

ABSTRACT

Title of Thesis: PHYTOSCREENING FOR PER- AND
POLYFLUOROALKYL SUBSTANCES
(PFAS) NEAR FORT GEORGE G. MEADE

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Per- and polyfluoroalkyl substances (PFASs) are a class of contaminants with growing concerns regarding their toxicity to humans, their high persistence, and their ubiquity in the environment. Many U.S. military bases that historically used PFAS-based Aqueous Film-Forming Foams (AFFFs) are now being investigated by the U.S. Department of Defense (DOD) to address potential releases into the environment, especially into groundwater. This study explores a preliminary screening approach to delineate the plume of groundwater contamination—phytoscreening—which uses the analysis of plant tissue to detect subsurface contamination. For phytoscreening applications, this study evaluated three tree species: red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), and willow oak (*Quercus phellos*). Sites were selected in proximity to a point source, Fort George G. Meade, in MD, USA. At each tree, samples were collected from the leaves, twigs, trunk (n=54, 9 trees), and soil surface (0-10 cm) (n=18) to characterize PFAS accumulation between each plant tissue. A linear mixed effects model with

censored data (LMEC) was used to analyze the effects of species and plant tissue. Of the 15 compounds detected in groundwater at Fort Meade, red maple and willow oak captured 7 compounds each, while sweetgum captured 3. Red maple and willow oak accumulated higher $\sum_{27}$ PFAS concentrations than sweetgum. PFAS accumulation also varied by plant tissue, with twigs showing the highest concentrations, followed by the trunk, and then leaf. Results indicate that the plant species and tissue type play a critical role in the accumulation of PFAS in trees.

PHYTOSCREENING FOR PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) NEAR FORT
GEORGE G. MEADE

by

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Chapter 1: Introduction

1.1. Overview

Per- and polyfluoroalkyl substances (PFASs) are a class of synthetic organic compounds that contain at least one carbon atom whose hydrogens have been replaced by C–F bonds (Buck et al., 2011). The high stability of the C–F bond imparts a high resistance to chemical and thermal degradation, making these compounds useful for a wide range of industrial and commercial applications (Buck et al., 2011). Concerns have emerged regarding their toxicity to humans (Itoh et al., 2016) and wildlife (Thalheimer et al., 2017), persistence, and ubiquity in the environment (Brennan et al., 2021).

One important source of PFAS in the environment is Aqueous Film-Forming Foams (AFFFs). Example mechanisms of release include extinguishing hydrocarbon fires, firefighting training, accidental release, and disposal (Thalheimer et al., 2017). Currently, the U.S. Air Force (USAF) is conducting investigations to identify and remediate AFFF-impacted sites—such as Fort George G. Meade (a.k.a. Fort Meade) in Maryland (Hunter Anderson et al., 2019). A critical step in these investigations will be evaluating the extent of impact (Wang et al., 2017; Würth et al., 2023).

Conventional methods used to investigate the spatial extent of contamination typically feature drilling and sampling boreholes followed by laboratory analysis (Liao et al., 2018). However, this process is time-consuming, labor-intensive, and expensive to execute. Consequently, fewer sampling points are collected, resulting in lower-resolution data (Liao et al., 2018). An emerging technology that addresses some of these drawbacks is Direct Push (DP) technology. This sampling method uses small-diameter hollow steel rods that are pushed and/or hammered into the ground. At the end of the rod, attachments are used to sample soil, soil gas, or

groundwater (Dietrich and Leven, 2009). Though DP technology can be cheaper than conventional methods, it is still expensive. The method is also invasive since DP technology uses rods that can disrupt the natural hydrology of a soil (Liao et al., 2018).

An alternative approach to delineating the plume of contamination is phytoscreening—a technique that uses the sampling of plant tissues to determine subsurface contamination. The technique can function as a screening tool to identify the most polluted areas of a site before analyzing these areas with more intensive methods (Burken et al., 2011). Phytoscreening can be a simple, fast, and cost-effective method compared to traditional groundwater sampling because of the little equipment required and the short time frame in which many samples can be collected (Balouet, 2015). Though primarily applied to volatile organic contaminants (VOCs) (Leoncini et al., 2022), evidence exists that the technique can also be applied to PFAS (Gobelius et al., 2017; Würth et al., 2023).

In this study, three tree species common to the area surrounding Fort Meade were selected: red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), and willow oak (*Quercus phellos*). The purposes of this study were to (i) evaluate the efficacy of phytoscreening of PFAS for select species in proximity to a point source, Fort Meade, and to (ii) identify potential differences in PFAS accumulation between plant tissues (i.e., leaves, twigs, trunk).

1.2. Research Questions and Hypotheses

1. Which tree species are the best candidates for phytoscreening of PFAS?

H₀: There is no significant difference between PFAS concentrations of different species.

H_A: There is a significant difference between PFAS concentrations of at least one species compared to the other species.

2. Within the context of phytoscreening, which plant tissue (i.e., trunk, twig, leaf) is the best candidate for sampling of PFAS?

H₀: There is no significant difference between PFAS concentrations of different plant tissues.

H_A: At least one plant tissue accumulates higher PFAS concentrations than other plant tissues.

1.3. Literature Review

The following literature review will define and characterize PFAS compounds, focusing on their use in firefighting foams. The most relevant mechanisms associated with PFAS fate and transport surrounding their use in extinguishing fires and all applicable environments will be explored. These environments include primary (point) sources such as AFFF use in firefighting, landfill leachate, and wastewater treatment plant effluent and secondary (non-point) sources such as atmospheric deposition and runoff.

1.3.1 History of PFAS

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organic compounds that contain at least one carbon atom whose hydrogens have been replaced by C–F bonds. Perfluoroalkyl substances contain carbon chains that are completely fluorinated whereas those of polyfluorinated substances are incompletely fluorinated (Buck et al., 2011). The key component of PFAS is the C–F bond, which is the most stable in organic chemistry (O’Hagan, 2008). The C–F bond has a high thermal and chemical stability, making these compounds stable under a broad range of environmental conditions. Many of these compounds are also excellent surfactants, particularly at low concentrations (Kissa and Kissa, 2001). These compound characteristics make PFAS useful for a wide range of industrial and commercial applications (Buck et al., 2011). At least 4,000 unique PFAS compounds have been manufactured since their invention in the 1930s (Brennan et al., 2021), though some estimates reach 12,000 (U.S. Environmental Protection Agency, 2021). By the 1940s, PFAS usage had expanded rapidly, spanning a variety of industrial and commercial applications. Example commercial uses include paints, coatings, textile treatments, and aqueous film-forming foams (AFFFs) (Kissa and Kissa,

2001). Example industrial uses include chrome plating, electronic manufacturing, and oil recovery (Langenbach and Wilson, 2021).

This stability, combined with their widespread use, has led to their ubiquity across the globe (Brennan et al., 2021). This includes all continents in addition to remote areas such as the arctic and the Tibetan plateau. Numerous studies have shown that various PFAS are present in remote areas far from any known sources (Brusseau et al., 2020).

Rules and Regulations. For many years, the level of contamination present in the environment was unknown, which was due, in part, to the inability of the existing methods to detect specific compounds at environmentally relevant concentrations. Method developments enabled researchers to analyze for these compounds in new media like wildlife (Giesy and Kannan, 2001). In 2001, perfluorooctane sulfonate (PFOS) was detected in several wildlife from across the globe, including the Arctic, Baltic Sea, Mediterranean Sea, and Ganges River (Giesy and Kannan, 2001). This finding, in conjunction with other studies linking PFAS exposure to negative health outcomes, fueled international discussion, regulation, and policy throughout the early 2000s (Sunderland et al., 2019; Langenbach and Wilson, 2021). During this time, the U.S. focused mainly on the most prevalent PFAS compounds, PFOS and perfluorooctanoic acid (PFOA) (Sunderland et al., 2019; Brennan et al., 2021). By 2002, the world's leading manufacturer of PFASs (3M) voluntarily phased out production of PFOA in addition to PFASs containing 6, 8, and 10 carbon atoms (3M, 2000). In 2006, the U.S. EPA established the PFOA Stewardship Program, which invited eight major PFOA manufacturers to reduce their emissions by 95% by 2010 (U.S. Environmental Protection Agency and Office of Chemical Safety and Pollution Prevention, 2016). The phase-out is thought to have been mainly successful, given that PFAS blood serum levels steadily decreased throughout the 2000s (Olsen et al., 2017).

In 2009, PFOS was added to the Stockholm Convention on Persistent Organic Pollutants (POPs). Though the policy does have limited regulatory power, the convention still plays a key international role in restricting persistent and bioaccumulative compounds (International Pollutants Elimination Network, 2019; Sunderland et al., 2019).

In 2022, the Environmental Protection Agency (EPA) issued a drinking water health advisory for PFOA at 0.004 parts per trillion (ppt) and for PFOS at 0.02 ppt, with these values being based on studies examining adverse health effects to human PFAS exposure (U.S. Environmental Protection Agency, 2022a).

On April 10, 2024, the U.S. Environmental Protection Agency established the nation's first legally enforceable limits on PFAS in drinking water. The final National Primary Drinking Water Regulation (NPDWR) sets limits, or Maximum Contaminant Levels (MCLs), for six PFAS. The limits for PFOA and PFOS are each 4.0 ppt, while the limits for PFHxS (perfluorohexane sulfonate), PFNA, and HFPO-DA (or GenX) are each 10 ppt. PFAS mixtures containing two or more of PFBS (perfluorobutane sulfonate), PFHxS, PFNA, and HFPO-DA will be limited using an EPA-provided equation (Furlow, 2024).

In response to environmental concerns surrounding PFAS, some chemical manufacturers began producing replacement chemicals which include Gen-X and ADONA (PFOA replacements) and 9Cl-PF3ONS (PFOS replacement used in China) (Nguyen et al., 2020). As a result, these compounds have been more frequently detected in the U.S., Europe, and China (Nguyen et al., 2020).

1.3.2 Classification and Terminology

Though the PFAS group encompasses thousands of compounds, all compounds can be classified as either a polymer or non-polymer substance. This review will focus mainly on non-polymer PFAS, since these are the compounds used in AFFF formulations. Non-polymer PFAS can be subdivided into perfluorinated substances and polyfluorinated substances, as defined earlier in Section 1 (Buck et al., 2011). Within the family of perfluorinated substances, there are several subclasses, which include perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), perfluoroalkane sulfonyl fluorides (PASFs), and perfluoroalkyl ether carboxylic and sulfonic acids (PFECAs and PFESAs). Examples from each class are shown in Figure 1. Compounds within the subclasses of PFCAs and PFSAs have garnered the most attention from regulators worldwide. PFECAs and PFESAs include many compounds engineered for the purpose of replacing PFCAs and PFSAs. Though lesser known, PASFs ($C_nF_{2n}SO_2F$) are important reactants used to manufacture many other compounds (Wang et al., 2017).

The other major family within non-polymer PFAS are polyfluorinated substances. This family can be divided into perfluoroalkane sulfonamido substances and fluorotelomer-based substances, which each encompass a wide range of subclasses. Specifically, perfluoroalkane sulfonamido substances include N-alkyl perfluoroalkane sulfonamides (e.g., N-methyl perfluorooctane sulfonamide [MeFOSA], $C_8F_{17}SO_2N(CH_3)H$) and other sulfonamido substances. Fluorotelomer-based substances refer to compounds produced using the fluorotelomerization process (See Section 1.3.5). This group includes the n:2 fluorotelomer alcohols (n:2 FTOHs, $C_nF_{2n+1}CH_2CH_2OH$), which serve as both building blocks for liquid-repellant coatings as well as final ingredients used in other products, such as AFFFs. Examples of all chemical classes are shown in Figure 1.

Beyond their chemical classes, there are many different ways to categorize and group contaminants. One such grouping emphasizes the status of the contaminant's use. Specifically, 'legacy' compounds are those that were historically produced and used in numerous developed countries but were discontinued or banned. Often, these compounds persist in the environment and continue to cause harm despite their discontinued production/use (Brase et al., 2021). In contrast, 'alternate' or 'replacement' compounds were created to replace legacy compounds (Brase et al., 2021). When these terms are applied to the PFAS class, 'legacy PFAS' refer to long-chain PFAAs (perfluoroalkyl acids; e.g., PFOS, PFOA) while 'replacement PFAS' refer to short-chain PFAAs (e.g., perfluorobutanoic acid [PFBA], perfluoropentanoic acid [PFPeA]) and polyfluorinated compounds (Brase et al., 2021; Richter et al., 2021).

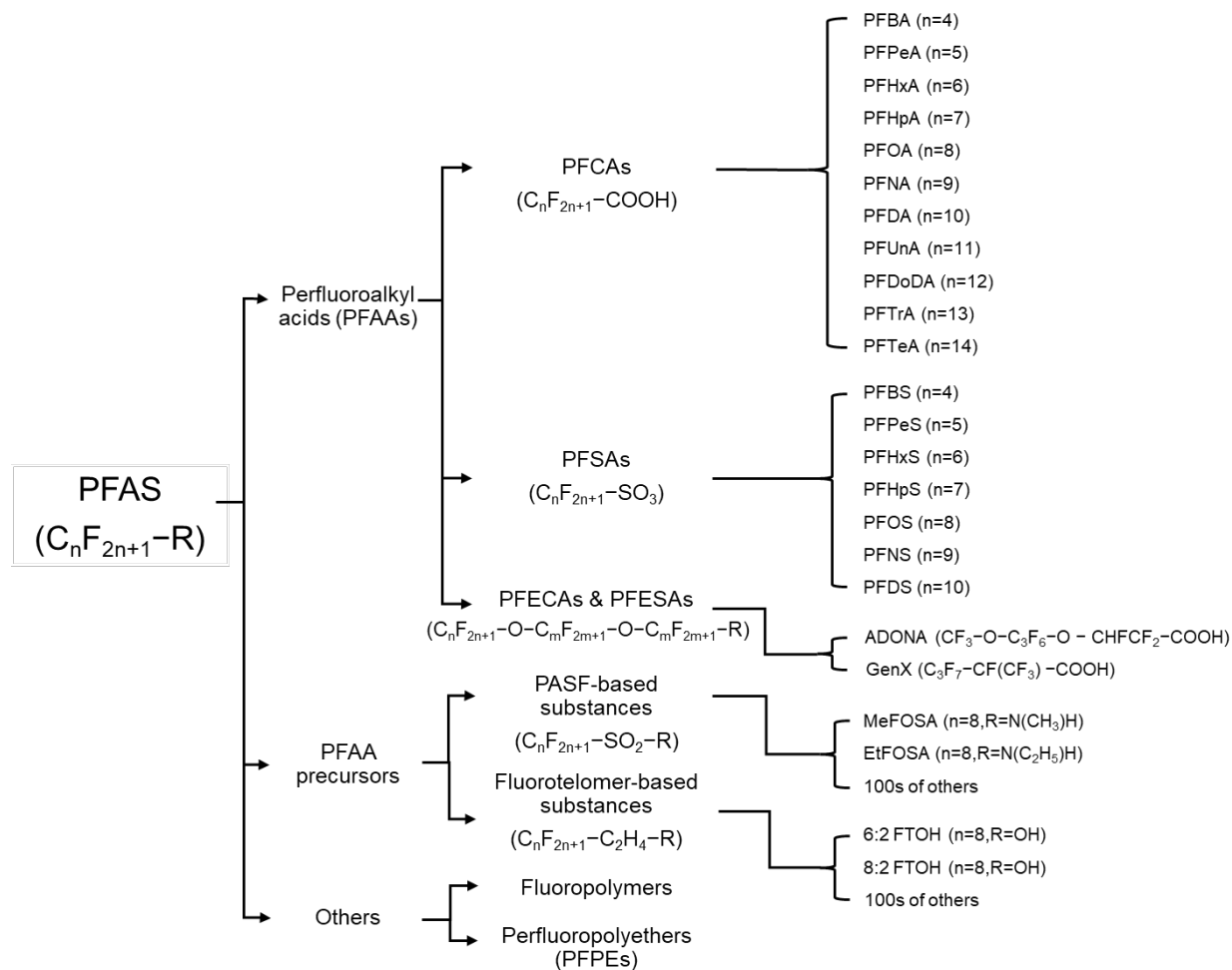


Figure 1. Diagram of PFASs, showing compounds included in the targeted analysis of this study. Modified from (Wang et al., 2017).

Another way to group PFAS is by their environmental fate. In natural conditions, most PFAS are either non-degradable compounds or degradable compounds resulting in highly stable end products. Thus, PFAS can be grouped into non-degradable ‘terminal’ compounds or into ‘precursors’ which degrade to form terminal compounds (Wang et al., 2017; Cousins et al., 2020). An example of the degradation of precursors into terminal products is the transformation of fluorotelomer alcohols ($C_nF_{2n+1}CHCH_2OH$) into PFAAs ($C_nF_{2n+1}COOH$) (Cousins et al., 2020). This example also displays a pattern common to many transformations. The highly stable perfluoroalkyl moiety ($C_nF_{2n+1}-$) present in the original precursor remains intact in the terminal product. In addition to the perfluoroalkyl moiety, the perfluoroether moiety ($C_nF_{2n+1}-O-C_mF_{2m+1}-$) is extremely stable, which is why PFAAs and perfluoropolyethers (PFPEs) are common terminal products (Wang et al., 2017).

1.3.3 Human Health Concern

In the past two decades, PFAS compounds have been gaining much attention because of their toxicity to humans (Itoh et al., 2016). Long-chain PFAS, especially, have raised health concerns given that they are more bioaccumulative and toxic than short-chain PFAS. ‘Long-chain’ typically describes compounds containing eight or more perfluorinated carbons. More specifically, long-chain PFCAs have eight or more carbons, whereas long-chain PFSA have six or more carbons (Buck et al., 2011). The reason for this distinction is that PFSA compounds are known to be more bioaccumulative and toxic than their PFCA counterparts equal in length (Kempisty et al., 2018).

Similarly, long-chain PFAS are concluded to be present in the blood of most Americans (Olsen et al., 2017; Centers for Disease Control and Prevention, 2022) with some of the those most at risk being firefighters exposed to AFFFs (Thalheimer et al., 2017). Several studies

investigated a variety of health parameters of a population living near the DuPont Washington Works fluorotelomer plant in West Virginia. These studies established that PFOA exposure was associated with high cholesterol, thyroid disease, pregnancy-induced hypertension, ulcerative colitis, and kidney and testicular cancer (Lopez-Espinosa et al., 2012; Barry et al., 2013; Steenland et al., 2013; Darrow et al., 2013; Sunderland et al., 2019).

Though perfluorinated compounds are often studied due to the availability of chemical standards—they account for 30-70% of total organic fluorine in some populations, indicating that other PFAS classes are important contributors to contamination in humans (Yeung et al., 2008). Because much is unknown about their effect on the human body (Fenton et al., 2021), research on the health effects of PFAS on humans and wildlife is ongoing (Narizzano et al., 2022). As a result, several toxic effects have been evidenced with a high degree of certainty. For example, PFAS exposure is associated with a decreased immune response, notably with antibodies against diphtheria and tetanus. Grandjean et al. (2012) measured the blood serum levels of children several times from birth to age 13. From ages 5 to 7, doubled serum PFOS, PFOA, and PFHxA (perfluorohexanoic acid) concentrations were associated with a 49% decrease in antibody concentrations (95% CI, -67% to -23%). A follow-up study confirmed that at age 13, increasing PFAS concentrations were still associated with a decreased immune response (Grandjean et al., 2017). A recent systematic review investigated these immunotoxicity effects even further and found that overall, antibody concentrations were inversely correlated to serum PFOA, PFOS, and PFHxS concentrations (Crawford et al., 2023). To conclude, PFOA and PFOS cause a decreased immune response in children.

PFAS exposure is also associated with altered liver and thyroid function, decreased birth weight, and harmful female reproductive system effects (Fenton et al., 2021; Rickard et al., 2022).

1.3.4 Persistence in the environment

The persistence of PFAS can be attributed to the strength of the C–F bond, especially in the perfluoroalkyl ($C_nF_{2n+1}-$) and perfluoroether ($C_nF_{2n+1}-O-C_mF_{2m+1}-$) moieties, which are completely fluorinated (Wang et al., 2017). Perfluorinated compounds are generally less susceptible to biodegradation because they lack non-fluorinated functional groups, which are more reactive than the fluorinated chain (Huang and Jaffé, 2019). This non-reactivity makes them highly persistent under natural conditions (Wang et al., 2017).

Despite the persistence of perfluorinated compounds in the environment, some experiments have demonstrated the ability of bacteria to biodegrade PFCAs in a laboratory setting (Huang and Jaffé, 2019). Similarly, Taniyasu et al. (2013) recorded the photolysis of PFOS in the field, which more broadly evidenced the possibility of abiotic degradation of perfluorinated compounds. Notably, the experiment was located at a site on Mt. Mauna Kea at 4200 m above sea level, which receives stronger solar radiation (~40% higher) than typically experienced at mean sea level due to high altitude and good air quality (which includes less suspended particles and urban air pollutants). To our knowledge, no other field studies have observed the photolysis of PFOS. Generally, biotic and abiotic degradation of perfluorinated compounds in the literature is rare.

As mentioned previously, PFAS precursor are compounds that can undergo abiotic or biotic transformations to form more stable forms of PFAS, like perfluorinated carboxylic acids (PFCAs) (Huang and Jaffé, 2019). Precursor compounds are less prevalent in the literature

compared to legacy compounds (Fenton et al., 2021), yet they are critically important contributors to legacy contamination. They can also make up a significant portion of total contamination. For instance, precursors and their intermediates contributed up to 86% of the total PFAS measured in sludge from wastewater treatment plants in Sweden (Eriksson et al., 2017).

In lab studies, precursors have been demonstrated to transform to stable PFAS through the following processes: hydrolysis (Washington and Jenkins, 2015), oxidation (Dombrowski et al., 2018), reduction, hydroxylation, and dehydroxylation (Cui et al., 2020). However, the processes that drive their transformation within natural systems are not well understood.

Some chemical structures make precursors more prone to transformation and/or degradation in the environment. To find more specific chemical features responsible for degradability, Che et al. (2021) reviewed the literature on microbially mediated transformations of short-chain fluorinated carboxylic acids (FCAs). They found all compounds undergoing transformation met at least one of two requirements: 1) a C–H bond at the alpha carbon position or 2) an sp^2 C–F bond (a double bonded C) at the beta-position (Che et al., 2021). Thus, PFAS that contain C–H bonds at the alpha carbon are more susceptible to microbial transformation and defluorination than their saturated counterparts. Similarly, unsaturated carbons—double (C=C) or triple bonded (C≡C) carbons—at the β -position serve as weak points for microbial attack. Both features allow for reactions that yield unstable intermediates that can ultimately result in cleavage of the C–F bond and the shortening of the parent compound. Both n:2 fluorotelomer alcohols (FTOHs) and carboxylic acids (FTCAs) contain the α -C–H bonds described above. These compounds (where n = 4, 6, 8) can lose 2 C–F bonds in the environment. Specifically, the maximum aerobic defluorination degree for 6:2 is 16% (2 of 13 F atoms) and for 8:2 is 12% (2

of 17 F). However, this defluorination is limited because it yields a perfluorinated product more recalcitrant than its precursor (Che et al., 2021).

1.3.5 PFAS in AFFF

There are many sources of PFAS in the environment including industrial activity, atmospheric deposition, application of biosolids, and residential sources (National Academies of Sciences, Engineering, and Medicine, 2023). One of the most important contributors to PFAS contamination in the environment is AFFFs (Hu et al., 2016). Historically, AFFFs have been released to the environment during emergency response efforts, firefighting training, equipment testing, disposal, or accidental release (Thalheimer et al., 2017; Yan et al., 2024). In fact, military fire training areas have been demonstrated to be an important source of PFAS in public water supplies across the U.S. (Hu et al., 2016).

AFFFs are formulated to extinguish Class B fires, which are fires fueled by highly flammable or combustible liquids, such as crude oil or gasoline. The capable surfactant abilities of PFAS make AFFFs effective at separating the fuel from the air, and their stability at high temperatures prevents their degradation (Thalheimer et al., 2017).

AFFFs were originally developed by the U.S. navy in the 1960s and have since been used both by military and civilian firefighting operations, especially where large amounts of hydrocarbon fuels are stored (Thalheimer et al., 2017). In practice, AFFF concentrates are mixed with water at their point of use by a proportioning nozzle. The resulting ‘foaming solution’—or ‘premix’—is then dispensed by the nozzle, where the mixture becomes foam, hence the name “film-forming” (Thalheimer et al., 2017; Kempisty et al., 2018). Figure 2 shows the general composition of an AFFF concentrate and the composition of the diluted foaming solution.

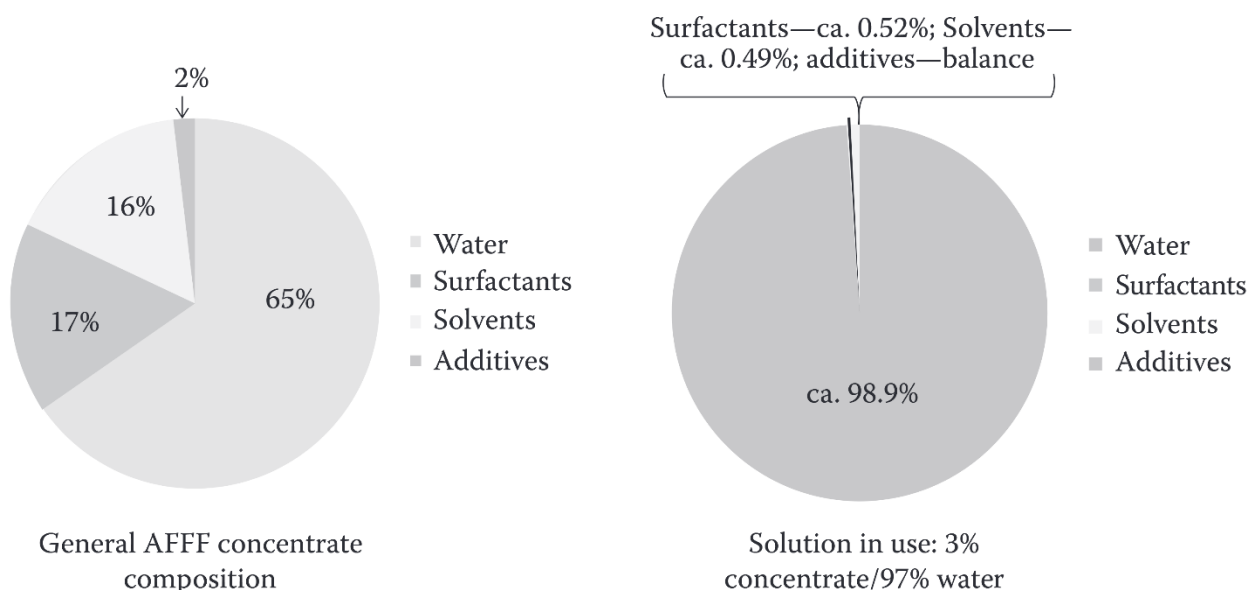


Figure 2. Composition of a 3% AFFF concentrate (left) and the diluted mixture in use (right) (Kempisty et al., 2018).

Though all AFFF concentrates' exact formulations are proprietary, their compositions are known to consist of about 1-3% PFAS with the remaining solution being made up of water, hydrocarbon surfactants, organic solvents, and other additives. For instance, alcohol-resistant AFFFs (AR-AFFFs) contain a gel-like additive designed to prevent the degradation of foam when exposed to polar solvents, like alcohols or ketones (Kempisty et al., 2018).

ECF-based vs. FT-based AFFF products. The two main ways that fluorosurfactants are produced commercially are electrochemical fluorination (ECF) and fluorotelomerization (FT) (Faust, 2023). The compounds yielded by each method differ in the number of carbons and the isomer profiles. Regarding the number of carbons, ECF yields perfluoroalkyl compounds with carbon chains that have even and odd lengths, while FT yields carbon chains with solely even lengths (Kempisty et al., 2018). Regarding the isomeric profiles, ECF yields a mixture of linear and branched isomers whereas FT yield mostly linear isomers (Faust, 2023).

Many of the compounds present in ECF-based AFFF formulations are C6–C8 PFASs (i.e. PFHxS [C6], perfluoroheptane sulfonate [PFHpS] [C7], and PFOS [C8]) (Backe et al., 2013). The most prevalent compounds in FT-based AFFF formulations contain either C6 or C8 perfluoroalkyl moieties (e.g. 6:2 fluorotelomer sulfonic acid [6:2 FTSA] or 8:2 fluorotelomer sulfonic acid [8:2 FTSA]) (Kempisty et al., 2018). However, both production methods yield AFFFs containing complex mixtures. In fact, between ECF-based and FT-based AFFFs, more than 40 classes of compounds have been discovered, and more classes are still being discovered today (Dasu et al., 2022).

For decades, AFFF formulations contained many long-chain PFAS until 3M voluntarily phased them out of production in 2002. Initially, long-chain fluorotelomer-based formulations (i.e., having 8 or more carbons, such as 10:2 fluorotelomer sulfonic acid [8:2 FTSA] or 8:2 fluorotelomer alcohol [8:2 FTOH]) fulfilled the need for alternative firefighting foams. However, these fluorotelomers were found to degrade into more stable forms of PFAS, such as PFOA. Once known that PFOA is persistent in the environment, the U.S. EPA led a voluntary global effort called the U.S. EPA 2010/2015 PFOA Stewardship program. The program invited manufacturers to reduce plant emissions and product content of PFOA, PFOA precursors, and related homologues by 95% by 2010 and 100% by 2015 (Thalheimer et al., 2017). The pressure to discontinue production of PFOA, combined with the existing ban on PFOS, accelerated research on alternative foams. These alternatives consist of fluorinated and fluorine-free products (Thalheimer et al., 2017). Examples of these products are shown in Table 1. Some fluorine-free Class B firefighting foams are available on the market (Glüge et al., 2020), which are being used in Europe and Australia, but they do not meet the North American military specification used by

Table 1. Types of Class B foams. Modified from (Thomas, n.d.).

All Class B Foams	
Fluorinated foams	Fluorine-free foams
Aqueous film-forming foam (AFFF)	Protein foam
Alcohol-resistant aqueous film-forming foam (AR-AFFF)	Alcohol-resistant protein foam (AR-P)
Film-forming fluoroprotein foam (FFFP)	Synthetic fluorine-free foam (FFF)
Alcohol-resistant film-forming fluoroprotein foam (AR-FFFP)	Synthetic alcohol-resistant fluorine-free foam (AR-FFF)

the U.S. Department of Defense, MIL-F-24385 (Kempisty et al., 2018). Thus, most installations still use PFAS-based foams because of their efficacy and rapid ability to knock down fires (Thalheimer et al., 2017). However, the industry has pivoted away from long-chain PFAS-based foams and towards short-chain fluorotelomer foams, which are manufactured using telomerization (Thalheimer et al., 2017). Yet, short-chain have disadvantages since they are more mobile in the environment and more difficult to remove from drinking water (Cousins et al., 2016).

Though many efforts have been made to characterize AFFF formulations, using this knowledge to identify the primary source of contamination in the field can be challenging due to the proprietary nature of the AFFF products, the varying hydrology of contaminated sites, the chemical transformation of precursors into perfluorinated products, and the tendency of PFAS to partition within environmental media. Indeed, PFAS profiles at the source of contamination can change drastically over time due to the transformation of precursors. For instance, Yan et al. (2024) found that primary precursors constituted a significant percentage (<50%) of PFAS in AFFFs used at a firefighting training site. However, in the nearby groundwater analyzed, primary precursors made up only 0.7% of total PFAS mass (Yan et al., 2024).

AFFF Impacts to Soil and Water. Because AFFF products are commonly released directly into the environment, soil is an important medium to study. Indeed, the soils at AFFF source zones are important reservoirs for PFAS (Yan et al., 2024) and are predicted to be a source of PFAS long-term (Brusseau et al., 2020). Currently, soil contamination of PFAS is widespread due to atmospheric deposition of PFAS. Soil is also the medium in which land plants grow and uptake PFAS, which is an exposure pathway for much wildlife.

Fate and Transport. Generally, AFFFs that are released into the environment adsorb preferentially to surface soil and tend to remain at shallower depths (Anderson, 2021; Yan et al., 2024). Most depth-dependent studies have shown that PFAS levels are highest at the surface and decrease exponentially with depth (Brusseau et al., 2020). Several soil processes act to retain PFAS throughout the profile and include sorption, partition, and complexation reactions (Bolan et al., 2021). Sorption is an electrostatic interaction with charged soil surfaces. Partition is a hydrophobic interaction with organic substrates like organic matter. Complexation occurs when PFAS interact with dissolved organic matter, forming soluble PFAS-organic matter complexes (Bolan et al., 2021). More broadly, the processes that determine the retention of nonpolymeric PFAS in the vadose zone are numerous and complex (Anderson, 2021). For PFAAs, a few more examples of important processes include the following: hydrophobic interactions with SOM; electrostatic interactions with charged clay minerals, including iron (Fe) and aluminum (Al) oxyhydroxides (oxides and hydroxides) (even to the point of chemical sorption [chemisorption]); and air–water interface partitioning that increases retention (Anderson, 2021). Thus, PFAS transport through unsaturated soil is nonideal (Anderson, 2021).

Though several processes act to retain PFAS in soil, some will inevitably leach farther into the soil profile and into groundwater (Yan et al., 2024). Once in groundwater, these

compounds can drain into surface water. PFAS at the soil surface can also be transported directly into surface water through surface runoff (Yan et al., 2024). The most prevalent PFAS reported in the literature remain PFOS and PFOA, though other compounds have reached a similar state of importance. PFOS, PFOA, PFHxS and PFHxA are the four compounds with the highest number of detections in surface soil from AFFF-impacted sites in the U.S. (Brusseau et al., 2020).

PFAS Transport in Air. Though the highest reported PFAS concentrations are typically found in soil, PFAS are also widespread in air (Pfoenhauer et al., 2022) and water across the globe (Johnson et al., 2022). The hydrologic cycle is a crucial mechanism driving long-range transport of PFAS to remote areas of the globe (Johnson et al., 2022) and continues to be an important mechanism for PFAS movement from AFFF-impacted sites today .

Though atmospheric deposition of PFAS is much less understood than other media such as surface water or soil, it plays a critical role in PFAS transport. PFAS are released into the atmosphere through emissions from manufacturing facilities, landfills and wastewater treatment plants, firefighting, vehicle exhaust, and indoor products (Pfoenhauer et al., 2022; Faust, 2023).

Behavior. Aerosols are defined as small particles suspended in the air, and they often consist of multiple components. When interacting with particulate matter, higher vapor pressure compounds (like FTOHs) tend to remain in the gas phase while lower vapor pressure compounds tend to partition to particulate matter (Faust, 2023). Short chain PFAS tend to partition to the aqueous phase of aerosols while long chain PFAS tend to adsorb to the surface (Faust, 2023). Generally, compounds with higher chain lengths tend to partition from the gas phase to the particle phase (Faust, 2023).

Some field studies have gleaned the partitioning behavior of PFAS according to their chemical class. According to Ahrens et al. (2012), the following classes increase in their tendency to partition to the solid phase: FTACs (fluorotelomer acrylates) < FTOHs < FASAs (perfluoroalkane sulfonamides) < PFASs (perfluoroalkane sulfonic acids) < FASEs (perfluoroalkane sulfonamido ethanols). Another study by Paragot et al. (2020) used a different methodology and found the following similar trend: FASAs/FASEs < PFCAs < PFASs.

Wet deposition. According to both field and modelling studies, PFAS are present in wet deposition around the world. Shimizu et al. (2021) studied PFOA, PFOS, and four PFECAs and found that wet deposition removed both gas-phase and particle-bound compounds ten times more effectively than dry deposition. Thus, regardless of PFAS presence in the gas-phase or particle-bound, precipitation is an effective scavenger for PFAS (Shimizu et al., 2021). Of course, the importance of wet vs. dry deposition will vary depending on a given location's climate. To further explore the mechanisms that drive PFAS incorporation into precipitation, several studies compared PFAS levels in the initial precipitation to PFAS levels in precipitation later in the event (Kwok et al., 2010; Sachi et al., 2013; Shimizu et al., 2021). These studies showed that measured PFAS concentrations are higher during the first few millimeters of a precipitation event than during later in the event (Shimizu et al., 2021). This finding suggests that in-cloud scavenging (rain-out) might be a higher driver of PFAS incorporation than below-cloud scavenging (washout). However, the exact mechanisms driving partitioning into precipitation are still debated (Shimizu et al., 2021).

To our knowledge only two studies have directly measured PFAS in dry deposition (Yao et al., 2016; Shimizu et al., 2021). Furthermore, only Shimizu et al. (2021) has directly compared

wet vs. dry deposition. Thus, there is a large research gap in how dry deposition drives PFAS transport.

1.3.6 Phytoscreening

History. Using plants as indicators for their environment is not a novel concept. For instance, the Romans inferred a shallow groundwater table from the presence of willow or poplar trees and used this info when placing drinking wells (Balouet, 2015). Only recently have trees been considered as bioindicators of groundwater contamination. Vrobley et al. (1999) was the first to show that analysis of tree-cores could be used to delineate shallow groundwater contaminated by chlorinated ethenes (CEs). The technique was first called ‘phytoscreening’ by Sorek et al. (2008), who defined it as a screening method for detecting VOC contamination of the sub-surface. Since then, the technique has been explored for several contaminant classes including volatile organic compounds (VOCs; e.g. BTEX or chlorinated hydrocarbons) and heavy metals (Cantonati et al., 2020). A sizable body of literature has been written around using trees as bioindicators of groundwater contamination (Balouet, 2015; Cantonati et al., 2020). Table 2 shows the factors which have been demonstrated to influence the efficacy of phytoscreening in chloroethenes.

Table 2: Potential factors affecting the effectiveness of phytoscreening in chloroethenes. Modified from Leoncini et al. (2022).

Topic	Factors	Measuring unit
Hydrogeology	Depth to water table (DWT)	m b.g.l (below ground level)
	Average aquifer thickness (b)	m
	Aquifer hydraulic conductivity (K)	m/s
Tree identity and anatomy	Family	e.g., Salicaceae
	Xylem structure	e.g., coniferous
	Tree diameter at breast height (DBH)	cm
Sampling and extraction methodology	Trunk drilling length (L)	cm
	Sampling height (H)	cm a.g.l. (above ground level)
	Extraction method	e.g., methanol extraction

Application. The USGS has previously used phytoscreening as a main reconnaissance method for the extent of subsurface contamination by chloroethenes (Schumacher et al., 2004). One hundred trees were studied (Balouet, 2015), and the results informed subsequent soil and groundwater sampling, saving time and costs associated with site assessment (Schumacher et al., 2004). Other applications of phytoscreening have included the following: detecting and/or delineating groundwater contamination; dating contamination events; monitoring groundwater contamination of naturally attenuating or remediated sites; and detect degradation of parent contaminants into daughter compounds (Cantonati et al., 2020).

Purpose. Sorek et al. (2008) highlighted phytoscreening as a tool to delineate subsurface plumes, which reduces costs and time associated with site characterization. Specifically, phytoscreening could potentially be used as a preliminary screening tool in urban settings where

the installation of monitoring wells can be expensive or difficult. Similarly, passive monitoring equipment are susceptible to interference in these settings (Sorek et al., 2008). One advantage of tree-sampling is that the zone of sampling is broader than the area typically sampled in the soil collection (Burken et al., 2011). After the plume of contamination is delineated, decision makers are better equipped to determine those most at risk of groundwater well contamination.

1.3.7 Mechanism of Uptake

Vascular plants develop root systems that contain a tremendous amount of surface area that interfaces with the soil (Burken et al., 2011). As roots draw soil pore water into the transpiration stream, dissolved contaminants will also be transported. Based on their physical and chemical properties, contaminants will translocate into higher plant parts (i.e., branches, leaves) and validate the uptake of these contaminants by the tree wood and sap as a reflection of soil chemistry within the tree system (Burken et al., 2011).

Organic compounds are taken up by plants either through the aqueous or vapor phases of soil (Collins et al., 2006). Chemicals enter deeper into the root either between intracellular fluid (symplastic movement) or between cell walls (apoplastic movement). To reach plant xylem, chemicals must penetrate several layers: the epidermis, cortex, endodermis, and pericycle. At maturity, the endodermis contains a suberized Casparian strip, which inhibits apoplastic movement, forcing compounds to pass through the plasma membrane and cytoplasm (Collins et al., 2006). While the endodermis effectively prevents absorption of many contaminants, the developing endodermis at the root tip lacks the Casparian strip, enabling some chemicals to bypass this cell layer (Yang Song et al., 2011). The importance of the Casparian strip in PFAS uptake has also been repeatedly evidenced in the literature; plants with no or few Casparian

strips (e.g. radish and carrot) are associated with a higher translocation factor (TF) (Mei et al., 2021).

Though soil uptake mainly drives absorption of PFAS into plants, foliar uptake can still be an important mechanism driving accumulation of organic compounds (Collins et al., 2006). Some volatile and semivolatile compounds will volatilize from the soil and can enter plants, diffusing either through the cuticle or the stomata (Jiao et al., 2021). Some PFAS affect plant concentrations via aerial deposition (Jiao et al., 2021). PFAS precursors and their degradation products (e.g. FTOHs, FOSEs) are the compounds most likely to be accumulated through this mechanism given their semivolatile nature (Jiao et al., 2021). In summary, the mechanisms responsible for plant uptake of PFAS are volatilization, atmospheric deposition, and most importantly, absorption through roots.

Factors affecting soil uptake. Most PFAS plant uptake studies focus on agricultural plants with an emphasis on human health, but few have examined potential factors driving uptake. A recent study examined four urban weeds and measured several foliar and root traits to determine which were correlated with PFAS uptake (Zhi et al., 2022). The compounds studied included 5 PFCAs, 3 PFSAAs, 1 FTSA, and 1 replacement PFAS (HFPO-DA). They measured many leaf traits including leaf chlorophyll content, leaf area and mass, leaf carbon (C), nitrogen (N), and phosphorus (P) content, and C:N, C:P, and N:P ratios. Root traits measured included root length, surface area, surface area to length ratio, average diameter, and length per volume. Of the parameters measured, the leaf area and average root diameter were most strongly correlated with plant uptake. Therefore, plant species with small leaves and fine roots absorbed more PFAS from the soil than species with large leaves and coarse roots.

Species dependency of PFAS uptake. The literature strongly evidences that PFAS uptake differs by species, and various attempts have been made to explain these findings (Lesmeister et al., 2021). To elucidate the factors that drive uptake of PFAS in plants, many of these studies calculate bioaccumulation factors (BAFs), which relate the concentration of a compound within a plant to the concentration in the environment. The first explanation for observed differences between species is that plant tissues sampled between different species can differ in function, altering their uptake patterns (Lesmeister et al., 2021). For instance, though the potato's tubers and the carrot's taproot are both located in the soil, the potato serves as a storage organ, while the carrot serves as both a storage organ and a root. Therefore, the carrot root interfaces more directly with the soil, increasing the likelihood of PFAS uptake. In contrast, PFAS compounds that reach a potato tuber must bypass several barriers including the transition from xylem to phloem (Lechner and Knapp, 2011). The second explanation is that physiological mechanisms unique to certain plant groups could drive differences in uptake. The Cucurbitaceae family, for example, contains protein-like substances within the xylem sap that promote uptake and translocation of PFOA (Murano et al., 2010). Lechner and Knapp (2011) posited that protein-like substance within the xylem sap of Cucurbitaceae promoted higher BAFs for PFOA in cucumbers (average 0.88) than in carrots (average 0.53) and tomatoes (average 0.40) (Murano et al., 2010). Similarly, differences in protein and lipid content have been evidenced to explain some of the observed differences in PFAS levels between plants (Liu et al., 2017; Zhou et al., 2020). For instance, in a study that included seven different species (maize, soy, radish, mung beans, lettuce, alfalfa, and ryegrass), Wen et al. (2018) implemented a principal component analysis that was able to explain 55% of the differences in EtFOSAA uptake among the included species by the protein content ($p > 0.05$). This accuracy increased to 85% when the lipid content

was included as a parameter (Wen et al., 2018). Thus, several explanations for differences in uptake between species have been offered in the literature. Notably, these studies focus on agricultural crops rather than trees. However, some findings could be useful for selecting species and tree tissues suitable for phytoscreening.

1.3.8 PFAS in Trees

From the existing literature on PFAS translocation in agricultural plants, it is unclear how PFAS translocation might differ in trees. Yet, a few studies exploring PFAS remediation strategies have tested the adsorptive properties of modified and unmodified wood pulp. Harris et al. (2022) found that unmodified wood pulp had an adsorption capacity of ~4.9 mg/g and ~5 mg/g for PFOA and PFOS, respectively, when left to equilibrate for 24 hours. Li et al. (2022) extracted cellulose fibers and lignin in corn stover (residual biomass left after harvest) and used the components to create a mixture that mimicked cellulose fibrils. They found the adsorption capacity for PFOA and PFOS was 3.9 mg/g and 225 mg/g, respectively. These values reflect extraordinarily high amounts of PFAS adsorbed given background concentrations for PFOA and PFOS in water are often on the scale of ng/g or ppt, which is magnitudes lower than mg/g. However, the data poorly fit the pseudo-second order model from which these values were derived as these mixtures were not the primary focus of the study (PFOA, $R^2 = 0.193$; PFOS, $R^2 = 0.150$). Though neither of these adsorption studies were conducted on living wood tissue, their findings might have important implications for the understanding of PFAS movement through xylem, namely, that the potentially high affinity of PFOA and PFOS for woody tissue might inhibit their translocation throughout trees.

Chapter 2: Evaluating Phytoscreening Efficacy of Select Species

2.1 Introduction

Per- and polyfluoroalkyl substances (PFASs) are a class of synthetic organic compounds that contain at least one carbon atom whose hydrogens have been replaced by C–F bonds (Buck et al., 2011). The high stability of the C–F bond imparts a high resistance to chemical and thermal degradation, making these compounds useful for a wide range of industrial and commercial applications (Buck et al., 2011). Concerns have emerged regarding their toxicity to humans (Itoh et al., 2016) and wildlife (Thalheimer et al., 2017), persistence, and ubiquity in the environment (Brennan et al., 2021).

Aqueous Fire Fighting Foams (AFFFs) are an important source of PFAS in the environment. Example mechanisms of release include extinguishing hydrocarbon fires, firefighting training, accidental release, and disposal (Thalheimer et al., 2017). Currently, the U.S. Air Force (USAF) is conducting investigations to identify and remediate AFFF-impacted sites—such as Fort George G. Meade (a.k.a. Fort Meade) in Maryland (Hunter Anderson et al., 2019). A critical step in these investigations will be evaluating the extent of impact (Wang et al., 2017; Würth et al., 2023).

An alternative approach to delineating the plume of contamination is phytoscreening—a technique that uses the sampling of plant tissues to determine subsurface contamination. The technique can function as a screening tool to identify the most polluted areas of a site before analyzing these areas with more intensive methods (Burken et al., 2011). Phytoscreening can be a simple, fast, and cost-effective method compared to traditional groundwater sampling because of the little equipment required and the short time frame in which many samples can be collected (Balouet, 2015). Though primarily applied to volatile organic contaminants (VOCs) (Leoncini et

al., 2022), evidence exists that the technique can also be applied to PFAS (Gobelius et al., 2017; Würth et al., 2023).

In this study, three tree species common to the area surrounding Fort Meade were selected: red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), and willow oak (*Quercus phellos*). The purposes of this study were to (i) evaluate the efficacy of phytoscreening of PFAS for select species in proximity to a point source, Fort Meade and to (ii) identify potential differences in PFAS uptake between species.

2.2. Materials and Methods

2.2.1 Chemicals and Materials

Isotope-labeled PFAS mixtures (MPFAC-HIF-ES and MPFAC-HIF-IS) and non-labeled calibration standards (PFAC30PAR) were provided by Wellington Laboratories (Overland Park, KS). This analyte list was chosen because of its inclusion of precursors, legacy PFAS common to AFFF-impacted sites, and replacement compounds. Methanol (HPLC grade), acetone (HPLC grade), ammonium hydroxide (ACS+ grade), and acetic acid (ACS grade) were purchased from Fisher Scientific. Solid-phase extraction (SPE) was performed using Supelclean ENVI-Carb (3 mL) cartridges.

2.2.2 Site Information

Fort Meade is located in Maryland, USA, between Washington D.C. and Baltimore. The fort is a confirmed source of PFAS associated with AFFFs due to previous and current firefighting related activities (U.S. Department of Defense, 2022b).

Table 3: Shows the AFFF (Aqueous Film-Forming Foam) history at each Area of Potential Interest (AOPI) investigated by the U.S. Department of Defense (2022a).

AOPI	Site Description	AFFF Application	Application Frequency	Application Window
1	900 Area – AFFF Equipment Testing Area	Fire hose training; foam inductor testing	>3	1980s and 1990s
2	2300 Area – AFFF Equipment Testing Area	Fire hose training; foam inductor testing	~130	1980s and 1990s
3	Salt Dome	Fire dep training release	1	NA
4	Building 4230 – Former Fire Station	Fire equipment testing, Fire truck washing, AFFF storage	Many	early 1980s to 2001
5	Railroad Avenue – Fire Equipment Testing Area	Fire equipment testing, Firehose testing and training exercises, AFFF storage	Many	late 1970s to late 1990s
6	Building 6619 – Current Fire Station	Fire equipment testing (fire truck washing)	Many	2001 to unknown
7	Closed Sanitary Landfill Cell 1 (FGGM-17)	Disposal of empty 5-gal buckets of AFFF	Many	early 1980s to 1996 (closure)
8	Closed Sanitary Landfill Cell 2 (FGGM-17)	Disposal of empty 5-gal buckets of AFFF	Many	early 1980s to 1996 (closure)
9	Closed Sanitary Landfill Cell 3 (FGGM-97)	Disposal of empty 5-gal buckets of AFFF	Many	early 1980s to 1976 (closure)
10	Parade Ground Area	Fire trucks stocked with AFFF parked here	Unknown	Unknown
11	Buildings 3486 and 3488 – Detached Fire Department Support Buildings	Fire dep pumper truck stocked with AFFF was parked here	Unknown	Unknown

Because on-base sampling was restricted due to access limitations, sampling sites were selected due south of Fort Meade at the Patuxent Research Refuge. Patuxent Research Refuge was established in 1936 and serves as both a wildlife refuge and research center. In 1991, 7,600 acres (3,076 hectares) of land was transferred from Fort Meade to Patuxent Research Refuge.

The land underlying both Fort Meade and Patuxent Refuge consists of mostly flat topography with scattered waterbodies (U.S. Department of the Army, 2020). This area is located over the Patapsco and Patuxent aquifers, which lie about 280 and 500 ft below ground surface (b.g.s.), respectively (Raffensperger et al., 2010). Another existing aquifer is the water-table aquifer, which includes shallow groundwater (<30 ft b.g.s.) that exists alongside local rivers and wetlands and contributes to their water supply (U.S. Fish and Wildlife Service, 2022).

2.2.3 Sample Collection

The year before site selection, several sites around the Fort were surveyed to determine the most common species to the area. The following summer, these sites were revisited, and several had become inaccessible, prompting a decision to sample from the area south of Fort Meade—within Patuxent Research Refuge—an ideal sampling location due to its location within the path of groundwater flowing off-base. New sites were surveyed, and tree species that were common to all sites, easily identifiable, and accessible were chosen. This included red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), and willow oak (*Quercus phellos*). All three tree species were represented at three sites, totaling nine trees. The presence of all species at each site helped account for site variability. Site and tree locations are shown in Figure 3.



Figure 3. This map displays the three sites and their corresponding media sampled in 2023. Each Area of Potential Interest (AOI) was investigated by the DOD in 2020.

To assess vertical distribution of PFAS within each tree, sampled media included surface soil, a trunk core, twigs, and leaves. A soil probe was used to collect composite surface soil samples (0-10 cm) about 110 cm from the base of each tree (Figure 4A & B) (Gobelius et al., 2017). Tree cores were collected using an increment borer (diameter 5.5 mm, Haglof) at breast height (Figure 4C). Leaves and twigs were sampled from the most easily accessible points (Figure 4D). This vertical sequence of sampling occurred on two sides of each tree. At each of the three sites, subsurface samples were taken from 30-40 cm and 100-110 cm using a soil auger. The sampling protocol performed at each site is visualized in Figure 5.

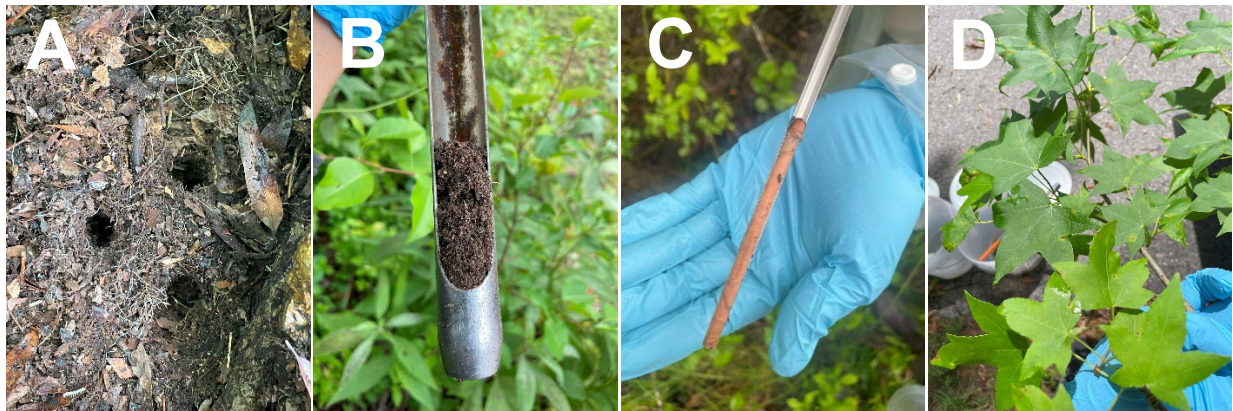


Figure 4. Display of surface soil (A and B), core (C), and twig and leaf (D) samples.

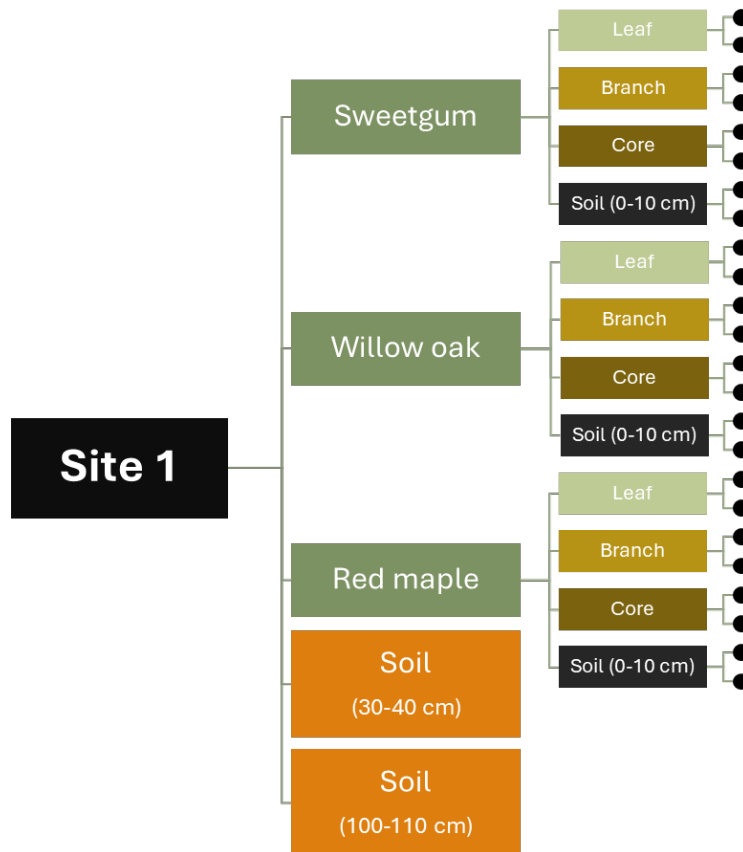


Figure 5. The sampling scheme for plant and soil samples at a single site, which was repeated at two other site locations. Black dots indicate samples taken from two different sides of the same tree.

All plant ($n = 27$) and soil ($n = 18$) samples were collected in high-density polyethylene (HDPE) bottles using nitrile gloves. Sampling equipment was decontaminated by washing with tap water and Alconox detergent, two rinses of tap water and then a rinse with PFAS-free deionized water. Sampling occurred from June 22-25, 2023, and samples were stored at 0 - 6 °C until analysis in August 2023.

2.2.4 Sample Preparation and Extraction

The analysis included 27 PFAS compounds (Table 4) and included all analytes previously recorded in groundwater at Fort Meade (U.S. Department of Defense, 2022b). All plant and soil samples were analyzed as solids according to EPA Method 1633 (3rd draft) with one modification (U.S. Environmental Protection Agency, 2022b). All solid samples were freeze-dried for, at minimum, 5 hours. Plant samples were homogenized in a grinder and soil samples by thorough mixing. Plant homogenate (1-2 g) and dried soil homogenate (5 g) were spiked with an extracted internal standard (EIS) mix before extracting with 0.3% methanolic ammonium hydroxide. Extracts from solid samples were diluted in DI water to be further handled as water samples. This modification was made for efficiency and had been previously demonstrated to avoid negatively impacting analyte recoveries.

2.2.5 Cleanup and Analysis

Extracts (10 mL) were diluted with 250 mL DI water and 2 mL 5% formic acid. The mixture underwent solid-phase extraction (SPE) with WAX Oasis™ cartridges at a flow rate of 5 mL/min. The remainder of the protocol followed method 1633 for aqueous samples—continuing from section 12.2.1. with the exception of the substitution Supelclean ENVI-Carb™ cartridges for cleanup to enable in-line processing without centrifugation to remove loose carbon.

Table 4: Name, abbreviation, and registered CAS number of PFAS analytes included in analysis.

Analyte	Abbreviation	CAS
Perfluoroalkyl carboxylic acids (PFCAs)		
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnDA	2058-94-8
Perfluorododecanoic acid	PFDoDA	307-55-1
Perfluorotridecanoic acid	PFTTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	376-06-7
Perfluoroalkyl sulfonic acids (PFSAAs)		
Perfluorobutane sulfonic acid	PFBS	375-73-5
Perfluoropentane sulfonic acid	PFPeS	2706-91-4
Perfluorohexane sulfonic acid	PFHxS	355-46-4
Perfluoroheptane sulfonic acid	PFHpS	375-92-8
Perfluorooctane sulfonic acid	PFOS	1763-23-1
Perfluorononane sulfonic acid	PFNS	68259-12-1
Perfluorodecane sulfonic acid	PFDS	335-77-3
Fluorotelomer sulfonic acids (FTSs)		
4:2 Fluorotelomer sulfonic acid	4:2 FTSA	757124-72-4
6:2 Fluorotelomer sulfonic acid	6:2 FTSA	27619-97-2
8:2 Fluorotelomer sulfonic acid	8:2 FTSA	39108-34-4
Perfluoroalkyl sulfamido substances (FASAs)		
N-Methylperfluorooctane sulfonamidoacetic acid	N-MeFOSAA	2355-31-9
N-Ethyl perfluorooctane sulfonamidoacetic acid	N-EtFOSAA	2991-50-6
Perfluorooctanesulfonamide	PFOSA	754-91-6
Per- and Polyfluoroether carboxylic acids		
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4
6:2 chlorinated polyfluorinated ether sulfonate	6:2 Cl-PFAES	3606-19-6

Targeted analysis was performed by an ultrahigh performance liquid chromatograph (UHPLC) coupled to a triple quadrupole (QQQ) mass spectrometer (Thermo TSQ Quantis,), equipped with a C18 column (1.7 μm , 50 x 2.1 mm; Waters Acquity BEH), and ran in multiple reaction monitoring (MRM) mode.

2.2.6 Quality Assurance and Control

Method reporting limits (MRLs) for all PFAS were determined using the lowest point calibration standard with replicate precision <20% Relative Standard Deviation (RSD) (Bangma et al., 2023). All data below the MRL were excluded from analysis. Ottawa sand served as the blank matrix. PFHxA and PFOS were observed >MRL in one of the two blanks, though PFPeA was observed at >17 ng/g in both blanks, indicating a contamination of the Ottawa sand. On more than two occasions PFBA, PFDA, PFPeA, and PFTrDA strayed >30% from the spiked concentrations of the Continuing Calibration Verification (CCV) standards. All equipment blanks, field trip blanks, and lab DI water, were found to be <MRL.

2.2.7 Data Handling and Analysis

Within the context of phytoscreening, each plant species was evaluated for its ability to uptake the same compounds detected in groundwater. In practice, each species was compared by the number of different compounds detected, the detection frequency of these compounds, and the concentration of these compounds. Thus, species containing the highest number of compounds present in nearby groundwater, the highest detection frequency of these compounds, and the highest concentrations of these compounds were considered better candidates for phytoscreening. Similarly, the different plant tissues were compared by their detection frequency and concentration.

To compare species and different plant tissues by PFAS detection frequency and concentration, a linear mixed-effects model with censored data (LMEC) was used. Linear mixed-effects models are well suited for datasets with small sample sizes and missing observations (Shoari and Dubé, 2018). The R package ‘lme4’ was used because it is designed to handle datasets where a number of observations fall below a certain threshold, such as the MRL in this study. To avoid overparameterization and to simplify the interpretation of results, the fixed effects—species and plant tissue—were analyzed in separate models with the random effect—site location—used for both. The species parameter was analyzed without grouping by plant tissue and vice versa. Both individual PFAS compounds and total $\sum_{27}$ PFAS concentrations were estimated using Restricted Maximum Likelihood Estimation (REML) (Vaida and Liu, 2012). The data were $\log_{10}$ transformed prior to the analysis, and the model results were back-transformed to enable interpretation.

All groundwater data was sourced from a DOD report that extensively investigated PFAS contamination in groundwater ($n = 25$), surface water, sediment, and soil across the base (U.S. Department of Defense, 2022b).

2.3. Results

2.3.1 Research Question 1: Evaluating Species for Phytoscreening

Table 5 displays the PFAS compounds present in groundwater at Fort Meade and in each species. The table shows, of the 15 compounds found in the groundwater at Fort Meade, 7 were detected in red maple (6:2 FTSA, PFBA, PFBS, PFHxA, PFOA, PFOS, and PFDA), 7 were detected in willow oak (6:2 FTSA, PFBA, PFBS, PFHxS, PFOA, PFOS, and PFDA), and 3 were detected in sweetgum (PFBA, PFHxS, and PFOS). Though the same number of compounds were found in red maple and willow oak, each species differed by the compounds detected. Specifically, PFHxA was detected in red maple but not in willow oak. Similarly, PFHxS was detected in willow oak but not in red maple. Interestingly, in all tree species, several compounds were detected in plant tissue but not detected in the soil (neither at surface nor subsurface depths). For instance, 6:2 FTSA and PFBS were detected in red maple tissue but not in the underlying soil. Other examples are noted in Table 5.

Table 5. Displays the PFAS analytes detected in groundwater at Fort Meade that were also present at concentrations > MRL (Method Reporting Limit) in each species. These groupings were made regardless of site location or plant tissue sampled.

Groundwater	Red maple	Sweetgum	Willow oak
6:2 FTSA	✓*		✓*
8:2 FTSA			
N-EtFOSAA			
N-MeFOSAA			
PFBA	✓	✓	✓
PFBS	✓*		✓*
PFPeA			
PFHxA	✓		
PFHxS		✓*	✓*
PFHpA			
PFOA	✓		✓
PFOS	✓	✓	✓
PFNA			
PFDA	✓		✓
PFUnDA			
Total	7	3	7

* Indicates compounds that were detected in plant tissue but not in the soil.

Figure 6 compares each species by the total number of PFAS compounds detected. The x-axis displays each species, and the y-axis shows the total number of detections for a given species, regardless of the site location, plant tissue sampled, or which side of the tree was sampled. This comparison could be made without bias because the same number of samples were collected for each species. The total number of detections displayed for each species is subdivided into different colors that correspond to different PFAS compounds. Figure 6 reveals the number of detections were higher for red maple (24) and willow oak (24) than they were for sweetgum (3). The figure also displays the diversity of compounds captured between red maple and willow oak.

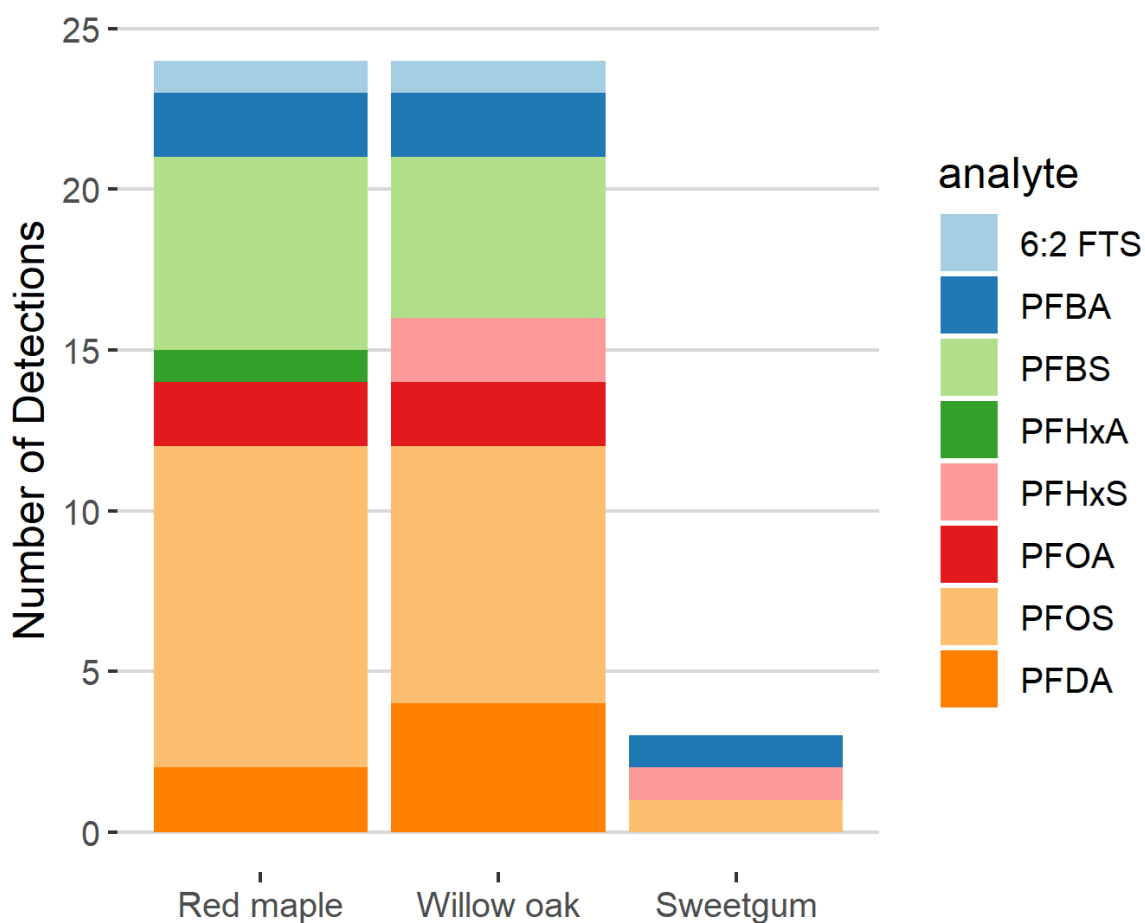


Figure 6. A comparison of each species by the number of times a compound was detected over the method reporting limit (MRL). This analysis was made across all plant samples of a given species, regardless of site location, plant tissue sampled, or the side of the tree sampled. The number of plant samples (n=18) collected for each species was the same.

In addition to the number of detections, the concentration of each compound is important when determining the best species for phytoscreening. The LMEC model incorporates both aspects to determine the effect of each species on PFAS accumulated.

Total $\sum_{27}$ PFAS. Figure 7A compares the impact of species type on the total $\sum_{27}$ PFAS concentrations across all sites. Representing a given species, each bar indicates a 95% confidence interval calculated using the LMEC model (see Data Handling and Analysis); bars that do not overlap indicate statistical significance between species. Positive values indicate that a given species accumulated more PFAS. Thus, red maple and willow oak accumulated significantly more PFAS than sweetgum. This finding is consistent with the previously reported number of detections for sweetgum, which was much lower than red maple and willow oak.

Individual PFAS compounds. To compare the concentrations of individual PFAS compounds between species, the analysis was limited to compounds detected in at least one tree from each species. This criterion included only PFOS and PFBA.

Figure 7B compares each species by their accumulation of PFOS and PFBA. The overall trend for these two compounds remains like that observed for total $\sum_{27}$ PFAS: red maple and willow oak accumulated more PFAS than sweetgum. However, by comparing PFOS concentrations to those of PFBA, all species accumulated significantly higher concentrations of PFBA than PFOS.

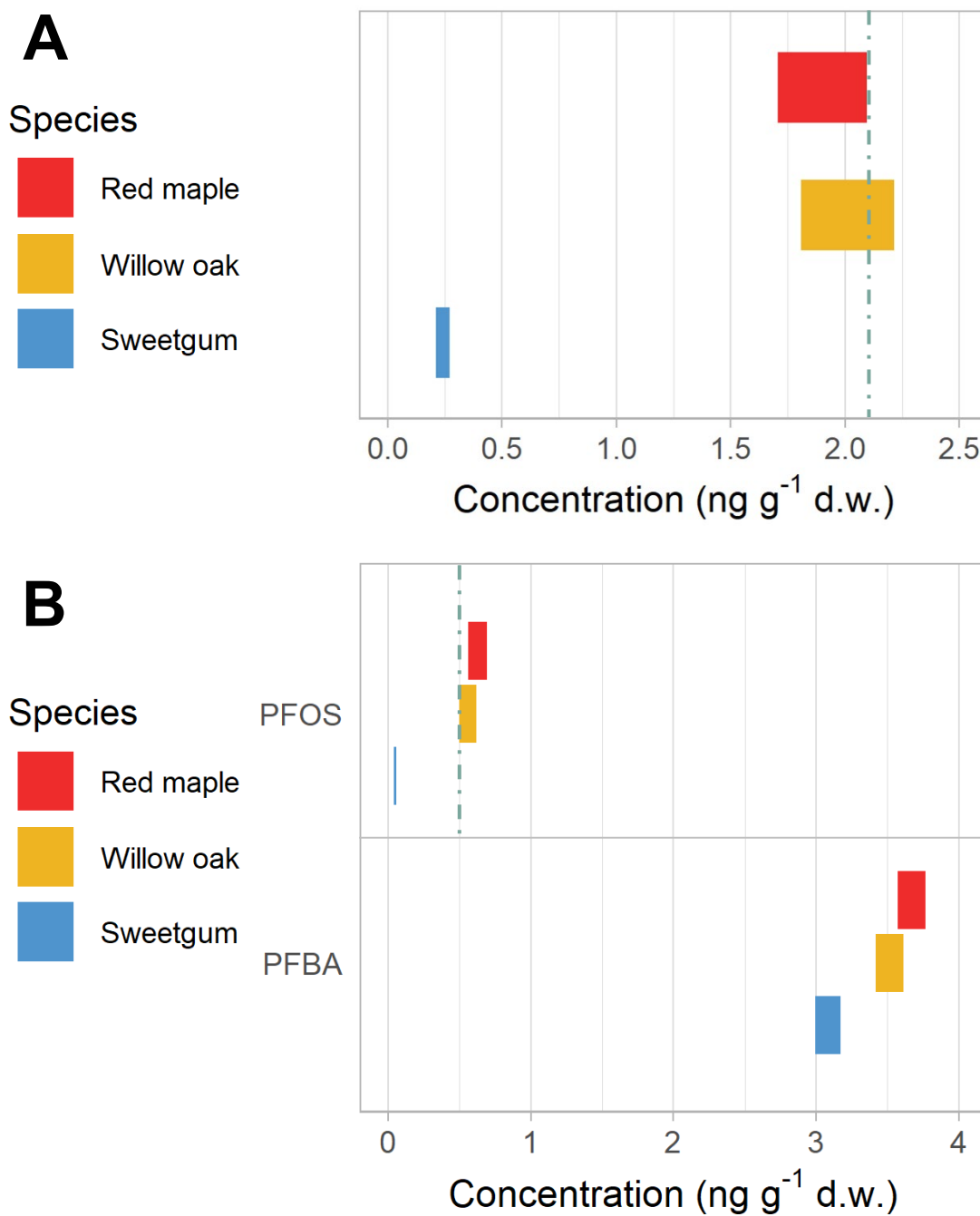


Figure 7. Results are displayed for total Σ_{27} PFAS (A) and individual compound (B) concentrations. The vertical dashed line indicates the average per-compound method reporting limit (MRL) (2.15 ng/g) (A) and the compound-specific MRL (B). The MRL of PFBA (5 ng/g) is not shown. Bars of species that do not vertically overlap are significantly different.

2.3.2 Research Question 2: Evaluating Plant Tissue for Phytoscreening

Total $\sum_{27}$ PFAS. Of the three plant tissues sampled (i.e., leaves, twigs, and trunk cores), the twigs accumulated the highest amounts of total $\sum_{27}$ PFAS. Twigs accumulated significantly more total $\sum_{27}$ PFAS than leaves and accumulated more total $\sum_{27}$ PFAS than cores, though this difference was not statistically significant (Figure 8A). Similarly, cores accumulated more total $\sum_{27}$ PFAS than leaves, though this difference was not statistically significant.

Individual compounds. Compounds that met the previously mentioned criteria (See Data Handling and Analysis) for further analysis were PFBS and PFOS. Figure 8B illustrates that for both compounds, twig and trunk tissue accumulated significantly more PFAS than the leaves. This pattern is similar to that observed for total $\sum_{27}$ PFAS. Because of the sparsity of data over the MRL, these two compounds are likely driving the results observed for total $\sum_{27}$ PFAS.

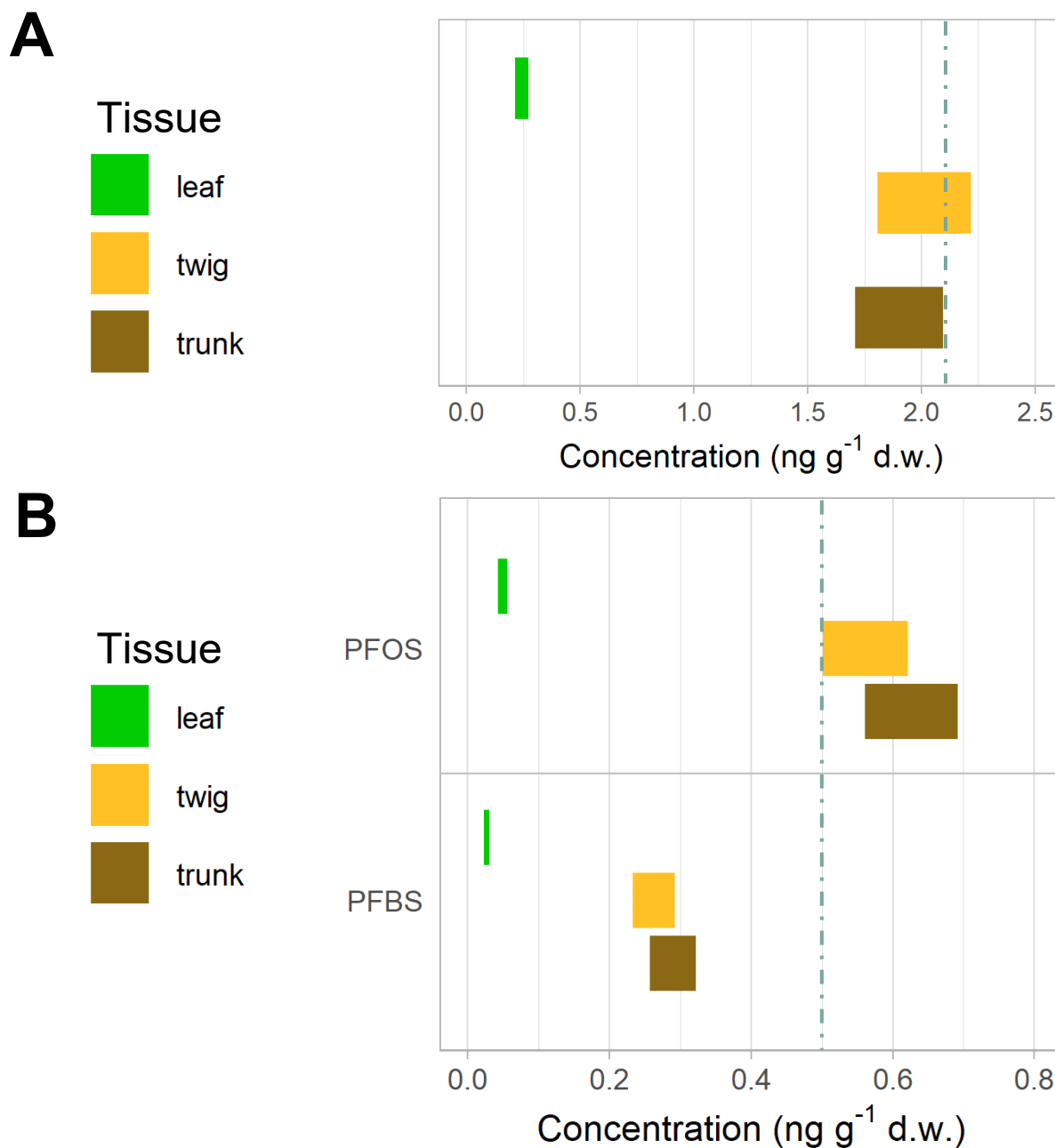


Figure 8. Results are displayed for total Σ_{27} PFAS (A) and individual compound (B) concentrations. The vertical dashed line indicates the average per-compound method reporting limit (MRL) (2.15 ng/g) (A) and the compound-specific MRL (B). Bars of tissues that do not vertically overlap are significantly different.

2.3.3 Other interesting findings

To explore how PFAS accumulation might vary between different sides of the same tree, samples were collected from two different sides of each tree. The results are found in Figure 9, where different sides of each tree are denoted by '1' and '2'. All trees in which PFAS were detected are shown on the x-axis, where 'G' represents sweetgum, 'K' is willow oak, and 'M' is red maple.

Data that fell below the MRL are not shown. Visually, this phenomenon is observed as the absence of a vertical bar on the graph. Therefore, on several instances, PFAS were present at high concentrations on one side of a tree but fell below the reporting threshold on the opposite side of the same tree. For example, in one tree, total $\sum_{27}$ PFAS concentrations reached approximately 14 ng/g (d.w.) in one side of the trunk but were not detected on the opposite side of the trunk.

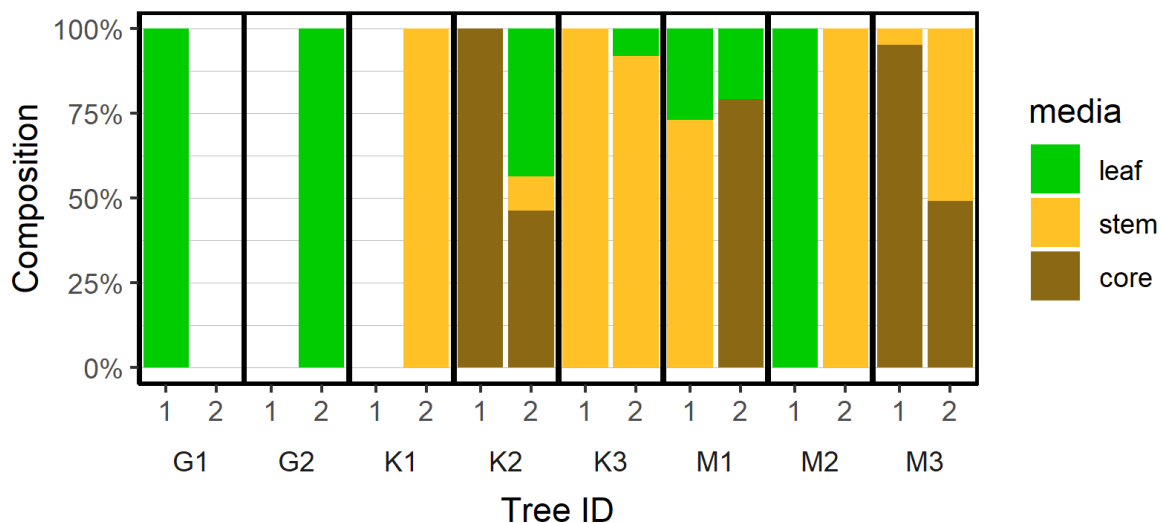


Figure 9. Comparison of plant compartment in which total $\sum_{27}$ PFAS was found. The y-axis shows the proportion of total $\sum_{27}$ PFAS detected by plant compartment. The x-axis shows all trees where PFAS was detected, where 'G' represents sweetgum, 'K' is willow oak, and 'M' is red maple. The x-axis also compares total $\sum_{27}$ PFAS from each side of the tree, where 1 and 2 represent different sides of the same tree. Samples below the MRL are not shown.

Figure 9 also displays the vertical distribution PFAS within sampled trees. To explore the vertical distribution of PFAS, different plant parts (i.e., trunk cores, twigs, leaves) were collected in a vertical line that followed the upward movement of water through xylem elements on a given side of a tree. In the figure, the plant parts are differentiated by color. Because compounds translocated from the roots to the leaves must pass through the trunk, trunk PFAS concentrations were assumed to be predictive of leaf concentrations. However, the figure shows that the accumulation of PFAS in trunk tissue did not necessarily ensure those compounds would be transported to the twigs or leaves. Similarly, some PFAS were detected in the leaves but not in the underlying twig or trunk tissue. Thus, this data set did not evidence the assumption that PFAS detected in trunk tissue would directly predict PFAS detected in the above twig and leaf tissue.

2.4. Discussion

2.4.1 Research Question 1: Evaluating Species for Phytoscreening

The objective of the study was to determine which of the sampled species are good candidates for phytoscreening of PFAS. By comparing each species by the number of compounds accumulated and their concentrations, it is clear that red maple and willow oak accumulated more PFAS than sweetgum, making red maple and willow oak better candidates for phytoscreening. More broadly, the observed differences between species validates species dependence in the context of phytoscreening. The higher accumulation of PFAS by red maple and willow oak is important, especially when considering the detection limits of modern analytical methods. Though some labs possess equipment capable of detecting PFAS at environmentally relevant concentrations—on the scale of parts per trillion—such equipment can be a financial barrier for many smaller labs. Therefore, exploring species, such as red maple and

willow oak, which are effective at accumulating PFAS, can serve to bridge the resource gap between larger and smaller labs.

Though red maple and willow oak captured several of the same compounds detected in groundwater at Fort Meade (i.e., 6:2 FTSA, PFBA, PFBS, PFOA, PFOS, PFDA), each species also managed to capture a compound not found in the other. This compound was PFHxA for red maple and PFHxS for willow oak. Furthermore, when selecting a plant species to sample for a phytoscreening survey, choosing to sample from several different species could be advantageous in that it could increase the variety of compounds detected. This is especially relevant for AFFF-impacted sites, since the mixtures of PFAS found in AFFFs are typically complex (Thalheimer et al., 2017).

As mentioned previously, all tree species contained several compounds that were detected in plant tissue but not detected in soil. These compounds are likely present in the soil but at levels below the MRL. The widespread presence of PFAS in soil is well-established, considering measurable amounts of PFOS and PFOA in soil are common, even in remote regions far from any known PFAS source (Brusseau et al., 2020).

All PFAAs included in this study (e.g., PFBS, PFOS, PFOA) are not suspected to have transformed within the plant due to the high stability of the perfluoroalkyl moiety (Wang et al., 2017). However, PFCA and PFSA precursors are known to degrade into PFCAs and PFSA respectively, contributing to higher observed levels over time. For instance, the PFOS precursor, N-EtFOSAA, was shown to degrade to PFOS in several agricultural plants over a period of 10 days (Wen et al., 2018). This degradation also results in decreased levels of precursors (e.g. 6:2 FTSA, 8:2 FTSA) over time. In other words, the observed levels of PFAAs in this study are a result of plant uptake from soil followed by translocation, as well as in vivo plant degradation of

precursors (Wen et al., 2018). To better characterize the proportion of PFAS precursors in a sample, various semiquantitative approaches could be used in the future. These include the total oxidizable precursor (TOP) assay, total organofluorine via combustion ion chromatography, and total fluorine via particle-induced gamma-ray emission (PIGE) spectroscopy (Sharifan et al., 2021).

Another reason for the discrepancy observed between compounds detected in plant tissue vs. those in soil is that these compounds could have been present at high concentrations in the soil but were not captured in the sample area. Composite surface soil samples were collected within a small vicinity of each other (~5 in), and subsurface samples were collected from a single borehole at each site. Because of the heterogeneity of soil, these soil samples might not have sufficiently captured PFAS concentrations representative of the wider area surrounding the base of each tree.

As mentioned previously, all species accumulated several of the same compounds found in groundwater at Fort Meade. Each species accumulated at least one compound that was not detected in the surrounding surface or subsurface soil. The presence of these compounds in sampled trees could be indicative of compound uptake directly from groundwater, assuming that these compounds are present in groundwater at these sites. This assumption is partly based on known site characteristics, that groundwater from under the base is estimated to flow down-gradient in the direction of all three sampling sites. Specifically, sites 1, 2, and 3 are located at 0.25 km, 0.86 km, and 2.4 km, respectively, from the nearest groundwater well sampled at Fort Meade. The assumption that these compounds could be transported from AFFF activity at Fort Meade is also based on the behavior of perfluorinated compounds, which are extremely persistent, able to resist many forms of degradation (Huang and Jaffé, 2019). It is also well

established that these compounds are highly soluble and able to migrate large distances once transferred from soil to groundwater (Davis et al., 2007; Post et al., 2012).

Plant vs. Groundwater Concentrations. PFAS levels in plants were higher than those in nearby groundwater. Nearby groundwater well $\sum_{18}$ PFAS concentrations ranged from 0.02-3.84 ng/mL, but total plant $\sum_{27}$ PFAS concentrations ranged from 1.03-15.96 ng/g dry weight (d.w.). Adjusted for moisture content, total plant $\sum_{27}$ PFAS concentrations were still comparably higher, ranging from 0.43-10.72 ng/g (w.w.), the maximum of which is approximately 35% higher than the highest measured groundwater $\sum_{18}$ PFAS concentration. While this study included nine more analytes than the DOD groundwater investigation, the higher number of targeted analytes in this study cannot account for the higher concentrations, since only one analyte, PFDoDA, was detected in plant samples that was not detected in groundwater. The elevated concentrations in plants relative to groundwater can partly be explained by the bioaccumulative behavior of these compounds (Zhi et al., 2022). Most analytes detected were PFAAs, which are ionized at environmental pH ranges (Vierke et al., 2013), are generally non-volatile, and tend to remain in the plant leaves once translocated (Blaine et al., 2014). Samples were collected in June, providing the sampled trees with available time to transpire by the time of sampling. Furthermore, because all included species are deciduous, non-volatile compounds that reach the leaves of the sampled trees are annually deposited on the soil surface as leaf litter. In theory, compounds could bio-magnify within the forest system as more PFAS are taken up from various sources. This phenomenon of PFAA deposition back to the soil could potentially serve as the primary contributor of higher PFAS concentrations detected in plants than those detected in nearby groundwater. This observation could serve as an additional validation marker for the use of phytoscreening in assessing the presence of PFAS when an analytical laboratory does not have

the full capabilities to reach sensitivity thresholds on the scale of parts per trillion. However, more research is needed in this area to determine the extent to which PFAS can accumulate on a systemic scale. Additional research is needed to help quantify the lower limits of PFAS in plant tissue for human health exposure and agricultural practices.

Limitations. When evaluating the efficacy of phytoscreening, one approach is to test the fidelity of plant data to groundwater data. This can be done quantitatively (e.g., the correlation of PFAS concentrations between both media) or qualitatively (e.g., what analytes are present in both media). Due to the challenges of obtaining sampling access close to groundwater wells, this study examined the efficacy of phytoscreening qualitatively. Other limitations of the study were the small number of trees sampled (9) and low detection rate. In many samples, the compound concentrations fell below the limit of quantitation / MRL, increasing the difficulty of drawing inferences about the factors analyzed. Future work can more quantitatively explore PFAS uptake from groundwater in these species by sampling from trees near accessible groundwater wells.

2.4.2 Research Question 2: Evaluating Plant Tissues for Phytoscreening

The second main objective of the study was to determine which plant tissues are the best candidates for PFAS sampling. The results indicate that regardless of PFAS origin (e.g., groundwater, surface water, or atmospheric deposition), the twigs accumulated the highest levels of PFAS in the included species. The second-best plant tissue to sample in this regard is the tree trunk. Worth noting is that especially for mature trees, the trunk is the most practical tissue to sample considering its accessibility. Across all included species, the leaves accumulated the least amount of PFAS compounds while woody tissues accumulated the highest. This finding demonstrates that the plant tissue can play a critical role in PFAS accumulation in trees.

This is somewhat surprising given that the mechanism of PFAS translocation within plants is mostly unilateral in the direction of leaves (Blaine et al., 2014). Thus, over the course of a growing season, leaves could reflect higher concentrations as more compounds are deposited. Instead, woody tissues accumulated more PFAS than leaf tissue. The most likely explanation for the higher observed PFAS levels in woody tissues is that woody tissues are generally older than the leaves. If PFAS levels in soil pore water were historically higher than present levels, then woody tissue might better reflect higher concentrations than the leaves, which are shed annually. It is also plausible that leaves harvested later in the growing season would have higher PFAS concentrations.

Limitations. PFAS detected in twigs or leaves of the sampled trees did not always ensure that PFAS was detected in the trunk from the same side of the tree. Because water in plant tissues at higher elevations must pass through the trunk, trunk concentrations should be predictive of concentrations in higher plant tissues. However, this relationship was not always observed. One limitation of this finding pertains to the method used to sample the plant tissues. When plant tissues on one side of each tree were sampled, the branch was sampled first, and then the trunk was sampled in vertical alignment with the branch. This was done to sample as close as possible in line with the upward movement of water from the sampled trunk to the branches. However, no instrumentation was used to follow the vertical direction of transpiration; this vertical sequence was visually estimated. Therefore, it is possible that the water found in the leaf and twig samples passed through trunk xylem elements different from those sampled. This potential discrepancy might explain why PFAS was detected above the MRL in some leaves and branches but not in the trunk.

2.4.3 Other interesting findings

The data show for samples taken at the same height, different sides of the tree could contain markedly different PFAS levels. Such large discrepancies of organic contaminant concentrations within the same trunk height have been observed in previous studies. At a Superfund site in New Haven, Missouri, several trees contained PCE concentrations that differed around the base of the same trunk as much as fivefold (Schumacher et al., 2004). The primary difference in this example is that PCEs are known to diffuse out of the trunk, a phenomenon not observed—nor suspected—with nonvolatile PFAS. The observed difference in concentration between different sides of the same tree is likely due to variation of soil PFAS concentrations. Future research could use this study's dataset to explore potential correlations between soil and plant PFAS.

References

- 3M. 2000. Letter to US EPA Re: phase-out plan for POSF-based products.
- Ahrens, L., T. Harner, M. Shoeib, D.A. Lane, and J.G. Murphy. 2012. Improved Characterization of Gas–Particle Partitioning for Per- and Polyfluoroalkyl Substances in the Atmosphere Using Annular Diffusion Denuder Samplers. *Environ. Sci. Technol.* 46(13): 7199–7206. doi: 10.1021/es300898s.
- Anderson, R.H. 2021. The Case for Direct Measures of Soil-to-Groundwater Contaminant Mass Discharge at AFFF-Impacted Sites. *Environ. Sci. Technol.* 55(10): 6580–6583. doi: 10.1021/acs.est.1c01543.
- Backe, W.J., T.C. Day, and J.A. Field. 2013. Zwitterionic, Cationic, and Anionic Fluorinated Chemicals in Aqueous Film Forming Foam Formulations and Groundwater from U.S. Military Bases by Nonaqueous Large-Volume Injection HPLC-MS/MS. *Environ. Sci. Technol.* 47(10): 5226–5234. doi: 10.1021/es3034999.
- Balouet, J. 2015. Pollution investigation by trees.
- Bangma, J., J. McCord, N. Giffard, K. Buckman, J. Petali, et al. 2023. Analytical method interferences for perfluoropentanoic acid (PFPeA) and perfluorobutanoic acid (PFBA) in biological and environmental samples. *Chemosphere* 315: 137722. doi: 10.1016/j.chemosphere.2022.137722.
- Barry, V., A. Winqvist, and K. Steenland. 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. *Environ. Health Perspect.* 121(11–12): 1313–1318. doi: 10.1289/ehp.1306615.
- Blaine, A.C., C.D. Rich, E.M. Sedlacko, L.S. Hundal, K. Kumar, et al. 2014. Perfluoroalkyl Acid Distribution in Various Plant Compartments of Edible Crops Grown in Biosolids-Amended soils. *Environ. Sci. Technol.* 48(14): 7858–7865. doi: 10.1021/es500016s.
- Bolan, N., B. Sarkar, Y. Yan, Q. Li, H. Wijesekara, et al. 2021. Remediation of poly- and perfluoroalkyl substances (PFAS) contaminated soils – To mobilize or to immobilize or to degrade? *J. Hazard. Mater.* 401: 123892. doi: 10.1016/j.jhazmat.2020.123892.
- Brase, R.A., E.J. Mullin, and D.C. Spink. 2021. Legacy and Emerging Per- and Polyfluoroalkyl Substances: Analytical Techniques, Environmental Fate, and Health Effects. *International Journal of Molecular Sciences* 22(3): 995. doi: 10.3390/ijms22030995.
- Brennan, N.M., A.T. Evans, M.K. Fritz, S.A. Peak, and H.E. von Holst. 2021. Trends in the Regulation of Per- and Polyfluoroalkyl Substances (PFAS): A Scoping Review. *International Journal of Environmental Research and Public Health* 18(20): 10900. doi: 10.3390/ijerph182010900.

- Brusseau, M.L., R.H. Anderson, and B. Guo. 2020. PFAS Concentrations in Soils: Background Levels versus Contaminated Sites. *Sci. Total Environ.* 740: 140017. doi: 10.1016/j.scitotenv.2020.140017.
- Buck, R.C., J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, et al. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management* 7(4): 513–541. doi: 10.1002/ieam.258.
- Burken, J.G., D.A. Vroblesky, and J.C. Balouet. 2011. Phytoforensics, Dendrochemistry, and Phytoscreening: New Green Tools for Delineating Contaminants from Past and Present. *Environ. Sci. Technol.* 45(15): 6218–6226. doi: 10.1021/es2005286.
- Cantonati, M., L.E. Stevens, S. Segadelli, A.E. Springer, N. Goldscheider, et al. 2020. Ecohydrogeology: The interdisciplinary convergence needed to improve the study and stewardship of springs and other groundwater-dependent habitats, biota, and ecosystems. *Ecological Indicators* 110: 105803. doi: 10.1016/j.ecolind.2019.105803.
- Centers for Disease Control and Prevention. 2022. National Report on Human Exposure to Environmental Chemicals. https://www.cdc.gov/exposurereport/data_tables.html (accessed 21 March 2023).
- Che, S., B. Jin, Z. Liu, Y. Yu, J. Liu, et al. 2021. Structure-Specific Aerobic Defluorination of Short-Chain Fluorinated Carboxylic Acids by Activated Sludge Communities. *Environ. Sci. Technol. Lett.* 8(8): 668–674. doi: 10.1021/acs.estlett.1c00511.
- Collins, C., M. Fryer, and A. Grosso. 2006. Plant Uptake of Non-Ionic Organic Chemicals. *Environ. Sci. Technol.* 40(1): 45–52. doi: 10.1021/es0508166.
- Cousins, I.T., J.C. DeWitt, J. Glüge, G. Goldenman, D. Herzke, et al. 2020. The high persistence of PFAS is sufficient for their management as a chemical class. *Environ. Sci.: Processes Impacts* 22(12): 2307–2312. doi: 10.1039/D0EM00355G.
- Cousins, I.T., R. Vestergren, Z. Wang, M. Scheringer, and M.S. McLachlan. 2016. The precautionary principle and chemicals management: The example of perfluoroalkyl acids in groundwater. *Environment International* 94: 331–340. doi: 10.1016/j.envint.2016.04.044.
- Crawford, L., S.A. Halperin, M.W. Dzierlenga, B. Skidmore, M.W. Linakis, et al. 2023. Systematic review and meta-analysis of epidemiologic data on vaccine response in relation to exposure to five principal perfluoroalkyl substances. *Environment International* 172: 107734. doi: 10.1016/j.envint.2023.107734.
- Cui, J., P. Gao, and Y. Deng. 2020. Destruction of Per- and Polyfluoroalkyl Substances (PFAS) with Advanced Reduction Processes (ARPs): A Critical Review. *Environ. Sci. Technol.* 54(7): 3752–3766. doi: 10.1021/acs.est.9b05565.

- Darrow, L.A., C.R. Stein, and K. Steenland. 2013. Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. *Environ Health Perspect* 121(10): 1207–1213. doi: 10.1289/ehp.1206372.
- Dasu, K., X. Xia, D. Siriwardena, T.P. Klupinski, and B. Seay. 2022. Concentration profiles of per- and polyfluoroalkyl substances in major sources to the environment. *Journal of Environmental Management* 301: 113879. doi: 10.1016/j.jenvman.2021.113879.
- Davis, K.L., M.D. Aucoin, B.S. Larsen, M.A. Kaiser, and A.S. Hartten. 2007. Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility. *Chemosphere* 67(10): 2011–2019. doi: 10.1016/j.chemosphere.2006.11.049.
- Dietrich, P., and C. Leven. 2009. Direct push-technologies. In: Kirsch, R., editor, *Groundwater Geophysics: A Tool for Hydrogeology*. Springer, Berlin, Heidelberg. p. 347–366
- Dombrowski, P.M., P. Kakarla, W. Caldicott, Y. Chin, V. Sadeghi, et al. 2018. Technology review and evaluation of different chemical oxidation conditions on treatability of PFAS. *Remediation Journal* 28(2): 135–150. doi: 10.1002/rem.21555.
- Eriksson, U., P. Haglund, and A. Kärrman. 2017. Contribution of precursor compounds to the release of per- and polyfluoroalkyl substances (PFASs) from waste water treatment plants (WWTPs). *Journal of Environmental Sciences* 61: 80–90. doi: 10.1016/j.jes.2017.05.004.
- Faust, J.A. 2023. PFAS on atmospheric aerosol particles: a review. *Environ Sci Process Impacts* 25(2): 133–150. doi: 10.1039/d2em00002d.
- Fenton, S.E., A. Ducatman, A. Boobis, J.C. DeWitt, C. Lau, et al. 2021. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environmental Toxicology and Chemistry* 40(3): 606–630. doi: 10.1002/etc.4890.
- Furlow, B. 2024. US EPA sets historic new restrictions on toxic PFAS in drinking water. *The Lancet Oncology* 25(5): e181. doi: 10.1016/S1470-2045(24)00215-8.
- Giesy, J.P., and K. Kannan. 2001. Global Distribution of Perfluorooctane Sulfonate in Wildlife. *Environ. Sci. Technol.* 35(7): 1339–1342. doi: 10.1021/es001834k.
- Glüge, J., M. Scheringer, I.T. Cousins, J.C. DeWitt, G. Goldenman, et al. 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environ. Sci.: Processes Impacts* 22(12): 2345–2373. doi: 10.1039/D0EM00291G.
- Gobelius, L., J. Lewis, and L. Ahrens. 2017. Plant Uptake of Per- and Polyfluoroalkyl Substances at a Contaminated Fire Training Facility to Evaluate the Phytoremediation Potential of Various Plant Species. *Environ. Sci. Technol.* 51(21): 12602–12610. doi: 10.1021/acs.est.7b02926.

- Grandjean, P., E.W. Andersen, E. Budtz-Jørgensen, F. Nielsen, K. Mølbak, et al. 2012. Serum Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. *JAMA* 307(4). doi: 10.1001/jama.2011.2034.
- Grandjean, P., C. Heilmann, P. Weihe, F. Nielsen, U.B. Mogensen, et al. 2017. Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. *Environmental Health Perspectives* 125(7): 077018. doi: 10.1289/EHP275.
- Harris, J.T., G.D. de la Garza, A.M. Devlin, and A.J. McNeil. 2022. Rapid Removal of Poly- and Perfluoroalkyl Substances with Quaternized Wood Pulp. *ACS ES&T Water*. doi: 10.1021/acsestwater.1c00396.
- Hu, X.C., D.Q. Andrews, A.B. Lindstrom, T.A. Bruton, L.A. Schaider, et al. 2016. Detection of Poly- and Perfluoroalkyl Substances (PFASs) in U.S. Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants. *Environ. Sci. Technol. Lett.* 3(10): 344–350. doi: 10.1021/acs.estlett.6b00260.
- Huang, S., and P.R. Jaffé. 2019. Defluorination of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) by *Acidimicrobium* sp. Strain A6. *Environ. Sci. Technol.* 53(19): 11410–11419. doi: 10.1021/acs.est.9b04047.
- Hunter Anderson, R., D.T. Adamson, and H.F. Stroo. 2019. Partitioning of poly- and perfluoroalkyl substances from soil to groundwater within aqueous film-forming foam source zones. *Journal of Contaminant Hydrology* 220: 59–65. doi: 10.1016/j.jconhyd.2018.11.011.
- International Pollutants Elimination Network. 2019. PFAS Pollution Across the Middle East and Asia. International Pollutants Elimination Network, Stockholm, Sweden.
- Itoh, S., A. Araki, T. Mitsui, C. Miyashita, H. Goudarzi, et al. 2016. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. *Environ Int* 94: 51–59. doi: 10.1016/j.envint.2016.05.011.
- Jiao, X., Q. Shi, and J. Gan. 2021. Uptake, accumulation and metabolism of PFASs in plants and health perspectives: A critical review. *Crit. Rev. Environ. Sci. Technol.* 51(23): 2745–2776. doi: 10.1080/10643389.2020.1809219.
- Johnson, G.R., M.L. Brusseau, K.C. Carroll, G.R. Tick, and C.M. Duncan. 2022. Global distributions, source-type dependencies, and concentration ranges of per- and polyfluoroalkyl substances in groundwater. *Science of The Total Environment* 841: 156602. doi: 10.1016/j.scitotenv.2022.156602.
- Kempisty, D.M., Y. Xing, and L. Racz, editors. 2018. *Perfluoroalkyl Substances in the Environment: Theory, Practice, and Innovation*. CRC Press, Boca Raton.
- Kissa, E., and E. Kissa. 2001. *Fluorinated surfactants and repellents*. 2nd ed., rev.expanded. Marcel Dekker, New York.

- Kwok, K.Y., S. Taniyasu, L.W.Y. Yeung, M.B. Murphy, P.K.S. Lam, et al. 2010. Flux of Perfluorinated Chemicals through Wet Deposition in Japan, the United States, And Several Other Countries. *Environ. Sci. Technol.* 44(18): 7043–7049. doi: 10.1021/es101170c.
- Langenbach, B., and M. Wilson. 2021. Per- and Polyfluoroalkyl Substances (PFAS): Significance and Considerations within the Regulatory Framework of the USA. *International Journal of Environmental Research and Public Health* 18(21): 11142. doi: 10.3390/ijerph182111142.
- Lechner, M., and H. Knapp. 2011. Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (*Daucus carota* ssp. *Sativus*), Potatoes (*Solanum tuberosum*), and Cucumbers (*Cucumis Sativus*). *J. Agric. Food Chem.* 59(20): 11011–11018. doi: 10.1021/jf201355y.
- Leoncini, C., M. Filippini, J. Nascimbene, and A. Gargini. 2022. A quantitative review and meta-analysis on phytoscreening applied to aquifers contaminated by chlorinated ethenes. *Science of The Total Environment* 817: 153005. doi: 10.1016/j.scitotenv.2022.153005.
- Lesmeister, L., F.T. Lange, J. Breuer, A. Biegel-Engler, E. Giese, et al. 2021. Extending the knowledge about PFAS bioaccumulation factors for agricultural plants – A review. *Science of The Total Environment* 766: 142640. doi: 10.1016/j.scitotenv.2020.142640.
- Li, J., X. Li, Y. Da, J. Yu, B. Long, et al. 2022. Sustainable environmental remediation via biomimetic multifunctional lignocellulosic nano-framework. *Nat Commun* 13(1): 4368. doi: 10.1038/s41467-022-31881-5.
- Liao, Q., Y. Deng, X. Shi, Y. Sun, W. Duan, et al. 2018. Delineation of contaminant plume for an inorganic contaminated site using electrical resistivity tomography: comparison with direct-push technique. *Environ Monit Assess* 190(4): 187. doi: 10.1007/s10661-018-6560-3.
- Liu, Z., Y. Lu, Y. Shi, P. Wang, K. Jones, et al. 2017. Crop bioaccumulation and human exposure of perfluoroalkyl acids through multi-media transport from a mega fluorochemical industrial park, China. *Environment International* 106: 37–47. doi: 10.1016/j.envint.2017.05.014.
- Lopez-Espinosa, M.-J., D. Mondal, B. Armstrong, M.S. Bloom, and T. Fletcher. 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. *Environ Health Perspect* 120(7): 1036–1041. doi: 10.1289/ehp.1104370.
- Mei, W., H. Sun, M. Song, L. Jaing, Y. Li, et al. 2021. Per- and polyfluoroalkyl substances (PFASs) in the soil–plant system: Sorption, root uptake, and translocation. *Environment International* 156(2021). doi: 10.1016/j.envint.2021.106642.

- Murano, H., T. Otani, and N. Seike. 2010. Dieldrin-dissolving abilities of the xylem saps of several plant families, particularly *Cucurbita pepo* L. *Environmental Toxicology and Chemistry* 29(10): 2269–2277. doi: 10.1002/etc.288.
- Narizzano, A.M., E.M. Lent, J.M. Hanson, A.G. East, M.E. Bohannon, et al. 2022. Reproductive and developmental toxicity of perfluorooctane sulfonate (PFOS) in the white-footed mouse (*Peromyscus leucopus*). *Reproductive Toxicology* 113: 120–127. doi: 10.1016/j.reprotox.2022.08.011.
- National Academies of Sciences, Engineering, and Medicine. 2023. PFAS Source Differentiation Guide for Airports. The National Academies Press, Washington, D.C.
- Nguyen, T.M.H., J. Bräunig, K. Thompson, J. Thompson, S. Kabiri, et al. 2020. Influences of Chemical Properties, Soil Properties, and Solution pH on Soil–Water Partitioning Coefficients of Per- and Polyfluoroalkyl Substances (PFASs). *Environ. Sci. Technol.* 54(24): 15883–15892. doi: 10.1021/acs.est.0c05705.
- O’Hagan, D. 2008. Understanding organofluorine chemistry. An introduction to the C–F bond. *Chemical Society Reviews* 37(2): 308–319. doi: 10.1039/B711844A.
- Olsen, G.W., D.C. Mair, C.C. Lange, L.M. Harrington, T.R. Church, et al. 2017. Per- and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000–2015. *Environmental Research* 157: 87–95. doi: 10.1016/j.envres.2017.05.013.
- Paragot, N., J. Bečanová, P. Karásková, R. Prokeš, J. Klánová, et al. 2020. Multi-year atmospheric concentrations of per- and polyfluoroalkyl substances (PFASs) at a background site in central Europe. *Environmental Pollution* 265: 114851. doi: 10.1016/j.envpol.2020.114851.
- Pfotenhauer, D., E. Sellers, M. Olson, K. Praedel, and M. Shafer. 2022. PFAS concentrations and deposition in precipitation: An intensive 5-month study at National Atmospheric Deposition Program – National trends sites (NADP-NTN) across Wisconsin, USA. *Atmospheric Environment* 291: 119368. doi: 10.1016/j.atmosenv.2022.119368.
- Post, G.B., P.D. Cohn, and K.R. Cooper. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature. *Environmental Research* 116: 93–117. doi: 10.1016/j.envres.2012.03.007.
- Raffensperger, J., B. Fleming, W. Banks, M. Horn, M. Nardi, et al. 2010. Simulation of Groundwater Flow to Assess Future Withdrawals Associated with Base Realignment and Closure (BRAC) at Fort George G. Meade, Maryland.
- Richter, L., A. Cordner, and P. Brown. 2021. Producing Ignorance Through Regulatory Structure: The Case of Per- and Polyfluoroalkyl Substances (PFAS). *Sociological Perspectives* 64(4): 631–656. doi: 10.1177/0731121420964827.

- Rickard, B.P., I. Rizvi, and S.E. Fenton. 2022. Per- and poly-fluoroalkyl substances (PFAS) and female reproductive outcomes: PFAS elimination, endocrine-mediated effects, and disease. *Toxicology* 465: 153031. doi: 10.1016/j.tox.2021.153031.
- Sachi, T., Y. Nobuyoshi, H.-B. Moon, K.Y. Kwok, P.K.S. Lam, et al. 2013. Does wet precipitation represent local and regional atmospheric transportation by perfluorinated alkyl substances? *Environment International* 55: 25–32. doi: 10.1016/j.envint.2013.02.005.
- Schumacher, J.G., G.C. Struckhoff, and J.G. Burken. 2004. Assessment of Subsurface Chlorinated Solvent Contamination Using Tree Cores at the Front Street Site and a Former Dry Cleaning Facility at the Riverfront Superfund Site, New Haven, Missouri, 1999–2003.
- Sharifan, H., M. Bagheri, D. Wang, J.G. Burken, C.P. Higgins, et al. 2021. Fate and transport of per- and polyfluoroalkyl substances (PFASs) in the vadose zone. *Science of The Total Environment* 771: 145427. doi: 10.1016/j.scitotenv.2021.145427.
- Shimizu, M.S., R. Mott, A. Potter, J. Zhou, K. Baumann, et al. 2021. Atmospheric Deposition and Annual Flux of Legacy Perfluoroalkyl Substances and Replacement Perfluoroalkyl Ether Carboxylic Acids in Wilmington, NC, USA. *Environ. Sci. Technol. Lett.* 8(5): 366–372. doi: 10.1021/acs.estlett.1c00251.
- Shoari, N., and J.-S. Dubé. 2018. Toward improved analysis of concentration data: Embracing nondetects. *Environmental Toxicology and Chemistry* 37(3): 643–656. doi: 10.1002/etc.4046.
- Sorek, A., N. Atzmon, O. Dahan, Z. Gerstl, L. Kushisin, et al. 2008. “Phytoscreening”: The Use of Trees for Discovering Subsurface Contamination by VOCs. *Environ. Sci. Technol.* 42(2): 536–542. doi: 10.1021/es072014b.
- Steenland, K., L. Zhao, A. Winquist, and C. Parks. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. *Environ Health Perspect* 121(8): 900–905. doi: 10.1289/ehp.1206449.
- Sunderland, E.M., X.C. Hu, C. Dassuncao, A.K. Tokranov, C.C. Wagner, et al. 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. *J Expo Sci Environ Epidemiol* 29(2): 131–147. doi: 10.1038/s41370-018-0094-1.
- Taniyasu, S., N. Yamashita, E. Yamazaki, G. Petrick, and K. Kannan. 2013. The environmental photolysis of perfluorooctanesulfonate, perfluorooctanoate, and related fluorochemicals. *Chemosphere* 90(5): 1686–1692. doi: 10.1016/j.chemosphere.2012.09.065.
- Thalheimer, A.H., L.B. McConney, I.K. Kalinovich, A.V. Pigott, J.D. Franz, et al. 2017. Use and Potential Impacts of AFFF Containing PFASs at Airports. Transportation Research Board, Washington, D.C.

- Thomas, T. n.d. Firefighting Foam. <https://pfas-1.itrcweb.org/3-firefighting-foams/> (accessed 5 February 2025).
- U.S. Department of Defense. 2022a. Final Fourth Five-Year Review Report for Tipton Airfield Parcel.
- U.S. Department of Defense. 2022b. Final Preliminary Assessment and Site Inspection for Fort George G. Meade and Phoenix Military Reservation, Maryland.
- U.S. Department of the Army. 2020. Ft Meade 2020 Final Site Management Plan.
- U.S. Environmental Protection Agency. 2021. PFAS Master List of PFAS Substances. <https://comptox.epa.gov/dashboard/chemical-lists/pfasmaster> (accessed 22 April 2024).
- U.S. Environmental Protection Agency. 2022a. Drinking Water Health Advisories for PFAS Fact Sheet for Communities. <https://www.epa.gov/system/files/documents/2022-06/drinking-water-ha-pfas-factsheet-communities.pdf> (accessed 23 April 2023).
- U.S. Environmental Protection Agency. 2022b. 3rd Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.
- U.S. Environmental Protection Agency and Office of Chemical Safety and Pollution Prevention. 2016. Fact Sheet: 2010/2015 PFOA Stewardship Program. <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program> (accessed 10 April 2023).
- U.S. Fish and Wildlife Service. 2022. Contaminant Assessment Process Report for Patuxent Research Refuge - 2021.
- Vaida, F., and L. Liu. 2012. Imec: Linear mixed-effects models with censored responses. R package version 1.
- Vierke, L., U. Berger, and I.T. Cousins. 2013. Estimation of the Acid Dissociation Constant of Perfluoroalkyl Carboxylic Acids through an Experimental Investigation of their Water-to-Air Transport. *Environ. Sci. Technol.* 47(19): 11032–11039. doi: 10.1021/es402691z.
- Vroblesky, D.A., C.T. Nietch, and J.T. Morris. 1999. Chlorinated Ethenes from Groundwater in Tree Trunks. *Environ. Sci. Technol.* 33(3): 510–515. doi: 10.1021/es980848b.
- Wang, Z., J.C. DeWitt, C.P. Higgins, and I.T. Cousins. 2017. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environ. Sci. Technol.* 51(5): 2508–2518. doi: 10.1021/acs.est.6b04806.
- Washington, J.W., and T.M. Jenkins. 2015. Abiotic Hydrolysis of Fluorotelomer-Based Polymers as a Source of Perfluorocarboxylates at the Global Scale. *Environ. Sci. Technol.* 49(24): 14129–14135. doi: 10.1021/acs.est.5b03686.

- Wen, B., Y. Pan, X. Shi, H. Zhang, X. Hu, et al. 2018. Behavior of N-ethyl perfluorooctane sulfonamido acetic acid (N-EtFOSAA) in biosolids amended soil-plant microcosms of seven plant species: Accumulation and degradation. *Science of The Total Environment* 642: 366–373. doi: 10.1016/j.scitotenv.2018.06.073.
- Würth, A., M. Mechler, K. Menberg, M.A. Ikipinar, P. Martus, et al. 2023. Phytoscreening for Per- and Polyfluoroalkyl Substances at a Contaminated Site in Germany. *Environ. Sci. Technol.* 57(10): 4122–4132. doi: 10.1021/acs.est.2c04519.
- Yan, P.-F., S. Dong, K.D. Pennell, and N.L. Cápiro. 2024. A review of the occurrence and microbial transformation of per- and polyfluoroalkyl substances (PFAS) in aqueous film-forming foam (AFFF)-impacted environments. *Science of The Total Environment* 927: 171883. doi: 10.1016/j.scitotenv.2024.171883.
- Yang Song, Astha Tuladhar, and Naosuke Nii. 2011. Anatomical Development of Cell Structure Including Casparian Strip during Root Growth in Grapevines. *Journal of the Japanese Society for Horticultural Science* 80(2): 164–168. doi: 10.2503/jjshs1.80.164.
- Yao, Y., S. Chang, H. Sun, Z. Gan, H. Hu, et al. 2016. Neutral and ionic per- and polyfluoroalkyl substances (PFASs) in atmospheric and dry deposition samples over a source region (Tianjin, China). *Environmental Pollution* 212: 449–456. doi: 10.1016/j.envpol.2016.02.023.
- Yeung, L.W.Y., Y. Miyake, S. Taniyasu, Y. Wang, H. Yu, et al. 2008. Perfluorinated Compounds and Total and Extractable Organic Fluorine in Human Blood Samples from China. *Environ. Sci. Technol.* 42(21): 8140–8145. doi: 10.1021/es800631n.
- Zhi, Y., H. Lu, K.D. Grieger, G. Munoz, W. Li, et al. 2022. Bioaccumulation and Translocation of 6:2 Fluorotelomer Sulfonate, GenX, and Perfluoroalkyl Acids by Urban Spontaneous Plants. *ACS EST Eng.* 2(7): 1169–1178. doi: 10.1021/acsestengg.1c00423.
- Zhou, J., M. Li, J. Li, Z. Shao, Y. Liu, et al. 2020. Bioavailability and Bioaccumulation of 6:2 Fluorotelomer Sulfonate, 6:2 Chlorinated Polyfluoroalkyl Ether Sulfonates, and Perfluorophosphinates in a Soil–Plant System. *J. Agric. Food Chem.* 68(15): 4325–4334. doi: 10.1021/acs.jafc.0c00542.