fe(II) (μg/g)	2.2	1.0
Nitrate Nitrogen (μg/g)	59	40
C/N ratio	11.3	7.8
Water holding capacity at 1/3 bar (%)	19.8	30.4
*Microbial biomass-C (μg/g)	8.4 ~ 10	8.0 ~ 9.7
Sampling location	McGill University, Macdonald Campus, Montreal, QC	Parc Elgar, Montreal, QB.

* Microbial biomass C was determined based on the soil ATP level at the last days of incubation; an ATP to soil biomass conversion factor of 10 ~ 12 μmol ATP/g biomass-C proposed by Contin et al.[11] was applied.

Table B.4 Background levels of PFAS in the non-spiked soils.

Analyte	Concentration, ng/g-dry soil (Average of 3 replicates ± standard deviation)	
	Sandy loam soil (M soil)	**Loam soil (P soil)**
PFBS	0.563±0.032	0.783±0.055
PFOS	0.400±0.573	0.130±0.225
PFDS	0.764±0.038	n.d.
PFHxA	0.289±0.172	0.330±0.124
PFHpA	0.202±0.026	0.216±0.031
PFOA	0.634±0.257	0.565±0.109
PFNA	0.153±0.018	0.128±0.006
EtFOSA	n.d.	n.d.
FOSA	n.d.	n.d.
FOSAA	n.d.	n.d.
PFOSB	n.d.	n.d.
PFOAB	n.d.	n.d.
PFOSAm	n.d.	n.d.

(n.d.: not detected)

Table B.5 Details on the analytical methods.

Instrument	Dionex UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer		
Ionization	Positive and negative heated electrospray		
Acquisition mode	Full scan MS mode (R: 70,000 at m/z = 200) t-MS^2 mode		
Analytical Column	Thermo C18 Hypersil aQ Gold column, 1.9 µm, 100 x 2.1mm		
Delay Column	Thermo Hypercarb trap column, 7 µm, 20 x 2.1 mm,		
Column Temperature	40°C		
Mobile Phases	A: 0.1% formic acid in LCMS water B: 0.1% formic acid in acetonitrile		
Gradient Profile	Time (min)	Percentage B	Flow Rate (mL/min)
	0.0	10	0.550
	7.5	72.5	0.550
	8.5	100	0.550
	12.5	100	0.550
	12.6	10	0.550
	14.5	10 (Stop)	0.550
Injection Volume	10 µL (Full scan) 10 µL (t-MS^2)		

Table B.6 List of quantifiable PFASs in full-scan mode.

Acronym	$[M+H]^+$ or $[M-H]^-$	Theoretical m/z	Observed m/z	Error (ppm)	Retention time (min)
PFOA	$[C_8F_{15}O_2]^-$	412.96643	412.96711	1.647	5.27
PFOS	$[C_8F_{17}SO_3]^-$	498.93022	498.93128	2.125	6.46
EtFOSA	$[C_{10}F_{17}H_5NSO_2]^-$	525.97750	525.97819	1.312	8.41
FOSAA	$[C_{10}F_{17}H_3NSO_4]^-$	555.95168	555.95195	0.486	7.46
FOSA	$[C_8F_{17}HNSO_2]^-$	497.94620	497.94774	3.093	7.56
PFOSAm	$[C_{13}F_{17}H_{14}N_2SO_2]^+$	585.04990	585.04893	-1.658	6.34
PFOSB	$[C_{15}F_{17}H_{16}N_2SO_4]^+$	643.05538	643.05483	-0.855	6.32
PFOAB	$[C_{15}H_{16}F_{15}N_2O_3]^+$	557.09159	557.09349	3.411	5.38
Internal standards					
MPFOA	$[^{13}C_4C_4F_{15}O_2]^-$	416.97985	416.98049	1.535	5.27
MPFOS	$[^{13}C_4C_4F_{17}SO_3]^-$	502.94364	502.94431	1.332	6.46
d-EtFOSA-M	$[C_{10}F_{17}D_5NSO_2]^-$	531.00830	531.01093	4.953	8.41
M8FOSA-I	$[^{13}C_8F_{17}HNSO_2]^-$	505.97249	505.97464	4.249	7.56
PFOAAmS	$[C_{15}H_{16}F_{15}N_2O]^+$	513.10176	513.10183	0.136	5.49

Table B.7 Summary of retention time (RT), instrumental limit of detection (iLOD), instrumental limit of quantification (iLOQ), method limit of detection (mLOD), method limit of quantification (iLOQ), linearity range (ng/mL) and determination coefficient (R2) of calibration curves for the targeted analytes.

Analyte	RT (min)	iLOD (ng/mL)	iLOQ (ng/mL)	mLOD (ng g^{-1})	mLOQ (ng g^{-1})	Linear range (ng/mL)	R^2
PFOSB	6.32	0.07	0.10	0.56	0.80	0.20-50	0.992
PFOAB	5.38	0.08	0.10	0.64	0.80	0.10-50	0.999
PFOSAm	6.34	0.01	0.05	0.06	0.29	0.050-50	1.000
EtFOSA	8.41	0.01	0.05	0.06	0.29	0.025-50	0.997
FOSA	7.56	0.01	0.05	0.06	0.29	0.025-50	0.993
FOSAA	7.46	0.02	0.05	0.03	0.07	0.050-50	0.998
PFOS	6.46	0.02	0.05	0.03	0.07	0.025-50	0.993
PFOA	5.27	0.009	0.02	0.01	0.03	0.025-50	0.996

Table B.8 Spike recoveries (average ± standard deviation, n = 3) of parent compounds and their quantifiable biotransformation products in two soils.

Analytes	Live M (sandy loam) soil	Live P (loam) soil
PFOSB	84±4%	83±2%
PFOAB	84±5%	83±2%
PFOSAm	81±3%	82±4%
FOSA	83±2%	82±1%
EtFOSA	73±3%	74±3%
FOSAA	80±3%	73±4%
PFOS	91±2%	89±7%
PFOA	106±2%	97±6%

Table B.9 Absolute and effective matrix effects in the live soil matrix.

(a) **Absolute matrix effects (%)** of internal standards in the live soil matrix.

Internal standard	Matrix effect % Live M (sandy loam) soil	Matrix effect % Live P (loam) soil
M-PFOA	5	10
M-PFOS	9	13
M8FOSA-I	-8	-8
d-N-EtFOSA-M	7	7
PFOAAmS*	4	-1

*Used as IS for PFOSB and PFOAA.

(b) Effective matrix effects (%) of native analytes in the live soil matrix.

Analyte	Matrix effect % Live M (sandy loam) soil	Matrix effect % Live P (loam) soil
PFOSB	-8.6	-5.2
PFOAB	3.3	-6.3
PFOSAm	-4.0	-0.2
FOSA	3.3	-8.6
EtFOSA	-2.0	4.6
FOSAA	-2.6	-7.3
PFOS	-2.5	-2.1
PFOA	-0.1	-0.2

Table B.10 Whole-method accuracy, intraday and interday precision.

Analyte	Whole-method accuracy (%)	Intraday precision (%) RSD (n=5)	Interday precision (%) RSD (n=15)
PFOSB	89.8-111.7	2.0	1.4
PFOAB	89.2-108.9	1.9	2.3
PFOSAm	92.0-110.6	6.7	12.4
FOSA	92.6-108.5	2.7	11.1
EtFOSA	93.0-109.3	2.8	9.9
FOSAA	91.4-111.1	4.8	7.7
PFOS	92.9-106.8	2.9	13.2
PFOA	90.6-111.0	1.8	8.7

Table B.11 The concentrations of total ATP in both live and sterile soils.

Soil treatments	Relative Light Unit (RLU)	ATP level (pg ATP/g-soil)	Estimated Soil Biomass* (µg biomass-C/g-soil)
Live M soil for PFOAB/PFOSB study	17000	42746	8.4 ~ 10
Sterile M soil for PFOSAm study	29	72.9	0.014 ~ 0.017
Sterile M soil for PFOSB study	104	261.5	0.052 ~ 0.062
Live P soil for PFOAB/PFOSB study	16235	40822	8.0 ~ 9.7
Sterile P soil for PFOSAm study	22	55.3	0.011 ~ 0.013
Sterile P soil for PFOSB study	24	60.3	0.012 ~ 0.014

* Calculation was performed based on the ATP to soil biomass conversion factor of 10 ~ 12 µmol ATP/g biomass-C proposed by Contin et al.[7]

Table B.12 (a) Comparison of results of different kinetic models for biotransformation of PFOAB in the sandy loam (M) soil. (b) Summary of the kinetics parameters of fitting the proposed pathways (Figure 4 and S5) to PFOAB, PFOSB and PFOSAm experiment data in live M soil.

(a)

Model	# Data sets	# Parameters, n	Degrees of freedom, m	x^2_{error}
SFO	24	2	6	3.670
DFOP	24	4	4	4.190
HS	24	4	5	4.190
FOMC	24	3	5	3.915

*The SFO model fits the best due to the lowest x^2_{error}.Type equation here.

(b)

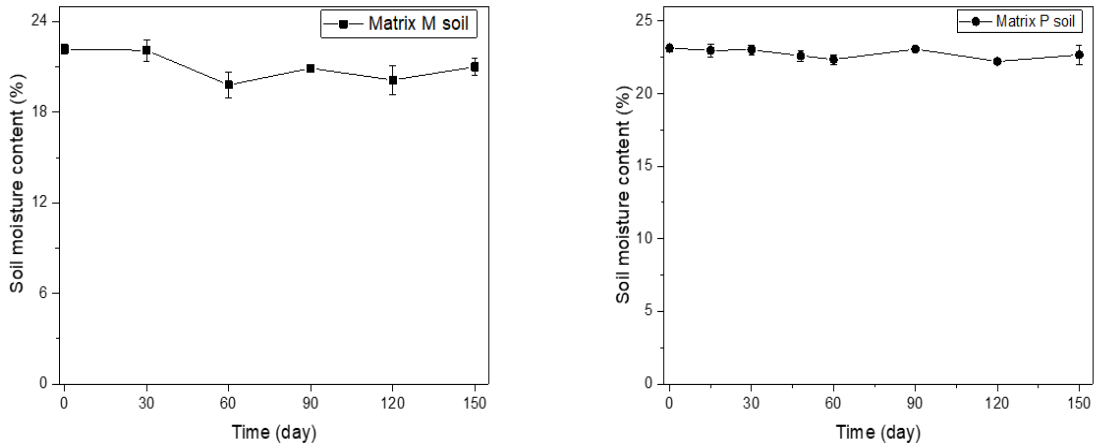
Parent compound	Biotransformation step	Formation fraction, FF		Parent compound	DT_{50}	R^2
		Average	SD			
PFOAB	PFOAB→PFOAAm	0.0736	0.0332	PFOAB	265.8	0.8831
	PFOAAm→PFOA	*0.0000*	*11.669*	PFOAAm	14.1	0.9655
	PFOAB→PFOA	0.8041	2.2669	PFOA	>1000	0.8444
				Overall Goodness of Fit		0.9935
PFOSB	PFOSB→PFOSAm	1.000	0.5018	PFOSB	674.7	0.2697
	PFOSB→PFOS	*0.000*	*0.4036*	PFOSAm	15.7	0.9973
	PFOSAm→FOSAA	0.0012	0.0004	FOSAA	139.0	0.7969
	PFOSAm→FOSA	0.0232	0.0055	FOSA	>1000	0.9479
	FOSAA→FOSA	1.000	0.0000	PFOS	>1000	0.9544
	FOSA→PFOS	0.1399	0.0000			
	PFOSAm→PFOS	0.0358	0.0076			
				Overall Goodness of Fit		0.9849
PFOSAm	PFOSAm→FOSAA	0.0002	0.0000	PFOSAm	47.5	0.9696
	PFOSAm→FOSA	0.0952	0.0206	FOSAA	>1000	0.9397
	PFOSAm→PFOS	0.0317	0.0057	FOSA	>1000	0.9674
	FOSAA→FOSA	1.000	0.000	PFOS	>1000	0.9716
	FOSA→PFOS	*0.000*	*0.000*			
				Overall Goodness of Fit		0.9950

Table B.13 Transformation product yields from each source (PFOSB, PFOSAm impurity) in both types of live and sterile soils by day 90.

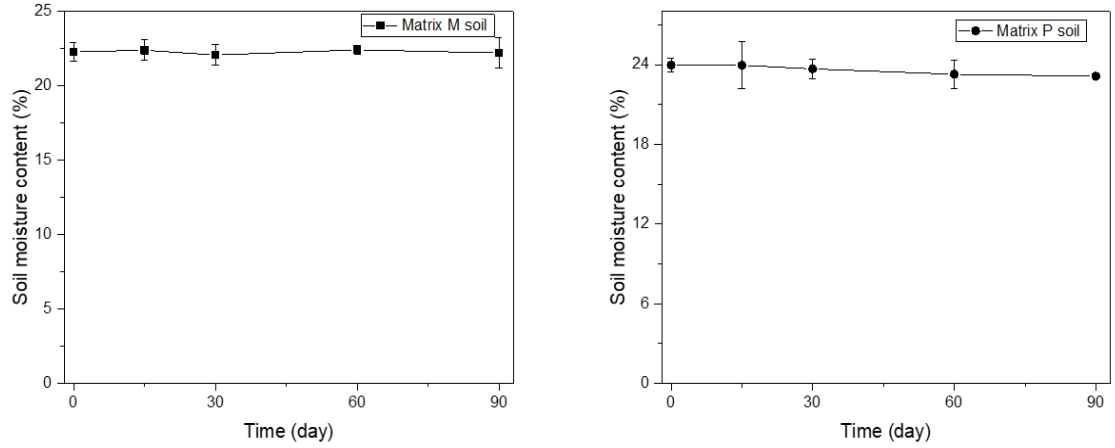
Soil name	Product yields from PFOSAm impurity (mol%)			Product yields from PFOSB (mol%)		
	FOSA	FOSAA	PFOS	FOSA	FOSAA	PFOS
Live M soil	0.80±0.067	0.001±0.000	0.27±0.007	0.52±0.045	0.064±0.004	1.53± 0.027
Sterile M soil	0.038±0.002	0	0.008±0.000	0.14±0.012	0.007±0.002	0.033±0.016
Live P soil	0.007±0.000	0	0.005±0.001	0.068±0.002	0.010±0.001	0.070±0.013
Sterile P soil	0.007±0.001	0	0.001±0.000	0.37±0.008	0.029±0.001	0.019±0.000

Table B.14 Details on qualitatively detected abiotic and biotic transformation products from PFOAB, PFOSB, and PFOSAm.

Parent compound	Number	Proposed formula	M, $[M+H]^+$ or $[M-H]^-$	ESI mode	Theoretical m/z	Observed m/z	Error (ppm)	RT (min)	Confidence level
PFOAB (PFOAAm impurity)	#1	$F(CF_2)_7CONH(CH_2)_3N(CH_3)_2$	$C_{13}F_{15}N_2OH_{14}^+$	ESI+	499.08611	499.08667	1.1	5.43	level 2b
	#2	$F(CF_2)_7CONH(CH_2)_3NH(CH_3)$	$C_{12}F_{15}N_2OH_{12}^+$	ESI+	485.07046	485.07190	3.0	5.39	level 3
	#3	$F(CF_2)_7CONH(CH_2)_3NH_2$	$C_{11}F_{15}N_2OH_{10}^+$	ESI+	471.05481	471.05667	3.9	5.23	level 4
	#4	$F(CF_2)_7CONH(CH_2)_2COOH$	$C_{11}F_{15}NO_3H_5^-$	ESI-	484.00244	484.00479	4.9	6.67	level 2b
	#5	$F(CF_2)_7CONHCH_2CH(OH)CH_2N^+(CH_3)_2CH_2COOH$	$C_{15}F_{15}N_2O_4H_{16}^+$	ESI+	573.08651	573.08813	2.8	5.57	level 4
PFOSB (with PFOSAm impurity)	#6	$F(CF_2)_8SO_2NH(CH_2)_3NH(CH_3)$	$C_{12}F_{17}SN_2O_2H_{12}^+$	ESI+	571.03425	571.03540	2.0	6.26	level 3
	#7	$F(CF_2)_8SO_2NH_2(CH_2)_2NH_2$	$C_{11}F_{17}SN_2O_2H_{10}^+$	ESI+	557.01860	557.01984	2.2	6.20	level 4
	#8	$F(CF_2)_8SO_2NH_2(CH_2)_2COOH$	$C_{11}F_{17}SNO_4H_5^-$	ESI-	569.96623	569.96906	5.0	7.59	level 2b
	#9	$F(CF_2)_8SO_2H$	$C_8F_{17}SO_2^-$	ESI-	482.93421	482.93649	4.7	6.11	level 2b
PFOSB	#10	$F(CF_2)_8SO_2NH(CH_2)_2CH(OH)N^+(CH_3)_2CH_2COOH$ or $F(CF_2)_8SO_2NHCH_2CH(OH)CH_2N^+(CH_3)_2CH_2COOH$ or $F(CF_2)_8SO_2NHCH(OH)(CH_2)_2N^+(CH_3)_2CH_2COOH$	$C_{15}F_{17}SN_2O_5H_{16}^+$	ESI+	659.05030	659.05072	0.6	6.16	level 4
	#11	$F(CF_2)_8SO_2NH(CH_2)_3NH^+(CH_3)CH_2COOH$	$C_{14}F_{17}SN_2O_4H_{14}^+$	ESI+	629.03973	629.04114	2.2	6.20	level 4



a) Soil moisture content in the two live soils (n = 3) during PFOSB/PFOAB transformation.



(b) Soil moisture content in the two live soils (n = 3)) during PFOSAm transformation.

Figure B.1 Soil moisture contents during the incubation of PFOAB/PFOSB and PFOSAm, as measured in the live matrix control vessels**.**

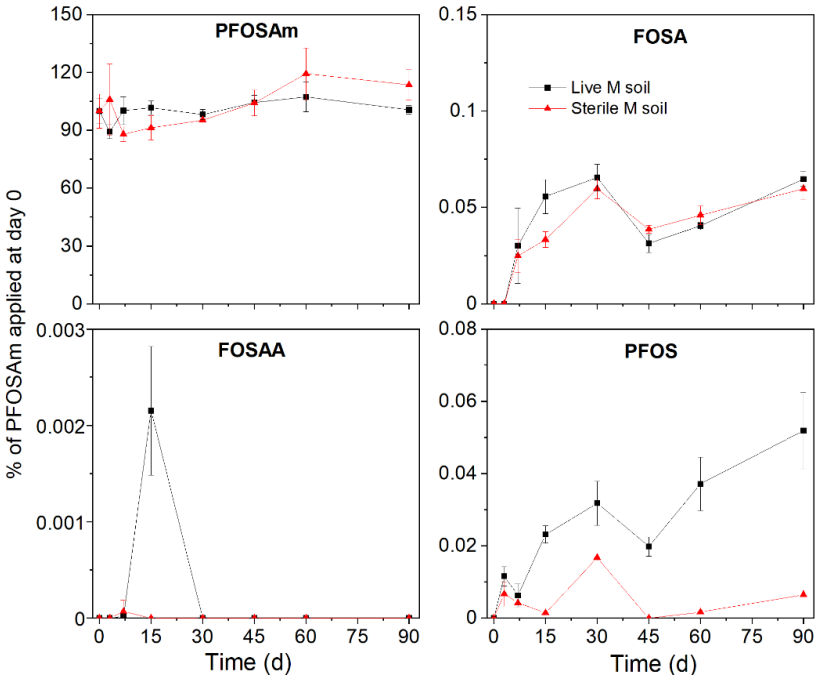


Figure B.2 Concentration profiles of PFOSAm and its quantitative transformation products, including FOSA, FOSAA and PFOS, in aerobic live and sterile P soil.

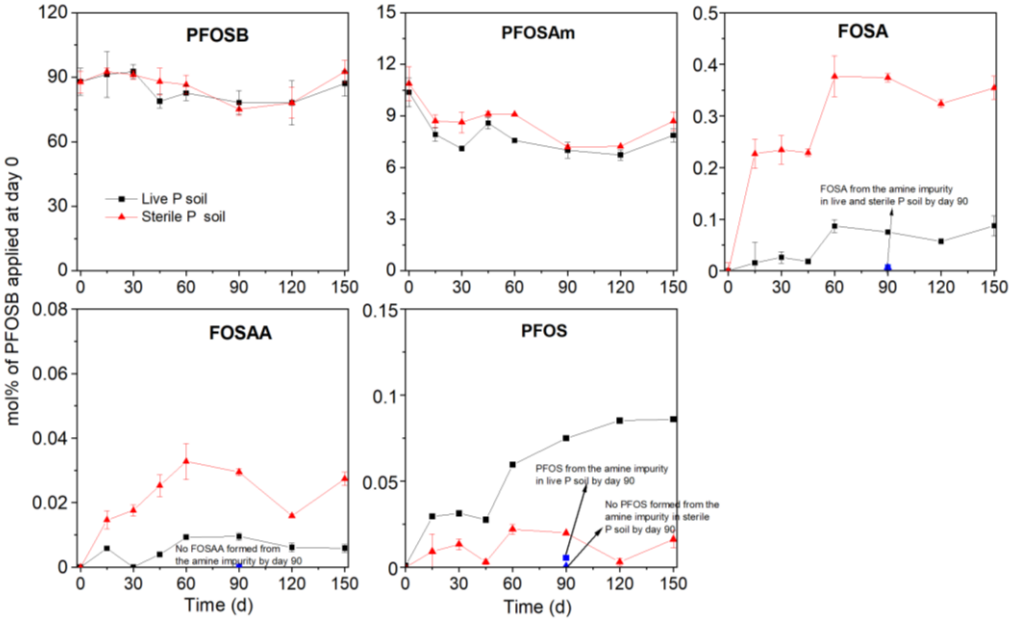


Figure B.3 Concentration profiles of PFOSB, PFOSAm impurity and their quantitative transformation product in live and sterile P soils.

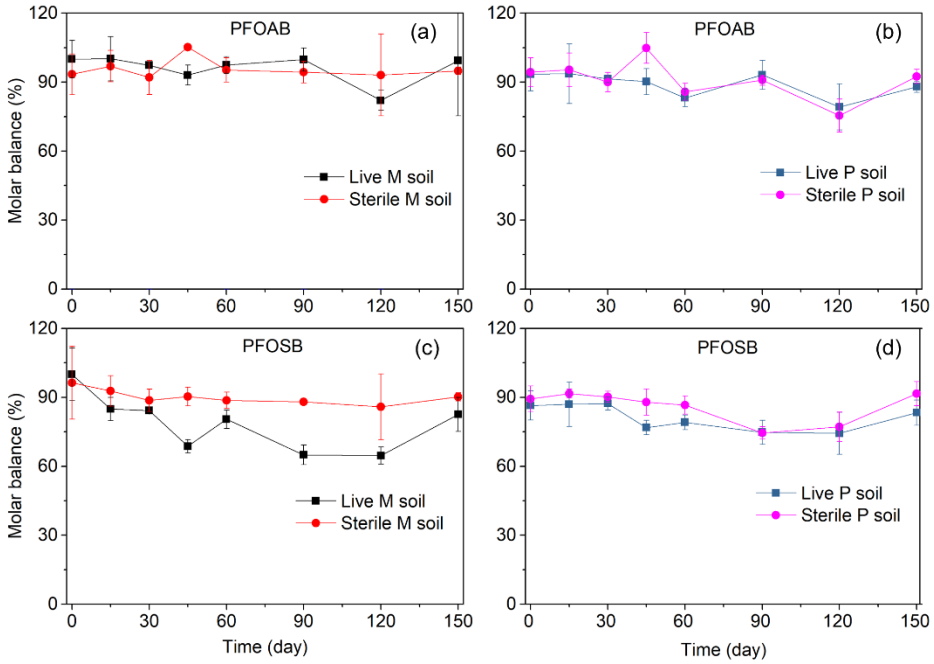


Figure B.4 The molar balance of PFOAB and its quantifiable transformation products in M soil (a) and P soil (b). The molar balance of PFOSB and its quantifiable transformation products in M soil (c) and P soil (d).

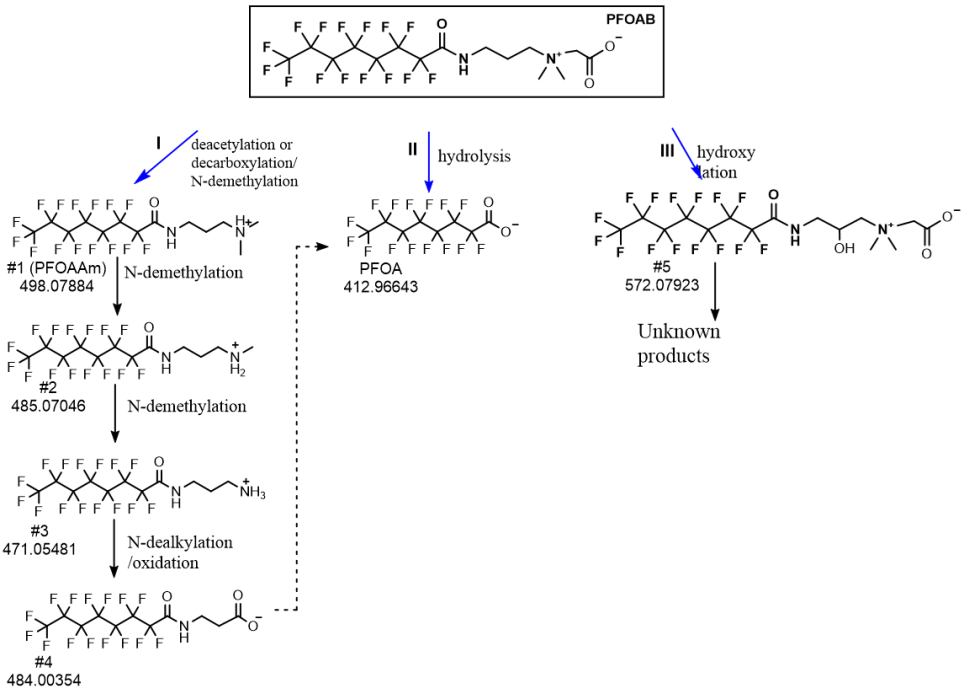
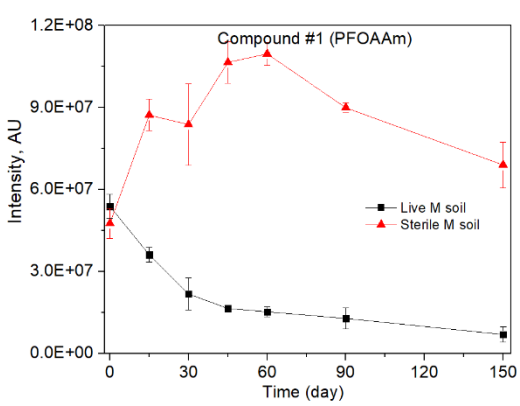


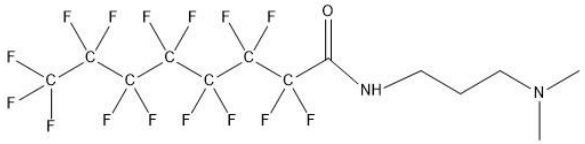
Figure B.5 Proposed abiotic and biotic transformation pathways of PFOAB in aerobic soils. The dashed line refers to a hypothetical multiple-step pathway.

PFOAB degradation in the sandy loam (M) soil

The first panel is the extracted chromatogram of masses within a 5 ppm window of the scouted m/z. The second panel is the predicted exact mass and monoisotopic intensity distribution of the scouted compound. The third panel is the extracted mass spectrum from the largest peak in the first panel.

Compound #1 (PFOAAm)





The increase trend followed by decrease trend in the sterile soil indicates the production of this compound as abiotic degradation product, the much faster decrease of its concentration in the live soil suggests its biotic transformation.

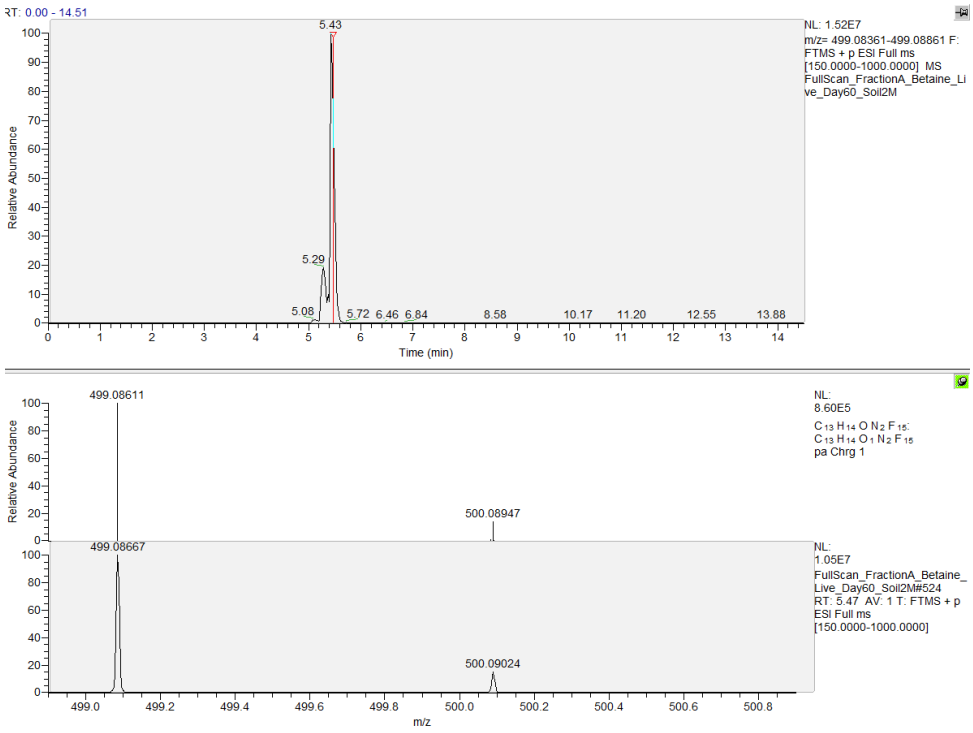
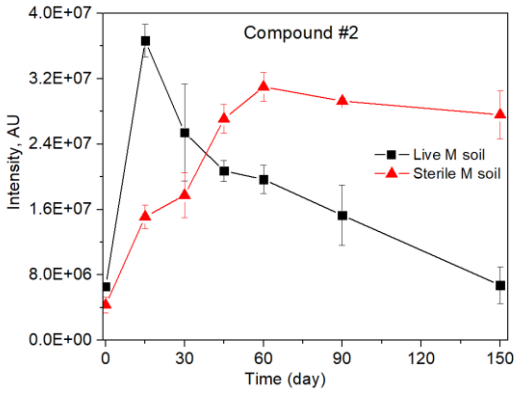
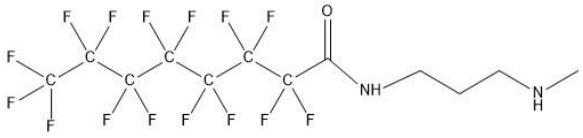


Figure B.6 Chromatogram and mass spectra of polyfluoroalkyl Compounds detected in the sandy loam (M) soil.

Figure B.6 (a) (continued).

Compound #2





The much faster increase and decrease of concentration of compound #2 in live M soil compared with sterile M soil suggests it is likely a biotransformation product.

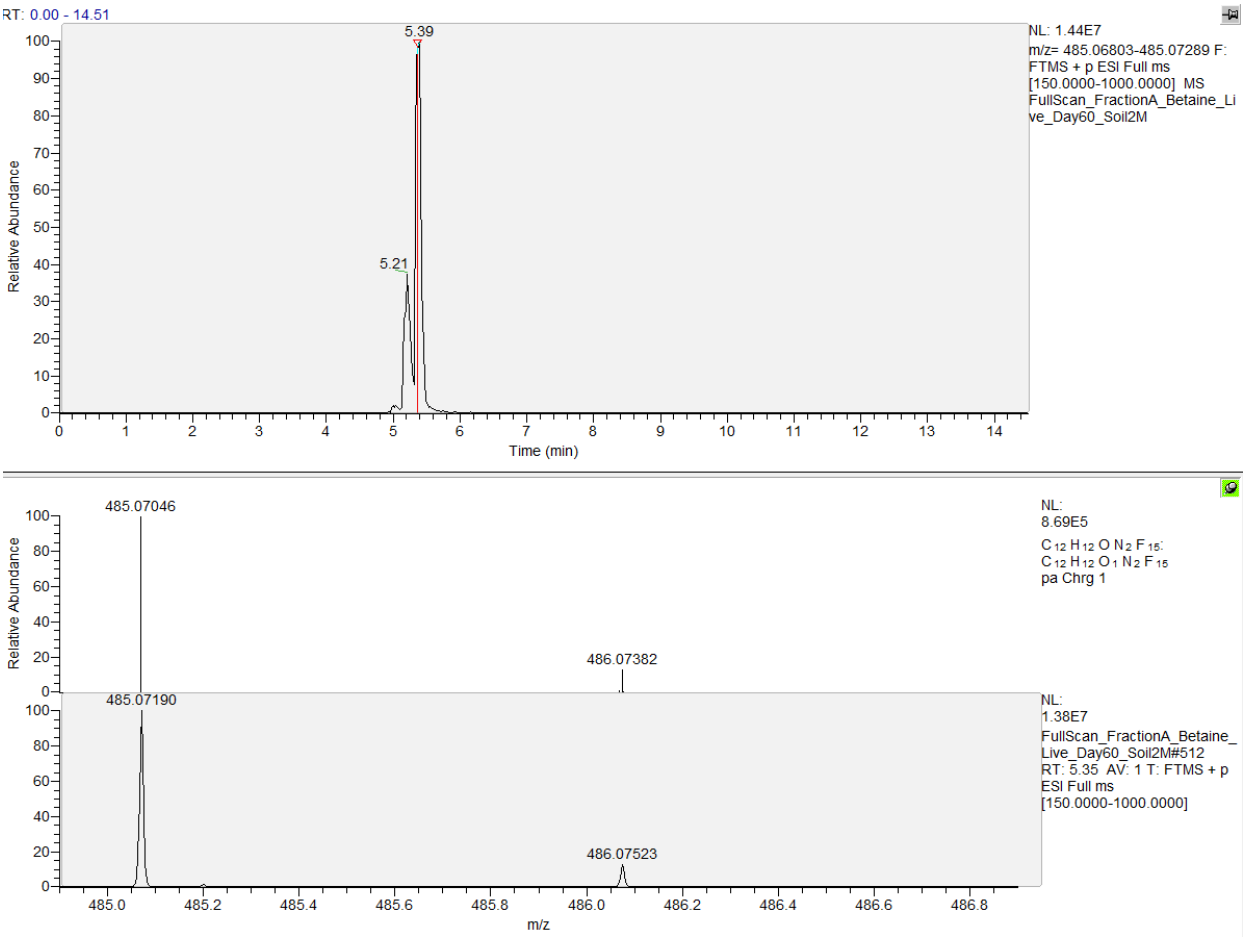
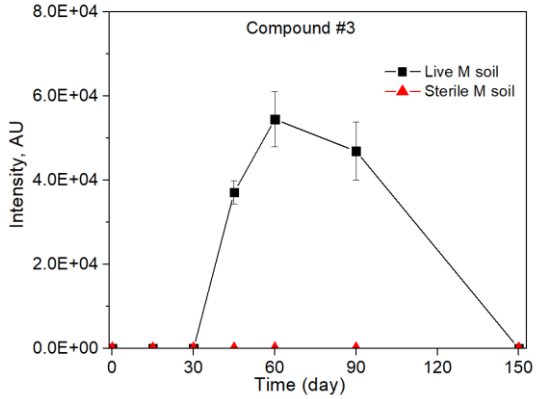
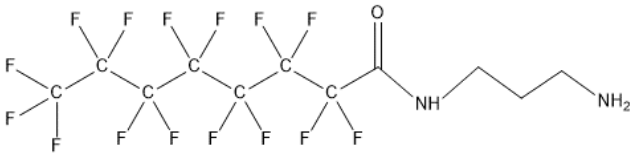


Figure B.6 (b) (continued)

Compound #3





The increasing trend followed by decreasing trend in live M soil suggests it is likely a biotransformation product.

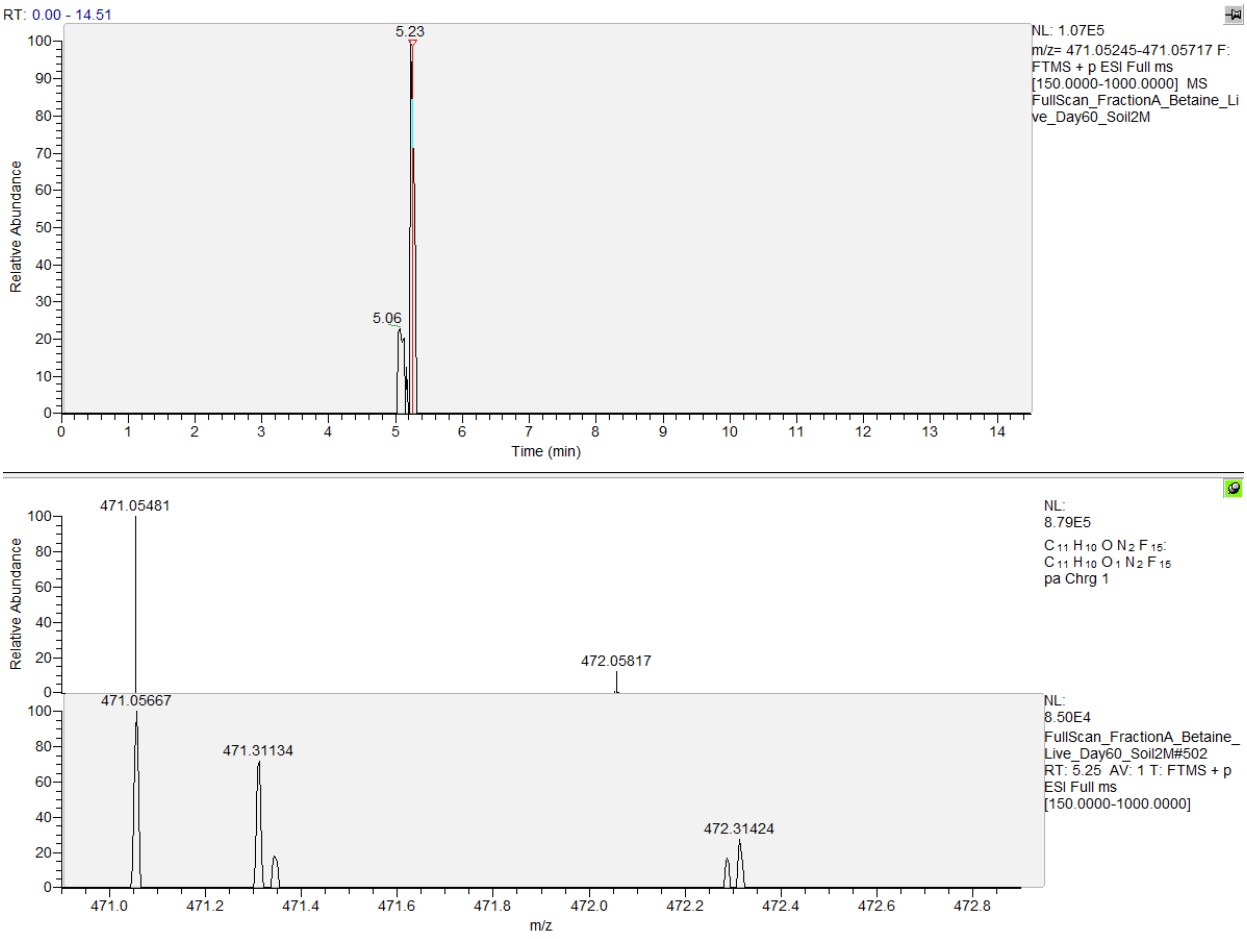
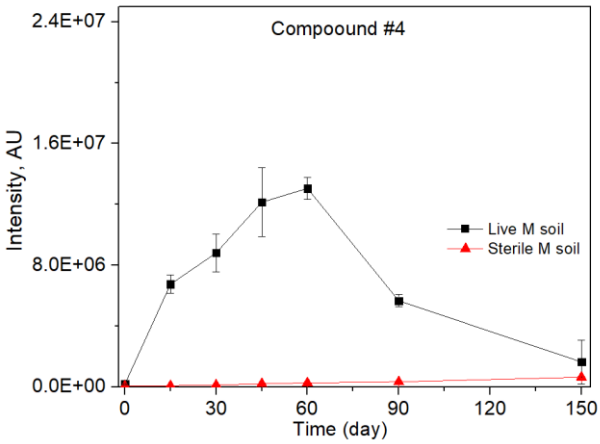
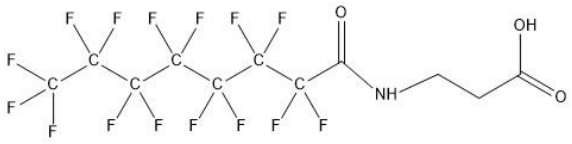


Figure B.6 (c) (continued)

Compound #4





The significantly high concentration of compound #4 in live M soil and the slight increase of its concentration in sterile M soil suggests that this compound is both an abiotic and biotic transformation product.

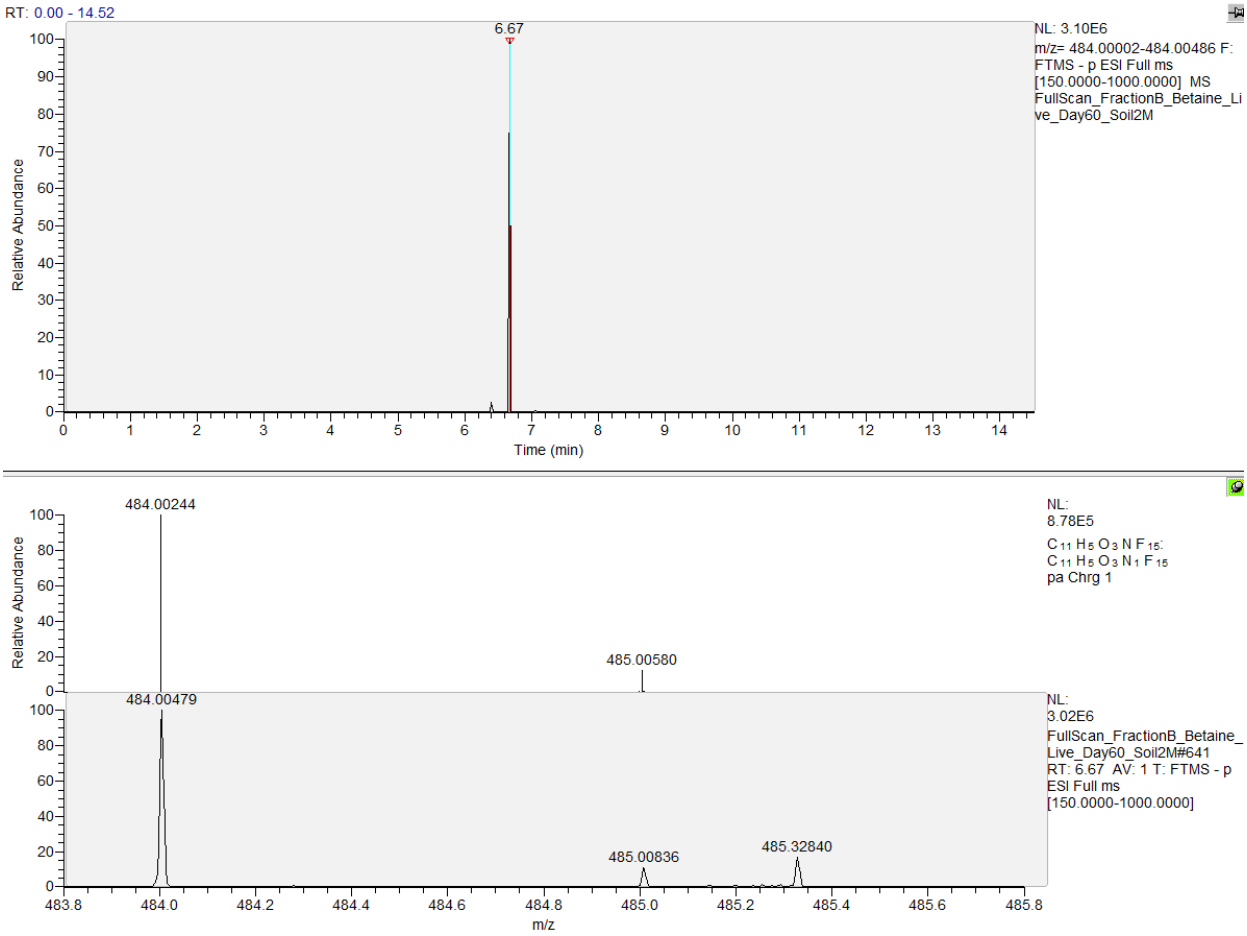
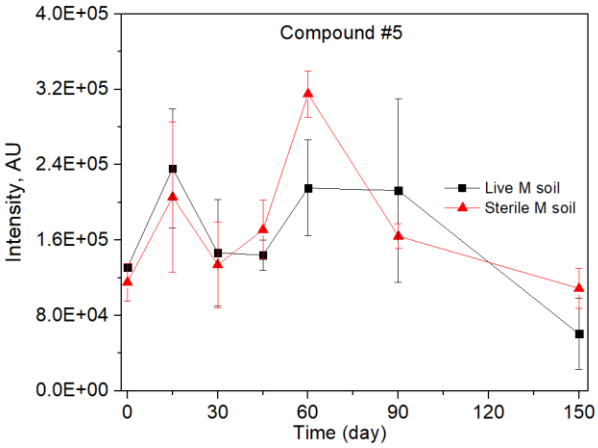
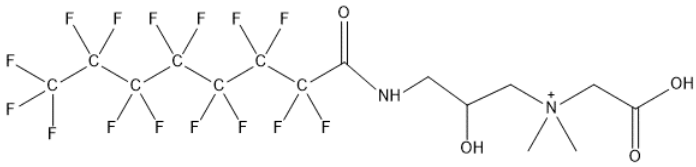


Figure B.6 (d) (continued)

Compound #5





The increasing and decreasing trend of compound #5 in sterile M soil suggests it is likely an abiotic transformation product. The lower concentration in live M soil may be due to its faster transformation to downstream products.

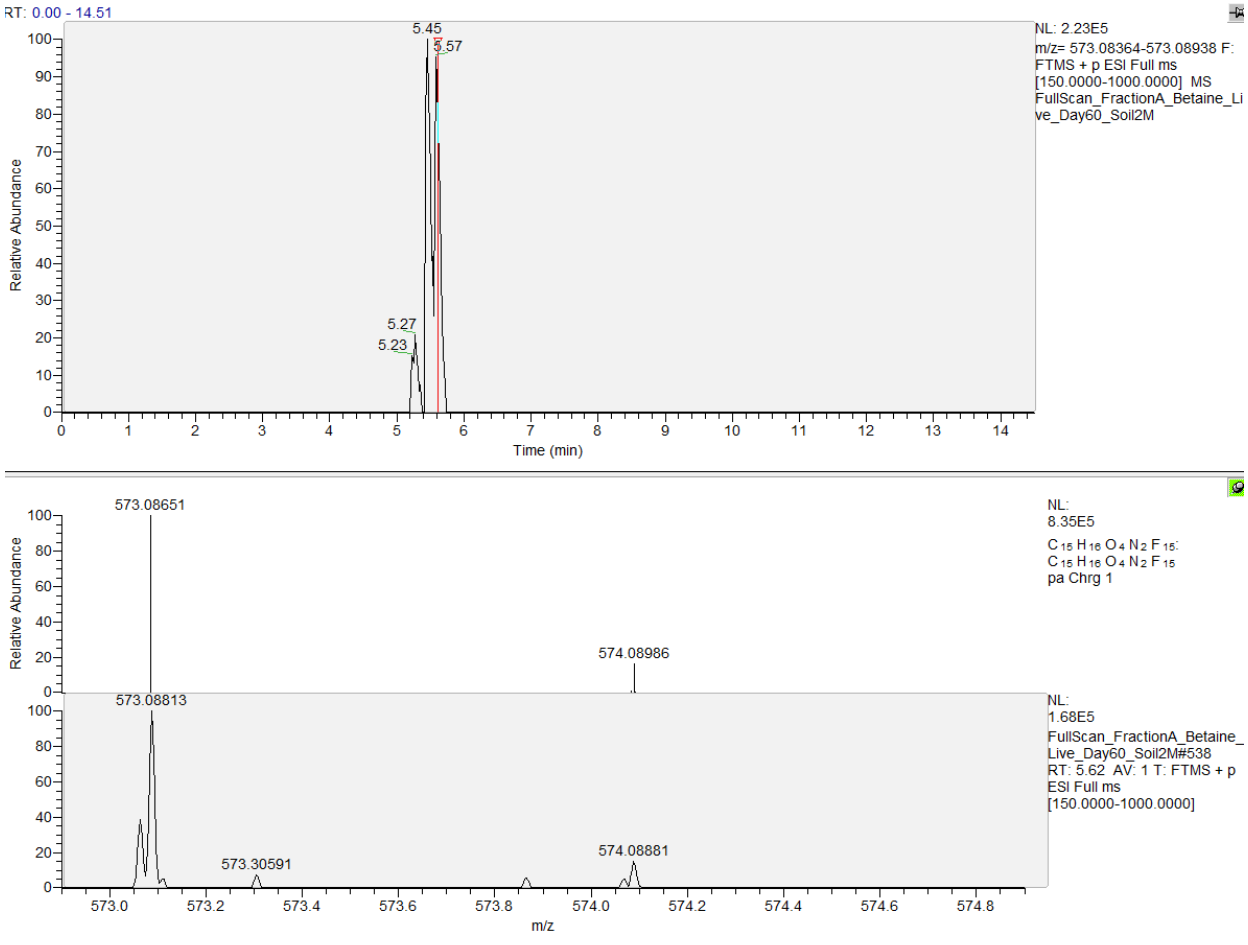
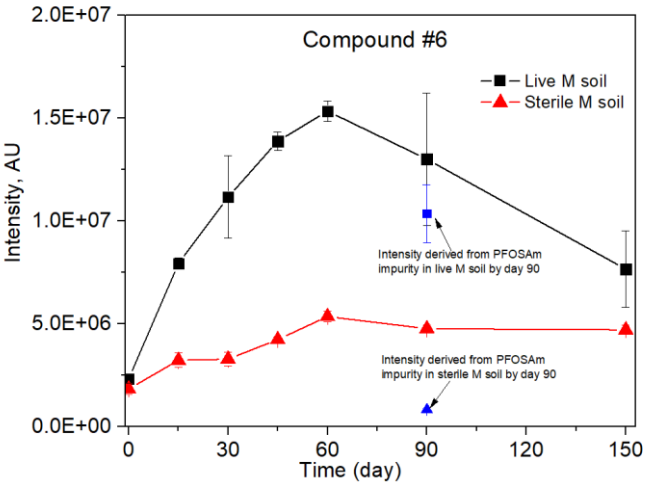
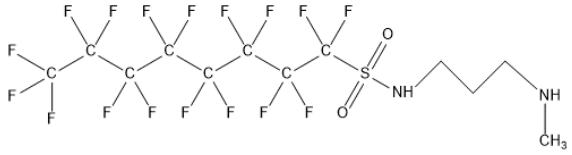


Figure B.6 (e) (continued)

Compound #6





Compound #6 was also present in sterile control samples. The much faster increase in live M soil suggests it is likely both an abiotic and biotic transformation product.

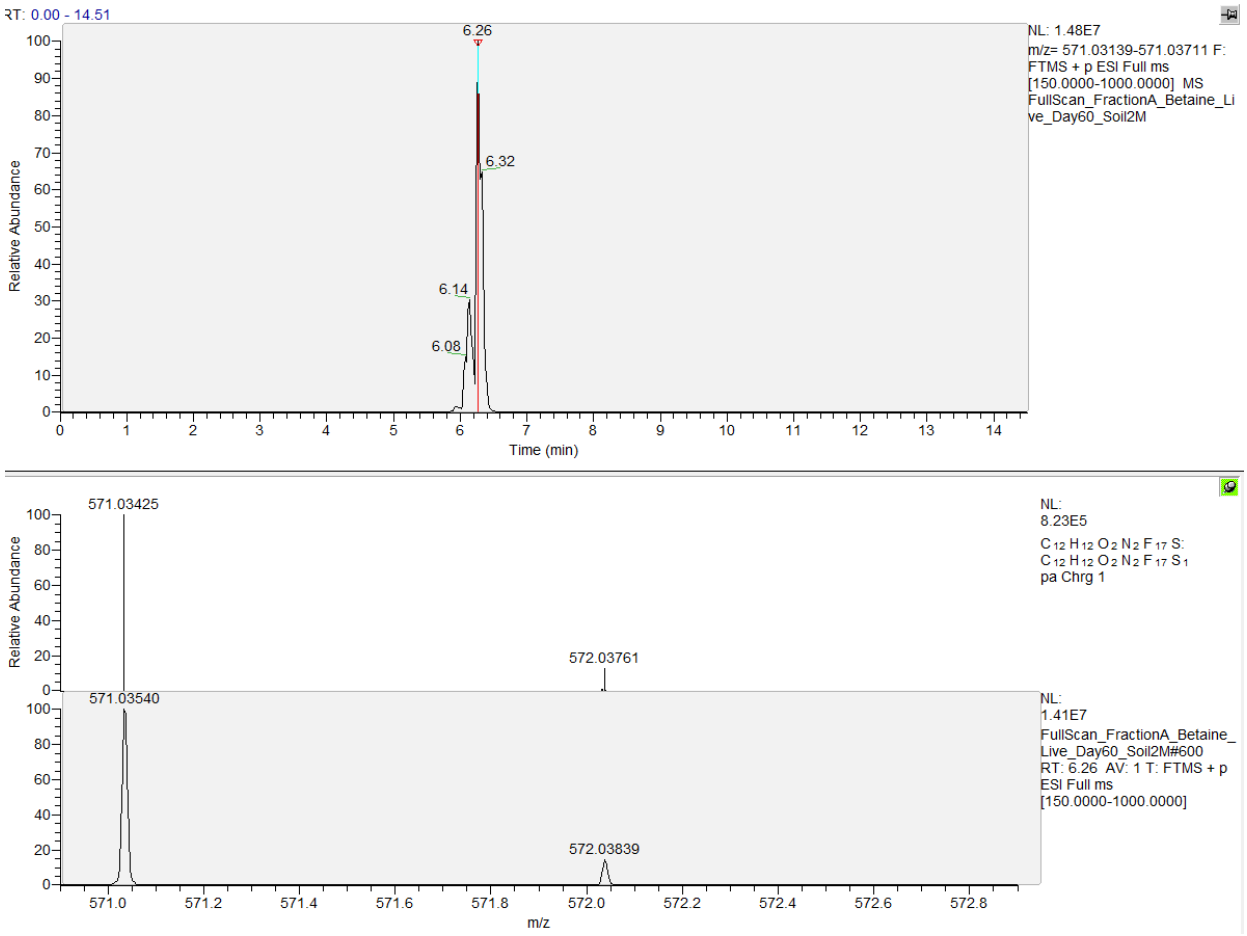
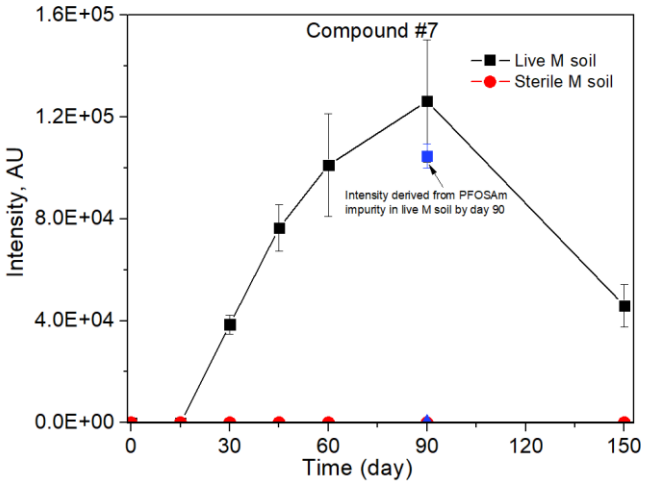
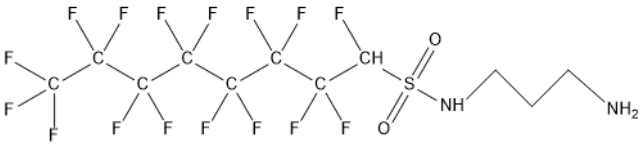


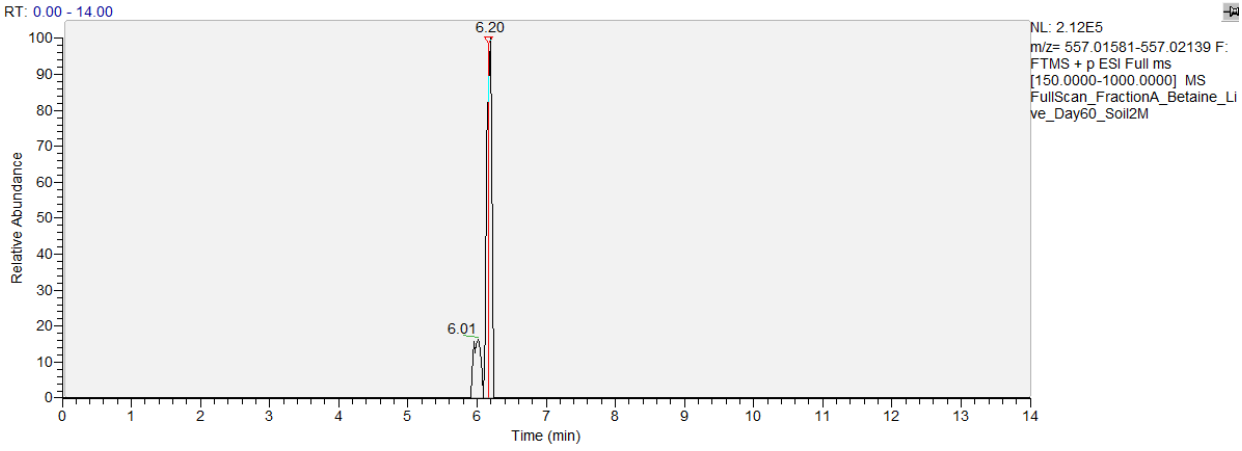
Figure B.6 (f) (continued)

Compound #7





The only presence of compound #7 in the live soil suggests it is likely a biotransformation product.



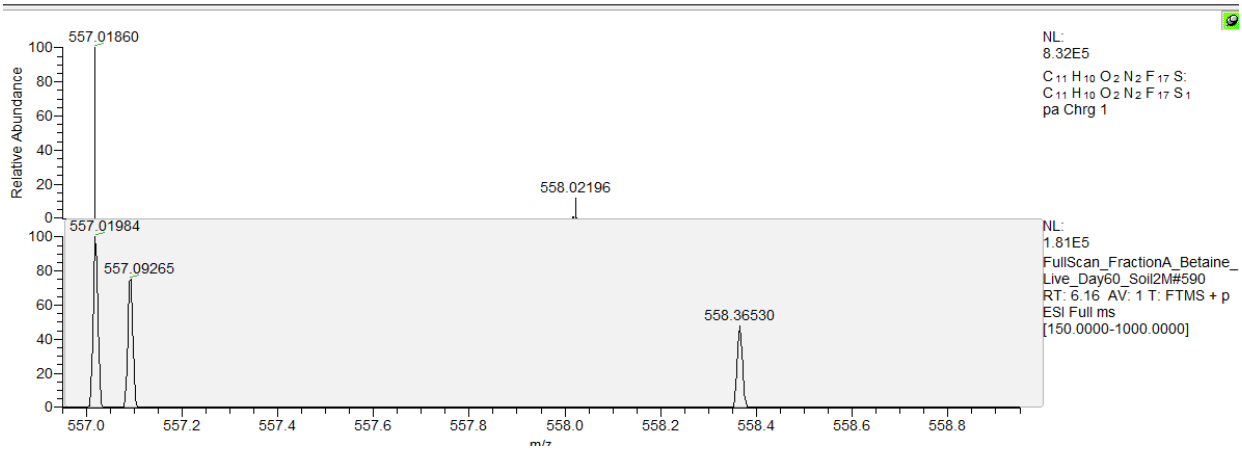
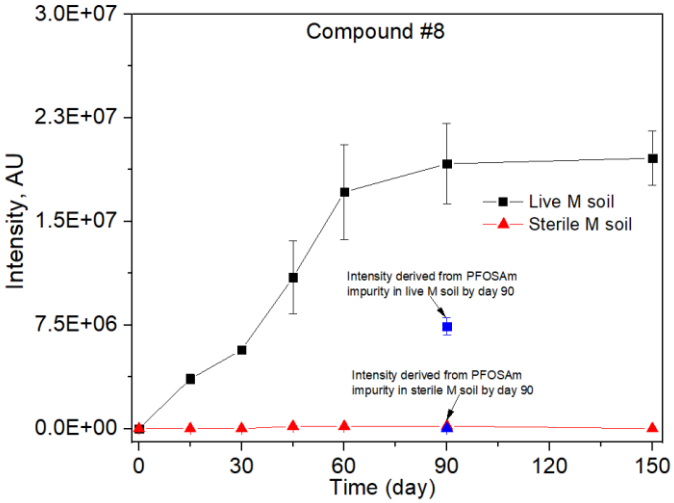
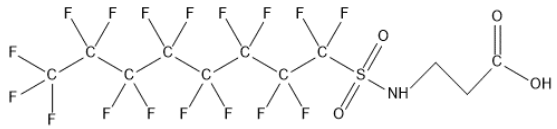


Figure B.6 (g) (continued)

Compound #8





The significantly higher concentration of compound #8 in live M soil compared with sterile M soil suggests it is likely both an abiotic and biotic transformation .product.

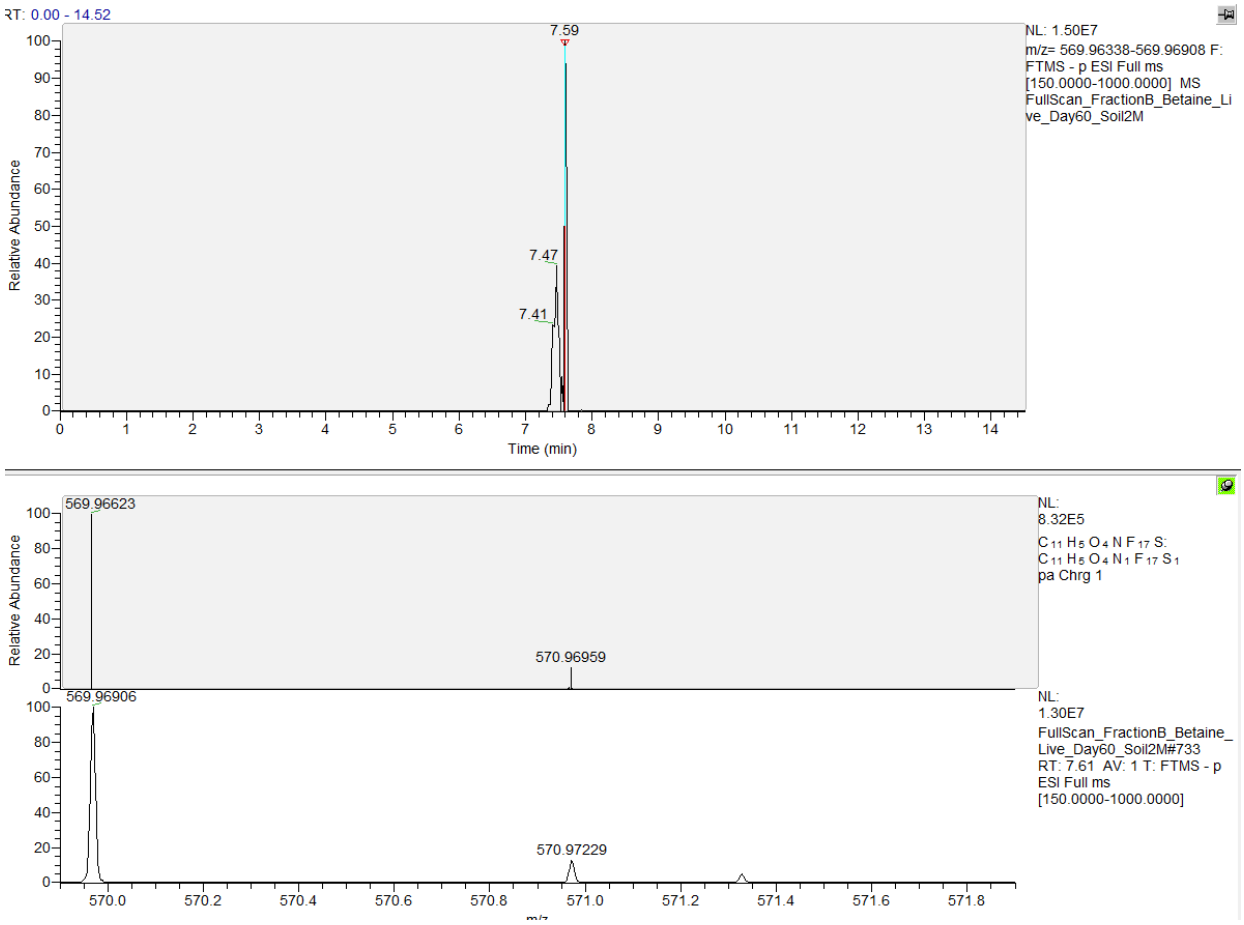
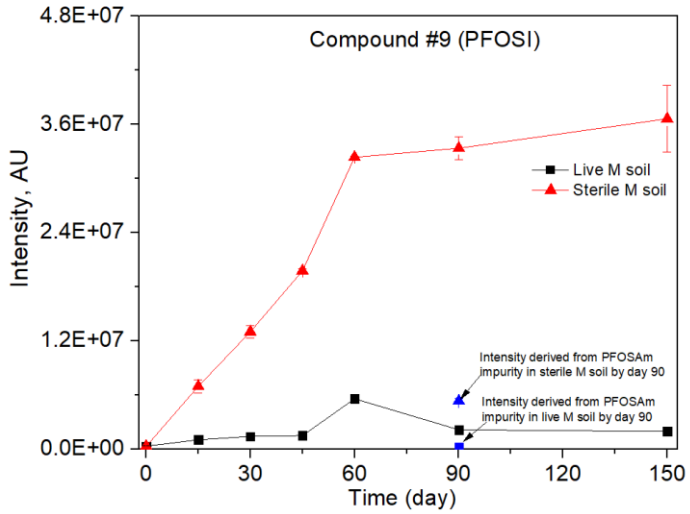
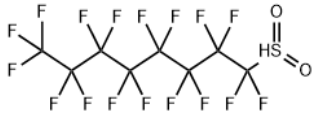


Figure B.6 (h) (continued)

Compound #9 (PFOSI)





The slightly higher level of compound #9 in live compared with sterile soil suggests it is likely both an abiotic and biotic transformation product.

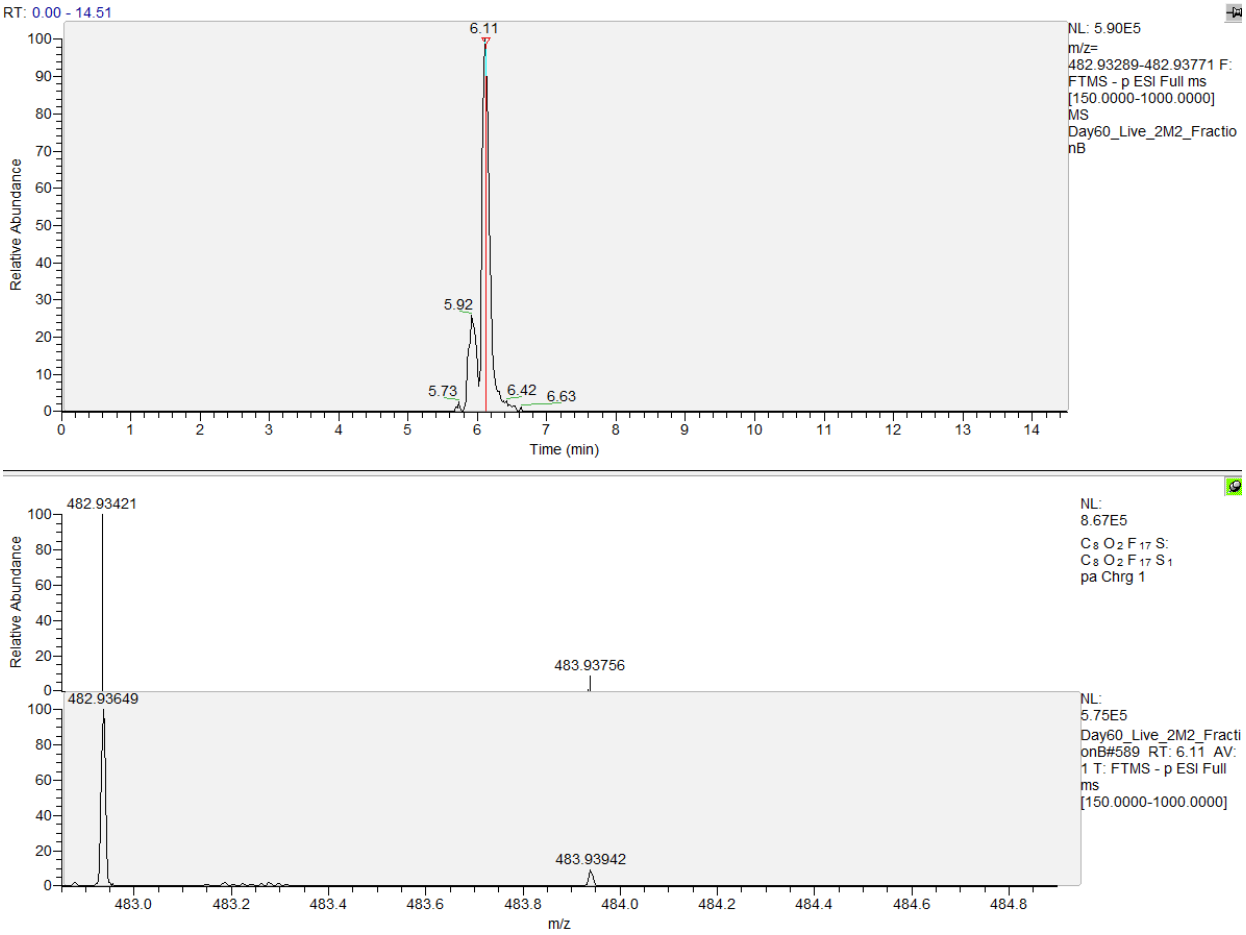
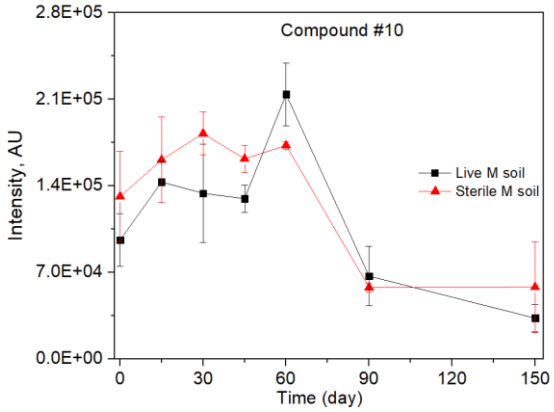
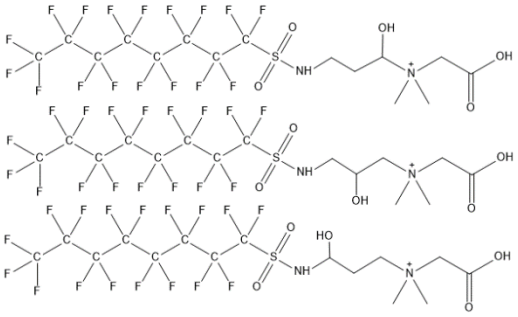


Figure B.6 (i) (continued)

Compound #10





The slightly higher level of compound #10 in live M soil compared with sterile M soil suggests it is likely both an abiotic and biotic transformation product.

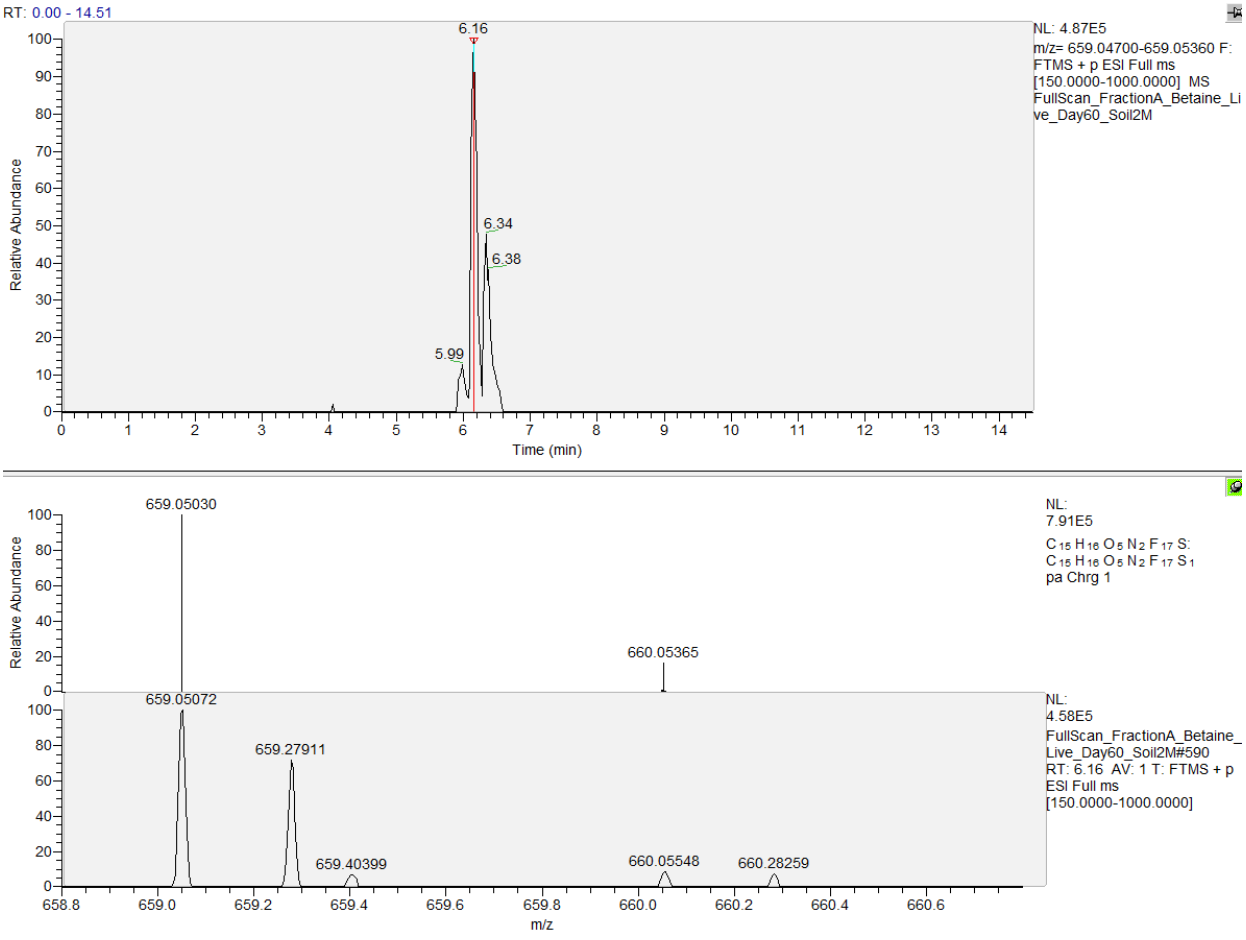
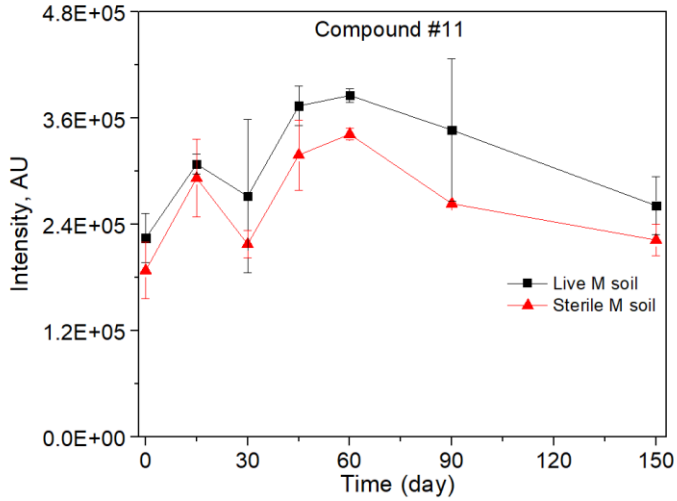
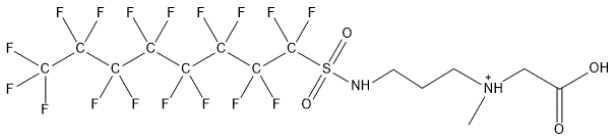


Figure B.6 (j) (continued)

Compound #11





The higher concentration of compound #11 in live M soil compared with sterile M soil suggest it is likely an abiotic and biotic transformation product.

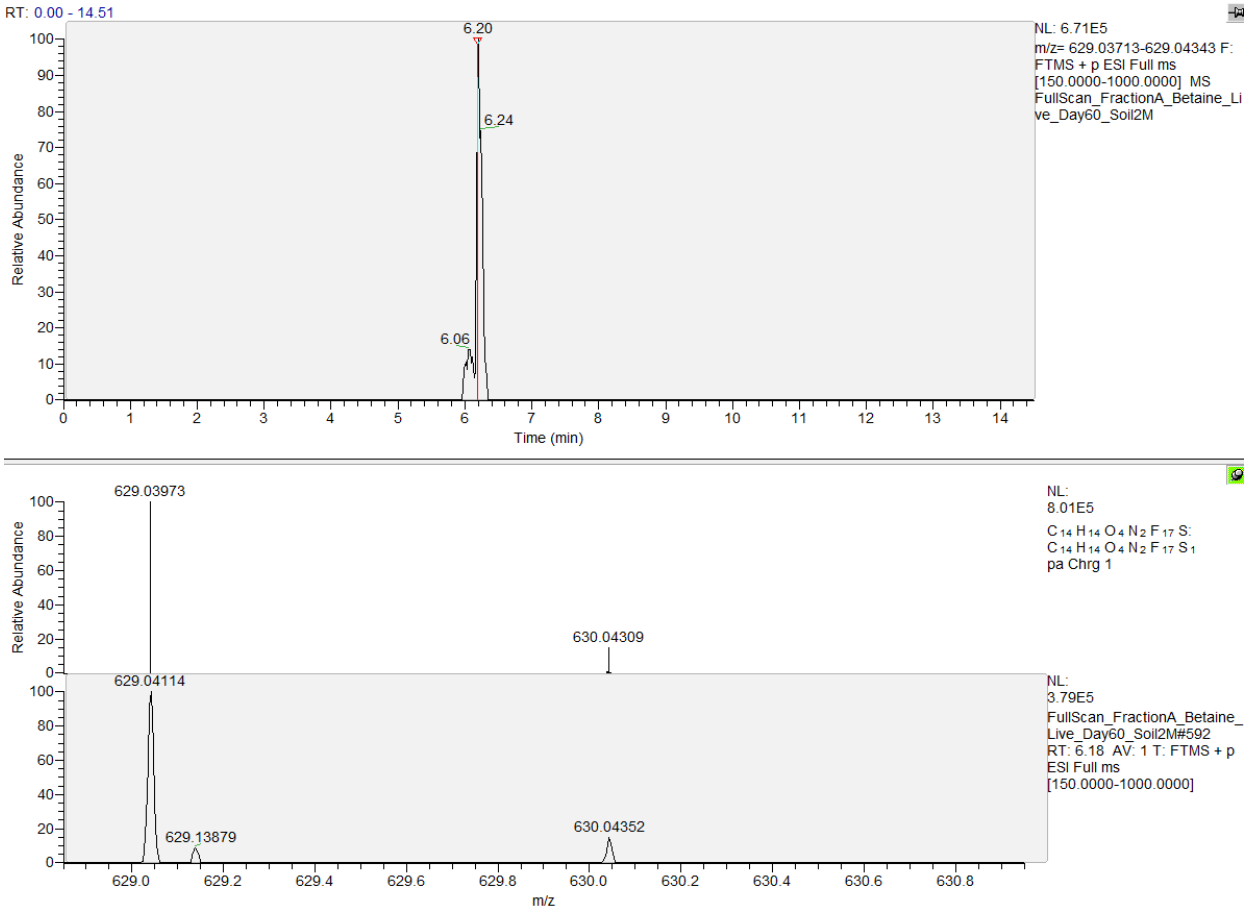
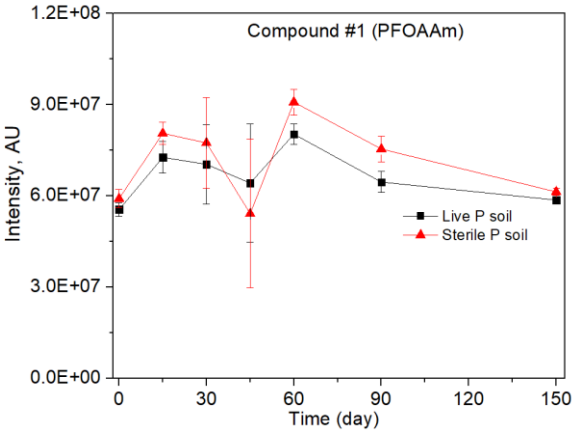
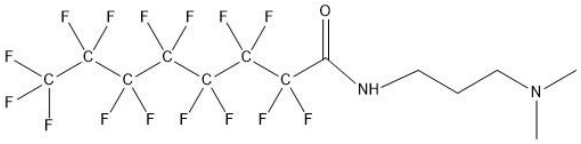


Figure B.6 (k) (continued)

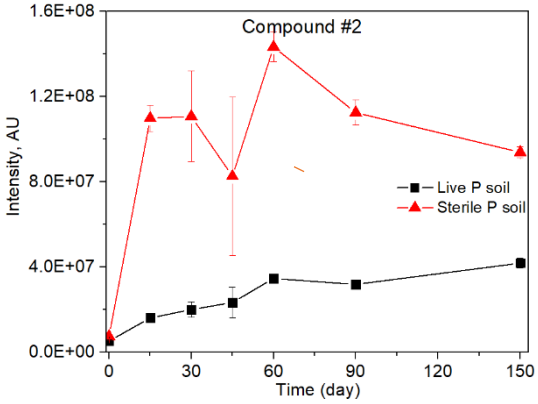
PFOAB degradation in loam (P) soil

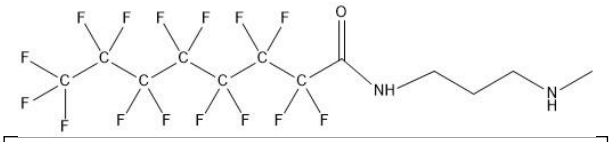
Compound #1 (PFOAAm), #2, #3 #4 and #5



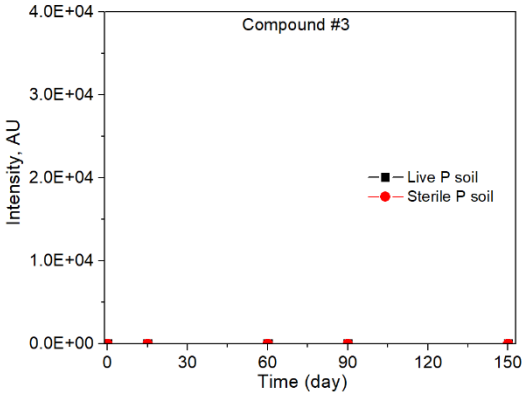


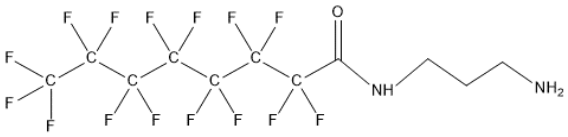
Compound #1 was also present in sterile control samples. The slightly faster increase in the sterile control suggests that it is likely an abiotic transformation product from PFOAB. The lower concentration in live soil is possible due to its fast transformation to further downstream products.



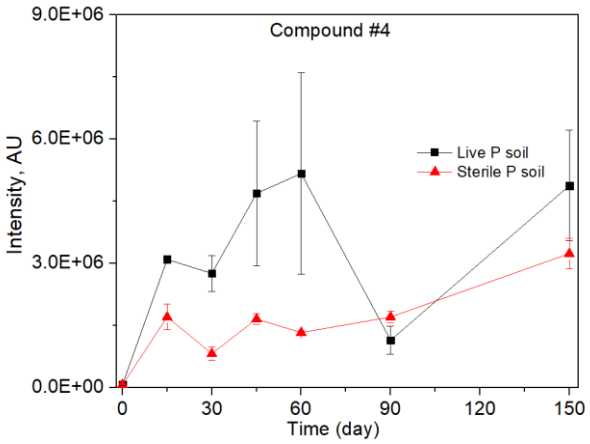


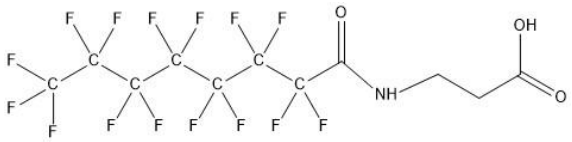
Compound #2 was also present in sterile control samples. The much faster increase in the sterile control suggests that it is likely an abiotic transformation product. The lower concentration in the live soil may be due to its degradation to further downstream products, thus it is also likely a biotransformation product.



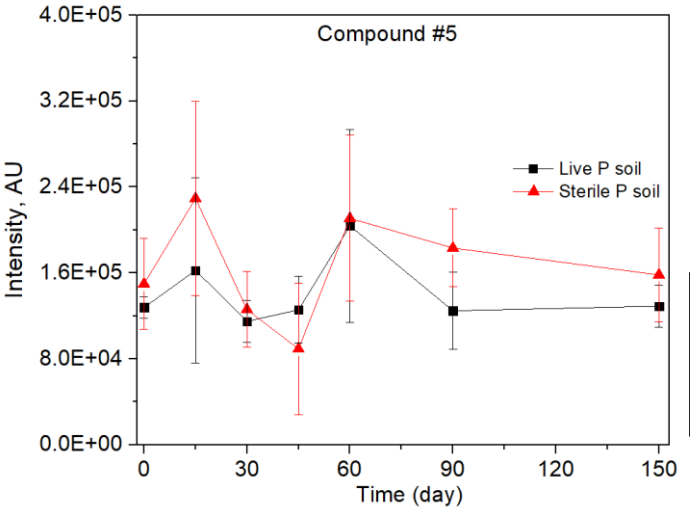


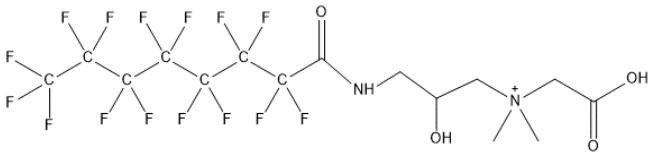
Compound #3 is not detectable in both live and sterile P soil, which may be due to its low concentration or its fast transformation to further downstream products.





Compound #4 was also present in sterile control samples. The much faster increase in the live soil suggests it is likely a biotransformation product.



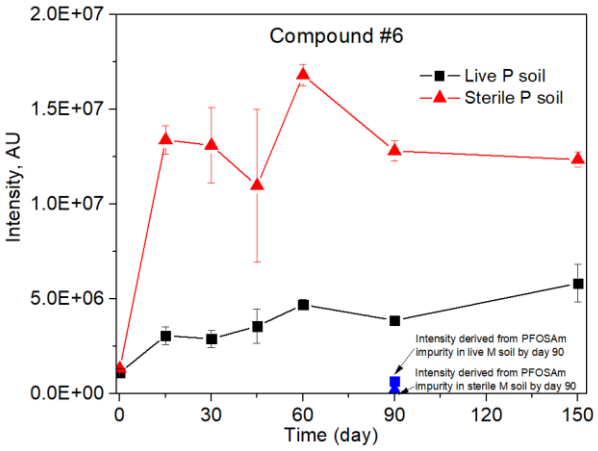


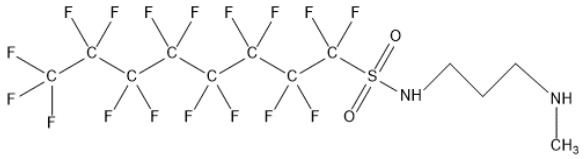
The increasing and decreasing trend of concentration of compound #5 in sterile soil suggests it is likely an abiotic transformation product. The low concentration in live soil is probably due to its fast transformation to further downstream products.

Figure B.6 (l) Chromatogram of compounds #1, #2 #3, #4 and #5 in **live and sterile P soil.**

PFOSB degradation in loam (P) soil

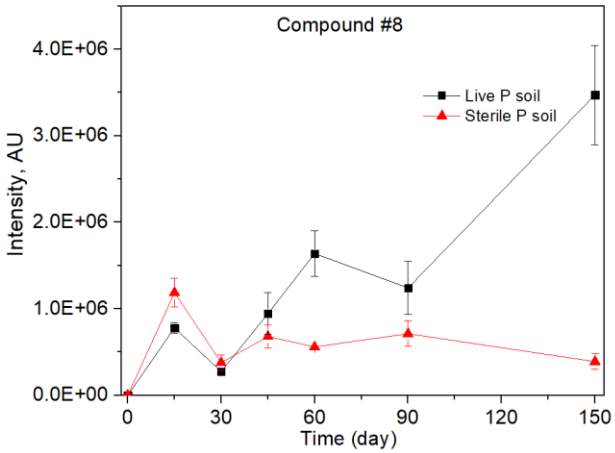
Compound #6, # 8, #9 and #10

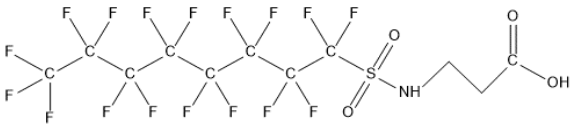




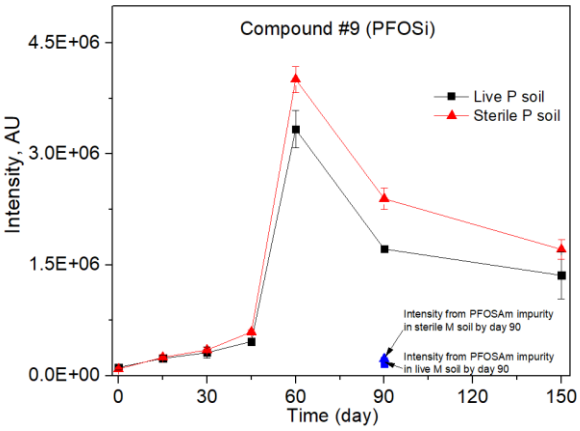
Compound#6 was also present in sterile control samples. The much faster increase in the sterile control suggests that it is likely an abiotic transformation product. The lower concentration in the live soil is possible due to its further degradation to further downstream products by soil microorganisms.

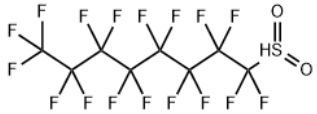
Note: compound#7 was not detectable in both live and sterile soils during the PFOSB incubation in the loam soil.



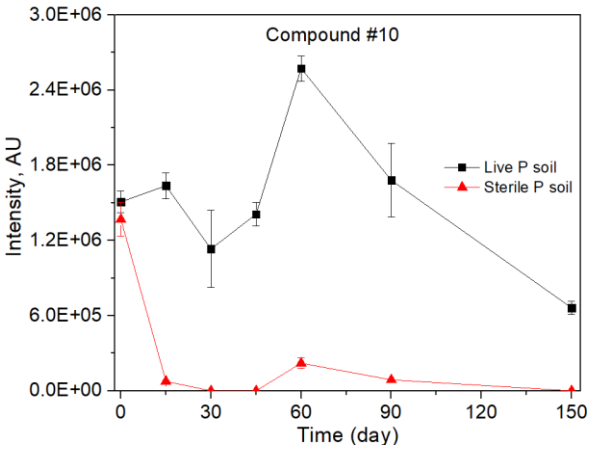


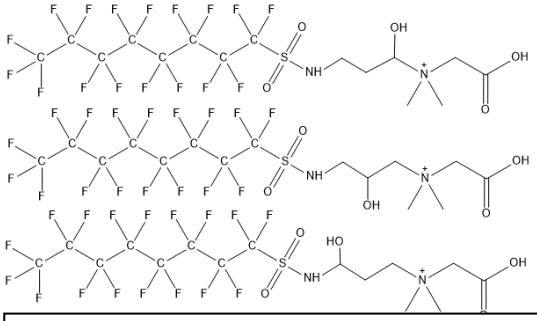
The faster increase of concentration of compound #8 in live P compared with sterile P soil suggest that it is both an abiotic and biotic product.



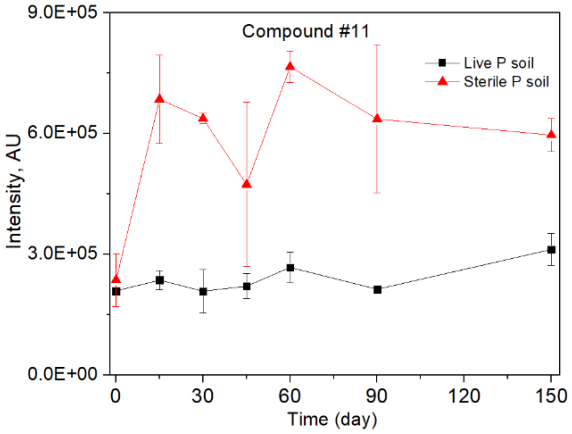


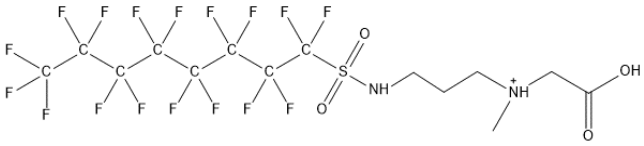
The increasing trend followed by decreasing trend of concentration of compound #9 suggests that it is an abiotic transformation product, the low concentration in live soil is probably due to its fast transformation to further downstream products.





Compound #10, the increase phase followed by decrease phase observed only in live soil suggests it is likely a biotic transformation product in this loam soil.

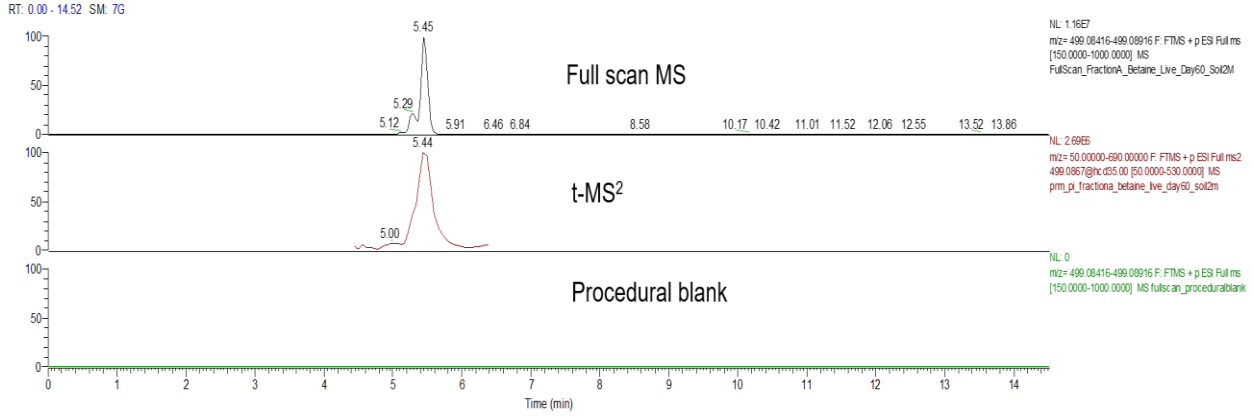


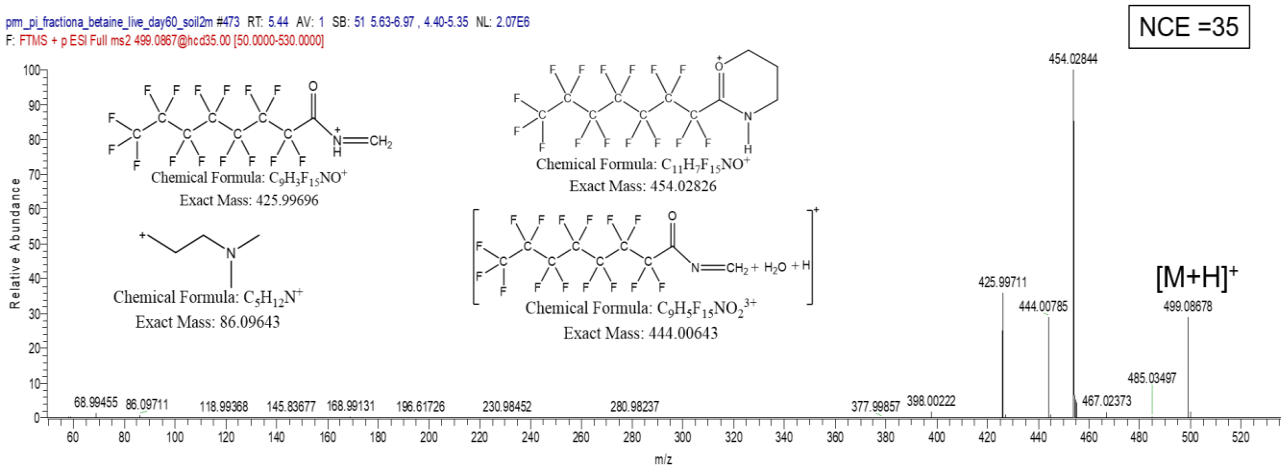


For compound #11, the increasing trend followed by decreasing trend in sterile soil suggests it is likely an abiotic transformation product. The lower concentration in live soil may be due to its further degradation into further downstream products by soil microorganisms.

Figure B.6 (m) Chromatogram of detected polyfluoroalkyl compound#6, #8, #9, #10 and #11in live P and sterile P soil.

Compound #1 (PFOAAm)



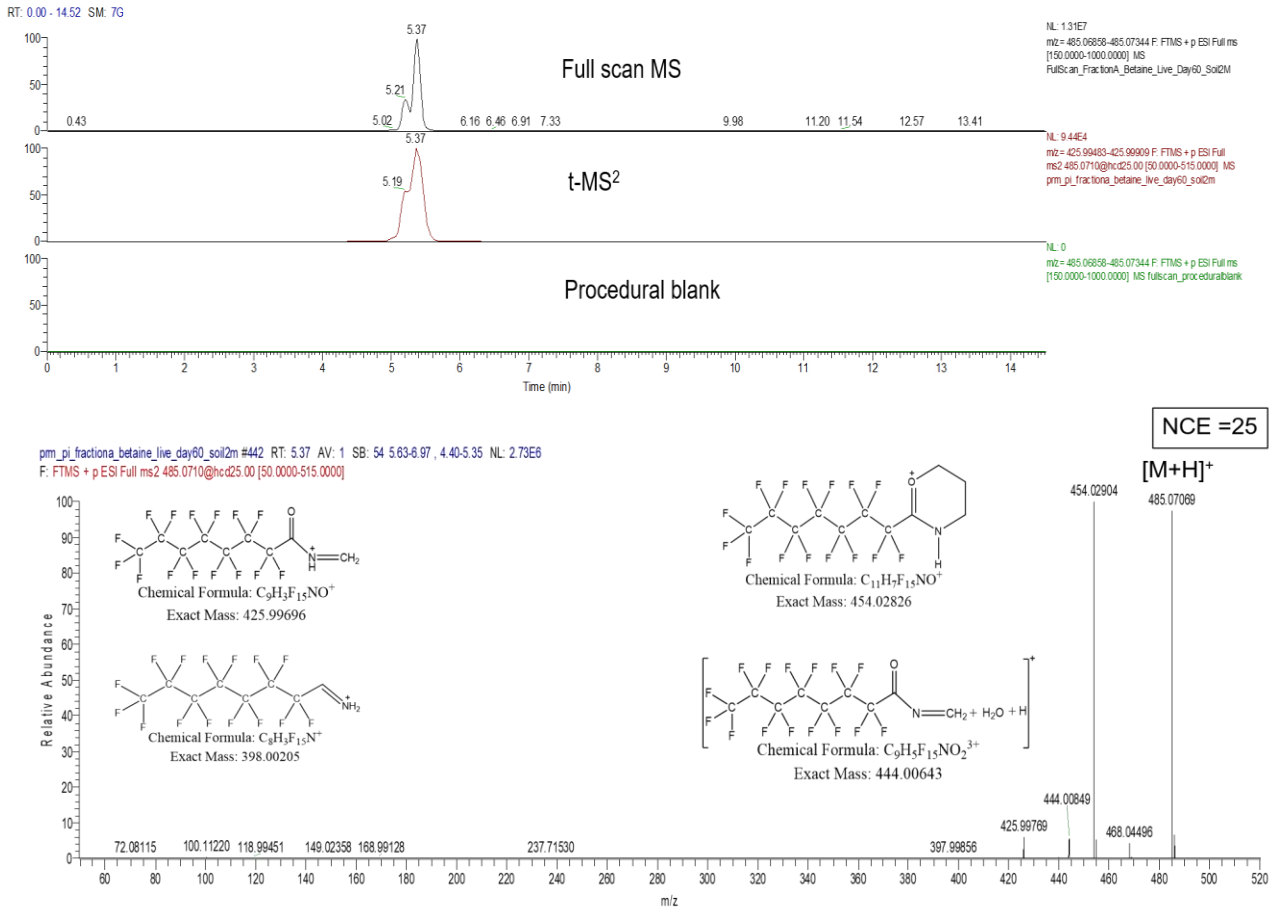


	Theoretical m/z	Observed m/z	Error (ppm)
$[M+H]^+$	499.08611	499.08678	1.342
Fragments	454.02826	454.02844	0.396
	444.00643	444.00785	3.198
	425.99696	425.99711	0.352
	86.09643	86.09711	7.898

Figure B.7 t-MS^2 spectra of positively identified polyfluoroalkyl compounds.

Figure B.7 (a) (continued).

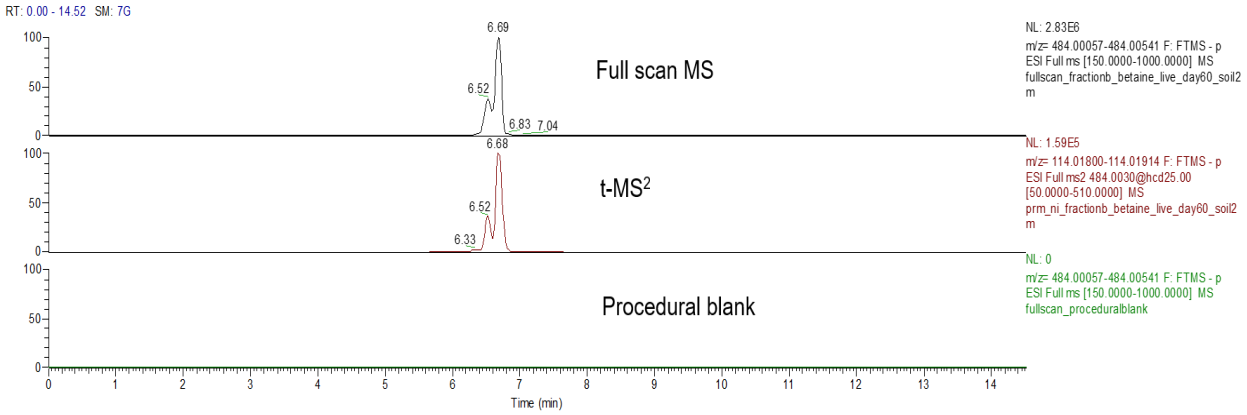
Compound #2

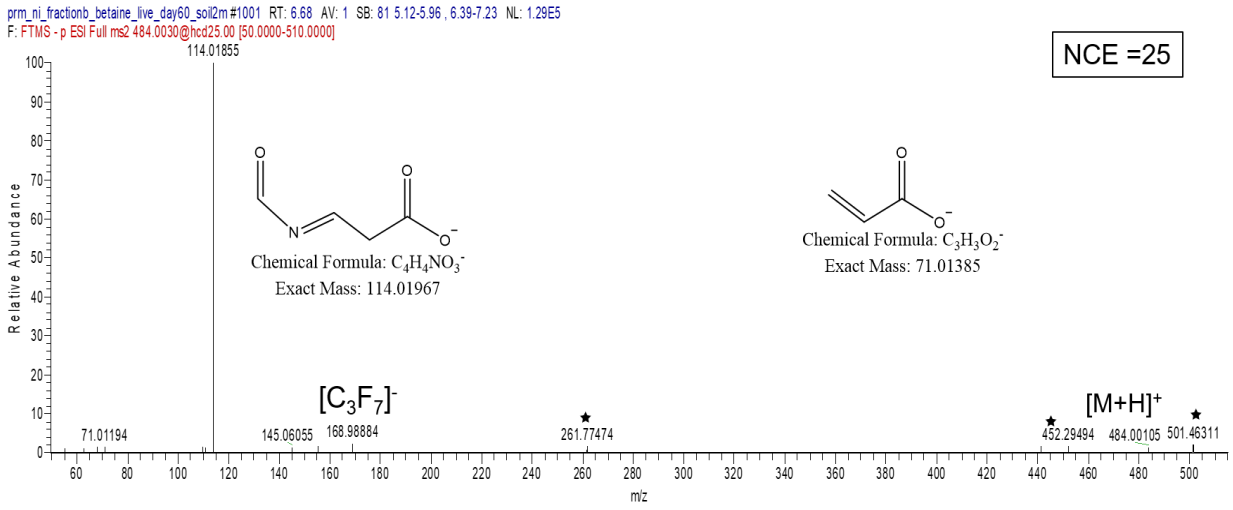


	Theoretical m/z	Observed m/z	Error (ppm)
$[M+H]^+$	485.07046	485.07069	0.474
Fragments	454.02826	454.02904	1.718
	444.00643	444.00849	4.640
	425.99696	425.99769	1.714
	398.00205	397.99856	-8.769

Figure B.7 (b) (continued).

Compound #4

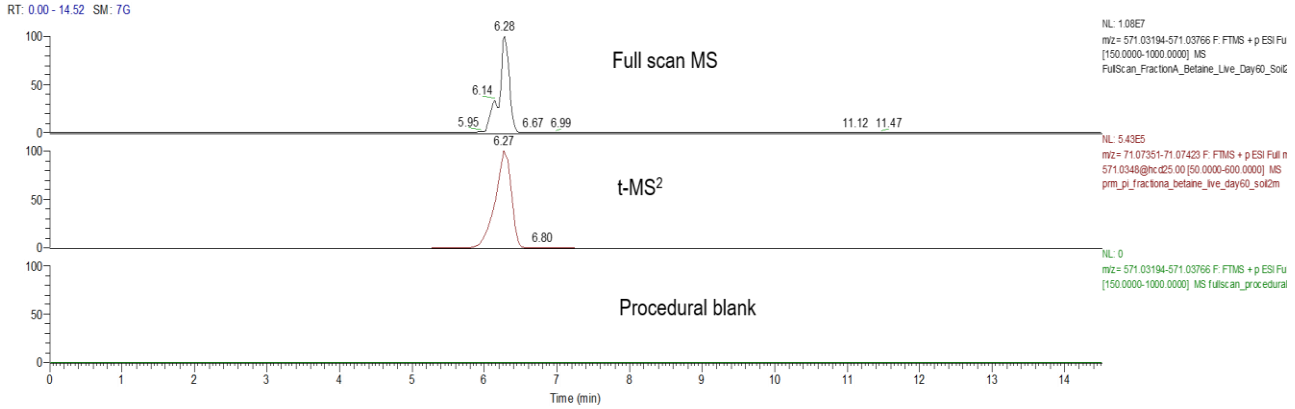


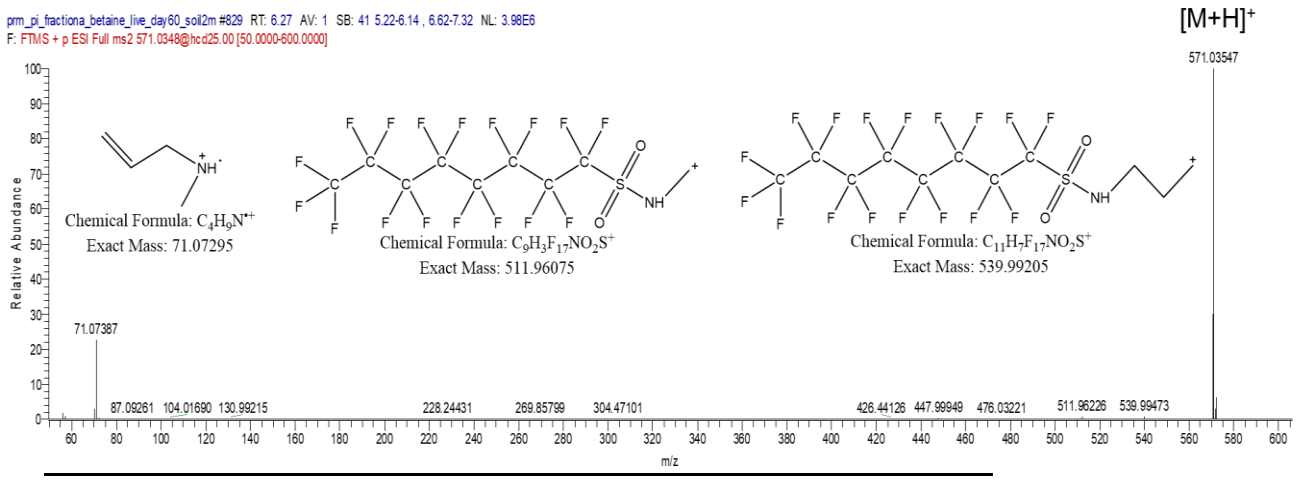


	Theoretical m/z	Observed m/z	Error (ppm)
$[M-H]^-$	484.00354	484.0011	-5.145
Fragments	114.01967	114.0186	-9.384
	168.98883	168.9891	1.379

Figure B.7 (c) (continued).

Compound #6

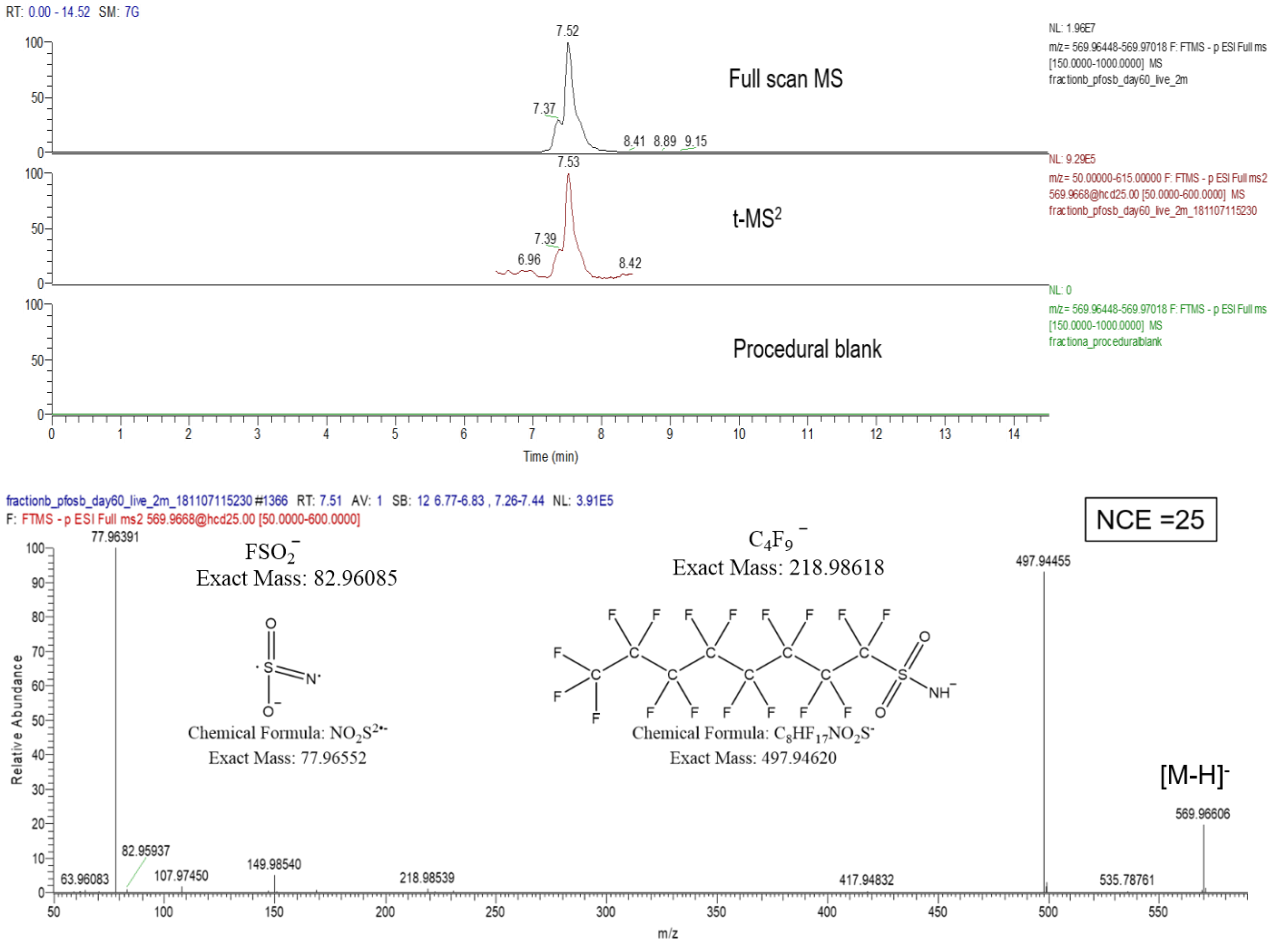




	Theoretical m/z	Observed m/z	Error (ppm)
$[M+H]^+$	571.03425	571.03547	2.136
Fragments	511.96075	511.96226	2.949
	539.99205	539.99473	4.963
	71.07295	71.07387	12.944

Figure B.7 (d) (continued).

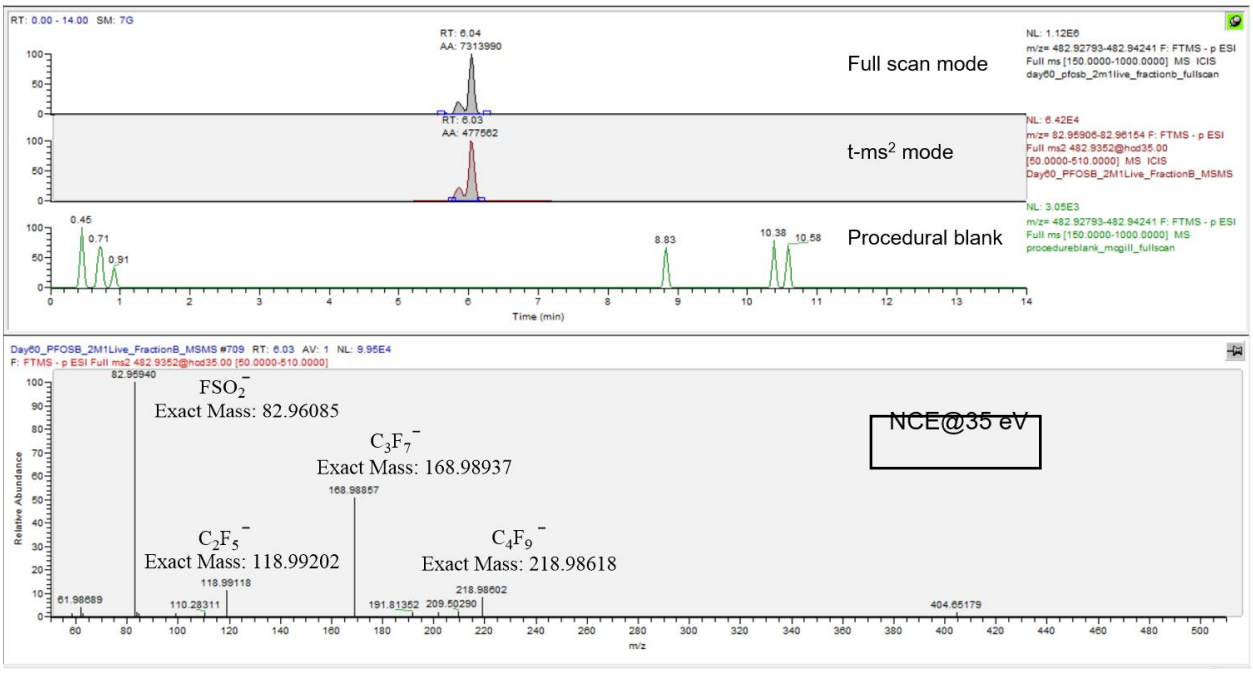
Compound #8



	Theoretical m/z	Observed m/z	Error (ppm)
$[M-H]^-$	569.96733	569.96620	-1.983
Fragment 1	77.96552	77.96392	-20.522
Fragment 2	497.94620	497.94456	-3.294
Fragment 3	82.96085	82.95937	-17.840
Fragment 4	218.98618	218.98539	-3.608

Figure B.7 (e) continued.

Compound #9



	Theoretical m/z	Observed m/z	Error (ppm)
Fragment 1	82.96085	82.95941	-10.728
Fragment 2	118.99202	118.99109	-7.816
Fragment 3	168.98937	168.98855	-4.852
Fragment 4	218.98618	218.98602	-0.731

Figure B.7 (f).

Text B.11 References

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Appendix C. Supplemental information for Chapter 5

Text C.1 Additional information on chemicals and materials

For the native standards (listed in Table C.4a), 2:3 FTCA, 5:2 sFTOH, and 5:2 ketone with a purity of 95-98% were obtained from Synquest Laboratories (Alachua, FL, USA), while a mixture of PFAAs (2 μg mL^{-1} as an acid or salt) and other native PFAS standards were obtained from Wellington Laboratories (Guelph, ON, Canada) and all had >98% purity. The isotopically labeled internal standards (listed in Table C.4b) were also obtained from Wellington Laboratories (Guelph, ON, Canada). Perfluorooctane aminoalkyl ammonium salt (PFOAAmS), custom synthesized at Beijing Surfactant Institute (Peking, China), was used as an internal standard for positive mode native analytes.

HPLC-grade acetonitrile (ACN) and methanol (MeOH), LC/MS-grade water, formic acid, acetic acid, ammonium hydroxide (NH_4OH), and ammonium fluoride (NH_4F) were purchased from Fisher Scientific (Whitby, ON, Canada). Ammonium acetate (purity ≥ 98%) was acquired from Sigma-Aldrich (St. Louis, MO, USA). Nitrogen (N_2) (purity 99.998%) was from Praxair distribution Inc. (Montreal, QC, Canada). The high-speed vortex (LP Vortex Mixer) was from Fisher Scientific (Whitby, ON, Canada).

Text C.2 Soil and headspace sample preparation

The soil samples at each sampling time point were were extracted by a previously reported method allowing improved extraction of zwitterionic and cationic PFAS.[1, 2] Specifically, 2 ml of 400 mM of ammonium acetate in methanol were added to each tube, and the tubes were high-speed vortexed for 0.5 min and ultrasonicated for 10 min followed by centrifugation at 5000 rpm for 5 min and then the supernatant was pipetted out. The same extraction steps were repeated twice (i.e. 3 extraction cycles in total), and the three extracts were combined. The combined extracts (~5.5 mL) were passed through Supelclean ENVI-Carb (500 mg/6 mL) cartridges previously conditioned with MeOH, with the clean eluate being directly recovered in a new 15-mL PP tube. After all the extracts had been transferred and eluted, the tube was rinsed with 0.5 mL of MeOH, and the rinse fraction was transferred to the cartridges. A final rinse step of the cartridges with 1 mL of MeOH was also performed. The volume of the soil extracts was then adjusted to 7.0 mL with methanol. 0.5 mL of extract (labeled as “Fraction O”) was aliquoted while the remaining 6.5 mL extract was

concentrated by nitrogen evaporation (45 ºC) to 1 mL (labeled as "Fraction A"). Both fractions were stored at -20 ºC in the freezer. Before instrumental analysis, 150 µl of each fraction was separately added with 50 µl of a 10-ng/mL internal standard solution mixture. The exact sample mass intake, final extract volume, and effective dilution factor (resulting from the preparation procedures and/or 1.33-fold dilution from aliquoting and addition of internal standards) were duly considered in the quantification procedure to convert the concentrations determined in the LC-MS injection vial (ng/mL) into concentrations in the soil sample (ng/g dw). Notably, fraction O was used for the analysis of both parent compounds (e.g., n:3 FTBs, n:1:2 FTBs, etc) and volatile transformation products (e.g., alcohols, ketones, etc) in soil extracts, while fraction A for the analysis of other potential biotic and/or abiotic transformation products.

An aliquot (150 µl) of the headspace extract stored in the freezer was taken out and added with 50 µl of a 10 ng/mL internal standard solution mixture right before instrument analysis.

Text C.3 Procedures for target, suspect screening, and nontarget analysis

First, select soil and headspace extracts or 5000 times-diluted Ansulite AFFF solution were submitted to a full scan mode UHPLC-HRMS analysis, with separate acquisitions for negative and positive ionization modes to maximize the number of points per chromatographic peak.[1] The maximum injection time of ions in the C-trap was set at 50 ms, and automated gain control at 3E6. The resolution of full-scan mode analysis was 7,0000 FWHM at m/z 200. Nontarget analysis (the workflow diagram shown in Figure C.2) was then performed to search for all PFAS (including both parent compounds and possible transformation products) present in the select samples.

Nontarget analysis. XCMS online (The Scripps Research Institute, La Jolla, USA) was used for preprocessing the full scan data by performing peak detection, filtering, and alignment. Specifically, Xcalibur raw files of the above samples were inputted pairwise with a procedural blank into XCMS Online (The Scripps Research Institute, La Jolla, USA) to eliminate the blank background, with the features at specific signal intensity (absolute peak area $\geq 10^5$) retained. For each sample, the generated Excel data frame of peak lists (accurate m/z, retention time, and signal intensity) was subject to mass defect filtering[2-4] using an in-house script programmed with

Anaconda (Python distribution). The measured mass from IUPAC mass scale was converted to Kendrick mass scale,[2] and extracted peaks with CF_2-normalized mass defects 0.85~1 or 0~0.15 were retained.[3] Additional rules were adopted from PFAS nontarget literature: the observation of ascending retention times for homolog series and the exclusion of dimers, adducts, and isotopes potentially corresponding to the same entity.[3, 4]

Following peak-picking, an automated library search (in-house script) was conducted within ± 15 ppm by comparing m/z features to general PFAS Excel databases and previously reported lists for AFFF-derived PFAS.[4, 5] The PFAS databases include: 1) the Norman Network PFAS Suspect List (available at https://www.norman-network.com/? q=node/236); 2) the OECD's New Comprehensive Global Database for PFASs, available at: http://www.oecd. org/chemicalsafety/portal-perfluorinated-chemicals/; 3) USEPA Comptox Chemistry Dashboard, available at: https://comptox.epa.gov/dashboard/chemical_lists /EPAPFASRL; 4) KEMI, available online at https://www.norman-network.com/?q=suspect-list-exchange.

Next, the soil and headspace samples at each sampling point or 5000 times-diluted Ansulite AFFF solution were submitted to another full-scan mode analysis with a polarity switching electrospray ionization. Target analysis was performed for the quantification of the parent compounds (e.g., 5:3 FTB, 5:1:2 FTB, 8:2 FTSA) and predicted metabolites with available authentic standards (listed in Table C.4), while suspect screening for the qualitative analysis of other suspected transformation products without available standards (Table C.5), including the ones predicted by EAWAG's BBD[6] and previous literature[7, 8] and additional PFAS identified by nontarget analysis.

Target analysis. The identification of target analytes relied on matching retention times (± 0.1 min) with certified standards, peak intensities superior to the set threshold (absolute peak area ≥ 1×10^5), exact mass accuracy with a tolerance of ± 10 ppm, and lack of detectable levels in the procedural/solvent blank or live soil matrix controls. The low absolute and effective matrix effects (Text C.3 and Figure C.5) in soils indicate the negligible influence of soil matrix on the instrument responses, therefore, solvent-based calibration curves were used for the quantification of PFAS with available standards. 9 calibration levels (0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, and 50 ng mL^{-1}) of native analytes with a constant concentration of internal standards (final: 2.5 ng mL^{-1}) were

included. Inverse-weighted (1/x) linear regressions were performed, with linearity range and determination coefficients (R^2) determined (shown in Table C.8).

Suspect screening. The identification of suspects relied on exact mass accuracy (tolerance ± 15 ppm), the isotopic pattern distribution, intensity for the extracted LC-MS chromatogram (absolute peak area) higher than 1×10^5, and lack of detectable levels should also be confirmed in the following controls: i) LC-MS procedural blanks; ii) live matrix controls and iii) sterile controls. Other factors, including consistent retention times among homologous series (e.g., ca. +0.5 to +0.8 min for each additional -CF_2 moiety in a series of PFAS homologs with the current C_{18} column and gradient elution program) and chromatographic peak shapes (e.g., presence of only linear isomers for FT-based PFAS) were also considered. Putative molecular formulae of the suspect PFAS were assigned using the "Elemental Composition" tool in Xcalibur based on exact mass accuracy (error <15 ppm), isotopic pattern distribution, and general elemental composition.[4, 9]

High-resolution MS/MS analysis. Select soil extracts or 5000 times-diluted Ansulite AFFF solution were rejected under target MS/MS mode (t-MS^2 mode, resolution of 70,000 FWHM) on the Orbitrap Q-Exactive). Select compounds among each class were inputted in the inclusion list (PRM acquisition mode) with normalized collision energies tested at different levels (between 20 and 50%). Spectrum elucidation was aided with MS/MS fragmentation patterns reported in PFAS literature[10, 11] and in-silico prediction (Mass Frontier, for positive mode PFAS). Other factors, including the observation of consistent retention time patterns among homologous series and chromatographic peak shapes (e.g., presence of only linear isomers for FT-based PFAS) were also considered. Identification confidence levels were finally assigned as per Schymanski.[12]

Text C.4 The selection of optimal mobile phase for instrument analysis method

Since the analysis of the volatile analytes [including fluorotelomer alcohols (e.g., 5:2 sFTOH, 6:2 FTOH, etc.) and ketones (e.g., 5:2 ketone)], short-chain polyfluoroalkyl and perfluoroalkyl acids (e.g., 1:3 FTCA, 2:3 FTCA, PFPrA) prove challenging, seven different mobile phases, and two different source temperatures (listed below in Table C.6) were tested for the analysis of FTBs and their potential quantitative transformation products (listed in Table C.4).

The analytical results (Figure C.3) showed that: (1) 5:3 FTB and 5:1:2 FTB were robustly detected across all conditions with excellent peak shapes; (2) The FT-based alcohols (e.g. 5:2 sFTOH) were very sensitive to the mobile phase type: the alcohols have a high signal when using acetate buffered phases (Condition 2, 4, 5 and 6), while they were barely or not detected when using formic acid phases (Condition 1), NH_4OH aqueous phase (Condition 3) or NH_4F phases (Condition 7); (3) 5:2 ketone had a very broad peak or a peak with huge tailing under acidic mobile phase conditions (Condition 2 and 6), while its peak was more focused and symmetric under Condition 5; (4) The short-chain carboxylates (e.g., PFPrA/PFBA) eluted in the dead time with the NH_4OH aqueous mobile phases (Condition 3 or 4); (5) The 1:3 acid was undetected with all acetate/acetic acid phases due to high baseline (Condition 2, 4-6). Overall, Condition 5 was finally chosen as the mobile phase for analysis of soil extracts and headspace elutes since it had good signal responses for 5:2 sFTOH, 5:2 ketone, and all other compounds except 1:3 acid (its peak can not be observed under this condition).

Regardless of mobile phase condition, the signal response of FT-based alcohols was approximately 2-3 times higher at a low auxiliary gas heater temperature (150°C) compared with a higher temperature (350°C), while all other analytes, including ketone, had a better response at 350°C. Therefore, an auxiliary gas heater temperature of 350°C was used during the instrument analysis of the soil and headspace extracts.

Text C.5 Details on soil microbial community analysis method

PCR amplification. After DNA extraction of soil samples, the V4 hypervariable region of bacterial 16S rRNA genes was amplified by polymerase chain reaction (PCR) using 341 (forward) 5´-TACGGGAGGCAGCAG-3´ and 806 (reverse) 5´-GGACTACVSGGGTATCTAAT-3´ primers. Reaction mixtures (20 µL) contained 1× GoTaq Green Master Mix (Promega, Madison, Wisconsin, USA), 0.5 µmol/L of each forward and reverse primer, and 5 µL template DNA. Thermocycling conditions included an initial denaturation at 95 °C for 4 min, 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 45 s, and 72 °C for 2 min, with a final extension at 72 °C for 5 min. Agencourt AMPure XP beads (Beckman–Coulter, Brea, Calif.) were

used to remove DNA fragments smaller than 100 bp before the second round of PCR with adaptor and Ion Xpress barcoded primers [341 (forward), 5´-CCATCTCATCCCTGCGTGTCTCCGACTCAG[barcode]TACGGGAGGCAGCAG-3´; 806 (reverse), 5´-CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATGGACTACVSGGGTATCTAAT-3´). Thermocycling conditions for the second round of PCR were the same as for the first round of PCR, except the annealing temperature was adjusted to 65 °C and the program consisted of 20 cycles. A second clean-up was then completed using Agencourt AMPure XP beads (Beckman–Coulter).

Sequencing data processing. Sequencing data were processed using AMPtk v1.5.1 for quality filtering,[13] operational taxonomic unit (OTU) clustering at 97% sequence identity, and to assign taxonomies using the Greengenes 16S rRNA gene database.[14] The resulting dataset was processed in R.[15] Samples with less than 9,900 sequencing reads were deleted from the dataset and the remaining samples were randomly subsampled to obtain equal numbers of sequencing reads between samples (9,933 reads) using the package *phyloseq.*[16] MDS plots based on Bray-Curtis dissimilarity between samples were prepared using the *vegan* and *ggplot2* packages in R,[17, 18] to evaluate differences in microbial community composition between samples.

Text C.6 Spike recovery tests

The spike recovery tests for FTBs, Ansulite AFFF solution mixture, and their quantitative transformation products were performed, and the detailed procedures were described as follows.

Soil extract spiked before extraction. Native PFAS (5:3 FTB, 5:1:2 FTB, and their quantifiable transformation products) were spiked to a triplicate of live and sterile M soil samples at a concentration of 100 ng/g (dw) for parent compounds (5:3 FTB, 5:1:2 FTB) and volatile transformation products (5:2 ketone and 5:2 sFTOH), and 5 ng/g for quantifiable biotransformation products.

Briefly, approximately 1 g dry weight (dw) of live and sterile M soils were added into 15-ml centrifuge tubes, and the soils were adjusted to a gravimetric moisture content of 23% (~80% of water holding capacity at 1/3 bar). Then a set of soil samples were spiked with 20 µl of a methanol stock mixture solution (containing 5 ppm of 5:3 FTB, 5:1:2 FTB, 5:2 sFTOH, and 5:2 ketone) to result in an initial spiked concentration of 100 ng/g dw**,** followed by spiking with 20 ul of another methanolic solution mixture (containing 0.25 ppm of their postulated biotransformation products including 5:3 FTCA, 4:3 FTCA, 3:3 FTCA, 2:3 FTCA, 6:2 FTCA, 6:2 FTUCA, C_3-C_{14} PFCA, C_4, C_6, C_8, and C_{10} PFSA) to result in an initial spiked concentration of 5 ng/g dw. The set of tubes that were initially spiked with PFAS was referred to as "Spiked Before". Note that another set of soil samples not spiked initially with PFAS was subject to the same extraction and cleanup procedure as the spiked samples; such tubes were referred to as "Spiked After" and will be later used to provide a post-extraction matrix-matched reference for calculating the recovery.

Following a wait time of 2 h, the soils in the PFAS-spiked tubes were extracted and cleaned up in the same fashion as described in Text S2. The polished extracts were adjusted to a volume of 7.0 mL with methanol. An aliquot of the extract (0.5 mL, labeled as "Fraction O") was taken out, while the remaining 6.5 mL extract was concentrated by nitrogen evaporation (45 ^{o}C) and finally adjusted to a volume of 1 mL (labeled as "Fraction A"). Both fractions were stored in the freezer at -20 °C until instrumental analysis. Notably, fraction O was used for the analysis of both parent compounds (e.g., 5:3 FTB, 5:1:2 FTB, etc) and volatile transformation products (e.g., 5:2 sFTOH, 5:2 ketone), while fraction A for the analysis of other potential transformation products (e.g., PFCA, FTCA, FTUCA, etc).

Following brief vortexing (0.25 min), a 150-µL aliquot of each fraction was introduced into a 250-µL LC-MS injection vial, along with 50 µL of a 10-ng mL^{-1} internal standard solution mixture, Subsequently, the extracts were submitted to ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometer (UHPLC-HRMS) analysis.

Soil extract spiked at the end of the preparation. A triplicate of live and sterile M soil not initially fortified with PFAS was subject to the same extraction and cleanup procedures, and the resulting soil extracts were spiked post sample preparation with an equivalent amount of native PFAS (5:3

FTB, 5:1:2 FTB, and their quantifiable transformation products) at the last step (i.e., into the injection vial). It is important to note that the matrix dilution (resulting from the final spiking for "Spiked After" samples) is kept rigorously the same as for the "Spiked Before" samples. The "Spiked After" samples were indeed constructed by combining 50 µL of native solution (at an appropriate concentration to reflect the expected concentration to be the same as for the final "Spiked Before" extract), 50 µL of a 10-ng mL^{-1} internal standard solution, and 100 µL of non-spiked filtered soil extract into an injection vial. Hence, matrix effects, if any, would not be expected to be different between the "Spiked Before" and "Spiked After" samples, allowing a legitimate comparison for the recovery determination.

Non-spiked reference. A triplicate of live and sterile M soil samples not spiked with PFAS was also extracted and cleaned up using an Envi-carb cartridge in the same fashion. No native standards were added to these samples. These samples were referred to as "non-spiked" samples. For these soil extracts, internal standards were also added at the end of the preparation procedure to the LC-MS injection vials for a final concentration of 2.5 ng mL^{-1} each.

Calculation of the recovery. The recovery of native analytes from the soil samples (i.e. live or sterile M soil) was determined as follows:

$$\text{Recovery (\%)} = 100\times \frac{\text{SB}-\text{NS}}{\text{SA}-\text{NS}} \quad (1)$$

Where SB is the analyte to IS response ratio of the sample spiked at the start of the preparation procedure with native analytes ("Spiked Before" samples), SA is the analyte to IS response ratio of the sample spiked at the end of the preparation procedure with native analytes ("Spiked After" samples), and NS is the analyte to IS response ratio of the reference (non-spiked samples).

The recovery test of Ansulite AFFF-derived PFAS components and their potential quantitative transformation products were performed in the same way, while the major difference is that the soils were spiked with a different methanolic solution mixture for both the soil extract spiked before extraction and soil extract spiked at the end of the preparation. Specifically, for the former, 20 ul of a methanolic solution mixture containing Ansulite AFFF (409.5-fold dilution) as well as native standards (7:3 FTCA, 8:2 FTUCA, and 7:2 sFTOH) was spiked into live and sterile soils to

result in an initial concentration of 200 ng/g for 5:1:2 FTB (the concentrations of other PFAS components can be calculated correspondingly) and 5 ng/g for the 7:3 FTCA, 8:2 FTUCA, and 7:2 sFTOH. For the latter, an aliquot of the soil extract after cleanup (0.5 out of 7 ml) was spiked with the 5733-fold diluted Ansulite foam solution, while another aliquot of soil extracts after nitrogen evaporation was spiked with the native standards mixture containing 7:3 FTCA, 8:2 FTUCA, and 7:2 sFTOH**.**

Text C.7 Assessment of matrix effects

Matrix-matched and matrix-free calibration curves. Calibration curves were generated in the matrix-free solvent (MeOH) or live soil final extracts by spiking native analytes at 9 calibration levels covering > 2 orders of magnitude (0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, and 50 ng mL^{-1}) while the concentration of the internal standards was kept constant (2.5 ng mL^{-1}). Using 9 intermediate spiking solutions, we constructed matrix-matched calibration levels in such a way that the soil matrix concentration would be constant between the different calibration levels; additionally, the matrix dilution factor of the calibration curve levels was equivalent to that of the samples as per our procedure defined in Text C.2. Inverse-weighted (1/x) linear regressions were generated by plotting the native analyte to internal standard peak area ratio (y-axis) as a function of native analyte to internal standard spiked concentration (x-axis).

Effective matrix effect (at the instrumental analysis stage). The soil matrix was subject to the same extraction and cleanup procedure as described in Text C.2. Native PFASs (5:3 FTB, 5:1:2 FTB, and their quantifiable transformation products) and isotope-labeled ISs were spiked post-extraction to create matrix-matched calibration curves. Meanwhile, a clean solvent spiked with native PFAS analytes was also used to produce a matrix-free calibration curve. The slopes of the resulting matrix-matched calibration curves were then compared to those prepared in clean solvent to assess the effective matrix effects at the instrumental stage.

As reported,[11] instrumental matrix effects were evaluated by comparing solvent-based native analyte to internal standard area ratios (S) to those of soil extracts spiked post-extraction (M) but corrected by the non-spiked sample initial contribution (ref), as described hereafter:[19]

$$\text{Effective matrix effect} = 100\times \left(\frac{\text{M}-\text{ref}}{\text{S}} - 1\right)$$

Text C.8 Persistence of 5:3 FTB and 5:1:2 FTB mixture in aerobic soil

The evidence supporting the high persistence of these two compounds is shown in Figure C.6 and discussed as follows. (1) the concentrations of 5:3 FTB and 5:1:2 FTB remained unchanged in live and sterile M soils between days 0 and 120 (Figure C.6). (2) The likely biotransformation products, which showed higher levels on Day 120 than on Day 0 in the live soil but not in the sterile soil or soil matrix control, were 5:3 FTCA (increase by 0.001mol%), PFPeA (increase by 0.03 mol %), and PFHxA (increase by 0.01 mol%). Although the formation of the products is in contrast with their non-formation in the single betaine incubation experiments, the increases were insignificant. The starting concentration of betaine compounds was twice those in single betaine experiments, so we hypothesize that the different starting concentrations might have an impact. Furthermore, nontarget analysis and suspect screening did not reveal the presence of any other potential transformation products. We once again observed increases in FBSA and PFBS concentrations over 120 d in the live CA-M soil only, by 0.39 and 1.60 mol%, respectively. The yields were slightly higher than those in the single betaine experiments, but it is hard to pinpoint any causes for the slightly higher yields because of the lack of identified origins of these precursor compounds. Lastly, satisfactory molar balance in live and sterile CA-M soil (100~104 mol% and 124~125 mol%, respectively) over the 120-d incubation was achieved, shown in Figure C.8c.

Table C.1 The properties of four soils used for the biotransformation study.

	CA-M surface soil	**CA-L surface soil**	**US-F surface soil**	**US-G subsurface soil**
Sampling location	McGill University, Macdonald Campus, Montreal, QC	Lac Du Bois Grasslands, BC, Canada	5000 SR26 W, West Lafayette, IN, 47906, USA	5000 SR26 W, West Lafayette, IN 47906, USA
Soil depth (mbgs)	0-0.15	0-0.2	0.05-0.15	0.30-0.38
Textural Class	Sandy loam	Sandy loam	Loam	Loam
Sand percentage	64.9	63	38	37
Silt percentage	26.0	28	48	43
Clay percentage	9.1	9	14	20
Organic matter (%)	3.8	2.6	4.1	1.8
pH	7.2	7.7	5.1	6.7
Cation Exchange Capacity (CEC, meq/100g)	18.9	14.0	15.1	10.6
Phosphate (μg/g)	90	10	10	29
Potassium (μg/g)	158	832	118	78
Magnesium (μg/g)	142	953	103	294
Calcium (μg/g)	3260	800	630	1350
Sodium (μg/g)	31	7	7	11
Aluminium (μg/g)	465	606	807	872
Iron(μg/g)	10.9	50	76	64
Nitrate Nitrogen (μg/g)	59	9	7	3
C/N ratio	11.3	17.5	14.8	10.7
Water holding capacity at 1/3 bar (%)	19.8	21.8	28.0	28.0
Solvita CO_2-C (μg/g)	82	33	69	20
*Microbial biomass (μg/g)	1830	760	1550	470

*Microbial biomass is calculated based on the Solvita CO_2 burst results (https://solvita.com/soil/potential-min-n-calc/).

Table C.2 PFAS background levels in four different soils.

PFAS analyte	PFAS concentrations in soils (ng/g dw)			
	CA-M soil	CA-L soil	US-F soil	US-G soil
PFPrA	ND	ND	ND	ND
PFBA	0.04±0.02	0.16±0.01	0.50±0.05	0.03±0.01
PFPeA	ND	ND	ND	ND
PFHxA	ND	ND	ND	ND
PFHpA	ND	ND	ND	ND
PFOA	0.34±0.14	ND	ND	ND
PFNA	ND	ND	ND	ND
PFDA	ND	ND	ND	ND
PFUnA	ND	ND	ND	ND
PFDoA	ND	ND	ND	ND
PFTrA	ND	ND	ND	ND
PFTeDA	ND	ND	ND	ND
PFHxDA	ND	ND	ND	ND
PFPrS	ND	ND	ND	ND
PFBS	ND	ND	ND	ND
PFPeS	ND	ND	ND	ND
PFHxS	ND	ND	ND	ND
PFHpS	ND	ND	ND	ND
PFOS	0.90±0.07	ND	ND	ND
PFNS	ND	ND	ND	ND
PFDS	0.89±0.05	ND	ND	ND
PFDoS	ND	ND	ND	ND
3:3 FTCA	ND	ND	ND	ND
4:3 FTCA	ND	ND	ND	ND
5:3 FTCA	ND	ND	ND	ND
7:3 FTCA	ND	ND	ND	ND
6:2 FTUA	ND	ND	ND	ND
8:2 FTUA	ND	ND	ND	ND
10:2 FTUA	ND	ND	ND	ND
4:2 FTS	ND	ND	ND	ND
6:2 FTS	ND	ND	ND	ND
8:2 FTS	ND	ND	ND	ND
10:2 FTS	ND	ND	ND	ND
6:2 FTAB	ND	ND	ND	ND
5:3 FTB	ND	ND	ND	ND
5:1:2 FTB	ND	ND	ND	ND
FBSA	ND	ND	ND	ND
FH_XSA	ND	ND	ND	ND
FOSA	ND	ND	ND	ND
FOSAA	ND	ND	ND	ND
MeFOSAA	ND	ND	ND	ND
EtFOSA	ND	ND	ND	ND
MeFOSA	ND	ND	ND	ND
EtFOSAA	ND	ND	ND	ND
PFHxSAm(AmPr-FHxSA)	ND	ND	ND	ND
PFHxSAmS (N-TAmP-FHxSA)	ND	ND	ND	ND
PFOAB (N-CMAmP-FOAd)	ND	ND	ND	ND
PFOANO (N-OxAmP-FOAd)	ND	ND	ND	ND
PFOSAm (AmPr-FOSA)	ND	ND	ND	ND
PFOSB (N-CMAmP-FOSA)	ND	ND	ND	ND
PFOSNO (N-OxAmP-FOSA)	ND	ND	ND	ND
PFOSAmS (N-TAmP-FOSA)	ND	ND	ND	ND
PFECHS	ND	ND	ND	ND

Table C.2 The initial PFAS concentrations in Ansulite AFFF-spiked live and sterile soils.

PFAS components	Initial concentration in soils (ng/g dw)	Molar fraction (%)
5:1:2 FTB	800.0	38.33
7:1:2 FTB	767.8	29.88
9:1:2 FTB	192.5	9.45
5:3 FTB	189.0	6.88
7:3 FTB	170.8	6.31
9:3 FTB	61.7	2.08
11:1:2 FTB	46.9	1.33
4:1:3 FTB	20.9	1.09
8:2 FTS	19.2	0.75
6:4 FTB	17.2	0.75
11:3 FTB	17.2	0.50
6:1:3 FTB	16.6	0.69
8:4 FTB	14.4	0.52
6:2 FTB	9.1	0.42
10:2 FTB	6.6	0.25
8:1:3 FTB	5.7	0.20
10:4 FTB	4.7	0.14
4:4 FTB	4.3	0.24
13:1:2 FTB	3.1	0.08
13:3 FTB	2.7	0.07
10:1:3 FTB	1.3	0.04
10:2 FTS	0.3	0.01
6:2 FTS	0.2	0.01

Table C.3 Native standards and isotope-labeled internal standards (IS) used for the FTB and Ansulite transformation experiments.

(a) List of native standards used for quantification.

No.	Acronym	Full name	$[M+H]^+$ or $[M-H]^-$	Theoretical m/z	Observed m/z	Error (ppm)	RT (min)	IS used	RT of IS (min)	Commercial Sources
1	5:3 FTB	5:3 fluorotelomer betaine	$[C_{12}F_{11}H_{15}NO_2]^+$	414.09271	414.09201	-1.7	5.18	PFOAAmS	5.99	
2	5:1:2 FTB	5:1:2 fluorotelomer betaine	$[C_{12}F_{12}H_{14}NO_2]^+$	432.08329	432.08258	-1.6	5.24	PFOAAmS	5.99	Wellington Laboratories (Guelph, ON, Canada)
3	6:2 FTS	6:2 fluorotelomer sulfonate	$[C_8F_{13}H_4SO_3]^-$	426.96756	426.96878	2.9	5.60	M2-6:2 FTS	5.60	
4	8:2 FTS	8:2 fluorotelomer sulfonate	$[C_{10}F_{17}H_4SO_3]^-$	526.96138	526.96259	2.3	6.24	M2-8:2 FTS	6.24	
5	2:3 FTCA	2:3 fluorotelomer carboxylate	$[C_5F_5H_4O_2]^-$	191.01314	191.01358	2.3	2.33	MPFHxA	4.70	Synquest Laboratories (Alachua, FL, USA)
6	3:3 FTCA	3:3 fluorotelomer carboxylate	$[C_6F_7H_4O_2]^-$	241.00995	241.01083	3.7	3.84	MPFHxA	4.70	
7	4:3 FTCA	4:3 fluorotelomer carboxylate	$[C_7F_9H_4O_2]^-$	291.00676	291.00806	4.5	4.65	MPFOA	5.64	
8	5:3 FTCA	5:3 fluorotelomer carboxylate	$[C_8F_{11}H_4O_2]^-$	341.00356	341.00482	3.7	5.26	MPFOA	5.64	
9	7:3 FTCA	7:3 fluorotelomer carboxylate	$[C_{10}F_{15}H_4O_2]^-$	440.99717	440.99860	3.2	5.90	MPFDA	6.27	
10	6:2 FTCA	6:2 fluorotelomer carboxylate	$[C_8H_3F_{13}O_2]^-$	376.98472	376.98077	-10.5	5.17	MFHEA	5.17	
11	8:2 FTCA	8:2 fluorotelomer carboxylate	$[C_{10}H_3F_{17}O_2]^-$	476.97833	476.97626	-4.3	6.07	MFHEA	5.17	
12	6:2 FTUCA	6:2 fluorotelomer unsaturated acid	$[C_8H_2F_{12}O_2]^-$	356.97849	356.97903	1.5	5.15	MFHUEA	5.15	
13	8:2 FTUCA	8:2 fluorotelomer unsaturated acid	$[C_{10}H_2F_{16}O_2]^-$	456.97210	456.97244	0.7	6.05	MFOUEA	6.04	
14	10:2 FTUCA	10:2 fluorotelomer unsaturated acid	$[C_{11}H_2F_{18}O_2]^-$	556.96571	556.9671	2.5	6.58-	MFOUEA	6.04	Wellington Laboratories (Guelph, ON, Canada)
15	PFPrA	Perfluoropropionoic acid	$[C_3F_5O_2]^-$	162.98225	162.98175	-3.1	1.10	MPFBA	2.72	
16	PFBA	Perfluorobutanoic acid	$[C_4F_7O_2]^-$	212.97947	212.97914	-1.5	2.72	MPFBA	2.72	
17	PFPeA	Perfluoropentanoic acid	$[C_5F_9O_2]^-$	262.97669	262.97662	-0.3	3.95	MPFHxA	4.70	
18	PFHxA	Perfluorohexanoic acid	$[C_6F_{11}O_2]^-$	312.97335	312.97369	1.1	4.70	MPFHxA	4.70	
19	PFHpA	Perfluoroheptanoic acid	$[C_7F_{13}O_2]^-$	362.97013	362.97067	1.5	5.23	MPFOA	5.64	
20	PFOA	Perfluorooctanoic acid	$[C_8F_{15}O_2]^-$	412.96735	412.96738	0.1	5.64	MPFOA	5.64	
21	PFNA	Perfluorononanoic acid	$[C_9F_{17}O_2]^-$	462.96457	462.96439	-0.4	5.99	MPFNA	5.99	
22	PFDA	Perfluorodecanoic acid	$[C_{10}F_{19}O_2]^-$	512.96179	512.96094	-1.7	6.26	MPFDA	6.27	
23	PFUnA	Perfluoroundecanoic acid	$[C_{11}F_{21}O_2]^-$	562.95860	562.95789	-1.3	6.52	MPFUnA	6.52	

24	PFDoA	Perfluorododecanoic acid	$[C_{12}F_{23}O_2]^-$	612.95540	612.95477	-1.0	6.73	MPFDoA	6.73	
25	PFTrDA	Perfluorotridecanoic acid	$[C_{13}F_{25}O_2]^-$	662.95221	662.95209	-0.2	6.90	MPFDoA	6.73	
26	PFTeDA	Perfluorotetradecanoic acid	$[C_{14}F_{27}O_2]^-$	712.94901	712.94934	0.5	7.06	MPFDoA	6.73	
27	PFBS	Perfluorobutane sulfonate	$[C_4F_9SO_3]^-$	298.94326	298.9436	1.1	4.09	MPFHxS	5.18	Wellington Laboratories (Guelph, ON, Canada)
28	PFHxS	Perfluorohexane sulfonate	$[C_6F_{13}SO_3]^-$	398.93712	398.93747	0.9	5.18	MPFHxS	5.18	
29	PFOS	Perfluorooctane sulfonate	$[C_8F_{17}SO_3]^-$	498.93126	498.9313	0.1	5.89	MPFOS	5.89	
30	PFDS	Perfluorodecane sulfonate	$[C_{10}F_{21}SO_3]^-$	598.92487	598.92529	0.7	6.40	MPFOS	5.89	
31	6:2 FTOH	6:2 fluorotelomer alcohol	$[C_{10}F_{13}H_8O_3]^-$*	423.02658	423.02975	7.5	6.19	MPFOA	5.64	
32	8:2 FTOH	8:2 fluorotelomer alcohol	$[C_{12}F_{17}H_8O_3]^-$*	523.02019	523.0203	0.1	6.77	MPFOA	5.64	
33	5:2 sFTOH	5:2 fluorotelomer secondary alcohol	$[C_9F_{11}H_8O_3]^-$*	373.02978	373.03018	1.1	5.98	MPFOA	5.64	
34	7:2 sFTOH	7:2 fluorotelomer secondary alcohol	$[C_{11}F_{15}H_8O_3]^-$*	473.02339	473.0232	-0.4	6.71	MPFDA	6.27	Synquest Laboratories (Alachua, FL, USA)
35	5:2 ketone	5:2 fluorotelomer ketone	$[C_7F_{11}OH_2]^-$	310.99299	310.99362	2.0	5.90	MPFOA	5.64	
36	FBSA	Perfluorobutane sulfonamide	$[C_4F_9SO_2NH]^-$	297.95843	297.95934	3.1	4.64	M8FOSA	6.34	
37	FHxSA	Perfluorohexane sulfonamide	$[C_6F_{13}SO_2NH]^-$	397.95204	397.95288	2.1	5.69	M8FOSA	6.34	Wellington Laboratories (Guelph, ON, Canada)
38	FOSA	Perfluorooctane sulfonamide	$[C_8F_{17}SO_2NH]^-$	497.94631	497.9465	0.4	6.33	M8FOSA	6.34	

Note: The red star (*) represents the acetate adduct. 5:2 sFTOH $[C7F11H5O+CH3COO]^-$, 7;2 sFTOH $[C9F15H5O+CH3COO]^-$, 6:2 FTOH $[C8F13H5O+CH3COO]^-$, 8:2 FTOH$[C10F17H5O+CH3COO]^-$.

(b) List of isotope-labeled internal standards (IS) used for quantification.

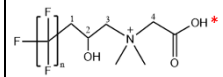
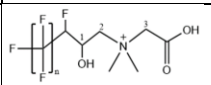
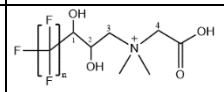
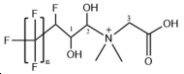
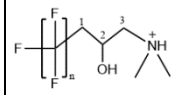
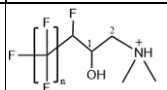
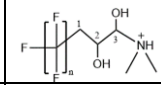
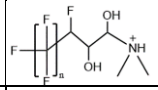
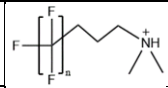
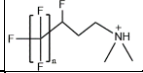
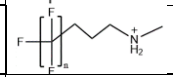
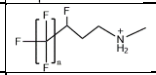
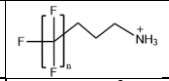
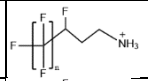
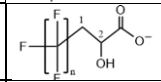
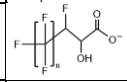
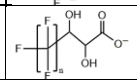
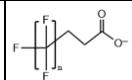
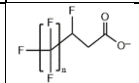
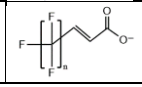
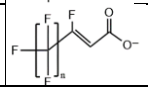
Acronym	Full Name	Formula	Theoretical mz	Observed mz	Mass error (ppm)	RT (min)	Analysis mode
MPFBA	Perfluoro-n-[1,2,3,4-$^{13C}_4$] butanoic acid	$[^{13C}_4F_7O_2]^-$	216.99177	216.99344	7.7	2.72	
MPFHxA	Perfluoro-n-[1,2-$^{13C}_2$] hexanoic acid	$[^{12}C_3{}^{13C}_2F_9O_2]^-$	314.98039	314.98050	0.3	4.70	
MPFOA	Perfluoro-n-[1,2,3,4-$^{13C}_4$] octanoic acid	$[^{12}C_4{}^{13C}_4F_{15}O_2]^-$	416.97975	416.98096	2.9	5.64	
MPFNA	Perfluoro-n-[1,2,3,4,5-^{13}C5] nonanoic acid	$[^{12}C_4{}^{13C}_5F_{17}O_2]^-$	467.97969	467.98096	2.7	5.99	
MPFDA	Perfluoro-n-[1,2-^{13}C2] decanoic acid	$[^{12}C_8{}^{13C}_2F_{19}O_2]^-$	514.96640	514.96783	2.8	6.27	
MPFUnA	Perfluoro-n-[1,2-^{13}C2] undecanoic acid	$[^{12}C_9{}^{13C}_2F_{21}O_2]^-$	564.96326	564.96484	2.8	6.52	
MPFDoA	Perfluoro-n-[1,2-^{13}C2] dodecanoic acid	$[^{12}C_{10}{}^{13C}_2F_{23}O_2]^-$	614.96041	614.96185	2.3	6.73	
MPFHxS	Sodium perfluoro-1-hexane[^{18}O2]sulfonate	$[C_6F_{13}S^{18}O_2{}^{16}O]^-$	402.94505	402.94598	2.3	5.18	
MPFOS	Sodium perfluoro-1-[1,2,3,4-^{13}C4]octanesulfonate	$[^{12}C_4{}^{13C}_4F_{17}SO_3]^-$	502.94334	502.94485	3.0	5.89	
M8FOSA	Perfluoro-1-[^{13}C8] octane sulfonamide	$[^{13C}_8F_{17}NHSO_2]^-$	505.97249	505.97305	1.1	6.34	ESI (-)
M6:2 FTUA (MFHUEA)	2H-Perfluoro-[1,2-^{13}C2]-2-octenoic acid	$[^{13C}_2C_8F_{17}H_4SO_3]^-$	358.98520	358.98682	4.5	5.15	
M8:2 FTUA (MFOUEA)	2H-perfluoro-[1,2-^{13}C2]-1-decenoic acid	$[^{12}C_6{}^{13C}_2F_{12}HO_2]^-$	458.97881	458.97891	0.2	6.04	
M6:2 FTCA (MFHEA)	2-Perfluorohexyl-[1,2-^{13}C2]-ethanoic acid	$[^{12}C_6{}^{13C}_2F_{13}H_2O_2]^-$	378.99142	378.99295	4.0	5.17	
M2-6:2FTS	1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate	$[^{12}C_6{}^{13C}_2F_{13}H_4SO_3]^-$	428.97537	428.97580	1.0	5.60	
M2-8:2FTS	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13C}_2$]-decane sulfonate	$[^{12}C_8{}^3C_2F_{17}H_4SO_3]^-$	528.96898	528.96808	-1.7	6.24	
PFOAAmS*	Perfluorooctane amidoalkyl ammonium	$[C_{14}H_{16}F_{15}N_2O]^+$	513.10176	513.10229	1.0	5.99	ESI (+)

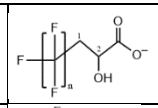
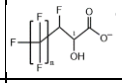
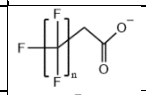
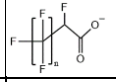
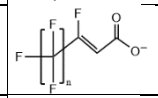
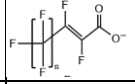
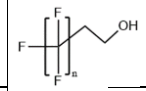
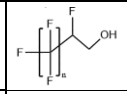
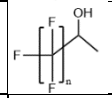
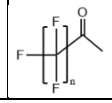
Note:

1. The red star (*) means the PFOAAmS was used as the internal standard of positive mode PFAS analytes due to the lack of isotope-labeled IS.

2. Except for PFOAAmS, which was custom synthesized by Beijing Surfactant Institute (Peking, China), all the other standards were purchased from Wellington Laboratories (Guelph, ON, Canada)

Table C.4 Potential qualitative transformation products from either pure betaine (5:3 FTB, or 5:1:2 FTB) or AFFF-derived n:3/n:1:2 FTBs (n = 5, 7, 9, 11 and 13) in aerobic soils.

PFAS classes	Analyte (for parent n:3 FTB, n=5, 7, 9, 11, 13)	Structure	Chemical formula (n=5)	Theoretical m/z (n=5)	Analyte (for parent n:1:2 FTB, n=5, 7, 9, 11, 13)	Structure	Chemical formula (n=5)	Theoretical m/z (n=5)
Hydroxylated betaine	n:3 OH-FTB (n=1~13)		$C_{12}H_{15}F_{11}NO_3^+$	430.08708	n:1:2 OH-FTB (n=1~13)		$C_{12}H_{14}F_{12}NO_3^+$	448.07766
Dihydroxylated betaine	n:3 diOH-FTB (n=1~13)		$C_{12}H_{15}F_{11}NO_4^+$	446.08199	n:1:2 diOH-FTB (n=1~13)		$C_{12}H_{14}F_{12}NO_4^+$	464.07312
Hydroxylated tertiary amine	OH-n:3 FTA (n=1~13)		$C_{10}H_{13}F_{11}NO^+$	372.08160	OH-n:1:2 FTA (n=1~13)		$C_{10}H_{12}F_{12}NO^+$	390.07273
Dihydroxylated tertiary amine	diOH-n:3 FTA (n=1~13)		$C_{10}H_{13}F_{11}NO_2^+$	388.07652	diOH-n:1:2 FTA (n=1~13)		$C_{10}H_{12}F_{12}NO_2^+$	406.06709
Tertiary amine	n:3 FTA (n=1~13)		$C_{10}H_{13}F_{11}N^+$	356.08669	n:1:2 FTA (n=1~13)		$C_{10}H_{12}F_{12}N^+$	374.07726
Secondary amine	n:3 demethyl-FTA (n=1~13)		$C_9H_{11}F_{11}N^+$	342.07104	n:1:2 demethyl-FTA (n=1~13)		$C_9H_{10}F_{12}N^+$	360.06161
Primary amine	n:3 didemethyl-FTA (n=1~13)		$C_8H_9F_{11}N^+$	328.05539	n:1:2 didemethyl-FTA (n=1~13)		$C_8H_8F_{12}N^+$	346.04596
Hydroxylated-n:3/n:1:2 FTCA	OH-n:3 FTCA (n=1~13)		$C_8H_4F_{11}O_3^-$	356.99903	OH-n:1:2 FTCA (n=1~13)		$C_8H_3F_{12}O_3^-$	374.98961
Dihydroxylated - n:3/n:1:2 FTCA	diOH-n:3 FTCA (n=1~13)		$C_8H_4F_{11}O_4^-$	372.99394	NA	NA	NA	
n:3/n:1:2 FTCA	n:3 FTCA (n=1~13)		$C_8H_4F_{11}O_2^-$	341.00411	n:1:2 FTCA(n=1~13)		$C_8H_3F_{12}O_2^-$	358.99469
n:3/n:1:2 FTUCA	n:3 FTUCA (n=1~13)		$C_8H_2F_{11}O_2^-$	338.98846	n:1:2 FTUCA(n=1~13)		$C_8HF_{12}O_2^-$	356.97904

OH n:3/n:1:2 FTCA	α-OH n:3 FTCA *or* β-OH n:3 FTCA(n=1~13)		$C_8H_4F_{11}O_3^-$	356.99903	α-OH n:1:2 FTCA *or* β-OH n:1:2 FTCA(n=1~13)		$C_8H_3F_{12}O_3^-$	374.98961
n:2/n:1:1 FTCA	n:2 FTCA (n=1~13)		$C_7H_2F_{11}O_2^-$	326.98846	2H-PFCA or n:1:1 FTCA (n=1~13)		$C_7HF_{12}O_2^-$	344.97904
n:2/n:1:1 FTUCA	n:2 FTUCA (n=1~13)		$C_8HF_{12}O_2^-$	356.97904	n:1:1 FTUCA (n=1~13)		$C_8F_{13}O_2^-$	374.96962
n:2/n:1:1 FTOH	n:2 FTOH (n=1~13)		$C_7H_4F_{11}O^-$	313.00920	n:1:1 FTOH (n=1~13)		$C_7H_4F_{12}O^-$	332.00760
n:2/n:1:1 sFTOH	n:2 sFTOH (n=1~13)		$C_7H_4F_{11}O^-$	313.00865	NA	NA	NA	NA
n:2/n:1:2 ketone	n:2 ketone (n=1~13)		$C_7H_3F_{11}O$	312.00082	NA	NA	NA	NA

Note: The pure betaine (5:3/5:1:2 FTB) was expected to lead to qualitative transformation products with fluorinated carbon chain length less than or equal to five (n=1~5), while qualitative transformation products with different fluorinated chain lengths (n=1~13) were expected to be formed from the Ansulite AFFF containing a mixture of n:3/n:1:2 FTB (n=5, 7, 9, 11and 13).

The volatile PFAS potentially formed from n:3 or n:1:2 FTB are marked in blue color. NA: not available.

The red asterisk (*) represents that the hydroxyl group (-OH) can occur in different positions (labeled as 1, 2, 3, and 4 on the compound structures).

Table C.5 The seven mobile phases and two source temperatures tested to determine the instrument analysis method.

Condition No.	Mobile phase	Source temperature
1	A: H_2O with 0.1% formic acid B: Acetonitrile with 0.1% formic acid	150°C, 350 °C
2	A: H_2O with 0.15% acetic acid B: Acetonitrile with 0.15% acetic acid	150°C, 350 °C
3	A: H_2O with 0.1% NH_4OH B: MeOH	150°C, 350 °C
4	A: H_2O with 0.1% NH_4OH B: 80%MeOH/20%ACN with 10 mM Ammonium acetate	150°C, 350 °C
5	**A: H_2O with 10 mM Ammonium acetate** **B: 80%MeOH/20%ACN with 10 mM Ammonium acetate**	**150°C, 350 °C**
6	A: H_2O with 2 mM Ammonium acetate B: 9n%ACN/n%H_2O with 2 mM Ammonium acetate	150°C, 350 °C
7	A: H_2O with 0.1 mM NH_4F B: MeOH with 0.1 mM NH_4F	150°C, 350°C

Table C.6 Details on the instrument analytical methods.

Instrument	Dionex UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer		
Ionization	Positive and negative heated electrospray		
Acquisition mode	Full scan MS mode t-MS^2 mode		
Analytical Column	Thermo C18 Hypersil aQ Gold column, 1.9 μm, 100 x 2.1mm		
Delay Column	Thermo Hypercarb trap column, 7 μm, 20 x 2.1 mm,		
Column Temperature	50°C		
Auxiliary gas heater temperature	350°C		
Mobile Phases	A: HPLC-water with 10mM NH_4CH_3COOH B: 80%MeOH/20%ACN with 10mM NH_4CH_3COOH.		
Gradient Profile	Time (min) 0.0 6.5 9.0 11.0 11.1	Percentage B 10 100 100 10 Stop	Flow Rate (mL/min) 0.450 0.450 0.450 0.450
Injection Volume	10 μL (Full scan) 10 μL (t-MS^2)		
Orbitrap MS parameters	AGC target (maximum capacity in C-trap): 3×10^6, Maximum injection time: 50 ms.		
The heated electrospray ionization source parameters	Sheath gas flow rate: 40 arbitrary units (a.u.), Auxiliary gas flow rate: 15 a.u., Sweep gas flow rate: 0 a.u., Capillary temperature: 320°c, Vaporizer temperature: 350°c, Auxiliary gas heater temperature: 350°C. Spray voltage: -4 kV or +4 kV.		
Full scan mode parameters	Resolution: 70,000 full width at half maximum (FWHM) at m/z 200. Mass scan range: m/z 100–1000.		
t-MS^2 mode parameters	Resolution: 70,000 FWHM. Normalized collision energy (NCE): 20−50%.		

Note: The Dionex Ultimate 3000 LC chain was controlled via the Chromeleon 7.2 Software (Thermo Fisher Scientific, Waltham, MA, USA, and Dionex Softron GMbH part of Thermo Fisher Scientific, Germany).

Before each injection, the injection needle was rinsed with i) a 1:1:1 volumetric mixture of acetonitrile, methanol, and isopropanol and ii) HPLC water containing 10mM NH_4CH_3COOH.

Table C.7 Summary of determination coefficient (R^2) of calibration curves, linearity range (ng/mL), the instrument limit of detection (iLOD), the instrument limit of quantification (iLOQ), method limit of detection (mLOD), method limit of quantification (mLOQ) for the targeted analytes.

PFAS analyte	Solvent-based calibration curve	R^2	Linear range (ng/mL)	iLOD (ng/mL)	iLOQ (ng/mL)	mLOD in soil (ng/g dw)	mLOQ in soil (ng/g dw)	mLOD in headspace extract (ng/mL)	mLOQ in headspace extract (ng/mL)
PFPrA	y=1.06848x+0.02156	0.9959	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFBA	y=1.36340x+0.99839	0.9912	0.1-100	0.03	0.10	0.04	0.15	0.04	0.13
PFPeA	y=0.96598x+0.07382	0.9913	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFHxA	y=0.96657x+0.08533	0.9907	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFHpA	y=1.60986x+0.18620	0.9922	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFOA	y=1.47818x+0.05890	0.9962	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFNA	y=1.39582x+0.04725	0.9961	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
PFDA	y=1.29580x+0.05884	0.9936	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
PFUnA	y=1.22997x+0.06505	0.9926	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
PFDoA	y=1.28891x+0.04448	0.9949	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
PFTrDA	y=1.10263x+0.02132	0.9953	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
PFTeDA	y=0.84371x+0.04145	0.9957	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
2:3 FTCA	y=0.26684x-0.00898	0.9948	0.1-100	0.03	0.10	0.04	0.15	0.04	0.13
3:3 FTCA	y=0.31349x+0.01102	0.9962	0.1-100	0.03	0.10	0.04	0.15	0.04	0.13
4:3 FTCA	y=1.07915x+0.01446	0.9949	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
5:3 FTCA	y=0.57292+0.00399	0.9969	0.05-100	0.02	0.05	0.03	0.07	0.03	0.07
7:3 FTCA	y=0.43458x+0.04273	0.9920	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
6:2 FTUCA	y=1.42206x+0.01809	0.9960	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
8:2 FTUCA	y=1.36137x+0.15576	0.9976	0.05-20	0.02	0.05	0.03	0.07	0.03	0.07
10:2 FTUCA	y=0.79561x+0.58629	0.9904	0.5-20	0.15	0.50	0.21	0.72	0.20	0.67
6:2 FTCA	y=1.01015x+0.13787	0.9902	0.2-50	0.06	0.20	0.08	0.29	0.08	0.27
5:2s FTOH	y=0.01346x+0.02686	0.9902	2-800	0.60	2.00	5.59	18.62	0.80	2.66
7:2 sFTOH	y=0.02815x-0.00088	0.9917	0.5-100	0.15	0.50	1.40	4.66	0.20	0.67
5:2 ketone	y=0.29478x-0.06379	0.9973	0.5-100	0.15	0.50	1.40	4.66	0.20	0.67

5:3 FTB	y=0.45663x-0.00207	0.9944	0.05-100	0.02	0.05	0.15	0.47	0.03	0.07
5:1:2 FTB	y=0.31972x+0.02581	0.9931	0.2-100	0.06	0.20	0.56	1.86	0.08	0.27
6:2 FTS	y=1.41310x+0.33791	0.9934	0.05-50	0.02	0.05	0.03	0.07	0.03	0.07
8:2 FTS	y=1.82163x+0.06705	0.9900	0.05-10	0.02	0.05	0.03	0.07	0.03	0.07
PFBS	y=1.73545x+0.29475	0.9954	0.05-50	0.04	0.05	0.05	0.07	0.05	0.07
PFHxS	y=1.50717x+0.19855	0.9953	0.05-50	0.03	0.05	0.04	0.07	0.04	0.07
PFOS	y=1.31106x+0.18236	0.9918	0.05-50	0.03	0.05	0.04	0.07	0.04	0.07
PFDS	y=1.13067x+0.12645	0.9924	0.05-50	0.03	0.05	0.04	0.07	0.04	0.07

Table C.8 Whole-method accuracy, intraday, and interday precision.

Analyte	Whole-method accuracy (%)			Intraday precision (%) RSD (n=5)	Interday precision (%) RSD (n=15)
	Min	Max	Average		
PFPrA	87.1	148.8	115.5	11.4	21.6
PFBA	92.7	152.2	119.5	12.5	20.7
PFPeA	81.4	118.1	97.8	4.3	12.1
PFHxA	86.1	115.0	98.7	3.2	9.9
PFHpA	90.0	104.0	95.1	0.3	4.3
PFOA	89.8	104.2	97.1	2.6	4.2
PFNA	91.5	105.6	97.9	1.5	4.7
PFDA	85.0	105.0	92.3	2.5	6.9
PFUnA	89.9	160.3	103.6	8.4	18.7
PFDoA	77.6	117.3	95.4	13.4	12.8
PFTrDA	78.6	163.8	107.7	31.1	26.5
PFTeDA	79.7	162.6	108.4	21.0	24.3
PFHxDA	78.5	139.6	104.4	12.8	22.7
2:3 FTCA	73.7	133.0	97.4	9.0	16.1
3:3 FTCA	88.4	111.9	99.4	4.9	6.8
4:3 FTCA	88.4	112.5	99.2	6.8	7.9
5:3 FTCA	85.3	117.9	101.0	10.7	10.1
7:3 FTCA	69.1	107.3	86.3	6.1	13.4
6:2 FTUCA	75.5	177.6	106.8	9.1	24.9
8:2 FTUCA	92.8	112.2	98.8	2.6	5.3
10:2 FTUCA	96.1	112.6	104.0	3.6	5.9
6:2 FTCA	82.2	130.9	99.5	5.1	12.7
5:3 FTB	77.5	112.2	94.9	5.2	10.1
5:1:2 FTB	76.6	119.9	97.7	7.6	18.6
6:2 FTS	91.4	113.6	100.5	2.9	6.4
8:2 FTS	75.1	105.4	87.3	-7.1	8.6
5:2 sFTOH	53.1	128.5	101.0	17.7	21.0
7:2 sFTOH	74.6	118.9	93.1	7.9	13.5
5:2 ketone	84.7	176.3	121.8	13.9	30.5
PFBS	92.2	105.7	99.5	2.2	4.0
PFHxS	87.6	104.5	98.4	2.6	5.1
PFOS	91.4	103.1	97.8	-2.2	3.3

PFDS	95.8	106.5	101.8	2.3		4.1

Table C.9 Details on qualitatively PFAS detected in Ansulite AFFF.

No.	PFAS Class	Acronym	Chemical Formula	Theoretical m/z	Observed m/z	Mass error, ppm	RT, min	Ionization mode	Compounds used for quantification	IS used	Identification confidence level
1	n:3 FTB (n=7, 9, 11, 13)	7:3 FTB	$C_{14}F_{15}H_{15}NO_2^+$	514.08632	514.08447	3.6	6.10	ESI+	5:3 FTB	PFOAAmS	2a
		9:3 FTB	$C_{16}F_{19}H_{15}NO_2^+$	614.07993	614.07733	4.2	6.68	ESI+	5:3 FTB	PFOAAmS	2a
		11:3 FTB	$C_{18}F_{23}H_{15}NO_2^+$	714.07354	714.07043	4.4	7.10	ESI+	5:3 FTB	PFOAAmS	2a
		13:3 FTB	$C_{20}F_{27}H_{15}NO_2^+$	814.06716	814.06262	5.6	7.60	ESI+	5:3 FTB	PFOAAmS	2a
2	n:1:2 FTB (n=7, 9, 11, 13)	7:1:2 FTB	$C_{14}F_{16}H_{14}NO_2^+$	532.07711	532.07483	4.3	6.15	ESI+	5:1:2 FTB	PFOAAmS	2a
		9:1:2 FTB	$C_{16}F_{20}H_{14}NO_2^+$	632.07093	632.06775	5.0	6.72	ESI+	5:1:2 FTB	PFOAAmS	2a
		11:1:2 FTB	$C_{18}F_{24}H_{14}NO_2^+$	732.06475	732.06110	5.0	7.14	ESI+	5:1:2 FTB	PFOAAmS	2a
		13:1:2 FTB	$C_{20}F_{28}H_{14}NO_2^+$	832.05857	832.05481	4.5	7.64	ESI+	5:1:2 FTB	PFOAAmS	2a
3	n:2 FTB (n=6, 8, 10)	6:2 FTB	$C_{12}F_{13}H_{13}NO_2^+$	450.07387	450.07214	3.8	4.70	ESI+	5:3 FTB	PFOAAmS	2a
		8:2 FTB	$C_{14}F_{17}H_{13}NO_2^+$	500.07078	500.07422	-6.9	5.33	ESI+	5:3 FTB	PFOAAmS	2a
		10:2 FTB	$C_{16}F_{21}H_{13}NO_2^+$	550.06769	550.06512	4.7	5.80	ESI+	5:3 FTB	PFOAAmS	2a
4	n:4 FTB (n=6, 8, 10)	6:4 FTB	$C_{14}F_{13}H_{17}NO_2^+$	478.10496	478.10333	3.4	5.96	ESI+	5:3 FTB	PFOAAmS	2a
		8:4 FTB	$C_{16}F_{17}H_{17}NO_2^+$	578.09878	578.09662	3.7	6.54	ESI+	5:3 FTB	PFOAAmS	2a
		10:4 FTB	$C_{18}F_{21}H_{17}NO_2^+$	678.09260	678.08887	5.5	7.37	ESI+	5:3 FTB	PFOAAmS	2a
5	n:1:3 FTB (n=4, 6, 8, 10)	4:1:3 FTB	$C_{12}H_{16}F_{10}NO_2^+$	396.10491	396.10103	-9.8	4.90	ESI+	5:1:2 FTB	PFOAAmS	2b
		6:1:3 FTB	$C_{14}H_{16}F_{14}NO_2^+$	496.09853	496.09441	-8.3	5.82	ESI+	5:1:2 FTB	PFOAAmS	2b
		8:1:3 FTB	$C_{16}H_{16}F_{18}NO_2^+$	596.09233	596.08723	-8.6	6.43	ESI+	5:1:2 FTB	PFOAAmS	2b
		10:1:3 FTB	$C_{18}H_{16}F_{22}NO_2^+$	696.08575	696.08065	-7.3	6.85	ESI+	5:1:2 FTB	PFOAAmS	2b
6	n:2 FTS (n=10)	10:2 FTS	$C_{12}H_4O_3SF_{21}^-$	626.95513	626.95573	1.0	6.59	ESI-	8:2 FTS	M2-8:2 FTS	2a

Table C.10 The concentration of different PFAS components in the Ansulite AFFF.

PFAS components	Full name	Concentration (mg/L)
5:1:2 FTB (or 6:2 H-FTB)	5:1:2 (or 6:2 hydrogen substituted-) fluorotelomer betaine	3473.91
7:1:2 FTB (or 8:2 H-FTB)	7:1:2 (or 8:2 hydrogen substituted-) fluorotelomer betaine	3334.22
9:1:2 FTB (or 10:2 H-FTB)	9:1:2 (or 10:2 hydrogen substituted-) fluorotelomer betaine	835.87
5:3 FTB	5:3 fluorotelomer betaine	820.74
7:3 FTB	7:3 fluorotelomer betaine	741.82
9:3 FTB	9:3 fluorotelomer betaine	267.87
11:1:2 FTB (or 12:2 H-FTB)	11:1:2 (or 12:2 hydrogen substituted-) fluorotelomer betaine	203.83
4:1:3 FTB (or 5:3 H-FTB)	4:1:3 (or 5:3 hydrogen substituted-) fluorotelomer betaine	90.94
8:2 FTS	8:2 fluorotelomer sulfonate	83.18
6:4 FTB	6:4 fluorotelomer betaine	74.88
11:3 FTB	11:3 fluorotelomer betaine	74.66
6:1:3 FTB (or 7:3 H-FTB)	6:1:3 (or 7:3 hydrogen substituted-) fluorotelomer betaine	71.99
8:4 FTB	8:4 fluorotelomer betaine	62.68
6:2 FTB	6:2 fluorotelomer betaine	39.40
10:2 FTB	10:2 fluorotelomer betaine	28.70
8:1:3 FTB (or 9:3 H-FTB)	8:1:3 (or 9:3 hydrogen substituted-) fluorotelomer betaine	24.90
10:4 FTB	10:4 fluorotelomer betaine	20.38
4:4 FTB	4:4 fluorotelomer betaine	18.76
13:1:2 FTB (or 14:2 H-FTB)	13:1:2 (or 14:2 hydrogen substituted-) fluorotelomer betaine	13.34
13:3 FTB	13:3 fluorotelomer betaine	11.82
10:1:3 FTB (or 11:3 H-FTB)	10:1:3 (or 11:3 hydrogen substituted-) fluorotelomer betaine	5.51
10:2 FTS	10:2 fluorotelomer sulfonate	1.29
6:2 FTS	6:2 fluorotelomer sulfonate	0.79

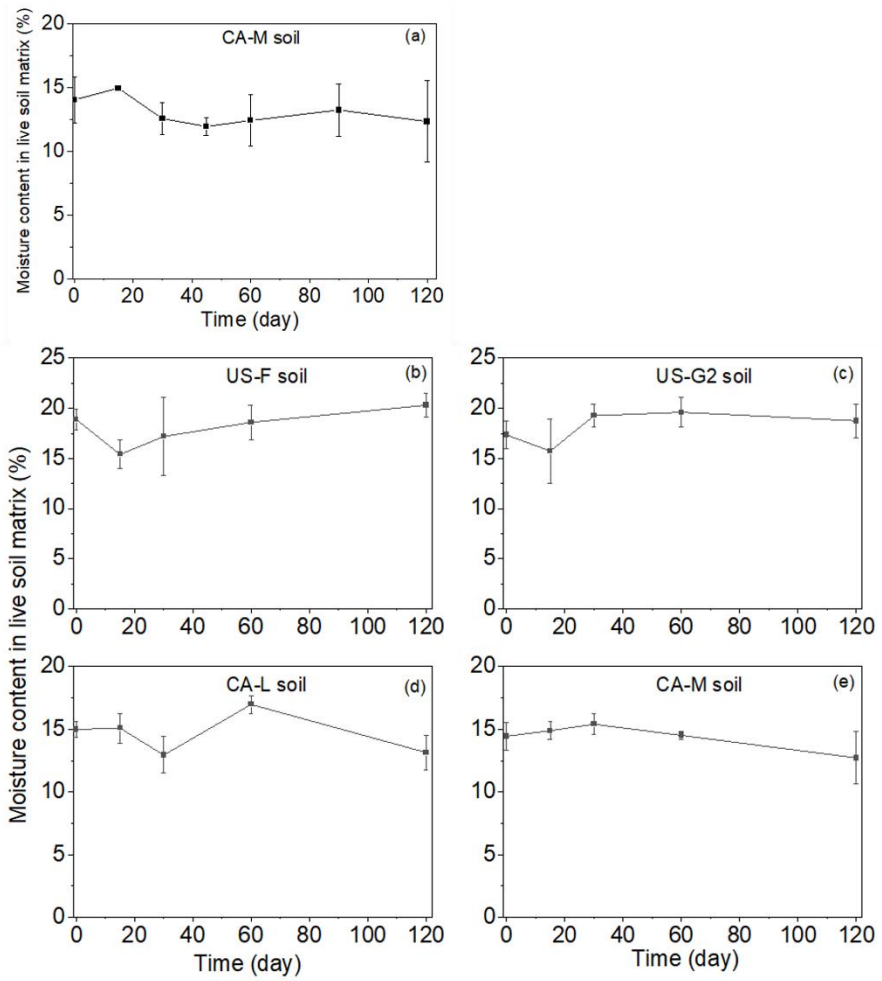


Figure C.1 The soil moisture contents were measured gravimetrically in live soil matrixes during the incubation of n:3 and n:1:2 FTBs (a) or the Ansulite AFFF (b-e).

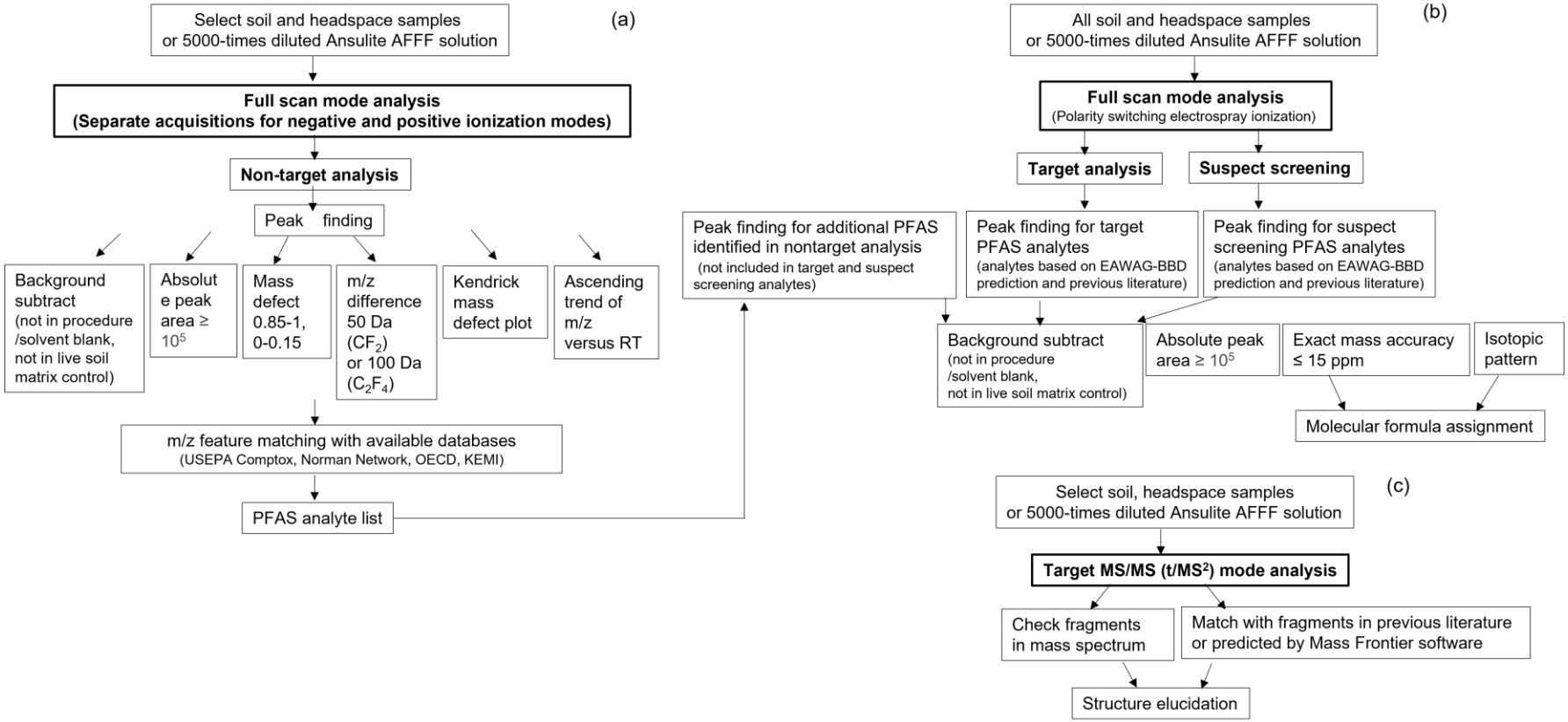
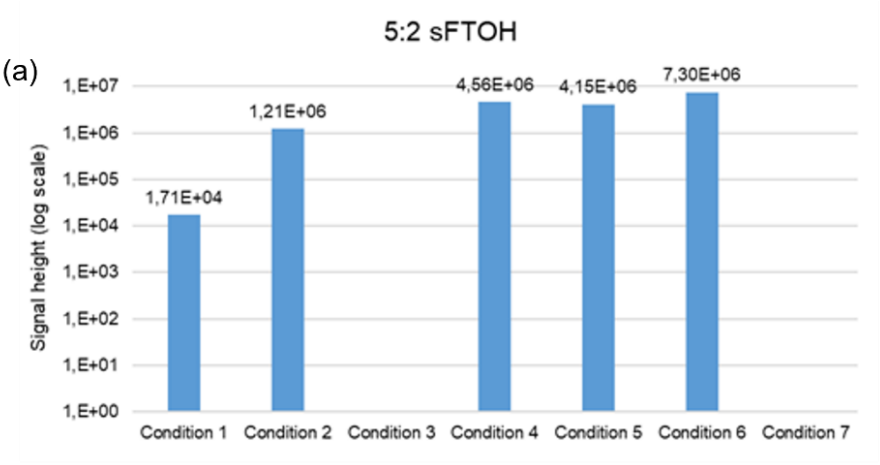


Figure C.2 Workflow diagram depicting the steps taken during nontarget (a), target, and suspect screening analysis (b, c) by UHPLC-HRMS; the procedures were proposed based on previous literature.[3, 4]



(b) Condition 5 (H_2O – MeOH/ACN, both $AceNH_4$ 10 mM)

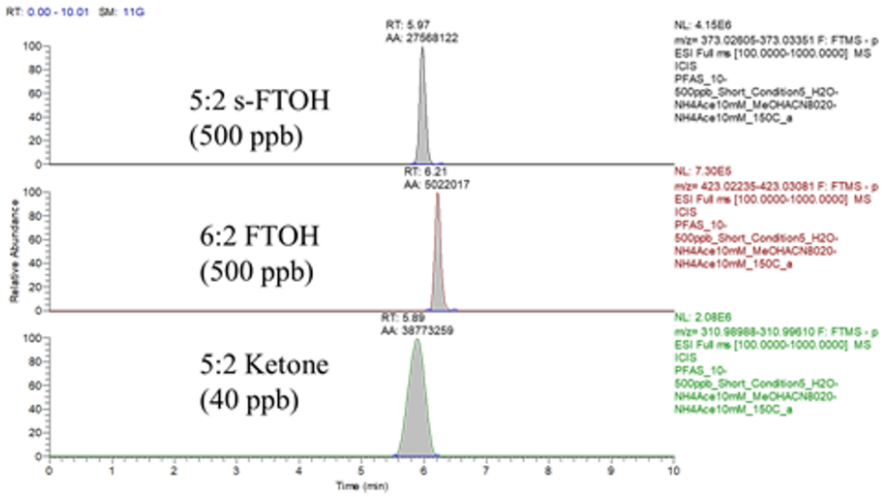


Figure C.3 (a) The absolute peak area of 5:2 sFTOH under different LC-HRMS instrumental conditions; (b) An illustration of chromatographic peak shapes for 5:2 sFTOH, 6:2 FTOH, and 5:2 ketone under Condition 5.

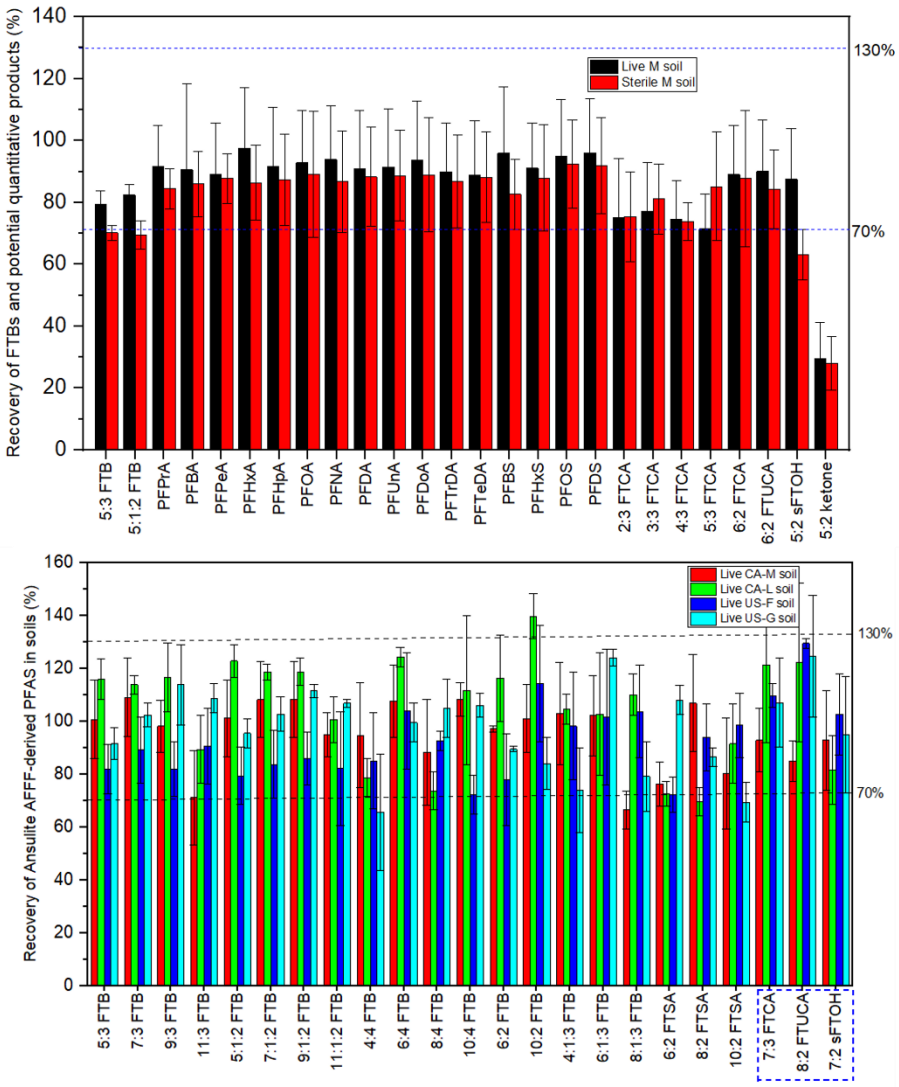


Figure C.4 Recovery of 5:3 FTB, 5:1:2 FTB, and their potential quantitative transformation products in live and sterile CA-M soil (a), and recovery of the Ansulite AFFF-derive PFAS and three other potential quantitative transformation products (marked with a blue box) in the four live soils (CA-M, CA-L, US-F, and US-G soil).

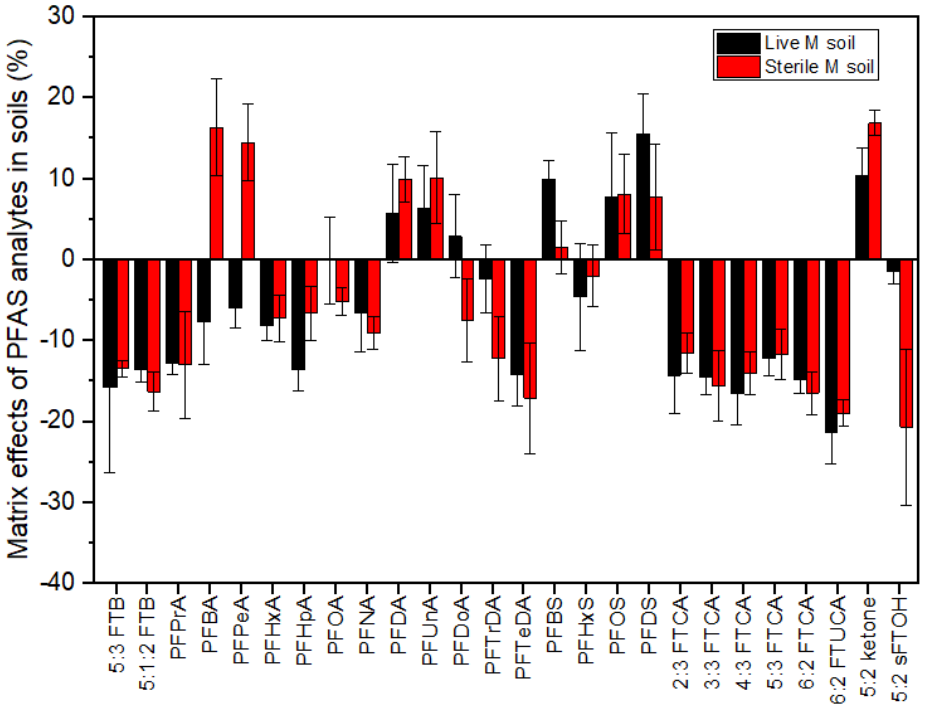


Figure C.5 Matrix effects of 5:3 FTB, 5:1:2 FTB, and other quantitative PFAS analytes monitored in live and sterile CA-M soils.

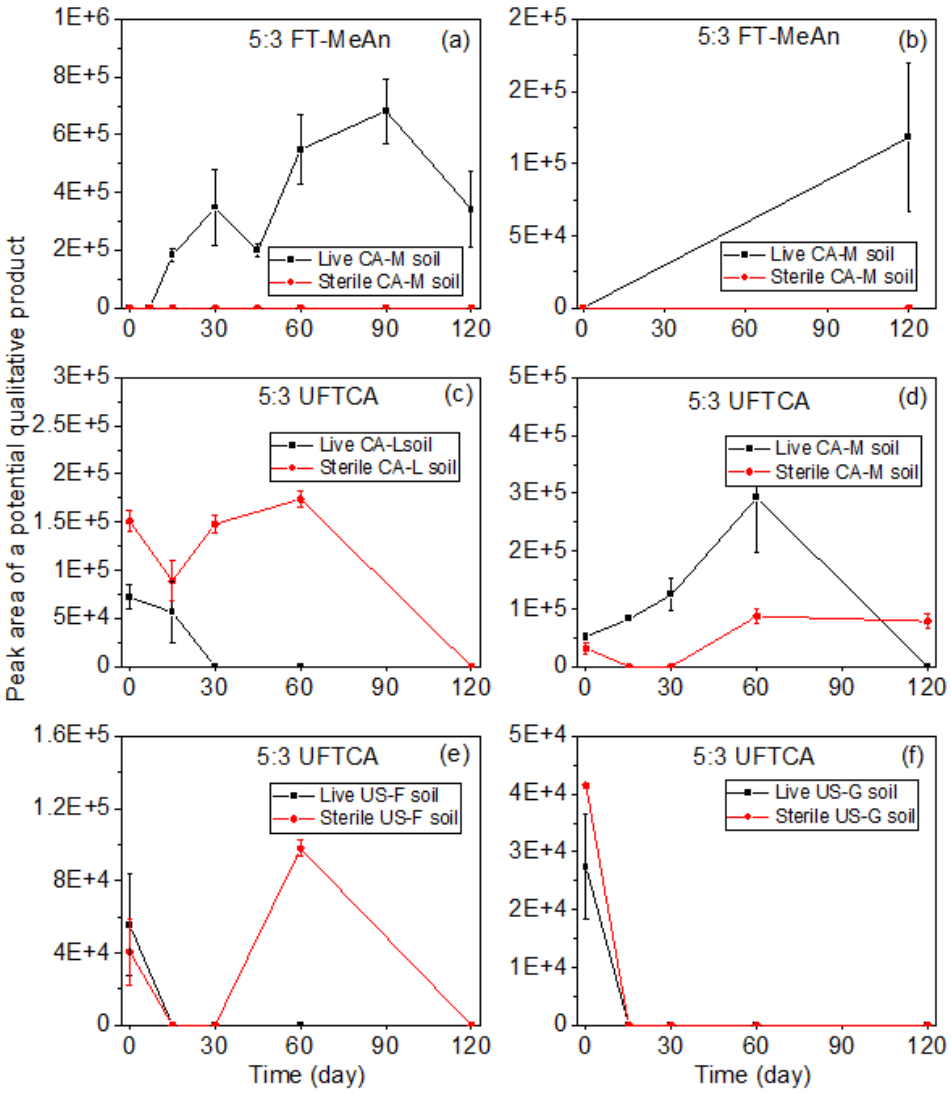


Figure C.6 Qualitative PFAS that were sporadically detected during the incubation of single 5:3 FTB (a), and mixture of 5:3 and 5:1:2 FTB (b) in CA-M soil or the incubation of Ansulite-AFFF (c-f) in four soils.

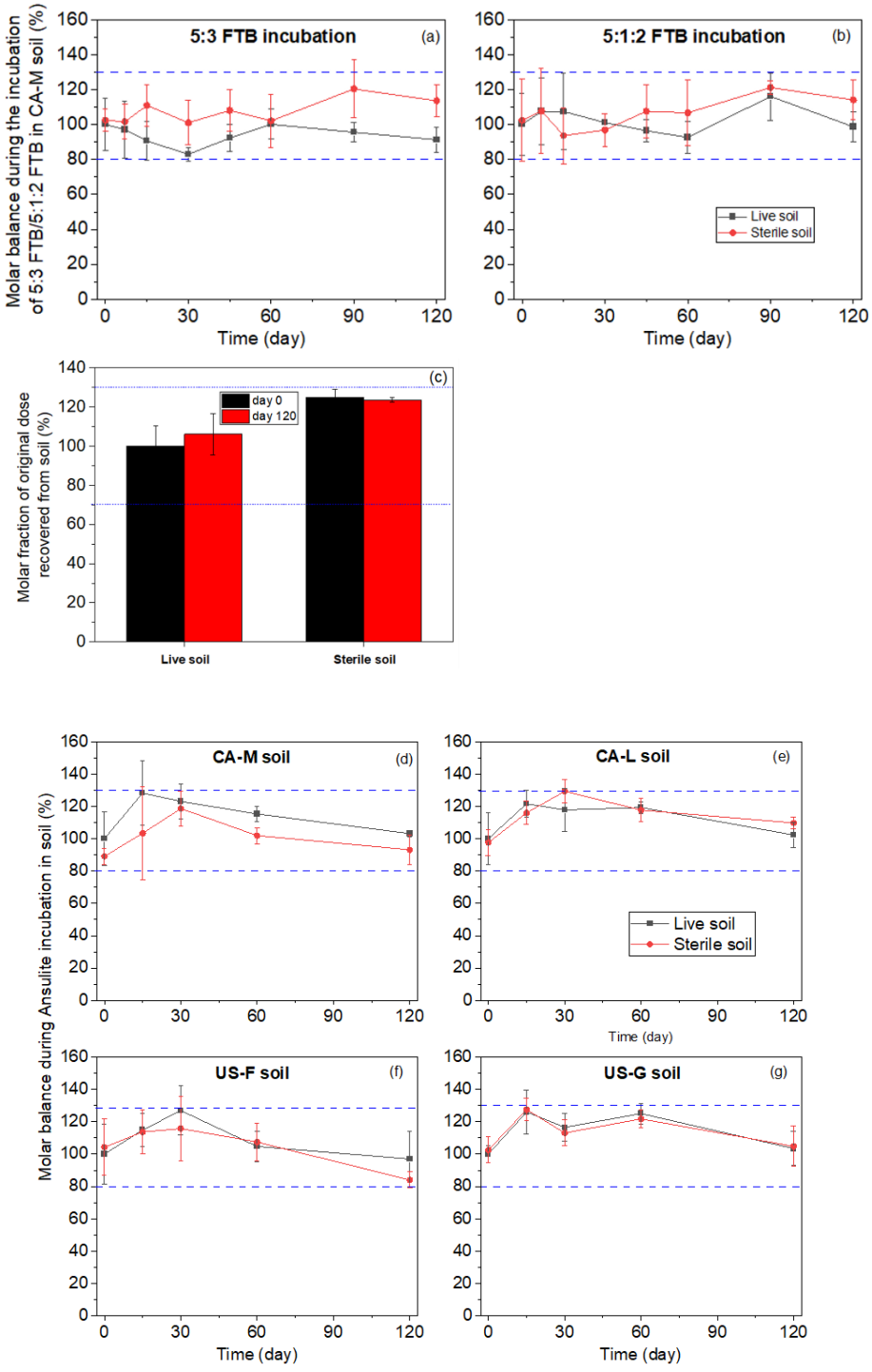


Figure C.7 The molar balance of parent compounds and quantitative transformation products in CA-M soil for (a) 5:3 FTB as the sole parent compound, (b) 5:1:2 FTB as the sole parent compound, (c) 5:3 and 5:1:2 FTB mixture as the parent compound, and in four soils for (d-g) Ansulite AFFF-derived PFAS as parent compounds.

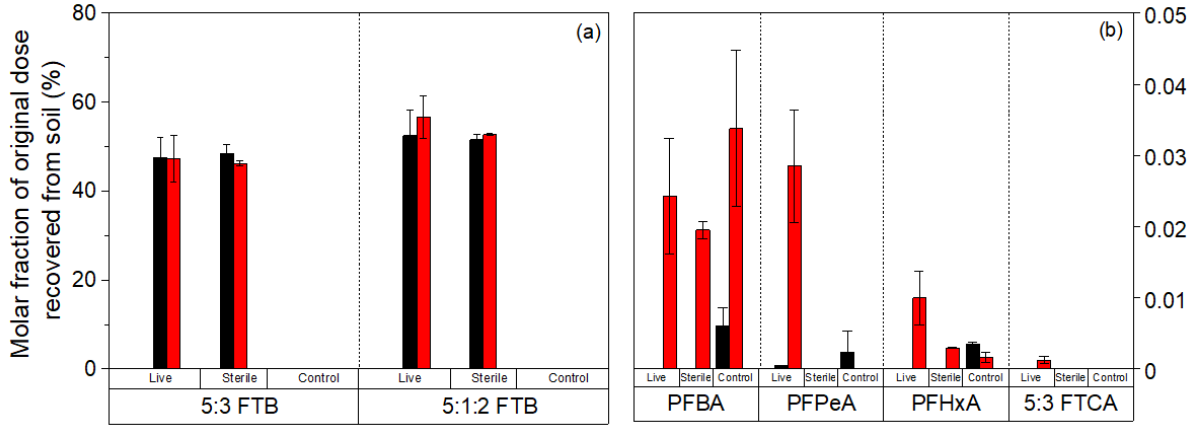


Figure C.8 Co-incubation of 5:3 FTB and 5:1:2 FTB in CA-M soil (black column – day 0; red column – day 120). (a) The molar fraction of parent 5:1:2 FTB and 5:3 FTB compounds relative to the total dose into the vessels, and (b) the molar fraction of potential quantitative transformation products including 5:3 FTCA, PFBA, PFPeA, and PFHxA.

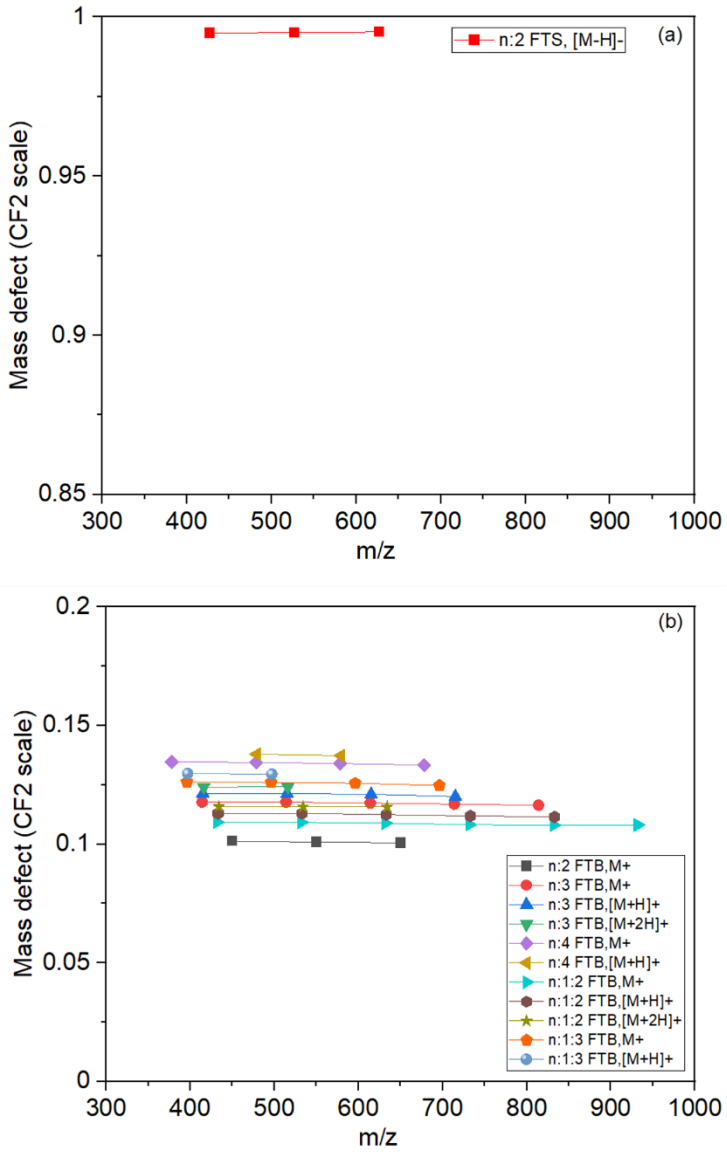


Figure C.9 The CF_2-normalized Kendrick mass defect plot for ESI (−) (a) and ESI (+) (b) data in 5000 times diluted Ansulite AFFF solution.

Notably, the m/z of M^+, $[M+H]^+$, $[M+2H]^+$ for several classes of FTB (e.g., n:3, n:1:2, n:4, n:1:3 FTB) were identified in the Ansulite AFFF under positive mode, M+ showed highest signal and was used for the semi-quantification.

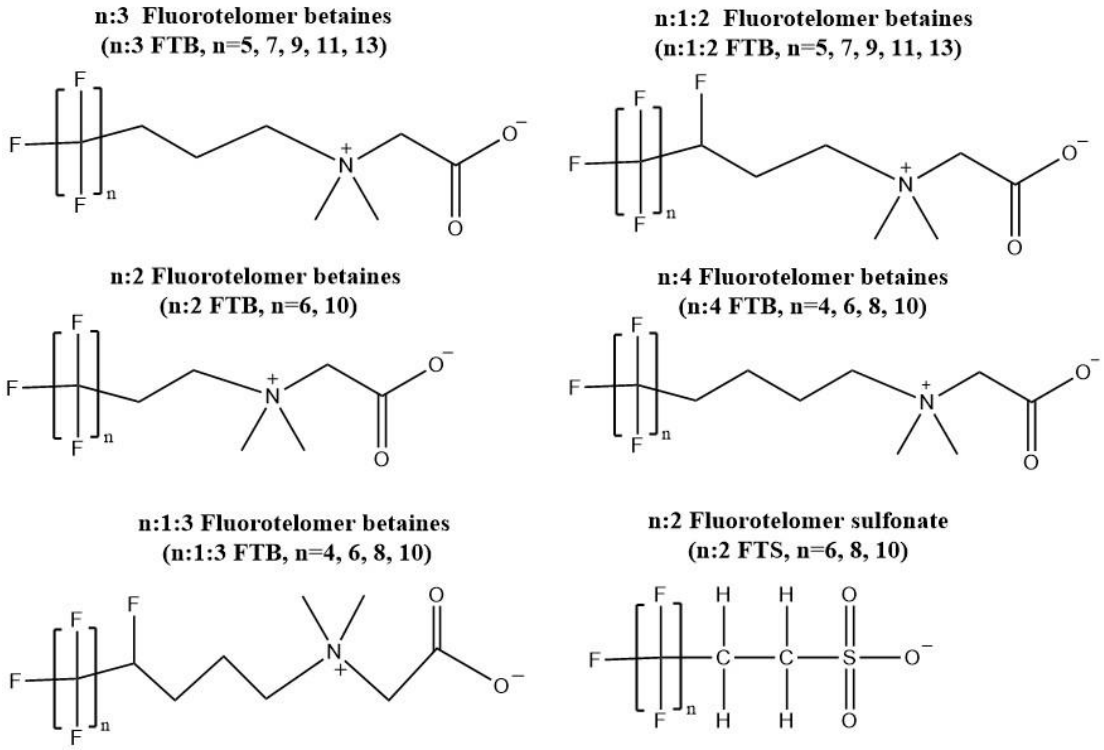
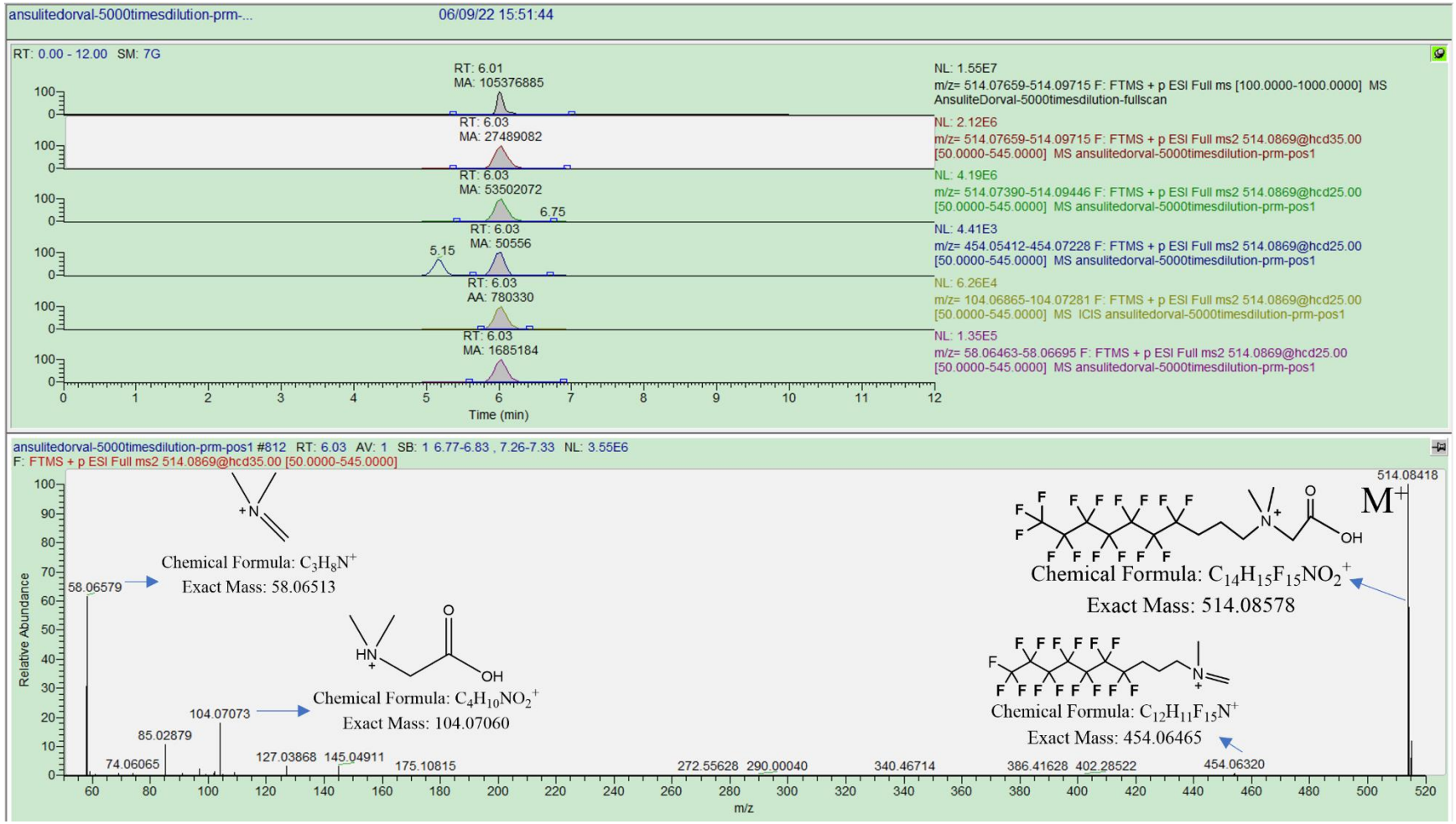
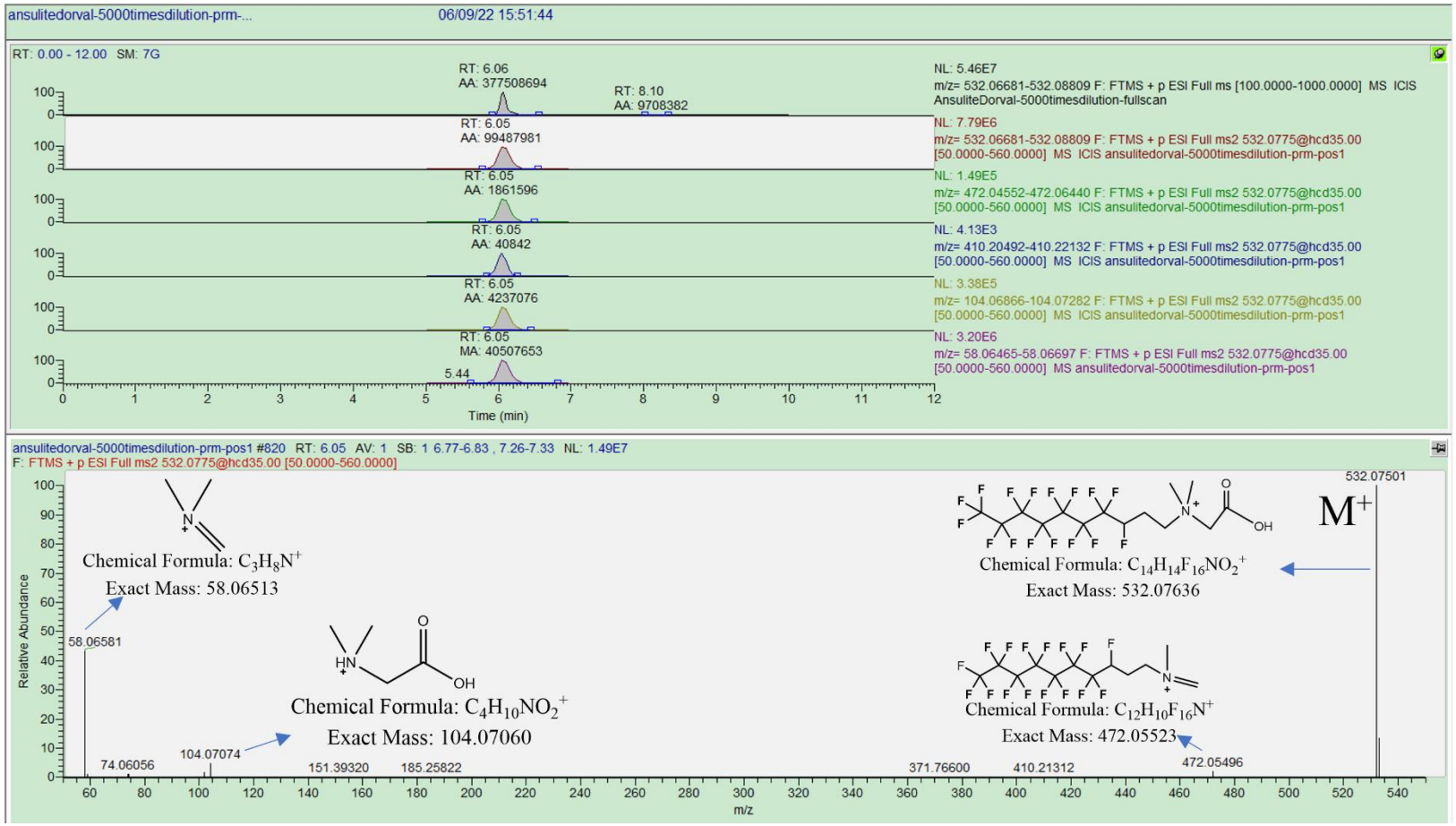


Figure C.10 The structure of PFAS components in the Ansulite AFFF.

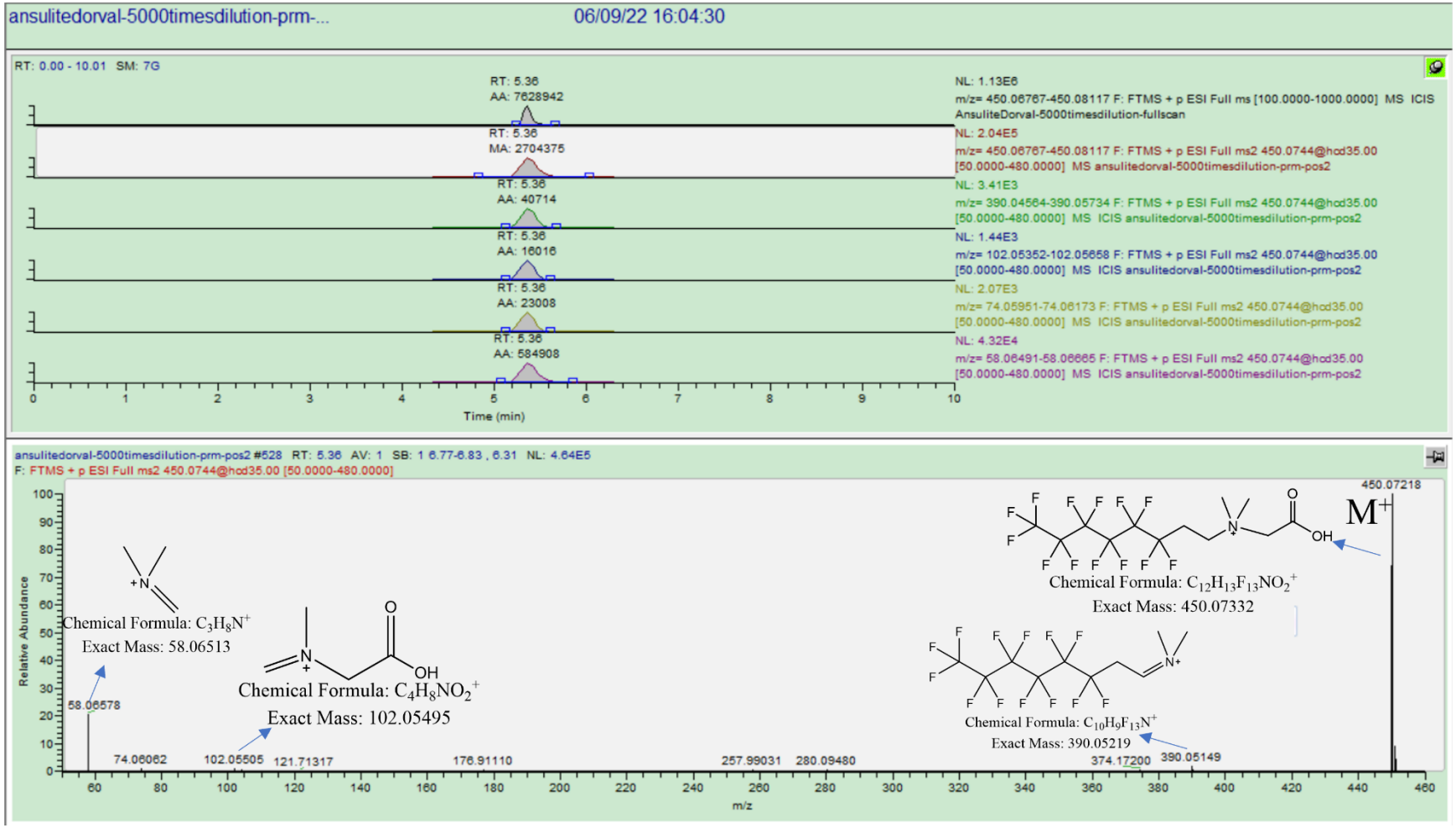
Figure C.11 The t-MS^2 mode spectra of qualitative PFAS in 5000-times diluted Ansulite solution or Ansulite-spiked live soils.



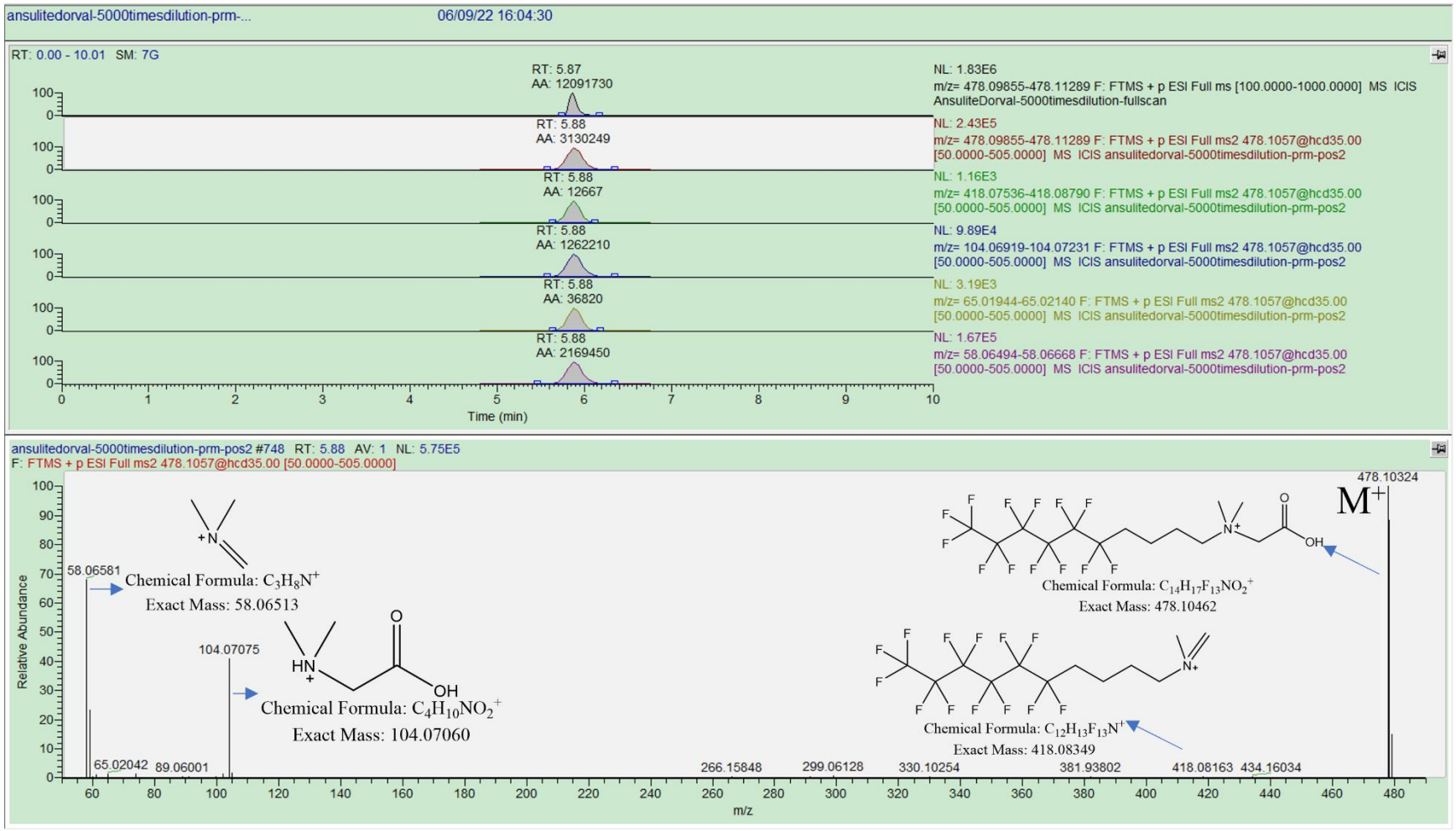
(a) 7:3 FTB.


ansulitedorval-5000timesdilution-prm-...
06/09/22 15:51:44
RT: 0.00 - 12.00 SM: 7G
RT: 6.06
AA: 377508694
RT: 8.10
AA: 9708382
RT: 6.05
AA: 99487981
RT: 6.05
AA: 1861596
RT: 6.05
AA: 40842
RT: 6.05
AA: 4237076
RT: 6.05
MA: 40507653
5.44
Time (min)
NL: 5.46E7
m/z= 532.06681-532.08809 F: FTMS + p ESI Full ms [100.0000-1000.0000] MS ICIS AnsuliteDorval-5000timesdilution-fullscan
NL: 7.79E6
m/z= 532.06681-532.08809 F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos1
NL: 1.49E5
m/z= 472.04552-472.06440 F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos1
NL: 4.13E3
m/z= 410.20492-410.22132 F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos1
NL: 3.38E5
m/z= 104.06866-104.07282 F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos1
NL: 3.20E6
m/z= 58.06465-58.06697 F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000] MS ansulitedorval-5000timesdilution-prm-pos1
ansulitedorval-5000timesdilution-prm-pos1 #820 RT: 6.05 AV: 1 SB: 1 6.77-6.83 , 7.26-7.33 NL: 1.49E7
F: FTMS + p ESI Full ms2 532.0775@hcd35.00 [50.0000-560.0000]
Chemical Formula: C3H8N+
Exact Mass: 58.06513
58.06581
Chemical Formula: C4H10NO2+
Exact Mass: 104.07060
104.07074
74.06056
151.39320
185.25822
Chemical Formula: C14H14F16NO2+
Exact Mass: 532.07636
532.07501
M+
Chemical Formula: C12H10F16N+
Exact Mass: 472.05523
472.05496
371.76600
410.21312
Relative Abundance
m/z

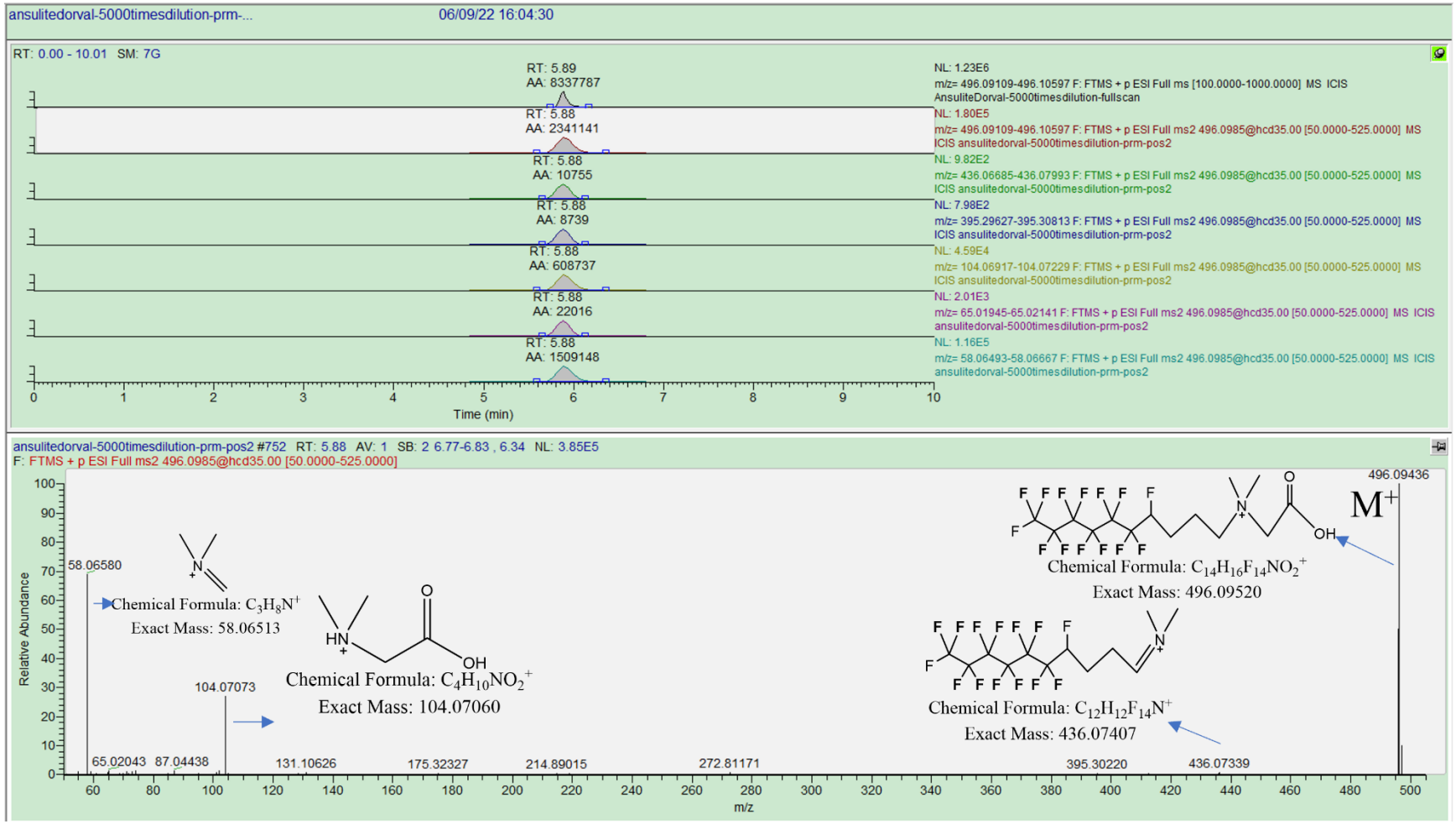
(b) 7:1:2 FTB.


ansulitedorval-5000timesdilution-prm-...
06/09/22 16:04:30
RT: 0.00 - 10.01 SM: 7G
RT: 5.36
AA: 7628942
NL: 1.13E6
m/z= 450.06767-450.08117 F: FTMS + p ESI Full ms [100.0000-1000.0000] MS ICIS AnsuliteDorval-5000timesdilution-fullscan
RT: 5.36
MA: 2704375
NL: 2.04E5
m/z= 450.06767-450.08117 F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000] MS ansulitedorval-5000timesdilution-prm-pos2
RT: 5.36
AA: 40714
NL: 3.41E3
m/z= 390.04564-390.05734 F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
RT: 5.36
AA: 16016
NL: 1.44E3
m/z= 102.05352-102.05658 F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
RT: 5.36
AA: 23008
NL: 2.07E3
m/z= 74.05951-74.06173 F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
RT: 5.36
AA: 584908
NL: 4.32E4
m/z= 58.06491-58.06665 F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
Time (min)
ansulitedorval-5000timesdilution-prm-pos2 #528 RT: 5.36 AV: 1 SB: 1 6.77-6.83 , 6.31 NL: 4.64E5
F: FTMS + p ESI Full ms2 450.0744@hcd35.00 [50.0000-480.0000]
Relative Abundance
m/z
450.07218
M+
Chemical Formula: C12H13F13NO2+
Exact Mass: 450.07332
Chemical Formula: C3H8N+
Exact Mass: 58.06513
Chemical Formula: C4H8NO2+
Exact Mass: 102.05495
Chemical Formula: C10H9F13N+
Exact Mass: 390.05219
58.06578
74.06062
102.05505
121.71317
176.91110
257.99031
280.09480
374.17200
390.05149

(c) 6:2 FTB.


ansulitedorval-5000timesdilution-prm-...
06/09/22 16:04:30
RT: 0.00 - 10.01 SM: 7G
RT: 5.87
AA: 12091730
RT: 5.88
AA: 3130249
RT: 5.88
AA: 12667
RT: 5.88
AA: 1262210
RT: 5.88
AA: 36820
RT: 5.88
AA: 2169450
Time (min)
NL: 1.83E6
m/z= 478.09855-478.11289 F: FTMS + p ESI Full ms [100.0000-1000.0000] MS ICIS AnsuliteDorval-5000timesdilution-fullscan
NL: 2.43E5
m/z= 478.09855-478.11289 F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 1.16E3
m/z= 418.07536-418.08790 F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 9.89E4
m/z= 104.06919-104.07231 F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 3.19E3
m/z= 65.01944-65.02140 F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 1.67E5
m/z= 58.06494-58.06668 F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
ansulitedorval-5000timesdilution-prm-pos2 #748 RT: 5.88 AV: 1 NL: 5.75E5
F: FTMS + p ESI Full ms2 478.1057@hcd35.00 [50.0000-505.0000]
Relative Abundance
m/z
58.06581
Chemical Formula: C3H8N+
Exact Mass: 58.06513
104.07075
Chemical Formula: C4H10NO2+
Exact Mass: 104.07060
65.02042
89.06001
266.15848
299.06128
330.10254
381.93802
418.08163
434.16034
478.10324
M+
Chemical Formula: C14H17F13NO2+
Exact Mass: 478.10462
Chemical Formula: C12H13F13N+
Exact Mass: 418.08349

(d) 6:4 FTB.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06/09/22 16:04:30
RT: 0.00 - 10.01 SM: 7G
RT: 5.89
AA: 8337787
RT: 5.88
AA: 2341141
RT: 5.88
AA: 10755
RT: 5.88
AA: 8739
RT: 5.88
AA: 608737
RT: 5.88
AA: 22016
RT: 5.88
AA: 1509148
Time (min)
NL: 1.23E6
m/z= 496.09109-496.10597 F: FTMS + p ESI Full ms [100.0000-1000.0000] MS ICIS AnsuliteDorval-5000timesdilution-fullscan
NL: 1.80E5
m/z= 496.09109-496.10597 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 9.82E2
m/z= 436.06685-436.07993 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 7.98E2
m/z= 395.29627-395.30813 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 4.59E4
m/z= 104.06917-104.07229 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 2.01E3
m/z= 65.01945-65.02141 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
NL: 1.16E5
m/z= 58.06493-58.06667 F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000] MS ICIS ansulitedorval-5000timesdilution-prm-pos2
ansulitedorval-5000timesdilution-prm-pos2 #752 RT: 5.88 AV: 1 SB: 2 6.77-6.83 , 6.34 NL: 3.85E5
F: FTMS + p ESI Full ms2 496.0985@hcd35.00 [50.0000-525.0000]
Relative Abundance
m/z
58.06580
Chemical Formula: $C_3H_8N^+$
Exact Mass: 58.06513
104.07073
Chemical Formula: $C_4H_{10}NO_2^+$
Exact Mass: 104.07060
65.02043
87.04438
131.10626
175.32327
214.89015
272.81171
395.30220
436.07339
496.09436
M^+
Chemical Formula: $C_{14}H_{16}F_{14}NO_2^+$
Exact Mass: 496.09520
Chemical Formula: $C_{12}H_{12}F_{14}N^+$
Exact Mass: 436.07407

(e) 6:1:3 FTB.

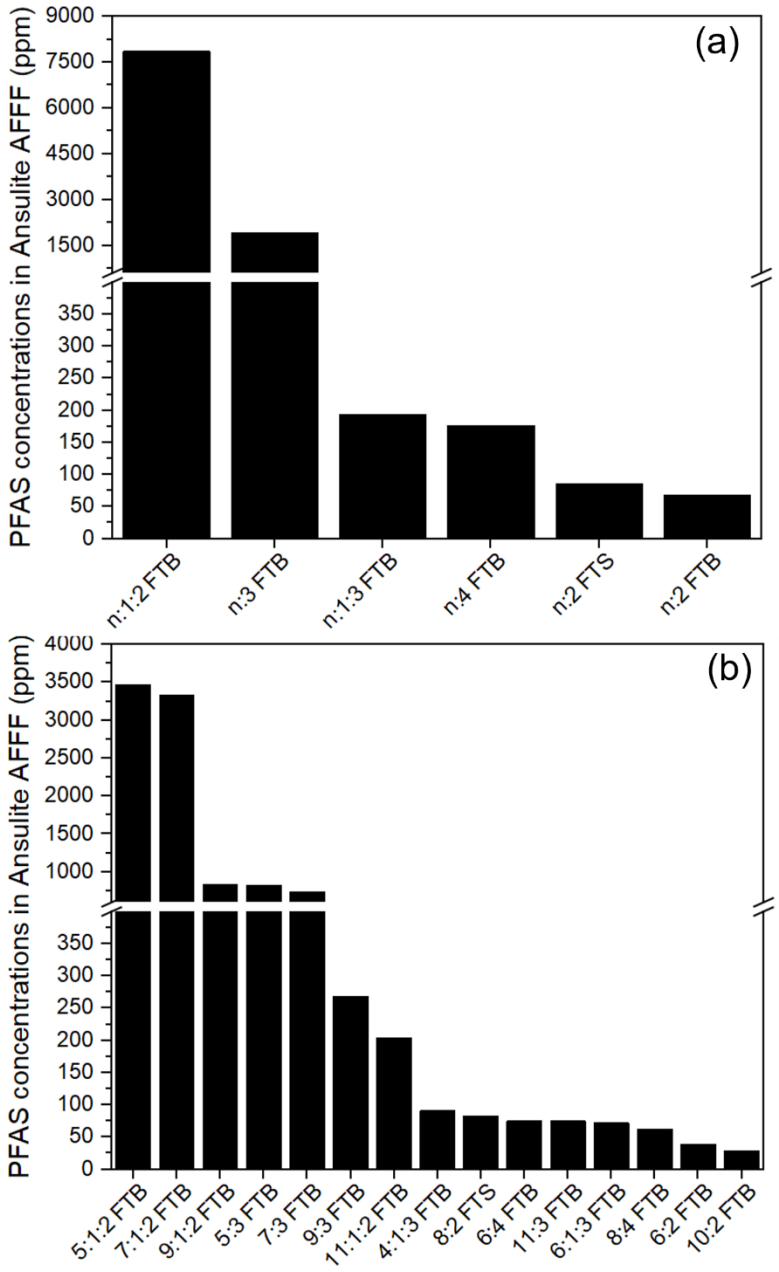
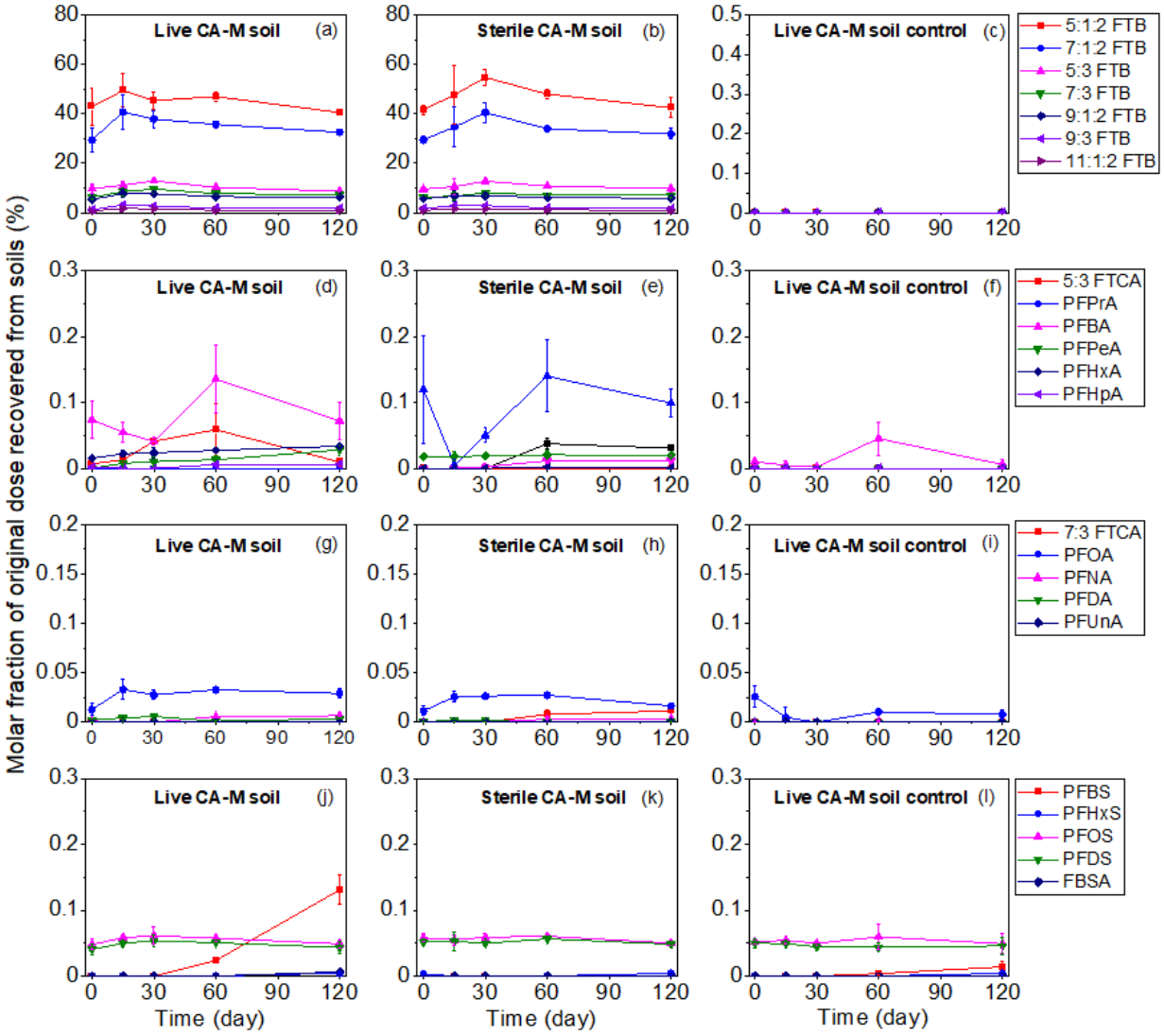


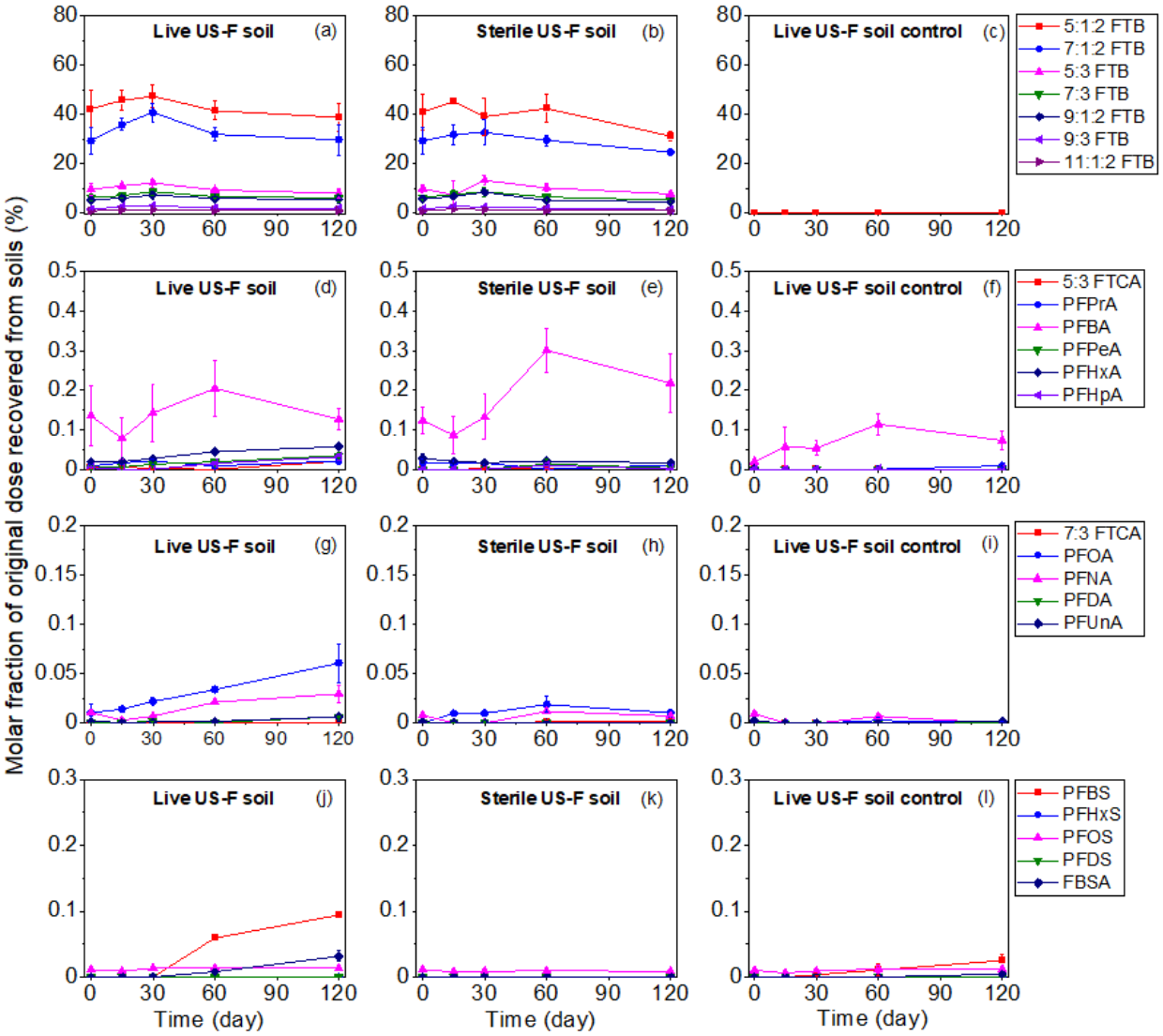
Figure C.12 Concentrations of different classes of PFAS identified in the Ansulite AFFF (top) and those of the top 15 most abundant PFAS (bottom).

Figure C.13 Concentration profiles of parent n:3 and n:1:2 FTBs (n = 5, 7, 9, 11), major PFAS contained in the Ansulite AFFF, and their potential transformation products, including short-chain polyfluoroalkyl acid and PFCA and long-chain polyfluoroalkyl acid and PFCA, in three other live and sterile soils and live soil matrix controls. PFSA and FASA(C4) concentration profiles in three other live and sterile soils and live soil matrix controls. CA-M (A), US-F (B), and US-G (C) soil.

(A) CA-M soil



(B) US-F soil


Live US-F soil (a)
Sterile US-F soil (b)
Live US-F soil control (c)
5:1:2 FTB
7:1:2 FTB
5:3 FTB
7:3 FTB
9:1:2 FTB
9:3 FTB
11:1:2 FTB
Live US-F soil (d)
Sterile US-F soil (e)
Live US-F soil control (f)
5:3 FTCA
PFPrA
PFBA
PFPeA
PFHxA
PFHpA
Live US-F soil (g)
Sterile US-F soil (h)
Live US-F soil control (i)
7:3 FTCA
PFOA
PFNA
PFDA
PFUnA
Live US-F soil (j)
Sterile US-F soil (k)
Live US-F soil control (l)
PFBS
PFHxS
PFOS
PFDS
FBSA
Molar fraction of original dose recovered from soils (%)
Time (day)

(C) US-G soil

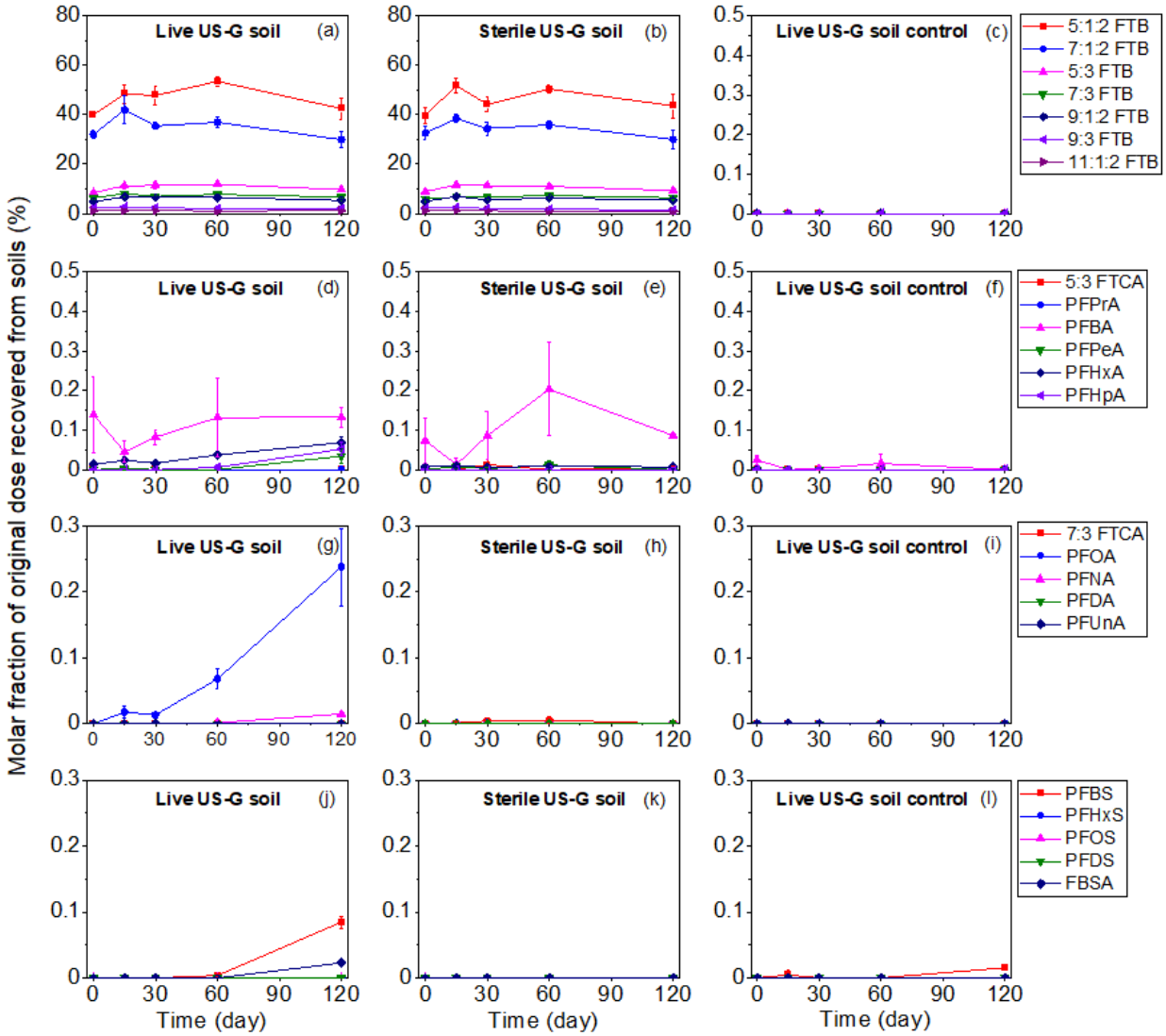
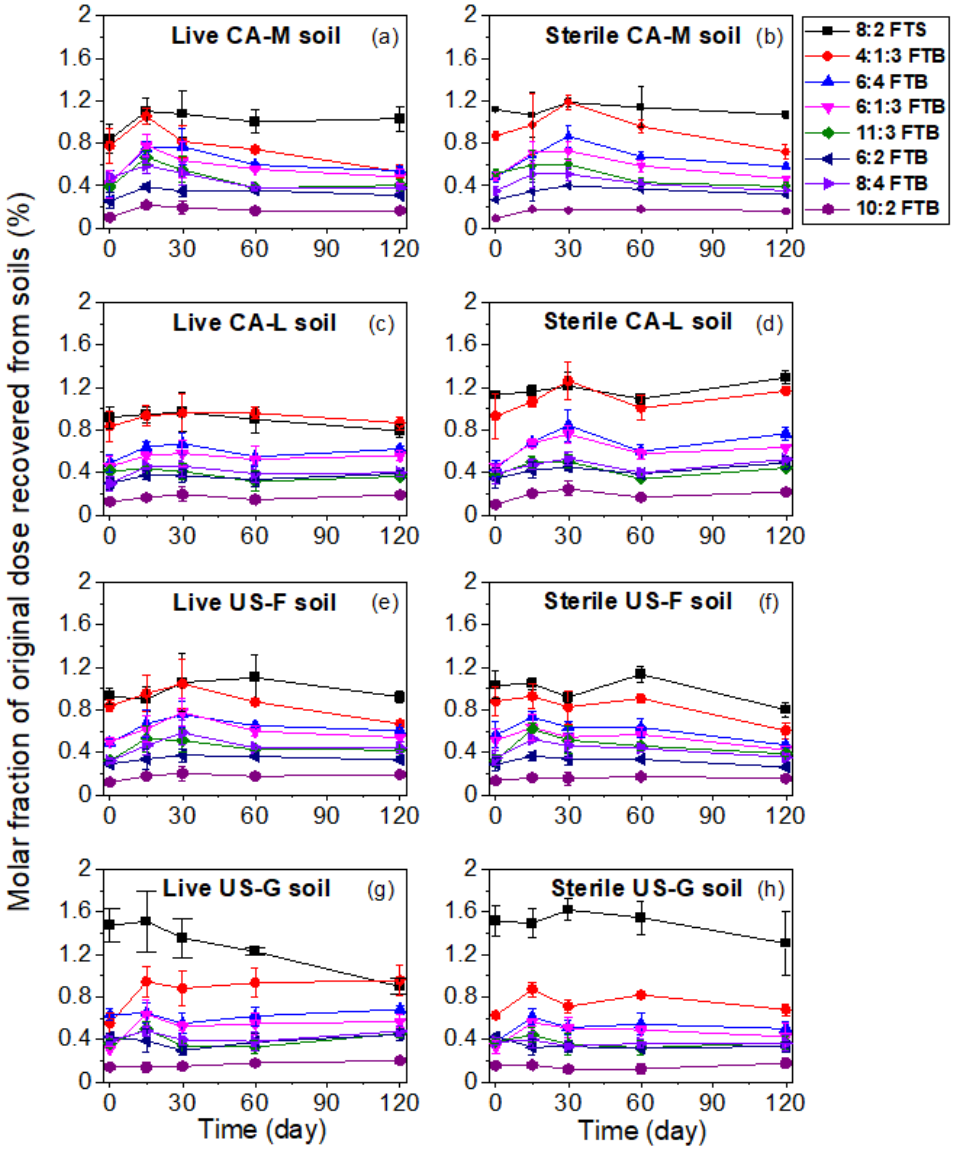

Live US-G soil (a)
Sterile US-G soil (b)
Live US-G soil control (c)
5:1:2 FTB
7:1:2 FTB
5:3 FTB
7:3 FTB
9:1:2 FTB
9:3 FTB
11:1:2 FTB
Live US-G soil (d)
Sterile US-G soil (e)
Live US-G soil control (f)
5:3 FTCA
PFPrA
PFBA
PFPeA
PFHxA
PFHpA
Live US-G soil (g)
Sterile US-G soil (h)
Live US-G soil control (i)
7:3 FTCA
PFOA
PFNA
PFDA
PFUnA
Live US-G soil (j)
Sterile US-G soil (k)
Live US-G soil control (l)
PFBS
PFHxS
PFOS
PFDS
FBSA
Molar fraction of original dose recovered from soils (%)
Time (day)

Figure C.14 Concentration profiles of the minor (A) and trace-level (B) precursors derived from the Ansulite AFFF in four live and sterile soils.

(A) Minor PFAS components derived from the Ansulite AFFF



(B). Trace-level PFAS components derived from the Ansulite AFFF.

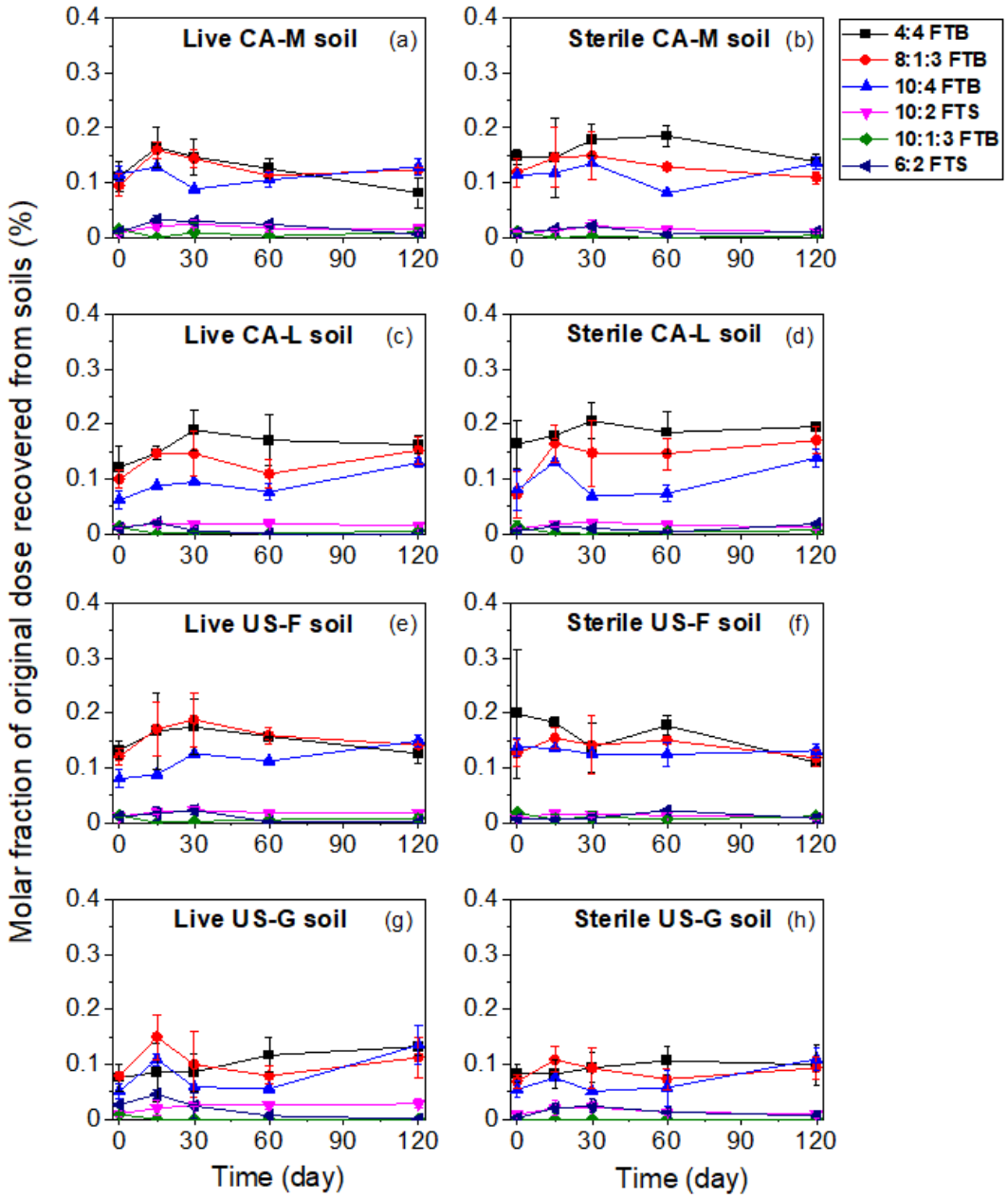
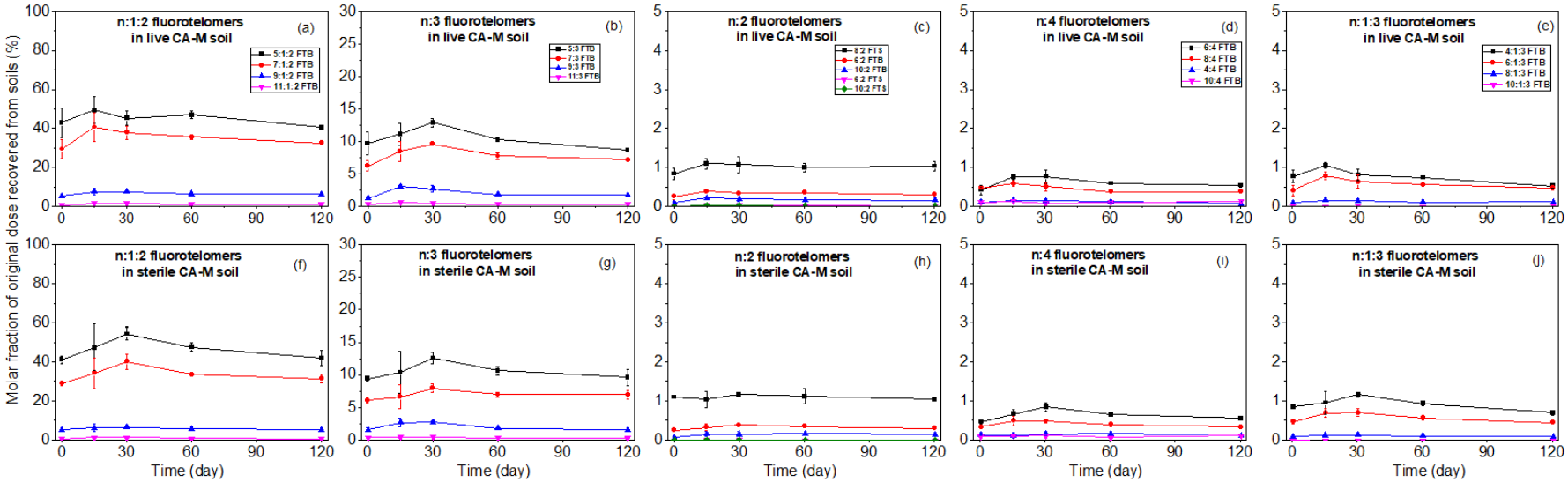
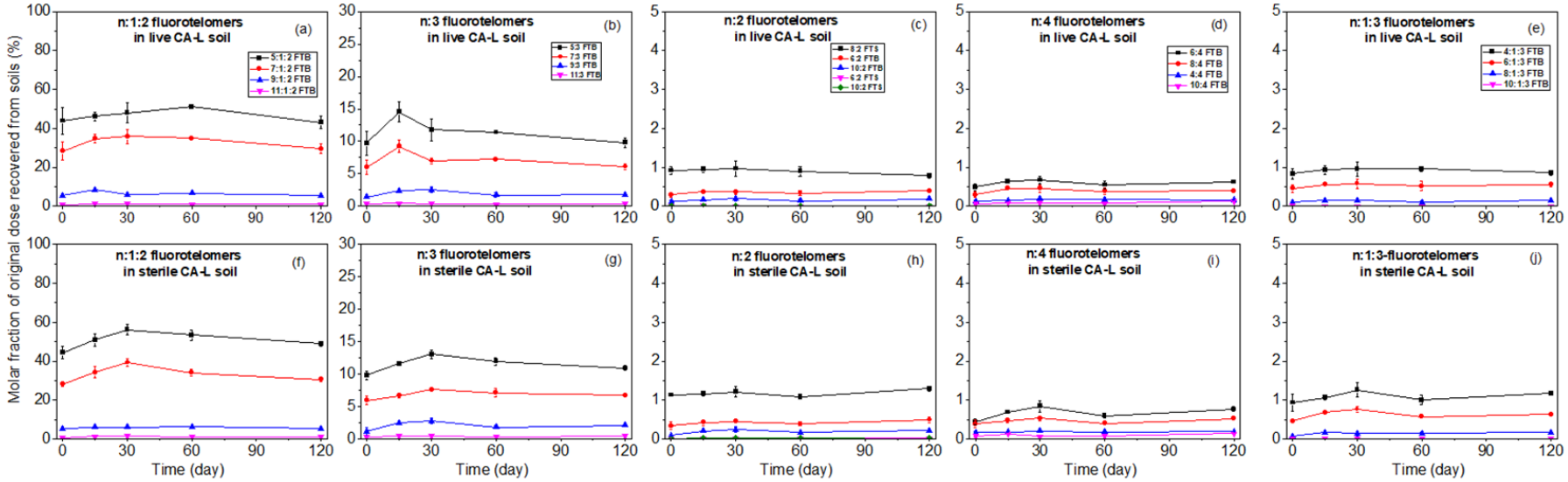


Figure C.15 Concentration profiles of AFFF-derived precursors with distinct polyfluoroalkyl chains in four live and sterile soils: CA-M (A), CA-L (B), US-F (C), and US-G (D) soils.

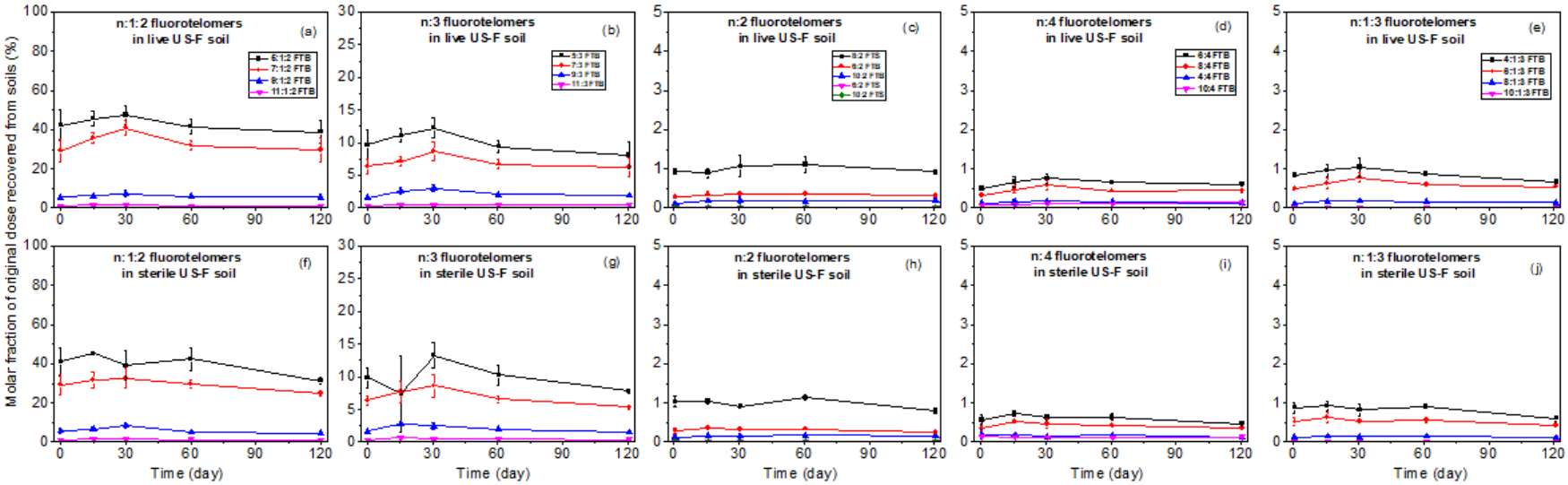
(A) In CA-M soil



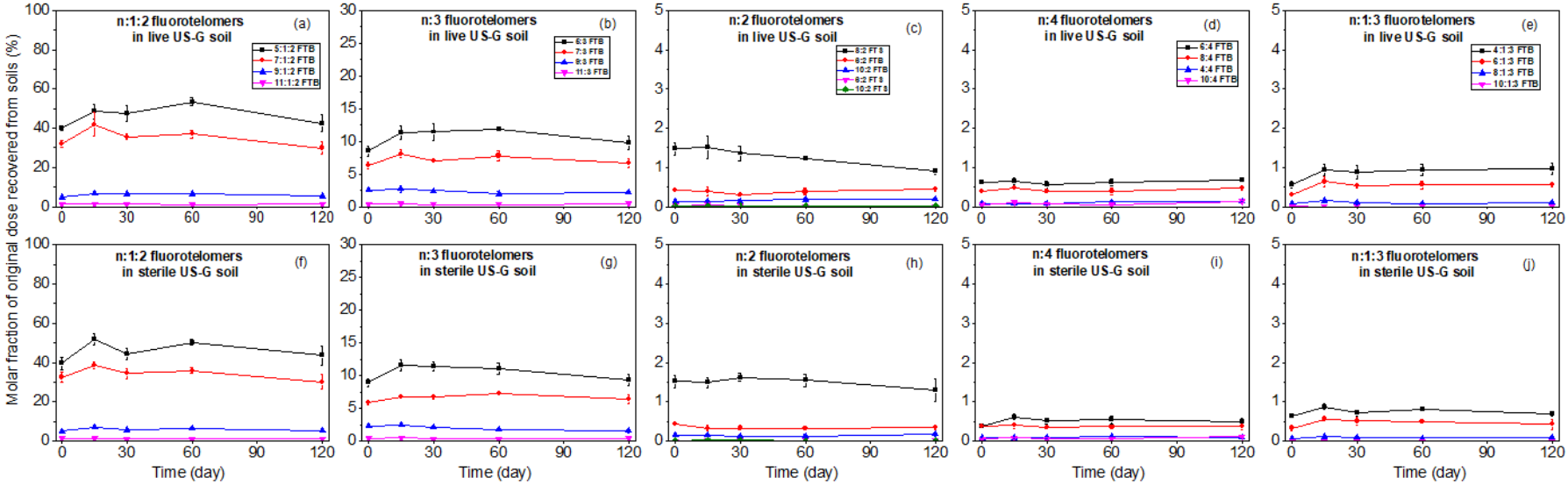
(B) In CA-L soil.


n:1:2 fluorotelomers in live CA-L soil
(a)
5:1:2 FTB
7:1:2 FTB
9:1:2 FTB
11:1:2 FTB
n:3 fluorotelomers in live CA-L soil
(b)
5:3 FTB
7:3 FTB
9:3 FTB
11:3 FTB
n:2 fluorotelomers in live CA-L soil
(c)
8:2 FTS
6:2 FTB
10:2 FTB
6:2 FTS
10:2 FTS
n:4 fluorotelomers in live CA-L soil
(d)
6:4 FTB
8:4 FTB
4:4 FTB
10:4 FTB
n:1:3 fluorotelomers in live CA-L soil
(e)
4:1:3 FTB
6:1:3 FTB
8:1:3 FTB
10:1:3 FTB
n:1:2 fluorotelomers in sterile CA-L soil
(f)
n:3 fluorotelomers in sterile CA-L soil
(g)
n:2 fluorotelomers in sterile CA-L soil
(h)
n:4 fluorotelomers in sterile CA-L soil
(i)
n:1:3-fluorotelomers in sterile CA-L soil
(j)
Molar fraction of original dose recovered from soils (%)
Time (day)

(C) In US-F soil.


n:1:2 fluorotelomers in live US-F soil
(a)
6:1:2 FTB
7:1:2 FTB
8:1:2 FTB
11:1:2FTB
n:3 fluorotelomers in live US-F soil
(b)
n:2 fluorotelomers in live US-F soil
(c)
n:4 fluorotelomers in live US-F soil
(d)
6:4 FTB
8:4 FTB
4:4 FTB
10:4 FTB
n:1:3 fluorotelomers in live US-F soil
(e)
4:1:3 FTB
6:1:3 FTB
8:1:3 FTB
10:1:3 FTB
n:1:2 fluorotelomers in sterile US-F soil
(f)
n:3 fluorotelomers in sterile US-F soil
(g)
n:2 fluorotelomers in sterile US-F soil
(h)
n:4 fluorotelomers in sterile US-F soil
(i)
n:1:3 fluorotelomers in sterile US-F soil
(j)
Molar fraction of original dose recovered from soils (%)
Time (day)

(D) In US-G soil


n:1:2 fluorotelomers in live US-G soil
(a)
5:1:2 FTB
7:1:2 FTB
9:1:2 FTB
11:1:2 FTB
n:3 fluorotelomers in live US-G soil
(b)
6:3 FTB
7:3 FTB
8:3 FTB
11:3 FTB
n:2 fluorotelomers in live US-G soil
(c)
8:2 FTB
10:2 FTB
n:4 fluorotelomers in live US-G soil
(d)
6:4 FTB
8:4 FTB
10:4 FTB
n:1:3 fluorotelomers in live US-G soil
(e)
4:1:3 FTB
6:1:3 FTB
8:1:3 FTB
10:1:3 FTB
n:1:2 fluorotelomers in sterile US-G soil
(f)
n:3 fluorotelomers in sterile US-G soil
(g)
n:2 fluorotelomers in sterile US-G soil
(h)
n:4 fluorotelomers in sterile US-G soil
(i)
n:1:3 fluorotelomers in sterile US-G soil
(j)
Molar fraction of original dose recovered from soils (%)
Time (day)

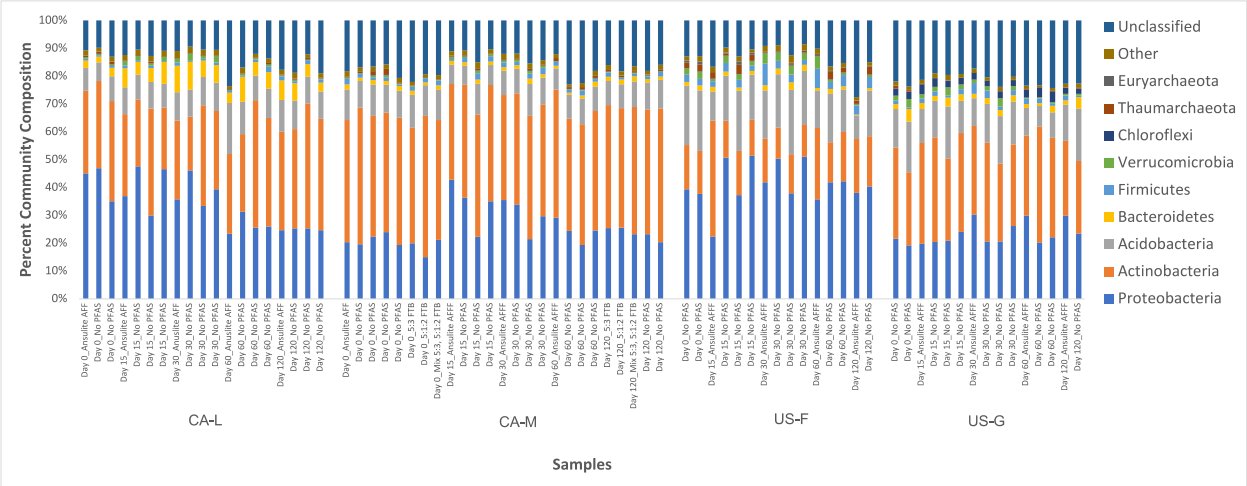


Figure C.16 Community composition plot of live soil samples, based on percent composition at the phylum level. Any phylum representing more than 1% of any sample was included in the bar plot, with all other phyla grouped as 'Other'. Samples are grouped by sample origin (CA-L, CA-M, US-F, and US-G) and are ordered left to right based on the sampling day, with treatments noted for each bar.

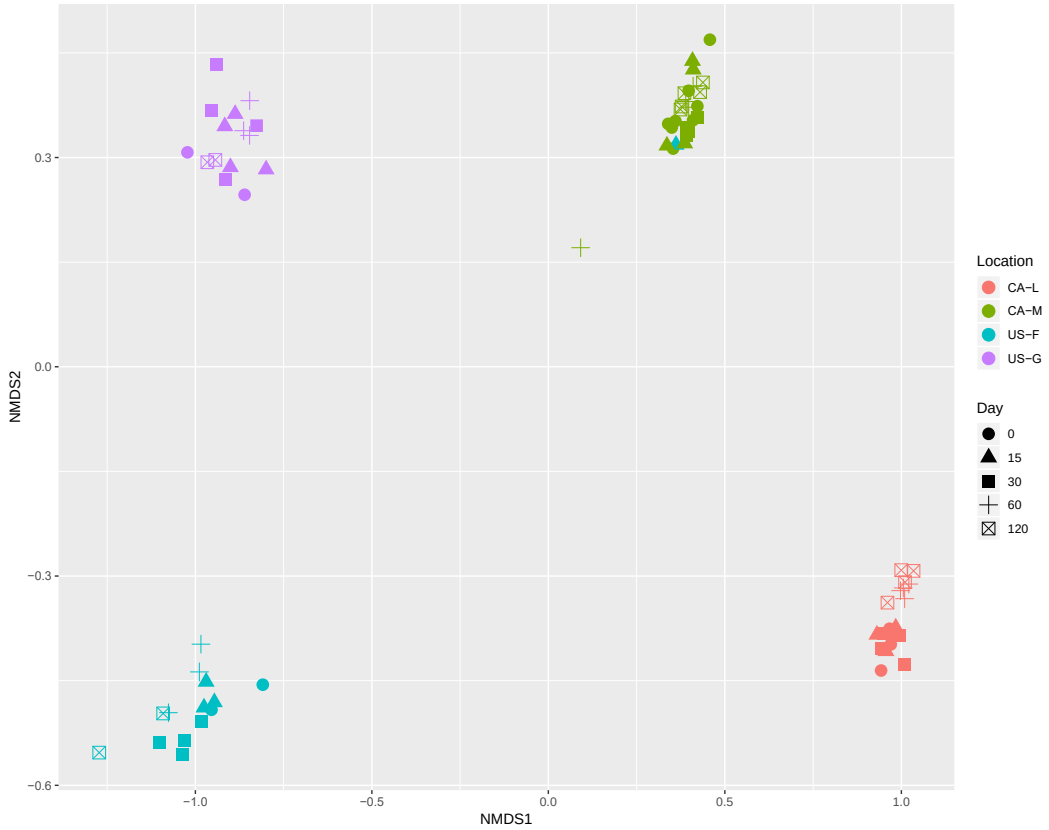


Figure C.17 MDS plot representing Bray-Curtis dissimilarity in community composition between live soil samples. Samples are colored by location (CA-L, CA-M, US-F, and US-G) and different shapes represent different sampling days.

Text C.9 References

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