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Accumulation of six PFAS compounds by woody and herbaceous plants: potential for phytoextraction

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ABSTRACT
Per and polyfluoroalkyl substances (PFAS) consist of a large group of compounds used to make products more resistant to stains, grease, and water and for fire suppression. They have been widely detected in the environment and exposure has been linked to adverse human health effects. Phytoremediation could be used to remediate PFAS-impacted sites, but there is little information on herbaceous and woody plant species uptake of PFAS compounds from soil. A greenhouse study evaluated the potential for eight herbaceous and seven woody plant species to absorb PFAS compounds. Six PFAS compounds: PFPeA, PFHxA, PFOA, PFBS, PFHxS, and PFOS were added weekly to irrigation water, and the plants grown for up to 14 weeks after an initial establishment period. Significant accumulation of all PFAS compounds occurred in at least one plant species. Mass recovery in above-ground tissue by the best performing plant ranged from a low of 3.8% for PFOS by *Festuca rubra* to a high of 42% for PFPeA by *Schedonorus arundinaceus*. Hyperaccumulation, defined as tissue/soil concentrations >10/1, was observed for all six PFAS compounds in at least one plant species. These results demonstrate the potential use of phytoremediation as a tool for remediating PFAS-contaminated sites.

KEYWORDS
Bioaccumulation factor; perfluoroalkyl; PFOS; phytoextraction; polyfluoroalkyl; uptake

Introduction

Per and polyfluoroalkyl substances (PFAS) represent a large group of synthetic compounds that have been used since the 1940s in various products to improve resistance to stains, grease, and water. Examples of products that contain PFAS include nonstick cookware, stain-resistant textiles, waterproof clothing, food packaging (Posner 2012; Kotthoff *et al.* 2015) as well as aqueous foam-forming foam (AFFF) used for fire suppression and fire training (Moody and Field 2000). Due to their ability to reduce friction, PFAS are also used in a variety of industries, including aerospace, automotive, building and construction, and electronics (Kissa 2001; Buck *et al.* 2011). The carbon-fluorine bond in PFAS compounds is not easily broken and PFAS compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) break down slowly in the environment and are characterized as persistent (Li *et al.* 2019). The persistence and extensive use of PFAS compounds in commercial products have resulted in widespread human exposure to PFAS as indicated by median PFOS and PFOA serum levels of 5.2 and 2.3 ng/mL, respectively (NHANES 2016). There is mounting evidence that exposure to some PFAS compounds leads to adverse health effects, including cancer, ulcerative colitis, and hypothyroidism (Lau *et al.* 2007; Ballesteros *et al.* 2017; Herrick *et al.* 2017; Steenland *et al.* 2018). Six PFAS are included in the United States Environmental Protection Agency (U.S. EPA) Unmonitored Contaminant Rule 3 List: (perfluoro-octanoic acid (PFOA), perfluoro-heptanoic acid (PFHpA), perfluoro-nonanoic acid (PFNA)) and three sulfonates (perfluoro-butane sulfonate (PFBS), perfluoro-hexane sulfonate (PFHxS), perfluoro-octane sulfonate (PFOS)), and the EPA recently established a health advisory level for total PFOS and PFOA of 0.07 ng/L. (EPA 2016).

At many industrial sites, PFAS-impacted groundwater is managed using conventional "pump and treat" remediation approaches that rely on extraction and above-ground treatment with granular activated carbon (GAC) or anion exchange resin (Espana *et al.* 2015; McNamara *et al.* 2018; Yu *et al.* 2009). Destruction methods, such as chemical oxidation, have been effective for PFOA treatment (Mitchell *et al.* 2014; Bruton and Sedlak 2017) but have shown minimal applicability for PFOS (Park *et al.* 2016). Biodegradation of PFAS precursors such as 6:2 and 8:2 fluorotelomer alcohols (FTOH) has been documented by several research groups (Liu *et al.* 2010; Royer *et al.* 2015) and recently, the transformation of PFOA and PFOS by *Acidimicrobium* sp. Strain A6 was reported, but the reaction rates are relatively slow (Huang and Jaffé 2019). Thus, there is an urgent need to develop viable remediation options for PFAS-impacted soils and aquifer formations.

On sites with shallow groundwater or soil contamination, uptake and transport of contaminant compounds into

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Supplemental data for this article can be accessed here.

Table 1. Herbaceous and woody plant species selected for greenhouse evaluation.

Scientific Name	Common Name
Herbaceous	
Amaranthus tricolor	Amaranth
Brassica juncea	Mustard
Cynodon dactylon	Bermudagrass
Esquisetum hyemale	Horsetail
Helianthus annus	Sunflower
Schedonorus arundinaceus	Tall fescue
Festuca rubra	Red fescue
Trifolium incarnatum	Crimson clover
Woody	
Betula nigra	River birch
Fraxinus pennsylvanica	Green ash
Liquidambar styraciflua	Sweetgum
Liriodendron tulipifera	Tulip poplar
Platanus occidentalis	Sycamore
Pinus taeda	Loblolly pine
Salix nigra	Black willow

above-ground portions of plants, where accumulated compounds can be harvested and treated, can be part of a viable remediation approach. This approach has been used for a variety of contaminants in many projects including remediation of sites contaminated with metals such as As, Cr, Cd Cu, Mn or Zn (Robinson and McIvor 2013; Kaur *et al.* 2018), some explosives (McCutcheon and Schnoor 2003; Rajaei and Seyedi 2018) and chlorinated solvents (Spriggs *et al.* 2003; Huang *et al.* 2014). Multiple studies have shown that a variety of agricultural crop plants accumulate PFAS compounds in both root and above-ground tissue (Navarro *et al.* 2017; Ghisi *et al.* 2019) and accumulation depends on a variety of factors including plant species (Navarro *et al.* 2017; Ghisi *et al.* 2019), PFAS group and chain length (Blaine *et al.* 2014; Ghisi *et al.* 2019), water or soil concentration (Blaine *et al.* 2014; Ghisi *et al.* 2019), the organic carbon content of the soil (Blaine *et al.* 2014), salinity and pH (Zhao *et al.* 2013). Organic carbon content, salinity, and pH all affect uptake through their effect on sorption/desorption from soil surfaces and the availability for uptake.

In agricultural crops, uptake and accumulation of PFAS in plant tissues presents a potential route of animal and human exposure. Plant uptake, however, also provides a potential opportunity for phytoremediation of PFAS-contaminated sites as part of an overall remediation strategy. For non-crop plants, Zhang *et al.* (2019) investigated the uptake and accumulation of seven PFAS compounds by the wetland species *Juncus effuses* and reported removal efficiencies from solution as high as 11.4% (mass basis) for spiked PFAS, but reported minimal translocation to above-ground components of the plant. Gobelius *et al.* (2017) measured the accumulation of 26 PFAS compounds in plants growing on a PFAS-contaminated fire training site near Stockholm, Sweden. Total PFAS concentrations in soil and groundwater of this site ranged from 16 to 160 ng/g dry weight (dw) and 1200–34,000 ng/L, respectively. Samples from different species and tissues of the local plant community were collected and analyzed. Plant tissue PFAS concentrations varied widely among plant species with the highest total PFAS concentrations in vegetative compartments. Up to 97 ng/g wet weight (ww) was found in *Betula pendula* leaves and 94 ng/g ww in *Picea abies* needles. Annual ground cover plants such as *Phegopteris connectilis* and *Aegopodium podagraria* and bushes like *Prunus padus* exhibited total PFAS concentrations of up to 6.9, 23, and 21 ng/g ww, respectively. The bioconcentration factors (BCFs; plant/soil ratios) were highest in foliage. A total whole-plant accumulation of up to 11 mg for *Betula* and 1.8 mg for *Picea* were observed (Gobelius *et al.* 2017).

Phytoremediation, particularly phytoaccumulation, provides a possible alternative to excavation and removal of PFAS-impacted soils, particularly for sites where contamination exists in near-surface soil or in shallow groundwater. However, there is limited information on the potential of various non-crop plants to absorb and translocate PFAS compounds into above-ground portions of the plant, or which plant species are best suited for use in phytoremediation of specific compounds. Thus, the objectives of this study were to:

1. Identify woody and herbaceous plant species that have the greatest potential for use in phytoremediation through phytoaccumulation of PFAS compounds
2. Determine which PFAS compounds are most likely to be effectively remediated by phytoaccumulation.

A greenhouse study was conducted to evaluate the potential for eight herbaceous and seven woody plant species to absorb PFAS compounds. Six PFAS compounds: PFPeA, PFHxA, PFOA, PFBS, PFHxS, and PFOS were added weekly to irrigation water, and the plants are grown for up to 14 weeks after an initial establishment period. Accumulation of all PFAS compounds was measured in samples of the plant tissue to evaluate the potential use of phytoremediation as a tool for remediating PFAS-contaminated sites.

Materials and methods

Plant species selection and greenhouse conditions

Eight herbaceous plant species and seven woody species (Table 1) were selected for testing in a greenhouse study designed to assess PFAS phytoaccumulation. These species were selected for evaluation based upon their prior successful use for phytoextraction of other contaminants and their occurrence on sites that are known to be PFAS contaminated. Seedlings of these plant species were planted in columns containing washed sand. Seeds of herbaceous species were purchased from commercial sources germinated and first propagated in shallow trays of potting soil before being transplanted into the columns. *Amaranthus tricolor*, *Brassica juncea*, *Helianthus annuus*, and *Trifolium incarnatum* seeds were obtained from Johnny's Selected Seeds (Winslow, ME). *Cynodon dactylon*, *Schedonorus arundinaceus*, and *Festuca rubra* seeds were obtained from Athens Seed Company (Watkinsville, GA). *Esquisetum hyemale* rootstock was obtained from Tennessee Wholesale Nursery (Altamont, TN).

Woody species were purchased from commercial nurseries as one-year-old bare-root seedlings. *Liriodendron*

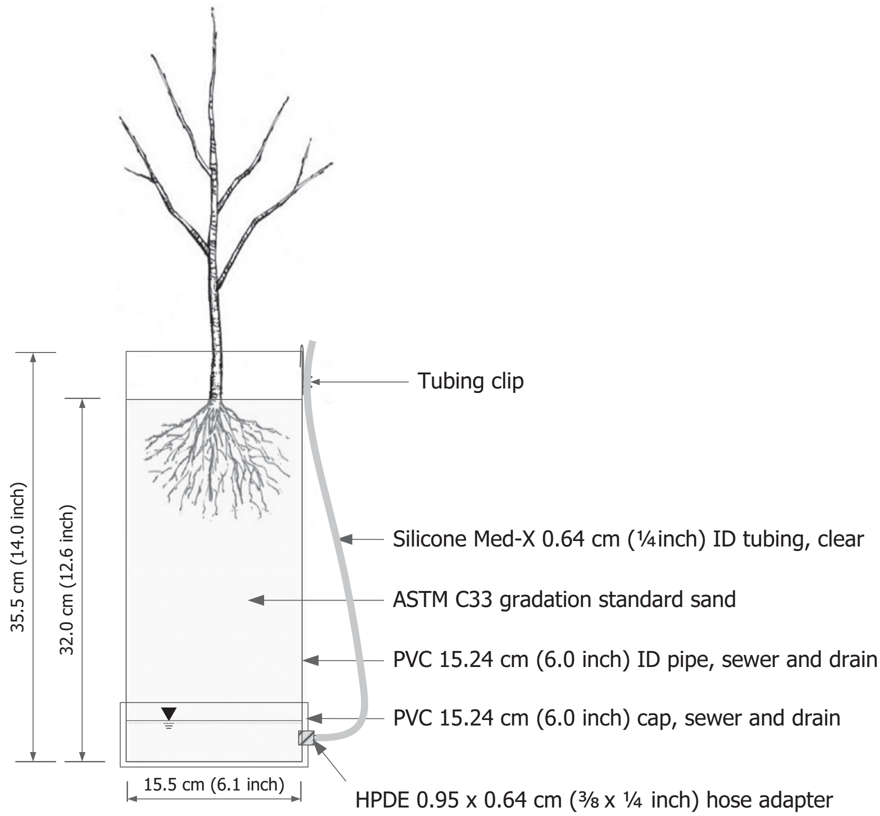

Figure 1. Schematic of a PVC column (growth chamber) used in greenhouse study of PFAS uptake by herbaceous and woody species.

tulipfera was purchased from Angel Creek Nursery (Bishop, GA) and *Salix nigra* was purchased from Tennessee Wholesale Nursery (Altamont, TN). Other tree species were purchased from ArborGen (Bellville, GA). These seedlings were planted directly into 15.2 cm diameter × 32 cm length polyvinylchloride (PVC) columns similar to those of Barcellos *et al.* (2016). Each PVC column was capped at the bottom and linked by an outlet valve for the collection of leachate and control of water levels within the columns. The outlet valve tubes were made of clear silicone tubing so water levels in the columns could be monitored via the fluid levels (Figure 1). Columns were filled with sand meeting ASTM C33 gradation standard with low CEC and minimal sorption capacity (Table 2). A nominal volume of 6 L of sand, which equates to 9 kg at an approximate bulk density of 1.5 g/cm^3 was added to each column. The study was located in a secured greenhouse that was temperature-controlled at 25 ± 3 °C and with a relative humidity target range of 70 ± 5%. Supplemental lighting was used to extend day length to 16 h during the autumn and winter experimental periods. Pests were controlled via biweekly applications of beneficial insects obtained from Evergreen Growers Supply, LLC (Clackamas, OR). The insects included *Chrysoperia* (lacewing) larvae and two spider mites: *Fallacis neoseiulus* and *Phytoseiulus persimilis*. These predatory larvae and mites were applied for the control of aphids and whiteflies.

Plants were grown for a 14 to 18-week establishment period during which time they were fertilized weekly with a complete medium solution that supplied plant-available N, P, K, Ca, Mg, S, B, Fe, Mn, Zn, Cu, and Mo in constituent salts of a Hoagland's solution (Hoagland and Arnon 1950). The solution was applied at an application rate of 100 mL of a solution prepared as 1.6 grams of Hoagland's solid media per liter of water. The solution was also applied to the no-plant control columns. Weekly fertilizer applications continued throughout the entirety of the study.

Table 2. Agricultural soil test data for the washed sand growth media prior to treatment application.

pH	Base saturation	CEC	Ca	Fe	K	Mg	Mn	Na	Ni	P	Zn	EC
S.U.	%	meq/100g			Mehlich 1 mg/kg (ppm)							µS/cm
5.91	92.8	0.30	37.7	52.2	6.14	7.15	19.2	3.5	0.05	1.61	1.07	50

S.U.: standard units; meq/100g: milliequivalents per 100 grams; mg/Kg: milligrams per kilogram; ppm: parts per million; µS/cm: micro siemens per centimeter.

Experimental design

Experimental units (columns) were allocated in a randomized block design within three replicate blocks. Blocks were physically located to distribute the treatments and replications over the greenhouse microenvironmental conditions. Randomization was constrained so that the tree species and herbaceous species were separately randomized to minimize the canopy interference of taller trees species on lower growing grasses and forbs. Treatments were a combination of 15 plant species with and without PFAS compound addition. Two no-plant, soil mix-only columns were included in each block. Additionally, four plant species exhibiting moderate

			Block 1								Block 2								Block 3				
Bn	Ls	Ls+	Pt+	Ls+S	Fp+	Pt	Po+	Sn	Fp	Ls+S	Po+	Bn	Fp+	Ls+	Sn+S	Bn+	Pt	Fp+	Sn	Ls+	Sn+S	Ls+S	Lt
Sn+S	Lt+	Sn+	Fp	Bn+	Lt	Po	Sn	Pt+	Lt+	Sn+	Pt	Ls	Lt	Po	Bn+	Pt+	Po	Sn+	Po+	Lt+	Bn	Fp	Ls
Ti+	C+	Ha	Fr+S	Fr	At+	Bj+	Cd+	C+	Ha+	Bj	Bj+	Fr	Ti	Fr	C	Cd	Eh	At	Ti				
Sa+	Cd+	Bj	Ti	Fr+	At	Eh	Cd+S	Fr+S	Sa	Sa+	Cd	Eh+	Eh+	Sa	C+	Bj+	Fr+	Cd+	Sa+				
C	Cd	Ha+	Cd+S	Sa	Eh	Ti	Eh	At	At+	Fr+	C	Ha	Ti+	At+	Bj	Ha+	Fr+S	Ha	Cd+S				

Air Flow →

Greenhouse Table

Herbaceous Species

C = *No plant control*
At = *Amaranthus tricolor*
Bj = *Brassica juncea*
Cd = *Cynodon dactylon*
Eh = *Esquisetum hyemale*
Sa = *Schedonorus arundinaceus*
Fr = *Festuca rubra*
Ha = *Helianthus annuus*
Ti = *Trifolium incarnatum*

Woody Species

C = *No plant control*
Bn = *Betula nigra*
Fp = *Fraxinus pennsylvanica*
Ls = *Liquidambar styraciflua*
Lt = *Liriodendron tulipifera*
Po = *Platanus occidentalis*
Pt = *Pinus taeda*
Sn = *Salix nigra*

Treatments

Xx No Treatment
Xx+ + PFAS Treatment
Xx+S + PFAS Treatment + Saline

Figure 2. Greenhouse blocking of PFAS phytoremediation study of eight herbaceous and seven woody species.

salt tolerance (*Salix nigra*, *Liquidambar styraciflua*, *Festuca rubra*, *Cynodon dactylon*) were treated in separate additional experimental units that utilized a saline irrigation solution. The saline irrigation solution contained 2.5 g/L gypsum ($CaSO_4{\cdot}2H_2O$) and 5 g/L of Epsom salt ($MgSO_4{\cdot}2H_2O$) mixed with deionized water. This water quality produced an electrical conductivity of approximately 4.5 dS cm^1. Thus, a total number of 36 experimental units (columns) comprised each block (Figure 2).

Contaminant dosing

Six PFAS compounds were chosen for testing. PFOS and PFOA (both 8-chain carbon PFAS compounds) were chosen because EPA has initiated steps to evaluate the need for maximum contaminant levels for these compounds and is beginning the necessary steps to propose designating PFOA and PFOS as "hazardous substances," per its February 2019 PFAS Action Plan. In addition, as part of the PFAS Action Plan, EPA is developing toxicity values for PFBS (4-chain). The other three PFAS compounds were chosen to provide a better spectrum of plant uptake rates along intermediate carbon chain lengths (e.g., PFHxA and PFHxS are 6-chain, PFPeA is 5-chain). The six PFAS compounds used for the contaminant dosing solution were sourced from Sigma-Aldrich (St. Louis, MO) with a minimum purity as follows:

Perfluorohexane sulfonic acid (PFHxS), 98%
Perfluorooctane sulfonic acid (PFOS), 98%
Perfluorooctanoic acid (PFOA), 96%
Perfluoropentanoic acid (PFPeA), 97%
Perfluorohexanoic acid (PFHxA), 97%
Perfluorobutane sulfonic acid (PFBS), 98%

A seventh compound, n-methyl perfluorooctane sulfonamide (MeFOSA), was included in the dosing solution but was only analyzed as detect or non-detect in later analyses. The contaminant dosing solution was an aqueous mix with nominal concentrations of 1 mg/L of each compound. To

Table 3. PFAS concentrations of dosing solution as determined by laboratory analysis.

PFAS compound	Analyte concentration (ng/L)	Mass per dose (µg)
PFPeA	1,600,000	160
PFHxA	2,100,000	210
PFOA	940,000	94
PFBS	920,000	92
PFHxS	890,000	89
PFOS	850,000	85
MeFOSA	Presence	

determine actual concentrations, a prepared contaminant solution was sampled using laboratory provided water sampling vials, and the sample shipped via overnight courier to Eurofins TestAmerica laboratory (West Sacramento, CA). The laboratory reported concentrations of the analytes are presented in Table 3. We used relatively high concentrations for dosing in this research due to the short-term nature of plant exposure. The selected nominal concentration of 1 mg/L was based on PFOS concentrations observed on contaminated sites. It was within the range of concentrations observed in soil that reached 9.7 g/Kg and surface water that reached 9.0 mg/L on a military site contaminated by the use of fire retardants (Anderson *et al.* 2016) and would result in soil concentrations after dosing near the midpoint of the range (5 to 290 µg/Kg) reported for a Georgia site receiving wastewater that had historically included PFAS compounds (US EPA 2010).

The contaminant and salinity dosing of the plants began once the plant species exhibited healthy growth following the 14 to 18-week establishment period. The dosing of the herbaceous species began on March 8, 2019. Tree species were first dosed on March 14, 2019. Contaminant solution treatments were applied weekly in 100 mL doses to the surface of each column using a syringe to distribute the solution evenly over the soil surface. When the columns contained too much water for healthy plant growth, the leachate was collected and reapplied after evapotranspiration made the application feasible. Nitrile gloves were worn during sampling.

Saline treatment columns were irrigated with 100 mL increments of saline irrigation water per week or greater if water levels in the columns allowed. Thus, the saline plots experienced a gradual increase in the salinity level during the course of the study.

Tissue sampling and analyses

An initial, partial round of plant tissue sampling took place after six doses had been applied to the herbaceous species and five doses had been applied to the tree species. Tissue samples were collected using clippers that were decontaminated between each sample using the following process: plant matter was wiped from clippers, clippers were washed in a Liquinox® and deionized water solution, clippers were rinsed with deionized water, clippers were rinsed in isopropyl alcohol and rinsed again in deionized water. Samples were placed in plastic bags and immediately placed on ice in a cooler. Prior to shipping, the samples were re-packed on fresh ice and shipped via overnight courier with chain-of-custody documentation.

The following six species were sampled during the initial sampling event: *Cynodon dactylon* (bermudagrass), *Festuca rubra* (red fescue), *Schedonorus arundinaceus* (tall fescue), *Trifolium incarnatum* (crimson clover), *Salix nigra* (black willow) and *Liquidambar styraciflua* (sweetgum). At the time of the initial sampling, the *Brassica juncea* (mustard) and *Helianthus annuus* (sunflower) plants had reached physiological maturity and the entire above-ground plants were harvested and stored in a laboratory freezer at −4 °C for subsequent analyses. Similarly, on May 14 *Amaranthus tricolor* was harvested and the samples frozen for later analyses as it had reached physiological maturity. Although harvested sooner, at the time of harvest, *Amaranthus* had been dosed twelve times.

The final vegetation sampling event was conducted from June 6 through June 14, 2019. At the time of the final sampling, a total of twelve contaminant doses had been applied to the remaining herbaceous species and a total of eleven doses had been applied to the tree species.

For the tree species, samples of the leaves and petioles were collected separately from woody samples of the main stem and branches. *Trifolium incarnatum* (crimson clover) was only sampled during the initial sampling event as the plants underwent senesce prior to the final sampling. In addition, the *Fraxinus pennsylvanica* (green ash) grew poorly and only three of the six plots generated adequate plant material for the collection of leaf/petiole samples.

Consistent with the initial sampling, plant tissue samples were shipped on ice via overnight delivery for analysis. This final sampling included the previously harvested *Brassica juncea*, *Heliantnhus annuus*, and *Amaranthus tricolor*. The remaining above-ground portions of the plants were harvested and dried in a forced-air oven at 60 °C for use in dry mass determination. For trees, leaf and petioles were separated from branches and stems and dried separately. Tissue samples were extracted using the procedure of Yoo *et al.* (2011).

Samples extraction and analysis

Approximately 1 g of each dried and homogenized plant sample was transferred to a 15 mL polypropylene centrifuge tube (Thermo Scientific, Waltham, MA), amended with 50 µL of a 100 ng/mL isotopically-labeled standards ($^{13}C_3$-PFBS, $^{13}C_5$-PFHxA, $^{13}C_3$-PFHxS, $^{13}C_8$-PFOA, $^{13}C_2$-PFOA, $^{13}C_8$-PFOS, $^{13}C_4$-PFOS, $^{13}C_5$-PFPeA) from Wellington Laboratories (MPFAC-C-ES, Park, Kansas) to estimate the recovery of PFAS during the extraction procedure. The extraction procedure was based on the method of Rankin *et al.* (2016). The procedure involved adding 0.4 mL of 2 M sodium hydroxide and then 8.5 mL of a 90:10 mixture of acetonitrile and ultrapure water, sonicating for 30 min in a water bath at 25 °C followed by end-over-end mixing for 1 h. The tubes were centrifuged at 5000 rpm for 5 min and the supernatant was transferred to 20 mL glass vials with polypropylene lines caps (DWK Life Sciences, Millville, NJ). The extraction was repeated, and the fluids combined into the 20 mL glass vial. The extract was evaporated to dryness using a Biotage TurboVap LV (Charlotte, NC) supplied with lab air.

Recovering the PFAS from each extract was accomplished by adding 4 mL of a tetrabutylammonium bisulfate (86868-100 G, Sigma-Aldrich, Saint Louis, MO) and sodium carbonate (223484-500 G, Sigma-Aldrich, Saint Louis, MO) mixture in ultrapure water to each 20 mL glass vial followed by 5 mL of methyl tert-butyl ether (MTBE). The vials were capped, vortexed for 5 sec and placed in a −80 °C freezer for 30 min to freeze the water fraction. The MTBE was poured off the frozen portion and into a separate 20 mL glass vial. The MTBE recovery step was repeated, and the combined MTBE was evaporated in the Biotage TurboVap LV. Each vial was amended with 1 mL of a 60:40 acetonitrile to ultrapure water solution that contained isotopically labeled internal standards from Wellington Laboratories (MPFAC-C-IS, Park, Kansas) to evaluate any matrix interference effects. A 1 mL sample was collected from each vial and passed through a polyvinylidene difluoride (PVDF) 0.2 µm syringe filter that had been pre-rinsed with methanol into a 2 mL autosampler vial.

Extraction recoveries from test tube samples without plant material ranged from 68% for PFBS to 96% for PFHxS. Extraction recoveries from samples containing plant tissue were more variable but typically ranged from 40 to 80%, but with several recoveries as low as 10% or as high as 300%. However, the concentrations reported herein were corrected for the extraction or internal standard recoveries because the concentrations of PFAS in the plant samples were many times in excess of the mass of the isotopically labeled compounds introduced into each sample to estimate extraction efficiencies. Tissue concentration and recovery data for each species and tissue type are provided in the supporting information.

The concentration of PFAS in each sample extract was determined using a Waters Acquity H-Class ultra-performance liquid chromatograph (UPLC) equipped with a Waters BEH C-18 column and a PFC column kit to eliminate contamination. The eluent gradient consisted of ammonium acetate in water or methanol connected to a Waters Xevo TQ-S micro mass spectrometer (MS/MS). The Waters Xevo TQ-S micro was operated in negative electrospray ionization using multiple reaction monitoring (MRM) mode tuned to a unit mass resolution to isolate precursor and product ions for quantitation (Table 1 Supplementary material). Mobile phases were prepared from LC-MS grade water, methanol, and ammonium acetate that were purchased from Honeywell Burdick & Jackson (Muskegon, MI). Instrument calibration in the μg/L concentration range was performed using certified calibration standards in the range from 5 to 500 ng/mL from Waters Corp. (Cat# 186004624, Milford, MA).

Calculation of bioconcentration factors (BCF)

The Bioconcentration Factor (BCF) is a key metric that can be used to assess the fitness of plant species to serve in a phytoremediation program through phytoextraction. Although originally applied to metal accumulation (Brooks *et al.* 1977), it has been expanded to include accumulation of other contaminants (McCutcheon and Schnoor 2003). There are several approaches for defining bioconcentration and identifying plants with potential for phytoremediation. Brooks *et al.* (1977) defined the term hyperaccumulator for plants that accumulate tissue concentrations of a contaminant that are two orders of magnitude or greater than tissue concentrations of plants that exclude the contaminant (excluder plants). They calculated the BCF as follows:

$$BCF_{plant} = C_{accumulator}/C_{excluder}$$

An alternative definition of a hyperaccumulator is based on the ratio of contaminant concentration in plant tissue to the contaminant concentration in the soil:

$$BCF_{soil} = C_{plant}/C_{soil}$$

A BCF >1 is considered to indicate accumulation. Although the definition is somewhat arbitrary, a BCF >10 can be considered a hyperaccumulator that may be particularly valuable for phytoremediation.

We calculated BCFs for each contaminant for all the species using both definitions. For calculating BCF_{plant}, we used *Pinus taeda* foliage concentrations grown in the + PFAS treatment to represent an excluding plant and compared final sampling tissue concentrations of other species to it. For calculating BCF_{soil}, we used measured tissue concentrations in the final sampling and estimated soil PFAS concentrations from the mass of soil in the columns and the amount of contaminant added in dosing solutions (Table 3). The mass of soil in each column was approximated by measuring the freeboard in each column to determine the final volume of sand media in each. The sand volumes were converted to a mass basis using a bulk density value of 1.5 g/cm^3. The mass of each PFAS constituent dosed to the treatment columns over the course of the study was used to determine the final soil concentration for each column by dividing the mass of PFAS dosed by the total mass of sand in the columns.

Statistical analyses

Growth and tissue concentrations of PFAS-treated and non-PFAS-treated plants were analyzed for each sampling period, species and tissue type by one-way ANOVA ($p = 0.05$) of the three replicate greenhouse blocks following tests of equal/unequal variance using SAS JMP Pro ver 14.1 (SAS Institute, Cary, NC). The effect of salinity was evaluated separately for the subset of plants that received the salinity treatments. Again, a one-way ANOVA in three replicate blocks was used after testing for equal/unequal variance.

Results

Tissue concentrations

A total of 46 samples from four herbaceous and two woody species were analyzed in the initial sampling event (after six doses of herbaceous and five doses of woody species) and a total of 128 samples were analyzed for the seven herbaceous and seven woody species in the second and final sampling event. Herbaceous plants, that grew throughout the entire experimental period received twelve doses of contaminant solution. *Brassica juncea*, *Helianthus annuus*, and *Trifolium incarnatum*, which matured before the end of the designed treatment period, received six doses. All woody species received eleven doses of contaminant solution at the time of the final harvest. Six PFAS that were evaluated (PFPeA, PFHxA, PFOA, PFBS, PFHxS, PFOS) accumulated in aboveground tissue and, with few exceptions, the differences in plant tissue concentrations between no PFAS control and PFAS treated plants were large and statistically significant at $p < 0.05$ (Tables 4 and 5). MeFOSA was only analyzed for presence or absence and was not detected.

Species-specific differences occurred in both observed tissue concentrations of individual PFAS and their pattern of accumulation. In general, tissue concentrations (ng/g) followed the trend: PFPeA > PFHxA > PFBS > PFOA > PFHxS > PFOS but there was some variation by plant. This trend is not completely consistent with literature that indicates the greatest plant uptake of shorter chain compounds (Krippner *et al.* 2015; Ghisi *et al.* 2019); however, the highest uptakes were observed with the 5-chain PFPeA compound and lowest was observed with the 8-chain PFOS compound. The herbaceous species *Equisetum hyemale*, *Amaranatus tricolor*, and *Festuca rubra* developed the greatest concentrations of most compounds ranging from a high of 21,882 ng/g for PFPeA to a low of 131 ng/g for PFHxA (Table 4). Most of the hardwoods (angiosperms) evaluated had significant foliage accumulation of one or more PFAS compounds. The greatest concentrations of most compounds were found in the foliage of *Liriodendron tulipfera*, *Salix nigra*, and *Betula nigra* (Table 5). In contrast, the one conifer tree species evaluated, *Pinus taeda*, exhibited relatively low foliage concentrations of PFAS compounds, < 105 ng/g for all compounds except PFPeA. For PFPeA, which generally was accumulated in foliage to the greatest extent of all PFAS evaluated, concentrations exceeding 30,000 ng/g occurred in *Amaranthus tricolor*, *Equisetum hyemale*,

Table 4. Mean tissue concentrations and standard errors (*SE*) for herbaceous plant species irrigated with and without addition of PFAS compounds during initial (6–7) and final (13–14) weeks of treatment.

Species	PFPeA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
	None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS
	ng/g											
	Initial sampling											
Cynodon dactylon	4	*1219	1	*821	8	*2846	1	*1162	1	*807	2	*287
	±1	±58	±0	±22	±5	±104	±0	±17	±0	±55	±0	±39
Festuca rubra	4	*1335	3	*556	35	*2334	7	*1784	5	*1409	6	*531
	±1	±111	±2	±36	±14	±203	±4	±168	±1	±151	±2	±52
Trifolium incarnatum	4	*1495	1	*572	13	*2493	3	*581	1	*449	1	NS42
	±1	±166	±0	±138	±3	±200	±2	±146	±1	±160	±0	±42
Festuca Arundinacea	5	*1280	2	*530	19	*2309	4	*493	5	*381	6	*79
	±0	±267	±1	±39	±3	±468	±1	±89	±1	±70	±3	±18
	Final sampling											
Festuca rubra	215	*21,882	131	*19,753	8	*3737	7	*6472	5	*4310	1	NS1146
	±74	±2143	±39	±1513	±4	±391	±5	±1714	±3	±489	0	±534
Cynodon dactylon	164	*4642	129	*4574	11	*588	42	*1672	10	*555	1	*220
	±58	±153	±54	±87	±10	±84	±26	±121	±8	±88	0	±13
Schedonorus arundinaceus	197	*14,780	156	*12,679	11	*1100	20	*4725	4	*1682	1	*264
	±18	±6294	11	±5873	±1	±327	±14	±2329	±2	±565	0	±80
Helianthus annuus	9	*3937	5	NS967	7	*361	3	*178	13	*276	120	NS78
	±8	±1770	±3	±499	±3	±35	±2	±10	±9	±33	±75	±74
Brassica juncea	5	*13,030	4	*8362	1	*1814	1	*1184	1	*969	1	*434
	±4	±3974	±3	±4098	0	±435	0	±650	0	±417	0	±102
Amaranthus tricolor	306	*38,121	204	*13,434	10	*5774	1	*326	5	*2865	39	*636
	±202	±18,208	±70	±5327	±4	±676	0	±43	±3	±172	±38	±145
Equisetum hyemale	237	*32,032	306	*23,531	13	*1533	3	*40	3	*279	1	*169
	±36	±2341	±7	±2050	±2	±136	±1	±5	±1	±14	±0	±28

Significant differences ($p = 0.05$) between plants without PFAS addition (None) and PFAS treated plants (+PFAS) are indicted by * and non-significant differences by NS for a one-way ANOVA following tests for equal/unequal variance.

Table 5. Mean tissue concentrations and standard errors (SE) for tree species irrigated with and without addition of PFAS compounds during initial (6–7) and final (13–14) weeks of treatment.

Species		PFPeA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
		None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS	None	+PFAS
		ng/g											
		Initial sampling											
Salix nigra	Foliage	1	*609	0	*143	1	*275	1	*227	1	*198	0	NS9
		±0	±124	±0	±48	±0	±55	0	±46	±0	±49	±0	±4
Liquidambar styraciflua	Woody	1	*782	0	*110	3	*842	1	NS65	1	*93	0	NS0
		±0	±298	±0	±18	±0	±334	±1	±40	±0	±41	±0	±0
		Final sampling											
Salix nigra	Foliage	67	*31,646	40	*19,001	1	*3442	1	*3271	1	*2562	1	*556
		±30	±3327	±16	±1166	±0	±241	±0	±1490	±0	±260	±0	±199
	Woody	5	*373	2	*186	1	*241	1	*11	1	*56	1	*32
		±3	±84	±1	±38	±0	±52	±0	±2	±0	±15	±0	±11
Fraxinus pennsylvanica	Foliage	56	*169	66	*690	1	NS 1	4	*816	3	*1353	1	NS1
		±1	±1	±7	±9	±0	±0	±3	±4	±2	±2	±0	±0
	Woody	4	NS379	2	NS 129	1	NS 23	1	NS76	1	NS87	1	NS21
		±1	±297	±1	±104	±0	±12	±0	±56	±0	±47	±0	±17
Pinus taeda	Foliage	9	*964	1	*93	1	*105	1	*41	1	*71	1	NS13
		±3	±191	±0	±36	±0	±49	±0	±18	±0	±29	±0	±7
	Woody	1	*3	1	NS3	1	NS3	1	NS1	1	NS1	1	NS2
		±0	±1	±0	±2	±0	±2	±0	±0	±0	±0	±0	±1
Betula nigra	Foliage	30	*28,496	33	*20,076	26	*5419	5	*1135	10	*3033	3	*1759
		±24	±3443	±19	±2086	±25	±1277	±4	±131	±9	±421	±2	±646
	Woody	1	NS686	1	*220	1	*178	1	*3	1	*42	1	*180
		±0	±482	±0	±98	±0	±31	±0	±1	±0	±13	±0	±75
Liquidambar styraciflua	Foliage	15	NS2070	47	NS1314	1	NS1330	1	*308	1	NS937	1	NS392
		±6	±1797	±16	±868	±0	±951	±0	±70	±0	±526	±0	±277
	Woody	1	NS981	1	NS350	1	*354	1	*7	1	*41	1	*72
		±0	±800	±0	±251	±0	±26	±0	±1	±0	±7	±0	±31
Platanus occidentalis	Foliage	14	*17,838	21	NS9227	1	*1123	1	NS1724	1	*968	1	*262
		±7	±4539	±7	±5070	±0	±396	±0	±1526	±0	±432	±0	±110
	Woody	2	*83	1	*55	1	*63	1	NS2	1	*15	1	NS16
		±1	±15	±0	±5	±0	±14	±0	±1	±0	±5	±0	±9
Liriodendron tulipifera	Foliage	26	*35,975	24	*17,938	1	*1382	3	*16,878	1	*2994	1	NS814
		±17	±10,731	±16	±7473	±0	±204	±2	±7151	±0	±839	±0	±463
	Woody	9	*1726	2	*1259	1	*70	1	*35	1	*56	1	*29
		±5	±882	±1	±532	±0	±35	±0	±14	±0	±23	±0	±10

Significant differences ($p = 0.05$) between plants without PFAS addition (none) and PFAS treated plants (+PFAS) are indicted by * and non-significant differences by NS for a one-way ANOVA following tests for equal/unequal variance.

Liriodendron tulipfera, and *Salix nigra*. Although significant accumulations of PFAS occurred in woody components of tree species, concentrations in wood were generally low, often several orders of magnitude lower than the concentration in the foliage of the same species.

Concentrations of PFAS observed in the initial sampling event for the four herbaceous and two woody species for which tissue was collected were much lower than in the final harvest. This observation indicates that PFAS continued to accumulate in plant tissue and that the observed concentrations do not represent a concentration maximum. Further, it indicates significant accumulation was observed across a spectrum of contaminant concentrations.

The influence of salinity treatments on PFAS accumulation is presented in Table 6. Although mean concentrations of most PFAS constituents were greater in the saline treatments (e.g., increases in PFOA and PFOS accumulations ranged from 44 to 344% above the contaminant treatments that did not receive salinity additions), only increases of PFOA and PFHxS in *Cynodon dactylon* were statistically significant.

Bioconcentration

Using BCF_{plant} approach with *Pinus taeda* considered an excluding species, four herbaceous and three tree species were identified as hyperaccumulators for at least one of the six PFAS compounds. *Festuca rubra*, *Schedonorus arundinaceus*, *Amaranthus tricolor*, *Esquisetum hyemale*, *Salix nigra*, *Betula nigra* and *Lirodendron tulipfera* were hyperaccumulators of PFHxA. *Festuca rubra*, *Schedonorus arundinaceus*, and *Lirodendron tulipfera* were hyperaccumulators of PFBS and *Betula nigra* was a hyperaccumulator of PFOS.

BCF_{soil} values are presented in Figures 3 and 4 for herbaceous and wood species, respectively. All seven herbaceous species and four of six tree species had $BCF_{soil} > 10$ for at least one of the six PFAS compounds evaluated. The greatest BCFs were generally found for PFPeA and PFHxA, and the lowest BCFs were for PFOS. However, two species, *Festuca rubra* and *Betula nigra*, had BCF >10 for PFOS. Additionally, the PFAS + salinity treatments showed BCF_{soil} >10 for *Festuca rubra*, *Betula nigra*, *Salix nigra*, and *Liqudambar styraciflua*.

Mass recovery

Mass recovery of applied compounds in biomass of herbaceous plants (Table 7) was as great as 42% for PFPeA and 28% for PFHxA by *Schedonorus arundinaceus*. *Festuca rubra* recovered the greatest amount of PFOA (11%), PFHxS (13%), and PFOS (4%). *Betula nigra* was generally the best performing tree species (Table 8) with recovery of PFPeA at 32%, PFHxA at 17%, PFOA at 10%, PFBS at 2%), PFHxS at 6% and PFOS at 3%. Several tree species recovered significantly more than *Betula* for specific compounds. For example, *Liriodendron tulipfera* recovered greater amounts of PFBS (20%) than *Betula nigra* (2%) underscoring the species-specific nature of uptake and accumulation of these

Table 6. Effect of saline treatment on PFAS concentrations of plant tissue in a subset of PFAS-treated plants.

		PFPeA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
Species		+PFAS	+PFAS + Saline	+PFAS	+PFAS + Saline	+PFAS	+PFAS + Saline	+PFAS	+PFAS + Saline	+PFAS	+PFAS + Saline	+PFAS	+PFAS + Saline
		ng/g											
Herbaceous													
Cynodon dactylon	Foliage	4642	^{NS}4984	4575	^{NS}4621	588	*817	1672	^{NS}1980	555	*852	220	^{NS}280
		±153	±129	±87	±396	±84	±92	±121	±211	±88	±129	±13	±7
Festuca rubra	Foliage	21,882	^{NS}24480	19,753	^{NS}25,262	3737	^{NS}5384	6472	^{NS}7342	4310	^{NS}5700	1146	^{NS}1871
		±2142	±6195	±151	±7375	±391	±2087	±1714	±3271	±489	±1941	±534	±290
Trees													
Liquidambar styraciflua	Foliage	2070	^{NS}6967	1314	^{NS}4803	1330	^{NS}2471	308	^{NS}1639	937	^{NS}2859	392	^{NS}1759
		±1797	±4966	±866	±3256	±951	±1163	±70	±1359	±526	±1320	±277	±1060
	Wood	981	^{NS}236	350	^{NS}152	354	*212	7	^{NS}30	41	^{NS}31	72	^{NS}165
		±800	±81	±251	±48	±26	±50	±1	±17	±7	±4	±31	±32
Salix nigra	Foliage	31,646	^{NS}27,958	19,001	^{NS} 12,055	3442	^{NS}3319	3271	^{NS}419	2562	^{NS}2932	556	^{NS}1108
		±3327	±5996	±1166	±3246	±241	±727	±1490	±84	±260	±1055	±199	±782
	Wood	374	^{NS} 71	186	^{NS}58	241	^{NS}218	11	^{NS}1	56	^{NS}40	32	^{NS}62
		±84	±53	±38	±21	±52	±83	±2	±1	±15	±29	±11	±59

Means and standard errors (±*SE*) are provided and significant differences ($p = 0.05$) between plants only treated with PFAS (+PFAS) and those also treated with saline solution (+PFAS, +Saline) are indicted by * and non-significant differences by ^{NS} for a one-way ANOVA following tests for equal/unequal variance.

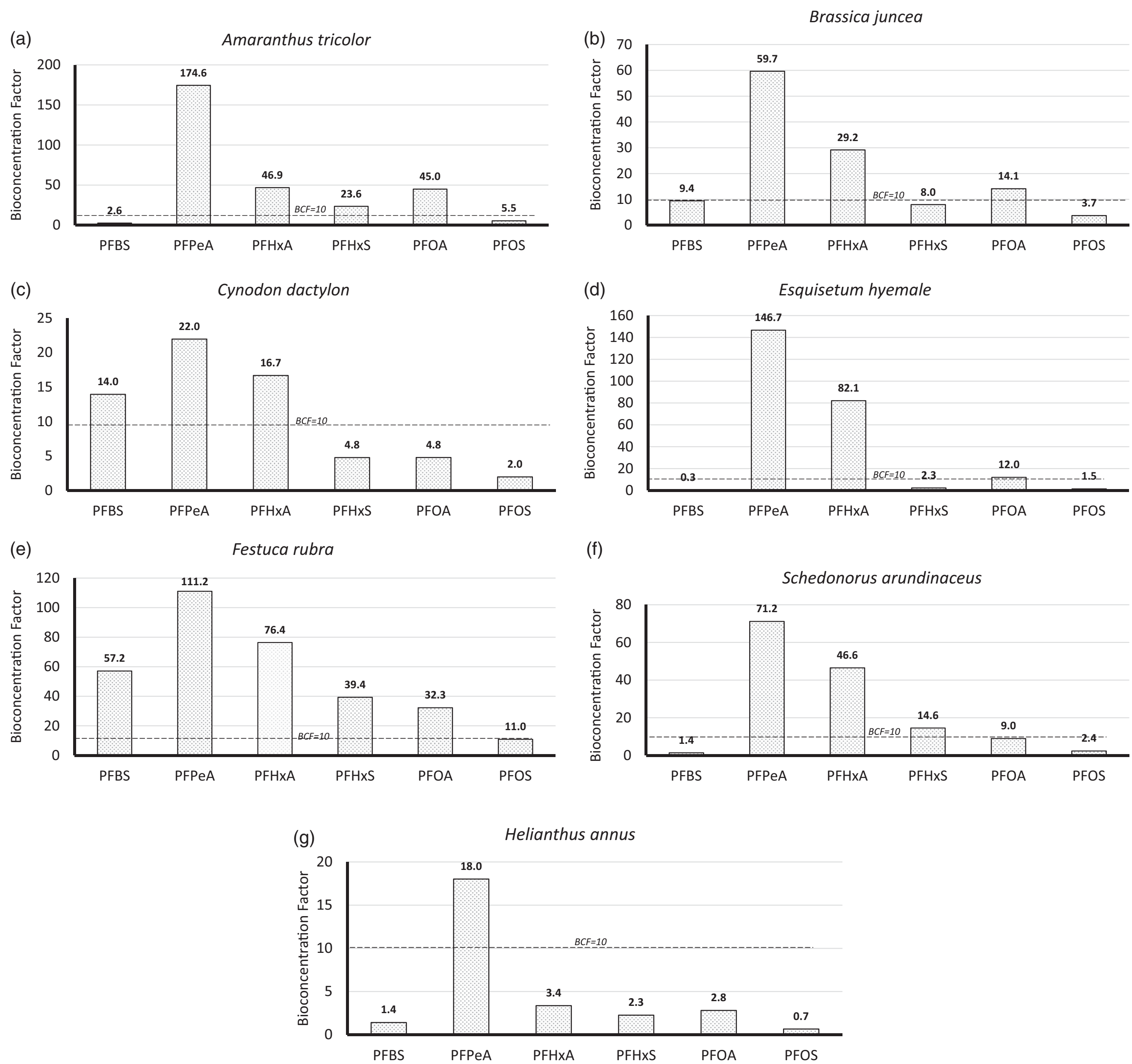

Figure 3. (a–g) bioconcentration factors (BCF_{soil}) of herbaceous plant species grown in a sand culture treated with six PFAS compounds.

compounds. For all three species, most recovery was in the foliage with only minor amounts accumulated in wood. We note that although concentrations in wood were much lower than concentrations in tree foliage, the increased mass of wood, as the trees age, may result in wood accumulation contributing significantly to overall mass recovery.

Discussion

These results provide strong evidence for phytoaccumulation of multiple PFAS compounds in aboveground components of herbaceous and woody plants and, thus, the potential for phytoremediation to be incorporated into remediation programs designed for PFAS-impacted sites. Although many of the species investigated accumulated one or more of the evaluated compounds, major differences occurred among species. *Festuca rubra* was the most effective species when overall contaminant accumulation was considered. It achieved hyperaccumulation (tissue concentration/soil concentration >10/1) of all six PFAS compounds including PFOA, PFOS, and PFBS over the course of the study (as well as after 24 days from initial contaminant dosing when the first sampling event was completed on April 1, 2018). This species had $BCFs_{soil}$ that ranged from 11.0 (PFOS) to 111.2 (PFPeA) based on soil contaminant concentrations. *Amaranthus tricolor*, *Equisetum hyemale*, and *Schedonorus arundinaceus* were the other herbaceous species that were found to exhibit above-average accumulation of multiple PFAS compounds. *Amaranthus tricolor* did not hyperaccumulate either PFBS or PFOS but it did hyperaccumulate the remaining four PFAS compounds and had one of the highest BCF_{soil} for PFOA (45.0) (Table 7).

Festuca rubra became a widely planted species during World War II due to demand for a seed-propagated turf-

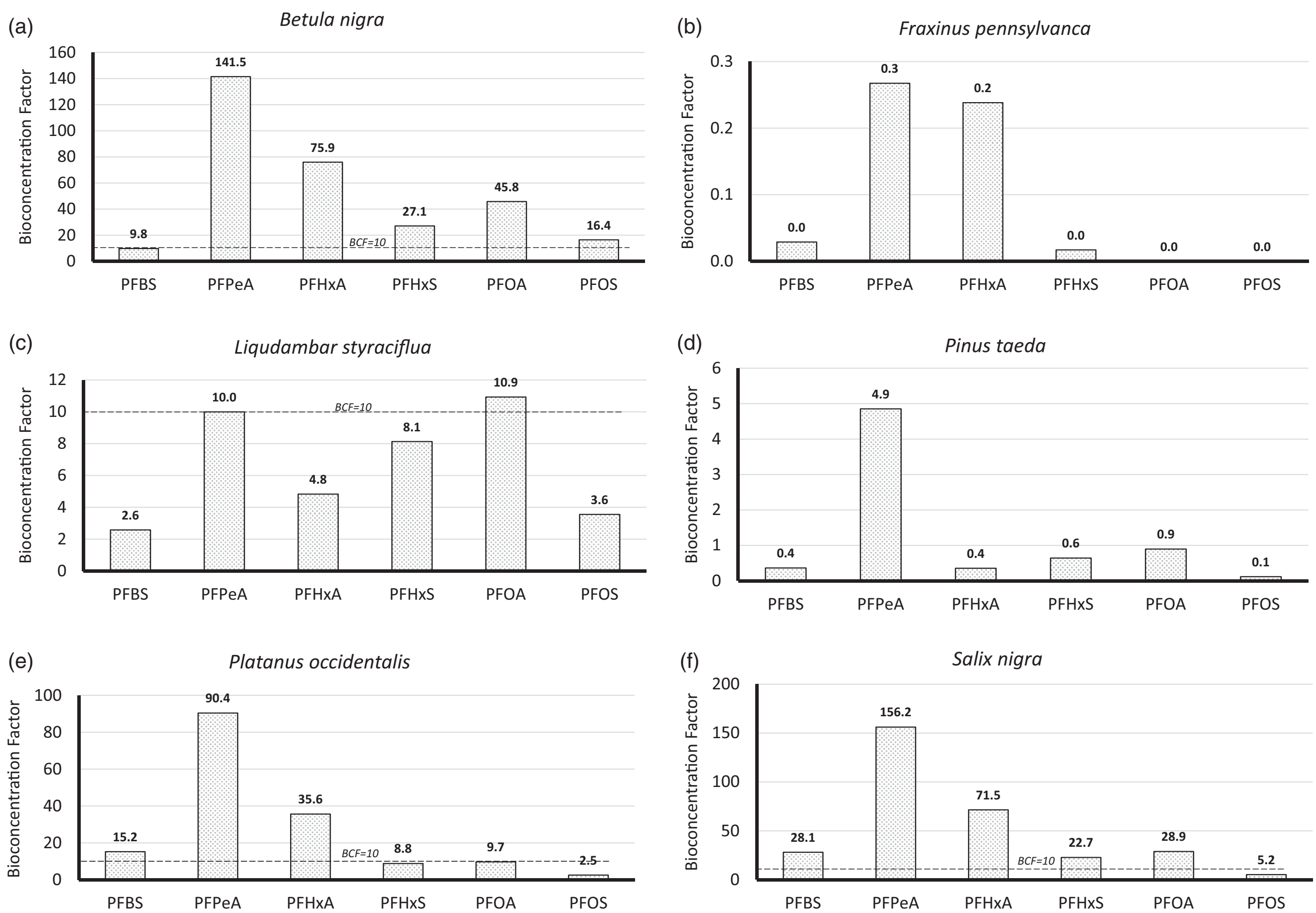

Figure 4. (a–f) bioconcentration factors (BCF_{soil}) of woody plant species grown in a sand culture treated with six PFAS compounds.

Table 7. Estimated average mass recovery of PFAS compounds by herbaceous plant species.

		PFPeA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
Species	Doses	µg	%	µg	%	µg	%	µg	%	µg	%	µg	%
Festuca rubra	12	717	37.4	652	25.9	122	10.8	224	20.3	141	13.2	39	3.8
Cynodon dactylon	12	434	22.6	427	16.9	55	4.9	156	14.1	51	4.8	20	2.0
Schedonorus arundinaceus	12	807	42.0	696	27.6	60	5.3	262	23.8	92	8.6	14	1.4
Equisetum hyemale	12	759	39.5	557	22.1	36	3.2	1	0.1	7	0.6	4	0.4
Helianthus annuus	6	52	5.5	8	0.6	4	0.8	2	0.4	3	0.6	1	0.2
Brassica juncea	6	114	11.8	72	5.7	15	2.7	9	1.7	8	1.6	4	0.7
Trifolium incarnatum	6	29	3.1	11	0.8	50	8.9	13	2.3	10	1.9	1	0.2
Amaranthus tricolor	9	446	30.9	153	8.1	66	7.7	4	0.4	1	4.0	0	0

Herbaceous plants received a different number of doses depending on how quickly the plant matured and required harvesting.

Table 8. Estimated average mass recovery of PFAS compounds in aboveground components of tree species after 11 doses of contaminant solution.

	PFPeA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
Species	µg	%	µg	%	µg	%	µg	%	µg	%	µg	%
Salix nigra												
Foliage	404	23.0	241	10.4	43	4.2	43	4.3	33	3.3	7	0.8
Woody	6	0.3	3	0.1	4	0.3	0	0.0	1	0.1	0	0.1
Pinus taeda												
Foliage	33	1.8	3	0.1	4	0.4	2	0.2	3	0.3	1	0.1
Woody	0	0	0	0	0	0	0	0	0	0	0	0
Betula nigra												
Foliage	561	31.9	400	17.3	103	10.0	22	2.1	58	5.9	31	3.3
Woody	23	1.3	7	0.3	4	0.4	0	0	1	0.1	5	0.6
Liquidambar styraciflua												
Foliage	22	1.2	16	0.7	16	1.5	5	0.4	12	1.2	5	0.5
Woody	17	1.0	6	0.3	6	0.6	0	0	1	0.1	1	0.1
Platanus occidentalis												
Foliage	583	33.1	298	12.9	38	3.6	54	5.4	32	3.3	9	1.0
Woody	4	0.2	3	0.1	3	0.3	0	0	1	0.1	1	0.1
Liriodendron tulipifera												
Foliage	401	22.8	192	8.3	16	1.6	200	19.8	35	3.6	9	1.0
Woody	22	1.2	17	0.7	1	0.1	0	0	1	0.1	0	0

forming grass that could be seeded on airfield strips and military bases throughout North America (Elliott and Baenziger 1977; Cole *et al.* 2002). This suitability for airfield sites is significant because the past use of aqueous film-forming foam (AFFF) fire suppressants, which contained large amounts of PFAS, particularly PFOS, was extensive at airfields and legacy PFAS impacts to soils and sediments are widespread (Anderson *et al.* 2016). When evaluated on the basis of BCF, there is a large difference in PFAS accumulation between *Festuca rubra* and *Schedonorus arundinaceus*. However, when evaluated on the basis of mass recovery (Table 7), *Schedonorus arundinaceus* is shown to be as effective or superior to *Festuca rubra* due to greater biomass growth during the experimental period.

Bioconcentration factors of the best performing tree species (based on foliage concentrations) were lower than $BCFs_{soil}$ of the best performing herbaceous species but were generally in the same overall range of the herbaceous plants. The best performing tree species was *Salix nigra*, which hyperaccumulated five of the six PFAS compounds in foliage. Only PFOS was not hyperaccumulated by this species. Under saline conditions; however, PFOS was also hyperaccumulated. In contrast to foliage, PFAS concentrations in the woody components of *Salix* were low and, in no case, significantly different from the untreated controls. *Betula nigra* foliage concentrations showed hyperaccumulation for all but PFBS, although this contaminant was also close to hyperaccumulation with a BCF of 9.8. *Betula nigra* foliage had the highest BCF_{soil} for PFOA observed in the study with a value of 45/1. Similar to *Salix nigra*, concentrations of most PFAS compounds in the woody component were negligible. *Liriodendron tulipfera* foliage had the highest BCF for any of the tested PFAS compounds with a value of 176/1 for PFPeA. PFAS concentrations were low in woody components of *Liriodendron*.

Three tree species, *Fraxinus pennsylvanica*, *Betula nigra* and *Liquidambar styraciflua* accumulated one or more PFAS compounds in woody components. These species may have particular value for remediation programs that combine the management of a woody crop with herbaceous species because of the potential to accumulate significant quantities of contaminants in woody tissue. One approach would involve a double-cropping system that entails the management of two remedial crops. For example, the cool season *Festuca rubra* could be interplanted with the *Betula nigra*. *Festuca* would grow during the fall through the spring period as a winter cover beneath the deciduous tree species. Then, as the trees break winter dormancy and begin to leaf-out, *Festuca* could be harvested. *Betula nigra* would then have the major phytoremediation role during the warmer summer months when *Festuca* is largely dormant. Leaves of the trees can be raked in the fall to remove contaminants accumulated in foliage and harvesting of the trees could be delayed for five or more years allowing contaminants to accumulate in the woody structure. A more intensive option to this approach uses elements of ultrashort rotation coppice silviculture practice. In this approach, both the herbaceous *Festuca rubra* and the woody tree would be harvested annually. Again, the cool season *Festuca rubra* could be harvested as the tree crop emerges from dormancy, and the hardwood is harvested in late summer or early autumn before leaf fall using specialized power scythes (Kopp *et al.* 1993). The hardwoods are allowed to regenerate each year from coppice and the need for replanting to replace mortality is manageable, even with annual harvests (e.g., <30–50% mortality after five successive annual harvests) (Kopp *et al.* 1993). Such a system would be expected to maximize contaminant removal.

In this study, the growth media consisted of low ionic exchange sieved sand with little organic matter content. This growth media-generated conditions where the PFAS compounds were more readily available for plant uptake than would be found in many field situations. Infield applications, sorption of PFAS on soil would limit uptake; however, practices that increase desorption from the soil and increase PFAS uptake could be employed. Reduction in soil organic carbon, which is associated with PFAS adsorption to soil (Chen *et al.* 2018) and can limit PFAS uptake, could be achieved by site management that includes elements of tillage and application of inorganic nitrogen fertilizer that speeds decomposition. The relationship between soil organic carbon is not strong for many PFASs compounds and other soil factors such as pH (Li *et al.* 2018) and salinity also affect sorption. Again, within limits, these factors can be managed to decrease soil sorption and increase uptake potential. Soil pH can be adjusted by lime amendment and use of lime or other salts can create greater ionic strength within the soil solution and could potentially displace bound PFAS compounds.

In interpreting these analytical results, it is important to note the In the case of PFOS, some recoveries were greater than 100%, possibly indicating that an interfering compound was present, which may have impacted the analysis of PFOS. With respect to PFOS, the largest recoveries exceeding 100% were observed in *Liquidambar styraciflua* at 1224% ± 490, *Helianthus annuus* at 362% ± 203, *Liriodendron tulipfera* at 261% ±38, *Betula nigra* at 759% ±193, and *Pinus taeda* at 182% ± 69. The relatively low concentrations of PFOS detected in *Helianthus annuus*, *Liriodendron tulipifera* and *Pinus taeda* make the significance of the interference finding less important because they showed little uptake regardless. With respect to *Betula nigra* and *Liquidambar styraciflua*, the corrected PFOS values would still approximate BCFs of 10; therefore, the adjusted values remain substantial. Although the tissue concentration data were not corrected for extraction efficiency, the raw data and recovery efficiencies are provided for each species and tissue type in the supporting information.

Conclusions

Overall, the results of this study demonstrate the potential use of phytoremediation as a tool for remediating PFAS contaminated sites. Accumulation of multiple PFAS compounds in above-ground portions of both herbaceous and woody plants was demonstrated. In particular, *Festuca rubra* was found to hyperaccumulate all of six of the evaluated contaminants. More than 25% of the PFPeA, PFHxA, and PFBS applied during the twelve-week dosing period were recovered in the above-ground biomass of this species. *Amaranthus tricolor*, *Equisetum hyemale*, and *Schedonorus arundinaceus* were other herbaceous species that were found to have an above-average accumulation of multiple PFAS compounds. Several tree species also accumulated significant amounts of one or more contaminants in foliage and, for three species, *Fraxinus pennsylvanica*, *Betula nigra* and *Liquidambar styraciflua* there was evidence for accumulation in woody components. These data suggest that phytoremediation systems that combine short-lived herbaceous plants with long-lived tree species could be developed and refined to maximize phytoremediation efficiency. Also, at least 10% of every contaminant except PFOS was recovered by in above-ground biomass by at least one tested plant species. Thus, there is the potential for phytoremediation to be useful in situations where complex contaminant mixtures occur in soil and shallow groundwater at PFAS impacted sites.

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