Bioaccumulation of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in earthworms (*Eisenia fetida*) from sorbent-amended soil

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Abstract

The use of aqueous film-forming foams (AFFFs) for extinguishing fuel fires has introduced poly- and perfluoroalkyl substances (PFAS) into different environmental media, including soil. Such contaminated sites are long-term sources of PFAS pollution, posing a threat to drinking water supplies in many locations. *In situ* soil stabilization using sorbent amendments, such as granular activated carbon and modified clay minerals, has demonstrated efficacy in immobilizing PFAS in contaminated soil. The measure is expected to be highly effective in reducing PFAS bioavailability to earthworms, the predominant soil invertebrates, but limited experimental evidence is available. Therefore, PFAS uptake from contaminated soils by earthworms (*Eisenia fetida*) following amendment was studied to address the knowledge gap. Two different amendments were evaluated for this study: coal-based activated carbon (F400) and a new modified clay-based adsorbent (FLUORO-SORB100®). Surface soil was collected locally and spiked with PFAS at ~ 100 ng/g dry weight to simulate a moderately contaminated soil, to which the sorbents were amended at different concentrations (0-4 w/w%). A field-collected AFFF-impacted soil with high PFAS levels was tested at one amendment concentration (4 w/w%). The mixture of representative PFAS included 4 perfluoroalkyl sulfonates (PFSAs), 6 perfluoroalkyl carboxylates (PFCAs), and 3 (n:2) fluorotelomer sulfonates (FTSAs). Earthworms were exposed to PFAS during the uptake phase test until the expected steady-state was reached (28 days). Both amendments resulted in reduced earthworm body burdens compared to the setup without amendment, with 4 w/w% amendment being the most effective, reducing body burdens of total PFAS by >95% in the spiked soil. Activated carbon performed slightly better at reducing body burdens in both soils in terms of total PFAS, possibly owing to minimal gut exposure; earthworms largely avoided ingesting the activated carbon probably due to its particle size. In clay-amended soil, both gut route and skin contact contributed to PFAS uptake by earthworms. Furthermore, a soil leaching test was performed at the end of the uptake phase to understand the mobility of PFAS following the soil amendment. The clay-based adsorbent performed better at immobilizing most analytes in the contaminated soil; however, in the spiked soil, clay was not as effective as activated carbon for short-chain PFCAs. Strong positive log-log relationships were observed between leachate concentrations and

earthworm body burdens for most PFAS analytes in the spiked soil. The finding suggests that although the leaching test evaluates PFAS mobility, the PFAS leachate concentrations might be correlated with pore water concentrations, thus partly explain the extent of earthworm body burdens. Overall, this study allows for a risk-based assessment strategy for the use of amendments for mitigating PFAS pollution of soils.

Résumé

L'utilisation de mousses à formation de pellicule aqueuse (mousses AFFF) pour éteindre les feux de carburant a introduit des substances per- et polyfluoroalkylées (SPFA) dans différents milieux environnementaux, y compris le sol. Ces milieux contaminés qui sont des sources de pollution à long terme par les SPFA, et menacent l'approvisionnement en eau potable dans de nombreux endroits. La stabilisation des sols *in situ* à l'aide d'amendements sorbants, tels que le charbon actif en granulées et les minéraux argileux modifiés, a démontré son efficacité à immobiliser les SFPA dans les sols contaminés. Cette mesure devrait s'avérer très efficace dans la réduction de la biodisponibilité des SPFA pour les vers de terre, le groupe prédominant d'invertébrés du sol, mais jusqu'à présent, peu de preuves expérimentales sont disponibles. Par conséquent, l'absorption des SPFA par des vers de terre (*Eisenia fetida*), dans des soils contaminés après amendement, a été étudiée pour combler le manque de connaissances. Deux amendements différents ont été effectués pour cette étude : le charbon actif à base de charbon (F400) et un nouvel adsorbant modifié à base d'argile (FLUORO-SORB100®). Le sol de surface a été collecté localement et dopé avec du SPFA à ~ 100 ng/g de poids sec pour simuler un sol modérément contaminé, auquel les sorbants ont été ajoutés à différentes concentrations d'amendement (0-4 p/p%). Un sol contaminé par les mousses AFFF, collecté sur le terrain et présentant des niveaux élevés de SPFA, a été testé à une concentration (4 % p/p). Le mélange de SPFA représentatives comprenait 4 sulfonates de perfluoroalcane (PFSA), 6 carboxylates d'alkyles perfluorés (PFCA) et 3 (n:2) sulfonates fluorotélomériques (FTSA). Les vers de terre ont été exposés aux SPFA pendant l'essai de la phase d'absorption jusqu'à ce que l'état d'équilibre prévu soit atteint (28 jours). Les deux amendements ont rapidement réduit la charge corporelle des vers de terre, l'amendement de 4 % p/p était le plus efficace, car il a permis à réduire la charge corporelle des SPFA totaux de plus de 95 % dans le sol dopé. Le charbon actif a légèrement mieux réussi à réduire la charge corporelle dans les deux sols, en terme de SPFA total, peut-être en raison d'une exposition intestinale minimale; les vers de terre ont largement évité d'ingérer le charbon actif, probablement à cause de la grande taille de particules.

Dans les sols avec argile, la voie intestinale et le contact cutané ont tous deux contribué à l'absorption de SPFA par les vers de terre. En outre, un test de lixiviation du sol a été effectué à la fin de la phase d'absorption pour comprendre la mobilité des SPFA à la suite de l'amendement du sol. L'adsorbant à base d'argile a mieux réussi à immobiliser la plupart des analytes dans le sol contaminé ; cependant, dans le sol enrichi, l'argile n'était pas aussi efficace que le charbon actif pour les PFCA à chaîne courte. De fortes relations log-log positives ont été observées entre les concentrations de lixiviat et la charge corporelle des vers de terre pour la plupart des analytes SPFA dans le sol dopé. La conclusion suggère que bien que le test de lixiviation évalue la mobilité des SPFA, les concentrations de lixiviat de SPFA pourraient être corrélées avec les concentrations d'eau interstitielle, ce qui expliquerait en partie l'étendue de la charge corporelle des vers de terre. Dans l'ensemble, cette étude permet d’avoir une stratégie d'évaluation des risques d'utilisation d'amendements afin d'atténuer la pollution des sols par les SPFA.

Thesis Structure and Contribution of Authors

This thesis is prepared in the traditional monograph style following the guidelines by the Graduate and Postdoctoral Studies office of McGill University. The thesis consists of an introduction (Chapter 1), a literature review (Chapter 2), the materials and methods (Chapter 3), the results and discussions (Chapter 4), and the conclusion (Chapter 5). The last section is followed by supplementary information.

The experimental design, protocol, and analyses were performed by the candidate Julie Jarjour, under the supervision of Dr. Jinxia Liu.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AC	Activated Carbon
AFFFs	Aqueous film-forming foams
ASTM	American Society for Testing and Materials
BSAF	Biota-soil accumulation factor
CH_3COONH_4	Ammonium acetate
CM	Carbonaceous materials
d.w.	Dry weight
ECF	Electrochemical fluorination
F400	Filtrasorb 400
FLUORO-SORB100®	FS100
FTAoS	Fluorotelomer thioether amido sulfonates
FTAs	Fire training areas
FTSAs	Fluorotelomer sulfonic acids
GAC	Granular activated carbon
K_d	Soil-water distribution coefficient
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
m.c.	Moisture content
MeOH	Methanol
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
OM	Organic matter
PFAAs	Perfluoroalkyl acids
PFAS	Polyfluoroalkyl and perfluoroalkyl substances
PFBA	Perfluorobutanoate
PFBS	Perfluorobutane sulfonate
PFCA	Perfluoroalkyl carboxylic acid
PFHpA	Perfluoroheptanoate
PFHpS	Perfluoroheptane sulfonate
PFHxA	Perfluorohexanoate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoate

PFOA	Perfluorooctanoate
PFOAAmS	Perfluorooctane amidoalkyl ammonium
PFOS	Perfluorooctane sulfonate
PFOSAmS	Perfluorooctane sulfonamidoalkyl ammonium
PFPeA	Perfluoropentanoate
PFSA	Perfluoroalkyl sulfonic acid
TCLP	Toxicity Characteristic Leaching Procedure
4:2 FTSA	4:2 Fluorotelomer sulfonate
6:2 FTAB	6:2 fluorotelomer sulfonamidoalkyl betaine
6:2 FTOH	6:2 fluorotelomer alcohol
6:2 FTSA	6:2 fluorotelomer sulfonate
8:2 FTSA	8:2 Fluorotelomer sulfonate

Chapter 1 Introduction

1.1 Background

Aqueous film-forming foams (AFFFs) have historically been used in firefighting training activities and emergency response at military bases, airports, hydrocarbon processing facilities, and municipality fire departments. AFFFs contain a diverse mixture of chemicals, including highly fluorinated surfactants, hydrocarbon surfactants, solvent, and water, in a relatively stable emulsion. The highly fluorinated surfactants present in AFFFs belong to a large chemical class, per- and polyfluorinated substances (PFAS), which reportedly represent more than 5000 different structures in commerce. Long-chain PFAS, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), have been subject to regulatory restrictions and phased out of production in recent years, due to their persistence, bioaccumulative potential, and toxicity [1-3]. PFOA has shown probable links to six major human diseases, including kidney and testicular cancer [4]. Many other PFAS structures have received increasing attention, but their impact on human health and the environment is less understood, compared to PFOS and PFOA.

Numerous surveys of AFFF-impacted soils and nearby water systems have indicated high levels of PFAS contamination [5-10]. Aside from PFOS and PFOA, other PFAS compounds and newly identified classes of PFAS have been discovered at impacted sites in recent years, comprised of cationic and neutral/zwitterionic compounds [5, 6, 11, 12] in addition to regularly identified anionic compounds. Examples of abundant anionic PFAS detected in such sites include PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFPeA), and 6:2 fluorotelomer sulfonate (FTSA), which are either present in AFFFs as effective ingredients, impurities, or as degradation products of other PFAS compounds of more complex structures [13]. A study by Milley et al. (2018) estimated that a total of 152 airports in Canada are likely contaminated with PFAS from the use of AFFFs [14], while the number of impacted sites in military installations and private sectors is expected to be no smaller but remains unknown. Although PFAS used in AFFFs contain hydrophilic functional groups and have

been detected in surface and groundwater surrounding AFFF sites, soils can retain a significant amount of PFAS [15]. The exact mechanisms are not clear and hypothesized to be due to strong ionic interactions between charged groups on PFAS with soil components and air-water interfacial retention of PFAS with surface-active properties. AFFF-impacted soils are thus significant long-term sources of PFASs to groundwater, surface water, and surrounding ecosystem biota.

Multiple *in situ* or *ex situ* remediation or mitigation methods are being developed to reduce PFAS leaching from source zones [16]. An easy to implement technology is *in situ* stabilization, which involves mixing appropriate sorbent materials into contaminated soils to immobilize PFAS and reduce leaching or availability to biota. Granular activated carbon (GAC) – known for its high sorption capacity and capabilities for hydrophobic contaminants – has shown effective immobilization of PFOS, PFOA, and PFHxS in soils in laboratory studies [17, 18]. RemBind® (made in Australia), a mixture of activated carbon, aluminum hydroxide, kaolin clay, and other proprietary additives, has been tested in field-scale and successfully immobilized 20 analytes in 14 fire training area soils [19]. The presence of multiple components with a varying mode of actions are probably responsible for the promising results. Modified clay, created through intercalating smectite clays with cationic surfactants, has recently gained attention for its sorption potential [20, 21]. Modifying smectite minerals with cationic surfactants convert their hydrophilic silicate surface to a lipophilic one, thus enhancing their sorption capacity. A commercial clay adsorbent MatCARE™ (made in Australia) effectively reduced aqueous concentrations of PFOS in soil [20], but the efficacy for reducing other PFAS is not known. A study by Wang et al. (2019) evaluated leachate reduction using modified bentonite, FLUORO-SORB100® (FS100, made in the US) for a broad suite of PFAS and found it achieved greater removal efficiency than GAC for anionic and neutral/ zwitterionic PFAS [22]. While GAC performed better for cationic PFAS, the removal efficiency of the FS100 clay was enhanced by the addition of natural bentonite clay, confirming the potential of clay minerals for the immobilization of PFAS [22].

As chemical leaching from contaminated soil is mitigated through soil amendment, strong sorption of the contaminants to solid or amendments often results in a concurrent decline in the bioavailability of the contaminants. Bioaccumulation reduction in soil biota such as plants and earthworms following the amendment of biochar and activated carbon has been documented for other organic contaminants, including polycyclic aromatic hydrocarbons [23], polychlorinated dibenzo-p-dioxins/dibenzofurans [24, 25], atrazine [26], pentachlorophenol [27], hexabromocyclododecanes (HBCDs) [28], chlorobenzenes [29], and others. PFAS differs from other hydrophobic contaminants in their low affinity to lipids or fatty tissues but a high affinity for proteins [30]. Such characteristics may impact the bioavailability of PFAS to organisms upon the sorbent amendment in a distinct way. A study by Xia et al. (2012) observed decreased concentrations of six PFAS compounds (up to 66-97%) in larvae of a benthic organism *Chironomus plumosus* with increased carbonaceous materials [31]. The reduced aqueous phase concentrations of PFAS were considered to have resulted in a decrease in their bioavailability.

Little work has addressed the bioavailability of PFAS following the addition of sorbents to soils in the published literature. Bioaccumulation is commonly used as a measure of bioavailability. A study by Bräunig et al. (2016) performed a bioaccumulation test in wheatgrass and earthworms following RemBind® amendment. Up to a 30-fold decrease in the accumulation of PFOS in wheatgrass in one soil was observed [32]. Since earthworms closely interact with soil and constitute a large portion of invertebrate biomass in soil, they are an important medium for the uptake of chemicals in terrestrial food chains. Earthworms in contaminated soils can uptake chemicals via two routes: dermal contact to soil pore water or ingestion of soil particles. Bioaccumulation of legacy PFAS in the earthworm *Eisenia fetida* from contaminated or spiked soil has been widely studied [33-37], however fewer studies exist on novel compounds [38].

1.2 Thesis objectives

The addition of modified clays (including FS100) in soils has been demonstrated to be an effective immobilization measure to reduce the leaching of a range of PFAS from contaminated soils of various textural classes. Therefore, I hypothesize:

1) The same clay material can concurrently reduce the bioavailability of a variety of PFAS to earthworms;
2) The efficacy of sorbent amendment would vary with the type and concentration of the sorbent materials;
3) The reduction in PFAS bioavailability would vary with the type and molecular size of PFAS in a way consistent with their sorption affinity for soils.

The study was conducted to verify the above hypotheses. In particular, the modified clay was compared to GAC, which has been commonly used for benchmarking purposes for sorption or desorption studies. The overarching goal of the study is to provide experimental evidence to support the large-scale application of the low-cost FS100 *in situ* stabilization of PFAS in AFFF-impacted soils. The specific objectives were:

1) To evaluate the bioavailability of PFAS by performing a kinetic earthworm uptake bioaccumulation test in spiked and AFFF-impacted soils amended with different mass concentrations of activated carbon (F400) and a proprietary modified clay (FS100);
2) To determine the mobility of PFAS from the amended soils by employing a modified leaching test following the bioaccumulation test;
3) To elucidate potential relationships between decreased leachate concentrations and bioaccumulation;
4) To establish a preliminary understanding if the reduction of PFAS bioaccumulation is consistent with their sorption affinity for soils.

Chapter 2 Literature Review

2.1 PFAS definition and chemistry

Per- and polyfluoroalkyl substances (PFAS) are a group of human-made organic fluorochemicals consisting of a non-polar per- or polyfluoroalkyl chain and a non-fluorinated polar end group. These compounds possess various attractive physicochemical properties, which has led to their use as surface-active agents in both commercial and industrial products since the 1940s [39]. The lipophobic and hydrophobic tail and hydrophilic end groups result in optimal surfactant properties. The carbon-fluorine bond is very strong and even further strengthened when the carbon is fully fluorinated. In PFAS molecules, the perfluoroalkyl tail demonstrates high thermal stability and chemical resistance. PFAS have been used in a wide variety of applications, including cosmetics, non-stick cookware, food packaging, stain and water-resistant textiles and apparel, electronic manufacturing, and aqueous film-forming foams (AFFFs).

PFAS follows the general chemistry structure C_nF_{2n+1} –R, with the values of n ranging from 3 to 18. Perfluoroalkyl substances are aliphatic compounds comprised of a fully fluorinated carbon chain tail where all hydrogens on all carbons have been replaced by fluorines, except carbons associated with the functional group head, whereas polyfluoroalkyl substances are not fully fluorinated. Polar groups of PFAS can be anionic, cationic, nonionic, and amphoteric and constitute different moieties such as carboxylates, sulfonates, phosphates, quaternary ammonium, betaines, amides, sulfonamides, etc [40]. Perfluoroalkyl acids (PFAAs) are the most commonly studied class of PFAS, which are divided into two groups: perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). In the natural aquatic and terrestrial environment, PFAAs are persistent and resistant to chemical or biological degradation. Therefore, PFAAs are considered as terminal PFAS since, under environmental conditions, polyfluoroalkyl compounds may undergo biotic or abiotic transformation to lose the hydrocarbon functionality to form PFAAs. Thus, polyfluoroalkyl PFAS are also commonly referred to as “precursors” to PFAAs. Due to having greater bioaccumulative potential – greater ability to concentrate in living organisms or tissue – than their shorter chain analogues,

long-chain PFSAs ($n \geq 6$) and PFCAs ($n \geq 7$) have been of particular concern in the regulatory and scientific community.

PFAS can be manufactured by two main processes: electrochemical fluorination (ECF) and telomerization. ECF subjects a starting raw material (e.g. octane sulfonyl fluoride, $C_8H_{17}SO_2F$ or octanoyl fluoride, $C_7H_{15}COF$) to electrolysis in anhydrous hydrogen fluoride resulting in the replacement of hydrogen atoms of the material by fluorine atoms. This process creates a mixture of linear and branched isomers of perfluorinated isomers of the starting material. Perfluorooctane sulfonate (PFOS) and its salts, perfluorooctanoate (PFOA) and its salts, sulfonamides, and sulfonamido alcohols are some examples of PFAS compounds produced by ECF [39]. The eight-carbon chain length was dominant in older chemicals; though other chain lengths have also been commonly identified. Telomerization is a chemical polymerization process used to create polyfluoroalkyl substances by yielding intermediates, fluorotelomer iodide ($C_nF_{2n+1}CH_2CH_2I$) or fluorotelomer alcohol ($C_nF_{2n+1}CH_2CH_2OH$), that serve as initial materials to synthesize fluorotelomer based substances. The two main groups of polyfluorinated substances – PFAA precursors – are fluorotelomer based substances and perfluoroalkyl sulfonamido substances (ECF-derived). The structures of the PFAS evaluated in this thesis are illustrated in **Figure 1**, which are among the most widely detected and abundant structures. PFCAs, PFSAs, and fluorotelomer sulfonic acids (FTSAs) are commonly termed “legacy” PFAS compounds since their behavior is more widely understood than other newly reported compounds, termed “novel” or “emerging”, even though they have all been used for decades. A prominent example of “novel” PFAS is 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB), which was found widespread only recently in surface water and even drinking water [7]. It is noted that dozens of PFAS classes and several hundred distinct structures are possible for AFFFs [12]. The propriety nature of many structures, in particular those of the precursors, and the lack of analytical tools have prevented researchers and regulators from detecting them in the environment for decades until very recently. However, the lack of chemical standards and insufficient understanding of their environmental behaviours prevent a thorough study.

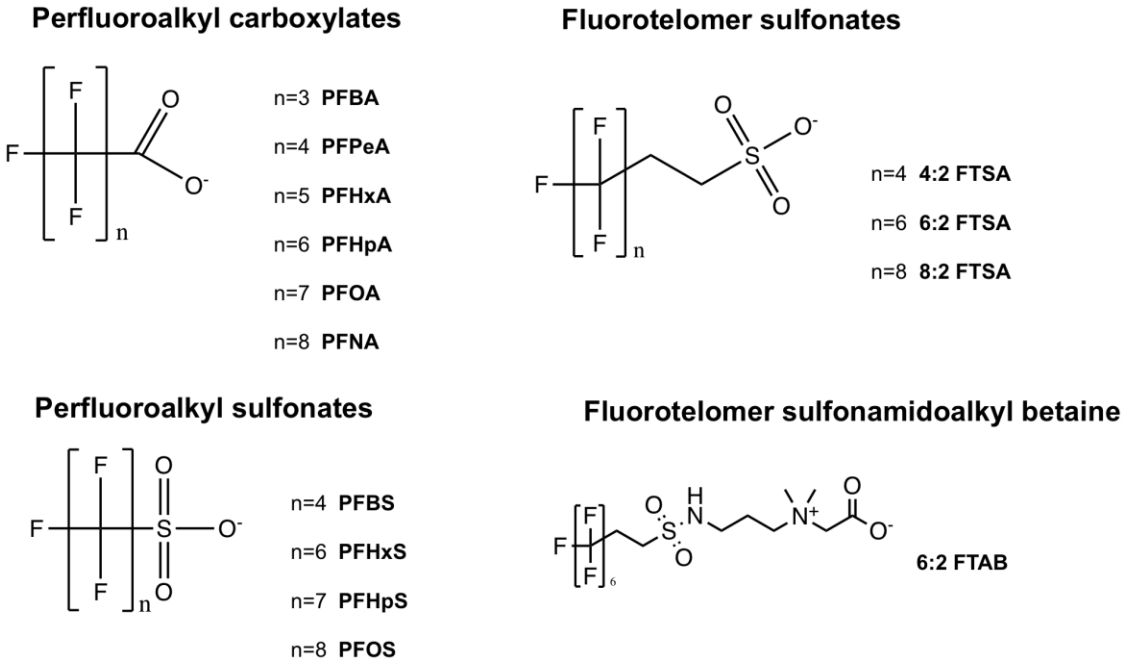

Figure 1 Structures of major classes of PFAS found in the environment and also studied in the project.

2.2 Concern

PFAS can be globally detected in different environmental media, including water, air, sediments, and soils [41-44]. They have also been detected in biota and human blood and breast milk [45, 46]. Due to their mobility in the environment, PFAS have even been identified in marine environmental media and biota in the Arctic regions [47].

Most regulation has focused on two long-chain PFAAs, PFOA and PFOS, which are particularly alarming due to their high persistence, bioaccumulative potential, and toxicity [1, 2, 48]. A recent study reported levels of PFOS/ PFOA in water supplies linked to 6 million residents in the United States (US) exceeded the US Environmental Protection Agency (EPA) lifetime health advisory level for drinking water of 70 ng/L [49]. Studies have shown associations of human exposure to some PFAS compounds with increased cancer risks, immunotoxic effects, liver damage, hormone interference, and

developmental and reproductive effects [4, 50-53]. Estimated half-lives in humans may reach up to the order of several years: 4.3-5.0 years for PFOS and 2.1-3.8 years for PFOA [54].

2.3 Phase-out and regulations

Gradual phase-out of the production and use of several PFAS compounds, specifically PFOS and PFOA and their precursor substances, has been committed to by international efforts in recent years. By 2002, PFOS was phased out of production by the primary manufacturer in the United States, 3M, and a 4-carbon analogue has been used since. In 2006, eight other major fluorotelomer manufacturers agreed on phasing out PFOA production and related precursor chemicals by 2015. The new replacement fluorotelomer chemicals are either of a short-chain version of the previous generations or new ether-based compounds such as GenX (ammonium perfluoro-2-propoxypropionate) that generally have lower hydrophobicity than phase-out chemicals. Most European Union countries have also banned the production and limited the use of PFOS since 2008. In 2009, PFOS and its salts and precursors were added as a persistent organic pollutant to the *Stockholm Convention* with certain exemptions for use [55]. Currently, in Canada, PFOS and its salts, PFOA, its salts, and precursors, and PFCAs (n 8-20) are subject to the Prohibition of Certain Toxic Substances Regulations, 2012. Drinking water guidelines have also been developed in Canada for PFOS and PFOA with maximum acceptable concentrations of 0.2 µg/L and 0.6 µg/L, respectively [56]. Despite higher regulatory values in Canada than in the US, few drinking water samples in Canada have demonstrated levels that would exceed the US EPA lifetime health advisory level. Due to regulatory pressure, manufacturers have developed replacement compounds; however, the new alternatives, despite being less hydrophobic and probably less bioaccumulative, may still pose particular risks because of extreme chemical persistence [57]. The higher environmental mobility of the alternatives is also a concern. Nonetheless, the circulation of PFAS remains in the environment due to continued unregulated use, development and use of replacement compounds, and contamination from historical uses.

2.4 Environmental fate and transport

2.4.1 Sources and exposure routes

Elevated levels of PFAS contamination have resulted largely from the use of AFFFs for emergency fire response or at fire training areas (FTAs). Other known sources include various industries that use or produce PFASs (e.g., fluorochemical plants, metal plating facilities, electronics manufacturers, etc.), wastewater treatment plants, and landfills. AFFFs historically used to extinguish hydrocarbon fuel fires constitute a diverse mixture of PFAS, although their formulations remain proprietary. Efforts have been undertaken to try to classify formulations and PFAS mixtures at impacted sites, which have found a significant portion of formulations to contain PFAA precursors [8, 58]. The use of AFFFs has resulted in contamination of soil, surface water, and groundwater on or near treated sites [8, 12, 13, 59, 60]. Additionally, manufacturing facilities producing PFAS or materials requiring PFAS as processing aids or in the final product have contributed to large discharges of PFAS in wastewater. This has led to large amounts of PFAS being detected in downstream drinking water wells [61]. Sources may also include leaching from industrial waste, consumer goods, or sewage sludge containing PFAS that have been disposed of in landfills. Application of biosolids from contaminated wastewater to agricultural fields is also a contributing factor to contamination of soil, surface water, groundwater, and agricultural products [62, 63].

Ingestion of PFAS is regarded to be the primary route of exposure to the general public through the consumption of contaminated food and water [64, 65]. Atmospheric emissions from releasing sources may also constitute a large source of exposure to PFAS in neighboring populations through inhalation. Dermal contact is likely a minor contributor to exposure in humans as the compounds cannot be easily absorbed by the skin [66].

2.4.2 Properties, fate, and behavior

Since most PFAAs have low pKa values (estimated at -3.27 and -0.5 for PFOS [67] and PFOA [68], respectively), they dissociate into their anionic form at environmentally relevant pH values. PFAS are also highly persistent in environmental conditions due to their perfluoroalkyl or perfluoroether moieties. While some of these compounds can

undergo biotic and abiotic transformation, the terminal transformation products are persistent PFAAs. Varying chemical structures of PFAS lead to significantly different physicochemical properties [69]. The perfluoroalkyl chain length and terminal functional group type govern their behavior in environmental media. Environmental conditions also strongly influence their behavior, such as pH, the fraction of organic carbon (OC), matrix electrostatic potential, particle composition, and size distribution, presence of dissolved natural organic matter (NOM), presence of co-contaminants, ionic strength of water, and rainfall enhanced leaching [70-72]. To date, the behavior of PFAAs, including PFCAs and PFSAs, has been most widely studied.

Most PFAS exhibit high water solubility and low vapor pressure, thus resulting in minimal air partitioning with some exceptions. Increased aqueous solubility occurs with decreased perfluoroalkyl chain; however, saline water may decrease their solubility. Possible partitioning mechanisms include hydrophobic and lipophobic interactions (driven by matrix interactions with the perfluoroalkyl chains), electrostatic interactions (driven by the functional group charge), and interfacial behavior (driven by the competing head and tail). Due to their presence in the anionic form at environmental pH, PFAAs are mobile in groundwater. In soils and sediments, their sorption increases linearly with increasing perfluoroalkyl chain lengths (C_6 or more), with PFSAs exhibiting stronger sorption than PFCAs of equal chain length [73]. Anionic PFAS can associate with soil OC; however, zwitterionic and cationic PFAS do not show a positive correlation between soil-water distribution coefficient (K_d) and OC [74]. Moreover, soil solution chemistry may affect the behavior of PFAAs, with decreased pH and increased ionic strength resulting in increased sorption and retardation [73, 75].

2.5 Occurrence

The current thesis primarily focuses on AFFF-derived pollution due to its scale and direct impact on groundwater and drinking water supplies. Historically contaminated sites are still regarded as point sources of PFAS due to continued leaching [59, 76]. The recent detection of legacy and novel PFAS in environmental matrices at AFFF-impacted sites

suggests a need to continue to evaluate the extent of risks and to develop solutions to minimize further distribution [12, 59, 60, 76, 77].

Numerous studies have been published regarding PFAS contamination in soil, groundwater, and surface water [5, 7-10, 12] – background levels are also detectable in supposed clean soils. Soil is an important medium through which the transfer of chemicals into other environmental matrices such as groundwater, air, or biota is possible. PFOS and PFOA concentrations in affected surface soil were measured at a high of 373,000 ng/g and 50,000 ng/g, respectively [5]. One study estimates a total of 152 airports in Canada are likely contaminated with PFAS from the use of AFFFs [14].

2.6 Biotransformation in soils

Perfluorinated PFAAs have not been observed to undergo transformation or complete mineralization under environmental conditions, likely owed to the high strength of the C-F bond, lack of enzymatic systems in the environment, and absence or shielding of structures available for a biological attack. However, polyfluorinated PFAS may be mediated by biotic or abiotic reactions to form terminal recalcitrant PFAAs. The transformation of the precursors is regarded as an important source of PFAAs in the environment [8, 78]. Possible processes include hydrolysis, photolysis, oxidation, and biotransformation.

2.6.1 Abiotic transformation

Hydrolysis of some precursors with hydrolyzable functional groups can produce intermediates that may further biotransform to form PFAAs [79]. Polyfluoroalkyl sulfonamide- and fluorotelomer based PFAA precursors can undergo indirect photolysis to produce PFAAs [80, 81]. The indirect photolysis of fluorotelomer alcohol is particularly important due to its contribution to the atmospheric deposition of PFCAs [82]. Oxidation by hydroxyl radicals has been observed for perfluoroalkyl sulfonamides and fluorotelomer-derived precursors in the atmosphere and natural waters [83, 84]. Notably,

abiotic transformations may occur very slowly, with an example of a 50-year half-life for the hydrolysis of fluorotelomer derived precursors at environmental pH [85].

2.7 Bioaccumulation

Many commonly observed PFAS are subject to bioaccumulation with the potential to enter and concentrate in higher trophic levels (biomagnify). Bioaccumulation is a sum of processes by which chemicals are taken up by an organism leading to increased levels in its tissue. Several studies have evaluated the bioaccumulation potential of various PFAS in aquatic and terrestrial organisms [34, 38, 96-99]. Bioaccumulation of PFAAs is generally influenced by fluorinated chain lengths, with longer chain homologues being more bioaccumulative. In certain cases, shorter chain PFAAs have demonstrated greater bioaccumulation potential than long-chain PFAAs in edible parts of crops such as leaves, stems, and fruits [62, 100]. PFSAs are usually more bioaccumulative than PFCAs of the same perfluoroalkyl chain length. PFAS molecules are known to have an affinity to bind to proteins in organisms; in contrast, most persistent organic pollutants preferentially partition into lipids [30].

2.7.1 Earthworm relevance

Terrestrial invertebrates such as earthworms closely interact with soil and constitute a large portion of invertebrate biomass in soil, which makes them an important medium for the uptake of chemicals in terrestrial food chains. They are commonly used in laboratory investigations to test the bioaccumulation of chemicals in soils. The earthworm subspecies *Eisenia fetida* and *Eisenia andrei*, which belong to the family Lumbricidae, are recommended test species for testing bioaccumulation from soil [101]. Earthworms can take up chemicals through two different routes: dermal contact to soil pore water or ingestion of soil particles. Biota-soil accumulation factor (BSAF) is a typical parameter used to describe bioaccumulation of biota in soil, which is calculated as the chemical concentration in the soil divided by that of the biota (worm) at steady-state. For certain compounds, the BSAF is normalized to the lipid content of the worm, but since PFAS do not preferentially partition into lipids, reporting the normalized value may not be relevant.

BSAFs can also be normalized to organic carbon; however, PFAS bioaccumulation and sorption are greatly influenced by several soil parameters [70]. Authors may choose to normalize for ease of comparison with literature values. A duration of around 28 days is sufficient to reach a steady-state for most PFAS [35]. Standard protocols have been developed to evaluate the fate of contaminants via bioaccumulation in earthworms, such as the American Society for Testing and Materials (ASTM) standard E1676-12 [102] and Organisation for Economic Co-operation and Development (OECD) test number 317 [101].

The bioaccumulation of select legacy PFAS in earthworms has been reported in the literature [33, 35-37, 103]. Either field contaminated soils or spiked soils were used in the studies. The general trend observed was that BSAFs have a chain length dependency for PFCAs (C_7-C_{12}) and were overall greater for PFSAs. A recently published study by Munoz et al. (2020) evaluated bioaccumulation of a large subset of PFAS, including novel zwitterionic fluorotelomers, and found a fluoroalkyl chain length dependency for fluorotelomer betaines, a group of PFASs only recently detected in AFFF sites but can be present at very high levels [38]. Notably, the study reported the significant presence of fluorotelomer sulfonates and fluorotelomer betaines in field-collected worms, which was explained by the slow biotransformation in soil and limited metabolization in earthworms. Metabolism of ECF-based precursors has been observed in terrestrial invertebrates; however, fluorotelomers metabolism has not [104, 105]. The work by Munoz et al. (2020) was the first study that looked at the bioaccumulation of FTSAs in earthworms. The bioaccumulation potential of FTSAs in terrestrial biota needs to be better understood since some FTSAs are abundant in AFFF-impacted sites. BSAF values can greatly vary and seem to be dependent on several factors: soil concentration, soil characteristics, duration of exposure, and analyte [35]. In addition to being bioaccumulative in earthworms, PFOS or PFOA in high concentrations can also cause adverse effects in earthworms such as oxidative stress, DNA damage, weight reductions, and fatality [106, 107]. In a 14-day acute toxicity test of *Eisenia fetida* in artificial soil substrate, no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values for PFOS of 77 mg/kg and 141 mg/kg, respectively, were derived [108].

2.7.2 Biotic transformation

Many polyfluoroalkyl substances have the capacity to aerobically biotransform into PFAAs. Several studies have also shown the possibility of certain precursors to undergo anaerobic transformation [86, 87]. Biotransformation of fluorotelomer-based precursors occurs through the degradation of the non-fluorinated segment to produce a mixture of shorter-chain PFCAs [88, 89]. On the other hand, biotransformation of ECF-derived precursors such as perfluoroalkyl amides or sulfonamides also occurs through the transformation of the non-fluorinated moiety to produce PFSAs with the same perfluoroalkyl chain length [90, 91], without any de-fluorination.

In some cases, polyfluoroalkyl compounds are produced as intermediates that also exhibit persistent behavior in select environmental compartments. For instance, 5:3 acid was found to be the terminal stable transformation product of 6:2 fluorotelomer alcohol (6:2 FTOH) in sludge and soil, which shows surprising high persistence despite the propyl moiety [92, 93]. To date, several PFAS commonly detected in AFFF formulations have been studied for their biotransformation behavior, such as 6:2 fluorotelomer sulfonate (6:2 FTSA), fluorotelomer thioether amido sulfonates (FTAoS), 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB), perfluorooctane sulfonamidoalkyl ammonium (PFOSAmS) and perfluorooctane amidoalkyl ammonium (PFOAAmS) [89, 94, 95].

2.8 Effect of sorbents on PFAS immobilization in soils

Stabilization using the addition of sorbents to soils has been evaluated as a means to immobilize PFAS in contaminated soils or sediments [16]. Either *in situ* mixing or *ex situ* stabilization reduces the leaching potential of PFAS from source zones into groundwater or surface water. Through adsorption, the sorbent reduces the liquid phase concentration of PFAS. Two main interactions can take place between the sorbent and PFAS: hydrophobic interactions between the amendment non-polar functional groups and electronegative carbon-fluorine chain and electrostatic interactions between the negative charge on PFAS functional group and positive charges on the sorbent. Several materials

have been tested, with carbon and mineral treatment showing the most promise. A patented sorbent mixture, RemBind®, made up of activated carbon, aluminum hydroxide, kaolin clay, and other proprietary additives, has gained popularity for PFAS stabilization [19]. Field-scale have been employed and immobilized 20 analytes in 14 FTA soils. A 2.5-10% RemBind® amendment resulted in 95 to >99% PFOS reduction and 40 to 99% reduction of total PFAS depending on the soil type. Granular activated carbon (GAC) alone has demonstrated effectiveness in immobilizing several analytes such as PFOS, PFOA, and perfluorohexane sulfonate (PFHxS) [17, 18]. One study using a modified clay showed great extents of PFOS immobilization [20] – greater than 99% reduction in PFOS in aqueous phase/leachate with a 10% amendment to the soil. Modifying clay minerals with cationic surfactants converts their hydrophilic silicate surface to lipophilic one, thus enhancing their sorption capacity. Wang et al. (2019) demonstrated the effectiveness of another modified clay adsorbent FS100 (image in **Figure S1**) in immobilizing a large suite of PFAS in AFFF-impacted soils [22]. Solution pH and ionic strength of the leachate had little impact on removal efficiencies, although sorbent or soil surface charges may be impacted where at lower pH (less aggressive leaching conditions), sorbent or soil surface charges may become more positive which would enhance PFAS sorption. Since studies on PFAS remediation using modified clay are limited, it is important to establish the effectiveness of clay amendments for different soil profiles.

While the investigation of sorption is relevant for immobilization, long-term leachability and bioaccumulation of PFAS are also necessary in understanding the environmental fate of PFAS and ecological safety following sorbent amendment to soils. Long-term leachability is yet to be validated on a field-scale; a study is underway in the US that is monitoring leachability over a few years [16].

2.8.1 Effect of carbonaceous materials and sorbents on bioaccumulation of PFAS

Bioaccumulation of several PFAS in sediment-dwelling organisms with the amendment of carbonaceous materials (CM), which may have otherwise been naturally occurring, has been evaluated [31, 109]. Materials included carbon nanotubes, ash, chars, and fullerene.

Most of these resulted in reduced BSAF values by 66-97% with a 1.5% amendment. One study by Zhai et al. (2016) showed increased contributions of the ingestion route to bioaccumulation of PFAS with the addition of fullerene, suggesting solubilization of CM-associated PFAS by larval digestive juice of *Chironomus plumosus* [109]. One unpublished study has looked at the influence of the amendments of RemBind® on the bioaccumulation of PFAS, specifically PFOS, PFHxS, PFOA, and perfluorohexanoate (PFHxA), in earthworms and plants, finding a 30-fold decrease of PFOS in wheatgrass at a 25% sorbent concentration and reduced earthworm body burdens (exact values not reported in the conference proceeding) [32]. The literature on bioaccumulation of PFAS in solely activated carbon or modified clay amended soils for remediation purposes has not yet been published to the knowledge of this author.

Chapter 3 Methodology

3.1 Materials

Analytical standards were obtained from Wellington Laboratories, Inc. (Guelph, ON, Canada). The chemicals prepared for the spike solution were obtained from SynQuest Laboratories, Inc (Alachua, FL, USA). Analytes include four perfluoroalkyl sulfonates (PFSAs, n = 4, 6, 7, and 8), six perfluoroalkyl carboxylates (PFCAs, n = 3, 4, 5, 6, 7, and 8), three (n:2) fluorotelomer sulfonic acids (FTSAs, n = 4, 6, and 8), and 6:2 FTAB. 6:2 FTAB was custom synthesized at the Shanghai Institute of Organic Chemistry (Shanghai, China). PFAS structures are displayed in **Figure 1**, and further detailed chemical information of the native and isotope labelled standards can be seen in **Table S1** and **Table S2**.

HPLC-grade water, HPLC-grade methanol, and LC-MS grade formic acid were obtained from Fisher Scientific (Whitby, ON, Canada). LC-MS grade acetonitrile was obtained from VWR International (Radnor, PA, USA). The deionized water was acquired from LabChem (Zelienople, PA, USA). Ammonium acetate (purity ≥ 98%) was from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (25-30% in water) was from Fisher Scientific (Whitby, ON, Canada). Nitrogen gas was from Praxair (Mississauga, ON, Canada). Supelclean ENVI-Carb cartridges (250 mg/6 mL) were obtained from Supelco (Bellefonte, PA, USA) and Strata-X-AW 33um Polymeric Weak Anion (200 mg/6 mL) were obtained from Phenomenex, Inc (Torrence, CA, USA).

3.2 Soil and sorbents

Two soils were used in this study. An AFFF-contaminated soil, CAN 1, was donated by Environment Canada (stored at -20°C). The other soil, MC, was collected from the Macdonald Campus Farm (McGill University, Ste-Anne-de-Bellevue, QC, Canada, 45.406629, -73.936320) from the top 15 cm layer. The metal shovel and plastic containers used for soil collection were pre-cleaned with methanol. The soils were characterized by A&L Canada Laboratories Inc. (London, ON, Canada), with the summary of soil properties presented in **Table 1**. Additional soil properties are summarized in **Table S3**. All soils

were air-dried in a laboratory without known PFAS sources for two weeks, sieved (2 mm), and stored at room temperature before use.

Table 1 Soil Properties

Soil ID	Textural Class	Sand %	Silt %	Clay %	Organic Matter %	CEC (meq/100g)	pH
CAN 1	Loamy Sand	89.2	0.8	10	0.4	32.6	8
MC	Sandy Loam	68.3	16.4	15.3	2.5 (1.96 total organic carbon)	11.6	7.2

A commercial granular activated carbon Filtrasorb 400 (F400) was obtained from Calgon Carbon (Pittsburgh, PA, USA). A proprietary bentonite-based clay modified with quaternary ammonium surfactants named FLUORO-SORB100® (FS100) was obtained from Minerals Technologies Inc (IL, USA). Sorbent characterization details can be found in previous studies [22, 110]. Both sorbents were sieved (2 mm) and stored at room temperature before use.

3.3 Soil and amendment treatment and spike

The MC soil was weighed out into eight different 2-L high-density polyethylene (HDPE) containers. The soil in each container was spiked in three different spots with a methanolic PFAS mixture to achieve an approximate concentration of 100 ng/g dry weight (d.w.) of individual analytes. The resulting total PFAS concentrations are below expected NOEC and LOEC levels [108]. Two additional MC soil setups were prepared: a solvent control (clean soil without worm) and soil control (spiked soil without worm) for sampling at the beginning and end of the experiment. The containers were shaken by hand and left to allow methanol to evaporate overnight under a fume hood. The MC soil was moistened to ~10% moisture content (m.c.) and was left to equilibrate overnight. CAN 1 soil was also weighed out and transferred into two containers and moistened to 20% (m.c.). Moisture content was pre-determined by observing earthworm burrowing behavior in the soils.

Following the spike and moistening, each container was first mixed by hand using a metal spatula and subsequently mixed for 15 minutes using a rotary mixer. The soils were left to equilibrate overnight and mixed again each for 15 minutes for two additional days. The soils were left to equilibrate for 4 more days. The two sorbents, F400 and FS100, were then added at 0, 0.5, 1, and 4% dry mass concentrations to the MC soils. These concentrations were selected as they fall between typical soil amendment application rates [17, 18, 20, 111], and previous leachability tests [22] demonstrated the effectiveness of the lower range concentration at reducing PFAS leachate levels. The CAN 1 soils were amended with 4% of each sorbent. Each setup was then mixed in the rotary mixer for 20 minutes and left to equilibrate for two months. The MC soils were pre-moistened before the bioaccumulation test to 13 % m.c., and all soils were mixed by hand using a spatula.

3.4 Bioaccumulation test (uptake phase)

The bioaccumulation test was performed according to OECD standard Test No. 317: Bioaccumulation in Terrestrial Oligochaetes, with slight modifications. Mature *Eisenia fetida* earthworms, with visible clitellum, were obtained from Merlan Scientific Ltd (Mississauga, ON, Canada). The worms were left to depurate overnight – purging the gut on a moist filter paper in a covered petri dish – and acclimated in a separate batch of clean MC soil for 24 hours. After acclimation, the worms were rinsed, dried (gently patted with a Kimwipe), and left to depurate overnight. About 55 g d.w. of the moistened and aged soil was weighed and distributed into each 120 mL PP jar for different sampling time points: Day 3, 7, 14, 21, and 28. Jars were prepared in triplicates. Earthworms were placed individually to avoid further reproduction. The earthworms were rinsed, dried, weighed, and distributed individually to each vessel. Each vessel was covered with parafilm punctured with small holes for air circulation. The test was carried out in a light-dark cycle of 16 hours light and 8 hours dark at room temperature. No food was provided to the worms during the test. Soils and earthworms were sampled for background levels and initial spiked PFAS concentrations at Day 0. Soils were moistened throughout the uptake phase to maintain initial moisture levels. At each time point, earthworms were subsampled: rinsed, dried, weighed, depurated overnight, and weighed again after

depuration. Earthworms were individually stored in 20 mL glass vials then frozen at -20°C. Soils were also subsampled at each time point and stored in 20 mL glass vials at -20°C.

Soil and earthworm samples were freeze-dried at -55°C for 24 hours (preceded by one-day storage at -80ºC) and stored at -20°C until further processing. Additional soils were also subsampled and stored in the fridge at 15°C for subsequent leachability tests.

3.5 Leaching test

An in-house method was developed for determining the PFAS leaching from soil based on the US EPA test 1311, and the modifications were necessary to account for the properties of PFAS and utilization of appropriate pH levels [22]. Day 28 soils stored in the fridge were removed, air-dried overnight in aluminum trays, and homogenized using a pestle. Five grams of soil were added into 60 mL HDPE bottles, and 50 mL deionized water was added to achieve a water:solid ratio of 10:1 (mL:g). The bottles were shaken in a horizontal shaker at 150 rpm for 8 days at 20°C in the dark. 30 mL of the liquid was subsampled into 50 mL polypropylene (PP) bottles, centrifuged, and subjected to solid-phase extraction (SPE) for further concentration. SPE was conducted according to the method by Herman et al. (2018) [112] for PFAS water extractions with slight modifications. Briefly, Strata-X-AW cartridges were conditioned using 2 x 4 mL of 0.2% ammonium hydroxide (NH_4OH) in methanol (MeOH) and 2 x 4 mL of HPLC-grade water at a rate of 1 drop/s. Water samples were then loaded into the cartridge. The cartridges were subsequently dried for 1 hour under vacuum. After drying, the samples were eluted using 2 x 4 mL of 0.2% NH_4OH in methanol and recovered in 15 mL PP tubes. Eluents were then concentrated to 0.4 mL under a gentle nitrogen stream at a low-temperature setting and stored at -20°C until analysis.

3.6 Extraction (soil and earthworm)

Earthworm extractions were performed according to Munoz et al. (2020) [38]. Freeze-dried earthworms were individually crushed with a pestle and mortar, weighed, and placed into 15 mL PP tubes. The tools were cleaned with methanol in between each

sample. The samples were then subjected to three extraction cycles, and each involved the following steps: addition of 3 mL of 7 mM of NH_4OH in (MeOH), vortexing for 30 seconds, ultrasonication for 10 minutes, centrifugation at 5000 rpm for 5 min, and transfer of supernatant to new 15 mL PP tubes. The combined supernatants were subsequently concentrated to 2 mL under a gentle stream of nitrogen and low-temperature setting and submitted for clean-up procedure using ENVI-CARB cartridges conditioned with 5 mL MeOH. The samples were loaded in the cartridges, the tubes were rinsed with 2 x 0.5 mL of MeOH and loaded, and the cartridges were rinsed with 2 mL of MeOH. The filtrates were collected into 15 mL PP tubes. The filtrates were evaporated to 3 mL under a gentle nitrogen stream and low-temperature setting, with several exceptions where filtrates were evaporated to 5 mL or greater (e.g. for non-amended soils), to achieve PFAS levels within instrumental analysis range. Samples were stored at -20°C until analysis.

Soil extractions were also performed, according to Munoz et al. (2020) [38]. Freeze-dried soil samples were homogenized, and 1 g (d.w.) of each sample was transferred into a 15 mL PP tube. The samples were then subjected to three extraction cycles, and each involved the following steps: addition of 4 mL of 400 mM of ammonium acetate (CH_3COONH_4) in MeOH, vortexing for 30 seconds, ultrasonication for 10 minutes, centrifugation at 5000 rpm for 5 min, and transfer of supernatant to new 15 mL PP tubes. The combined supernatants were submitted for clean-up procedure using ENVI-CARB cartridges conditioned with 5 mL MeOH. The samples were loaded in the cartridges, the tubes were rinsed with 2 x 0.5 mL of MeOH and loaded, and the cartridges were rinsed with 2 mL of MeOH. The filtrates were collected into 15 mL PP tubes. The filtrates were evaporated to 10 mL under a gentle nitrogen stream and low-temperature setting and stored at -20°C until analysis.

3.7 Instrumental analysis

All samples were aliquoted and combined with internal standards prior to instrumental analysis. The final internal standard concentration of 5 ng/L for all analytes was used except for 6:2 FTSA and 8:2 FTSA (at 2.6 ng/L) to avoid instrumental cross-talk. Quantitation was achieved using a Shimadzu ultra-high-performance liquid

chromatography (UHPLC) system coupled to an AB Sciex 5500 Qtrap mass spectrometer (MS). The MS was operated in scheduled multiple reaction monitoring (MRM) mode, with positive and negative electrospray ionization. The separation was achieved by a Thermo Hypersil Gold C18 column (1.9 µm, 100 mm × 2.1 mm), and a second delay column was placed before the injection port to separate out the PFAS leaching from internal parts of the UHPLC. Additional details on the instrumental method are provided in **Table S4** and **Table S5**.

3.8 Data analysis

A biota-soil bioaccumulation factor (BSAF) was calculated for various soils at Day 28 of the uptake phase – expected steady state – as follows:

$$BSAF = {C_{worm}}/{C_{soil}} \quad (1)$$

where C_{worm} (ng/g) and C_{soil} (ng/g) are the concentrations of PFAS in the earthworm and soil respectively reported in dry weight.

Reduction of PFAS in leachate with the addition of sorbents was calculated as follows:

$$\text{Reduction of PFAS (\%)} = 100 \times {(C_{NA} - C_A)}/{C_{NA}} \quad (2)$$

where C_{NA} is the concentration of PFAS from the leachate of non-amended soil, and C_A is the concentration of PFAS from the leachate of sorbent amended soil.

The data are expressed as means with standard deviation. Single-factor analysis of variance (ANOVA) and paired t-test were performed to test the significant differences between treatments.

3.9 Quality assurance/ control

Quality control measures for the bioaccumulation test were performed according to OECD standards. Procedural blanks were prepared alongside different sample preparations. All sample preparations were made in triplicates. A clean MC soil (spiked only with methanol) was prepared as a solvent control for sampling at the start and end of the experiment.

Soil and earthworm recovery tests (details in **Supplementary Information**) were performed to validate the extraction methodology. The recoveries obtained were in the range of 88-112% in soils. Analytical issues were encountered for recoveries of PFCAs in earthworms; however, remaining analytes exhibited recoveries of 92-107%. Future tests will be performed to verify the recovery of PFCAs. Background PFAS concentrations in worms were below the quantification limit for all compounds except for PFHpA for two out of the four earthworms (15.1 ± 8.2 ng/g). Peaks under quantification limits or no peaks were found for analytes in background MC soil.

Internal standardization with inverse-weighted linear regression was employed for quantification using fitted calibration curves. At least 6 points were fitted to achieve appropriate linearity coefficients R^2 (>0.99) and suitable accuracy (80-120%). The instrument limit of quantification varied depending on the compound and but ranged between 0.1-0.2 ppb. A quality control sample of known concentration was also injected throughout the instrumental run to ensure bias is within limits (± 20%). Quality control samples did not pass consistently for PFBS and 4:2 FTSA across various analysis batches; therefore, results for these analytes may be slightly overestimated. A combined effect of low signal response and potential experimental artefact from using diluted 6:2 FTAB for spiking the MC soils resulted in irreproducible results, so the 6:2 FTAB results are not reported in this thesis.

Chapter 4 Research Findings

4.1 Soil extracts

Soil extract concentrations of all analytes before the addition of the amendments were determined (see **Table S6**). The average concentration of individual PFAS analytes in each spiked MC set-up ranged from 107-122 ng/g d.w. The contaminated CAN 1 soil had exceptionally high levels of PFHxS, PFOS, 6:2 FTSA, and 8:2 FTSA at concentrations of 441, 44930, 566, and 1049 ng/g d.w., respectively. Concentrations of PFHpS, PFHxA, and PFOA were also relatively high, at concentrations of 73 ng/g, 180 ng/g, and 81 ng/g, respectively.

PFAS levels in soil extracts were also measured at the end of the uptake phase (Day 28) to derive expected steady-state BSAF values (**Table S7**). Notably, 4:2 FTSA, 6:2 FTSA, and 8:2 FTSA soil concentrations in non-amended MC soil at the end of the uptake phase significantly decreased ($p < 0.05$) compared to initial spiked concentrations. Slightly greater reductions were observed in the non-amended soil with earthworms. This decrease may be explained by the biotransformation of these compounds over the long ageing duration of two months prior to the uptake test. The biotransformation also possibly resulted in increased PFPeA concentrations (18-29%). A recent study of 6:2 FTSA degradation in aerobic river sediment showed stable transformation products of PFPeA, PFHxA, and 5:3 acid [113]. Another pure culture study demonstrated the necessity of sulfur-limiting conditions for the breakdown of 6:2 FTSA [114]. A further soil analysis may be carried out to confirm the sulfur level in the soil. FTSA biotransformation is expected in aerobic soils, but experimental evidence has been lacking. Transformation products for 4:2 FTSA and 8:2 FTSA in soils have not been reported, but they likely follow the same pattern as 6:2 FTSA, with the main difference being the disappearance kinetics of three compounds of different hydrophobicity. As the remaining analytes were in close range to initial concentrations (± 20%) in non-amended soils, the impact of FTSA biotransformation on determining other analytes and their associated BSAF was considered minimal.

Although soil extracts for the amended set-ups by Day 28 are expected to be slightly lower due to strong sorption of PFAS to the amendments, concentrations of several analytes actually increased from the time of initial sampling (see **Table S7**) for soil extract concentrations at Day 28). A similar phenomenon was observed by Hale et al. (2017) in a leachate test where aqueous concentrations with sorbent amendment resulted in slightly higher concentrations than without amendment [17]. The extent of sorbent-associated PFAS in different sorbents may result in different solvent-extractable amounts. Extraction methods for soil with sorbents need to be optimized, or correction methods may be used to achieve reliable PFAS concentrations in amended soils from solvent extracts.

4.2 Bioaccumulation kinetics

Normal burrowing was observed for all earthworms, and one mortality was observed (in MC soil with 4% FS100) among all the set-ups. Two earthworms did not appear to be healthy upon sampling as their movement was minimal, possibly due to inappropriate moisture rationing. Earthworm wet weight body masses decreased by an average of 18%. No significant difference between wet weight reductions at each time point was found between the amendment treatment groups ($p > 0.05$). Soil contaminants can be taken up by earthworms through various routes, including adsorption of pore water and ingestion of soil or food. Uptake through the gut – passive diffusion mechanism resulted from the feeding of particles – is thought to dominate over pore water adsorption for hydrophobic organic compounds with log $K_{ow} > 6$ [115].

Kinetic data for the uptake phase were obtained for all analytes and illustrated in **Figure S2**-**Figure S7** (values in **Table S8**-**Table S12**). In the MC soil without amendments, earthworm body burdens of most PFAS analytes appeared to reach peak values towards the end of the uptake phase. For certain PFCAs, including PFBA, PFPeA, and PFHxA, body burdens declined after reaching peak values. Different FTSA biotransformation rates in the soils may have contributed to different uptake extents of short-chain PFCAs. Body burdens for FTSAs also appear to have reached their maximum values, although

4:2 FTSA barely accumulated in the earthworms. Since no major deviations from the expected kinetic trend occurred for 6:2 FTSA and 8:2 FTSA, it is assumed that most of the biotransformation was attained before the start of the uptake phase for the non-amended MC soil. However, decreases were observed between PFAS body burdens for both compounds from Day 21 to Day 28. Steady-state concentrations in earthworms cannot be statistically confirmed since not enough data points were taken (within ± 20% at an interval of at least two days for three consecutive time points); however, previous studies showed that 28 days was sufficient to reach the steady-state for most PFAS in the absence of soil amendments [35, 37, 38].

Overall, the addition of sorbent quickly reduced the bioavailability of PFAS in the MC soils (**Figure S2**-**Figure S4**). Higher sorbent concentrations in most cases resulted in lower PFAS body burden throughout the uptake phase duration (uptake order: 0%>0.5%>1%>4%). The least fluctuations in body burdens were observed at the 4% concentration for most analytes. Most earthworm body burdens in the amended set-ups minimally fluctuated past their apparent peaks.

PFSAs body burdens in the amended soils, both with F400 and FS100, showed little change after Day 14. Fluctuations of body burdens in amended soils were more apparent for PFCAs, mainly at the lower amendment concentrations of 0.5 and 1%. At the lower F-400 amendment of 0.5%, several PFCA body burdens fluctuated greatly past their apparent peak values (attained around Day 7), including PFPeA, PFHxA, and PFHpA. Earthworm body burdens for those three analytes at the lower amendment concentration of 0.5% surpassed 1% in some instances. Earthworm body burdens of PFCAs in FS100 amended soils also underwent large fluctuations past apparent peaks with the three amendment concentrations for PFHxA and PFHpA, leading body burdens at 0.5% amendment concentration to surpass 1% for several time points. Variabilities are expected due to the potential uneven mixing of soils with sorbents.

Body burden peak values of 6:2 FTSA and 8:2 FTSA were reached with minimal fluctuations for the different concentrations of F400. Body burden values for 4:2 and 6:2

FTSA at lower FS100 clay concentrations surpassed those of non-amended soils at several time points; however, the body burden ended up decreasing by Day 28. Since this was observed only with FS100, it is suspected that soil biotransformation may have occurred at different rates in the sorbent amended soil. Fluctuations in body burdens with amendments were apparent for 4:2 FTSA; however, since the body burdens were in the low range, slight changes in soil concentrations may have significantly impacted the uptake.

In the contaminated CAN 1 soil (**Figure S5**-**Figure S7**), for most analytes at a 4% concentration of F400, a plateau was reached with minimal body burden fluctuations after the rapid initial uptake phase. However, the 4% FS100 amendment led to declines or large fluctuations in body burdens after peak values were reached. The declines in earthworm body burdens suggest a decrease in bioavailability over time and that a longer duration would be needed to evaluate the differences in reductions at steady-state between the two sorbents. The exact role of desorption within the earthworm gut is difficult to elucidate within the earthworms, and significant metabolism is not expected based on the soil extract data. Chai et al. (2011) attributed lower reduction efficiencies to larger sized particle distribution of sorbents as earthworms may avoid ingestion of larger particles [24]. Additionally, upon ingestion of particles, sorbent-associated contaminants can desorb by gut mediated processes. Smaller diffusion distances of smaller particles can result in more rapid uptake and release compared to larger ones. Ingestion of smaller sized clay or AC particles could explain the observed fluctuations.

4.3 Biota-soil bioaccumulation factors

BSAF values in non-amended spiked soil are presented in **Figure 2** and **Table S13**. BSAFs for PFCAs are lower than PFSAs at equal perfluorinated chain length and increase with increasing chain length for PFCAs with C_6 to C_8 – trends are also in agreement with those in literature [34, 35, 37, 38]. PFBA and PFPeA did not follow the expected chain length dependency trend. Bräunig et al. (2019) also observed a lack of trend of BSAFs for short-chain PFCAs in earthworms exposed to AFFF-impacted soils,

which was attributed to different mechanisms of sorption of lower chain PFAS [116]. Higgins and Guelfo (2013) found that $\log K_d$ of PFBA and PFPeA did not follow the trend of increased $\log K_d$ with chain length, suggesting ion exchange and steric effect as potentially important mechanisms that mediate preferential sorption of small PFCA molecules to soils [117]. Transformation of fluorotelomer sulfonates (e.g., 6:2 FTSA) in the MC soil may have also partly contributed to the lack of dependency on chain length for the short-chain PFCAs.

The BSAFs of other PFSAs were greater than that of PFOS, with PFHxS being the greatest at a value of 214 (see **Table S13**). No trend was observed with increasing chain length, and BSAF value followed the order of PFHxS>PFBS>PFHpS>PFOS. While a few studies observed a chain length dependency for PFSAs, others found a decreasing trend or no trend [32, 35, 104, 116]. Rich et al. (2015) attributed the decreased bioaccumulation with the chain length of PFSAs to the larger sulfonate head group relative to PFCAs and the stronger sorption of PFSAs to soil, making them less bioavailable [35]. Additionally, short-chain PFAS binding affinities to proteins may be different than those of long-chain [118].

The BSAF of PFOS in this study of 99.6 for the non-amended soil is greater than most values reported in the literature for earthworms exposed to spiked or field soils, for which values ranging between 1-75 for various soil concentrations were reported [33, 35, 119]. This BSAF is in close range with the study by Rich et al. (2015), which found a BSAF value of 75 for an AFFF-impacted soil with a comparable PFOS concentration of 187 ng/g and organic carbon fraction of 1.4%, which is only slightly lower than 1.96% in the present study [35]. Wen et al. (2014) employed regression to correlate soil parameters to bioaccumulation of PFOS and PFOA in earthworms and demonstrated that PFAS concentration had a positive influence (i.e., lowering BASF) and OM had a negative influence, while soil pH and clay content had little influence on bioaccumulation [36]. OM has been found to limit plant uptake of PFAS in plants [2, 120, 121], and may also have a similar influence on the uptake by earthworms. Bioaccumulation of PFAS has been hypothesized to be concentration-dependent, and this hypothesis was tested by several

authors who found BSAF values to be lower for higher concentrations in green mussels and earthworms [36, 37, 122, 123]. Liu et al. (2011) compared the phenomenon to a nonlinear adsorption mechanism where limited binding sites for PFAS exist in earthworms [123]. At lower soil concentrations, there are more binding sites, which eventually become saturated when PFAS concentration increases to result in decline BSAF values. Therefore, the most plausible explanation for the high BASF for PFOS in the study is the combination of the relatively low organic matter/ carbon fraction of the MC soil and the relatively low starting PFOS concentration. The BSAF of PFOS was 7.7 fold greater than that of PFOA. The BSAFs for 6:2 FTSA (151) and 8:2 FTSA (153) were much greater than that of 4:2 FTSA (0.59) and in the same range as for PFSAs (99.6-214).

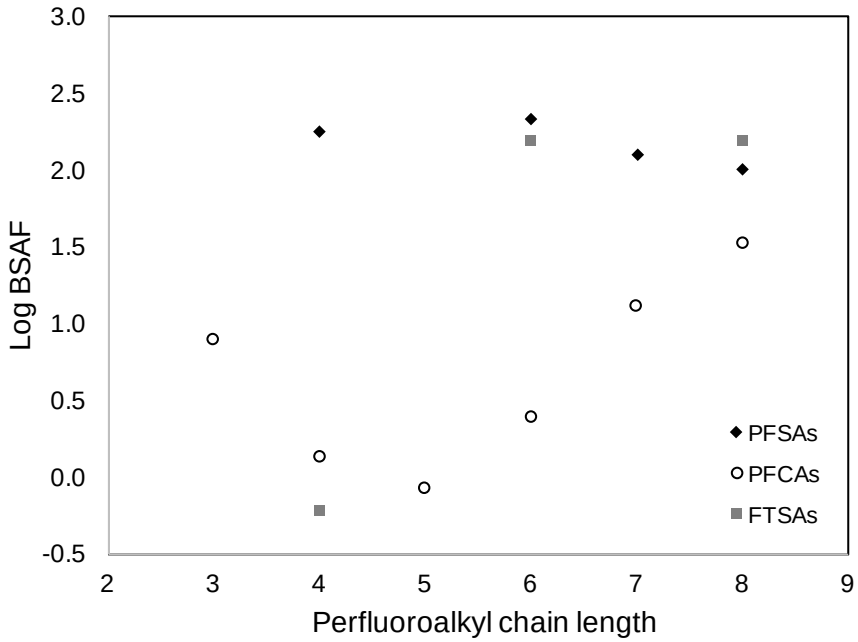

Figure 2 Relationship between perfluoroalkyl chain length and log of bioaccumulation factor (BSAF) of MC non-amended soils for three PFAS classes spiked at 100 ng/g d.w. (individual analyte concentration).

BSAFs of PFOS in MC and CAN 1 soil with 4% sorbent amendment were also calculated to determine rough estimates of BSAF for the abundant PFAS using Day 28 soil concentrations. In the MC spiked soils, initial concentrations of PFOS at ~ 100 ng/g resulted in BSAF values of 1.1 and 2.3 with a 4% amendment of F400 AC and FS100

clay, respectively. In the contaminated CAN 1 soil, an initial concentration of 44930 ng/g PFOS with a 4% amendment of F400 AC and FS100 clay resulted in BSAF values of 0.3 and 0.7, respectively. The BSAF concentration dependency on soil contaminant concentrations can be observed in the conditions where highly contaminated soils resulted in lower BSAF values. BSAFs of PFOS in both MC and CAN 1 soils amended with F400 were approximately 2-fold less than the soils amended with FS100.

A study by Bräunig et al. (2016) studied the effect of the addition of a commercial adsorbent to AFFF-impacted soils on bioaccumulation in earthworms. At a concentration of 25% of the sorbent, initial PFOS concentrations of 2193 ng/g and 13,362 ng/g resulted in approximate BSAFs of 0.03 and 0.1, respectively [32]. The concentration dependency phenomenon does not appear to apply with the addition of amendments in this case as a large increase in initial soil concentrations led to greater BSAFs. The BSAFs of PFOS are also less than those of this study. However, amendments in high concentrations should be applied with caution due to potential risks to bacteria and invertebrates [124, 125].

4.4 Effect of sorbent concentration and type on the uptake by earthworms

By the end of the uptake phase (Day 28), the application of both F400 AC and FS100 clay achieved reductions in earthworm body burdens (>80% total PFAS) in the MC soil. The application of FS100 clay to the MC soil at a concentration rate of 0.5%, 1%, and 4% resulted in total PFAS body burden reductions of 90, 92, and 97%, respectively, while the application of F400 activated carbon resulted in a reduction of 80, 92 and 99% of total PFAS, respectively. As displayed in **Figure 3**, increased amendment concentrations led to reduced earthworm body burdens of individual analytes in the MC soils, with 4 % being the most effective. The amendment concentration-response of the remaining analytes not commonly detected in AFFF-contaminated sites can be seen in **Figure S8**. The body burden trends with amendments generally followed the same trend as the BSAF without amendments.

Increasing sorbent concentrations in some cases did not result in decreased body burdens (see **Figure S9**). In F400 amended soils, a change in 1% to 4% led to slightly increased PFPeA, 0.5 to 1% led to increased PFHxA, 0.5 to 1 % resulted in little change in PFHpA, and 1 to 4% resulted in little change in 4:2 FTSA. FS100 clay amendment resulted in higher burdens of 4:2 FTSA at 4% relative to 1%, and little to no change was observed with the increase from 0.5% to 1% for 6:2 and 8:2 FTSA.

Comparable reductions in earthworm body burdens were achieved for most analytes at a 4% concentration of F400 and FS100 in the MC soil, yet F400 performed slightly better. Select analytes – expected to be abundant in contaminated sites – including PFOS, PFOA, and PFHxS, exhibited similar body burden reductions in the MC soil at the 4% sorbent concentration: 98%, 94%, and 99%, respectively, using FS100 and 99%, 98%, and 99%, respectively using F400. Similar body burden reductions were also observed for other analytes, including PFBS, PFHpS, PFHpA, and PFNA. FS100 performed least efficiently for 6:2 FTSA at 69% reduction of body burden in comparison with 92% with F400 at 4%.

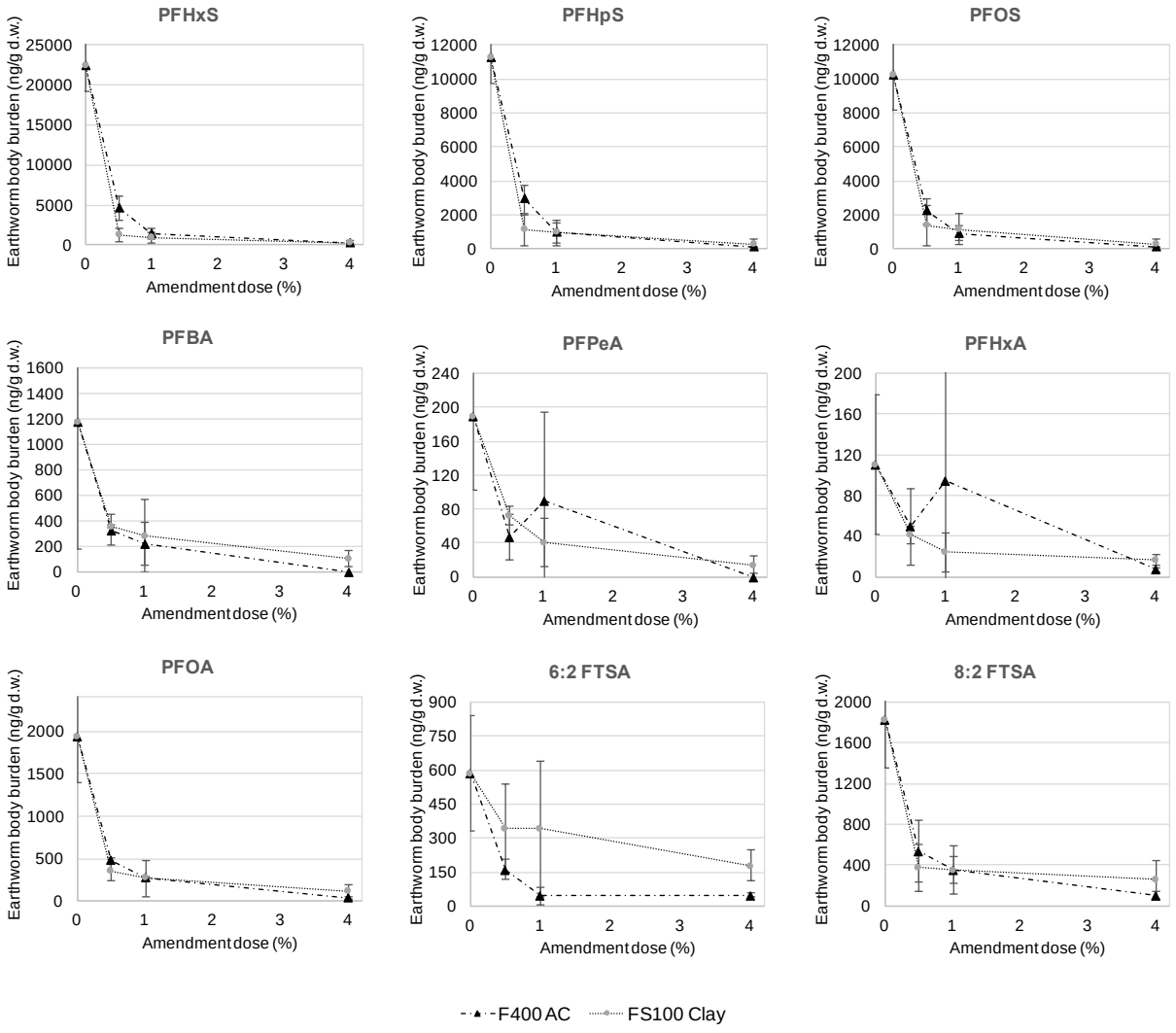

Figure 3 Earthworm body burden (ng/g d.w.) at the end of the uptake phase at different amendment concentrations of activated carbon (F400) and clay (FS100) for various PFAS analytes in MC soil. Error bars represent the standard deviation of triplicate samples.

In the CAN 1 soil, by Day 28, F400 AC resulted in greater body burden reductions for most of the abundant analytes (see **Table S14**). PFHxS, PFHpS, PFOS, PFOA, 6:2 FTSA, and 8:2 FTSA body burden concentrations with the F400 amendment were 1.4, 2.6, 3.7, 1.3, 5.0, and 3.3- fold less than those with the FS100 amendment. However, the PFHxA body burden with FS100 was 3.2-fold less than that with F400. At the greater soil PFAS concentrations, the difference in the sorbent effectiveness in reducing body burden was more apparent where the F400 performed better relative to FS100 for most analytes

(except PFHxA). This difference may be a result of the avoidance of larger particles by the earthworms and the greater accessibility of PFAS in the gut due to their hydrophilic groups. Additionally, interactions may have occurred between the surface-active agents in the gut and the surfactant intercalant used in the modified clay, leading the PFAS to become more bioavailable. Moreover, since deviations from the kinetic trend were observed in the FS100 amended CAN 1 soil, the Day 28 results may not be sufficient for a comparison of the effectiveness between the two sorbents.

4.5 Effect of sorbent concentrations on PFAS leaching

Leachate values from subsampled soils on Day 28 were obtained and are reported in **Table S15**. Soil leachate values in non-amended MC soil matched sorption trends in literature where longer chain PFAAs sorb more strongly to soil [73] – observed by normalizing leachate values to soil concentrations at the end of the uptake phase. The trend for FTSAs is difficult to discern due to soil transformations, although previous study show increased $\log K_d$ from 6:2 FTSA to 8:2 FTSA meaning the latter is more strongly associated with soil [126], which matches the findings in this study. Leachability data for non-amended CAN 1 soils can be seen in a previous study [22].

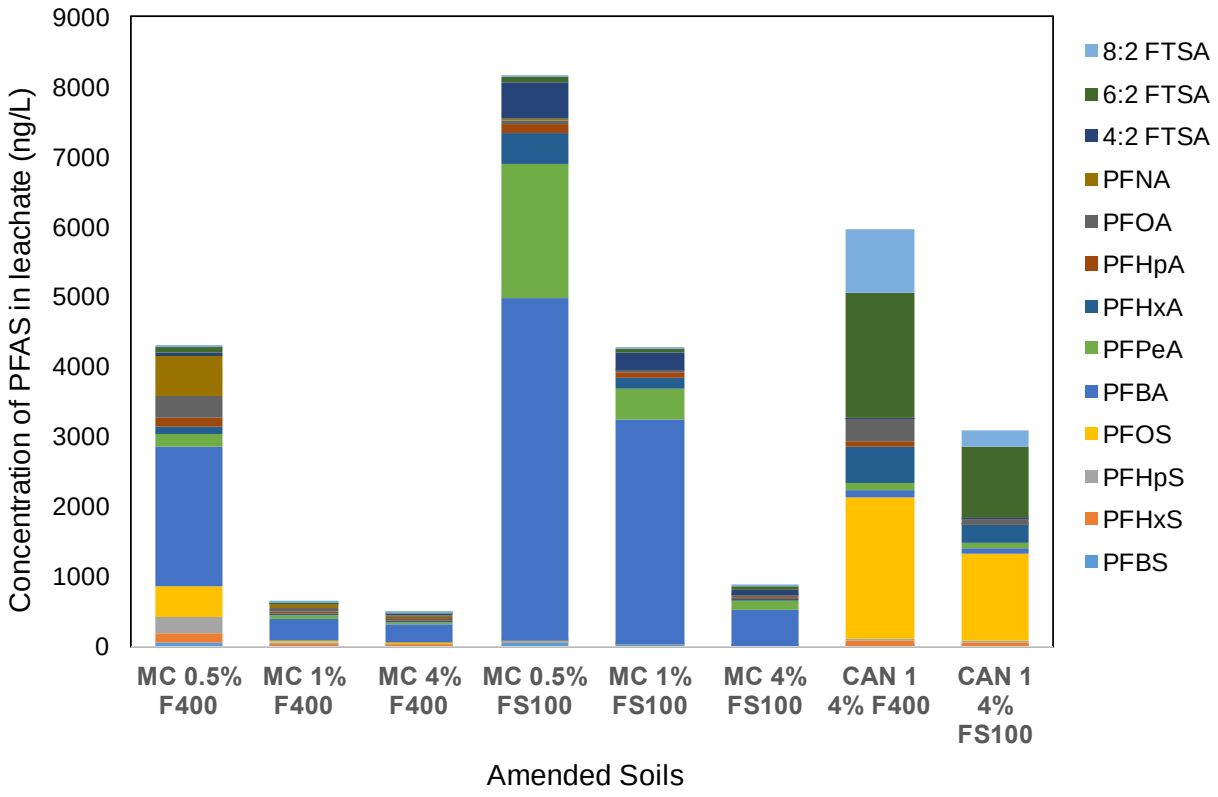

Figure 4 Comparison of PFAS soil concentrations in leachate (ng/L) between the amended MC and CAN 1 soils with activated carbon F400 and clay FS100.

PFAS leachate concentrations in amended MC and CAN 1 soils are presented in **Figure 4**. Reductions of individual PFAS analytes in the amended soils can be seen in **Figure 5** and **Figure S10**. Adding sorbents reduced the total PFAS leachate concentrations in the MC soil by >92%. Increased sorbent concentration resulted in greater reduction of total PFAS where a 4% concentration achieved >98% reduction using both sorbents. Total leachate reductions were comparable between both sorbents at the 4% concentration in the MC soil. While F400 resulted in greater leachate reductions of total PFAS concentrations in the MC soil, FS100 performed better relative to F400 in the CAN 1 soil.

A 4% amendment of FS100 achieved greater leachate reductions for most analytes relative to F400 in CAN 1 soil. This also matches results from previous findings for amended CAN 1 soil with 0.5% of FS109 [22]. Another study by Hale et al. (2016)

employed a batch leach test and observed similar extents of body burden reduction, 94% to > 99%, of total PFAS (11 analytes) using an amendment of 3% activated carbon in an AFFF-impacted soil [17].

The FS100 clay was more effective than F400 at reducing leachate concentrations of the long-chain PFSAs (C_6-C_8) and PFCAs (C_6-C_8), and 8:2 FTSA in both MC and CAN1 soils. However, comparable reductions were achieved for individual analytes at the 4% sorbent concentration in MC soil. F400 was more effective than FS100 for the short-chain PFAAs and 6:2 FTSA in the spiked MC soil but less effective than FS100 in the contaminated CAN 1 soil. Soil properties and potential preferential sorption mechanisms play a role in the extent of sorption of short and long-chain compounds, resulting in the different effectiveness of FS100 in the two soils. Sorbent surface chemistry and physical properties dictate adsorption. Sorption mechanisms of PFAS on AC have been explained by mainly hydrophobic and electrostatic interactions. AC's high surface area is also important in its effectiveness. The modified clay consists of exchangeable interlayer sites of 2:1 type Na-bentonite where quaternary ammonium surfactants have been intercalated. A recent study elucidated the dominant interactions in aqueous solution as electrostatic (between negatively charged PFAS head groups and intercalated cations), fluorophilic (lateral interaction among PFAS), and hydrophobic (between the PFAS C-F chain and intercalant hydrocarbon chain) [21].

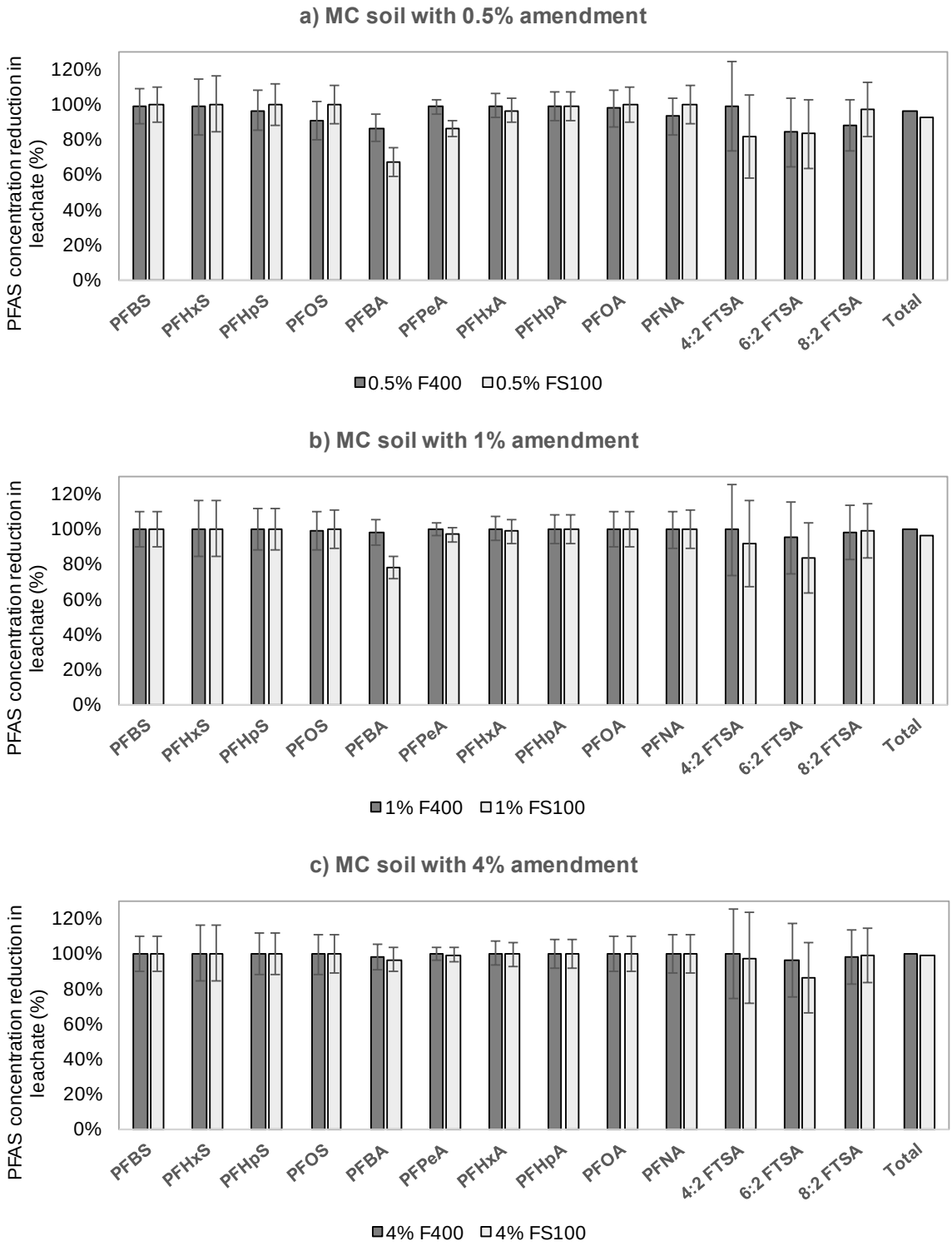

Figure 5 Percentage reduction of PFAS in leachate in MC soil with **a)** 0.5%, **b)** 1%, and **c)** 4% amendment concentration. Error bars represent the standard deviation of triplicate samples.

4.6 Relationship between sorbent concentration, leaching, and earthworm uptake

If the main exposure pathway to biota is via the aqueous phase, pore water measurements would be useful for estimating bioavailability. There are four basic methods of determining the levels of contaminants in soil pore water, (1) high-speed centrifugation-filtration, (2) low (negative-)-pressure Rhizon™ samplers, (3) high-pressure soil squeezing, and (4) equilibration of dilute soil suspensions [127]. As earthworms resided in unsaturated soils, the first and three methods would be appropriate ways to determine the PFAS in pore water to determine what the earthworms were exposed to. However, as the apparatus required for the first three measurements were not available; therefore, method (4) was used instead to determine the levels of leachable PFAS in dilute soil suspensions. Method (4) is susceptible to the soil:water ratio, so the modified EPA method 1311 was used in the study as the soil:water ratio is fixed and allows comparison to other studies. Strictly speaking, the leachability measurements do not consider exposure processes, but still give a reasonable prediction of what could be in pore water.

In **Figure S11**, the logarithm of the earthworm body burdens is plotted against the logarithm of PFAS in soil leachates per chemical class for the MC soil. Strong positive log-log relationships were observed for most individual analytes and also for both amendments (R^2 values in **Table S16**). The lines were better fitted to the FS100 data where R^2 values were >0.85 for all analytes except 6:2 FTSA. The R^2 values for the F400 data were >0.78 for all analytes except PFPeA, PFHxA, and PFHpA, which had lower values. Several data points may have been skewed due to soil transformations, the contribution of other exposure mechanisms, and experimental artefacts; however, overall as leachability decreases with increasing amendment concentration, earthworm concentrations decreased at a consistent rate. The slopes of individual analytes show a potential relationship between the two parameters. While leachate concentrations alone do not explain the extent of earthworm uptake of individual analytes, PFAS in both leachate and earthworms decreased with amendment concentration at similar rates.

Chapter 5 Conclusion and Summary

The study evaluated the bioavailability of PFAS to earthworms (*Eisenia fetida*) in PFAS-spiked and AFFF-impacted soils amended with different concentrations of activated carbon (F400) and a proprietary modified clay (FS100). Such data are necessary for assessing the advantages and limitations of implementing *in situ* soil stabilization, which is expected to be applied on a large scale in the future to reduce the environmental and ecological risk of AFFF-impacted sites. Bioaccumulation, as a measure of bioavailability, is an important and useful tool for a risk-based site assessment that encompasses eco-toxicological risks. PFAS under evaluation included perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFSAs), and fluorotelomer sulfonates (FTSAs), which are the most dominant PFAS observed in the environment.

In the experimental soils, both activated carbon and the modified clay reduced earthworm bioaccumulation of most of the PFAS at an amendment concentration as low as 0.5%. In the PFAS-spiked soil that simulates a moderately contaminated scenario, the highest sorbent concentration applied (4%) reduced the PFAS earthworm body burden (total PFAS) by 99% and 97% with the activated carbon and clay, respectively. In the field contaminated soil, the activated carbon also resulted in lower earthworm body burdens for most individual analytes than those with the clay amendment. The slightly better performance of activated carbon was suspected to be attributed to the difference in PFAS exposure routes. Based on the absence of black particles in the depurated gut content, it is hypothesized that earthworms avoided the ingestion of activated carbon particles but did not avoid the clay particles. Thus, earthworm gut exposure likely contributed to the greater desorption of PFAS from the smaller sized clay particles. Although the different uptake kinetics supports the hypothesis, further studies are necessary. The data collected also allowed the determination of the bioaccumulation factors for each PFAS. The lack of chain length dependency in PFSAs on earthworm bioaccumulation, consistent with the literature, is still unclear and must be further studied.

The parallel PFAS leachability showed that the 4% amendment was most effective in reducing PFAS leaching, and comparable total PFAS leachate reductions (>99%) were

observed for the two sorbents. The amendment as low as 0.5% achieved effective immobilization with >95% reductions of total PFAS in leachate. The modified clay was more effective at immobilizing long-chain PFAS in both spiked and contaminated soils. The activated carbon performed better for short-chain compounds only in the spiked soil, but not in the contaminated soil. Differences in the performance of the sorbents for long vs. short-chain compounds in the two soils are suggestive of the occurrence of preferential sorption mechanisms in the soils. The results were consistent with a prior study that shows the modified clay, in general, excelled at reducing PFAS leaching. Given that the modified clays still have not been extensively tested as a sorbent for PFAS, the use of this new material must be tested in various soil profiles to validate superior effectiveness over activated carbon. Reductions in PFAS leachate concentration and earthworm body burdens follow a log-log relationship, suggesting the leachate concentration may proportionally approximate the PFAS pore water concentration.

The results suggest that for future field applications, activated carbon and the modified clay each have certain advantages but also limitations. A single mortality was observed in the amended soils, but future studies are necessary to assess potential toxicity to earthworms, especially for higher amendment concentration (e.g., it is common to see 5 ~10% in field applications). Additionally, the environmental conditions that result in greater absorption of PFAS in earthworms or biota must be better understood. The study also raised additional questions that are worthy of future investigations. 1) It is important to understand how the biotransformation of PFAS carried out by indigenous microorganisms affects the estimation of bioaccumulation potential in field studies. AFFF-impacted sites could be highly concentrated with the polyfluorinated PFAS that are prone to partial breakdown. 2) Steady-state of PFAS concentrations in the earthworms could not be confirmed due to limited time points; however, large fluctuations that occurred suggest that 28 days might not be sufficient to observe steady-state in earthworms following sorbent amendment. 3) Long-term effects of using either sorbent to reduce PFAS leachability and bioavailability in contaminated soils require thorough evaluations.

Supplementary Information

Materials and methods

Spike recovery procedure for soil and earthworms

The spike recovery was determined according to the protocol of Munoz et al. (2018) using the extraction methods of Munoz et al. (2018) for soil and Munoz et al. (2020) for earthworms. The MC soil was used to evaluate the soil spike recovery.

Individual freeze-dried earthworm (~ 40 mg d.w.) and soil homogenates (1 g d.w.) were placed in 15 mL PP tubes (both prepared in triplicates). The samples were spiked with 10 ng of the PFAS mixture (50 uL of a 200 ng/mL methanolic solution), lightly vortexed, equilibrated overnight, and subjected to extractions specified in the Methodology section. These are the “spiked before” (SB) samples. Additional “spiked after” (SA) and “non-spiked” (NS) were prepared in parallel (in triplicates for soil and earthworms). SA samples were prepared by spiking the soil or earthworm homogenates with 50 uL MeoH, subjecting the samples to extractions, and spiking them with 10 ng of the PFAS mixture at the end. NS samples were prepared by spiking the homogenates with 50 uL MeOH before and after the extraction procedure. Finally, all the samples were lightly vortexed and a subsample of the extract solution was aliquoted and combined with internal standards. The percent of PFAS recovered was calculated according to the following equation:

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

where the values of SB, SA, and NS represent the native analyte to internal standard ratio of their respective samples.

Table S1 List of PFAS used.

Acronym	Name	Formula	m/z
PFBS	Perfluorobutane sulfonate	$C_4F_9SO_3^-$	298.94326
PFHxS	Perfluorohexane sulfonate	$C_6F_{13}SO_3^-$	398.93712
PFHpS	Perfluoroheptane sulfonate	$C_7F_{15}SO_3^-$	448.93286
PFOS	Perfluorooctane sulfonate	$C_8F_{17}SO_3^-$	498.93126
PFBA	Perfluorobutanoate	$C_3F_7COO^-$	212.97947
PFPeA	Perfluoropentanoate	$C_4F_9COO^-$	262.97669
PFHxA	Perfluorohexanoate	$C_5F_{11}COO^-$	312.97335
PFHpA	Perfluoroheptanoate	$C_6F_{13}COO^-$	362.97013
PFOA	Perfluorooctanoate	$C_7F_{15}COO^-$	412.96714
PFNA	Perfluorononanoate	$C_8F_{17}COO^-$	462.96414
4:2 FTSA	4:2 Fluorotelomer sulfonate	$C_4F_9(CH_2)_2SO_3^-$	326.97374
6:2 FTSA	6:2 Fluorotelomer sulfonate	$C_6F_{13}(CH_2)_2SO_3^-$	426.96866
8:2 FTSA	8:2 Fluorotelomer sulfonate	$C_8F_{17}(CH_2)_2SO_3^-$	526.96097
6:2 FTAB	Fluorotelomer sulfonamide alkylbetaine	$[C_{15}F_{13}H_{20}N_2SO_4]^+$	571.09362

Table S2 List of mass labeled internal standards used.

Acronym 1	Acronym 2	Name	m/z
$^{13}C_4$-PFBA	MPFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	216.99177
$^{13}C_5$-PFHxA	MPFHxA	Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	317.99046
$^{13}C_8$-PFOA	MPFOA	Perfluoro-n-[$^{13}C_8$]octanoic acid	420.99272
$^{13}C_9$-PFNA	MPFNA	Perfluoro-n-[$^{13}C_9$]nonanoic acid	471.99288
$^{13}C_3$-PFHxS	MPFHxS	Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	401.94612
$^{13}C_8$-PFOS	MPFOS	Perfluoro-1-[$^{13}C_8$]octanesulfonate	506.95641
$^{13}C_2$-6:2 FtSA	M6:2 FTSA	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	428.97537
$^{13}C_2$-8:2 FtSA	M8:2 FTSA	1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	528.96898

Table S3 Additional soil properties of collected MC soil and AFFF-contaminated CAN 1 soil.

ID.	Element Composition, ppm						Percent Base Saturation, %					K/Mg Ratio	Saturation P, %	Saturation Al, %	Water Holding Capacity at 1/3 bar, %
	P	K	Mg	Ca	Na	Al	K	Mg	Ca	H	Na				
CAN 1	8	20	110	6250	92	33	0.2	2.8	95.8	-	1.2	0.07	1	0	
MC	42	86	251	1730	29	1111	1.9	18	74.5	4.5	1.1	0.11	5	0.1	14.8

Table S4 Instrumental method details.

Instrument	Shimadzu Nexera ultra-high-performance liquid chromatography system coupled to an AB Sciex 5500 Qtrap mass spectrometer
Software	The Dionex Ultimate 3000 LC (Chromeleon 7.2) coupled with Q-Exactive Orbitrap mass spectrometer (Xcalibur 2.3)
Ionization	Heated electrospray ionization source; polarity-switching mode
Acquisition mode	Scheduled multiple reaction monitoring (MRM)
Analytical column	Thermo Hypersil Gold C18 column (100 mm × 2.1 mm; 1.9 µm particle size)
Delay column	Thermo Hypercarb column (20 mm × 2.1 mm; 7 µm particle size)
Column Temperature	40°C
Mobile Phases	A: 0.1% formic acid in HPLC-water B: 0.1% formic acid in LCMS-acetonitrile Flow rate 0.55 mL/min
Gradient Profile	Time (min) % B 0.0 10 7.0 72.5 8.5 100 12.5 100 12.6 10 15.50
Injection Volume	7 µL
Source/ gas	Sheath gas flow rate 40 arbitrary units (a.u.) Aux gas flow rate 15 a.u. Sweep gas flow rate 0 a.u. Capillary temperature 320 °C Vaporizer temperature 350 °C Spray Voltage either -4 kV or +4 kV (fast polarity-switching mode)
Orbitrap parameters	Resolution 70,000 at 200 m/z AGC target 3e6 Maximum Inject Time 50 ms Scan range 150–1000 m/z
Calibration	Linear regression, inverse weighing (1/x)

Table S5 LC-MS transitions, ionization mode, and retention times of native and mass-labeled PFAS analytes.

Compound	Ionization mode	Transition	Qualifying Transition	RT (min)	Internal Standard	Internal Standard Transition	RT (min)
PFBS	–MRM	299 > 80	299 > 99	4.61	MPFHxS	403 > 103	6.15
PFHxS	–MRM	399 > 80	399 > 99	6.15	MPFHxS	403 > 103	6.15
PFHpS	–MRM	499 > 80	499 > 99	6.83	MPFOS	503 > 80	7.51
PFOS	–MRM	549 > 80	549 > 99	7.51	MPFOS	503 > 80	7.51
PFBA	–MRM	213 > 169		2.6	MPFBA	217 > 172	2.6
PFPeA	–MRM	263 > 219		3.72	MPFHxA	315 > 270	4.58
PFHxA	–MRM	313 > 269	313 > 119	4.58	MPFHxA	315 > 270	4.58
PFHpA	–MRM	363 > 319	363 > 169	5.31	MPFOA	417 > 372	5.99
PFOA	–MRM	413 > 369	413 > 169	5.99	MPFOA	417 > 372	5.99
PFNA	–MRM	463 > 419	463 > 219	6.67	MPFNA	468 > 423	6.67
4:2 FTSA	–MRM	327 > 80	327 > 307	4.27	M6:2 FTSA	429 > 81	5.64
6:2 FTSA	–MRM	427 > 80	427 > 407	5.64	M6:2 FTSA	429 > 81	5.64
8:2 FTSA	–MRM	527 > 80	527 > 507	6.94	M8:2 FTSA	529 > 81	6.94

Table S6 Concentration of PFAS in soils (ng/g d.w.) before ageing and the start of bioaccumulation experiments.

	PFAS initial concentration in soils (ng/g d.w.) prior to amendment									
	MC worm control	**MC 0%**	**MC 0.5% F400**	**MC 1% F400**	**MC 4% F400**	**MC 0.5% FS100**	**MC 1% FS100**	**MC 4% FS100**	**CAN 1 4% FS100**	**CAN 1 4% F400**
PFBS	97 ± 2.2	95 ± 3.8	100 ± 3.5	104 ± 4.9	113 ± 14	94 ± 5.4	96 ± 7.4	103 ± 10	9.3 ± 1.6	8.5 ± 1.6
PFHxS	115 ± 0.6	114 ± 4.6	121 ± 8.2	135 ± 6.8	125 ± 10	114 ± 11	112 ± 8	122 ± 13	455 ± 44	427 ± 39
PFHpS	89 ± 1.4	85 ± 0.1	94 ± 6.3	104 ± 0.7	102 ± 1.4	96 ± 16	93 ± 6.4	91 ± 4.9	72 ± 4.2	75 ± 0.4
PFOS	100 ± 0.4	99 ± 4.3	109 ± 8.3	116 ± 2.2	114 ± 3.6	111 ± 16	109 ± 12	107 ± 2.1	44740 ± 865	45120 ± 22
PFBA	138 ± 7.8	142 ± 5.4	143 ± 0.3	130 ± 8.3	144 ± 0.7	133 ± 1.2	137 ± 11	142 ± 8.3	12 ± 0.3	11 ± 0.9
PFPeA	117 ± 0.8	121 ± 3.1	124 ± 3	115 ± 0.8	121 ± 11	109 ± 1.6	117 ± 7.4	117 ± 7.0	30 ± 0.3	29 ± 1.0
PFHxA	130 ± 4.9	137 ± 7.2	132 ± 5.3	139 ± 1.2	137 ± 15	133 ± 6.8	135 ± 8.7	137 ± 16	183 ± 9.4	177 ± 5
PFHpA	117 ± 1.3	128 ± 4.0	120 ± 0.2	136 ± 3.7	147 ± 7	127 ± 3.3	142 ± 8.9	139 ± 17	16 ± 0.5	14 ± 1.9
PFOA	121 ± 11	132 ± 2.6	133 ± 6	143 ± 8.7	134 ± 20	136 ± 8.0	128 ± 2.4	133 ± 14	78 ± 3.6	85 ± 0.2
PFNA	122 ± 5.0	117 ± 6.1	128 ± 14	138 ± 3.7	142 ± 1.4	132 ± 12	125 ± 8.1	139 ± 18	12 ± 0.1	11 ± 0.2
4:2 FTSA	86 ± 3.1	81 ± 8.4	91 ± 8.3	103 ± 4.9	112 ± 0.0	98 ± 10	104 ± 6.1	99 ± 8.6	2.8 ± 0.1	2.6 ± 0.5
6:2 FTSA	70 ± 5.7	64 ± 3.2	81 ± 1.1	90 ± 8	99 ± 8.4	84 ± 6.1	85 ± 1.5	86 ± 11	573 ± 39	559 ± 45
8:2 FTSA	82 ± 2.5	79 ± 3.4	89 ± 0.1	88 ± 2.2	93 ± 9.3	87 ± 7.4	87 ± 4	84 ± 14	1059 ± 49	1038 ± 2.9

Table S7 Concentration of PFAS in soils (ng/g d.w.) from individual vessels at the end of the 28-day bioaccumulation uptake phase.

PFAS concentrations in soils at the end of the uptake phase (ng/g d.w.)

	MC worm control	MC 0%	MC 0.5% F400	MC 1% F400	MC 4% F400	MC 0.5% FS100	MC 1% FS100	MC 4% FS100	CAN 1 4% FS100	CAN 1 4% F400
PFBS	111 ± 7.2	96 ± 11	78 ± 52	119 ± 19	100 ± 7.6	122 ± 6.6	117 ± 11	145 ± 35	12 ± 1.1	4.7 ± 0.5
PFHxS	127 ± 4.5	104 ± 5.3	82 ± 43	108 ± 14	97 ± 8.3	132 ± 12	128 ± 8.4	145 ± 27	501 ± 95	287 ± 5.2
PFHpS	97 ± 4	90 ± 4.1	67 ± 21	75 ± 9.4	70 ± 6.8	103 ± 3.8	107 ± 2.9	102 ± 7.6	70 ± 6.3	43 ± 2.5
PFOS	111 ± 7.1	102 ± 1.5	84 ± 21	90 ± 6.7	77 ± 7.3	114 ± 5.2	118 ± 1.1	101 ± 7	43147 ± 1989	27751 ± 1245
PFBA	154 ± 14	151 ± 3.8	86 ± 57	138 ± 15.9	128 ± 20	165 ± 28	127 ± 13	121 ± 1.7	12 ± 0.3	10 ± 0.6
PFPeA	151 ± 14	142 ± 4.4	83 ± 53	124 ± 14	108 ± 18	155 ± 12	126 ± 5.1	126 ± 16	33 ± 2.4	26 ± 1.0
PFHxA	137 ± 7.7	136 ± 3.9	93 ± 58	132 ± 8.5	117 ± 17	155 ± 7.1	138 ± 6.7	130 ± 3.2	198 ± 19	229 ± 11
PFHpA	133 ± 8	139 ± 3.5	89 ± 42	131 ± 16	121 ± 21	160 ± 17	128 ± 1.4	150 ± 11	22 ± 1.6	17 ± 0.5
PFOA	138 ± 9.1	150 ± 1.7	98 ± 35	124 ± 4.8	116 ± 15	154 ± 9.1	136 ± 2	153 ± 6.2	94 ± 3.9	77 ± 4.8
PFNA	137 ± 16	134 ± 8.1	107 ± 30	117 ± 11	112 ± 11	141 ± 5.7	144 ± 1.6	146 ± 10	13 ± 0.5	9 ± 0.7
4:2 FTSA	49 ± 7.2	37 ± 4.3	44 ± 29	74 ± 14	75 ± 15	83 ± 11	84 ± 5.2	90 ± 2.9	4.3 ± 0.5	2.8 ± 0.2
6:2 FTSA	8 ± 2.3	3.9 ± 0.4	18 ± 8.7	35 ± 5.1	50 ± 10	30 ± 2.7	41 ± 4.1	63 ± 4.1	814 ± 48	658 ± 67
8:2 FTSA	20.9 ± 2.9	12 ± 1.3	19 ± 8.7	33 ± 5.2	47 ± 8.7	44 ± 3.4	51 ± 3.3	68 ± 3.5	1139 ± 57	782 ± 56

Table S8 Earthworm body burden (ng/g d.w.) of PFAS in non-amended and amended MC soil at Day 3 of the uptake phase.

Earthworm PFAS body burdens in MC soils including standard deviation

	0%	0.5%		1%		4%	
		F400	FS100	F400	FS100	F400	FS100
PFBS	5001 ± 1819	458 ± 11	313 ± 97	437 ± 17	402 ± 128	127 ± 23	94 ± 69
PFHxS	4282 ± 1265	763 ± 173	283 ± 65	489 ± 65	350 ± 108	116 ± 20	95 ± 68
PFHpS	1687 ± 404	517 ± 139	180 ± 22	316 ± 43	269 ± 79	60 ± 12	59 ± 47
PFOS	1637 ± 369	612 ± 199	219 ± 27	380 ± 56	278 ± 74	69 ± 18	80 ± 59
PFBA	442 ± 136	179 ± 92	119 ± 58	205 ± 96	127 ± 23	54 ± 31	34 ± 0.15
PFPeA	278 ± 158	51 ± 36	62 ± 38	48 ± 45	36 ± 25	19 ± 15	16 ± 4.3
PFHxA	207 ± 174	40 ± 20	43 ± 30	21 ± 25	21 ± 14	17 ± 12	18 ± 5.6
PFHpA	241 ± 151	92 ± 15	47 ± 34	52 ± 37	45 ± 4.0	30 ± 1.7	28 ± 0.33
PFOA	703 ± 274	238 ± 89	94 ± 24	127 ± 59	153 ± 66	49 ± 18	44 ± 11
PFNA	1217 ± 342	482 ± 185	162 ± 34	287 ± 62	264 ± 93	62 ± 18	70 ± 36
4:2 FTSA	58 ± 32	12 ± 1.1	28 ± 5.9	21 ± 12	31 ± 19	NA	24 ± 18
6:2 FTSA	208 ± 50	87 ± 23	90 ± 3	94 ± 16	201 ± 24	39 ± 14	61 ± 49
8:2 FTSA	541 ± 157	223 ± 66	136 ± 51	184 ± 43	247 ± 128	143 ± 67	249 ± 185

NA refers to sample concentration values below detection limits

Table S9 Earthworm body burden (ng/g d.w.) of PFAS in non-amended and amended MC soil at Day 7 of the uptake phase.

Earthworm PFAS body burdens in MC soils including standard deviation

	0%	0.5%		1%		4%	
		F400	FS100	F400	FS100	F400	FS100
PFBS	7704 ± 3500	2900 ± 734	7245 ± 10371	777 ± 239	1409 ± 1020	253 ± 56	442 ± 150
PFHxS	7380 ± 1425	4195 ± 428	3877 ± 4821	899 ± 311	1089 ± 813	190 ± 10	261 ± 108
PFHpS	3974 ± 16	2119 ± 368	1731 ± 1902	457 ± 84	799 ± 636	96 ± 14	171 ± 90
PFOS	4828 ± 1709	1848 ± 528	1750 ± 1690	467 ± 74	926 ± 814	103 ± 12	176 ± 81
PFBA	799 ± 5.7	402 ± 263	309 ± 87	139 ± 90	297 ± 69	42 ± 12	109 ± 59
PFPeA	231 ± 73	117 ± 70	115 ± 59	46 ± 55	63 ± 11	NA	23 ± 3.9
PFHxA	137 ± 149	122 ± 14	86 ± 73	58 ± 66	28 ± 1.3	NA	19 ± 5.0
PFHpA	349 ± 274	327 ± 119	216 ± 180	93 ± 67	112 ± 16	19 ± 10	46 ± 21
PFOA	1040 ± 353	641 ± 53	536 ± 412	180 ± 105	264 ± 105	22 ± 14	98 ± 64
PFNA	2272 ± 102	1252 ± 400	1195 ± 1006	310 ± 124	558 ± 363	39 ± 15	184 ± 108
4:2 FTSA	133 ± 87	31 ± 9.3	38 ± 14	13 ± 2.7	321 ± 317	23 ± 18	147 ± 140
6:2 FTSA	513 ± 94	196 ± 100	342 ± 98	73 ± 36	375 ± 249	65 ± 10	158 ± 72
8:2 FTSA	974 ± 112	507 ± 199	572 ± 403	197 ± 45	336 ± 264	93 ± 23	159 ± 43

NA refers to sample concentration values below detection limits

Table S10 Earthworm body burden (ng/g d.w.) of PFAS in non-amended and amended MC soil at Day 14 of the uptake phase.

Earthworm PFAS body burdens in MC soils including standard deviation

	0%	0.5%		1%		4%	
		F400	FS100	F400	FS100	F400	FS100
PFBS	16009 ± 1906	1861 ± 327	1584 ± 556	1708 ± 404	853 ± 247	168 ± 49	428 ± 282
PFHxS	13477 ± 2999	4126 ± 388	2255 ± 695	2030 ± 356	1082 ± 333	199 ± 37	402 ± 293
PFHpS	5955 ± 1907	1945 ± 148	2220 ± 852	1007 ± 138	952 ± 292	90 ± 18	329 ± 263
PFOS	4843 ± 1830	1515 ± 175	2548 ± 1112	856 ± 129	1059 ± 321	89 ± 27	313 ± 239
PFBA	1429 ± 831	360 ± 278	658 ± 307	360 ± 241	128 ± 55	47 ± 32	92 ± 11
PFPeA	474 ± 89	NA	101 ± 28	56 ± 31	24 ± 2.3	NA	22 ± 10
PFHxA	261 ± 119	15 ± 5.6	43 ± 20	33 ± 24	12 ± 6	8.5 ± 2	25 ± 14
PFHpA	350 ± 177	50 ± 19	116 ± 90	63 ± 15	37 ± 8	11 ± 10	44 ± 38
PFOA	954 ± 326	216 ± 168	574 ± 287	193 ± 83	195 ± 87	27 ± 24	185 ± 129
PFNA	2285 ± 1200	460 ± 279	1275 ± 472	416 ± 206	508 ± 176	43 ± 25	368 ± 254
4:2 FTSA	35 ± 12	NA	73 ± 36	NA	181 ± 148	16 ± 3.5	40 ± 25
6:2 FTSA	466 ± 287	223 ± 32	702 ± 165	126 ± 65	401 ± 114	50 ± 16	273 ± 149
8:2 FTSA	1011 ± 282	474 ± 92	638 ± 209	364 ± 134	321 ± 47	76 ± 14	344 ± 248

NA refers to sample concentration values below detection limits

Table S11 Earthworm body burden (ng/g d.w.) of PFAS in non-amended and amended MC soil at Day 21 of the uptake phase.

Earthworm PFAS body burdens in MC soils including standard deviation

	0%	0.5%		1%		4%	
		F400	FS100	F400	FS100	F400	FS100
PFBS	16144 ± 1874	2203 ± 440	1807 ± 276	981 ± 190	1680 ± 917	219 ± 120	369 ± 109
PFHxS	18074 ± 6015	5997 ± 1805	2207 ± 445	1558 ± 448	1534 ± 761	173 ± 94	220 ± 120
PFHpS	9333 ± 5014	3254 ± 1007	2090 ± 357	1063 ± 302	1518 ± 852	75 ± 37	161 ± 89
PFOS	8101 ± 5777	2281 ± 556	2439 ± 409	1090 ± 392	1827 ± 985	72 ± 30	180 ± 94
PFBA	1655 ± 735	290 ± 139	521 ± 308	215 ± 94	455 ± 369	86 ± 61	161 ± 68
PFPeA	337 ± 100	56 ± 27	120 ± 29	130 ± 152	75 ± 48	NA	63 ± 51
PFHxA	156 ± 43	52 ± 6.2	81 ± 20	133 ± 162	63 ± 23	14 ± 9.4	80 ± 74
PFHpA	365 ± 136	146 ± 50	232 ± 38	130 ± 112	207 ± 103	24 ± 20	100 ± 63
PFOA	1797 ± 1445	636 ± 230	770 ± 97	169 ± 80	583 ± 328	37 ± 39	168 ± 93
PFNA	4304 ± 3615	1266 ± 229	1569 ± 225	388 ± 107	1111 ± 575	42 ± 32	226 ± 121
4:2 FTSA	82 ± 44	14 ± 4.7	56 ± 33	60 ± 83	63 ± 25	15 ± 12	47 ± 53
6:2 FTSA	747 ± 590	204 ± 54	733 ± 18	110 ± 18	540 ± 199	43 ± 4.3	120 ± 45
8:2 FTSA	2085 ± 1741	517 ± 27	608 ± 87	252 ± 66	560 ± 280	73 ± 28	246 ± 166

NA refers to sample concentration values below detection limits

Table S12 Earthworm body burden (ng/g d.w.) of PFAS in non-amended and amended MC soil at Day 28 of the uptake phase.

Earthworm PFAS body burdens in MC soils including standard deviation

	0%	0.5%		1%		4%	
		F400	FS100	F400	FS100	F400	FS100
PFBS	16746 ± 986	1685 ± 306	862 ± 518	881 ± 237	658 ± 405	229 ± 61	273 ± 292
PFHxS	22328 ± 3122	4582 ± 1485	1255 ± 788	1444 ± 635	924 ± 688	180 ± 71	260 ± 313
PFHpS	11218 ± 1493	2921 ± 839	1110 ± 914	927 ± 564	927 ± 766	93 ± 31	246 ± 308
PFOS	10174 ± 1997	2193 ± 761	1326 ± 1189	918 ± 438	1152 ± 931	84 ± 21	236 ± 302
PFBA	1176 ± 1000	329 ± 124	356 ± 13	220 ± 168	282 ± 284	NA	104 ± 59
PFPeA	190 ± 88	47 ± 26	73 ± 11	89 ± 104	41 ± 28	NA	14 ± 11
PFHxA	111 ± 68	49 ± 38	41 ± 8.4	94 ± 113	24 ± 19	7.0 ± 1.9	17 ± 5.7
PFHpA	334 ± 101	92 ± 32	126 ± 23	95 ± 50	80 ± 65	20 ± 11	33 ± 17
PFOA	1929 ± 534	482 ± 31	345 ± 101	266 ± 28	265 ± 220	42 ± 16	111 ± 86
PFNA	4417 ± 115	1163 ± 259	724 ± 402	497 ± 210	584 ± 482	62 ± 22	252 ± 221
4:2 FTSA	22 ± 21	7.1 ± 2.3	19 ± 13	NA	NA	NA	4.6 ± 2.2
6:2 FTSA	585 ± 254	161 ± 44	344 ± 196	44 ± 39	341 ± 299	48 ± 8	179 ± 69
8:2 FTSA	1827 ± 477	538 ± 303	379 ± 231	353 ± 133	358 ± 240	103 ± 37	258 ± 193

NA refers to sample concentration values below detection limits

Table S13 Biota-soil bioaccumulation factors derived for non-amended MC soil.

BSAF values ($g_{d.w.}$ earthworm / $g_{d.w.}$ soil)			
Perfluoroalkyl chain length	**PFSAs**	**PFCAs**	**FTSAs**
3		7.8 ± 6.6	
4	174 ± 22	1.3 ± 0.6	0.6 ± 0.6
5		0.8 ± 0.5	
6	214 ± 32	2.4 ± 0.7	151 ± 67
7	124 ± 17	13 ± 3.6	
8	100 ± 20	33 ± 2.2	153 ± 43

Table S14 Earthworm body burden concentrations at the end of the uptake phase in amended CAN 1 soil with 4% FS100 and 4% F400.

Earthworm body burden concentrations in amended CAN1 soil (ng/g d.w.)		
	4% FS100	**4% F400**
PFBS	68 ± 24	12 ± 1.2
PFHxS	293 ± 146	215 ± 81
PFHpS	26 ± 17	10 ± 4.9
PFOS	32283 ± 32822	8711 ± 3290
PFBA	NA	NA
PFPeA	NA	NA
PFHxA	11 ± 3.1	34 ± 36
PFHpA	NA	NA
PFOA	31 ± 18	24 ± 19
PFNA	15 ± 6.7	7.4 ± 4.8
4:2 FTSA	NA	NA
6:2 FTSA	1782 ± 270	353 ± 96
8:2 FTSA	2537 ± 488	762 ± 150

Table S15 Concentration of PFAS in leachate water (ng/L) taken from soils after the 28-day bioaccumulation uptake phase.

	PFAS leachate concentrations (ng/g d.w.)									
	MC worm control	**MC 0%**	**MC 0.5% F400**	**MC 1% F400**	**MC 4% F400**	**MC 0.5% FS100**	**MC 1% FS100**	**MC 4% FS100**	**CAN 1 4% FS100**	**CAN 1 4% F400**
PFBS	7880 ± 144	7573 ± 537	61 ± 4,0	13 ± 8.9	11 ± 13	53 ± 7.7	23 ± 3.9	5.9 ± 0.7	2.3 ± 0.4	1.2 ± 0.1
PFHxS	10187 ± 244	9067 ± 1022	119 ± 30	20 ± 14	16 ± 21	16 ± 6.0	7.9 ± 1.7	3.5 ± 0.1	67 ± 22	90 ± 9.0
PFHpS	7053 ± 311	6400 ± 525	235 ± 68	17 ± 13	12 ± 13	11 ± 2.8	4.5 ± 1.2	1.7 ± 0.7	2.4 ± 1.1	4.3 ± 0.4
PFOS	5307 ± 115	4667 ± 363	448 ± 93	40 ± 34	29 ± 40	14 ± 2.8	6.8 ± 0.2	3.7 ± 1.2	1249 ± 1041	2021 ± 136
PFBA	14000 ± 1211	14640 ± 730	1984 ± 511	308 ± 191	240 ± 271	4880 ± 781	3189 ± 212	512 ± 98	83 ± 28	103 ± 10
PFPeA	12307 ± 227	13587 ± 384	186 ± 6.8	47 ± 30	35 ± 42	1932 ± 368	459 ± 44	122 ± 30	72 ± 25	114 ± 8.4
PFHxA	10640 ± 183	11640 ± 560	115 ± 4.2	28 ± 20	22 ± 27	424 ± 9	155 ± 24	38 ± 6.9	254 ± 110	529 ± 38
PFHpA	10973 ± 384	11760 ± 680	119 ± 21	35 ± 11	30 ± 29	128 ± 9	59 ± 8.6	18 ± 10	20 ± 7.6	82 ± 6.4
PFOA	11267 ± 197	11907 ± 862	298 ± 74	35 ± 25	22 ± 27	59 ± 11	25 ± 2.6	12 ± 3.0	78 ± 31	290 ± 30
PFNA	8960 ± 174	8547 ± 647	595 ± 122	48 ± 32	35 ± 42	29 ± 11	16 ± 4.7	8.1 ± 3.0	1.9 ± 0.4	12 ± 1.8
4:2 FTSA	3693 ± 44	2845 ± 520	38 ± 2.3	10 ± 6.6	7.6 ± 9.5	517 ± 60	243 ± 39	74 ± 16	9.2 ± 2.0	12 ± 2.0
6:2 FTSA	777 ± 66	436 ± 65	70 ± 8.8	23 ± 11	16 ± 8.8	74 ± 12	72 ± 18	60 ± 19	1010 ± 356	1787 ± 289
8:2 FTSA	426 ± 26	178 ± 19	21 ± 4.0	4.1 ± 1.4	3.5 ± 0.9	5.2 ± 2.0	2.7 ± 1.0	2 ± 0.3	250 ± 64	912 ± 218

Table S16 Parameters of the fitted linear equation of log earthworm body burden concentrations versus log soil leachate concentrations of amended MC soil.

Parameters of linear equation y = mx + b and R^2 of log earthworm body burdens versus log soil leachate concentrations

	F400 Activated Carbon			FS100 Modified Clay		
	m	**b**	**R^2**	**m**	**b**	**R^2**
PFBS	0.56	2.09	0.91	0.57	2.00	1.00
PFHxS	0.61	2.06	0.78	0.51	2.36	0.96
PFHpS	0.63	1.79	0.81	0.41	2.52	0.93
PFOS	0.76	1.28	0.81	0.43	2.47	0.85
PFBA	0.44	1.20	0.93	0.70	0.07	0.96
PFPeA	0.18	1.49	0.57	0.53	0.11	0.98
PFHxA	0.25	1.09	0.32	0.33	0.70	0.99
PFHpA	0.35	1.14	0.70	0.32	1.27	0.89
PFOA	0.50	1.32	0.81	0.37	1.80	0.95
PFNA	0.63	1.24	0.81	0.37	2.23	0.94
4:2 FTSA	-	-	-	0.33	0[a]	0.69
6:2 FTSA	0.80	0.66	0.98	0.43	1.64	0.69
8:2 FTSA	0.60	1.93	0.85	0.42	2.32	0.99

Data points were not fitted for 4:2 FTSA with F400 amendment as only two earthworm body burden values were available for fitting and the remaining values were below detection limits

[a] Trendline was fitted to 0 as the intercept was negative without fitting (negative value is not possible as actual experimental value)

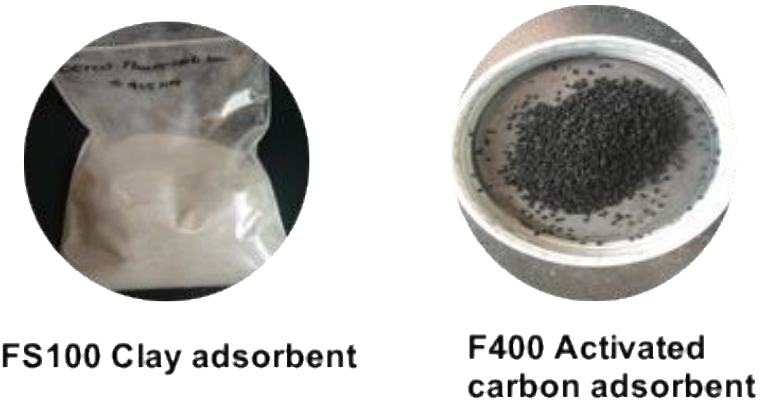

Figure S1 Images of the adsorbents: FS100 modified clay adsorbent on the left and F400 activated carbon on the right.

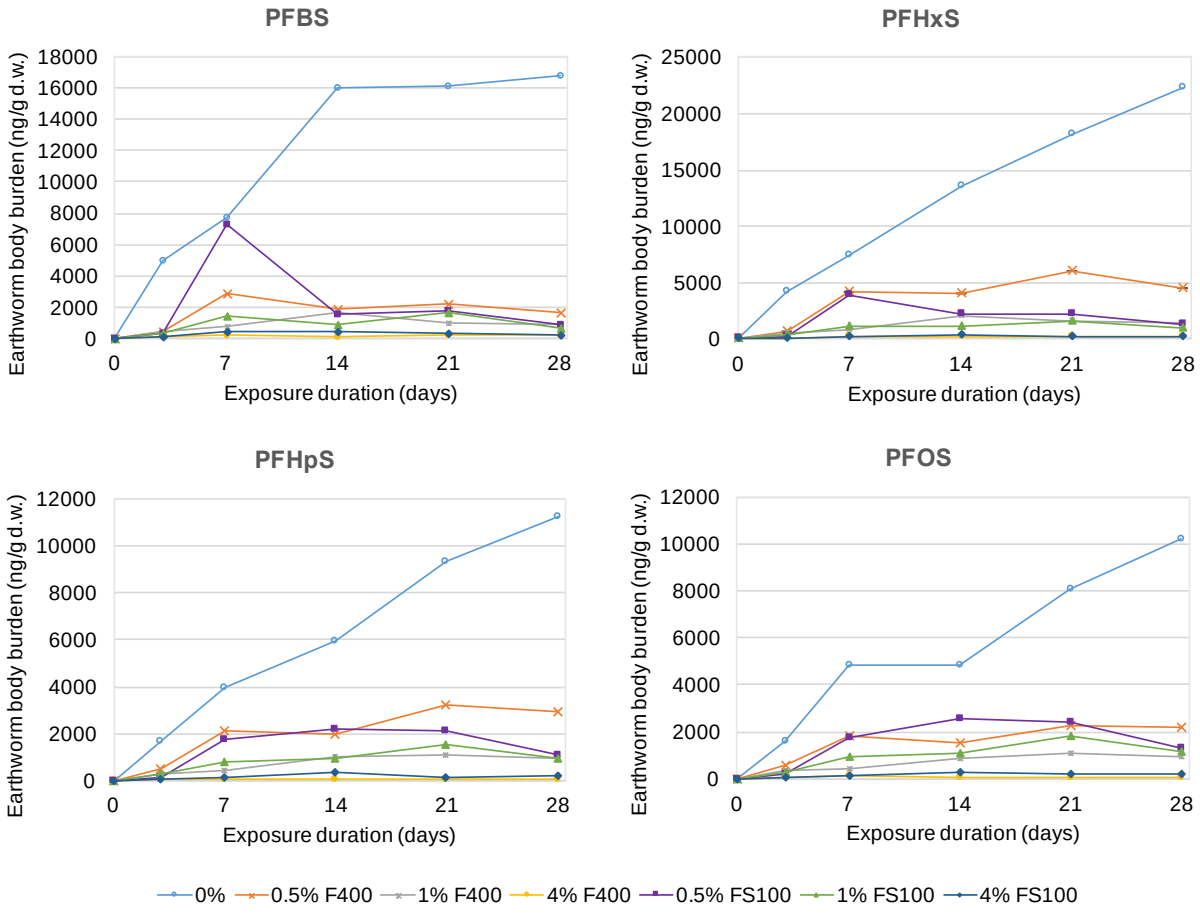

Figure S2 Earthworm uptake kinetics of PFSAs in MC soils.

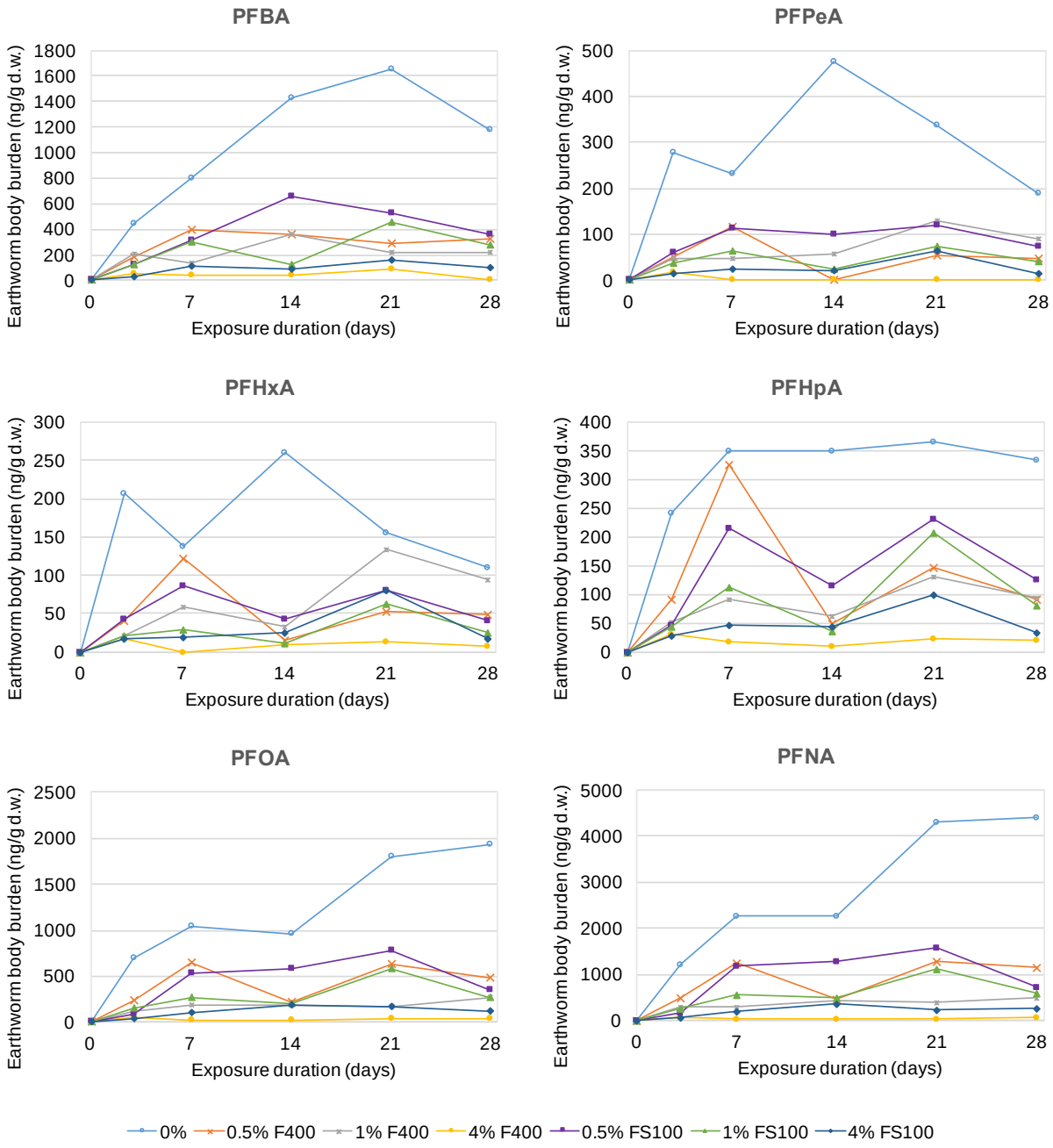

Figure S3 Earthworm uptake kinetics of PFCAs in MC soils.

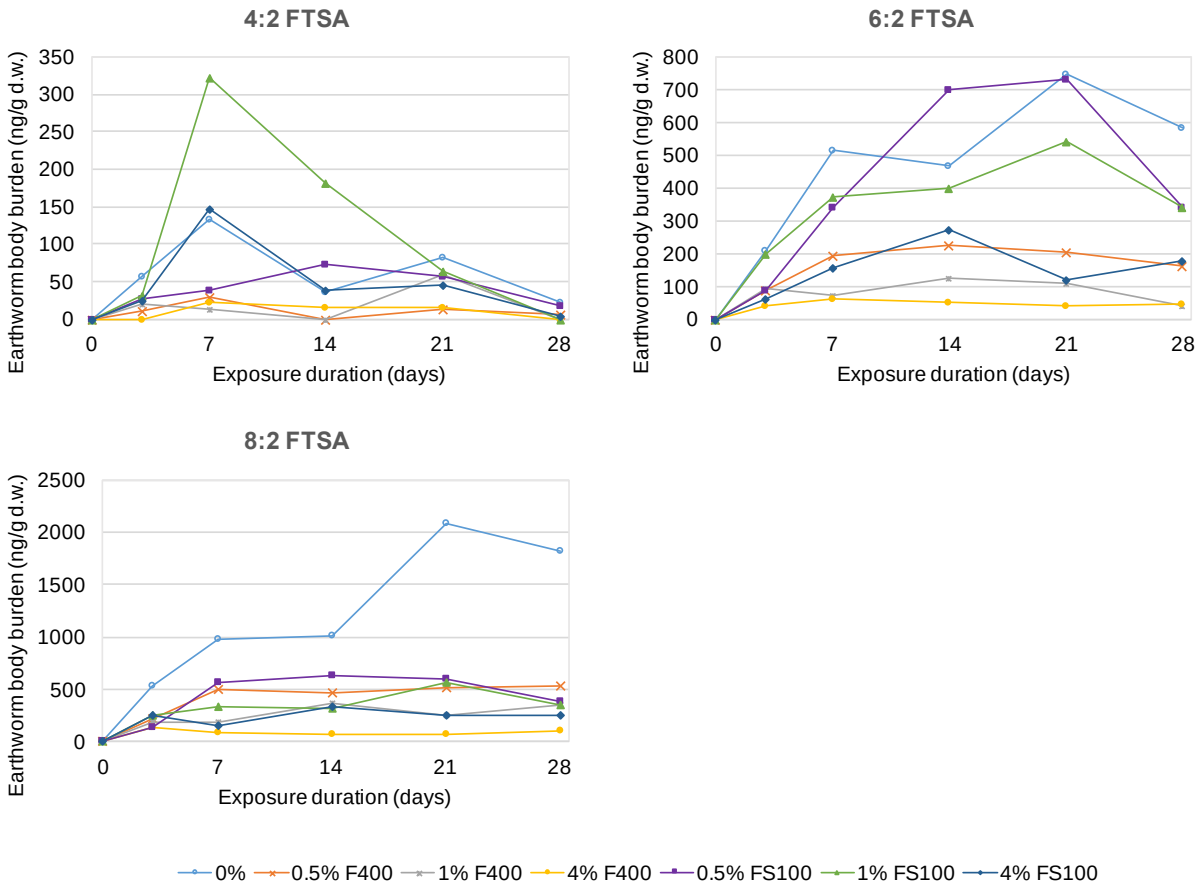

Figure S4 Earthworm uptake kinetics of FTSAs in MC soils.

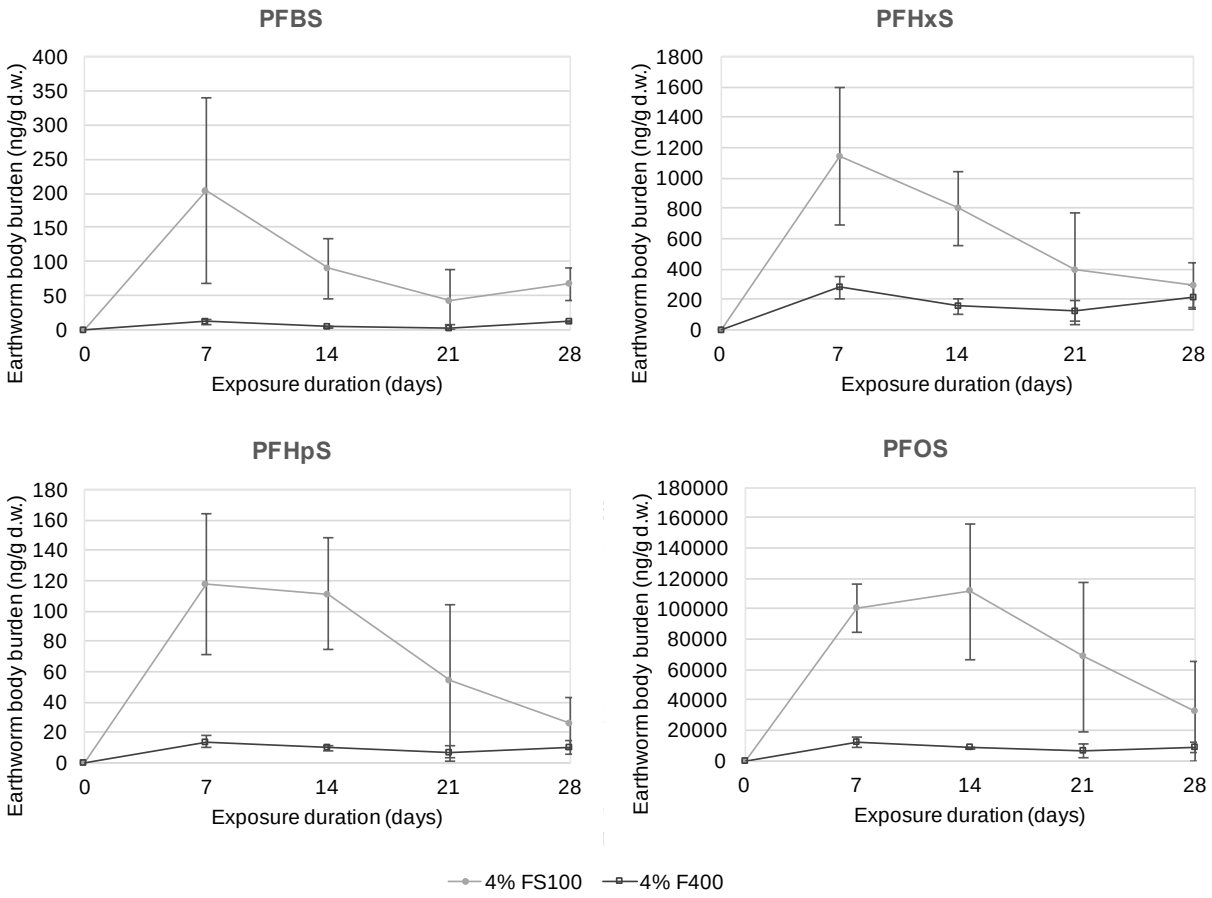

Figure S5 Earthworm uptake kinetics of PFSAs in CAN1 soils.

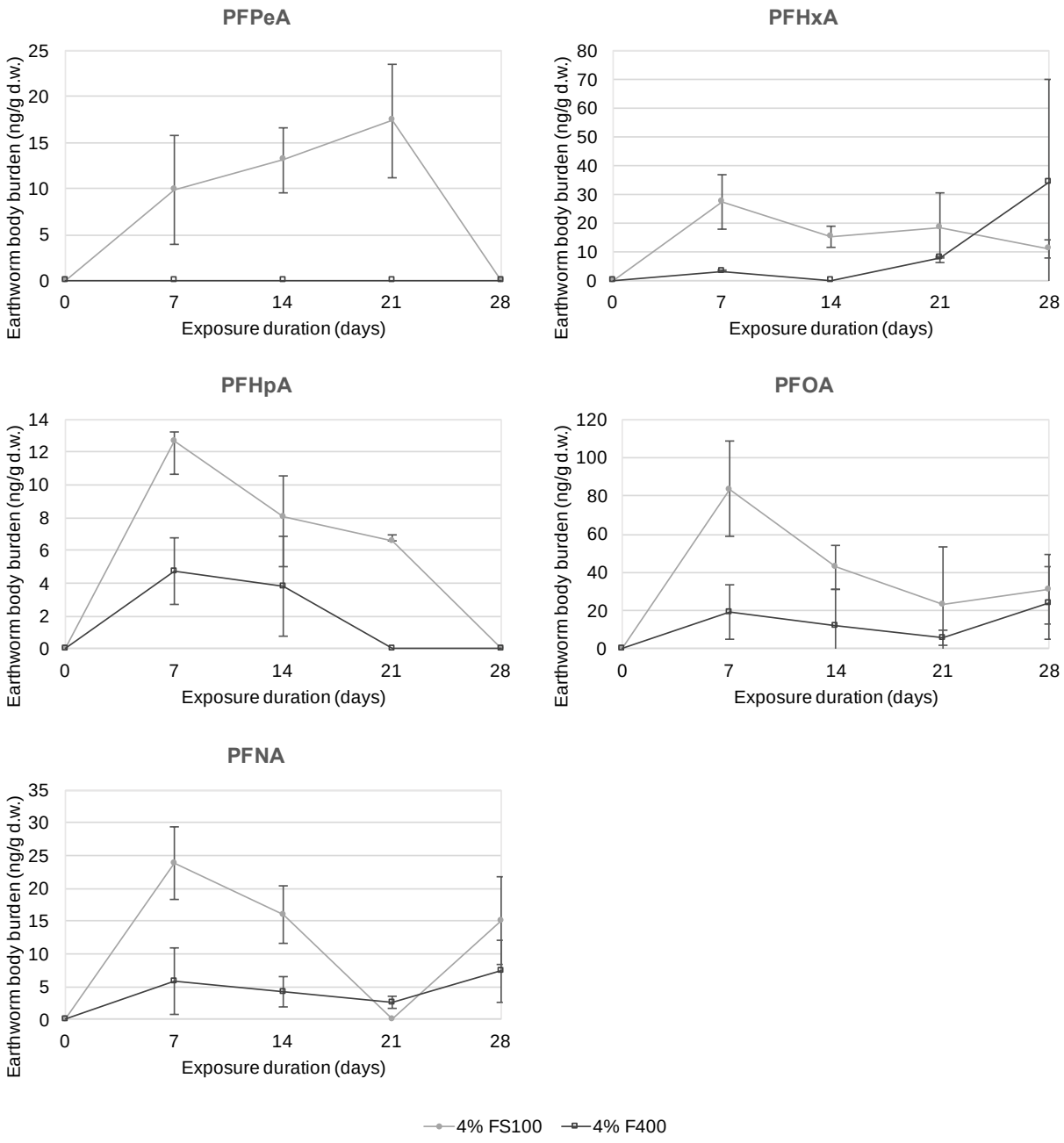

Figure S6 Earthworm uptake kinetics of PFCAs in CAN1 soils.

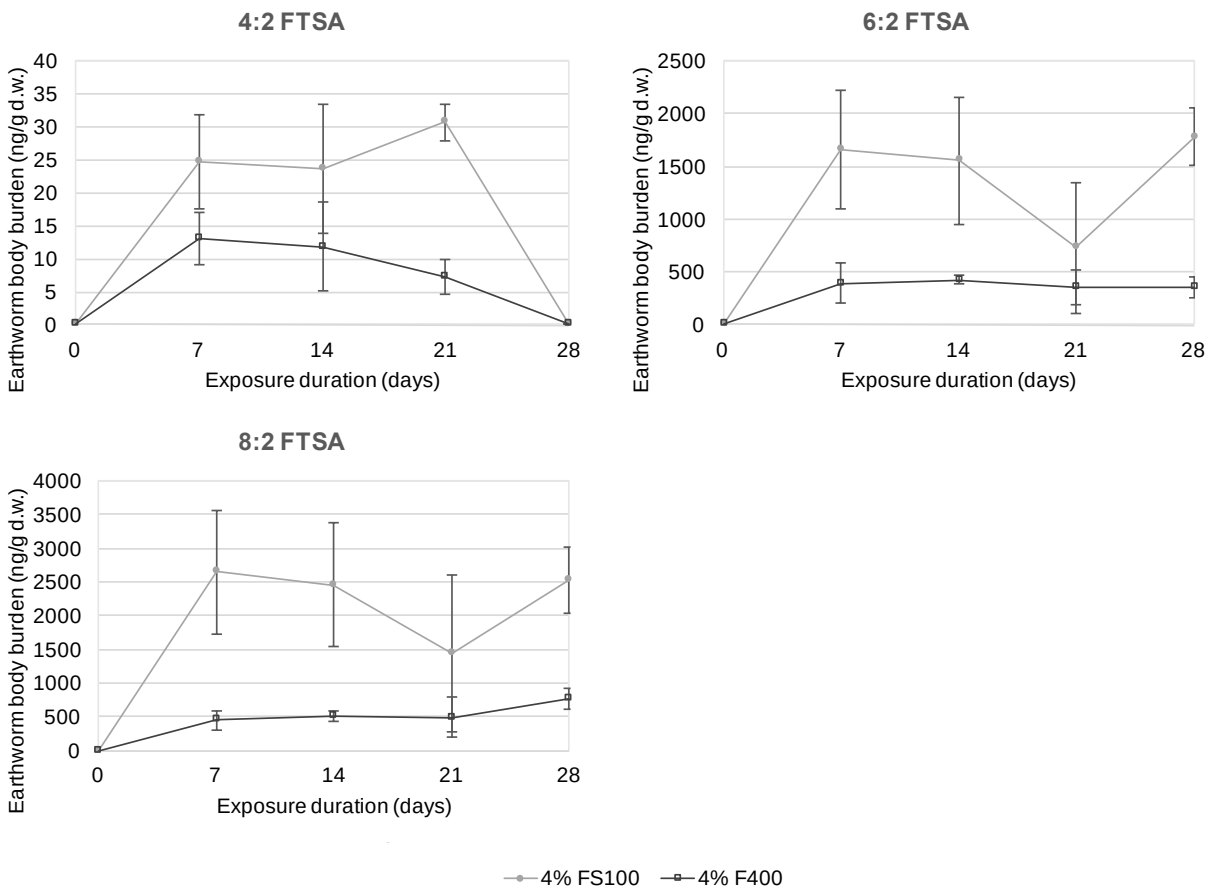

Figure S7 Earthworm uptake kinetics of FTSAs in CAN1 soils.

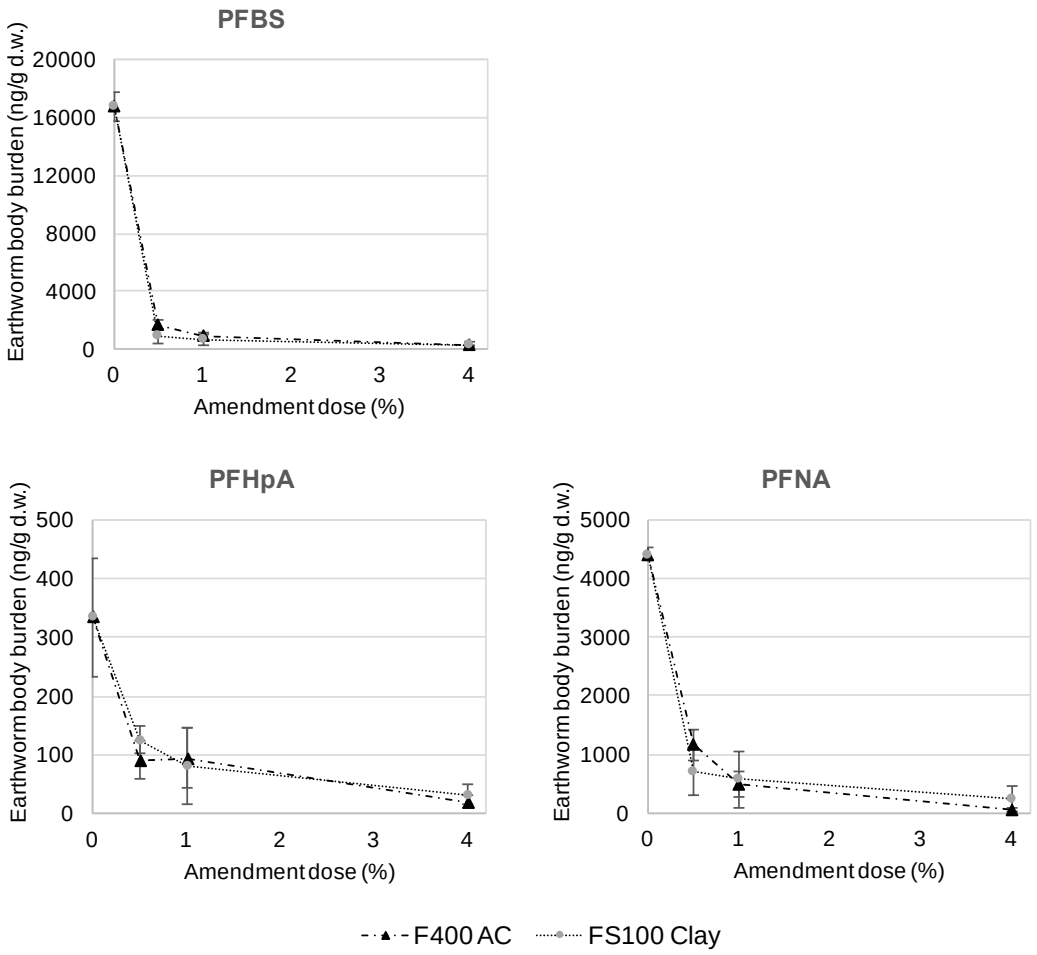

Figure S8 Earthworm body burden (ng/g d.w.) at the end of the uptake phase at different amendment concentrations of activated carbon (F400) and clay (FS100) for remaining PFAS analytes in MC soil. 4:2 FTSA was excluded due to multiple undetected instrumental peaks (suggesting concentrations close to zero).

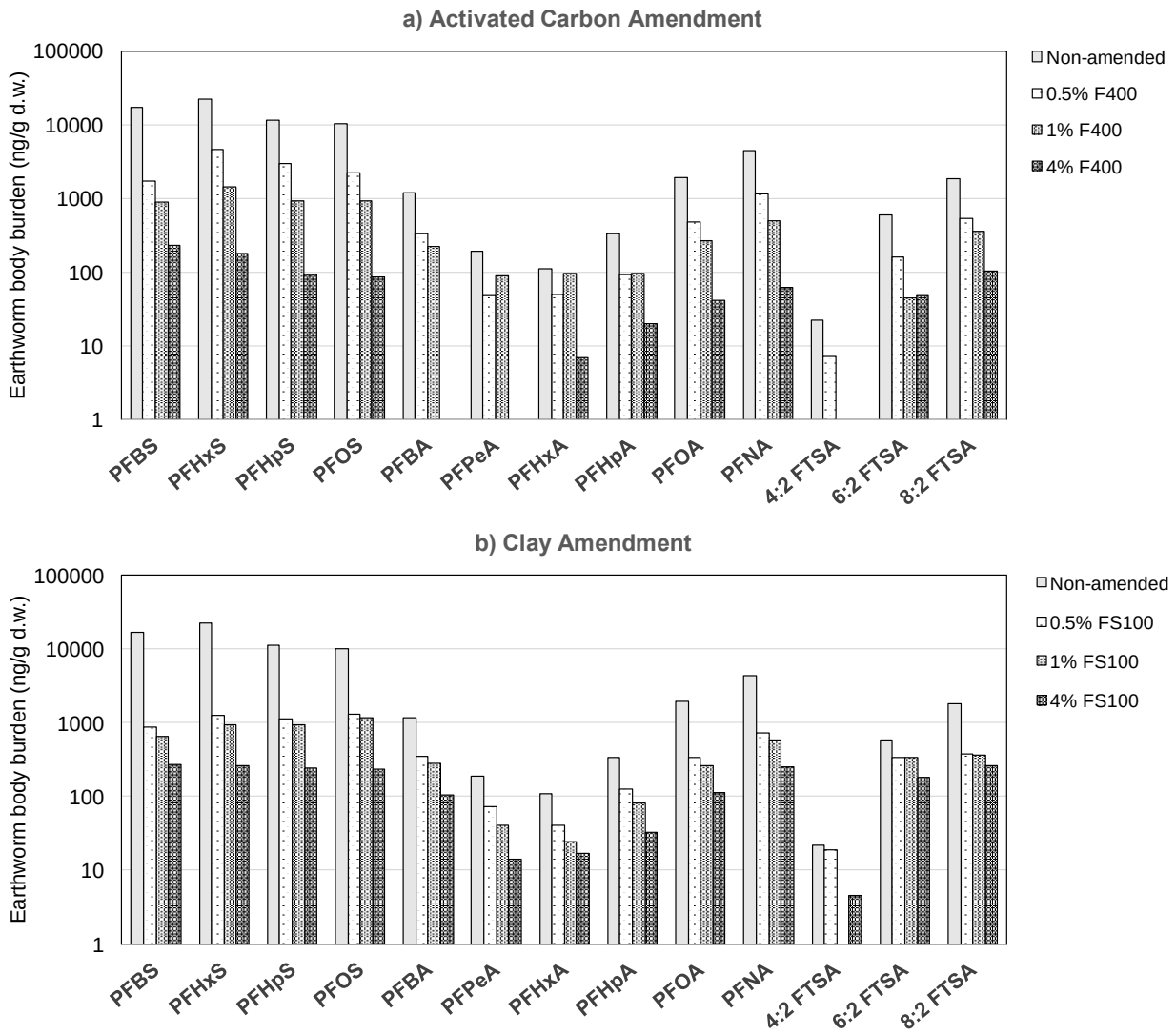

Figure S9 Earthworm body burden values (ng/g d.w.) at Day 28 for individual PFAS analytes in non-amended and amended MC soils with 0.5%, 1%, and 4% concentration of **a)** activated carbon (F400) and **b)** modified clay (FS100).

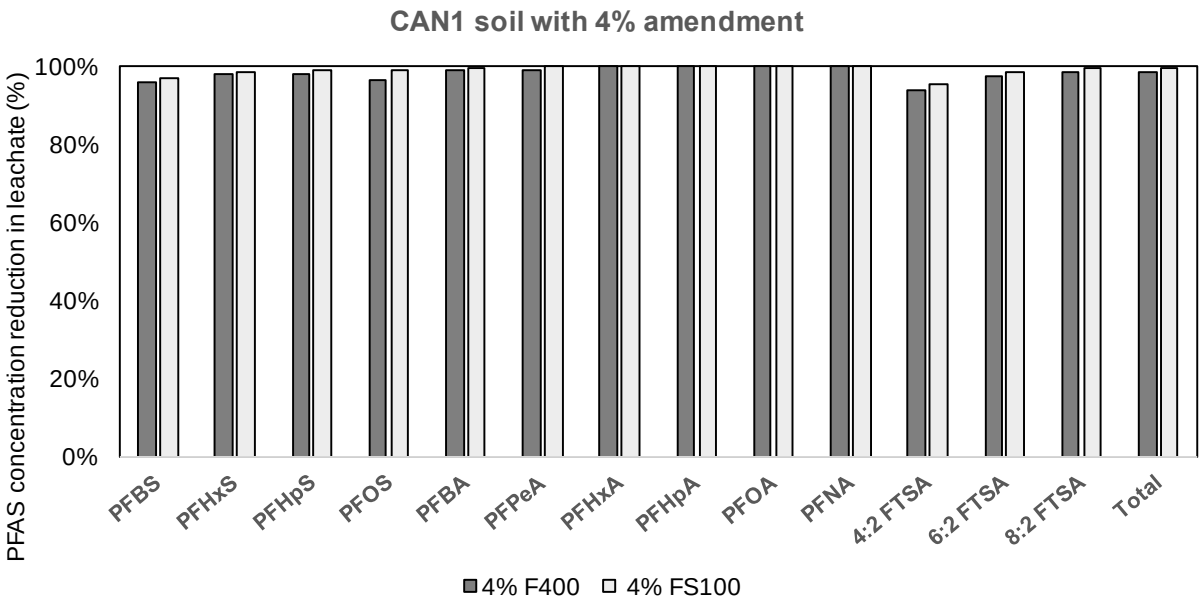

Figure S10 Percentage reduction of PFAS in leachate in CAN 1 soil with 4% amendment as compared with non-amended soil leachate from the previous study by Wang (2019).

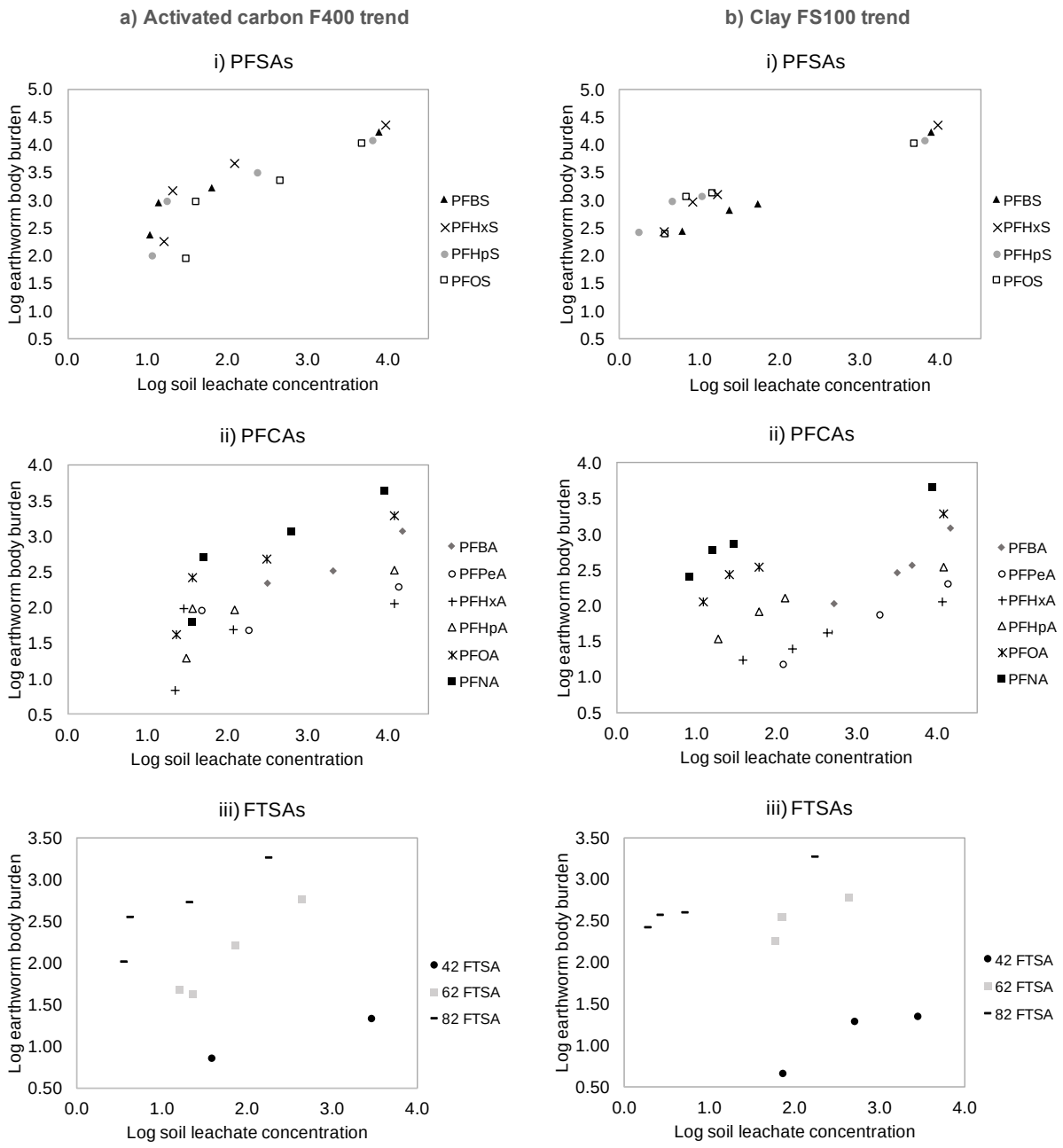

Figure S11 Relationship between log soil leachate concentrations and log earthworm body burdens. Trend displays the values with the varying amendment concentrations of **a)** activated carbon F400 and **b)** modified clay FS100; (Left to right: 4% to 0% amendment concentration).

List of References

1. Munoz, G., et al., *Environmental occurrence of perfluoroalkyl acids and novel fluorotelomer surfactants in the freshwater fish Catostomus commersonii and sediments following firefighting foam deployment at the Lac-Mégantic railway accident.* Environmental science & technology, 2017. **51**(3): p. 1231-1240.

2. Wen, B., et al., *Field study on the uptake and translocation of perfluoroalkyl acids (PFAAs) by wheat (Triticum aestivum L.) grown in biosolids-amended soils.* Environmental pollution, 2014. **184**: p. 547-554.

3. Wolf, C.J., et al., *Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4–C12 compounds.* Reproductive toxicology, 2012. **33**(4): p. 546-551.

4. Barry, V., A. Winquist, and K. Steenland, *Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant.* Environmental health perspectives, 2013. **121**(11-12).

5. Brusseau, M.L., R.H. Anderson, and B. Guo, *PFAS concentrations in soils: Background levels versus contaminated sites.* Science of The Total Environment, 2020: p. 140017.

6. Xiao, F., *Emerging poly-and perfluoroalkyl substances in the aquatic environment: A review of current literature.* Water research, 2017. **124**: p. 482-495.

7. D'Agostino, L.A. and S.A. Mabury, *Certain perfluoroalkyl and polyfluoroalkyl substances associated with aqueous film forming foam are widespread in Canadian surface waters.* Environmental Science & Technology, 2017. **51**(23): p. 13603-13613.

8. Houtz, E.F., et al., *Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil.* Environmental science & technology, 2013. **47**(15): p. 8187-95.

9. Hu, X.C., et al., *Detection of poly-and perfluoroalkyl substances (PFASs) in US drinking water linked to industrial sites, military fire training areas, and wastewater treatment plants.* Environmental science & technology letters, 2016. **3**(10): p. 344-350.

10. Munoz, G., et al., *Occurrence survey and spatial distribution of perfluoroalkyl and polyfluoroalkyl surfactants in groundwater, surface water, and sediments from tropical environments.* Science of the Total Environment, 2017. **607**: p. 243-252.

11. Baduel, C., et al., *Discovery of novel per-and polyfluoroalkyl substances (PFASs) at a fire fighting training ground and preliminary investigation of their fate and mobility.* Chemosphere, 2017. **185**: p. 1030-1038.

12. Barzen-Hanson, K.A., et al., *Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater.* Environmental science & technology, 2017. **51**(4): p. 2047-2057.

13. Mejia-Avendaño, S., et al., *Novel fluoroalkylated surfactants in soils following firefighting foam deployment during the Lac-Megantic railway accident.* Environmental science & technology, 2017. **51**(15): p. 8313-8323.

14. Milley, S.A., et al., *Estimating the number of airports potentially contaminated with perfluoroalkyl and polyfluoroalkyl substances from aqueous film forming foam: A Canadian example.* Journal of environmental management, 2018. **222**: p. 122-131.

15. Nickerson, A., et al., *Enhanced extraction of AFFF-associated PFASs from source zone soils.* Environmental Science & Technology, 2020. **54**(8): p. 4952-4962.

16. Ross, I., et al., *A review of emerging technologies for remediation of PFASs.* Remediation Journal, 2018. **28**(2): p. 101-126.

17. Hale, S.E., et al., *Sorbent amendment as a remediation strategy to reduce PFAS mobility and leaching in a contaminated sandy soil from a Norwegian firefighting training facility.* CHEM Chemosphere, 2017. **171**: p. 9-18.

18. Kupryianchyk, D., et al., *Treatment of sites contaminated with perfluorinated compounds using biochar amendment.* Chemosphere, 2016. **142**: p. 35-40.

19. R., S. and M. R. *Immobilisation of per- and polyfluorinated alkyl substances (PFAS) in 14 soils from airport sites across Australia*. in *Battelle International Bioremediation Symposium*. 2017. Miami, FL.

20. Das, P., et al., *Remediation of Perfluorooctane Sulfonate in Contaminated Soils by Modified Clay Adsorbenta Risk-Based Approach.* Water Air Soil Pollut Water, Air, & Soil Pollution : An International Journal of Environmental Pollution, 2013. **224**(12): p. 1-14.

21. Yan, B., et al., *Molecular Mechanisms of Per-and Polyfluoroalkyl Substances on a Modified Clay: A Combined Experimental and Molecular Simulation Study.* Water Research, 2020: p. 116166.

22. Wang, C., *Modified Clays as Effective Soil Amendment to Reduce Leaching of Per- and Polyfluoroalkyl Substances from Contaminated Soils*, in *Department of Civil Engineering and Applied Mechanics*. 2019, McGill University: Montreal, QC, Canada.

23. Beesley, L., E. Moreno-Jiménez, and J.L. Gomez-Eyles, *Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil.* Environmental pollution, 2010. **158**(6): p. 2282-2287.

24. Chai, Y., et al., *Effectiveness of activated carbon and biochar in reducing the availability of polychlorinated dibenzo-p-dioxins/dibenzofurans in soils.* Environmental science & technology, 2012. **46**(2): p. 1035-1043.

25. Fagervold, S.K., et al., *Bioaccumulation of polychlorinated dibenzo-p-dioxins/dibenzofurans in E. fetida from floodplain soils and the effect of activated carbon amendment.* Environmental science & technology, 2010. **44**(14): p. 5546-5552.

26. Wang, F., et al., *Species-dependent effects of biochar amendment on bioaccumulation of atrazine in earthworms.* Environmental pollution, 2014. **186**: p. 241-247.

27. Wen, B., et al., *Immobilization of pentachlorophenol in soil using carbonaceous material amendments.* Environmental Pollution, 2009. **157**(3): p. 968-974.

28. Li, B., et al., *Effects of the amendment of biochars and carbon nanotubes on the bioavailability of hexabromocyclododecanes (HBCDs) in soil to ecologically different species of earthworms.* Environmental Pollution, 2017. **222**: p. 191-200.

29. Song, Y., et al., *Immobilization of chlorobenzenes in soil using wheat straw biochar.* Journal of agricultural and food chemistry, 2013. **61**(18): p. 4210-4217.

30. Ng, C.A. and K. Hungerbühler, *Bioconcentration of Perfluorinated Alkyl Acids: How Important Is Specific Binding?* Environmental Science & Technology, 2013. **47**(13): p. 7214-7223.

31. Xia, X., et al., *Effects of carbon nanotubes, chars, and ash on bioaccumulation of perfluorochemicals by Chironomus plumosus larvae in sediment.* Environmental science & technology, 2012. **46**(22): p. 12467-75.

32. Bräunig, J., C. Baduel, and J. Mueller. *Influence of a commercial adsorbent on the leaching behaviour and bioavailability of selected perfluoroalkyl acids (PFAAs) from soil impacted by AFFFs*. in *International Conference on Emerging Contaminants (EmCon)*. 2016. Sydney, Australia.

33. Das, P., M. Megharaj, and R. Naidu, *Perfluorooctane sulfonate release pattern from soils of fire training areas in Australia and its bioaccumulation potential in the earthworm Eisenia fetida.* Environ Sci Pollut Res Environmental Science and Pollution Research, 2015. **22**(12): p. 8902-8910.

34. Navarro, I., et al., *Bioaccumulation of emerging organic compounds (perfluoroalkyl substances and halogenated flame retardants) by earthworm in biosolid amended soils.* Environmental Research, 2016. **149**: p. 32-39.

35. Rich, C.D., et al., *Bioaccumulation of perfluoroalkyl acids by earthworms (Eisenia fetida) exposed to contaminated soils.* Environmental science & technology, 2015. **49**(2): p. 881-8.

36. Wen, B., et al., *Bioavailability of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in biosolids-amended soils to earthworms (Eisenia fetida).* Chemosphere, 2015. **118**: p. 361-366.

37. Zhao, S., et al., *Bioaccumulation of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkane sulfonates (PFSAs) by earthworms (Eisenia fetida) in soil.* Environmental pollution., 2013. **179**: p. 45-52.

38. Munoz, G., et al., *Bioaccumulation of Zwitterionic Polyfluoroalkyl Substances in Earthworms Exposed to Aqueous Film-Forming Foam Impacted Soils.* Environmental Science & Technology, 2020. **54**(3): p. 1687-1697.

39. Kissa, E., *Fluorinated surfactants and repellents*. 2001, New York: Marcel Dekker.

40. Buck, R.C., et al., *Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins.* Integrated Environmental Assessment and Management, 2011. **7**(4): p. 513-41.

41. Kim, S.-K. and K. Kannan, *Perfluorinated Acids in Air, Rain, Snow, Surface Runoff, and Lakes: Relative Importance of Pathways to Contamination of Urban Lakes.* Environmental Science & Technology, 2007. **41**(24): p. 8328-8334.

42. Rankin, K., et al., *A North American and global survey of perfluoroalkyl substances in surface soils: Distribution patterns and mode of occurrence.* Chemosphere, 2016. **161**: p. 333-341.

43. Sharma, B.M., et al., *Perfluoroalkyl substances (PFAS) in river and ground/drinking water of the Ganges River basin: emissions and implications for human exposure.* Environmental pollution, 2016. **208**: p. 704-713.

44. Lee, H. and S.A. Mabury, *Global Distribution of Polyfluoroalkyl and Perfluoroalkyl Substances and their Transformation Products in Environmental Solids*, in *Transformation Products of Emerging Contaminants in the Environment*. 2014. p. 797-826.

45. Giesy, J.P. and K. Kannan, *Global Distribution of Perfluorooctane Sulfonate in Wildlife.* Environmental Science & Technology, 2001. **35**(7): p. 1339-1342.

46. Kärrman, A., et al., *Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma.* Environment International, 2007. **33**(6): p. 782-788.

47. Kelly, B.C., et al., *Perfluoroalkyl Contaminants in an Arctic Marine Food Web: Trophic Magnification and Wildlife Exposure.* Environmental Science & Technology, 2009. **43**(11): p. 4037-4043.

48. Wolf, C.J., et al., *Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARa) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds.* Reproductive toxicology (Elmsford, N.Y.), 2012. **33**(4): p. 546-51.

49. Hu, X.C., et al., *Detection of Poly- and Perfluoroalkyl Substances (PFASs) in U.S. Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants.* Environmental Science & Technology Letters, 2016. **3**(10): p. 344-350.

50. Darrow, L.A., et al., *Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community.* Environmental health perspectives, 2016. **124**(8): p. 1227-33.

51. Stein, C.R., et al., *Perfluorooctanoate exposure and major birth defects.* RTX Reproductive Toxicology, 2014. **47**: p. 15-20.

52. Jensen, A.A. and H. Leffers, *Emerging endocrine disrupters: perfluoroalkylated substances.* IJAN International Journal of Andrology, 2008. **31**(2): p. 161-169.

53. Eriksen, K.T., et al., *Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells.* MUTGEN Mut.Res.-Genetic Toxicology and Environmental Mutagenesis, 2010. **700**(1): p. 39-43.

54. Li, Y., et al., *Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water.* Occupational and environmental medicine, 2018. **75**(1): p. 46-51.

55. UNEP, *Listing of perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride, in The new POPs under the Stockholm Convention.*, in *SC-4/17*, UNEP, Editor. 2009: Geneva, Swizterland.

56. Canada, H., *Guidelines for Canadian Drinking Water Quality - Summary Table*. 2020.

57. Wang, Z., et al., *Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: Status quo, ongoing challenges and possible solutions.* E.I. Environment International, 2015. **75**: p. 172-179.

58. Place, B.J. and J.A. Field, *Identification of novel fluorochemicals in aqueous film-forming foams used by the U.S. military.* Environmental science & technology, 2012. **46**(13): p. 7120-7.

59. Filipovic, M., et al., *Historical usage of aqueous film forming foam: A case study of the widespread distribution of perfluoroalkyl acids from a military airport to groundwater, lakes, soils and fish.* CHEM Chemosphere, 2015. **129**: p. 39-45.

60. Gewurtz, S.B., et al., *High levels of perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario, Canada.* E.I. Environment International, 2014. **67**: p. 1-11.

61. Sun, M., et al., *Legacy and Emerging Perfluoroalkyl Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina.* Environmental Science & Technology Letters, 2016. **3**(12): p. 415-419.

62. Blaine, A.C., et al., *Perfluoroalkyl Acid Distribution in Various Plant Compartments of Edible Crops Grown in Biosolids-Amended soils.* Environmental Science & Technology, 2014. **48**(14): p. 7858-7865.

63. Lindstrom, A.B., et al., *Application of WWTP Biosolids and Resulting Perfluorinated Compound Contamination of Surface and Well Water in Decatur, Alabama, USA.* Environmental Science & Technology, 2011. **45**(19): p. 8015-8021.

64. Domingo, J.L. and M. Nadal, *Human exposure to per-and polyfluoroalkyl substances (PFAS) through drinking water: A review of the recent scientific literature.* Environmental research, 2019. **177**: p. 108648.

65. Tittlemier, S.A., et al., *Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging.* Journal of Agricultural and Food Chemistry, 2007. **55**(8): p. 3203-3210.

66. Franko, J., et al., *Dermal Penetration Potential of Perfluorooctanoic Acid (PFOA) in Human and Mouse Skin.* j toxicol environ health a Journal of Toxicology and Environmental Health Part A, 2012. **75**(1): p. 50-62.

67. Brooke, D., A. Footitt, and T. Nwaogu, *Environmental Risk Evaluation Report: Perflurooctanesulphonate (PFOS)*. 2004.

68. Goss, K.-U., *The pKa Values of PFOA and Other Highly Fluorinated Carboxylic Acids.* Environmental Science & Technology, 2008. **42**(2): p. 456-458.

69. Rayne, S. and K. Forest, *Perfluoroalkyl sulfonic and carboxylic acids: A critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods.* Journal of Environmental Science and Health, Part A, 2009. **44**(12): p. 1145-1199.

70. Li, Y., D.P. Oliver, and R.S. Kookana, *A critical analysis of published data to discern the role of soil and sediment properties in determining sorption of per and polyfluoroalkyl substances (PFASs).* Science of the Total Environment, 2018. **628**: p. 110-120.

71. Pereira, H.C., et al., *Sorption of perfluoroalkyl substances (PFASs) to an organic soil horizon–Effect of cation composition and pH.* Chemosphere, 2018. **207**: p. 183-191.

72. Guelfo, J.L., et al., *Evaluation and management strategies for per-and polyfluoroalkyl substances (PFASs) in drinking water aquifers: perspectives from impacted US Northeast communities.* Environmental health perspectives, 2018. **126**(6): p. 065001.

73. Higgins, C.P. and R.G. Luthy, *Sorption of Perfluorinated Surfactants on Sediments.* Environmental Science & Technology, 2006. **40**(23): p. 7251-7256.

74. Mejia-Avendaño, S., et al., *Sorption of polyfluoroalkyl surfactants on surface soils: Effect of molecular structures, soil properties, and solution chemistry.* Environmental Science & Technology, 2020.

75. McKenzie, E.R., et al., *Effects of Chemical Oxidants on Perfluoroalkyl Acid Transport in One-Dimensional Porous Media Columns.* Environmental Science & Technology, 2015. **49**(3): p. 1681-1689.

76. Baduel, C., C.J. Paxman, and J.F. Mueller, *Perfluoroalkyl substances in a firefighting training ground (FTG), distribution and potential future release.* HAZMAT Journal of Hazardous Materials, 2015. **296**: p. 46-53.

77. Anderson, R.H., et al., *Occurrence of select perfluoroalkyl substances at U.S. Air Force aqueous film-forming foam release sites other than fire-training areas: Field-validation of critical fate and transport properties.* Chemosphere, 2016. **150**: p. 678-685.

78. Liu, J. and S.M. Avendaño, *Microbial degradation of polyfluoroalkyl chemicals in the environment: a review.* Environment international, 2013. **61**: p. 98-114.

79. Martin, J.W., et al., *PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure?* Journal of Environmental Monitoring, 2010. **12**(11): p. 1979-2004.

80. Gauthier, S.A. and S.A. Mabury, *Aqueous photolysis of 8: 2 fluorotelomer alcohol.* Environmental Toxicology and Chemistry: An International Journal, 2005. **24**(8): p. 1837-1846.

81. Plumlee, M.H., K. McNeill, and M. Reinhard, *Indirect photolysis of perfluorochemicals: hydroxyl radical-initiated oxidation of N-ethyl perfluorooctane sulfonamido acetate (N-EtFOSAA) and other perfluoroalkanesulfonamides.* Environmental science & technology, 2009. **43**(10): p. 3662-3668.

82. Yarwood, G., et al., *High-resolution atmospheric modeling of fluorotelomer alcohols and perfluorocarboxylic acids in the North American troposphere.* Environmental science & technology, 2007. **41**(16): p. 5756-5762.

83. Martin, J.W., et al., *Atmospheric chemistry of perfluoroalkanesulfonamides: kinetic and product studies of the O.H. radical and Cl atom initiated oxidation of N-ethyl perfluorobutanesulfonamide.* Environmental science & technology, 2006. **40**(3): p. 864-872.

84. Wallington, T., et al., *Formation of C7F15COOH (PFOA) and other perfluorocarboxylic acids during the atmospheric oxidation of 8: 2 fluorotelomer alcohol.* Environmental science & technology, 2006. **40**(3): p. 924-930.

85. Washington, J.W. and T.M. Jenkins, *Abiotic hydrolysis of fluorotelomer-based polymers as a source of perfluorocarboxylates at the global scale.* Environmental science & technology, 2015. **49**(24): p. 14129-14135.

86. Zhang, S., et al., *6: 2 and 8: 2 fluorotelomer alcohol anaerobic biotransformation in digester sludge from a WWTP under methanogenic conditions.* Environmental science & technology, 2013. **47**(9): p. 4227-4235.

87. Allred, B.M., et al., *Physical and biological release of poly-and perfluoroalkyl substances (PFASs) from municipal solid waste in anaerobic model landfill reactors.* Environmental science & technology, 2015. **49**(13): p. 7648-7656.

88. D'Agostino, L.A. and S.A. Mabury, *Aerobic biodegradation of 2 fluorotelomer sulfonamide–based aqueous film–forming foam components produces perfluoroalkyl carboxylates.* Environmental toxicology and chemistry, 2017. **36**(8): p. 2012-2021.

89. Harding-Marjanovic, K.C., et al., *Aerobic biotransformation of fluorotelomer thioether amido sulfonate (Lodyne) in AFFF-amended microcosms.* Environmental science & technology, 2015. **49**(13): p. 7666-7674.

90. Avendano, S.M. and J. Liu, *Production of PFOS from aerobic soil biotransformation of two perfluoroalkyl sulfonamide derivatives.* Chemosphere, 2015. **119**: p. 1084-1090.

91. Zhang, L., et al., *Kinetic analysis of aerobic biotransformation pathways of a perfluorooctane sulfonate (PFOS) precursor in distinctly different soils.* Environmental Pollution, 2017. **229**: p. 159-167.

92. Liu, J., et al., *6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture.* Chemosphere, 2010. **78**(4): p. 437-444.

93. Wang, N., et al., *5: 3 Polyfluorinated acid aerobic biotransformation in activated sludge via novel "one-carbon removal pathways".* Chemosphere, 2012. **87**(5): p. 527-534.

94. Mejia-Avendaño, S., et al., *Generation of perfluoroalkyl acids from aerobic biotransformation of quaternary ammonium polyfluoroalkyl surfactants.* Environmental science & technology, 2016. **50**(18): p. 9923-9932.

95. Wang, N., et al., *6: 2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants.* Chemosphere, 2011. **82**(6): p. 853-858.

96. Haukås, M., et al., *Bioaccumulation of per-and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web.* Environmental Pollution, 2007. **148**(1): p. 360-371.

97. Taylor, M.D., et al., *Bioaccumulation of perfluoroalkyl substances in exploited fish and crustaceans: Spatial trends across two estuarine systems.* Marine pollution bulletin, 2018. **131**: p. 303-313.

98. Xia, X., et al., *Comparing humic substance and protein compound effects on the bioaccumulation of perfluoroalkyl substances by Daphnia magna in water.* Chemosphere, 2015. **119**: p. 978-986.

99. Abercrombie, S.A., et al., *Larval amphibians rapidly bioaccumulate poly-and perfluoroalkyl substances.* Ecotoxicology and environmental safety, 2019. **178**: p. 137-145.

100. Krippner, J., et al., *Effects of chain length and pH on the uptake and distribution of perfluoroalkyl substances in maize (Zea mays).* CHEM Chemosphere, 2014. **94**: p. 85-90.

101. OECD, *Test No. 317: Bioaccumulation in Terrestrial Oligochaetes*. 2010.

102. E1676-12, A., *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm Eisenia Fetida and the Enchytraeid Potworm Enchytraeus albidus. E1676-12. ASTM International, West Conshohocken, PA*. 2012.

103. Navarro, I., et al., *Bioaccumulation of emerging organic compounds (perfluoroalkyl substances and halogenated flame retardants) by earthworm in biosolid amended soils.* Environmental research, 2016. **149**: p. 32-39.

104. Zhao, S., et al., *Behaviors of N-ethyl perfluorooctane sulfonamide ethanol (N-EtFOSE) in a soil-earthworm system: transformation and bioaccumulation.* Science of the Total Environment, 2016. **554**: p. 186-191.

105. Zhao, S., et al., *Different biotransformation behaviors of perfluorooctane sulfonamide in wheat (Triticum aestivum L.) from earthworms (Eisenia fetida).* Journal of Hazardous Materials, 2018. **346**: p. 191-198.

106. Xu, D., et al., *Antioxidant defense system responses and DNA damage of earthworms exposed to perfluorooctane sulfonate (PFOS).* Environmental Pollution, 2013. **174**: p. 121-127.

107. Zareitalabad, P., et al., *Dose-dependent reactions of Aporrectodea caliginosa to perfluorooctanoic acid and perfluorooctanesulfonic acid in soil.* Ecotoxicology and environmental safety, 2013. **95**: p. 39-43.

108. Sindermann, A., et al., *PFOS: an acute toxicity study with the earthworm in an artificial soil substrate.* Project Number 454-111. EPA Docket AR226, 2002. **1106**.

109. Zhai, Y., et al., *Role of ingestion route in the perfluoroalkyl substance bioaccumulation by Chironomus plumosus larvae in sediments amended with carbonaceous materials.* HAZMAT Journal of Hazardous Materials, 2016. **302**: p. 404-414.

110. Zhi, Y. and J. Liu, *Adsorption of perfluoroalkyl acids by carbonaceous adsorbents: Effect of carbon surface chemistry.* Environmental pollution (Barking, Essex : 1987), 2015. **202**: p. 168-76.

111. Sörengård, M., D.B. Kleja, and L. Ahrens, *Stabilization and solidification remediation of soil contaminated with poly-and perfluoroalkyl substances (PFASs).* Journal of hazardous materials, 2019. **367**: p. 639-646.

112. Kaboré, H.A., et al., *Worldwide drinking water occurrence and levels of newly-identified perfluoroalkyl and polyfluoroalkyl substances.* STOTEN Science of the Total Environment, 2018. **616-617**: p. 1089-1100.

113. Zhang, S., et al., *Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment.* Chemosphere, 2016. **154**: p. 224-230.

114. Shaw, D.M.J., et al., *Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by Gordonia sp. strain NB4-1Y under sulfur-limiting conditions.* Science of The Total Environment, 2019. **647**: p. 690-698.

115. Jager, T., et al., *Elucidating the routes of exposure for organic chemicals in the earthworm, Eisenia andrei (Oligochaeta).* Environmental science & technology, 2003. **37**(15): p. 3399-3404.

116. Bräunig, J., et al., *Leaching and bioavailability of selected perfluoroalkyl acids (PFAAs) from soil contaminated by firefighting activities.* Science of the Total Environment, 2019. **646**: p. 471-479.

117. Guelfo, J.L. and C.P. Higgins, *Subsurface Transport Potential of Perfluoroalkyl Acids at Aqueous Film-Forming Foam (AFFF)-Impacted Sites.* Environmental Science & Technology, 2013. **47**(9): p. 4164-4171.

118. Hebert, P.C. and L.A. MacManus-Spencer, *Development of a Fluorescence Model for the Binding of Medium- to Long-Chain Perfluoroalkyl Acids to Human Serum Albumin Through a Mechanistic Evaluation of Spectroscopic Evidence.* Analytical Chemistry, 2010. **82**(15): p. 6463-6471.

119. Zhao, L., et al., *Sequestration and bioavailability of perfluoroalkyl acids (PFAAs) in soils: Implications for their underestimated risk.* Science of The Total Environment, 2016. **572**: p. 169-176.

120. Yoo, H., et al., *Quantitative determination of perfluorochemicals and fluorotelomer alcohols in plants from biosolid-amended fields using LC/MS/MS and GC/MS.* Environmental science & technology, 2011. **45**(19): p. 7985-7990.

121. Zhao, S., et al., *Mutual impacts of wheat (Triticum aestivum L.) and earthworms (Eisenia fetida) on the bioavailability of perfluoroalkyl substances (PFASs) in soil.* Environmental pollution, 2014. **184**: p. 495-501.

122. Karnjanapiboonwong, A., et al., *Perfluoroalkylsulfonic and carboxylic acids in earthworms (Eisenia fetida): Accumulation and effects results from spiked soils at PFAS concentrations bracketing environmental relevance.* Chemosphere, 2018. **199**: p. 168-173.

123. Liu, C., et al., *Novel Perspectives on the Bioaccumulation of PFCs – the Concentration Dependency.* Environmental Science & Technology, 2011. **45**(22): p. 9758-9764.

124. Hale, S.E., et al., *Short-term effect of the soil amendments activated carbon, biochar, and ferric oxyhydroxide on bacteria and invertebrates.* Environmental science & technology, 2013. **47**(15): p. 8674-8683.

125. Ren, X., et al., *Effect of exogenous carbonaceous materials on the bioavailability of organic pollutants and their ecological risks.* Soil Biology and Biochemistry, 2018. **116**: p. 70-81.

126. Barzen-Hanson, K.A., et al., *Sorption of fluorotelomer sulfonates, fluorotelomer sulfonamido betaines, and a fluorotelomer sulfonamido amine in national foam aqueous film-forming foam to soil.* Environmental Science & Technology, 2017. **51**(21): p. 12394-12404.

127. Di Bonito, M., et al., *CHAPTER TEN - OVERVIEW OF SELECTED SOIL PORE WATER EXTRACTION METHODS FOR THE DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN CONTAMINATED SOILS: OPERATIONAL AND TECHNICAL ASPECTS*, in *Environmental Geochemistry*, B. De Vivo, H.E. Belkin, and A. Lima, Editors. 2008, Elsevier: Amsterdam. p. 213-249.