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Phytoremediation of soil and groundwater contaminated with per- and polyfluoroalkyl substances (PFAS)

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic compounds recognised for their persistence, mobility and potential toxicity. This thesis examines the efficacy of phytoremediation as a potential technique for managing PFAS-contaminated soil and groundwater. First, an extraction and clean-up method was developed to measure PFAS in different plant tissues. A solid-liquid extraction method using methanol and ENVICarb cartridge as clean-up showed satisfactory performance and was selected for validation and application. PFAS were then analysed in plants, soil and groundwater at one landfill and three former fire training sites. The aim was to investigate the phytoextraction potential of trees growing at these sites. Plant tissue concentration and composition profiles highly depended on the soil and groundwater fingerprints. Birch and willow showed the highest PFAS concentrations in the field. Furthermore, the phytoextraction potential of five plants (i.e. sunflower, mustard, hemp, willow and poplar) was also investigated in pot experiments. Parameters such as species-specific uptake, bioaccumulation in different plant tissues, duration of PFAS exposure and effects of supplements were assessed. The supplements i.e. fertilizer, microbes and hormones had limited influence on plant concentration in some cases increased plant biomass, which in turn increased total mass PFAS removal by the plants. Willow and sunflower showed the highest PFAS removal efficiency of all investigated plants. The highest PFAS removal obtained was for short chain PFAS (C3 – C6), with up 34% removal by the plants after 90 days of exposure. These results can be useful for field application of phytoremediation. Further work is required to improve the efficiency of the method and to study the fate of PFAS in plant biomass following remediation.

Keywords: Bioremediation, emerging contaminants, microbial argumentation, phytohormones, poplar, willow, short-rotation coppice, sustainable remediation, PFAS, PFOS

Fytosanering av jord och grundvatten förorenade med per- och polyfluoralkylsubstanser (PFAS)

Sammanfattning

Per- och polyfluorerade alkylsubstanser (PFAS) är antropogena föreningar vilka är beständiga, rörliga och med en potentiell toxicitet. Denna avhandling undersöker effektiviteten hos fytoremediering som en potentiell teknik för hantering av jord och grundvatten förorenade med PFAS. Först utvecklades och validerades en metod för extraktion och rening för att mäta PFAS i olika vävnader hos växter. En metod med fast-vätske-extraktion med metanol och ENVICarb-patron för rening valdes på grund av dess tillfredsställande prestanda. PFAS analyserades vid en deponi och tre tidigare brandövningsplatser förorenade med PFAS för att undersöka fytoextraktionspotentialen hos träd som växte på dessa platser. PFAS-ämnen med kortare kedjor ackumulerades i skotten medan PFAS-ämnen med längre kedjor kvarstod bundna till rötterna. Björk och vide visade de högsta PFAS-koncentrationerna i fältstudierna. Fytoextraktionspotentialen hos fem växter (solros, senap, hampa, vide och poppel) undersöktes också i krukförsök. Parametrar som artspecifikt PFAS-upptag, exponeringstid, effekt av tillsatser och bioackumulering i olika vävnader hos växterna bedömdes. Tillsatserna d.v.s. gödningsmedel, mikrober och hormoner, ledde till en ökning av växtbiomassan, vilket i sin tur ökade den totala massreduceringen av PFAS genom växterna. Vide och solros visade den högsta effektiviteten av alla undersökta växter när det gällde avlägsnande av PFAS. Det högsta avlägsnandet av kortkedjade PFAS-ämnen (C3 - C6) uppnåddes, med upp till 34% reducering av dessa ämnen, av växterna efter 90 dagars exponering. Sammantaget visade alla testade växter en hög potential för fytoremediering, särskilt för kortkedjade PFAS-ämnen.

Nyckelord: Bioremediering, nya föroreningar, mikrobiell argumentation, fytohormoner, poppel, vide, snabbomsättningsskog, hållbar sanering, PFAS, PFOS.

Dedication

To my dad, Mwami Gerald M.M. Ssemanda!!!

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Nassazzi, W., Lai, F.Y. and Ahrens, L. (2022). A novel method for extracting, cleaning up and analysing per- and polyfluoroalkyl substances (PFAS) in different plant matrices using LC-MS/MS. *Journal of Chromatography B*, 1212, 123514.

II. Nassazzi, W., Lai, F.Y., Lewis, J., Jass, J., and Ahrens, L. Uptake of per- and polyfluoroalkyl substances by various plant species at four different contaminated sites in Sweden (manuscript).

III. Nassazzi, W., Wu, T.C., Jass, J., Lai, F.Y and Ahrens, L. (2023). Phytoextraction of per- and polyfluoroalkyl substances (PFAS) and the influence of supplements on the performance of short-rotation crops. *Environmental Pollution*, 333, 122038.

IV. Nassazzi, W., Guo, W., Bazabhe, Y., Tapase, S., Jaffe, D.B., Key, A.T., Lai, F.Y., Jass, J. and Ahrens, L. Phytoremediation of per- and polyfluoroalkyl substances (PFAS) using salix and poplar (manuscript).

The contribution of Winnie Nassazzi to the papers included in this thesis was as follows:

I. Planned the study together with the co-authors. Was responsible for laboratory analyses, data handling, interpretation, writing and submission.

II. Planned the study together with the co-authors. Responsible for laboratory analyses, data handling, interpretation, and writing.

III. As main supervisor of master student T.C. Wu, I designed the experiment with co-authors, and performed extensive data analysis, interpretation, writing and submission.

IV. Planned the study together with the co-authors. Had the responsibility for laboratory analyses, data handling, interpratation, and writing.

1. Introduction

A clean, safe, and wholesome environment is a fundamental human right that has increasingly gained global recognition. Consequently, exposure to toxic substances without consent becomes a human rights concern (UNEP 2019). Per- and polyfluoroalkyl substances (PFAS) is an emerging class of contaminants characterized by extremely high persistency, potential of bioaccumulation and toxicity to the environment and humans (Ahrens & Bundschuh 2014; Wang *et al.* 2017). Various regulations have been introduced to reduce PFAS emissions and exposure. Special emphasis has been placed on prohibiting long chain PFAS due to their high bioaccumulation and toxicity potential (EPA 2020). This has led to substituting long chain PFAS with short chain ones that are also still persistent and highly mobile (Ritter 2010). Short chain PFAS are very mobile and can contaminate drinking source areas, a key route of exposure for humans (Li *et al.* 2020). Historical and current emissions of long chain PFAS remain in soil, water and sediments, especially near their source areas (Ahrens *et al.* 2015). Remediation technologies are vital instruments that can reduce PFAS transport and its burden on the ecosystem. Phytoremediation, a process where plants absorb and assimilate contamination into their above-ground biomass or immobilize and reduce transportation of contaminates in the environment, is a potential PFAS remediation technique (EPA 2000). The technique has been greatly utilized for the remediation of heavy metals and nutrients from wetlands and other contaminated sites over the years (Mench *et al.* 2009; Mench *et al.* 2010). However, little is known about the feasibility of phytoremediation as a treatment technique for PFAS-contaminated soil and water.

2. Overall aim and specific objectives of the thesis

The thesis aimed to investigate and advance the understanding of phytoremediation as an effective and sustainable approach for the remediation of PFAS-contaminated environments.

Specific objectives were to:

- Develop an analytical method to quantify plant PFAS (Paper I).
- Investigate the PFAS phytoextraction potential of trees growing at contaminated sites (Paper II).
- Assess the mechanistic understanding of PFAS accumulation in plants from contaminated soil (Papers III and IV).
- Optimize PFAS uptake through i) assessing the phytoextraction potential of various plant species and ii) evaluating the impact of supplements on PFAS accumulation (Papers III and IV).

3. Background

3.1 Per- and polyfluoroalkyl substances

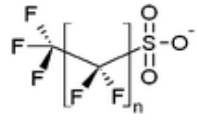
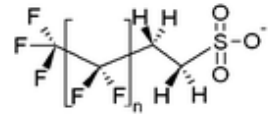
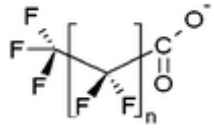
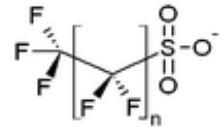
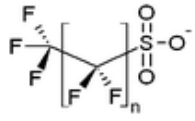
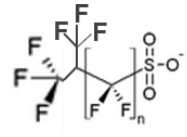
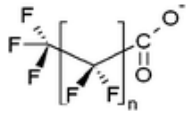
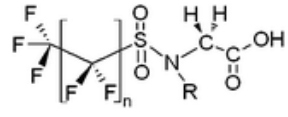
Per- and polyfluoroalkyl substances, or PFAS, are a diverse group of >4700 anthropogenic chemicals produced since the 1940s (Kissa 2001). They are characterised by a molecular structure with at least one fully fluorinated methyl (-CF3) or methylene (-CF2-) group (Table 1) (OECD 2021). The stability of the carbon-fluorine bonds generates recalcitrant compounds with low surface tension (Krafft & Riess 2015). Thus, several PFAS have been utilised in various industrial applications especially where water or oil repellency is required (OECD 2013). Common industrial applications of PFAS include producing electrical equipment, machinery manufacturing, building and construction, rubber, and plastic production. They are used as surfactants, firefighting foams, adhesives and sealants, cleaning and greasing solvents, air conditioners and finishing agents etc (Gluge *et al.* 2020). They can also be found in consumer products like cosmetics, non-stick cookware e.g. frying pans, food packaging, leather, cleaning products, pharmaceuticals, and textiles (Paul & Amin 2015; EPA 2021).

PFAS are characterised as either fully fluorinated compounds i.e. “per” or partially fluorinated unstable compounds i.e. “poly” fluoroalkyl substances that can be transformed into other stable forms (Table 1) (Buck *et al.* 2011). The fluorinated carbon backbone (hydrophobic tail) is often attached to a functional group (hydrophilic head). The fluorinated carbon back bone can also be linear or branched (with side groups attached to the main chain) in nature. Functional groups include anionic (e.g. sulfonates,

carboxylates and phosphates), cationic (e.g. ammonium) and neutral (glycols and sugars) moieties (Buck *et al.* 2012). Anionic PFAS are the most relevant category due to their extensive use and abundance in the environment. They can also exist as acids called perfluoroalkyl acids (PFAA) i.e. perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulphonic acid (PFSA) (Buck *et al.* 2011). PFAS are also categorised based on their chain length, i.e., short and long chained PFAS. When PFCA have 7 or more perfluorocarbon atoms, they are considered long chain PFAS. While PFSA are categorized as long chain PFAS when they contain 6 or more perfluorocarbon atoms. It is important to note that PFSA with the same carbon count as PFCA tend to have a higher potential for bioaccumulation and sorption to particles compared to PFCA, which explains the distinction in their definitions. Any other PFAS, excluding PFCA and PFSA, can be classified as long-chain if their perfluoroalkyl chain consists of 7 or more perfluorocarbon atoms (Buck *et al.* 2011). Aside the chain length, the functional group can also influence their sorption and bioaccumulation properties (Krafft & Riess 2015; Nguyen *et al.* 2020).

Some PFAS such as PFAA are acknowledged as chemicals of emerging concern due to their persistence, high mobility, bioaccumulative nature and potential toxicity to humans and other living organisms. PFAA are stable compounds that resist degradation at high temperature and chemical reaction with strong acids and bases (Krafft & Riess 2015). Biodegradation of PFAA is also limited and in some cases reported to be impossible especially because of the fluorinated moiety (Bolan *et al.* 2021b). PFAA and their precursors are ubiquitously distributed in the environment and have been found in almost all matrices and locations worldwide. Water soluble and volatile PFAS are transported to remote places by water currents and air transport. Some possible sinks include deep ocean water and sediment burial (OECD 2013; Brase *et al.* 2021). Long chained PFAA have been detected in human blood, lungs, liver, kidneys and urine (Daly *et al.* 2018; Fenton *et al.* 2020; Xu *et al.* 2020). They have also been found in sediments and biota such as fish (Leat *et al.* 2013; Abercrombie *et al.* 2019; Leon *et al.* 2020; Zheng *et al.* 2020). On the other hand, short chained PFAA are more mobile and can accumulate in plants and groundwater (Gobelius *et al.* 2017; Ghisi *et al.* 2019).

Table 1: Examples of Chemical structures of PFAS frequently found in the environment, n≥1

Perfluoroalkyl substance 	Polyfluoroalkyl substance 
PFCA 	PFSA 
Linear 	Branched 
Ionizable 	Neutral 

Sinclair *et al.* (2020) summarised the toxicity of PFAS on various aquatic and terrestrial organisms, i.e. zebrafish, trout, frogs, earthworms, vegetation, algae and humans. PFAS were observed to alter the sex, development and survival of the organisms mentioned above except vegetation. Some PFAS, for example, perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorobutanaote (PFBA), perfluoroooctanoate (PFOA) and 6:2 fluorotelomer sulfonate (6:2 FTSA), have been associated with high incidences of cancers like testicular cancer

among humans, immunotoxicity and kidney toxicity (Barry *et al.* 2013; Borg *et al.* 2013).

PFAS are directly introduced in the environment during their production and utilization, or indirectly emitted through the transformation of their precursors (Buck *et al.* 2011). Their sources in the environment can further be categorized as either point or non-point (diffuse) sources (Ahrens & Bundschuh 2014). Major point sources include wastewater treatment plants (WWTPs) (Wang *et al.* 2020; Golovko *et al.* 2021), manufacturing plants (Boiteux *et al.* 2017), landfills (Hamid *et al.* 2018; Masoner *et al.* 2020) and sites with firefighting activities e.g. airports and oil storage depots (Ahrens *et al.* 2015; Baduel *et al.* 2015b; Dauchy *et al.* 2017). Major nonpoint sources include atmospheric deposition (Barber *et al.* 2007; Cousins *et al.* 2022) and surface runoff from contaminated streets and fields (Codling *et al.* 2020). Soil, surface and groundwater contaminated with PFAS form major direct and indirect exposure pathways of these substances to humans and other living organisms in the ecosystem. Examples of direct PFAS exposure pathways to organisms include drinking water from contaminated sources, consumption of food grown on PFAS-contaminated soils, and dust (Vestergren & Cousins 2009; Banzhaf *et al.* 2017; Ghisi *et al.* 2019).

3.2 Land Remediation Techniques

As a result of the reports mentioned above on potential toxicity, persistence and mobility, various risk reduction approaches are being explored. Some PFAS manufacturers and users have volunteered to limit unessential production and use. Governments and regulatory agencies are also increasingly establishing stricter guidelines on the levels of PFAS in food, (drinking)water and soil (OECD 2015). In addition, large quantities of PFAS are still being emitted from old contaminated sites that act as PFAS reservoirs (Filipovic *et al.* 2015). These have emphasized the necessity for remediation. Several recent studies have focused on developing new or optimising existing PFAS remediation technologies. However, PFAS remediation is still challenging because of their persistance and existence as mixtures in the environment. The constituent PFAS in the mixtures possess variable physiochemical properties such as stability, sorption to surfaces and solubility. This has posed a significant challenge in identifying a comprehensive technique for managing all PFAS simultaneously

(Kucharzyk *et al.* 2017; Mahinroosta & Senevirathna 2020; Naidu *et al.* 2020). PFAS remediation technologies can be categorised according to their mode of action. These include; concentration, adsorption and degradation (Bolan *et al.* 2021b).

Concentration technologies isolate PFAS from a large mass/volume of contaminated media to produce a concentrate that is easier to dispose of or manage. Examples of the methods include foam fractionation (generation of bubbles to extract PFAS), membrane filtration and phytoremediation (Gobelius *et al.* 2017; Lee *et al.* 2022; Smith *et al.* 2023). These methods are effective for treating large volumes/areas of contaminated media but have the potential to generate secondary waste. Adsorption techniques are a group of methods that use a sorbent to immobilize and/or remove PFAS from contaminated media. This often relies on the hydrophobic properties of PFAS to trap and restrict their mobility. Such methods include stabilization and solidification using typically activated carbon, biochar or ion exchange (Sorengard *et al.* 2021; Sørmo *et al.* 2021; Liu *et al.* 2022). Degradation technologies refer to methods that breakdown or transform PFAS into preferably other non-harmful products. Examples include thermal treatment, oxidation processes, ball milling and bioremediation which agents such as heat, oxidative agents, steel balls and microorganisms are used to degrade PFAS (Zhang *et al.* 2013; Merino *et al.* 2016; Winchell *et al.* 2020).

Most of the above mentioned techniques are still under development and require further optimization to become effective PFAS remediation solutions. Only a few technologies have been tested in the field or successfully commercialized (Mahinroosta & Senevirathna 2020). This thesis exclusively concentrates on investigating phytoremediation as a potential method to explore its efficacy and applicability in PFAS remediation practices.

3.3 Phytoremediation

Phytoremediation is a reclamation technology that uses plants to clean-up contaminated water, soil, or sludge. It is an eco-friendly and cost-effective approach that relies on solar energy. Phytoremediation involves various mechanisms such as accumulation (phytoextraction), immobilisation (phytostabilization), toxin metabolism (phytodegradation), microbial degradation enhancement (phytostimulation), and contaminant volatilization

(phytovolatilization) (Figure 1). These mechanisms collectively contribute to the effectiveness of phytoremediation as a remediation technique (EPA 2000; Arthur *et al.* 2005). Remediation of heavy metals commonly occurs through phytoextraction while photodegradation is commonly relevant during the remediation of organic compounds (Mench *et al.* 2010). PFAS are recalcitrant compounds that cannot be biodegraded (Mahinroosta & Senevirathna 2020). Therefore, phytoextraction is potentially the most suitable mechanism for PFAS remediation.

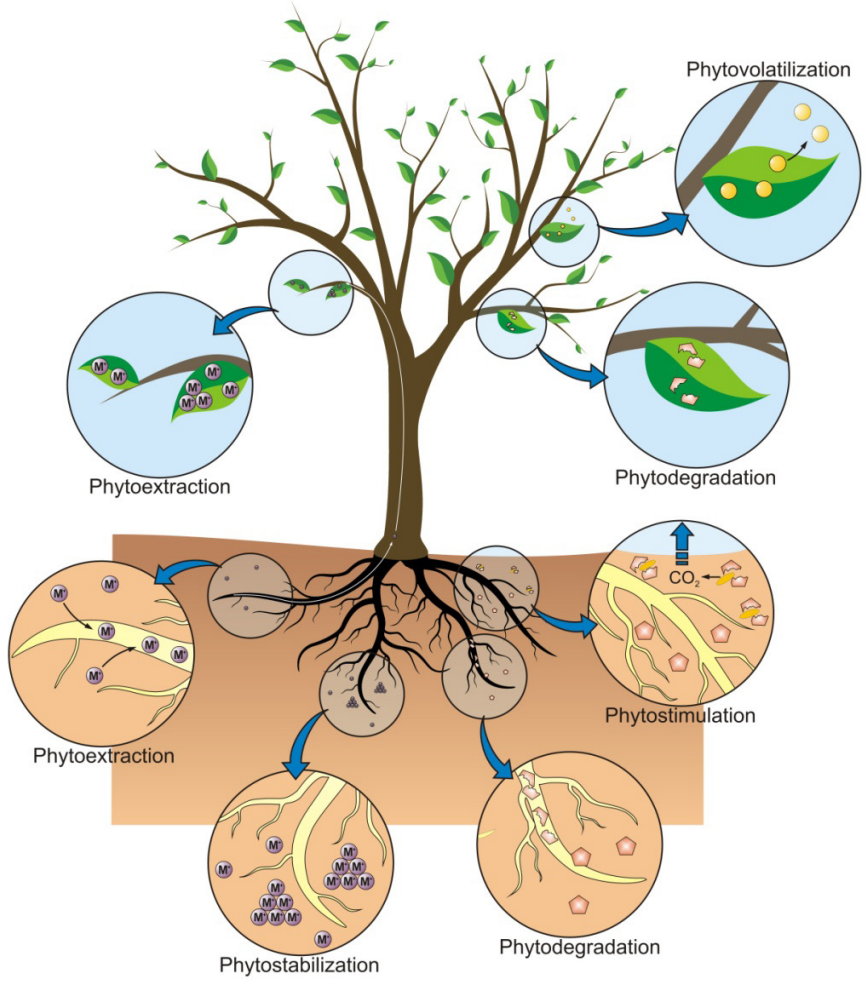


Figure 1: Mechanisms of phytoremediation. *Adopted from Favas et al. (2014)*.

PFAS uptake and distribution in plants

The mechanisms of PFAS uptake and distribution in plants are not yet properly understood but some studies have attempted to investigate the underlying processes (Felizeter *et al.* 2012; Wen *et al.* 2013; Qian *et al.* 2023). Plants involuntarily absorb PFAS from contaminated soil or water as they take up water and other nutrients essential for their growth through the roots. PFAS uptake by plant roots can either be through active transport or passive-diffusive processes (Wen *et al.* 2013). However, passive-diffusive processes are considered the most common uptake mechanism. This mechanism comprises of two components, i.e. equilibration of the aqueous phase in the plant root with the concentration in the surrounding solution and sorption of the chemical onto lipophilic root solids that can include lipids in membranes and walls (root epidermis) (Collins *et al.* 2006). Another possible root uptake mechanism of PFAS is the carrier-mediated processes, i.e. active or facilitated passive absorption. In a study where maize seedlings were cultivated in a PFOA and PFOS-contaminated nutrient solution, absorption of PFOA and PFOS was not competitive. This led to the proposition that PFOA and PFOS do not share a common transport mechanism. Uptake of PFOA by the maize root was an energy dependent active process, and anion channels may be involved in the uptake. On the other hand, PFOA uptake was a carrier-mediated passive process via aquaporins and an anion channel in root cell membranes (Wen *et al.* 2013).

On acquisition by the root, the PFAS follow the same transport pathway as water and nutrients, i.e. apoplastic pathway (diffusion between extracellular spaces), symplastic (movement through the plasmodesmata) and transcellular (movement though the plasma membrane) to the xylem. In the innermost layers of the root is the endodermis that protects for the plant from acquiring toxins and pathogens (Waisel 2002; Geldner 2013). The process of differentiation within the endodermis results in the development of two crucial structures: the Casparian strip, which blocks the apoplastic water transport pathways, and suberin lamellae, responsible for blocking the transcellular pathway (Doblas, 2017). It is assumed that the Casparian strip inhibits the transfer of longer chained PFAS from the root to the xylem due to their lipophilicity and size (Costello & Lee 2020). In the xylem, the transpiration stream creates a pressure used to translocate the compounds (especially the short chain PFAS) through the shoot to the leaves (Felizeter

et al., 2014). Longer chain PFAS have been found to accumulate in the roots mainly. Furthermore, an additional accumulation mechanism for roots is PFAS sorption to the surface tissue of the roots (Felizeter *et al.* 2014).

Factors affecting PFAS uptake and distribution

PFAS uptake and distribution in plants is highly influenced by their physicochemical properties i.e. perfluoroalkyl chain length and functional group. Both properties are strongly associated to the compounds' hydrophobicity and solubility. High hydrophobicity limits PFAS solubility, bioavailability and transportation within the plant while increasing sorption onto root surfaces and soil particles (Algreen *et al.* 2014; Qian *et al.* 2023).

Various plant related factors have also been demonstrated to determine PFAS uptake and distribution. Variations in PFAS bioaccumulation have been observed among crop species and genotypes (Xiang *et al.* 2018; Chen *et al.* 2019). Plants with high lipid: protein content, biomass production, and root exudates with high low molecular weight organic acids as well as amino acids have been proposed to have high PFAS affinity (Xiang *et al.* 2018; He *et al.* 2023; Qian *et al.* 2023).

The plant growth media can also significantly influence PFAS uptake. Soil factors such as pH, salinity, organic matter content, texture, growth media (water or soil) affect PFAS sorption and can limit bioavailability (Zhao *et al.* 2016; Xiang *et al.* 2018; Zhao *et al.* 2018). PFAS concentration was demostrated to be positively correlated with PFAS uptake (Wen *et al.* 2013).

3.4 PFAS analysis in plants

Analysis involves the determination and quantification of PFAS in various plant tissues. Precise and accurate measurement is important for assessing the uptake, accumulation, and potential effects of PFAS on plant health, food safety and monitoring. The analysis typically begins with sample collection and preparation, followed by extraction and clean-up (purification) of sample extracts for PFAS analysis. Collected plants are often cleaned, dried and homogenised before extraction (Felizeter *et al.* 2012; Gobelius *et al.* 2017; Muschket *et al.* 2020). Typically employed extraction techniques include solvent solid extraction (SLE), matrix solid phase dispersion (MSPD) and ion pairing extraction (IPE) to isolate PFAS from the plant matrix.

During SLE, solvents such as methanol and acetonitrile or their solutions with ammonium acetate, sodium hydroxide (NaOH) or water are often used to extract the targeted PFAS from the tissues (Bizkarguenaga *et al.* 2016; Navarro *et al.* 2017; Gobelius *et al.* 2018; Muschket *et al.* 2020). The process is often coupled with sonication or shaking to increase extraction efficiency. However, the extraction process is not very selective and is often combined with a purification step. Purification steps, such as solid-phase extraction (SPE) or dispersive solid-phase extraction (dSPE), are often performed to remove residual matrix components and improve selectivity and sensitivity. During SPE, target analytes interact with the sorbent (e.g. Oasis WAX), allowing for their retention while impurities are washed away (Bizkarguenaga *et al.* 2016; Eun *et al.* 2020). On the other hand, dSPE involves the addition of a mixture of solid-phase sorbents (e.g. activated carbon and magnesium sulphate) directly to the sample extract, to adsorb impurities. At the same time, the target analytes remain in solution. In some cases, both sample clean-up methods are employed (Gobelius *et al.* 2017; Muschket *et al.* 2020).

MSPD is a sample preparation method that involves simultaneous extraction and clean-up. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is the most common application of this method originally developed for pesticide analysis in fruits, and has been applied for PFAS analysis in plants (Stahl *et al.* 2013; Zhou *et al.* 2019). An extraction solvent typically, acetonitrile dissolves the targeted analytes which are then subject to the dSPE clean-up step. The method consumes less solvent and is simple to apply.

Some studies have employed ion pair extraction (IPE) using alkaline tetrabutylammonium hydroxide (TBAH), followed by liquid extraction using methyl ter-butyl ether (MTBE) (Felizeter *et al.* 2012; Wen *et al.* 2013; Zhao *et al.* 2018). This is proceeded by SPE clean-up using a mixture of Florisil and ENVICarb sorbent (Felizeter *et al.* 2012). However, extraction with MTBE, has been reported to have lower recoveries especially for longer PFAS (Xiang *et al.* 2017).

Subsequently, instrumental analysis is performed most commonly using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) (Felizeter *et al.* 2012; Gobelius *et al.* 2017; Gredelj *et al.* 2020) or high-resolution mass spectrometry (HRMS) (Just *et al.* 2022). These techniques allow for identifying and quantifying specific PFAS in plant

samples, providing insights into their presence and levels in different plant tissues.

Analyzing PFAS in biological matrices such as plants is challenging due to low concentrations, complex plant matrices, and potential matrix effects that can interfere with accurate measurements (Nakayama *et al.* 2019). Furthermore, several of the applied plant preparation and analytical protocols used were developed to analyse other sample matrices or compounds (Hansen *et al.* 2001; Powley *et al.* 2005; Mazzoni *et al.* 2016). Additionally, sample size and homogeneity, as well as background contamination, need to be carefully addressed during analysis.

3.5 Optimizing phytoremediation of PFAS.

A common reported shortfall of phytoremediation is the long duration required to restore the contaminated media to safe levels (Arthur *et al.* 2005). Various factors have been reported to improve the effeciency of the method especially for removal of heavy metals that can also be investigated for PFAS (Bolan *et al.* 2021a).

Strategic plant selection can improve the effeciency of the method. Hyperaccumulators (highly contaminant-concentrating plants) can be used for continuous or natural phytoextraction with high biomass plants (rapid growth and large plant material) can be utilized. Ideal plant candidates should grow on contaminated sites, accumulate or detoxify contaminants, and be easily integrated into phytoremediation efforts. Potential candidates can be identified by studying naturally occurring vegetation at contaminated sites (Mench *et al.* 2010; Yin *et al.* 2017).

Soil amendments, such as inorganic fertilizers, biowastes (compost, biosolids, biochar), hormones, and microorganisms, have been studied to enhance phytoremediation of heavy metals (Mench *et al.* 2010; Bolan *et al.* 2021b). These amendments improve plant survival and vigor, thus increasing phytoremediation efficiency. They modify the rhizosphere leading to mobilization or immobilization of contaminants (Haider *et al.* 2021). Inorganic fertilizers can influence contaminant speciation and bioavailability; acidic fertilizers like ammonium sulphate may increase uptake of positively charged contaminants, while alkaline fertilizers can reduce their bioavailability (Lan *et al.* 2020). Additionally, amendments like manures, compost, and biochar improve soil fertility, enhance soil microbial

diversity, and confer protection against plant infections (Radziemska *et al.* 2021).

Application of phytohormones has been noted to improve uptake of contaminants by affecting plant uptake of nutrients and water and stimulating photosynthesis. For instance, application of cytokinins increased the zinc and lead in sunflower shoots by 35% and 25%, respectively (Tassi *et al.* 2008). Foliar application of gibberellic acid and indoleacetic acid (IAA) along with fertilizer increased the phytoextraction of cadmium. This could be due to the increase in plant biomass that had strong correlation with contaminant accumulation (Hadi *et al.* 2021). Phytoextraction of toluene and formaldehyde was also enhanced by application IAA by 20% and 40% respectively (Ullah *et al.* 2020). Generally, phytohormones increase the production of plant defensive substances and chlorophyll which increased plants' survival and biomass production (Saleem *et al.* 2020; Chen *et al.* 2021; Hadi *et al.* 2021).

Over the years, plants have developed a wide range of mutualistic associations with bacteria and fungi, which enable plants to grow in stressful and nutrient deficient conditions. The composition of microbes is greatly determined by soil conditions, plant exudates and development of mutualistic associations, i.e. nitrogen fixing nodules or mycorrhizal root tips. Although there is a variety of microorganisms surrounding plants, only a subset of these organisms is able to colonize and form mutualistic relations with plants (Martin *et al.* 2017). The use of microorganisms to enhance phytoremediation is commonly referred to as microbial-assisted phytoremediation. Exogenous inoculation of plants with microorganisms has been observed to increase and improve phytoremediation (Escalante-Espinosa *et al.* 2005; Ye *et al.* 2014). The presence of PFAS is thought to increase the diversity of microbes in the soil while some microorganisms can also accumulate PFAS (Li *et al.* 2021). Diverse microbial communities can enhance contaminant retention in roots and help plants acquire sufficient nutrients and recycle organic matter (Radziemska et al., 2021).

Some studies report that applying amendments is more valuable to phytostabilization than phytoextraction. This is due to increased immobilization of contaminants in the rhizosphere and sorption onto root surfaces (Lan *et al.* 2020). Therefore, when selecting amendments, one should consider the type of phytoremediation strategy one intends to accomplish. Another common challenge associated with amendments

include exacerbating contamination as some amendments like biowaste are sources of PFAS and pathogenic microorganisms (Bolan *et al.* 2021a).

4. Materials and Methods

4.1 Overall study design

This thesis involves a combination of a field experiment and two pot experiments. The field experiment was used for application of the developed PFAS analytical method (**Paper I**) and to establish PFAS uptake in trees at contaminated sites **(Paper II)**. On the other hand, the pot experiments provided controlled environments to study specific factors influencing phytoremediation performance. One pot experiment focused on the effects of varying plant species and supplements on plant uptake **(Paper III)**. The second pot experiment explored temporal changes in PFAS plant accumulation from soil, addition of supplements and a mass balance was performed to investigate the fate of PFAS in the experimental setup **(Paper IV).**

4.2 Investigated plants

In **Paper I**, plant tissues from 10 plant species were collected from non PFAS contaminated sites in Uppsala. Studied plants included i.e. silver birch (*Betula pendula*), strawberry (*Fragaria spp*), Scots pine (*Pinus sylvestris*), rowan (*Sorbus aucuparia*), willow (Salix spp), Norway spruce (*Picea abies*), poplar (Populus spp), bird cherry (*Prunus padus*), common oak (*Quercus robur*), sycamore maple (*Acer pseudoplatanus*) growing at known locations without PFAS contamination in Sweden. The collected plant samples were pooled in two groups i.e leaves and needles (Group I) and twigs, stems and roots (Group II). The two sample groups were then used for method

development. Plant (birch and spruce) and soil samples from a PFAS-contaminated landfill site in Sweden were analysed for application.

In **Paper II,** plant, soil and water samples were collected from four PFAS-contaminated sites within the Stockholm-Uppsala region in Sweden. The sites included a landfill, a former military airport, and two fire training sites. Plant samples from seven plant species were assessed i.e. birch, pine, willow, spruce, poplar, rowan, alder (Alnus spp) and whitebeam (*Sorbus intermedia*).

In **Papers III** and **IV,** pot experiments were conducted in the greenhouse at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Experimental conditions in the greenhouse were set to 22 ºC during the day and 18 ºC at night, 16/8 h of light/dark cycle, 150 µmol light intensity and 50-60% relative humidity. PFAS-spiked organic potting soil was prepared to achieve a theoretical concentration of 1 mg kg^{-1} of each individual PFAS (**Paper III**) and 500 µg kg^{-1} and 250 µg kg^{-1} of each individual PFAS for willow and poplar respectively (**Paper IV**). In **paper III**, six-week seedlings of sunflower (*Helianthus annuus*), mustard (*Brassica juncea*) and hemp (*Cannabis sativa*) were planted in 1 kg wet weight (ww) of the PFAS-spiked soil over a period of 90 days. Tested supplements included: an inorganic fertilizer, commercial microbial supplement or a mixture of the inorganic fertilizer and microbial supplement. These were mixed in the irrigation water and applied throughout the experiment. In **Paper IV**, rooted cuttings of both willow (*Salix miyabeana*) and poplar (*Populus trichocarpa*) were also tested for their phytoextraction potential. The cuttings were also exposed to treatments including a microbial supplement and a phytohormone (naphthalene acetic acid) over a period of 90 days. In addition, the temporal changes in PFAS accumulation were investigated by harvesting willow grown on PFAS-spiked soil every month over a seven-month duration (210 days).

4.3 Chemicals

Throughout the studies, up to 24 PFAS were investigated comprising of C_3-C_{13} PFCA (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA), C_4-C_{10} PFSA (i.e. PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS), perfluorooctane sulfonamide (FOSA), methyl- and ethylperfluorooctane sulfonamidoacetic acid FOSAAs

(MeFOSAA, EtFOSAA), and 4:2, 6:2, 8:2 fluorotelomer sulfonates (4:2 FTSA, 6:2 FTSA, 8:2 FTSA). The target PFAS were both environmentally relevant and have shown potential uptake by plants (Gobelius *et al.* 2017; Ghisi *et al.* 2019). For quantification, their corresponding internal standards were used if available. Details can be found in each paper.

4.4 Sample preparation and analysis

All plant tissues and soil samples studied in this work were prepared using the method developed in **Paper I**.

In brief, the plant samples were cleaned using tap water, MilliQ water (2 times) and a mixture of MilliQ water and methanol (50:50; v,v; 2 times), and stored at -20 °C until extraction and analysis. Plant tissues were extracted using three cycles of extraction solvent. In each cycle, 3 mL of the extraction solvent was introduced, and vortexed for 1 min, followed by ultrasonication for 30 min, and centrifugation at 3000 rpm for 15 min. The resulting supernatants were combined, filtered through an ENVI-Carb cartridge (1 g, 12 mL), and collected in 15 mL polypropylene (PP) tubes. Subsequently, the cartridges were rinsed with 1 mL of the extraction solvent, and any residual solvents trapped within the cartridge were expelled by applying air pressure with a syringe. The wash solution was collected in the same tube as the purified extract in the PP tubes. The obtained extracts were concentrated to a final volume of 100 µL using nitrogen gas, then adjusted to a total volume of 500 µL by adding methanol. The reconstituted extracts were transferred to injection vials suitable for LC-MS/MS analysis.

Soil samples were also extracted as described above. Clean-up was performed using 250 mg ENVI-Carb cartridges. The extracts were then concentrated into 500 µL before instrumental analysis.

For **Papers I** and **IV**, methanol was used as the extraction solvent and analysed using the ultra-high pressure LC (SCIEX ExionLC AC system) coupled to MS/MS (SCIEX Triple Quad™ 3500) (UHPLC-MS/MS). The analytes were separated using the Phenomenex Gemini C18 column (50 mm × 2 mm, 3 µm). For **Papers II** and **III**, acetonitrile was used as the extraction solvent and analysed with UHPLC (Thermo Scientific LC system) coupled to a MS/MS (Quantiva TSQ; Thermo Fisher). The analytes were separated using a BEH-C18 column (1.7 µm, 50 mm, Waters). Details on the instrumental parameters and settings are available in each paper.

4.5 Quality Control and Quality Assurance

Quality control and assurance were done to ensure reliability and accuracy. Rigorous experimental protocols are followed, including cleaning all materials before utilization (rinsing with methanol or burning glass and metalware). Laboratory blanks, field blanks, replicates and reference samples were assessed. Quantification using calibration curves included in each run after every 10-15 samples were done (for details see **Paper I**).

Laboratory and field blanks were included in every extraction batch (10-15 samples). The method detection limits (MDL) and method quantification limits (MQL) (**Papers I** and **III**) were determined using a signal-to-noise (S/N) ratio of 3 and 10, respectively, in matrix spiked samples at low concentration levels (c = 5 ng g^{-1} dry weight (dw)). Matrix effects were determined as a ratio of the response of analytes in the presence of the matrix (post-extraction spike) to the response obtained from a pure solvent standard. The absolute recovery was determined as the percentage ratio of the peak area of pre-spike to the average peak area of post-spiked samples (for details see **Paper I**).

5. Results and discussion

5.1 PFAS analysis in plant matrices (Paper I)

An extraction and clean-up method was developed to analyse 24 different PFAS in plants. Six different extraction conditions were tested i.e. methanol, acetonitrile, a mixture of methanol and acetonitrile (50:50), methanol with 0.1% formic acid, acetonitrile with 0.1% formic acid and methanol with 400 mM ammonium acetate (buffer). There were statistically significant differences (ANOVA, $p<0.05$) amongst PFAS absolute recoveries of the tested extraction conditions for both sample groups I (leaves and needles) and II (stem, twigs and roots). Methanol showed acceptable performance (absolute recovery between 70-130%) for most PFAS in both sample groups and was therefore selected as the suitable extraction condition. Although acidification is often observed to improve extraction of polar organic substances in biological samples (Baduel *et al.* 2015a; Dürig *et al.* 2020), only slight improvements where observed in this study. Furthermore, acetonitrile a commonly utilised solvent for extraction of biological samples including plants (Navarro *et al.* 2017; Eun *et al.* 2020; Muschket *et al.* 2020), was observed to have the least absolute recoveries for the selected PFAS compared to methanol in this study.

A comparison amongst three clean-up methods was made i.e. ENVICarb cartridge, ENVICarb cartridge followed by WAX SPE and ENVICarb powder. Among the tested methods, ENVICarb cartridge was the most effective method in reducing matrix interferences, providing clean extracts without compromising the absolute recoveries (i.e. on average 86 ±11% and 85 ±11% for groups I and II). The method was also efficient, easy to use and thus was selected for validation and application for PFAS analysis of plants.

The clean-up using ENVICarb cartridge followed by WAX SPE was observed to have reduced matrix effects but compromised the absolute recoveries, with average absolute recoveries of 60 ± 7.9% and 72 ± 8.4% for group I and II, respectively. This is in agreement with results from previous studies where additional clean-up was used with WAX-SPE and CUNAX22Z-SPE (Muschket *et al.* 2020). The reduction in absolute recovery could be due to PFAS sorption to different materials used during sample preparation. Furthermore, clean-up with ENVICarb powder had the least performance for both matrix effects (−65 ± 40% and −78 ± 24% for groups I and II, respectively) and absolute recoveries (53 ± 8.2% and 65 ± 4.7% for groups I and II, respectively). The protocol used for sample clean-up using ENVICarb powder was adopted from a soil sample preparation protocol (Dalahmeh *et al.* 2018). This highlights the difference between method performance for PFAS analysis in soil and plant matrices and the need for extensive method optimization for analysis of PFAS in plant matrices.

5.2 PFAS uptake in trees at contaminated sites (Paper II)

PFAS distribution was assessed at four contaminated sites, i.e. landfill, former military airport, and two fire training sites (A-D). At each site, samples were collected from different areas (A1-2, B1-3, C1-3, D1-4). In total, 14 PFAS were detected that were categorised as; short chained PFCA (PFBA, PFPeA, PFHxA, PFHpA), long chain PFCA (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA), short chain PFSA (PFBS) and long chain PFSA (PFHxS, PFHpS and PFOS).

As illustrated in Figures 2-3, there was a variation in PFAS concentration and composition between the sites and within the different areas of the sites. Elevated PFAS concentrations were observed in groundwater and soil at all sites compared to background sites (Gobelius *et al.* 2018; Sörengård *et al.* 2022). PFAS concentration was highest in the soil and groundwater of site B and lowest at site A. Samples from areas B1, C1, D3 and D4 had the highest PFAS levels. The high PFAS concentration at area B1 can be explained by the fact that this site was a willow bed irrigated with landfill leachate for phytoremediation of heavy metals, while D3 and D4 were close to the PFAS source zones (Niarchos *et al.* 2023). These areas were, therefore, some of the

hotspots at their corresponding sites from where the PFAS concentration would diffuse to other areas. This agrees with previous studies in the literature identifying, landfills (Hamid *et al.* 2018) and fire training sites (Ahrens *et al.* 2015; Filipovic *et al.* 2015) as main point sources of PFAS to their surrounding environment. Lower PFAS concentrations were observed at the other areas of the contaminated sites.

Variations in PFAS composition in soil and groundwater were observed amongst the different sites and within different areas of the same site (Figures 2-3). Longer chain PFCA and PFSA were dominant in soil (i.e. up to 80% of ∑PFAS), while their shorter chain counterparts were mainly found in the groundwater (i.e. up to 90% ∑PFAS). This has been highly reported in the literature (Filipovic *et al.* 2015; Gobelius *et al.* 2017). The trend is strongly associated with increased PFAS sorption to sediment and organic matter with increased perfluoroalkyl chain length leading to reduce mobility and leaching to groundwater (Higgins & Luthy 2006; Campos Pereira *et al.* 2018).

For plants, PFAS concentration was generally highest in the foliage and fruit of the different plant species across all the sites (Figure 2). Sampling at site C was performed during late fall (November), therefore, no leaf samples could be collected from the deciduous plants at this site. The lowest PFAS concentration was observed for roots. Both PFAS concentration and composition in the plants highly corresponded with the PFAS concentration and composition of the soil and groundwater. PFAS concentration in the foliage, fruit, twig and stem (shoot) was predominantly made of the short chain PFAS. Some exceptions were noted, as plants on sites D3 and D4 showed higher concentrations of longer chain PFAS (i.e. up to 86% in leaves and 97% in roots). This can be mainly attributed to the areas' PFAS fingerprints in soil and groundwater (Niarchos *et al.* 2023). Preferential accumulation of short chain PFAS in plant shoots has been observed in various trees and other plant species (Zhou *et al.* 2019; Qian *et al.* 2023; Würth *et al.* 2023). It has been proposed that PFAS root uptake is influenced by the equilibrium between the root sap and the soil solution and the hydrophobicity of longer chain PFAS (Qian *et al.* 2023). Short chain PFAS are often more water soluble, bioavailable, easily penetrating the roots, and are transported upwards in the plants. However, the high fractions of longer chain PFAS in soil and/or groundwater at some investigated sites in this study led to increased uptake of these homologues. This could be due to an

increased concentration gradient between the plant root and the surrounding media. It could also be due to reduced interactive effects and competition from other PFAS. This result demonstrates the potential of phytoremediation for treatment of longer chain PFAS, that needs to be further explored. Furthermore, there also was a trend of substituting long chain PFAS with short chain PFAS (Wang *et al.* 2013). Therefore, the high affinity for short chain PFAS by plants also demonstrates the potential of phytoremediation for future field applications as most of the existing methods are unsuitable for treating short chain PFAS.

When comparing the uptake of PFAS in plant foliage, PFAS concentration was highest in the birch (34-476 ng g^{-1} dw) > willow (298 ng g^{-1} dw) > pine (13-179 ng g^{-1} dw). Previous screening studies in plants at PFAS-contaminated sites have revealed high PFAS concentrations in these species (Gobelius *et al.* 2017; Würth *et al.* 2023). The results demonstrated that plants at contaminated sites are highly capable of accumulating PFAS.

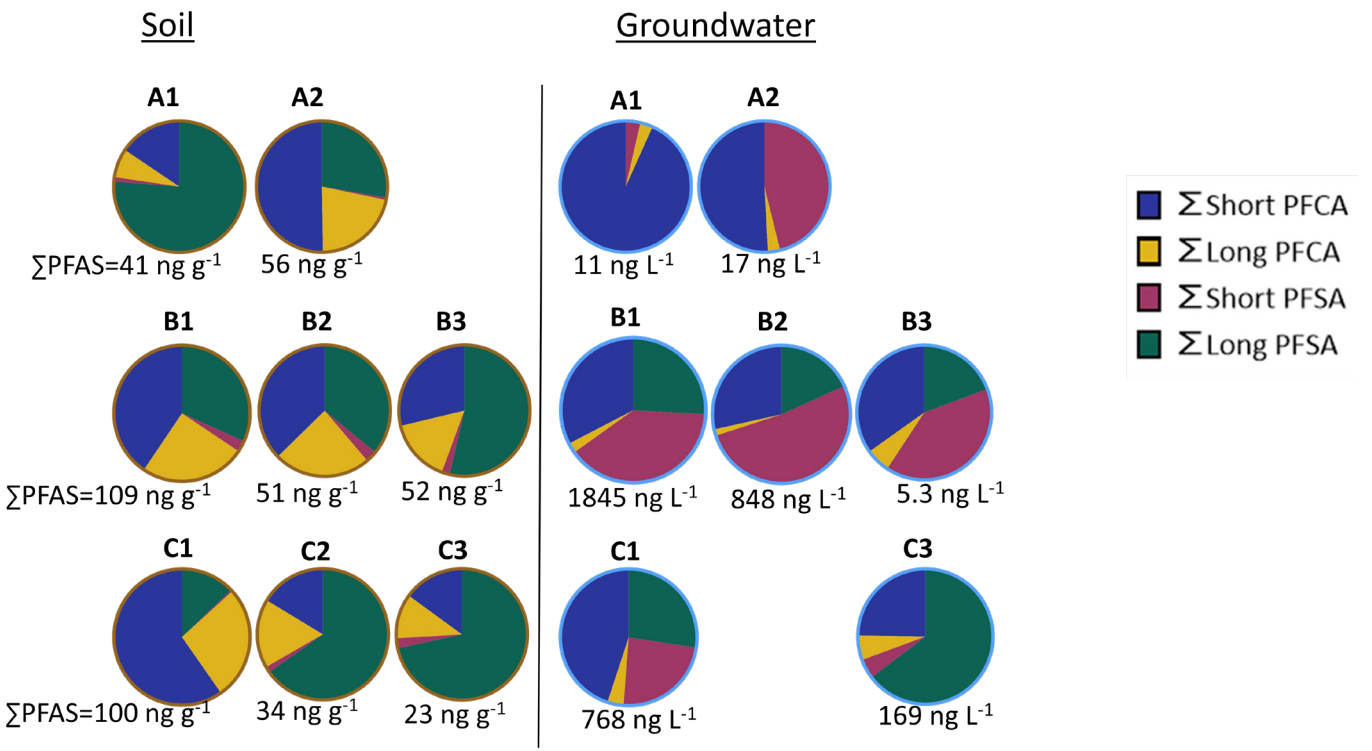


Figure 2: PFAS fingerprint at the PFAS-contaminated sites (A-C) with sampling areas (1-3) in the Stockholm-Uppsala region of Sweden.

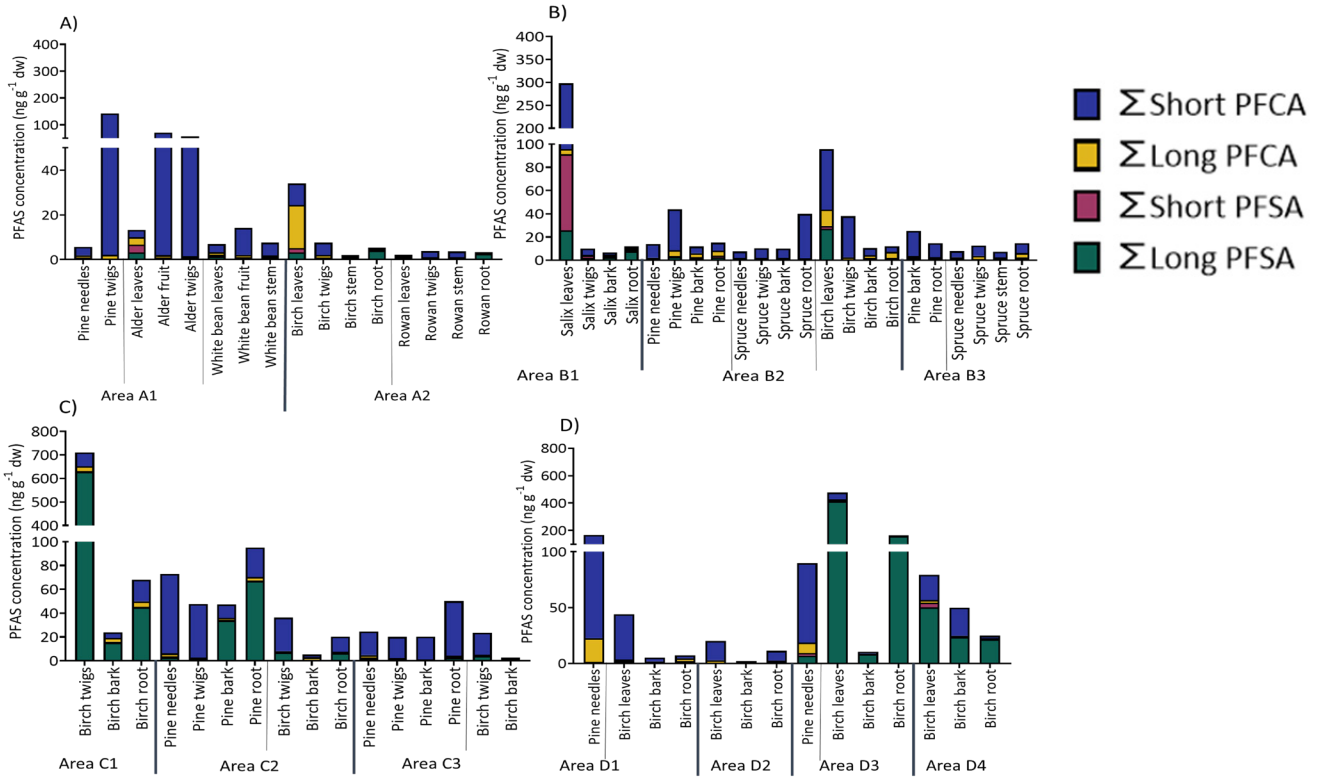


Figure 3: PFAS concentrations in the plant tissues collected at four different PFAS-contaminated sites (A-D) with sampling areas (A1-D4).

5.3 Optimizing PFAS phytoextraction (Papers III and IV)

Under controlled greenhouse conditions, PFAS accumulation was investigated for five plants (i.e. sunflower, mustard, hemp, willow and poplar) that have been previously investigated for phytoremediation of various pollutants, especially for heavy metals (Vangronsveld *et al.* 2009).

5.3.1 Temporal changes in PFAS uptake (Paper IV)

The concentration of 15 spiked PFAS was monitored in plants over 210 days. A slight increase in PFAS concentration was observed between 30 to 60 days. After 90 days, the concentration increased to a maximum for all PFAS. The highest increase was observed for PFBA (to an average concentration of 38±19 µg g^{-1} dw), PFPeA (31±14 µg g^{-1} dw), and PFBS (11±4.6 µg g-1 dw), (Figure 4). This could probably be due to increased plant water uptake and rapid growth. After this period, concentration was noted to decline and then plateau for several PFAS. Over time reduced available PFAS in soil, especially the short chain PFAS, were observed. This could mainly be due to leaching (see section 4.4). The observed dip/level off in plant concentration could also probably be due to PFAS dilution with in plant tissues. Previously, studies on temporal changes have been performed for short durations (a few hours to days) and often in hydroponics setups (Wen *et al.* 2013; García-Valcárcel *et al.* 2014). Comparing the results would be challenging due to mechanisms such as sorption to soil particles that could impact the outcomes but are absent in hydroponics experiments. Nevertheless, there were clear observations of linearly increased PFAS uptake with time for grass (García-Valcárcel *et al.* 2014). Another hydroponics study on maize revealed that PFOA and PFOS uptake peaked within the first 20 hours of exposure and plateued due to saturation (Wen *et al.* 2013).

Evaluating temporal changes in total mass PFAS uptake revealed an increase in accumulation with time from 9.8 to 594 µg dw from day 30 to day 210. A significant linear relationship (R^2=0.84, $p<0.0001$) between plant biomass a time was observed, which contributed to a continuous increase in PFAS mass accumulation. The results confirm the importance of biomass on PFAS uptake and phytoextraction (He *et al.* 2023).

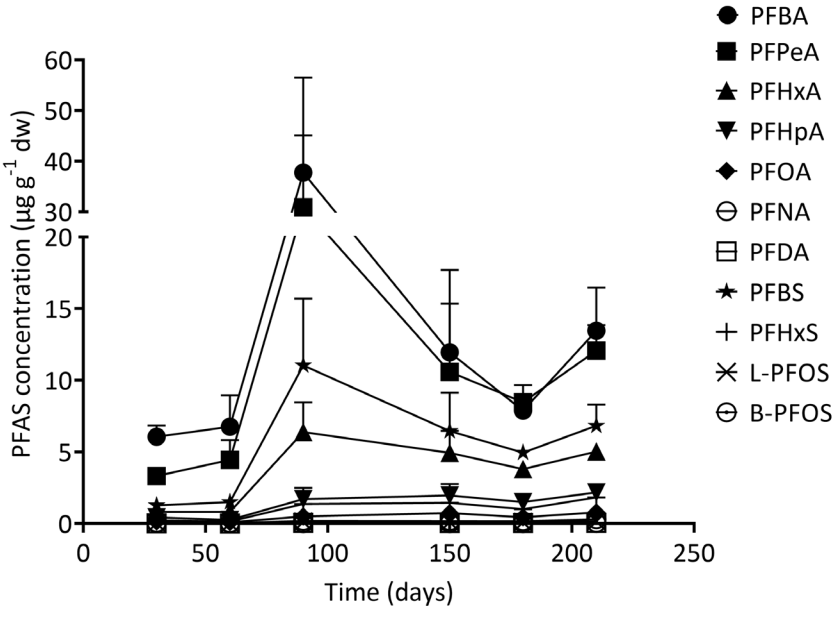


Figure 4: Temporal changes of individual PFAS in willow shoot (n =3) grown in PFAS spiked soil over 210 days.

5.3.2 PFAS accumulation in plants commonly used for phytoremediation

The bioconcentration factor (BCF) was used to assess the PFAS hyperaccumulation potential of the investigated plants after 90 days of exposure (Figure 5). It was determined as the ratio between plant concentration to soil concentration. It is generally agreed that a BCF value >1 indicates a plant's ability to accumulate a contaminant, while a BCF value >10 indicates a plant's ability to hyperaccumulate contaminants (Arthur *et al.* 2005; Huff *et al.* 2020). This concept was applied to the results of the pot experiments.

There was no statistical difference in the PFAS accumulation potential of the five investigated plant species. However, sunflower, mustard and hemp

had overall higher BCF values, ranging from 0.03±0.027 to 957±231 for individual PFAS. Willow and poplar had similar BCF values ranging from 0.09±0.002 to 224±225 for individual PFAS. Hemp demonstrated the ability to hyper-accumulate most investigated PFAS, i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDoDA, PFBS, and PFHxS. Both sunflower and mustard could hyperaccumulate at least five PFAS, i.e. PFBA, PFPeA, PFHxA, PFHpA and PFDoDA. Willow and poplar could only hyper-accumulate two PFAS, i.e. PFBA and PFPeA. The difference in the hyperaccumulation potential amongst the plants exposed to similar PFAS concentration, composition and duration is most probably determined by the plant species' characteristics (e.g. biomass production, uptake of water). Some studies have also demonstrated the influence of plant species and genotype on PFAS uptake and accumulation (Xiang *et al.* 2018; Chen *et al.* 2019). For several other PFAS, obtained BCF values were >1, indicating an acceptable level of PFAS accumulation in plants.

The physicochemical properties of PFAS also played a significant role in their uptake and accumulation in all plants. A negative linear correlation was observed after the log transformation of the BCF values with the perfluoroalkyl chain length ($p < 0.05$). This implies that PFAS accumulation in the species was highest for PFBA and reduced with each $–CF_2$ moiety added. PFCA of identical chain length as PFSA and FOSA had higher BCF values than their counterparts. PFAS of equivalent perfluoroalkyl chain length but different functional groups have a different molecular size. PFSA are often larger with stronger PFAS sorption than their PFCA counterparts. High PFAS sorption limits PFAS solubility and acropetal transportation in the plants (Nguyen *et al.* 2020; He *et al.* 2023). Previous studies also observed similar trends in various edible plants (Felizeter *et al.* 2012; Blaine *et al.* 2013; Krippner *et al.* 2015; Scher *et al.* 2018).

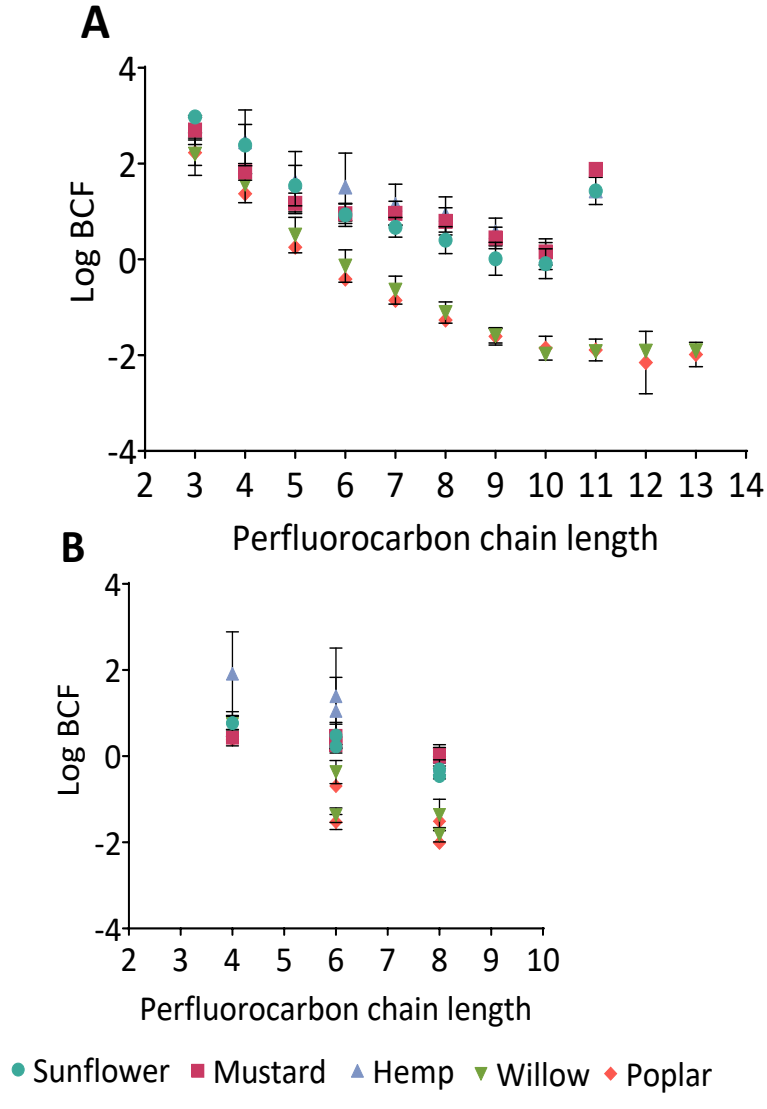


Figure 5: Relationship between bioconcentration factor (BCF) and perfluoroalkyl chain length of A) PFCA and B) PFSA for five plants grown in pot experiments after 90 days of PFAS exposure

5.3.3 The role of supplements to enhance PFAS uptake by plants

The enhancement of PFAS accumulation in plants was tested through inoculation of plants with microbes, adding fertilizer, and applying phytohormones (naphthalene acetic acid, NAA) (Figure 6). Diverging responses were obtained depending on the plant species and treatment.

Inoculation of plants with microorganisms had no effect on PFAS concentrations in plants in any of the performed experiments. A metagenomics analysis of the soil samples revealed that inoculation with the microbes didn't increase soil microbial diversity but the cultivation of plants on PFAS-contaminated improved microbial diversity. This could imply that the added microbes didn't successfully form any symbiotic relationships with the plants that could have improved the phytoextraction potential of PFAS in the plants. Future studies are required to identify and isolate microorganisms that improve PFAS phytoextraction. Nevertheless, the results demonstrated the benefit of phytoremediation to soil health.

Fertilizer application significantly (*t-test*) reduced PFAS uptake in all investigated plants based on concentration level but increased plant biomass. Therefore, PFAS uptake per mass with fertilizer application was similar to that obtained for the control (no addition of fertilizer). The reduction in plant PFAS concentration was probably due to increasing cation concentration that interacts with the PFAS and therefore reduced PFAS bioavailability (Cai *et al.* 2022). Applying the NAA, significantly (*t-test*) improved PFAS concentration of poplar but didn't affect PFAS concentration in willow. However, there was no significant difference (*t-test*) that was recorded in plant biomass. NAA is a synthetic hormone and a type of auxin known to promote plant growth, increase root exudates and increase contaminant uptake in certain plants (Israr & Sahi 2008; Hąc-Wydro *et al.* 2017; Hadi *et al.* 2021). The mechanism behind the observed increase in this study is not clearly understood. Further studies are required to investigate the underlying mechanisms and potential of the amendments with various plants.

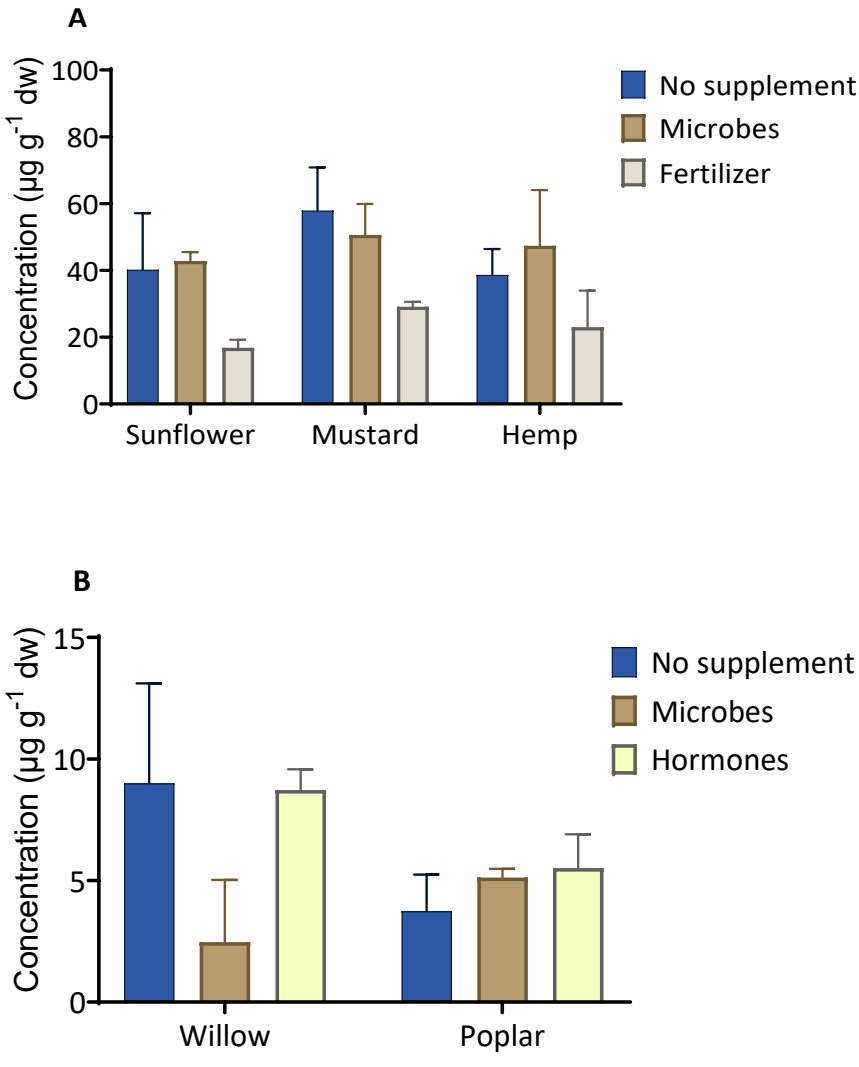


Figure 6: Average PFAS concentration (µg g^{-1} dw) for shoots of A) sunflower, mustard and hemp with supplements i.e. no supplement (control), microbes and fertilizer, and B) willow and poplar with supplements i.e. no supplement, microbes and hormones. All plants were grown on PFAS-spiked soil in triplicates.

5.4 Removal efficiency and mass balance (Papers III and IV)

Removal based on PFAS mass in percentages was determined for all the investigated plants from **Papers III and IV** (Table 2). The removal was determined as the percentage ratio of mass PFAS in plants to the mass PFAS in the soil at time zero (at planting). Sunflower and willow had the highest PFAS removal of 34±19-0.21±0.14% and 30±9.3-0.3±0.2% respectively, for individual PFAS. Furthermore, removal was the highest for the short chain PFAS, i.e. PFBA, PFPeA, PFHxA and PFBS for all plants (Table 2). Least PFAS removal was obtained for the longer chain PFAS. Previous studies have estimated the PFAS removal of plants. However, these studies were often performed briefly (≥14 days) or in hydroponics experimental setups (Zhang *et al.* 2019; Sharma *et al.* 2020; Greger *et al.* 2021). A study of various willow cultivars also obtained removal efficiency between 2-30% in leaves of willow grown in hydroponics experiments following eight days of PFAS exposure (Sharma *et al.* 2020).

Table 2: Summary of percentage (%) PFAS removal by the investigated plants grown in a pot experiment on PFAS spiked soil for 90 days. n=3, ±SD.

Compound	Sunflower	Mustard	Hemp	Willow	Poplar
PFBA	30±9.3	12±2.6	15±4.9	34±19	27±18
PFPeA	33±2.9	14±2.9	24±6.9	25±13	19±12
PFHxA	6.5±0.4	3.7±1.2	6.8±2.1	6.8±2.9	4.4±2.1
PFHpA	2.3±0.5	2.2±0.6	3.2±0.4	1.9±0.99	1.3±0.71
PFOA	1.0±0.5	1.2±0.4	1.3±0.2	0.61±0.24	0.55±0.18
PFNA	0.3±0.2	0.5±0.2	0.9±0.3	0.21±0.14	0.21±0.12
PFDA	0.5±0.2	1.1±0.3	2.2±0.8	0.086±0.049	0.11±0.08
PFBS	11±9.2	3.2±1.0	12±2.4	10±5.1	5.32±2.6
PFHxS	3.2±2.3	1.5±0.4	3.3±0.2	1.1±0.51	0.70±0.25
PFOS	0.5±0.4	0.4±0.1	0.5±0.2	0.27±0.16	0.18±0.11

In **Paper IV**, a mass balance was performed to understand the fate of PFAS in the pot experiment. Overall, the mean PFAS mass balance recovery after 90 days was 104±43% for willow. The recovered mass of PFAS was not statistically different (multiple t-tests) from 100%. However, the recovered mass of PFBA, PFPeA and PFBS was less than 100% with on average of

44±23, 51±19 and 76±24, respectively, for willow. This was due to the leaching of some PFAS during irrigation. Furthermore, losses of FOSA with on average of 62±8.9 for willow were observed while mass recovery of PFOS was significantly increased to an average of 112±22 for willow. This could be due to the transformation of FOSA to other products, including PFOS (Murakami *et al.* 2013). Similar results were observed for poplar. McLachlan *et al.* (2019) investigated the fate of PFAS in lysimeters filled with spiked soil and cultivated with vegetables. A significant loss of short chain PFAS after 72 days through leaching both with or without the plants was observed. This could imply that groundwater safety remains threatened due to the leaching of short chain PFAS. Future studies are needed to investigate combination techniques with adsorption technologies to reduce leaching and improve PFAS bioavailability.

6. Conclusions and Outlook

The main conclusions of this thesis with respect to the aims were as follows:

- A simple extraction and clean-up method was developed, validated and successfully applied for the analysis of 24 PFAS of various chain lengths and functional groups in plants. Extraction with methanol followed by ENVICarb cartridge as clean-up outperformed all tested methods and was selected for PFAS analysis.
- The investigation of various plants at PFAS-contaminated sites demonstrated PFAS uptake in trees growing at these sites. PFAS accumulation depended on individual PFAS, plant tissue, plant species and PFAS concentrations at the contaminated sites. All plants showed high uptake of PFAS demonstrating the suitability of phytoremediation for PFAS in the field.
- Results from the pot experiments demonstrated that PFAS removal was influenced by the physicochemical properties of PFAS (perfluoroalkyl chain length and functional group), PFAS concentration, plant characteristics (species and biomass), and soil conditions (nutrients, microbes, a phytohormone).
- Phytoextraction is most efficient for short chain PFAS, while phytostabilization is more efficient for the longer chain PFAS.
- Up to 34% short chain PFAS could be removed by plants following PFAS exposure showing the phytoremediation potential for short chain PFAS. Long chain PFAS were stabilized in the soil and roots, while there was a loss of short chain PFAS due to leaching. Several plant cycles might be needed until PFAS pollution is reduced to safe levels in soil and groundwater.

Effective measurement of PFAS in plants is important for proper environmental monitoring and assessment. However, plants are complex matrices that often possess strong matrix effects. Therefore, many analytical methods are often laborious with several steps compromising method performance. A simple and fast PFAS analytical method was developed to quantify PFAS in various plant matrices.

The presented also work demonstrated the potential of phytoremediation as a promising in situ method for remediation. Field screening and pot experiments were performed to evaluate the phytoextraction and phytostabilization potential of the plants as well as optimise PFAS uptake by testing various amendments. Long term field studies are required to assess the performance of the investigated plants, explore novel plant species and develop an effective remediation design.

Results from this thesis also demonstrated preferential accumulation of PFAS, and that amendments could potentially improve PFAS uptake in plants. Additional understanding of the interactions between amendments (microbes, hormones and fertilizers), dosage, time of application or interaction between amendments and PFAS is required. Further studies could also investigate integrated PFAS treatment approaches to improve the effectiveness of phytoremediation.

Plants are capable of hyperaccumulating PFAS from the groundwater, however, phytoremediation is applicable to soil and groundwater which are accessible by the root system. For deep soil and groundwater contamination, other treatment techniques might be needed. Alternatively, PFAS-contaminated groundwater or other water types can be used for irrigation of phytoremediation systems.

Following phytoremediation, the biomass can be converted to bioenergy while destroying the incorporated PFAS. Future studies on life cycle assessment and economic analysis to identify and manage potential environmental risks as well as maximise resource efficiency are required.

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Popular science summary

Driven by urbanization and an escalating demand for industrial and consumer products, vast quantities of chemicals are continuously produced and discharged into the environment. One category of such man-made chemicals is per- and polyfluoroalkyl substances (PFAS), which have emerged as a major environmental concern due to their widespread presence and potential health risks. PFAS exposure occurs not only through consumer products like makeup and cookware but also due to the application of PFAS-containing firefighting foams or emissions from landfills, leading to extensive land contamination. Consequently, PFAS from these sources can contaminate crops and groundwater, which are essential resources for food and drinking water, posing potential health risks.

Various remediation technologies are under development and testing to reclaim PFAS-contaminated soil and groundwater. In this thesis, a potential remediation technology called phytoremediation is explored. Phytoremediation is a remediation technique for contaminated soil and groundwater using specialized plants. It is characterized as a sustainable and eco-friendly solution to remediate large land areas cost-efficiently. It has been previously applied for managing soil contaminated with heavy metals and nutrients. However, little is known on the feasibility for PFAS remediation. Work in this thesis focused on developing a mechanistic understanding of phytoremediation of PFAS contamination and optimising the method for future application.

First, I developed a straightforward and user-friendly method with satisfactory performance for measuring PFAS levels in different plant species. This method allows for precise and accurate determination of PFAS quantities within the plants. Such measurements are crucial not only for assessing the risk of human and animal exposure to PFAS through food

consumption but also for identifying plants with a high potential to accumulate PFAS from the soil and groundwater for effective phytoremediation purposes. I collected samples from various PFAS-contaminated sites to identify plant species with the highest potential for PFAS accumulation. From this study, it was observed that several areas of these contaminated sites had high PFAS concentrations in the soil and groundwater as well as high PFAS concentrations in the plants at these sites indicating the high potential of phytoremediation. In order, to improve the phytoremediation potential of selected species, microbes, fertilizers and hormones were tested. Results revealed that PFAS uptake was species dependent with up to 34% removal of short chain PFAS within 90 days of plant exposure to PFAS-contaminated soil. Supplementation of plants with microbes, fertilizers or hormones increased plant biomass which in turn increases the total mass of PFAS removal. These results are important for future field studies and applications of phytoremediation.

Populärvetenskaplig sammanfattning

Drivet av urbanisering och en eskalerande efterfrågan på bekvämlighet, produceras och släpps stora mängder kemikalier ut i miljön. En kategori av sådana föreningar är per- och polyfluorerade alkylsubstanser (PFAS) som har blivit ett betydande miljömässigt skäl till oro på grund av deras utbredda förekomst och potentiella hälsorisker. Exponering för PFAS sker inte bara genom konsumentprodukter som smink och kokkärl, utan också via brandsläckningsskum som innehåller PFAS eller läckage från deponier, vilket leder till omfattande markförorening. Följaktligen kan PFAS från dessa källor förorena grödor och grundvatten, vilka är essentiella resurser för livsmedel och dricksvatten, och medföra potentiella hälsorisker.

Flera tekniker för sanering utvecklas och testas för att återställa PFAS-förorenad mark och grundvatten. I den här avhandlingen utforskas en potentiell saneringsteknik som kallas fytoremediering. Fytoremediering, användningen av specialiserade växter för att rena förorenad mark och grundvatten, utlovar en hållbar och miljövänlig lösning. Metoden kan användas för att sanera stora markområden och är kostnadseffektiv. Den har tidigare tillämpats för hantering av mark förorenad med tungmetaller och näringsämnen. Kunskapsläget för PFAS-sanering med metoden är dock begränsat. Arbetet i denna avhandling fokuserade på att utveckla en mekanistisk förståelse för metoden och optimera den för framtida tillämpningar.

Först utvecklade jag en enkel och användarvänlig metod med tillfredsställande prestanda för att mäta PFAS-nivåer i olika växtarter. Denna metod möjliggör exakt och noggrann bestämning av mängden PFAS i växterna. Sådana mätningar är avgörande inte bara för att bedöma risken för människor och djur att exponeras för PFAS genom livsmedelskonsumtion, utan också för att identifiera växter med hög potential att ackumulera PFAS

från marken för effektiva fytoremedieringsändamål. Jag samlade prover från olika PFAS-förorenade platser för att identifiera vilka växtarter som hade högst potential för PFAS-ackumulering. I studien observerades att flera områden på dessa förorenade platser hade höga PFAS-koncentrationer i marken och grundvattnet. De motsvarande växterna på dessa platser hade också högst PFAS-koncentration, vilket understryker deras potential för PFAS-sanering. För att förbättra fytoremedieringspotentialen hos utvalda växtarter testades mikroorganismer, gödningsmedel och hormoner. Resultaten visade att PFAS-upptag var artberoende, med upp till 34% avlägsnande efter 90 dagars exponering av växterna för PFAS. Tillsats av mikroorganismer, gödningsmedel eller hormoner ökade växtbiomassan, vilket i sin tur ökade massavlägsnandet av PFAS. Dessa resultat är viktiga för framtida fältstudier och tillämpningar av fytoremediering.

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A novel method for extraction, clean-up and analysis of per- and polyfluoroalkyl substances (PFAS) in different plant matrices using LC-MS/MS

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are chemicals of concern due to their persistence, bioaccumulation, and toxic properties. PFAS accumulation in plants poses a risk of human and animal exposure due to consumption of the affected plants, but also allows plants to be used in remediation of PFAS-contaminated soils and groundwater. Therefore, effective extraction, cleanup, and analytical methods for measuring PFAS concentrations in plants are fundamental for research on animal and environmental health. PFAS analysis in plant matrices is complex, due to high matrix interference, and scarcity of methods for analyzing different classes of PFAS. In this study, a simple sample preparation method for PFAS analysis in various plant tissues (leaves, needles, twigs, stems, roots from 10 different species) was developed and validated. Instrumental analysis was performed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The method was optimized considering six different extraction conditions and three different cleanup techniques. Methanol as extraction solvent, combined with 1 g ENVI carb cartridges, showed best performance among all extraction conditions and cleanup techniques tested. Method validation showed good recovery (90–120%), high within-day and between-day precision (<20% relative standard deviation), and low method detection limit (0.04–4.8 ng g^{-1} dry weight (dw)) for different plant matrices. In tests of the method on soil and different plant tissues of silver birch (*Betula pendula*) and Norway spruce (*Picea abies*) at a PFAS-contaminated site, 16 of 24 target PFAS were detected in plants and 17 in soil. ΣPFAS concentration in soil was 43 ng g^{-1} dw. PFAS distribution in silver birch tissues ranged from 7.1 ng g^{-1} dw in roots to 64 ng g^{-1} dw in leaves, and in Norway spruce from 14 ng g^{-1} dw in roots to 16 ng g^{-1} dw in needles. This novel method for PFAS analysis in plants can be valuable in future monitoring, process understanding, remediation, and risk assessments.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic micropollutants that are extremely persistent, bioaccumulative, and potentially toxic to humans and animals [1]. These substances are ubiquitous in humans and the environment [2–4], with previous studies reporting detection of PFAS in e.g., plants and crops at contaminated sites, agricultural fields treated with contaminated biosolids, reclaimed water, and aquatic environments [5–9].

PFAS accumulation in plants is important because plants are a major dietary component for humans and animals, but can also be used in remediation of contaminated sites [10]. Therefore, effective PFAS measurement in plant tissues is crucial for research, monitoring, formulation of remediation strategies, food safety, and regulation. Plants, like other biological matrices, are matrix-rich, which hinders effective extraction and quantification of contaminants [11]. Plants also contain complex elements such as phenolics and photosynthetic pigments, distinguishing them from other environmental and biological matrices [12]. Currently, only limited sample preparation methods for PFAS in plant matrices are available [13–16] and most existing methods have been optimized and validated for only a few compounds. The vast majority of methods used for PFAS analysis in plants to date have applied sample preparation protocols developed for other matrices, such as soil and sediment [17] or biota [18,19].

For extraction, several previous studies have applied solid–liquid extraction, solid-phase extraction (SPE), and the QuEChERS (quick,

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easy, cheap, effective, rugged, and safe) method for PFAS analysis, using polar solvents such as methanol, acetonitrile, methyl ter-butyl ether, or their solutions, with an acid, base, or buffer [5,20,21]. Commonly applied cleanup methods include ion pairing, SPE, activated carbon (as powder or cartridges) or magnesium sulfate, or a combination of SPE and activated carbon [8,22–24]. Several of these approaches have been used without comprehensive method optimization and validation for PFAS analysis in plants, which often poses challenges in obtaining high recovery and low matrix effects for some analysed PFAS [6,20,25]. To our knowledge, comprehensive optimization and validation of methods for PFAS analysis in plants is lacking and, in particular, no studies have investigated the occurrence of PFAS in plants growing at landfill sites.

The aim of this work was to develop and validate a simple extraction and cleanup method for analysis of five PFAS classes in multiple plant tissues, using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Specific objectives were to (i) evaluate the performance of six extraction conditions (acetonitrile, methanol, and their solutions with a weak acid or base), and three cleanup techniques (ENVI-Carb cartridge only, ENVI-Carb cartridge combined with WAX-SPE, and ENVI-Carb powder only); (ii) validate the optimized method; and (iii) apply the method to plant tissues and soil collected from a landfill site with known PFAS contamination as a pilot study of PFAS in landfill plants.

2. Methods

2.1. Chemicals and materials

Target PFAS ($n = 24$) comprised perfluoroalkyl carboxylates (PFCAs) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA), perfluoroalkanesulfonates (PFSAs) (PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS), perfluorooctanesulfonamide (FOSA), methyl- and ethylperfluorooctanesulfonamidoacetic acid FOSAAs (MeFOSAA, EtFOSAA), and 4:2, 6:2, 8:2 fluorotelomer sulfonate (FTSA) (Table S1 in Supporting Information (SI)). Mass-labelled internal standards (ISs) used were: $^{13}C_3$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_2$-PFTeDA, $^{13}C_3$-PFHxS, $^{13}C_8$-PFOS, $^{13}C_8$-FOSA, d_3-MeFOSAA, d_5-EtFOSAA (Table S1 in SI). Native standards (purity > 98%) and ISs (isotopic purity ≥ 99%) were obtained from Wellington Laboratories (Sweden).

Acetonitrile (ACN), methanol (MeOH), ammonium acetate (NH_4Ac) (≥99%), formic acid (FA) (≥98%), and glacial acetic acid (100%) of high analytical grade were obtained from Merck (LiChrosolv, Merck, Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). MilliQ water was generated by a Milli-Q IQ 7000 Ultrapure Water purification system filtered through a 0.22 μm Millipak Express membrane and an LC-Pak polishing unit (Merck Millipore, Billerica, MA, USA). Oasis solid phase extraction (SPE) WAX cartridges (150 mg, 6 cc, 30 μm) were obtained from Waters (New Bedford, MA, USA). ENVI-Carb cartridges (250 mg, 6 mL & 1 g, 12 mL (120–400 mesh, 100 $m^2\ g^{-1}$)) and ENVI carb powder (120–140 mesh, 100 $m^2\ g^{-1}$) were obtained from Sigma-Aldrich. Plant samples were milled in a blender (230 V; OBH Nordica, Sweden). Homogenization was performed using an overhead shaker (Reax 2, Heidolph, Germany) and an analog vortex mixer (VWR, Leuven, Belgium).

2.2. Sample collection

For method optimization and validation, plant samples were obtained from 10 common plant species at locations without PFAS contamination in the area of Uppsala, Sweden. The plant species were: silver birch (*Betula pendula)*, strawberry (*Fragaria* spp.), Scots pine (*Pinus sylvestris*), mountain ash (*Sorbus aucuparia)*, salix (*Salix* spp.), Norway spruce (*Picea abies*), poplar (*Populus* spp.), bird cherry (*Prunus padus)*, common oak (*Quercus robur)*, and sycamore maple (*Acer pseudoplatanus*).

The suitability of the method developed was tested using plant samples and soil samples collected from a PFAS-contaminated site in Stockholm, Sweden, on 10 October 2019. The site is a landfill, with silver birch and Norway spruce as the main tree vegetation. Tissues (needles, leaves, twigs, bark, roots) were collected separately from different trees ($n = 4$) and combined to produce composite samples for each tissue type. Soil samples were also collected from the same site ($n = 4$) and combined to make 1 composite sample. These composite samples were then analysed in duplicates.

Fresh plant tissue samples were cleaned with tap water (1 time), MilliQ water (2 times), and a mixture of MilliQ and MeOH (50:50; v:v) (2 times). The tissues were freeze-dried for 3 days and then homogenized and milled using a cleaned blender (cleaned 3 times with MilliQ water and 3 times with MeOH between samples).

2.3. Method optimization

The plant tissues collected for each species were sorted into foliage (leaves or needles), twigs, stems, and roots and then cleaned, freeze-dried, and milled using a blender as mentioned above. Thereafter, the leaves and needles (1:1; weight-based) were combined to form one matrix group called 'foliage' (Group I), while the twigs, stems, and roots (1:1:1; weight-based) were combined to form another matrix group called 'woody tissue' (Group II). Samples of these two matrix groups were shaken for 120 h using an overhead shaker, to obtain a respective homogenous mix. The mixes were then stored in the freezer at −20 °C until analysis.

For method optimization, three replicates of 1 g each were weighed into 15 mL PP tubes and spiked with a native PFAS mixture standard to a final concentration of 25 ng g^{-1} dry weight (dw) per compound and IS mixture (5 ng g^{-1} dw).

2.3.1. Extraction conditions

Six different solvents were tested for method validation: i) MeOH, ii) ACN, iii) MeOH:ACN (50:50; v/v), iv) MeOH with 0.1% formic acid (MeOH:FA), v) ACN with 0. 1% formic acid (ACN:FA), and vi) MeOH with 400 mM ammonium acetate (MeOH:NH_4Ac) (Fig. 1). These extraction solvents were applied to the two sample matrices (matrix groups I and II). Each extraction was performed using ultrasonication in three cycles and then the extracts were combined to one extract (for details, see Section 2.5). The combined extract was further cleaned up using an ENVI-carb cartridge prior to concentration and instrumental analysis.

2.3.2. Cleanup technique

Three cleanup methods using MeOH (i.e., best-performing solvent, see Section 3.1.1) as extraction solvent were tested: 1) ENVI-Carb cartridge only (ENVI-Carb cartridge), 2) ENVI-Carb cartridge combined with WAX-SPE (ENVI-Carb cartridge + WAX-SPE), and 3) ENVI-Carb powder only (ENVI-Carb powder) (Fig. 1).

For cleanup 1 (ENVI-Carb cartridge), the extracts (7 mL) were run directly through the ENVI-Carb cartridge (1 g, 12 mL) and collected in 15 mL PP tubes. The cartridges were washed with MeOH (1 mL) after use and then pressed with air using a syringe to collect the wash in the same vial with the extract. No cleaning and conditioning was done prior to using the cartridges.

For cleanup 2 (ENVI-Carb cartridge + WAX-SPE), the extracts were run through the ENVI-Carb cartridge (1 g, 12 mL) as described above, concentrated to 5 mL using nitrogen, and then diluted with 95 mL of Milli-Q water. SPE was performed with Oasis WAX cartridges (150 mg, 6 cc, 30 μm) preconditioned with 0.1% NH_4OH/MeOH (4 mL), MeOH (4 mL), and Milli-Q water (4 mL) sequentially. After sample loading, the cartridges were washed with 25 mM ammonium acetate in Milli-Q water (4 mL) and the WAX cartridges were centrifuged at 3000 rpm for 2 min. Elution was performed with MeOH (4 mL) and 0.1% NH_4OH/MeOH (4

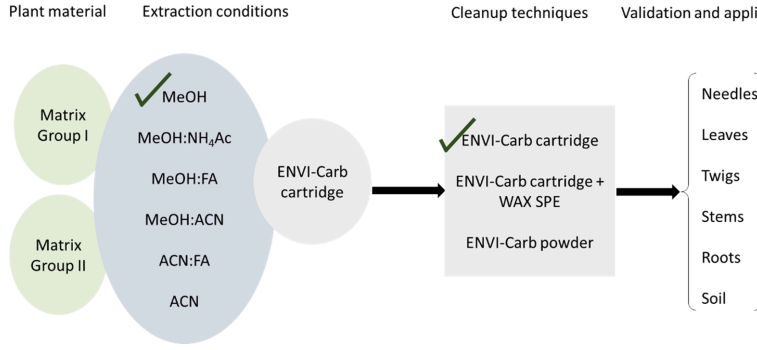

Fig. 1. Workflow used for evaluating the six extraction conditions and three cleanup techniques before validating and applying the method for PFAS analysis in different plant tissues and soil. The green check mark shows the extraction condition and cleanup technique that performed best in PFAS analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mL) in 15 mL PP tubes.

All extracts from cleanups 1 and 2 were concentrated to 100 μL using nitrogen and then topped up with methanol to obtain a total volume of 500 μL prior to instrumental analysis.

For cleanup 3 (ENVI-Carb powder), the combined extracts were concentrated to 500 μL and the concentrates were transferred to 2 mL Eppendorf centrifuge tubes containing 25 mg ENVI-Carb powder and 50 μL glacial acetic acid. The tube and its contents were vortexed, followed by 15 min of centrifugation at 4000 rpm. The supernatant was transferred to LC-MS injection vials for instrumental analysis.

2.4. Method validation

Samples (pre-spiked $n = 3$, post-spiked $n = 1$) from each of the matrix groups were used for validation of the optimized method and were analyzed on three different days. Method validation was based on the following parameters: procedural blanks, method detection limits (MDLs), method quantification limits (MQLs), relative recovery, linearity, and within-day and between-day precision (relative standard deviation, RSD(%)) for each of the matrix groups.

In total, six procedural blanks were prepared in the same way as natural samples, but without sample material. MDLs and MQLs were estimated using a signal to noise (S/N) ratio of 3 and 10, respectively, in matrix spiked samples at low concentration levels ($c = 5$ ng g^{-1} dw). Relative recovery was determined in triplicate for each matrix group.

Linearity was assessed using a nine-point calibration curve (0.01–100 ng mL^{-1}). For within-day precision, RSD (%) and between-day precision, RSD (%) was determined for the mean concentration of triplicate samples run on the same day and on three different days, at a medium concentration level ($c = 25$ ng g^{-1} dw). The criteria of acceptance for RSD (%) was $< 20\%$.

2.5. Method application

Procedural blanks, fortified samples, and duplicates were used in sample preparation and analysis. Methanol as extraction solvent and ENVI-Carb cartridge alone (cleanup 1) showed the best performance for analysis of PFAS (see Section 3.1.1). In brief, plant tissue (1 g dw) was spiked with 100 μL IS mixture, resulting in 5 ng g^{-1} dw for each IS. The plant tissues were extracted in three cycles using MeOH. During each of these cycles, 3 mL of extraction solvent was added and the samples were vortexed at high speed for 1 min, ultrasonicated for 30 min, and centrifuged at 3000 rpm for 15 min. Combined extracts were run through the ENVI-Carb cartridge (1 g, 12 mL) and collected in 15 mL PP tubes. The cartridges were washed with MeOH (1 mL) after use and then pressed with air using a syringe to collect all solvents trapped within the cartridge. The wash was collected in the same tube as the cleaned extract. The extracts were concentrated to 100 μL using nitrogen and then topped up with methanol to a total volume of 500 μL. The reconstituted extracts were transferred to LC-MS injection vials for instrumental analysis.

For soil samples, sample preparation and extraction was done as described above. Cleanup was performed using 250 mg ENVI-Carb cartridges [26]. The extracts were then concentrated to 500 mL prior to instrumental analysis.

2.6. LC-MS/MS analysis

Instrumental analysis was performed using ultra-high pressure liquid-chromatography (SCIEX ExionLC AC system) coupled to tandem mass spectrometry (SCIEX Triple Quad™ 3500) (UHPLC-MS/MS). The column oven was set to 40 °C, and 20 μL of sample were injected into a Phenomenex Kinetex C18 (30 × 2.1 mm, 1.7 μm) precolumn coupled to a Phenomenex Gemini C18 (50 mm × 2 mm, 3 μm) analytical column for chromatographic separation. The mobile phase consisted of MilliQ water with 10 mM ammonium acetate (A) and MeOH (B). The mobile phase gradient was as follows: 5% B, which was increased to 55% within the first 0.1 min, then further increased to 99% within 4.4 min, kept constant for the next 3.5 min, then decreased to 5% over 0.5 min and kept constant for the next 0.5 min. The flow rate was 0.6 mL min^{-1} and the total run time was 9 min. Information on optimized parameters for the ion source and MS/MS parameters is provided in Tables S2 and S3 in SI. The MS/MS was operated in scheduled multiple reaction monitoring (MRM) mode with negative electrospray ionization (Table S3 in SI). A nine-point calibration curve from 0.01 to 100 ng mL^{-1} was used for quantification. Data evaluation was performed using SciexOS software (2.0).

2.7. Data handling and statistical analyses

For comparison of the different treatments (i.e., extraction conditions and cleanup techniques and their combinations) regarding recovery and matrix effects, descriptive statistics (mean and standard deviation), analysis of variance (ANOVA), and t-tests (significance level, $\alpha = 0.05$) were computed in GraphPad Prism (version 9.2.0 (332)).

3. Results and discussion

3.1. Method optimization

3.1.1. Extraction conditions

Six different extraction conditions (i.e., MeOH or ACN, with or without the additives NH_4OH and 0.1% FA) were assessed for matrix group I (leaves and needles) and matrix group II (twigs, stems. and roots), based on their absolute recovery (Fig. 2). In general, MeOH performed better than ACN, with absolute recovery of 82 ± 12% (41–103%) and 89 ± 24% (64–187%) for matrix groups I and II, respectively. ACN gave generally lower recovery for matrix groups I and II, 61 ± 15% (range 11–79%) and 95 ± 61% (47–291%), respectively. In general, the 24 PFAS investigated showed good absolute recovery except for 6:2 FTSA, 8:2 FTSA, MeFOSAA, PFDA, PFTriDA, and PFTeDA. 6:2 FTSA had the lowest recovery (<50%) under all extraction conditions except MeOH:FA for matrix group I. Low recovery for long-chain PFCAs (PFDA, PFTriDA, PFTeDA) has been reported previously for different matrices, due to strong sorption of these compounds to surfaces, and for PFAS precursors, due to their potential degradation [11]. In matrix group II, PFTriDA showed the highest absolute recovery under all extraction conditions (>150%). High absolute recovery was also observed for PFDA with ACN extraction, MeFOSAA with MeOH:ACN extraction, and 8:2 FTSA with MeOH:ACN, ACN:FA, and ACN extraction.

Additives, especially FA (0.1%), improved the extraction efficiency of both MeOH and ACN for matrix group I, but not group II. For group I, MeOH:FA and ACN:FA generated average recovery of 102 ± 17% (69–139%) and 77 ± 25% (23–158%), respectively, for the targeted PFAS, values which were significantly ($p < 0.0001$) better than those achieved by MeOH and ACN without additives. Similar recovery was reported in a previous study using ACN for extraction with an acidification step (acetic acid) prior to cleanup (ENVICarb cartridges; 500 mg, 6 mL) for PFAS analysis in spinach, tomato, and corn tissue samples [27]. For group II, FA had significantly ($p < 0.0001$) lower extraction efficiency compared with using MeOH and ACN alone, with MeOH:FA giving 73 ± 6.5% recovery (62–91%) and ACN:FA 95 ± 61% (47–291%). MeOH:NH_4OH and MeOH:ACN performed better in extraction of group II than group I samples, but both conditions resulted in greater variation in absolute recovery (see below). For group I, absolute recovery was 100 ± 50 (20–287%) for MeOH:NH_4OH and 78 ± 23% (9.5–133%) for ACN:MeOH for group I. For group II, absolute recovery was slightly higher, 115 ± 67% (51–328%) for MeOH:NH_4OH and 107 ± 47% (41–221%) for ACN:MeOH. Munoz et al. [26] reported good recovery for extraction of soil using MeOH:NH_4OH with a cleanup (ENVICarb cartridges; 250 mg, 6 mL), as found in this study for group II samples.

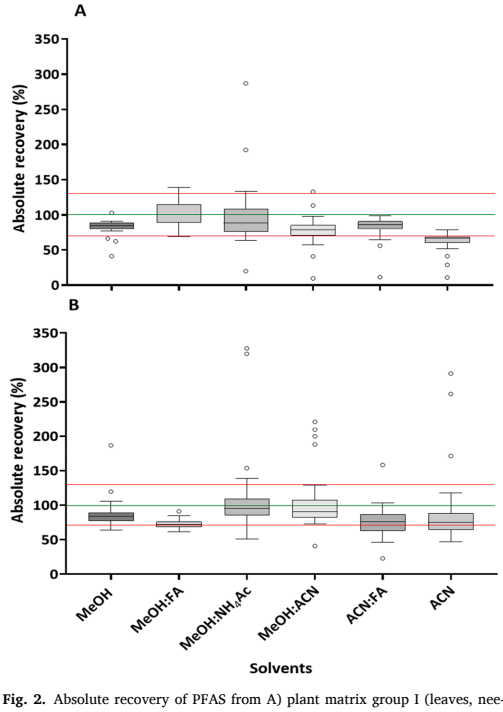

Fig. 2. Absolute recovery of PFAS from A) plant matrix group I (leaves, needles) and B) group II (twigs, stems, roots) under different extraction conditions.

ACN has been widely utilized as an extraction solvent for biological matrices [28] and several applied studies have used this solvent for extraction of PFAS in plants [16,24,27,29]. In a few studies, ACN has been mixed with water during extraction [22,23]. However, this is reported to generate lower recovery compared with using pure acidified organic solvents [28]. In the present study, ACN showed the worst performance of all extraction conditions tested.

Gobelius et al. [6] utilized MeOH and sodium hydroxide (NaOH) for PFAS extraction from plants and obtained absolute recovery of 12 ± 12% to 43 ± 26%. Similarly, Huff et al. [25] observed low absolute recovery (<10%) for several PFAS in plant extraction using MeOH and NaOH. In contrast, good performance was achieved when using MeOH or acidified MeOH for extraction in the present study. Baduel et al. [30] found that addition of acid or buffer improved recovery of acidic polar compounds, which is similar to our findings. Although both MeOH and MeOH:FA performed well for all 24 target PFAS, MeOH was ultimately selected as the extraction solvent in order to have a simple and consistent method for both matrix groups. The extraction method using MeOH was further tested on different cleanup techniques.

3.1.2. Cleanup techniques

For both matrix groups, three cleanup methods were tested: ENVICarb cartridge, ENVICarb cartridge + WAX-SPE, and ENVICarb powder. MeOH was selected as a suitable extraction solvent.

There was a significant difference ($p < 0.0001$) in absolute recovery obtained using the three cleanup methods for both matrix groups. ENVICarb cartridge had the highest absolute recovery, 86 ± 11% (61–119%) and 85 ± 11% (72–115%) for group I and II, respectively (Fig. 3). Use of two cleanup steps, i.e. ENVICarb cartridge + WAX-SPE, slightly improved the matrix effect (from −55 ± 51% to −36 ± 76 % for group I and from −58 ± 44% to −52 ± 53% for group II). However, it significantly reduced the absolute recovery to 60 ± 7.9% (42–72%) and 72 ± 8.4% (40–81%) for group I and II, respectively. Muschket et al. [16] made similar findings when using an additional cleanup step (WAX-SPE and CUNAX22Z-SPE) during sample preparation. ENVICarb powder is the most frequently used cleanup method for PFAS analysis [6,17] but showed the worst performance in this study, with absolute recovery of 53 ± 8.2% (25–60%) for group I and 65 ± 4.7% (59–76%) for group II. This can be explained by lack of proper method optimization (i.e. extraction solvent and ratio of powder to plant material/extract) despite extensive use of the ENVICarb powder to remove pigments from plant tissue in previous studies [6].

Although there were no major differences in matrix effects between the three cleanup methods, ENVICarb powder showed the strongest matrix effects (Fig. S2 in SI). For group I, matrix effects were −55 ± 51%, −36 ± 76%, and −65 ± 40% for ENVICarb cartridge, ENVICarb cartridge + WAX-SPE, and ENVICarb powder, respectively. For group II, the corresponding matrix effects were −58 ± 44%, −52 ± 53%, and −78 ± 24% for ENVICarb cartridge, ENVICarb cartridge + WAX-SPE,

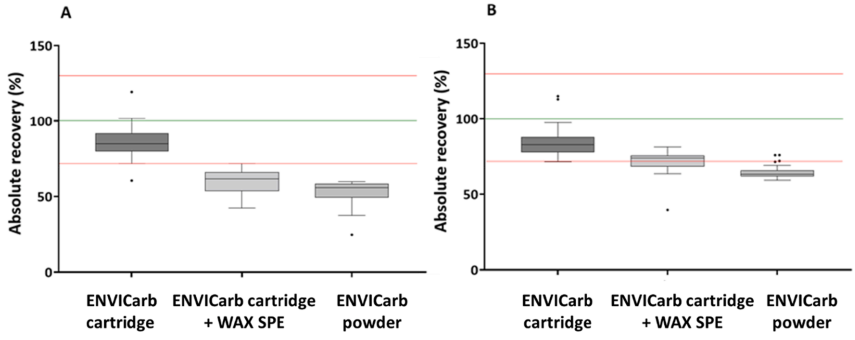

Fig. 3. Absolute recovery of PFAS for (A) matrix group I and (B) matrix group II when using three different cleanup techniques.

and ENVICarb powder, respectively. Based on the recovery and matrix effect results, ENVICarb cartridge was selected as the preferred cleanup method for both matrix groups.

3.2. Method validation

Method validation was performed with the selected extraction solvent (MeOH) and cleanup step (ENVICarb cartridge) (Table 1). None of the PFAS analyzed was detected consistently in the procedural blanks. PFBA (17 ng g^{-1} dw) was found in the matrix blanks (i.e., non-spiked samples) of group I. The MDLs and MQLs were determined from plant matrix samples spiked with low PFAS concentrations. The MDL range was 0.04–2.4 ng g^{-1} dw for group I and 0.1–4.8 ng g^{-1} for group II, while the MQL range was 0.1–8.1 ng g^{-1} dw for group I and 0.3–11 ng g^{-1} dw for group II (Table 1). The plant samples used were composite samples made up of tissues from different plant species on a dry matter basis, and not a single plant species. This represents the worst-case scenario, because the plants represented had different characteristics, with different levels of interferences. Therefore, the values obtained are applicable to other plants.

Relative recovery was within acceptable limits (70–130%) for all compounds except PFBA (140%), in matrix group II (Table 1). Recovery in this study was comparable to, but typically better than, that reported in the literature [13,16,27,29].

Our novel method showed linearity $R^2 \geq 0.99$ for the target compounds over a range of 0.01–100 ng mL^{-1} (Table 1). The precision of the method was < 20% for most target compounds in both matrix groups. For within-day precision, variations slightly above 20% were observed for PFBA and PFTrDA in group I and PFTriDA and 4:2 FTSA in group II, which showed RSD > 20%. For between-day precision, PFBA, PFHpA, PFHpS, PFNS, and PFDS in group 1 and PFBA, PFNS, and PFDS in group II had RSD > 20%.

3.3. Method applications

The novel method was used to investigate PFAS uptake and

Table 1
Validation data for analysis of 24 PFAS in plant matrix group I (leaves, needles) and group II (twigs, stems, roots) using MeOH as extraction solvent and ENVICarb cartridge cleanup.

		Matrix group I					Matrix group II				
Target compound	Linearity R^2	MDL (ng/g dw)	MQL (ng/g dw)	Relative recovery (%)	With-in day precision RSD (%)	Between-day precision RSD (%)	MDL (ng/g dw)	MQL (ng/g dw)	Relative recovery (%)	With-in day precision RSD (%)	Between-day precision RSD (%)
PFBA	0.990	0.05	0.18	115 ± 35	27	25	1.7	5.8	140 ± 25	2.9	31
PFPeA	0.993	2.4	8.1	104 ± 12	6.9	13	1.8	6.1	95 ± 3.3	7.7	13
PFHxA	0.996	0.93	3.1	105 ± 3.6	6.2	3.1	3.4	11	102 ± 5.5	4.7	4.6
PFHpA	0.998	0.27	0.91	108 ± 14	10	33	4.8	16	99 ± 7.5	4.7	32
PFOA	0.998	0.49	1.6	104 ± 4.3	5.1	9.9	0.60	2.0	98 ± 4.0	0.90	6.1
PFNA	0.996	1.3	4.2	102 ± 2.5	5.9	6.1	0.76	2.5	93 ± 12	7.4	7.7
PFDA	0.994	0.41	1.4	98 ± 2.9	7.0	5.6	0.79	2.6	102 ± 3.5	0.55	6.3
PFUnDA	0.990	0.60	2.0	101 ± 12	5.7	14	0.22	0.75	93 ± 9.5	6.5	17
PFDoDA	0.999	0.27	0.91	97 ± 3.5	2.9	1.3	0.20	0.68	94 ± 1.8	3.0	1.2
PFTriDA	0.993	0.21	0.70	95 ± 14	39	0.91	1.4	4.7	105 ± 78	26	14
PFTeDA	0.996	0.34	1.1	102 ± 1.4	2.9	9.2	1.9	6.4	95 ± 1.8	3.3	10
PFBS	0.999	0.22	0.74	96 ± 2.0	5.3	7.2	0.47	1.6	96 ± 6.2	5.0	5.9
PFPeS	0.999	0.19	0.62	101 ± 12	9.9	6.2	0.52	1.7	115 ± 15	12	7.1
PFHxS	0.999	0.43	1.4	97 ± 11	7.9	15	0.95	3.2	96 ± 12	17	5.7
PFHpS	0.997	0.14	0.45	107 ± 24	9.9	35	0.35	1.2	105 ± 15	10	12
PFOS	0.998	0.97	3.2	94 ± 2.2	2.4	14	0.92	3.1	99 ± 13	8.7	5.9
PFNS	0.995	0.18	0.59	109 ± 28	11	28	0.33	1.1	116 ± 32	14	37
PFDS	0.992	0.04	0.13	110 ± 13	15	36	0.20	0.68	110 ± 15	18	36
FOSA	0.999	0.21	0.69	106 ± 5.5	2.0	11	0.17	0.57	101 ± 4.2	3.0	8.0
EtFOSAA	0.999	0.06	0.21	104 ± 6.6	8.6	7.0	0.10	0.32	99 ± 6.3	3.4	5.9
MeFOSAA	0.999	0.17	0.56	107 ± 1.8	9.9	14	1.6	5.4	107 ± 32	18	7.9
4:2 FTSA	0.997	0.18	0.59	89 ± 27	17	40	0.24	0.81	93 ± 29	26	2.4
6:2 FTSA	0.998	0.07	0.24	101 ± 1.4	2.2	5.9	0.13	0.43	96 ± 11	9.1	6.2
8:2 FTSA	0.991	0.20	0.66	100 ± 1.2	5.3	2.1	0.31	1.0	98 ± 8.7	2.7	4.4

distribution in plant and soil samples from a PFAS-contaminated site (landfill) in Sweden. The main plant species at the site were silver birch and Norway spruce. Of the 24 PFAS analyzed, 16 were detected in plant samples (Fig. 4). Previous studies have typically only detected a few PFAS in plants (e.g., PFPeA, PFHxA, PFOA, PFHxS, PFOS, and 6:2 FTSA) [6]. Silver birch had the highest ΣPFAS concentrations, ranging from 7.1 ng g^{-1} dw in roots to 64 ng g^{-1} dw in leaves, while Norway spruce had ΣPFAS concentrations ranging from 14 ng g^{-1} dw in roots to 16 ng g^{-1} dw in needles. Foliage had the highest ΣPFAS concentration in both silver birch and Norway spruce (64 ng g^{-1} in leaves and 16 ng g^{-1} dw needles), followed by twigs (16 ng g^{-1} and 13 ng g^{-1} dw, respectively), bark (11 ng g^{-1}, 10 ng g^{-1} dw, respectively), and roots (7 ng g^{-1} and 14 ng g^{-1} dw, respectively). Particularly dominant PFAS in foliage included PFBA (on average 21% of ∑PFAS), PFHpA (5.8%), PFHxS (4.2%), and PFPeA (3.5%). Roots showed a different composition profile, dominated by the longer-chained PFAS i.e., PFOA (on average 2.1% of ∑PFAS), PFUnDA (2.0%), and PFDA (1.8%). This is in agreement with previous findings of higher concentrations of longer-chained PFAS in roots than in foliage [31]. In general, there are limited PFAS data available on silver birch and Norway spruce at contaminated sites, but our measured concentrations and composition profiles are in general agreement with those in a previous study [6]. Similar PFAS composition profiles, with dominance of short-chain PFAS, have been reported for other plants (vegetables, woody and other herbaceous plants) grown in PFAS-spiked soil [32], spiked water [25,33], and agricultural soils [24].

In soil samples, 17 of the 24 target PFAS were detected and ΣPFAS concentration was 43 ng g^{-1} dw. The PFAS composition profile in the soil differed from that in the two plant species, with PFSAs (PFOS, 17 ng g^{-1} dw, 38% of ∑PFASs) and PFHxS (10 ng g^{-1} dw, 22% of ∑PFAS) being the dominant PFAS in soil. This dominance of PFSAs (i.e., PFHxS and PFOS) in soil was not reflected in the PFAS composition profile of plant tissues, where PFCAs (especially PFBA) were the dominant PFAS. This can be explained by the low mobility of PFHxS and PFOS [31]. A previous study found that PFAS composition profile in plants was different from that in local soil, air, and rainwater [24]. However, Gobelius et al. [6] observed high PFOS concentrations in soil that were reflected in the PFAS distribution profile in plants.

4. Conclusions

A solid–liquid extraction method was developed for extraction of five different classes of PFAS from different plant species and a wide range of plant tissue samples. Methanol outperformed acetonitrile, despite the latter being the most commonly used extraction solvent for biological samples. Combining methanol with ENVICarb cartridges as a cleanup step produced a simple and novel sample preparation method. For most compounds, satisfactory validation parameters were obtained, illustrating good utility of the method for PFAS analysis.

The method was successfully applied to plant tissues of silver birch and Norway spruce from a PFAS-contaminated site, in a pilot investigation of PFAS uptake and distribution in plants at landfill sites. Several PFAS were detected in tissues of both plant species, especially the foliage, at concentration levels similar in magnitude to levels reported in previously [6]. The method presented can be used in future studies on dietary uptake of plant-related PFAS in animals and humans and on plant species for use in phytoremediation.

CRediT authorship contribution statement

Winnie Nassazzi: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Foon Yin Lai:** Conceptualization, Validation, Writing – review & editing, Supervision. **Lutz Ahrens:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision, Project administration.

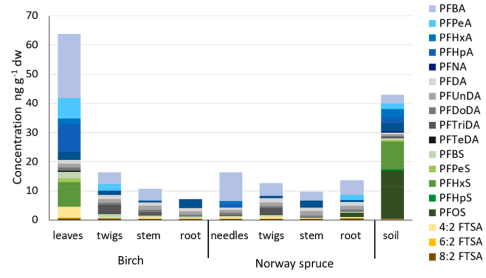

Fig. 4. Composition profiles and concentrations of PFAS in birch tissues, Norway spruce tissues, and soil at a landfill site in Sweden.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2022.123514.

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Supplementary information

Novel method for extraction, clean up, and analysis of per- and polyfluoroalkyl substances (PFAS) in different plant matrices using LC-MS/MS

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Contents

Table S1: Target analytes with their corresponding acronyms, internal standards and retention time

Compound	Acronym	Internal standard	Retention time RT (min)
Perfluorobutanoic acid	PFBA	$^{13}C_4$-PFBA	1.06
Perfluoropentanoic acid	PFPeA	$^{13}C_5$-PFPeA	1.27
Perfluorohexanoic acid	PFHxA	$^{13}C_5$-PFHxA	1.56
Perfluoroheptanoic acid	PFHpA	$^{13}C_4$-PFHpA	1.93
Perfluorooctanoic acid	PFOA	$^{13}C_8$-PFOA	2.32
Perfluorononanoic acid	PFNA	$^{13}C_9$-PFNA	2.71
Perfluorodecanoic acid	PFDA	$^{13}C_6$-PFDA	3.09
Perfluoroundecanoic acid	PFUnDA	$^{13}C_7$-PFUnDA	3.43
Perfluorododecanoic acid	PFDoDA	$^{13}C_3$-PFDoDA	3.74
Perfluorotridecanoic acid	PFTriDA	$^{13}C_2$-PFTeDA	4.03
Perfluorotetradecanoic acid	PFTeDA	$^{13}C_2$-PFTeDA	4.29
Perfluorobutane sulfonate	PFBS	$^{13}C_3$-PFBS	1.3
Perfluoropentane sulfonate	PFPeS	$^{13}C_3$-PFHxS	1.57
Perfluorohexane sulfonate	PFHxS	$^{13}C^3$-PFHxS	1.92
Perfluoroheptane sulfonate	PFHpS	$^{13}C^8$-PFOS	2.32
Perfluorooctane sulfonate	PFOS	$^{13}C^8$-PFOS	2.71
Perfluorononane sulfonate	PFNS	$^{13}C^8$-PFOS	3.07
Perfluorodecane sulfonate	PFDS	$^{13}C^8$-PFOS	3.41
Perfluorooctane sulfonamide	FOSA	$^{13}C_8$-FOSA	2.91
N-methyl-perfluorooctane sulfonamido acetic acid	MeFOSAA	D_3-MeFOSAA	3.26
N-ethyl-perfluorooctane sulfonamido acetic acid	EtFOSAA	D_5-EtFOSAA	3.44
4:2 Fluorotelomer sulfonic acid	4:2 FTSA	$^{13}C_2$-4:2 FTSA	1.53
6:2 Fluorotelomer sulfonate	6:2 FTSA	$^{13}C_2$-6:2 FTSA	2.28
8:2 Fluorotelomer sulfonate	8:2 FTSA	$^{13}C_2$-8:2 FTSA	3.08

Table S2: Optimized parameters of the ion source

Parameter	Value
Negative ion spray voltage	-3000 V
Curtain gas pressure	35 psi
Collision gas pressure	8 psi
Gas temperature	600 °C
Ion source gas 1 pressure	30 psi
Ion source gas 2 pressure	40 psi

Table S3: Information of the target analytes with their precursor mass, qualifier and quantifier ions with their corresponding collision energy, collision cell exit potential and declustering potential.

Compound	Precursor mass (Q1, m/z)	Quantifier (Q3, m/z)	Collision energy (V)	Collision cell exit potential (V)	Qualifier (Q3, m/z)	Collision energy (V)	Collision cell exit potential (V)	Declustering potential (V)
PFBA	213	168.9	-12	-5	-	-	-	-20
$^{13}C_4$-PFBA	217	172	-14	-9	-	-	-	-25
PFPeA	262.9	218.9	-12	-11	-	-	-	-5
$^{13}C_5$-PFPeA	268	223	-12	-9	-	-	-	-30
PFHxA	313	268.9	-10	-14	118.9	-10	-9	-35
$^{13}C_5$-PFHxA	318	273	-14	-13	-	-	-	-35
PFHpA	362.9	318.9	-14	-11	169	-24	-5	-40
$^{13}C_4$-PFHpA	367	172	-24	-7	-	-	-	-40
PFOA	413	369.1	-16	-15	169.1	-24	-5	-45
$^{13}C_8$-PFOA	421	376	-16	-17	-	-	-	-45
PFNA	463	219	-24	-9	168.9	-26	-9	-50
$^{13}C_9$-PFNA	472	172	-26	-9	-	-	-	-50
PFDA	513	268.9	-26	-11	218.9	-26	-9	-55
$^{13}C_6$-PFDA	519	474	-14	-19	-	-	-	-75
PFUnDA	563	519	-18	-9	268.9	-18	-11	-60
$^{13}C_7$-PFUnDA	570	525	-18	-9	-	-	-	-90
PFDoDA	613	569	-18	-11	318.9	-28	-13	-60
$^{13}C_3$-PFDoDA	615	570	-18	-11	-	-	-	-65
PFTriDA	662.9	618.9	-18	-11	319	-32	-11	-70
PFTeDA	713	668.9	-20	-9	368.8	-30	-13	-75
$^{13}C_2$-PFTeDA	715	670	-20	-13	-	-	-	-75
PFBS	299	80	-64	-7	99	-50	-9	-90
$^{13}C_3$-PFBS	302	80	-70	-5	-	-	-	-90

PFPeS	349	80	-76	-7	99	-66	-7	-100
PFHxS	399	79.9	-82	-5	99	-70	-7	-95
$^{13}C_3$-PFHxS	402	80	-86	-7	-	-	-	-105
PFHpS	448.9	80	-90	-7	99	-80	-7	-130
PFOS	499.2	80	-110	-7	98.9	-94	-9	-130
$^{13}C_8$-PFOS	507	80	-108	-7				-135
PFNS	549	80	-110	-7	99	-92	-7	-140
PFDS	598.9	80	-120	-7	99	-114	-7	-150
FOSA	498	78	-86	-7	-	-	-	-105
$^{13}C_8$-FOSA	506	77.9	-82	-7	-	-	-	-110
MeFOSAA	569.9	418.9	-28	-21	482.9	-22	-21	-90
D_3-MeFOSAA	573	419	-28	-19	-	-	-	-80
EtFOSAA	584	419	-28	-17	219	-36	-9	-85
D_5-EtFOSAA	589	419	-30	-15	-	-	-	-85
4:2 FTSA	327	307	-28	-11	287	-28	-11	-75
$^{13}C_2$-4:2 FTSA	329	309	-28	-13	-	-	-	-5
6:2 FTSA	427	81	-72	-7	-	-	-	-85
$^{13}C_2$-6:2 FTSA	429	81	-70	-7	-	-	-	-90
8:2 FTSA	527.2	506.9	-38	-15	81	-88	-7	-125
$^{13}C_2$-8:2 FTSA	529	81	-86	-7	-	-	-	-120

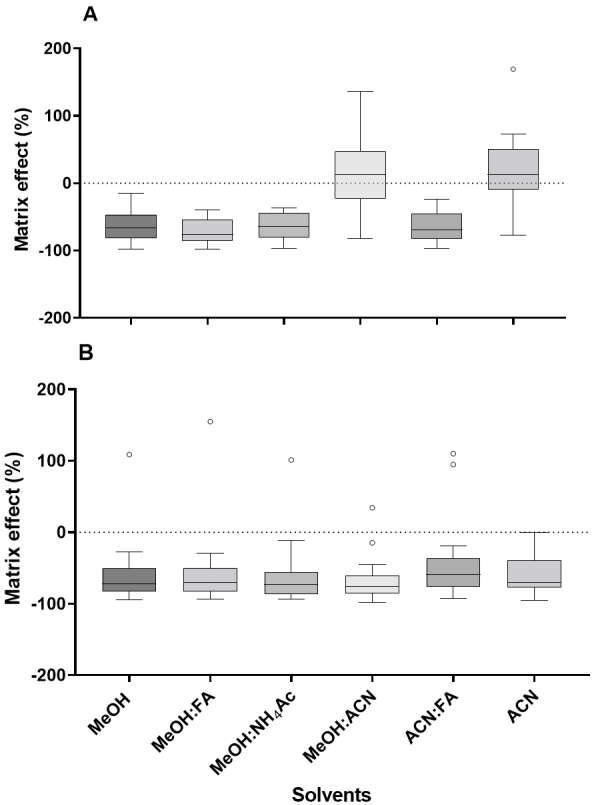

Figure S1: Matrix effect for A) group I (i.e leaves and needles) and B) group II (i.e. twigs, stems and roots) matrices under different extraction conditions.

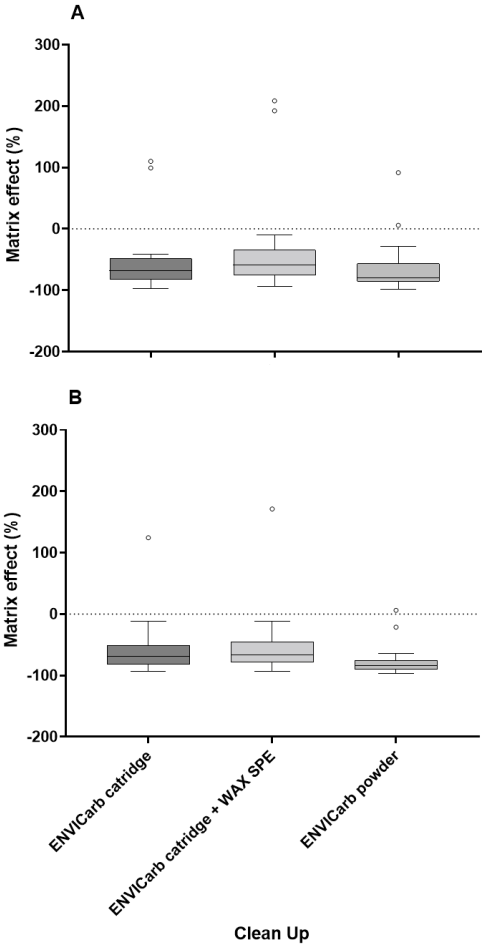

Figure S2: Matrix effects for A) group 1 and B) group II matrices when using three different cleanup techniques.

III

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Phytoextraction of per- and polyfluoroalkyl substances (PFAS) and the influence of supplements on the performance of short–rotation crops☆

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic compounds threatening water quality and food safety worldwide. Phytoremediation is a nature-based, cost-effective, and scalable solution with high potential for treating PFAS-contaminated sites. However, there is a large knowledge gap regarding choice of plant species and methods to enhance performance. This study assessed the PFAS phytoextraction potential of sunflower (*Helianthus annuus*), mustard (*Brassica juncea*), and industrial hemp (*Cannabis sativa*) in a greenhouse experiment, using inorganic fertilizer and a microbial mixture as supplements. PFAS concentrations were measured using UPLC-MS/MS, and bioconcentration factors for different plant tissues and removal efficiency were determined. Perfluoroalkyl carboxylic acid (PFCA) accumulation was 0.4–360 times higher than that of perfluoroalkyl sulfonic acid (PFSA) homologues of similar perfluorocarbon chain length. Inorganic fertilizer significantly ($p < 0.001$) reduced PFAS concentration in all plant tissues, whereas the microbial mixture tested did not affect PFAS concentration. PFAS uptake ranged from 0.2 to 33% per crop cycle. Overall, the potential number of crop cycles required for removal of 90% of individual PFAS ranged from six (PFPeA) to 232 (PFOA) using sunflower, 15 (PFPeA) to 466 (PFOS) using mustard and nine (PFPeA) to 420 (PFOS) using Hemp. In this study, the percentage of PFAS removal by plants was determined, and an estimation of the time required for PFAS phytoextraction was determined for the first time. This information is important for practical phytoremediation applications.

1. Introduction

Mass contamination of land with per- and polyfluoroalkyl substances (PFAS) mainly occurs from use of contaminated biosolids, firefighting activities using PFAS-containing aqueous film-forming foams (AFFF), landfilling, and atmospheric deposition. The contaminated land becomes a hotspot and source of PFAS for other parts of terrestrial and marine ecosystems (Hamid et al., 2018; Bolan et al., 2021a). Thus remediation remains a vital measure for managing the fate of PFAS at newly and historically contaminated sites. A wide array of PFAS remediation techniques are being developed and assessed (Naidu et al., 2020).

Phytoremediation is the utilization of plants to accumulate (phytoextraction), immobilize (phytostabilization), or destroy (phytodegradation) pollutants in a target medium (EPA, 2000). This technique is potentially useful for managing PFAS-contaminated sites (Kavusi et al., 2023). PFAS in plants have received much attention, as they are a potential hazard to human health. Some studies have focused on the uptake and transportation of perfluoroalkylacids (PFAA) and the degradation and uptake of PFAS precursors and their metabolites in edible plants (Bizkarguenaga et al., 2016; Blaine et al., 2014; Wen et al., 2014). Other studies have examined the phytotoxicity of PFAS by investigating the effects on plant growth, biomass, and various enzymes and genes (Chen et al., 2019; Zhang et al., 2019a). However, few studies have examined the potential of plants as a PFAS remediation strategy, although various review articles on the topic have been published (Kavusi et al., 2023; Lesmeister et al., 2021; Mayakaduwage et al., 2022).

Plants differ in their ability to accumulate PFAS and the success of a phytoremediation program is strongly determined by the plant species used (Mench et al., 2010; Ghisi et al., 2019). The potential of phytoremediation was first highlighted in a study investigating the fate of PFAS in plant species at a former firefighting site, where removal of up to 1.4 g of 26 PFAS per year was estimated for both silver birch and pine

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(Gobelius et al., 2017). A few subsequent studies have investigated the phytoremediation potential of plant species such as reed grass in wetlands (30–50% removal), *Juncus sarophorus* (9–11% PFOS removal), and other woody and herbaceous species in a greenhouse experiment (Huff et al., 2020; Zhu et al., 2022; Ferrario et al., 2022). Thus, there is a need to identify plant species with good ability to accumulate PFAS. Sunflower, mustard, and hemp have been used previously in heavy metal phytoremediation programs, mainly for their high biomass production, tolerance to environmental stress, and ability to hyperaccumulate contaminants (Nehnevajova et al., 2005; Rathore et al., 2019; Todde et al., 2022). These promising plant species need to be assessed for their phytoextraction potential when exposed to a wide range of PFAS prior to field application.

Furthermore, improving the plant growing environment increases accumulation of contaminants in plants (Vangronsveld et al., 2009; Bolan et al., 2021b), through increased bioavailability of the contaminants in the growing medium or increased plant survival and vigor (Mench et al., 2010; Vangronsveld et al., 2009; Mench et al., 2009). For example, aeration has been shown to increase the PFAS phytoextraction potential of duckweed grown on deionized water at pH 2.3 by up to 80% (Zhang and Liang, 2020). Soil additives such as chelating agents, fertilizers, and microbial supplements have been tested, especially at sites with heavy metal contamination (Radziemska et al., 2021; Wang et al., 2021; Haider et al., 2021). Application of supplements could increase plant biomass and water uptake which could in turn increase PFAS uptake, especially water-soluble PFAS. However, to our knowledge, no previous study has assessed the effect of soil supplements (i.e., inorganic fertilizers and microorganisms) on plant accumulation of PFAS.

This study evaluated the PFAS phytoextraction potential of three short rotation plants (sunflower (*Helianthus annuus*), mustard (*Brassica juncea*), and industrial hemp (*Cannabis sativa*)) in a pot experiment within a greenhouse set-up. Specific objectives were to: (i) determine PFAS concentrations and distribution in the different plants, (ii) evaluate the effect of inorganic fertilizer and a microbial supplement on PFAS uptake in the plants, (iii) estimate PFAS removal by the plants, and (iv) predict temporal changes in the concentrations of selected PFAS in soil hosting the different plant species.

2. Materials and methods

2.1. Chemicals

The target analytes comprised: 10 perfluoroalkyl carboxylic acids (PFCA), namely perfluorobutanoic acid (PFBA), perfluoropetanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohepatanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododadecanoic acid (PFDoDA), and perfluorotetradecanoic acid (PFTeDA); three perfluoroalkyl sulfonic acids (PFSA), namely perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS); and one perfluorooctanesulfonamide (FOSA) (Table S1 in Supporting Information (SI)). Nine mass-labelled internal standards (IS) were used ($^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, and $^{13}C_8$-FOSA) (Table S2 in SI). All above-mentioned compounds (with purity ≥99% were obtained from Wellington Laboratories (ON, Canada).

Methanol, acetonitrile, ammonium acetonitrile, and formic acid of high analytical grade were obtained from Sigma Aldrich (USA). Ultrapure water was obtained from a Milli-Q Advantage Ultrapure water purification system coupled with a 0.22 μm Millipak Express membrane and LC-Pak polishing unit from Merck Millipore (Billerica, USA).

2.2. Experimental design

The pot experiment was conducted in a greenhouse at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, with temperature of 22 °C during the day and 18 °C at night, light/dark cycle set to 16/8 h, light intensity 150 μmol, and 50–60% relative humidity. The experiment had a 3 x 4 factorial design, with three plants (sunflower, mustard, hemp) and four soil supplements (a microbe mixture, fertilizer, fertilizer + microbes, and a control (no fertilizer or microbes)) (Fig. 1). The growing medium consisted of organic potting soil (S-jord garden soil, Hasselfors company, Sweden) spiked to achieve a theoretical concentration of 1 mg kg^{-1} for each PFAS (for details, see text in SI). The spiked concentration is environmentally relevant and has been reported at various contaminated sites worldwide (Brusseau et al., 2020). Measured PFAS concentrations in soil at time point 0 was 1.5 ± 0.9 mg kg^{-1} for each PFAS. Seeds of sunflower, mustard, and hemp were pre-germinated for six weeks, and then transplanted (one per pot) in plastic pots with dimensions 13.7 x 13.7 × 23 cm (L x W x H) and 3 L volume, and containing 1 kg wet weight (ww) of PFAS-spiked soil. Each 3 x 4 experiment was performed in triplicate, resulting in a total of 36 pots (Fig. 1).

Irrigation water containing supplements was applied ad libitum to all pots throughout the experiment. For the treatment with fertilizer, a fertilizer solution containing (g L^{-1}): 51 N, 10 P, 43 K, 4 S, 3 Ca, 4 Mg, 0.17 Fe, 0.20 Mn, 0.10 B, 0.03 Zn, 0.015 Cu and 0.004 Mo obtained from Wallco Plant Nutrition (Cederroth International, Sweden) was used. For the treatment with microbes, a commercial microbial supplement (Tarantula Beneficial Bacterial Liquid fertilizer) containing *Arthrobacter globiformis*, *Bacillus brevis*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus thuringiensis canadiensis*, and *Paenibacillus polymyxa* was used. It was mixed with irrigation water in a ratio of 1:2 before application. For the fertilizer + microbes treatment, the microbial supplement was mixed with the fertilizer solution. Tap water was used to irrigate all control pots.

2.3. Sample preparation and analysis

All plants were harvested after three months of PFAS exposure and samples of each plant were divided into seeds, leaves, stem, and root. Water and soil samples were also collected. Preparation and extraction of plant and soil samples for PFAS was done using validated methods published elsewhere (Nassazzi et al., 2022) (details available in SI). Samples of irrigation water were extracted by solid phase extraction (SPE) using Oasis WAX cartridges (Waters, 150 mg, 6 mL, 30 μm) and the method can be found elsewhere (Gobelius et al., 2017). Branched isomers of PFOS and FOSA were quantified using the corresponding linear standards.

All samples were analyzed using an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) (Thermo Scientific Dionex Ultimate 3000 Pumps; TSQ Quantiva, Thermo Fisher Scientific, San Jose, CA USA). An Acquity UPLC BEH-C18 (2.1 × 50 mm, 1.7 μm particle size; Waters Corporation, Manchester, UK) analytical column was used for chromatographic separation. The data were evaluated using TraceFinder software (version 4.1, Thermo Fisher, USA) (details available in SI).

2.4. Quality control and assurance

Laboratory blanks, replicates, method detection limits (MDLs), linearity, and recovery were assessed. MDLs for plants were determined using a signal to noise ratio of 3 in matrix-spiked samples with a concentration of 5 ng g^{-1} dry weight (dw). The MDLs for water and soil samples were calculated based on average blank + 3xstandard deviation. A calibration curve with concentration ranging from 0.01 to 200 ng mL^{-1} for each PFAS was used for quantification. Correlation coeffients (R^2) of the calibration curve were used to determine the linearity. The relative recovery of the method was assessed using reference composite plant samples (pre-spike n = 3 and post-spike n = 3). The

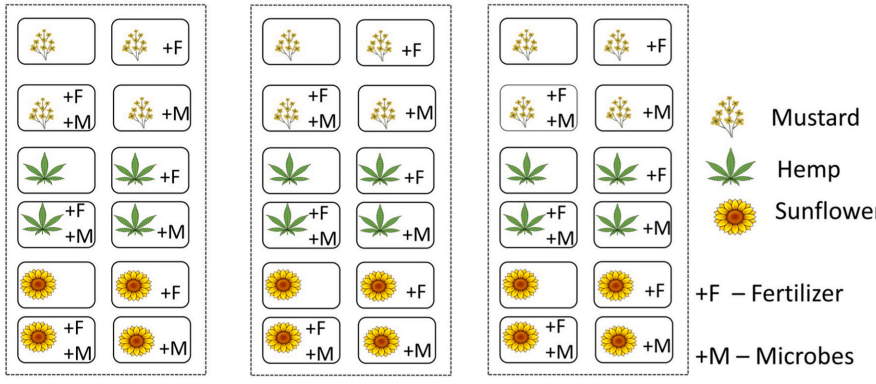

Fig. 1. Schematic diagram of the triplicate greenhouse experiment set-up of mustard, hemp, and sunflower pots, with and without fertilizer and microbe supplements.

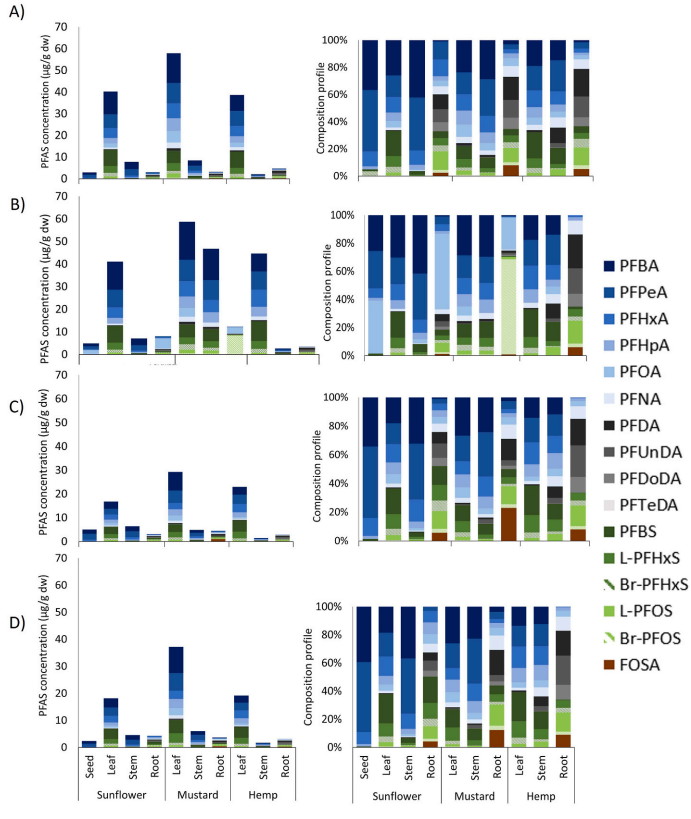

Fig. 2. Average PFAS concentration (µg g^{-1} dw) and composition profile (%) in different tissues (n = 3) of sunflower, mustard, and hemp grown in PFAS-spiked soil with different supplements: A) Untreated control, and supplementation with B) only microbes, C) both fertilizer and microbes, and D) only fertilizer.

composition of the reference samples can be found elsewhere (Nassazzi et al., 2022). Recoveries of the internal standards were also determined. Details on the MDLs, relative recovery values, and blank levels are available in Tables S3–S5 in SI.

2.5. Calculations

Plant concentration factors, representing the ability of different tissues (leaf, stem and root) to accumulate contaminants from soil, were calculated using the following equations:

$$\text{Leaf concentration factor (LCF)} = C_{leaf}/C_s \quad (1)$$

$$\text{Stem concentration factor (SCF)} = C_{stem}/C_s \quad (2)$$

$$\text{Root concentration factor (RCF)} = C_{root}/C_s \quad (3)$$

$$\text{Bioconcentration factor (BCF)} = C_p/C_s \quad (4)$$

where Cs is the PFAS concentration in soil (ng g^{-1} dw), C_{leaf}, C_{stem} and C_{root} is the PFAS concentration in the leaves, stem and root, respectively (ng g^{-1} dw), and C_p is the PFAS concentration in the whole plant (ng g^{-1} dw) at time of harvest.

Removal efficiency (r) was calculated as:

$$r = \frac{C_p\, M_p}{C_{si}\, M_s} \times 100 \quad (5)$$

where M_p is plant biomass (g dw), C_{si} is initial soil concentration (ng g^{-1} dw), and M_s is soil mass (g dw).

2.6. Statistics

Descriptive statistics (mean, standard deviation, and range), regression, correlation analyses, and data visualization were performed using GraphPad Prism (version 9.2.0 (332)). Statistical differences between means were evaluated using analysis of variance (ANOVA) at significance level $\alpha = 0.05$, using the R software.

3. Results and discussion

3.1. PFAS concentration in plants of the different species

Of the 14 target PFAS, 12 were detected in different tissues of sunflower, mustard, and hemp (Fig. 2, Tables S7–S8 in SI). ΣPFAS concentration was significantly higher (ANOVA, $p < 0.05$) in mustard than in sunflower and hemp in all treatments (Table S6 in SI). Without any supplement (control), mustard plants were observed to contain 2-7 times higher concentrations of some PFAS than sunflower (PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, FOSA) or hemp (PFOA, PFDoDA, PFOS, FOSA). A previous study investigating PFAS accumulation in various plants, including sunflower and mustard, found that the concentration of six PFAS was 3–6 times higher in mustard than in sunflower. Studies on other plants have also reported differences in PFAS uptake with plant species and genotypes (Blaine et al., 2014; Gobelius et al., 2017; Xiang et al., 2018). Variations in PFAS uptake are caused by plant anatomy and physiological traits such as biomass, transpiration rate, growth rate, root composition, and exudates (Sheoran et al., 2016).

ΣPFAS concentrations were also significantly different ($p < 0.001$) between the plant tissue groups and generally decreased in the order: leaf > stem > root ≈ seed (sunflower only). In the control, ΣPFAS concentrations in sunflower were within the range 0.018–11 μg g^{-1} dw in leaves, 0.003–3.3 μg g^{-1} dw in stems, 0.029–0.41 μg g^{-1} dw in roots, and 0.004–1.3 μg g^{-1} dw in seeds. The ΣPFAS concentration range in mustard was 0.015–14 μg g^{-1} dw in leaves, 0.005–2.4 μg g^{-1} dw in stems, and 0.069–0.54 μg g^{-1} dw in roots. In hemp, the ΣPFAS concentration range was 0.0008–7.4 μg g^{-1} dw in leaves, 0.007–0.48 μg g^{-1} dw in stems, and 0.07–0.96 μg g^{-1} dw in roots.

In general, the concentration of individual PFAS in the three plant species decreased in the order: PFBA > PFPeA > PFBS > PFHxA > PFHpA > PFHxS > PFOA > PFNA > PFOS > PFDA > PFUnDA > FOSA > PFDoDA. FOSA was only detected in the roots. Short-chain PFCA (i.e., PFBA, PFPeA, PFHxA, PFHpA) were the predominant PFAS accumulated in sunflower seeds (95% of ΣPFAS), and leaf (57–62%) and stem (52–95%) tissues in all three plant species. Sunflower stems had a lower proportion of PFSA (3.7% of ΣPFAS) than stems of mustard (14%) and hemp (21%). In contrast, the composition profile of roots was dominated by PFSA (27–31% of ΣPFAS) and long-chain PFCA (32–52%) homologues in all three plant species. This is consistent with previous findings for other plant species (Gredelj et al., 2020; Krippner et al., 2015). Some studies have also reported presence of PFAS in seeds of various cereals such as maize, wheat, rye, and canola in different experimental set-ups (Krippner et al., 2015; Stahl et al., 2013; Stahl et al., 2009). The variation in PFAS composition of different plant parts suggests that water-soluble and mobile short-chain PFAS are transported in the plant during water uptake and transpiration, and accumulate in upper plant parts. Hence, short-chain PFAS dominated in leaves and stems.

Use of a supplement (fertilizers, microbes, or fertilizer + microbes) significantly ($p < 0.001$) affected ΣPFAS concentrations in plants. Fertilizer application (with or without microbes) significantly ($p < 0.001$) reduced the ΣPFAS concentration in all plant tissues, by on average 19% (roots) to 49% (foliage) (Fig. 2C and D). A previous study involving supplementation of lettuce, tomato, and maize with biosolids to meet their nitrogen requirement observed increased concentrations of PFBA and PFPeA at high biosolid application rates to the soil (4 times the agronomic nitrogen requirement) (Blaine et al., 2013). However, this could have been due to more PFAS being applied with increasing biosolids application, rather than an effect of nitrogen on PFAS uptake.

Supplementation with the microbial mixture did not significantly affect PFAS concentration in any of the plant species studied ($p > 0.05$) (Fig. 2B). The microorganisms applied possess pesticidal effects, and also the ability to increase soil fertility and plant tolerance to stress (Hashem et al., 2019; Dobrzyński et al., 2022). These traits can enhance plant growth and survival, and could potentially increase PFAS concentration in the plant. However, this was not observed under the experimental conditions in the present study. The effect of PFAS on soil microbial communities and microbial PFAS remediation in the presence and absence of plants has been discussed in previous studies (Zhang et al., 2019b; Arslan and Gamal El-Din, 2021), but no published data are currently available on the effect of plant-microbial interactions on PFAS accumulation. Inoculation of plants with microorganisms has been shown to enhance the concentration of heavy metals in plants (Jankong et al., 2007; Alves et al., 2022), but more research is needed on PFAS uptake in plants.

3.2. Plant tissue-specific concentration factors

The bioaccumulation factors for leaf (LCF), stem (SCF) and root (RCF) of the different species were evaluated. ΣPFAS accumulation was generally highest in the order leaves > stem > roots. Observed LCF values for individual PFAS ranged between 0.6 (PFUnDA) and 2092 (PFBA) for sunflower, 0.13 (FOSA) and 1816 (PFBA) for mustard, and 0.033 (PFDoDA) and 2671 (PFBA) for hemp. Observed SCF for individual PFAS ranged between 0.1 (PFUnDA) and 656 (PFBA) for sunflower, 0.17 (PFDoDA) and 365 (PFBA) for mustard, and 0.2 (PFDoDA) and 197 (PFPeA) for hemp. Observed RCF for individual PFAS ranged between 0.37 (PFHxS) and 42 (PFPeA) for sunflower, 0.27 (PFHxS) and 12 (PFBA) for mustard, and 0.97 (PFBS) and 11 (PFUnDA) for hemp PFAS (for details, see Tables S9–S11 in SI). Thus LCF was higher than SCF or RCF, which is similar to previous findings (Navarro et al., 2017; Lechner and Knapp, 2011). This study is the first to report LCF and SCF

for 12 different PFAS in sunflower, mustard, and hemp.

The actual plant tissue concentration factors were generally higher than those previously reported for various edible plants (Ghisi et al., 2019), grass (Yoo et al., 2011), and forest trees (Gobelius et al., 2017). This could indicate that sunflower, mustard, and hemp have higher PFAS accumulation and uptake efficiency than previously studied plant species at similar PFAS concentration. However, plant concentration factors are influenced by PFAS bioavailability which is controlled by the physicochemical properties of PFAS, soil and plant factors (Lesmeister et al., 2021). Our results also revealed that PFAS uptake is dominated by roots, in which dissolved contaminants together with nutrients and water can be acropetally transported through the transpiration stream and accumulated in the leaves (Collins et al., 2006).

Linear regression plots of log-transformed data showed a significant decrease in LCF and SCF for PFCA with increasing perfluorocarbon chain length for all plant species studied ($p < 0.05$) (Fig. 3). Each addition of a perfluorocarbon moiety (CF_2) led to a decrease of 0.3–0.5 log units in both LCF or SCF. This is consistent with trends reported for vegetables and grass (Blaine et al., 2013; Yoo et al., 2011; Felizeter et al., 2012), and demonstrates the reliance of PFAS uptake and transport on their physiochemical properties. PFAS bioavailability in the soil is predominantly influenced by compound mobility, which can be predicted using the soil sorption coefficient (K_d) (Nguyen et al., 2020). A higher K_d value results in increased sorption, due to increases in both hydrophobicity and lipophilicity. Thus with each CF_2 added, both the absorption and transport of PFAS are reduced (Collins et al., 2006; Felizeter et al., 2014).

The LCF and SCF values for PFSA showed similar dependence on perfluorocarbon chain length as seen for PFCA. However, plant tissue accumulation of PFCA was 0.4–360 fold higher than for PFSA homologues of similar perfluorocarbon chain length. Although PFOA and PFOS uptake was observed to be a non-competitive process, a previous study found higher accumulation of PFOA compared with PFOS in wheat straw grown on biosolids-amended soil (Wen et al., 2014), which is in agreement with the results in this study. This can be explained by the physicochemical properties of PFSA molecules, which have a larger structure and stronger sorption to surfaces than PFCA molecules of similar perfluorocarbon chain length (Higgins and Luthy, 2006). In this study, FOSA, which has been shown to have higher K_d than PFOS and PFNA (Nguyen et al., 2020), was mainly found in the roots of all plants investigated. This implies that FOSA was strongly sorbed to the roots, which limited its transportation to the upper parts of the plant.

With regard to RCF, a different relationship with perfluorocarbon chain length was found for PFCA (Fig. 4). A significant decrease in RCF

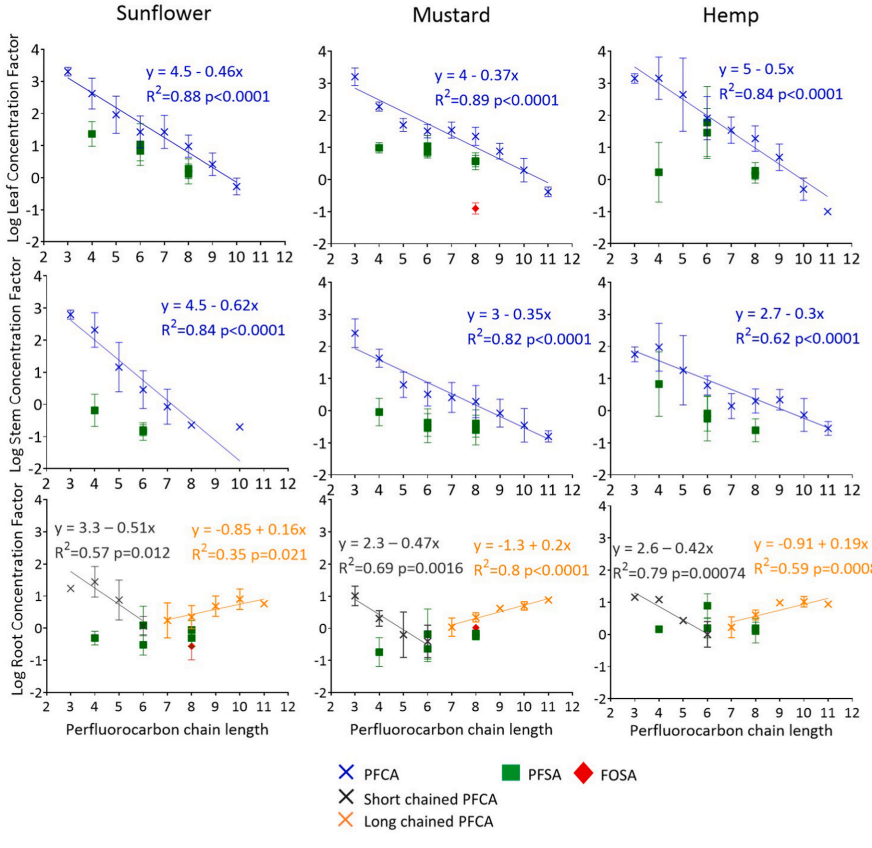

Fig. 3. Relationship between leaf concentration factor (LCF), stem concentration factor (SCF), and root concentration factor (RCF) and perfluorocarbon chain length for sunflower, mustard, and hemp plants grown on PFAS-spiked soil.

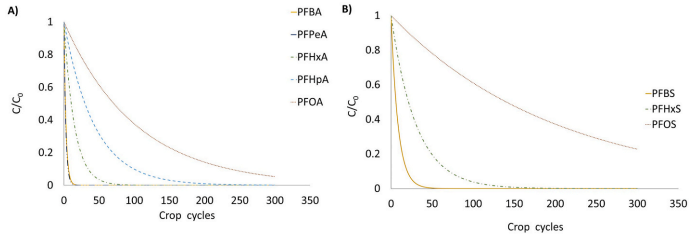

Fig. 4. Estimated number of crop cycles (1 crop cycle = 90 days) required to phytoextract the PFAS A) PFCA and B) PFSA from a contaminated site using sunflower.

with an increase in perfluorocarbon chain length was found for C_3 (PFBA) to C_6 (PFHpA) compounds ($p < 0.05$), but a significant increase for C_7 (PFOA) to C_{11} (PFDoDA) compounds ($p < 0.05$) in all plants. Thus RCF was lowest at C_6 (PFHpA) for all plants. A similar trend has been observed previously for hydroponically cultivated lettuce (*Lactuca sativa*) and for wheat (*Triticum aestivum*) in field experiments (Wen et al., 2014; Felizeter et al., 2012). The RCF values were also generally lower than both the LCF and SCF values. The low RCF observed for shorter-chain PFAS was probably due to their high mobility and continuous transportation to other plant tissues. Long-chain PFAS are structurally larger and more lipophilic than their short-chain counterparts (Buck et al., 2011), so limited amounts of long-chain PFAS are absorbed into the roots and there is limited transportation to other plant tissues (Costello and Lee, 2020). It should also be noted that all plant tissues in this study were thoroughly washed with water and MeOH (50:50) before analysis. Therefore, the results obtained mainly represent PFAS taken up by the roots, but it is possible that some PFAS were still sorbed onto root surfaces before washing and analysis. Studies using soil as the planting medium have generally found no relationship between RCF and chain length, especially at high PFAS concentrations (Blaine et al., 2014; Wen et al., 2014).

3.3. Species-specific accumulation

Bioconcentration factor, determined as the ratio of ΣPFAS concentration in the plant to ΣPFAS concentration in the soil at harvest, was used to assess and compare the overall PFAS accumulation and phytoextraction potential of the three plant species studied. Hemp had the highest BCF for ΣPFAS (0.05–1170), followed by sunflower (0.03–957) and mustard (0.19–590) (Tables S12–S13). BCF values >1 signify plant ability to accumulate a contaminant, while BCF values >10 indicate that the plant is a hyperaccumulator (Huff et al., 2020). Based on these thresholds, all three plant species tested were classified as hyperaccumulators of at least five compounds (PFBA, PFPeA, PFHxA, PFHpA, and PFDoDA). In addition, hemp was a hyperaccumulator of PFOA, PFNA, PFDA, PFBS, and PFHxS. A previous study assessing PFAS accumulation in both woody and herbaceous plants observed similar results, but found that sunflower only hyperaccumulated PFPeA among six compounds analyzed (Huff et al., 2020), in contrast to our results. In the same study, mustard was observed to have higher BCF values for all compounds except PFPeA than the BCF values found in our study. Other studies on vegetables and forest plants also report variations in plant BCFs, which they attribute to plant chemical composition (lipid:protein content) and the PFAS fingerprint of the growing medium (Gobelius et al., 2017; Xiang et al., 2018; Blaine et al., 2013; Wen et al., 2013).

3.4. Total plant burden

Total plant burden was determined as the absolute weight (in μg) of PFAS in plant biomass. Sunflower had the highest ΣPFAS burden (819 ± 262 μg per plant), followed by hemp (732 ± 111 μg). Despite mustard having high PFAS concentrations in plant tissues, it had the lowest ΣPFAS burden (417 ± 97 μg), which can be explained by the lower biomass of mustard plants compared with sunflower and hemp. At the time of harvest, hemp had not reached flowering, whereas sunflower and mustard had flowered. Therefore, the phytoextraction potential of hemp may not have been fully exploited in this study as plants probably did not attain full maturity. Mass PFAS distribution in different tissues was relatively similar between the plants (Table 1). Of the total PFAS mass (μg) found in the plants, C_3–C_9 perfluorocarbon PFAS were dominant in the shoot system (leaves and stem), while C_{10}–C_{11} perfluorocarbon PFAS were dominant in the root system, as also indicated by the LCF, SCF, and RCF values. The PFAS dominance in the shoot system could have positive implications for phytoremediation, as shoots are easier to harvest and complete root harvest can be difficult to achieve. Mustard and sunflower had 4-6 times more short-chain PFAS in their stems than hemp, which accumulated >90% of this group of compounds in the leaves. Only a small fraction (≤6%) of C_3–C_6 perfluorocarbon PFAS accumulated in seeds in sunflower.

3.5. Effect of fertilizer and microbial supplements

Plant response to the different supplements was examined using plant biomass, PFAS concentration in plant tissues, and effect on total plant burden. Sunflower (281 g ww, 54 g dw) and hemp (140 g ww, 47 g dw) produced more average biomass per plant than mustard (12 g ww, 7.9 g dw). Mustard had a much higher proportion of dry matter (up to 68%) than sunflower (19%) and hemp (34%). Addition of fertilizer was observed to increase plant biomass by 2- to 3-fold in sunflower and hemp, but slightly reduced the dry mass proportion for both species (from 34 to 18% for hemp, and from 19 to 15% for sunflower). There were no observable changes in biomass and dry matter content for mustard. The increase in biomass in sunflower and hemp did not result in an increase in PFAS accumulation. As previously noted (section 3.1), fertilizer application led to reduced PFAS concentration in plants. However, the greater biomass obtained for plants treated with fertilizer led to no significant difference in absolute PFAS mass in plants (ANOVA, $p < 0.05$) (Table S14 in SI). The mechanism for reduction of PFAS concentration in plants due to addition of inorganic fertilizers is not fully understood. However, possible reasons include (i) increased cation concentration that could reduce PFAS bioavailability (Cai et al., 2022), or (ii) increased water uptake, which led to dilution of contaminants in the plant. In the present study, use of the microbial supplement had no observable effects on biomass, dry matter content, or plant burden of PFAS. This is consistent with previous findings of increased plant biomass, but reduced heavy metal concentration, in rye (*Secale cereale*) supplemented with both inorganic fertilizers and microbes at a contaminated site in China (Chen et al., 2023). Further studies using metagenomics and root microscopy are needed to identify potential synergistic effects between specific organisms and plants, and their effect on PFAS accumulation.

Table 1
Distribution of individual PFAS based on the total burden in tissues of sunflower, mustard, and hemp, expressed as a percentage of their total PFAS uptake. A color gradient from green (highest) to red (lowest) represents the mass distribution.

	Sunflower (%)				Mustard (%)			Hemp (%)		
Compound	Seed	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
PFBA	4	61	35	0	64	36	0	94	6	0
PFPeA	6	46	47	1	56	43	1	91	9	0
PFHxA	3	65	30	2	67	32	1	94	6	0
PFHpA	2	76	21	1	73	26	1	94	5	1
PFOA	0	91	6	2	77	21	2	93	6	1
PFNA	0	88	5	7	71	24	5	84	13	3
PFDA	0	63	0	37	55	23	22	54	33	14
PFUnDA	0	18	2	81	28	19	53	16	41	43
PFDoDA	0	0	0	100	6	7	87	2	21	78
PFBS	0	94	5	1	72	27	2	95	5	1
L-PFHxS	0	95	4	2	79	19	3	95	3	1
Br-PFHxS	3	92	2	3	87	7	7	95	2	3
L-PFOS	0	86	1	13	71	19	10	75	15	9
Br-PFOS	0	89	0	11	75	17	8	88	0	12
FOSA	0	0	0	100	12	0	88	0	0	100

3.6. Phytoremediation potential

All plants grew without any visible abnormalities (such as chlorosis, stunting, or reduction in weight) despite the presence of PFAS, with or without supplements. This indicates that the species studied had high tolerance to PFAS contamination and could grow at PFAS-contaminated sites. Other studies have also observed no negative impact of PFAS on plant growth (Gobelius et al., 2017; Zhao et al., 2016), except at very high concentrations (e.g., 5–20 mg L^{-1}) (Chen et al., 2019; Wen et al., 2013). This is $\geq$ 10 times higher than the concentration used in this study and not realistic for PFAS-contaminated sites (Gobelius et al., 2017).

Individual PFAS uptake efficiency from soil was 0.2–33% for sunflower, 0.2–14% for mustard, and 0.2–24% for hemp, based on the PFAS concentrations in soil and all plant tissues (Table S15 in SI). For all plants, PFUnDA and PFDoDA had the lowest PFAS removal by plants, while the highest PFAS uptake efficiency was observed for PFPeA (14–33%), followed by PFBA (12–30%), PFBS (3.2–12%), PFHxA (3.7–6.8%), PFOA (1–1.1%), PFHxS (1.5–3.3%) and PFOS (0.4–0.5%).

The phytoremediation potential of the three plant species for individual PFAS was predicted based on crop cycles (1 crop cycle = 90 days of PFAS exposure), assuming constant PFAS uptake for subsequent crop cycles. Previous studies have reported an effect of PFAS concentration in the growing medium (e.g., water and soil) and PFAS concentration in plant tissues (Gobelius et al., 2017; Wen et al., 2013). However, there is no consensus on the influence of PFAS concentration on PFAS removal efficiency (Lesmeister et al., 2021; Wen et al., 2013). We, therefore, estimated the number of crop cycles required to phytoremediate soil with a similar PFAS concentration as tested in this experiment using sunflower (highest PFAS removal) and mustard (lowest PFAS removal). PFAS concentration after a cycle of phytoextraction was determined as C_i - rC_i, where C_i is initial PFAS concentration and r is percentage PFAS removal by the plant. PFAS concentration and number of cycles required were determined using an iterative approach.

For sunflower, shorter-chain PFAS required fewer crop cycles to reach 90% PFAS removal from soil. PFBA and PFPeA were estimated to require 6–7 crop cycles, PFHxA 34, PFHpA 96, PFOA 232, PFBS 20, PFHxS 70, and PFOS 458 crop cycles (Fig. 4 and Table S16 in SI). Estimated crop cycles required when using hemp increased as follows: PFPeA required 9 crop cycles, PFBA 14, PFBS 16, PFHxA 32, PFHxS 66, PFHpA 68, PFOA 165, PFOS 420. Similar findings were made for mustard, but this species generally was estimated to require more cycles than sunflower, i.e., PFBA required 19 crop cycles, PFPeA 15, PFHxA 60, PFHpA 100, PFOA 192, PFBS 20, PFHxS 70, and PFOS 466 crop cycles. The results highlighted the suitability of the method for media dominantly contaminated with short-chained PFAS. Furthermore, the results suggest that sunflower is a more suitable plant than mustard for phytoremediation of PFAS-contaminated sites, however, field experiments are required to verify these findings.

4. Conclusions

This study investigated the PFAS phytoextraction potential of sunflower, mustard, and hemp in greenhouse experiments. The results showed differences between the plant species in phytoremediation and PFAS-specific accumulation in different tissue types. All three species hyperaccumulated at least five of the target PFAS, and are thus potentially suitable for phytoremediation in the field. Treatments to optimize the phytoextraction potential of the species by using inorganic and microbial supplements gave only a limited improvement in PFAS uptake for all species. The estimated number of crop cycles required to remove individual PFAS from contaminated soil was lowest, i.e., removal efficiency was highest, for short-chain PFAS. This new information can be used in risk management and practical application of phytoremediation in the field. Harvested plant biomass can be used for energy production through which extracted PFAS can be degraded. However, life cycle analysis to determine and prevent potential negative environmental impacts of this process is needed for the future. Future studies should also examine other microbial species and the effects of microbial interactions with plants.

Credit author statement

Winnie Nassazzi: Conceptualization, Methodology, Data curation, Formal analysis, Writing-original draft, and editing. Tien-Chi Wu: Investigation, Data curation and Writing-review and editing, Jana Jass: Methodology, Writing-review and editing, Supervision, Foon Yin Lai: Conceptualization, Validation, Formal analysis, Writing-review and editing, Supervision. Lutz Ahrens: Conceptualization, Writing-review and editing, Supervision, Funding acquisition, and Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122038.

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Supplementary information

Phytoextraction of per- and poly-fluoroalkyl substances (PFAS) and the influence of supplements on the performance of short–rotation crops

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Contents

More details on soil preparation

A PFAS mixture (Table S1) was prepared to achieve a concentration of 1 μg g^{-1} soil for each PFAS. Aliquots of the soil (1 kg) were spiked with the PFAS mixture and shaken for 1 week in an overhead shaker (Heidolph Reax 2 overhead shaker, Germany) to obtain a homogenized mixture in the form of wet soil. The wet soil was then manually mixed using a shovel with the rest of the soil (32 kg) by adding 1 kg of unspiked soil at a time. The soil was continuously mixed to obtain a homogeneous mixture and then aged for two weeks in darkness at 4 °C in a refrigerator. Before planting, the soil was distributed into 3-L pots (n = 36), so that every pot had 1 kg wet weight (ww). During planting, soil samples were collected to estabilish the intial PFAS concetration in soil.

More details on plant and soil sample preparation for analysis

Freeze-dried and homogenized, plant and soil samples (2 g dry weight (dw)) were weighed into 50 mL PP-tubes, spiked with 50 µL of IS mixture (5 ng absolute for each IS), and extracted in three cycles with solid-liquid extraction using acetonitrile. The supernatants from the extraction cycles were collected and cleaned with 1 g ENVI-Carb cartridges before concentration to near dryness under a constant stream of nitrogen at room temperature. The extracts were reconstituted to 500 µL with 50:50 ultrapure water and methanol solution and then analysed.

Instrumental parameters

The temperature of the column oven was set at 40 °C. The system was equipped with a heated electrospray ion source with static spray voltage set at 2500 V negative mode.

Ultrapure water containing of 5 mM ammonium acetate (phase A) and acetonitrile (phase B) was used as the mobile phase. The gradient started at 0% of phase B and increased to 95% from 0.5 min to 8.0 min. This was maintained until 11 minutes, after which it returned to initial conditions. The total run time was 12 minutes.

Table S1: Target analytes with their corresponding classification, acronyms, and molecular formula

Compound	Abbreviation	Molecular formula
Perfluorinated carboxylic acids (PFCAs)		
Perfluorobutanoic acid	PFBA	$C_3F_7CO_2^-$
Perfluoropentanoic acid	PFPeA	$C_4F_9CO_2^-$
Perfluorohexanoic acid	PFHxA	$C_5F_{11}CO_2^-$
Perfluoroheptane acid	PFHpA	$C_6F_{13}CO_2^-$
Perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2^-$
Perfluorononanoic acid	PFNA	$C_8F_{17}CO_2^-$
Perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2^-$
Perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2^-$
Perfluorododecanoic acid	PFDoDA	$C_{11}F_{23}CO_2$
Perfluorotetradecanoic acid	PFTeDA	$C_{14}F_{27}CO_2^{--}$
Perfluoroalkyl sulfonic acids (PFSAs)		
Perfluorobutane sulfonic acids	PFBS	$C_4F_9SO_3^-$
Perflurohexane sulfonic acid	PFHxS	$C_6F_{13}SO_3^-$
Perfluorooctane sulfonic acids	PFOS	$C_8F_{17}SO_3^-$
Precusors		
Perfluorooctanesulfonamide	FOSA	$C_8F_{17}SO_2NH_2$

Table S2: Target analytes with their corresponding internal standards

Internal Standard	Corresponding PFAS for quantification
$^{13}C_2$-PFHxA	PFBS, PFPeA, PFHxA
$^{13}C_4$-PFOA	PFHpA, PFOA
$^{13}C_5$-PFNA	PFNA
$^{13}C_2$-PFDA	PFDA
$^{13}C_2$-PFUnDA	PFUnDA
$^{18}O_2$-PFHxS	PFHxS
$^{13}C_4$-PFOS	PFOS
$^{13}C_8$-FOSA	FOSA

Table S3: Recoveries for individual PFAS in the different matrices

Recovery	**Plant**		**Water**		**Soil**	
	(n = 117)		**(n = 4)**		**(n = 36)**	
	Mean	± SD	Mean	± SD	Mean	± SD
$^{13}C_2$-PFHxA	117	30	113	0.2	137	12
$^{13}C_4$-PFOA	124	25	105	0.1	138	11
$^{13}C_5$-PFNA	124	22	92	0.1	139	12
$^{13}C_2$-PFDA	117	22	66	0.1	125	9
$^{13}C_2$-PFUnDA	108	21	36	0.2	121	10
$^{18}O_2$-PFHxS	125	24	103	0.1	139	10
$^{13}C_4$-PFOS	120	17	75	0.1	130	11

Table S4: Linearity, relative standard deviation (RSD), method detection limits (MDL), and relative recovery for leaves (Group I) and seeds, stem, and roots (Group 2)

	Linearity	Group I			Group 2		
Compound	R^2	RSD (%)	MDL (ng/g dw)	Relative recovery (%)	RSD (%)	MDL (ng/g dw)	Relative recovery (%)
PFBA	0.996	2.4	0.017	160	11	2.2	196
PFPeA	0.995	8.9	2.3	84	7.9	1.8	89
PFHxA	0.919	5.6	1.2	99	2.6	1.9	114
PFHpA	0.986	2.4	0.19	98	6.7	7.9	112
PFOA	0.986	2.3	0.12	97	8.8	0.51	105
PFNA	0.996	3.0	0.20	102	1.7	0.66	100
PFDA	0.992	6.2	0.32	97	3.9	0.79	82
PFUnDA	0.994	7.1	0.20	103	9.2	0.25	101
PFDoDA	0.994	13	0.061	124	11	0.058	61
PFTriDA	0.995	6.8	0.058	59	3.1	0.049	122
PFTeDA	0.995	9.3	0.092	85	13	0.048	152
PFBS	0.992	1.7	0.87	83	17	0.18	105
PFHxS	0.995	4.7	0.051	90	12	0.23	112
PFOS	0.996	2.8	0.14	105	5.0	1.0	157
FOSA	0.998	11	0.17	94	4.3	0.79	96

Table S5: Blanks with their concentration for each PFAS and their corresponding MDLs for soil and water samples[a]

Blanks	**Method blanks**			**Soil blanks**		
	Millipore water (n=8)			**Soil (n=2)**		
	Blank ($\mu g\ mL^{-1}$)	MDLs ($\mu g\ mL^{-1}$)	MQLs ($\mu g\ mL^{-1}$)	Blank ($\mu g\ g^{-1}$)	MDLs ($\mu g\ g^{-1}$)	MQLs ($\mu g\ mL^{-1}$)
PFBA	0.1051	0.046	0.152	0.0049	0.007	0.024
PFPeA	0.0131	0.001	0.004	0.0003	ND	0.001
PFHxA	0.0068	0.001	0.002	0.0294	0.003	0.012
PFHpA	0.0064	0.001	0.003	0.0362	0.015	0.050
PFOA	0.0044	ND	0.001	0.0129	0.015	0.050
PFNA	0.0016	ND	0.001	0.0046	0.003	0.010
PFDA	0.0018	ND	0.001	0.2303	0.089	0.295
PFUnDA	0.0029	0.001	0.003	0.0110	0.007	0.023
PFDoDA	0.0033	0.001	0.002	0.0348	0.010	0.032
PFBS	0.0037	0.001	0.003	0.0003	ND	0.001
PFHxS	0.0016	0.001	0.002	0.0004	ND	0.001
B-PFOS	0.0009	ND	0.001	0.0000	ND	ND
L-PFOS	0.0009	ND	0.001	0.0008	ND	0.001

[a]ND = not detected.

Results

Table S6: Analysis of variance (ANOVA) to assess the effect of various parameters on PFAS concentration

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Compound	14	1676	119.71	45.6519	< 2.2e-16 ***
Tissue	3	1928.3	642.77	245.117	< 2.2e-16 ***
Treatment	3	162.1	54.05	20.6111	3.974e-13 ***
Plant species	2	114.3	57.13	21.786	4.498e-10 ***
Treatment: Plant	6	17.5	2.91	1.1109	0.3534
Residuals	1771	4644.1	2.62		

Significance level: ***<0.001, **<0.01, *<0.05.

Table S7: PFCA concentration (ng g^{-1} dw) for each plant tissue with or without supplement[a]

Plant	Tissue	Supplement	Replicate	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA
Sunflower	Seed	None	1	1284	1468	399	92	5.4	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	None	2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sunflower	Seed	None	3	870	1190	251	29	3.3	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	None	1	14429	10372	6995	3844	2251	712	154	18	<MDL
Sunflower	Leaf	None	2	8063	3642	2136	1119	1051	405	114	14	<MDL
Sunflower	Leaf	None	3	8884	4929	4792	2541	2510	1188	291	22	<MDL
Sunflower	Stem	None	1	2922	2341	702	328	59	22	<MDL	<MDL	<MDL
Sunflower	Stem	None	2	2378	2138	262	101	34	17	<MDL	6.4	<MDL
Sunflower	Stem	None	3	4541	4638	1509	401	87	17	<MDL	<MDL	<MDL
Sunflower	Root	None	1	87	281	328	112	80	100	239	252	203
Sunflower	Root	None	2	<MDL	357	201	91	107	162	295	249	205
Sunflower	Root	None	3	<MDL	551	586	91	192	264	454	363	194
Mustard	Leaf	None	1	10465	7784	4507	4017	3304	1683	670	100	11
Mustard	Leaf	None	2	15353	9511	8913	8002	6901	3210	1173	192	14
Mustard	Leaf	None	3	15168	10833	7437	5667	5700	3192	1284	221	20

Mustard	Stem	None	1	1193	1283	357	305	160	92	50	13	1.9
Mustard	Stem	None	2	2266	2161	950	563	375	231	126	35	5.3
Mustard	Stem	None	3	3846	3407	1804	1097	863	514	224	57	8.2
Mustard	Root	None	1	56	70	36	51	125	230	582	492	310
Mustard	Root	None	2	169	205	254	256	347	314	493	396	247
Mustard	Root	None	3	69	70	<MDL	35	84	222	556	362	244
Hemp	Leaf	None	1	8538	7491	4819	3045	2639	2366	873	33	<MDL
Hemp	Leaf	None	2	8704	8255	5945	2804	1481	705	193	14	<MDL
Hemp	Leaf	None	3	4723	4996	3642	3059	2041	1514	687	72	2.5
Hemp	Stem	None	1	401	482	157	69	64	232	378	96	6.5
Hemp	Stem	None	2	366	677	302	172	142	157	168	21	1.5
Hemp	Stem	None	3	155	272	149	87	59	82	162	82	13
Hemp	Root	None	1	<MDL	<MDL	<MDL	2.6	21	135	877	743	293
Hemp	Root	None	2	<MDL	<MDL	65	114	233	498	1084	661	356
Hemp	Root	None	3	72	219	220	147	179	360	906	771	269
Sunflower	Seed	Microbes	1	1745	1966	560	161	14	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Microbes	2	863	805	145	48	3.4	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Microbes	3	1152	1142	291	90	7.7	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Microbes	1	11488	7833	5831	3329	1903	668	143	13	<MDL
Sunflower	Leaf	Microbes	2	14174	6719	4569	2083	1625	574	130	12	<MDL
Sunflower	Leaf	Microbes	3	11499	8713	3719	2030	2029	786	245	27	<MDL
Sunflower	Stem	Microbes	1	2049	1876	622	339	89	33	<MDL	<MDL	<MDL
Sunflower	Stem	Microbes	2	4910	3398	1049	508	160	47	<MDL	6.7	<MDL
Sunflower	Stem	Microbes	3	1901	1704	343	133	56	30	<MDL	<MDL	<MDL
Sunflower	Root	Microbes	1	69	632	639	243	320	458	604	404	236
Sunflower	Root	Microbes	2	75	477	328	115	161	216	368	270	126
Sunflower	Root	Microbes	3	82	250	201	138	125	148	258	258	178
Mustard	Leaf	Microbes	1	21669	11920	8953	6357	5645	2945	1359	255	28
Mustard	Leaf	Microbes	1	16931	9430	7144	4876	4390	2550	1051	197	20
Mustard	Leaf	Microbes	2	11830	7118	4126	4026	3087	1407	538	90	10
Mustard	Leaf	Microbes	3	12859	9628	5151	4536	4135	1927	748	142	13

Mustard	Stem	Microbes	1	1952	2202	1020	574	322	232	125	36	5.0
Mustard	Stem	Microbes	2	1517	1985	600	306	215	159	97	37	8.8
Mustard	Stem	Microbes	3	1682	1906	786	419	312	188	122	35	6.9
Mustard	Root	Microbes	1	<MDL	<MDL	<MDL	1.7	3.4	<MDL	<MDL	<MDL	<MDL
Mustard	Root	Microbes	2	132	169	64	50	80	163	396	335	216
Hemp	Leaf	Microbes	1	9741	8645	8084	4836	3709	2046	469	21	<MDL
Hemp	Leaf	Microbes	2	7962	10808	10843	5535	3341	1894	597	32	<MDL
Hemp	Leaf	Microbes	3	5897	4938	3835	2253	1355	852	265	18	<MDL
Hemp	Stem	Microbes	1	524	753	418	241	158	278	407	66	6.1
Hemp	Stem	Microbes	2	428	745	353	111	91	189	327	77	6.7
Hemp	Stem	Microbes	3	151	212	62	34	33	66	104	35	2.3
Hemp	Root	Microbes	1	<MDL	<MDL	101	170	212	373	797	402	147
Hemp	Root	Microbes	2	<MDL	<MDL	<MDL	20	96	406	1194	1118	643
Hemp	Root	Microbes	3	<MDL	<MDL	52	67	124	275	576	419	179
Sunflower	Seed	Fertilizer	1	1117	1761	460	60	5.7	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer	3	2315	3227	800	135	14	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Fertilizer	1	1246	1532	2533	1490	1013	332	82	10	<MDL
Sunflower	Leaf	Fertilizer	2	6109	3617	2297	1269	1191	437	116	13	<MDL
Sunflower	Leaf	Fertilizer	3	1742	2171	1651	1277	1201	559	147	8.9	<MDL
Sunflower	Stem	Fertilizer	1	3190	3811	1620	297	68	28	<MDL	<MDL	<MDL
Sunflower	Stem	Fertilizer	2	1047	1211	431	229	105	56	40	15	<MDL
Sunflower	Stem	Fertilizer	3	1940	2472	901	342	168	72	30	7.5	<MDL
Sunflower	Root	Fertilizer	1	<MDL	<MDL	119	144	117	145	168	136	93
Sunflower	Root	Fertilizer	2	100	308	241	148	145	187	311	317	207
Sunflower	Root	Fertilizer	3	<MDL	<MDL	73	129	186	201	271	233	154
Mustard	Leaf	Fertilizer	1	6974	5052	2444	2717	2214	1026	307	52	4.8
Mustard	Leaf	Fertilizer	2	6995	5071	2970	2813	2155	1029	290	50	4.8
Mustard	Leaf	Fertilizer	3	9403	5581	2672	2359	2095	931	311	56	3.9
Mustard	Stem	Fertilizer	1	1047	1448	818	518	212	133	55	18	2.3
Mustard	Stem	Fertilizer	2	1556	1891	880	446	222	122	62	23	6.2
Mustard	Stem	Fertilizer	3	942	1214	362	235	163	92	57	22	5.5

Mustard	Root	Fertilizer	1	271	626	297	254	534	906	1328	1119	974
Mustard	Root	Fertilizer	2	76	113	91	70	88	136	206	224	160
Mustard	Root	Fertilizer	3	<MDL	<MDL	<MDL	39	85	291	465	300	247
Hemp	Leaf	Fertilizer	1	2027	2233	1512	747	266	154	57	7.2	<MDL
Hemp	Leaf	Fertilizer	2	3547	3920	4023	2283	627	472	211	23	<MDL
Hemp	Leaf	Fertilizer	3	4223	5587	5115	2806	1314	955	386	27	<MDL
Hemp	Stem	Fertilizer	1	152	202	143	103	57	81	91	32	5.7
Hemp	Stem	Fertilizer	2	199	255	203	192	70	103	122	51	5.2
Hemp	Stem	Fertilizer	3	191	229	208	203	117	145	149	44	3.4
Hemp	Root	Fertilizer	1	<MDL	<MDL	48	84	129	203	353	312	169
Hemp	Root	Fertilizer	2	<MDL	<MDL	<MDL	50	104	264	628	420	225
Hemp	Root	Fertilizer	3	<MDL	<MDL	<MDL	43	92	303	659	561	292
Sunflower	Seed	Fertilizer+Microbes	1	847	1137	291	50	3.8	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer+Microbes	2	1079	1583	220	39	3.9	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer+Microbes	3	874	821	108	20	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Fertilizer+Microbes	1	4370	4118	4223	1620	914	323	103	18	<MDL
Sunflower	Leaf	Fertilizer+Microbes	2	1960	2250	1763	969	1020	406	117	24	<MDL
Sunflower	Leaf	Fertilizer+Microbes	3	3758	2826	1530	728	534	178	50	8.4	<MDL
Sunflower	Stem	Fertilizer+Microbes	1	1831	1931	687	241	80	36	24	10	<MDL
Sunflower	Stem	Fertilizer+Microbes	2	1526	1948	454	163	80	41	26	11	2.4
Sunflower	Stem	Fertilizer+Microbes	3	1654	1487	343	115	47	17	<MDL	<MDL	<MDL
Sunflower	Root	Fertilizer+Microbes	1	<MDL	109	422	455	386	361	412	486	340
Sunflower	Root	Fertilizer+Microbes	2	<MDL	71	240	269	194	193	295	321	194
Sunflower	Root	Fertilizer+Microbes	3	<MDL	194	402	355	314	241	246	207	147
Mustard	Leaf	Fertilizer+Microbes	1	10206	7282	3167	3974	3532	1808	551	91	7.2
Mustard	Leaf	Fertilizer+Microbes	2	7695	5164	2420	2385	1662	716	212	41	4.3
Mustard	Leaf	Fertilizer+Microbes	3	11115	7134	3711	3570	2903	1311	360	54	3.8
Mustard	Stem	Fertilizer+Microbes	1	1192	1777	781	527	308	202	125	43	7.2
Mustard	Stem	Fertilizer+Microbes	2	1763	2393	833	616	313	165	90	29	3.3
Mustard	Stem	Fertilizer+Microbes	3	1172	1633	651	368	252	158	89	28	6.2
Mustard	Root	Fertilizer+Microbes	1	89	145	135	104	186	325	480	429	319

Mustard	Root	Fertilizer+Microbes	2	85	105	<MDL	42	107	237	488	441	376
Mustard	Root	Fertilizer+Microbes	3	236	311	187	223	327	575	1146	791	553
Hemp	Leaf	Fertilizer+Microbes	1	2323	2537	3019	2095	736	380	161	14	<MDL
Hemp	Leaf	Fertilizer+Microbes	2	3448	3634	3890	2312	954	438	163	15	<MDL
Hemp	Leaf	Fertilizer+Microbes	3	2005	2322	1861	1443	516	325	151	13	<MDL
Hemp	Stem	Fertilizer+Microbes	1	270	340	277	262	100	86	92	35	8.3
Hemp	Stem	Fertilizer+Microbes	2	291	377	339	230	104	168	201	61	12
Hemp	Stem	Fertilizer+Microbes	3	82	101	83	76	58	82	102	39	3.8
Hemp	Root	Fertilizer+Microbes	1	<MDL	<MDL	<MDL	95	142	252	539	545	297
Hemp	Root	Fertilizer+Microbes	2	<MDL	<MDL	<MDL	42	83	252	604	646	443
Hemp	Root	Fertilizer+Microbes	3	<MDL	<MDL	36	91	172	429	742	334	198

[a]MDL = method detection limit, [a]ND = not detected.

Table S8: PFSA concentration (ng g^{-1} dw) for each plant tissue with or without supplement[a]

Plant	Tissue	Supplement	Replicate	PFBS	PFHxS	PFHxS_br	PFOS	PFOS_br	FOSA
Sunflower	Seed	None	1	66	8.5	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	None	2	ND	ND	ND	ND	ND	ND
Sunflower	Seed	None	3	14	<MDL	200	<MDL	<MDL	<MDL
Sunflower	Leaf	None	1	10551	4152	4152	895	188	<MDL
Sunflower	Leaf	None	2	5953	2106	302	550	107	<MDL
Sunflower	Leaf	None	3	5983	3346	464	1269	223	<MDL
Sunflower	Stem	None	1	288	68	14	<MDL	<MDL	<MDL
Sunflower	Stem	None	2	100	43	6.7	28	<MDL	<MDL
Sunflower	Stem	None	3	226	75	8.6	<MDL	<MDL	<MDL
Sunflower	Root	None	1	199	122	23	261	38	59
Sunflower	Root	None	2	169	165	165	334	64	125
Sunflower	Root	None	3	84	202	202	640	90	32
Mustard	Leaf	None	1	3976	2509	2509	1190	260	27
Mustard	Leaf	None	2	5691	3928	567	2204	520	21
Mustard	Leaf	None	3	6855	4572	650	2235	657	34
Mustard	Stem	None	1	231	91	11	42	<MDL	<MDL

Mustard	Stem	None	2	417	177	27	132	26	<MDL
Mustard	Stem	None	3	1306	545	61	300	74	<MDL
Mustard	Root	None	1	51	102	10	348	62	281
Mustard	Root	None	2	351	326	326	350	81	213
Mustard	Root	None	3	46	64	64	317	63	273
Hemp	Leaf	None	1	8489	3067	3067	1192	163	<MDL
Hemp	Leaf	None	2	8083	2567	554	353	66	<MDL
Hemp	Leaf	None	3	5638	2631	523	814	122	<MDL
Hemp	Stem	None	1	298	37	6.6	157	<MDL	<MDL
Hemp	Stem	None	2	306	116	25	97	<MDL	<MDL
Hemp	Stem	None	3	142	44	10	62	<MDL	<MDL
Hemp	Root	None	1	<MDL	39	<MDL	418	<MDL	187
Hemp	Root	None	2	28	306	306	890	181	326
Hemp	Root	None	3	432	289	289	534	80	224
Sunflower	Seed	Microbes	1	94	14	14	<MDL	<MDL	<MDL
Sunflower	Seed	Microbes	2	35	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Microbes	3	50	11	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Microbes	1	6819	2720	2720	546	102	<MDL
Sunflower	Leaf	Microbes	2	5858	2822	403	675	122	<MDL
Sunflower	Leaf	Microbes	3	9835	3753	666	1077	200	<MDL
Sunflower	Stem	Microbes	1	250	84	13	<MDL	<MDL	<MDL
Sunflower	Stem	Microbes	2	646	208	31	45	<MDL	<MDL
Sunflower	Stem	Microbes	3	336	87	11	54	<MDL	<MDL
Sunflower	Root	Microbes	1	403	442	69	842	155	133
Sunflower	Root	Microbes	2	209	178	178	424	70	46
Sunflower	Root	Microbes	3	381	207	207	375	67	122
Mustard	Leaf	Microbes	1	6845	3790	3790	2281	569	45
Mustard	Leaf	Microbes	1	5384	2886	2886	1757	432	42
Mustard	Leaf	Microbes	2	4828	2578	372	1073	264	27
Mustard	Leaf	Microbes	3	5910	3332	499	1221	334	29
Mustard	Stem	Microbes	1	619	191	27	114	25	<MDL

Mustard	Stem	Microbes	2	391	166	8689	82	1837	<MDL
Mustard	Stem	Microbes	3	374	138	17	101	<MDL	<MDL
Mustard	Root	Microbes	1	7.7	<MDL	<MDL	<MDL	<MDL	<MDL
Mustard	Root	Microbes	2	87	95	8234	390	16602	219
Hemp	Leaf	Microbes	1	10047	3398	3398	664	94	<MDL
Hemp	Leaf	Microbes	2	10508	5109	1293	713	114	<MDL
Hemp	Leaf	Microbes	3	5700	1983	427	640	75	<MDL
Hemp	Stem	Microbes	1	569	184	35	247	31	<MDL
Hemp	Stem	Microbes	2	378	76	16	149	<MDL	<MDL
Hemp	Stem	Microbes	3	139	29	<MDL	60	<MDL	<MDL
Hemp	Root	Microbes	1	81	294	44	499	71	202
Hemp	Root	Microbes	2	7.7	87	87	774	127	313
Hemp	Root	Microbes	3	105	181	181	465	79	125
Sunflower	Seed	Fertilizer	1	41	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer	3	75	20	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Fertilizer	1	4371	1713	1713	466	91	<MDL
Sunflower	Leaf	Fertilizer	2	2141	1495	190	362	70	<MDL
Sunflower	Leaf	Fertilizer	3	2483	1982	267	896	167	<MDL
Sunflower	Stem	Fertilizer	1	273	86	9.3	33	<MDL	<MDL
Sunflower	Stem	Fertilizer	2	85	75	7.2	52	<MDL	26
Sunflower	Stem	Fertilizer	3	240	155	19	86	<MDL	<MDL
Sunflower	Root	Fertilizer	1	585	419	83	349	82	152
Sunflower	Root	Fertilizer	2	497	301	301	452	101	160
Sunflower	Root	Fertilizer	3	136	301	301	349	72	216
Mustard	Leaf	Fertilizer	1	3287	2109	2109	583	150	13
Mustard	Leaf	Fertilizer	2	3123	2168	286	607	176	8.5
Mustard	Leaf	Fertilizer	3	3339	2540	367	725	214	12
Mustard	Stem	Fertilizer	1	392	150	18	54	<MDL	<MDL
Mustard	Stem	Fertilizer	2	486	163	22	56	<MDL	<MDL
Mustard	Stem	Fertilizer	3	216	112	15	60	<MDL	<MDL
Mustard	Root	Fertilizer	1	558	458	59	1048	216	2619

Mustard	Root	Fertilizer	2	144	96	96	188	39	158
Mustard	Root	Fertilizer	3	51	69	69	435	90	309
Hemp	Leaf	Fertilizer	1	2598	1070	1070	114	16	<MDL
Hemp	Leaf	Fertilizer	2	4440	2883	533	382	45	<MDL
Hemp	Leaf	Fertilizer	3	7025	4474	966	782	99	<MDL
Hemp	Stem	Fertilizer	1	110	95	14	33	<MDL	<MDL
Hemp	Stem	Fertilizer	2	197	175	39	72	<MDL	<MDL
Hemp	Stem	Fertilizer	3	192	116	28	114	<MDL	<MDL
Hemp	Root	Fertilizer	1	12	188	28	372	74	212
Hemp	Root	Fertilizer	2	6.0	203	203	480	68	199
Hemp	Root	Fertilizer	3	<MDL	74	74	424	57	295
Sunflower	Seed	Fertilizer+Microbes	1	26	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer+Microbes	2	10	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Seed	Fertilizer+Microbes	3	15	<MDL	<MDL	<MDL	<MDL	<MDL
Sunflower	Leaf	Fertilizer+Microbes	1	4644	1967	1967	404	89	<MDL
Sunflower	Leaf	Fertilizer+Microbes	2	4280	2202	310	613	121	<MDL
Sunflower	Leaf	Fertilizer+Microbes	3	2377	960	131	255	40	<MDL
Sunflower	Stem	Fertilizer+Microbes	1	210	92	11	41	<MDL	<MDL
Sunflower	Stem	Fertilizer+Microbes	2	94	83	9.5	49	<MDL	<MDL
Sunflower	Stem	Fertilizer+Microbes	3	160	61	6.5	<MDL	<MDL	<MDL
Sunflower	Root	Fertilizer+Microbes	1	945	592	116	523	113	256
Sunflower	Root	Fertilizer+Microbes	2	484	368	368	315	63	156
Sunflower	Root	Fertilizer+Microbes	3	989	504	504	335	77	133
Mustard	Leaf	Fertilizer+Microbes	1	5425	4375	4375	1716	451	19
Mustard	Leaf	Fertilizer+Microbes	2	3260	2024	302	645	161	12
Mustard	Leaf	Fertilizer+Microbes	3	5875	4135	722	1441	371	14
Mustard	Stem	Fertilizer+Microbes	1	334	209	31	107	<MDL	<MDL
Mustard	Stem	Fertilizer+Microbes	2	762	316	40	103	27	<MDL
Mustard	Stem	Fertilizer+Microbes	3	413	184	27	96	<MDL	<MDL
Mustard	Root	Fertilizer+Microbes	1	329	196	23	493	87	271
Mustard	Root	Fertilizer+Microbes	2	62	102	102	374	72	561

Mustard	Root	Fertilizer+Microbes	3	302	307	307	756	155	546
Hemp	Leaf	Fertilizer+Microbes	1	3855	2653	2653	291	40	<MDL
Hemp	Leaf	Fertilizer+Microbes	2	4764	2292	429	253	35	<MDL
Hemp	Leaf	Fertilizer+Microbes	3	3199	1800	303	280	43	<MDL
Hemp	Stem	Fertilizer+Microbes	1	242	155	48	58	<MDL	<MDL
Hemp	Stem	Fertilizer+Microbes	2	343	143	26	69	<MDL	<MDL
Hemp	Stem	Fertilizer+Microbes	3	72	83	15	73	<MDL	<MDL
Hemp	Root	Fertilizer+Microbes	1	5.1	178	24	447	72	322
Hemp	Root	Fertilizer+Microbes	2	11	154	154	423	72	314
Hemp	Root	Fertilizer+Microbes	3	15	183	183	698	91	200

[a]MDL = method detection limit, [a]ND = not detected.

Table S9: Leaf concentration factor of all plant species investigated, as mean and standard deviation (SD)[a]

Compound	Sunflower		Mustard		Hemp	
	Mean	SD	Mean	SD	Mean	SD
PFBA	2092	693	1816	1108	1464	450
PFPeA	565	393	196	67	2579	2669
PFHxA	145	132	54	21	2671	4366
PFHpA	37	30	35	14	172	228
PFOA	42	47	38	18	47	47
PFNA	12	10	25	13	25	23
PFDA	3.3	2.9	8.5	4.3	6.4	5.0
PFUnDA	0.60	0.36	2.4	1.7	0.60	0.36
PFDoDA	0.0	0.0	0.43	0.2	0.033	0.058
PFBS	29	22	10	3.8	7.0	11
L-PFHxS	7.9	4.6	7.6	3.0	73	105
B-PFHxS	21	25	14	11	373	617
L-PFOS	2.2	1.4	4.0	1.6	2.1	1.2
B-PFOS	1.5	0.90	4.2	2.6	1.5	0.93
FOSA	ND		0.1	0.06	ND	

[a]ND = not detected.

Table S10: Stem concentration factor of all plant species investigated, as mean and standard deviation (SD)[a]

Compound	Sunflower		Mustard		Hemp	
	Mean	SD	Mean	SD	Mean	SD
PFBA	656	225	365	356	61	27
PFPeA	344	397	49	32	197	227
PFHxA	35	47	8.2	6.1	89	141
PFHpA	4.8	5.1	4.1	3.1	6.9	4.3
PFOA	1.4	1.7	3.7	3.6	1.7	1.1
PFNA	0.23	0.06	2.9	2.8	2.6	2.2
PFDA	ND	ND	1.1	0.97	2.7	2.1
PFUnDA	0.10	0.14	0.53	0.51	1.1	0.90
PFDoDA	ND	ND	0.17	0.06	0.20	0.20
PFBS	0.97	0.93	1.3	1.3	19	21
L-PFHxS	0.17	0.12	0.60	0.61	1.1	1.12
B-PFHxS	0.17	0.06	0.40	0.36	1.2	1.05
L-PFOS	ND	ND	0.37	0.38	0.30	0.20
B-PFOS	ND	ND	0.33	0.42	ND	ND
FOSA	ND	ND	ND	ND	ND	ND

[a]ND = not detected.

Table S11: Root concentration factor of all plant species investigated, as mean and standard deviation (SD)[a]

Compound	Sunflower		Mustard		Hemp	
	Mean	SD	Mean	SD	Mean	SD
PFBA	5.8	10	12	6.4	4.8	8.3
PFPeA	42	46	2.3	1.3	4.0	7.0
PFHxA	14	18	0.73	1.1	1.8	1.6
PFHpA	1.4	0.93	0.63	0.75	1.3	1.1
PFOA	3.0	3.8	1.3	0.90	2.0	1.4
PFNA	2.8	2.2	2.3	0.67	4.2	1.5
PFDA	5.8	4.1	4.2	1.1	9.9	2.2
PFUnDA	9.5	6.5	5.1	1.6	11	4.2
PFDoDA	5.8	0.15	7.7	1.1	8.8	1.3

PFBS	0.53	0.25	0.27	0.29	0.97	0.84
L-PFHxS	0.37	0.29	0.30	0.26	1.8	1.0
B-PFHxS	2.1	2.2	1.5	1.9	6.2	7.4
L-PFOS	1.0	0.75	0.70	0.10	1.7	0.81
B-PFOS	0.57	0.38	0.57	0.12	1.0	1.2
FOSA	0.37	0.31	1.1	0.25	1.0	0.58

[a]ND = not detected.

Table S12: Bioconcentration factors of all plant samples with replicates (n = 3) with each treatment[a]

Plant	Treatment	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA
Sunflower_1	None	780	216	27	7.4	3.3	1.2	0.4	0.4	28
Sunflower_2	None	1219	97	15	5.3	6.5	3.9	1.7	1.1	29
Sunflower_3	None	872	693	101	16	15	3.5	1.6	1.4	24
Mustard_1	None	262	47	8	5.2	4.8	3.1	1.7	0.8	74
Mustard_2	None	456	61	19	12	11	7.1	2.8	1.4	71
Mustard_3	None	1052	104	20	12	14	11	4.7	2.7	82
Hemp_1	None	717	841	3171	176	41	23	7.8	1.7	13
Hemp_2	None	777	2571	117	28	11	4.8	2.1	0.6	29
Hemp_3	None	316	96	15	6.8	6.0	5.9	3.2	1.7	50
Sunflower_1	Microbes	600	290	70	14	5.5	2.3	1.1	1.0	40
Sunflower_2	Microbes	984	69	8.0	2.5	1.8		0.4	0.48	19
Sunflower_3	Microbes	552	ND	32	7.0	5.8	2.6	1.5	1.6	46
Mustard_1	Microbes	513	107	22	8.5	6.2	3.8	1.2	0.31	0.048
Mustard_2	Microbes	285	55	10	6.0	5.5	3.2	1.5	0.73	0.58
Mustard_3	Microbes	907	137	22	13	11.3	4.7	1.6	0.44	0.10
Hemp_1	Microbes	875	694	109	26	14.8	8.4	3.4	0.94	35
Hemp_2	Microbes	ND	ND	1190	145	ND	6.5	2.5	0.69	44
Hemp_3	Microbes	354	102	19	7.4	6.7	3.8	2.0	1.0	24
Sunflower_1	Fertilizer	441	273	44	6.9	3.0	1.0	0.31	0.20	12

Sunflower_2	Fertilizer	568	142	38	11	9.1	4.1	1.3	0.75	30
Sunflower_3	Fertilizer	349	648	45	11	ND	3.0	1.2	0.68	24
Mustard_1	Fertilizer	215	43	10	6.9	7.0	4.6	3.2	3.5	405
Mustard_2	Fertilizer	253	54	12	7.3	6.0	2.9	0.9	0.6	0.50
Mustard_3	Fertilizer	240	51	9.4	5.7	5.4	2.4	1.1	0.5	0.37
Hemp_1	Fertilizer	237	260	13	3.6	1.6	1.2	0.8	0.5	25
Hemp_2	Fertilizer	438	365	116	14	2.9	2.5	1.3	0.4	0.18
Hemp_3	Fertilizer	388	673	45	7.8	3.6	2.6	1.2	0.4	0.18
Sunflower_1	Fertilizer+Microbes	423	223	27	6.0	3.1	1.8	1.1	1.8	50
Sunflower_2	Fertilizer+Microbes	282	185	17	3.8	3.5	1.7	0.9	0.9	31
Sunflower_3	Fertilizer+Microbes	ND	129	14	3.4	2.5	1.2	0.5	0.6	23
Mustard_1	Fertilizer+Microbes	579	67	13	10	9.2	5.6	2.5	1.7	106
Mustard_2	Fertilizer+Microbes	602	58	11	7.4	5.2	2.5	1.2	1.1	106
Mustard_3	Fertilizer+Microbes	275	62	14	7.3	7.4	4.0	2.0	1.3	1.0
Hemp_1	Fertilizer+Microbes	242	305	32	7.8	2.2	1.4	0.8	0.5	47
Hemp_2	Fertilizer+Microbes	329	273	40	8.9	3.8	2.4	1.4	0.7	42
Hemp_3	Fertilizer+Microbes	228	198	29	8.0	3.1	2.6	1.5	0.6	22

[a]ND = not detected.

Table S13: Bioconcentration factors of all plant samples with the corresponding replicates (n = 3) with each treatment[a]

Plant	Supplement	PFBS	L-PFHxS	B-PFHxS	L-PFOS	B-PFOS	FOSA
Sunflower_1	None	3.9	1.2	6.8	0.3	0.25	0.02
Sunflower_2	None	6.4	2.2	1.9	0.7	0.46	0.06
Sunflower_3	None	8.0	1.7	2.2	ND	0.37	0.01
Mustard_1	None	1.8	1.2	6.1	0.7	0.54	0.17
Mustard_2	None	2.4	1.8	2.0	1.0	0.90	0.14
Mustard_3	None	4.4	2.8	2.1	1.7	1.9	0.26
Hemp_1	None	488	78	428	1.6	1.0	0.01
Hemp_2	None	172	8.3	12	0.7	0.50	0.08

Hemp_3	None	6.7	2.2	2.9	0.7	0.41	0.06
Sunflower_1	Microbes	6.0	1.5	8.4	0.42	0.28	0.05
Sunflower_2	Microbes	2.4	0.85	0.83	0.40	0.21	0.02
Sunflower_3	Microbes	10	2.1	2.8	0.78	0.50	0.09
Mustard_1	Microbes	6.0	2.0	11	0.99	1.0	0.03
Mustard_2	Microbes	2.8	1.6	85	0.70	29	0.14
Mustard_3	Microbes	2.8	1.7	1.4	0.74	0.63	0.02
Hemp_1	Microbes	112	4.5	29	0.75	0.40	0.07
Hemp_2	Microbes	598	32	59	0.92	0.47	0.05
Hemp_3	Microbes	9.1	2.3	2.7	0.68	0.30	0.03
Sunflower_1	Fertilizer	2.2	0.81	4.1	0.25	0.16	0.05
Sunflower_2	Fertilizer	2.8	2.1	2.0	0.54	0.41	0.12
Sunflower_3	Fertilizer	3.0	1.9	2.2	0.76	0.45	0.07
Mustard_1	Fertilizer	1.4	1.2	5.0	0.74	0.67	2.5
Mustard_2	Fertilizer	1.9	1.4	1.3	0.53	0.55	0.09
Mustard_3	Fertilizer	1.5	1.5	1.3	0.64	0.56	0.25
Hemp_1	Fertilizer	57	1.7	10	0.27	0.14	0.06
Hemp_2	Fertilizer	865	6.8	7.5	0.74	0.34	0.04
Hemp_3	Fertilizer	924	5.0	6.5	0.82	0.33	0.06
Sunflower_1	Fertilizer+Microbes	2.6	0.95	4.3	0.32	0.22	0.09
Sunflower_2	Fertilizer+Microbes	2.3	1.2	1.5	0.38	0.24	0.06
Sunflower_3	Fertilizer+Microbes	1.5	0.59	1.1	0.18	ND	0.07
Mustard_1	Fertilizer+Microbes	2.5	2.2	11	1.2	0.92	0.24
Mustard_2	Fertilizer+Microbes	2.1	1.5	1.5	0.60	0.54	0.37
Mustard_3	Fertilizer+Microbes	2.5	2.1	2.6	1.2	1.01	0.42
Hemp_1	Fertilizer+Microbes	122	4.0	22	0.42	0.20	0.10
Hemp_2	Fertilizer+Microbes	119	2.6	3.0	0.32	0.14	0.05
Hemp_3	Fertilizer+Microbes	138	4.1	4.3	0.57	0.28	0.06

[a]ND = not detected.

Table S14: Analysis of variance (ANOVA) to assess the effect of various parameters on absolute PFAS mass in the plants

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Replicate	2	0.97	0.486	0.5516	0.5761
Tissue	3	322.48	107.492	121.9997	< 2.2e-16***
Treatment	3	0.53	0.176	0.2003	0.8962
Plant species	2	32.78	16.39	18.6018	1.01e-08***
Treatment x Plant	6	2.22	0.37	0.4195	0.8664
Residuals	1775	1563.93	0.881		

Significance level: ***<0.001.

Table S15: PFAS removal efficiency for each plant species without supplement

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFBS	PFHxS	PFOS
Sunflower	30±9.3	33±2.9	6.5±0.42	2.3±0.51	1.0±0.51	0.34±0.17	0.48±0.24	0.24±0.051	0.16±0.03	11±9.1	3.2±2.2	0.49±0.36
Mustard	12±2.6	14±2.9	3.7±1.2	2.2±0.62	1.2±0.37	0.55±0.17	1.1±0.27	0.43±0.062	0.19±0.0085	3.2±0.98	1.5±0.4	0.38±0.11
Hemp	15±4.9	24±6.9	6.8±2.1	3.2±0.39	1.3±0.15	0.93±0.33	2.2±0.83	0.66±0.45	0.17±0.13	12±2.4	3.3±0.22	0.53±0.16

Table S16: Estimated number of crop cycles required to reach 90 % PFAS removal from soil

Plant	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFBS	PFHxS	PFOS
Sunflower	7	6	34	96	232	20	70	458
Mustard	19	15	60	100	192	20	70	466
Hemp	14	9	32	68	165	16	66	420

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Per- and polyfluoroalkyl substances (PFAS) are persistent, mobile and potentially toxic anthropogenic compounds. This thesis explores the potential of phytoremediation for managing PFAS-contaminated soil and groundwater. Various plants, supplements and factors are examined to develop a nature-based PFAS remediation method. The results indicate good potential for PFAS remediation especially for short chain PFAS.

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