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Uptake of per- and polyfluoroalkyl substances (PFAS) into lettuce (*Lactuca sativa*), tall fescue (*Schedonorus arundinaceus*) and tomato (*Solanum lycopersicum*): A greenhouse experiment evaluating bioconcentration factors and testing the effect of intercropping

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ABSTRACT

The contamination of agricultural soils with per- and polyfluoroalkyl substances (PFAS), resulting from the application of biosolids and contaminated irrigation water, threatens both human health and the long-term viability of farming operations. While this is a known concern, less is known about factors associated with the transfer of PFAS from soils to crops and how transfer differs across crop compartments and species. The objectives of the present study were to 1) assess bioconcentration factors (BCFs) of lettuce (*Lactuca sativa* L.), tall fescue (*Schedonorous arundinaceus* [Screb.] Dumort), and tomato (*Solanum lycopersicum* L.) grown in a greenhouse using spiked potting mix, and 2) test the efficacy of intercropping as a phytomanagement strategy to influence facilitative and competitive interactions capable of altering PFAS uptake into plants.

We found distinct BCFs across species, compartments and compounds. Perfluorobutanoate (PFBA) edible portion BCFs were the highest in tomato fruit, being 3.5-fold higher than in lettuce leaves. For perfluorobutanesulfonate (PFBS), and perfluorooctanoate (PFOA) edible portion BCFs were highest in tall fescue, followed by lettuce, and lowest in tomatoes. BCFs for perfluorooctanesulfonate (PFOS) were not significantly different across species.

Across all crop compartments, aboveground BCFs significantly exceeded those of root BCFs ($p < 0.05$). Intercropped plant pairings only influenced PFBA uptake, reducing uptake of this compound into tomato fruit when paired with lettuce. While other intercropped pairings did alter uptake of PFAS into one or both species compared to monocropped pairings, the directionality of the trend showed that uptake generally increased in intercropped pairings, making this an impractical option for phytomanagement of contaminated agroecosystems.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are chemicals widely incorporated into industrial and consumer products. The stable carbon-fluorine bond, solubility in water, and amphiphilic properties of PFAS are characteristics that make them useful for products such as waterproof materials, aqueous film-forming foams, and non-stick pans (*History of PFAS and 3M*, n.d.; Verduzco & Wong, 2020). However, these are the same characteristics that result in their environmental persistence and the readiness of these compounds to bioaccumulate within crops and animals, with humans being no exception (Jogsten et al., 2009; Navarro et al., 2017; Stahl et al., 2018; Sznajder-Katarzyńska

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et al., 2019). This is of concern as studies link PFAS exposure to adverse human health impacts including kidney cancer, reduced immune response to vaccines, and decreased fetal growth (Beale et al., 2022; Fenton et al., 2021). A primary pathway of human PFAS consumption is through the consumption of crops and livestock products exposed to contaminated agricultural soils and irrigation water—a consequence of the historical spreading of biosolids as fertilizer, some of which contained high concentrations of PFAS (Fenton et al., 2021).

The state of Maine has some of the most developed guidelines for PFAS, but even there, guidance available to farmers regarding soil screening thresholds is limited to forage crops and based only on preliminary findings (Maine Center for Disease Control and Prevention, 2020). One barrier to expanding these guidelines is the large variation in bioconcentration factors (BCFs) of PFAS across and within crop types, found to be a function of factors including but not limited to crop physiology (water transport and storage, lipid content, protein content, growing days, root and leaf structures), soil sorption (organic matter, pH, particle size, and cation exchange capacity), chemical composition (chain length, functional group, biotransformation potential, and concentrations), mode of contamination, and the interactions therein (Battisti et al., 2024; Blaine et al., 2014; Costello & Lee, 2020; Felizeter et al., 2020; Lesmeister et al., 2021; Mei et al., 2021; Scearce et al., 2023). This work aims to target distinct plant physiologies: A leafy green, a grass, and a fruit vegetable, under like soil and contamination conditions to inform a broad range of growers about the risk associated with these three crop archetypes.

The drive to understand uptake mechanisms is coupled with the push to identify approaches to manage or remediate contaminated lands. In previous research, phytoextraction has received attention as an approach to remove PFAS from contaminated soils (He et al., 2023; Huff et al., 2020). Phytoextraction is nested within a broader category of processes called *phytomanagement*, which includes cropping strategies that result in minimal uptake of PFAS in edible portions, among other approaches (Evangelou & Robinson, 2022). Currently, studies on the phytomanagement of PFAS have not explored the influence of the crop neighbor, which has been shown to influence the uptake of nutrients and heavy metals in some plant species (Liu et al., 2023; Wan and Lei, 2018). Previous studies on crop neighbor interactions suggest that intercropped pairings may force biotic interactions, both competitive and facilitative (Maitra et al., 2019). For example, interspecific interactions of root exudates and soil microbes have been linked to safe and viable food production in systems contaminated by heavy metals, making this an approach of interest in the context of agroecosystems contaminated with PFAS (Bian et al., 2021; Liu et al., 2023).

The objectives of the present study were to 1) assess BCFs of crops from distinct families: Lettuce (*Lactuca sativa* L.), tall fescue (*Schedonorous arundinaceus* [Screb.] Dumort), and tomato (*Solanum lycopersicum* L.) grown in a greenhouse using spiked potting mix, and 2) test the efficacy of intercropping as a phytomanagement strategy to influence facilitative and competitive interactions capable of altering PFAS uptake into plants. To achieve this, transfer was measured in distinct plant compartments, including root, leaf, stem, and fruit, as applicable. To account for factors influencing uptake associated with soil sorption and chemical characteristics of PFAS, crops were grown in a potting mix spiked with perfluorobutanoate (PFBA), perfluorobutanesulfonate (PFBS), perfluorooctanoate (PFOA), and perfluorooctanesulfonate (PFOS).

2. Materials and methods

2.1. Greenhouse conditions

A greenhouse experiment was utilized to investigate the BCFs of PFAS from potting mix to crops from May 2023 to October 2023 in a greenhouse in Orono, ME (44.888769, −68.671153). Pots were arranged in a completely randomized design, with two crop individuals in each pot (Fig. 1A). There were monocropped (same species) treatments, where the number of replicates per treatment were n=4 for lettuce and n=3 for tomato and for tall fescue. There were also intercropped treatments where the number of replicates were n=4 for lettuce and tomato and n=3 for lettuce and tall fescue. Tomato and tall fescue were not considered under intercropping conditions due to sampling limitations. Because this study did not explore other pathways of contamination (i.e. air), nor did it explore impacts on plant physiology or yield, uncontaminated control pots were not included.

Seedlings were sown from May 16 to 19, 2023 and germinated in potting trays with a 50:50 ratio of unspiked Pro-Mix potting mix (Premier Tech and Home Garden, Quakertown, PA, USA) and vermiculite (PVP Industries, North Bloomfield, OH, USA). Varieties included 'Bluerock Classic' romaine lettuce (Johnny's Selected Seeds, Winslow, ME, USA), 'Mountain Fresh' tomatoes (Johnny's Selected Seeds), and 'Bronson' (pasture) tall fescue (Ernst Conservation Seeds, Meadville, PA, USA). Seedlings were transplanted by hand with nitrile powderless gloves into pots prepared with spiked potting mix after four to six weeks of growth (June 20, 2023). To ensure interaction of roots, plants within pots were spaced 10 centimeters apart, with 10 centimeters between each plant and the nearest pot edge.

Crop containers were adapted from Huff et al. (2020). This design allowed excess water to drain, facilitating maintenance of consistent soil moisture levels across replicates. Materials for these pots were reportedly PFAS-free, constructed from 30-centimeter lengths of polyvinyl chloride (PVC) pipes 30 centimeters in diameter and attached to a vinyl end cap using silicone sealant. Each pot was filled to a height of 5 centimeters with ~2 × 2-centimeter gravel and approximately 18 centimeters of spiked potting mix (3.175 kg, on a wet weight (w.w.) basis, Fig. 1C).

Pots were arranged by species treatment across two greenhouse benches and rotated front-to-back every two weeks beginning the second week of July to mitigate the effects of environmental variation in the greenhouse. To maintain a volumetric water content of 20 %, pots were assessed daily using a time domain reflectometry tensiometer fitted with 12.2-centimeter prongs (Spectrum Technologies, Aurora, IL, USA). Based on daily readings, pots were watered on an as-needed basis with PFAS-free irrigation water. Approximately 18 grams of soluble potash fertilizer (Intrepid Potash, CO, USA) were applied to each pot after lettuce harvest to address nutrient needs. Potting media was tested for PFAS, and none were detected except for 0.49 ng/g of NEtFOSE. No PFAS were detected in vermiculite or fertilizer used in the study.

2.2. Spiking

Potting mix was spiked with four PFAS compounds: PFBA, PFBS, and PFOA from Sigma Aldrich (Saint Louis, MO, USA), and PFOS from Agilent (Santa Clara, CA, USA) (Fig. 1B). All compounds were neat, with purities ranging from 95.5 % to 104.5 %. Percents greater than 100 account for the uncertainty associated with purity (mass of the pure chemical divided by the mass of the sample x 100). The target concentration of each compound per pot was 220 ng/g. To reach the target concentration, a 100 times concentrated stock solution was made by pipetting 11 μL of PFBA and PFBS and using sterilized forceps to weigh 0.021 (± 0.001) grams of PFOA and PFOS into 50 mL of deionized (DI) water in a fume hood. The concentrated solution was then diluted to become the working stock for each pot by adding 1.75 mL of stock solution to 248.25 milliliters of DI water. The 3.175 kg w.w. of potting media was primed with 1000 mL of pure DI water, and the watering solution with a concentration of 220 μg/L was then used to spike each pot. Under the fume hood, the potting media was mixed with the watering solution in a high-density polyethylene bucket, and then transferred into a PVC pot, wherein the crops were planted less than 48 h after spiking (Figure S1). Materials were cleaned between pot preparations. Quality control testing showed that potting media concentrations exceeded the target concentration with this method and were variable

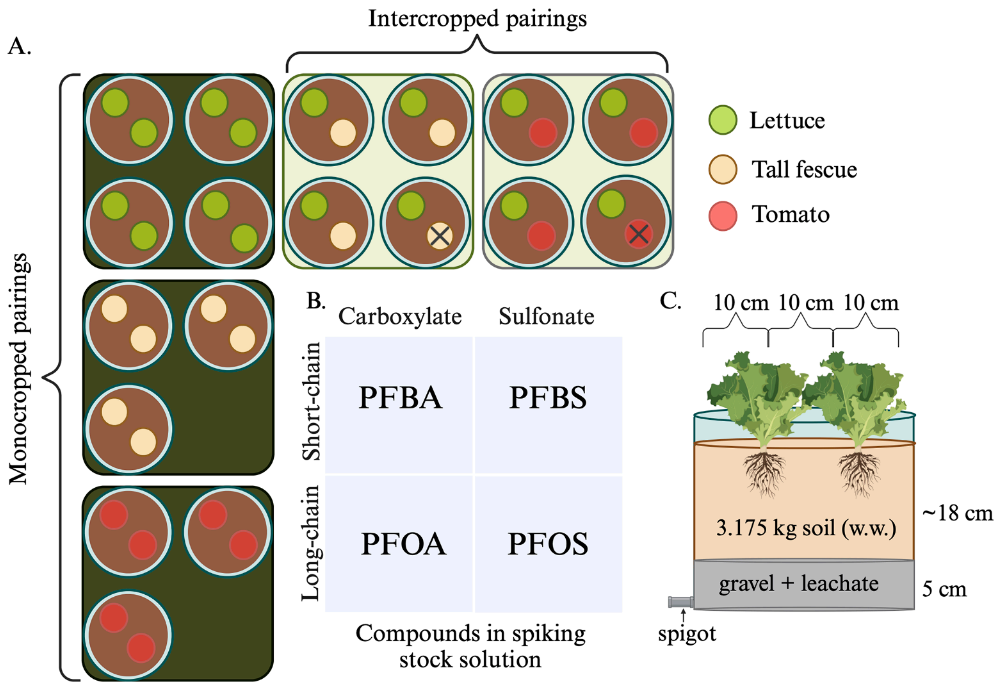

Fig. 1. A) Design of greenhouse experiment showing monocropped and intercropped plantings. Each small circle represents one crop individual within a pot represented by the larger circle. Plants marked with an "X" were not sampled. The sample arrangement was randomized; B) representation of the four compounds used in the potting mix spiking solution and their associated chemical characteristics (chain length and functional group); C) the pot design highlighting crop spacing, mass of potting mix, and pot dimensions.

(Table S1). To account for this, we focus this study on the ratio of PFAS concentration in the crop tissue relative to potting media, known as the BCF.

2.3. PFAS sampling

2.3.1. Crop tissue

Destructive sampling of crop tissue took place at crop maturity (lettuce at 42 days, tall fescue at 78 days, and tomato at 106 days). Tissue samples were separated into roots, leaves, stems, and fruits as applicable. In monocropped treatments, tissues were taken from individuals within each pot and combined into one composite sample per pot.

For all species, roots were removed from the potting media by hand. Researchers wore nitrile powderless gloves to carefully separate the potting media from the edge of the pot and worked inward to extract the root biomass with minimal breakage. To separate the above and belowground plant portions, the plant was cut with scissors along the potting media residue line. Roots were then rinsed of potting media by repeatedly dunking them in PFAS-free water and removing particles with forceps or gloved fingers, recognizing the infeasibility of the roots being completely clean of all potting media particles. At least five grams of roots (including coarse and fine hairs) were collected for each sample.

In monocropped pots for all species, composite leaf samples were collected by combining three leaves each from the bottom, middle, and top of both individual plants. In intercropped pots, six leaves were sampled from the bottom, middle, and top of the plant and combined for a total of 18 leaves per composite sample. Petioles were not included in leaf portions.

Tomato stems were sampled by cutting off all petioles until only the bare stem was present. The most mature fruits were chosen for sampling, all of which were judged as ripe or almost ripe based on color. As many ripe fruits as possible went into the composite sample for each pot, ranging from one to four fruits. Fruits with more than 20% damage were not sampled. Most damage was attributed to blossom end-rot, suspected to be a function of calcium deficiency.

2.3.2. Potting media

Samples of the potting media were collected immediately following destructive tissue sampling of each crop. Prior to collection, crops were harvested, and root samples were shaken into the pot for one minute to ensure that particles from the rhizosphere were included. Afterwards, a soil probe was used to sample three cores from the potting mix extending from the surface to the base of the potting media, where the gravel was present. These three cores were mixed, and a subsample was collected for PFAS analysis (Table S2).

2.4. PFAS analyses and quality control

All PFAS samples were analyzed according to EPA method 537.1 and modified for analysis of crop tissue and potting media by Pace Laboratories (Minneapolis, MN, USA). Crop tissue samples were ≥ 2 g each and potting mix samples were ≥ 5 g each. Crop and potting mix samples were cryo-milled and analyzed on a dry weight basis using LC/MS/MS. Two randomly selected tissue samples and one potting media sample were analyzed in duplicate for quality control. Additionally, an equipment blank was submitted at each sampling event. Equipment blanks used PFAS-free water to rinse equipment following between-sample cleaning protocols to check for cross-contamination between samples. One field blank was conducted by briefly exposing a sample of PFAS-free water to the greenhouse environment to ensure that the air was not further exposing crops to unintended contamination.

2.5. Calculations and statistical analyses

To calculate the BCFs of each PFAS compound in each crop compartment within each species, the following equation was used:

$$BCF = \frac{PFAS\ concentration\ in\ crop\ tissue\ \left(\frac{ng}{g}d.w.\right)}{PFAS\ concentration\ in\ potting\ media\ \left(\frac{ng}{g}d.w.\right)} \quad (1)$$

BCF is synonymous with the term *transfer factor* which is occasionally used in other literature (Costello & Lee, 2020; Scearce et al., 2023; Simones et al., 2024). The term BCF was chosen to be consistent with literature showing crop-compartment specific transfer factors of PFAS (Mei et al., 2021; Xu et al., 2022). To calculate BCFs with this equation, PFAS results below the practical quantitation limit (PQL), commonly denoted as NDs, were treated as zeros. Several assumptions were made in calculating BCFs including negligible biotransformation of compounds and the only pathway of contamination being from spiked potting media, not air or human contact.

All data analyses were performed in R (R Core Team, 2022). Normality was checked using Shapiro-Wilks normality tests, and p-values less than 0.05 were considered statistically different from the normal distribution. Prior to calculating BCF means, it was tested whether BCFs of monocropped and intercropped pairings were significantly different for each compound, compartment, and species using an Analysis of Variance (ANOVA), and results were verified with non-parametric Kruskal-Wallis tests in those instances where groups violated normality assumptions (Section 3.4). If not statistically different, monocropped and intercropped groups were combined for a more robust sample size from which mean BCFs were calculated.

Comparisons of BCFs between crops (Section 3.1), crop compartments (Section 3.2) and chemical characteristics (Section 3.3) were made using an ANOVA for parametric data and Kruskal-Wallis test for nonparametric data. In these tests, "pot" was added as a blocking term to account for variation within treatments followed by Tukey's HSD post-hoc tests, when applicable. Cross-compound comparisons may additionally have been confounded by high levels of variability within samples and zeroes used for results below the PQL. For additional information on methodology, please see the methods video found in Supplementary Material (Video S1).

3. Results

3.1. Species-level differences

Both PFAS compound and crop compartment influenced BCFs individually and interactively. Within edible portions (Table 1, bolded), PFBA BCFs were 3.5 times greater in tomato fruits than in lettuce leaves while no significant differences were identified between tomato fruits and tall fescue leaves. PFBS and PFOA BCFs were 5-100 times greater in tall fescue leaves than lettuce leaves and tomato fruits. No significant differences were detected in BCFs of PFOS across edible portions. Within each species, the mean edible portion BCFs of each compound were ranked as follows, although differences were not always statistically distinct: PFBA > PFBS > PFOA > PFOS.

When functionally similar compartments were compared between species, the order of BCFs from highest to lowest differed by crop compartment, compound and species (Table 1). In roots, transfer of short-chained compounds, PFBA and PFBS, was highest in lettuce and lowest in tall fescue (PFBA: $F = 13.5$, $p < 0.001$; PFBS: $F = 4.76$, $p = 0.02$), but long-chain PFOA and PFOS transfer was highest into tomato roots (PFOA: $F = 86.5$, $p < 0.001$, PFOS: $F = 30.7$, $p < 0.001$). PFAS transfer into leaves was greater in tomato than tall fescue and lettuce across chain lengths (PFBA: $F = 9.16$, $p < 0.001$; PFBS: $F = 198$, $p < 0.001$; PFOA: $F = 43.6$, $p < 0.001$, and PFOS: $F = 61.7$, $p < 0.001$). Results of TukeyHSD tests can be found in supplementary material (Figure S2).

Table 1
Summary of BCFs (mean ± standard deviation) of four PFAS compounds (PFBA, PFBS, PFOA, PFOS) in lettuce, tall fescue and tomato across compartments and compounds. Edible portions are bolded. Letters note TukeyHSD results comparing like compartments across species.

Species	Plant compartment	PFBA	PFBS	PFOA	PFOS
Lettuce	**Leaf**	**4.5 ± 2.5[b]**	**0.16 ± 0.09[c]**	**0.04 ± 0.02[b]**	**0.01 ± 0.02[b]**
	Root	1.1 ± 0.41[a]	0.070 ± 0.03[a]	0.05 ± 0.02[b]	0.05 ± 0.03[b]
Tall fescue	**Leaf**	**8.5 ± 3.6[b]**	**0.94 ± 0.45[b]**	**0.18 ± 0.14[b]**	**0.03 ± 0.05[b]**
	Root	0.03 ± 0.03[b]	0.01 ± 0.01[b]	0.02 ± 0.01[c]	0.04 ± 0.01[b]
Tomato	**Fruit**	**16 ± 14**	**0.01 ± 0.01**	**0.00 ± 0.01**	**0.00 ± 0.00**
	Stem	0.50 ± 0.55	0.01 ± 0.01	0.06 ± 0.04	0.04 ± 0.02
	Leaf	18 ± 12[a]	4.3 ± 0.73[a]	1.8 ± 0.79 [a]	0.23 ± 0.06[a]
	Root	0.39 ± 0.05[b]	0.06 ± 0.02[ab]	0.20 ± 0.02[a]	0.18 ± 0.02[a]

3.2. Crop compartment differences

BCFs within each species often showed greater transfer of PFAS into aboveground compartments than roots, but there were several exceptions, specifically among less mobile compounds (Fig. 2). In leaves of lettuce, tall fescue, and tomato, BCFs of short-chained PFBA and PFBS were higher in aboveground portions compared to roots. The picture became more complex for longer-chained molecules in lettuce and tall fescue. In lettuce, PFOA BCFs showed no difference by compartment, though PFOS BCFs were greater in roots than leaves. In both tall fescue and tomato, PFOA BCFs were greater in leaves than roots, but there was no statistically significant difference in PFOS BCFs. Tomato was the only crop to provide stem and fruit BCFs, which had lower BCFs of PFBS, PFOA and PFOS than roots, despite all these BCFs being greater in tomato leaves than roots.

3.3. Chain length and functional group influence BCFs

3.3.1. Trends across species

Across species and compartments, BCFs of short-chain compounds (PFBA and PFBS) were greater than long-chain counterparts (PFOA and PFOS) ($F = 16.2$, $p < 0.001$). However, there was no significant difference in BCFs of PFCAs versus PFSAs across crops and compartments ($F = 0.96$, $p = 0.33$).

Crops showed different levels of uptake by chain length and functional group within each compartment. For example, lettuce was the only crop to show greater short-chain than long-chain root BCFs (Table 2). Meanwhile, functional group differences of root BCFs were only identified in tomato roots, where PFCAs dominated. Tall fescue was the only species in which PFSA root BCF quantitatively exceeded PFCA root BCF, although the two were not significantly different from one another. In aboveground portions of all three species, leaf BCFs were greater among short-chain compounds than long-chain compounds. Lettuce, however, was the only species with a significant difference in functional groups in leaf BCFs, showing larger transfer of PFCAs than PFSAs. Like tomato roots, tomato stems showed no distinctions in transfer based on chain length, but did show a difference in transfer by functional groups, with greater stem BCFs of PFCAs than PFSAs (Table 2). In tomato fruits, transfer of short-chain compounds exceeded long-chain, but BCFs did not differ between functional groups (Table 2).

3.3.2. Trends within species

Within each species, chain length distributions differed across crop compartments. In lettuce, the transfer of short ($F = 8.26$, $p = 0.006$) and long-chain ($F = 14.9$, $p = 0.004$) compounds were higher in the leaves than the roots. Similarly, tall fescue transferred higher amounts of short-

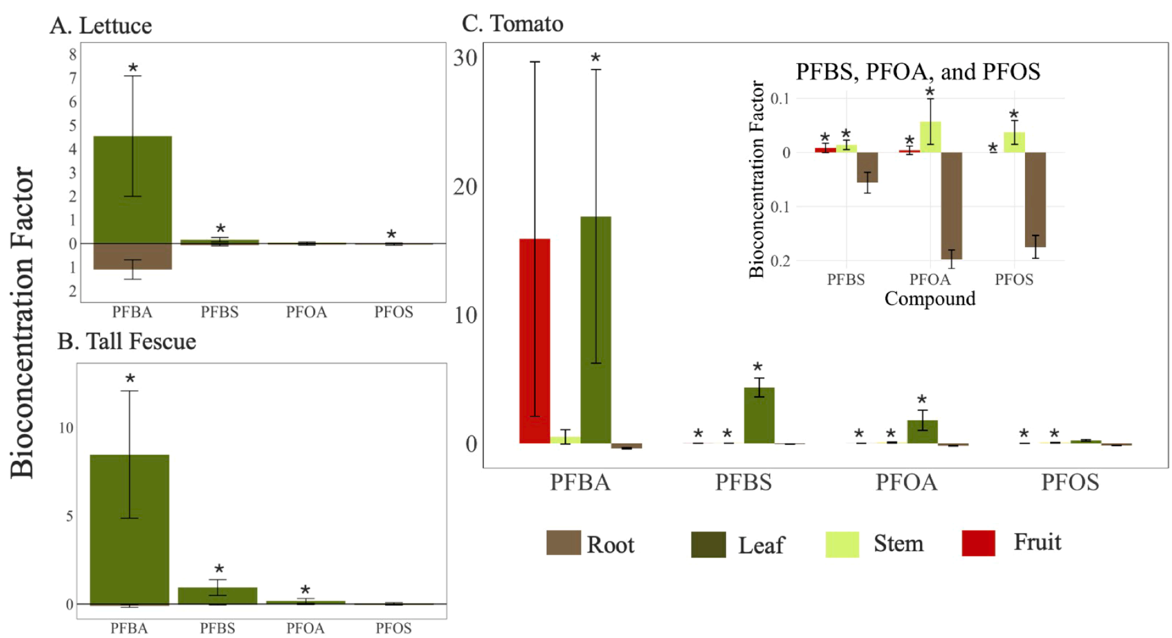

Fig. 2. BCFs for each plant compartment of monocropped lettuce (A), tall fescue (B), and tomato (C). Diverging y-axes on each plot show the mean ± standard deviation of aboveground (top) and belowground (bottom) BCFs. Asterisks indicate significant BCF differences ($p < 0.05$) in aboveground compartments compared to roots within compounds using Kruskal-Wallis tests. An inset on Fig. 2C aids in the visualization of smaller BCFs of PFBS, PFOA, and PFOS in tomato roots, stems, and fruits.

Table 2
Differences in crop uptake associated with chain length and functional group. Means and standard deviations of the BCFs are reported for each crop compartment within each species. Asterisks beside p values ($p < 0.05$) indicate significant differences in short versus long-chain or PFCA (C) versus PFSA (S) groups within a single crop compartment and species.

Species	Plant compartment	Chain length			Functional group		
		Type	BCF (mean ± SD)	p value	Type	BCF (mean ± SD)	p value
Lettuce	Leaf	Short	2.4 ± 2.8	< 0.001*	C	2.3 ± 2.9	0.005*
		Long	0.02 ± 0.57		S	0.08 ± 0.1	
	Root	Short	0.48 ± 0.58	< 0.001*	C	0.5 ± 0.6	0.06
		Long	0.05 ± 0.03		S	0.06 ± 0.03	
Tall fescue	Leaf	Short	4.7 ± 4.6	0.003*	C	4.3 ± 5.0	0.06
		Long	0.10 ± 0.13		S	0.48 ± 0.56	
	Root	Short	0.02 ± 0.02	0.34	C	0.02 ± 0.02	0.17
		Long	0.03 ± 0.02		S	0.02 ± 0.02	
Tomato	Fruit	Short	8.0 ±13	0.03*	C	7.9 ± 13	0.09
		Long	0.00 ± 0.01		S	0.00 ± 0.01	
	Stem	Short	0.25 ± 0.45	0.11	C	0.28 ± 0.44	0.01*
		Long	0.05 ± 0.03		S	0.03 ± 0.02	
	Leaf	Short	11 ± 10	0.004*	C	9.7 ± 11	0.08
		Long	1.0 ± 0.97		S	2.3 ± 2.2	
	Root	Short	0.23 ± 0.19	0.64	C	0.3 ± 0.11	0.01*
		Long	0.19 ± 0.02		S	0.12 ± 0.07	

chain compounds into leaves than roots ($F = 5.94$, $p = 0.03$). However, there was no distinction between BCFs of long-chain compounds in tall fescue ($F = 4.16$, $p = 0.05$). In tomato, short-chain transfer was higher in the leaf than the root, but otherwise there were no significant differences, suggesting that short-chain compounds were unevenly distributed across the crop. There was also evidence of uneven distribution of long-chain compounds, where transfer of long-chain PFAS into the leaves exceeded transfer into the roots, stem and fruit (p-adj = 0.02; p-adj = 0.003; p-adj < 0.001).

Similarly to chain length, functional groups were also distributed differently across crop compartments within species. While PFCAs and PFSAs were evenly distributed within lettuce, tall fescue and tomatoes showed uneven distribution. Specifically, tall fescue leaf PFCA transfer exceeded root transfer ($t = 14.7$, $p < 0.001$), despite there being no difference in PFSA transfer. Tomatoes showed greater BCFs of PFCAs in leaves compared to fruits ($t = -2.75$, p-adj = 0.02) and stems ($t = 3.5$, p-adj < 0.001). In tomatoes, PFSAs were greater in the fruits than the roots ($t = -3.05$, p-adj = 0.007). However, PFSA BCFs of the leaves exceeded those of the fruits ($t = 34$, p-adj < 0.001) and stems ($t = 34$, p-adj < 0.001).

3.4. Role of crop neighbor

Intercropping influenced BCFs for roots and edible portions, but these effects were limited to PFBA (Fig. 3). In lettuce, PFBA was the only compound showing significant differences across intercropping treatments in the roots, with lettuce/tall fescue pairings yielding the greatest lettuce root BCFs of PFBA ($F = 8.96$, $p = 0.02$). Lettuce leaves, however, had quantitatively but not significantly lower BCFs when the lettuce was planted alongside tall fescue, compared to lettuces in lettuce/lettuce and

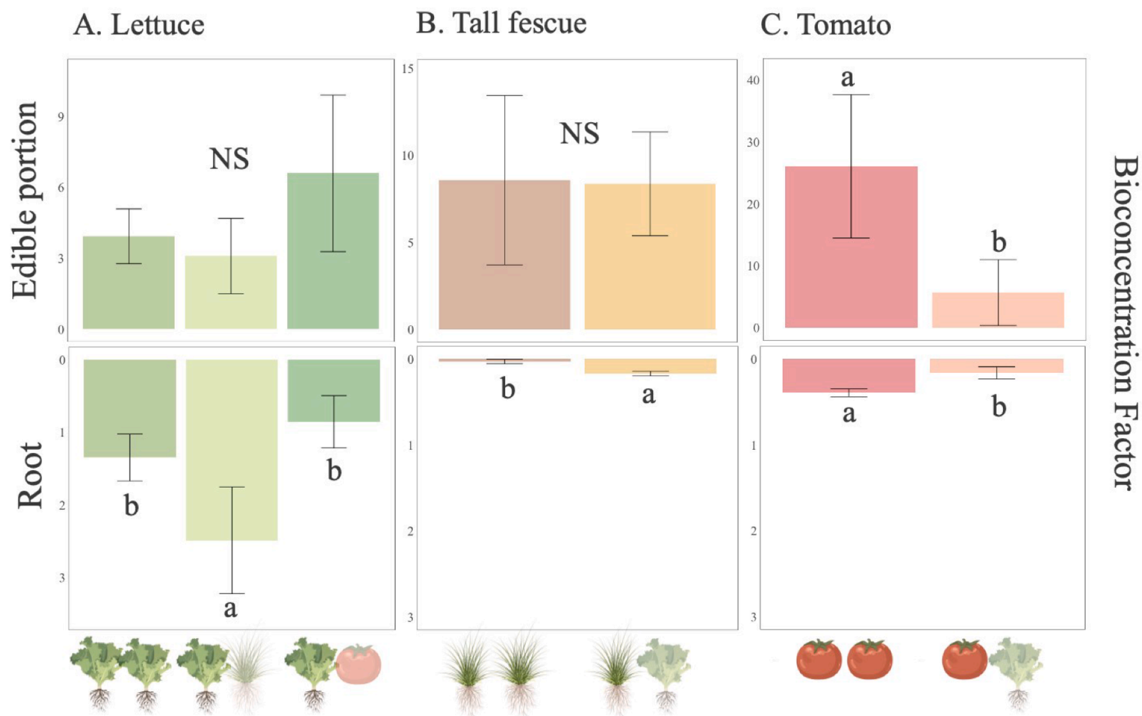

Fig. 3. BCFs of PFBA in edible portions (top) and roots (bottom) for lettuce (A), tall fescue (B) and tomato (C) under monocropped and intercropped conditions. The icons below each bar indicate the species pairings, and the leftmost icon of each pair is the focal species being reported by the bar. Each bar represents the mean, and the error bars indicate the standard deviation of the means. Letters indicate statistical differences ($p < 0.05$) between groups based on a two-way ANOVA followed by a TukeyHSD post-hoc test as applicable. NS indicates no significant differences between groups.

lettuce/tomato combinations.

In tall fescue, root BCFs of PFBA were significantly higher when tall fescue was planted with lettuce than when it was planted alongside another tall fescue ($F = 67.5$, $p = 0.003$). Root BCFs of other compounds showed no statistical differences as a function of crop pairing. There were also no influences of crop pairing on leaf BCFs of tall fescue.

In tomatoes, greater root BCFs were observed in monocropped compared to intercropped combinations. This trend was observed in both PFCA compounds, PFBA ($F = 19.1$, $p = 0.02$), and PFOA ($F = 168$, $p = 0.009$). There were no statistical differences in leaf or stem BCFs as a function of crop neighbors in tomatoes, but fruits were influenced by intercrop treatments. Notably, statistically significant differences in fruit BCFs as a function of crop pairings were observed in which lettuce/tomato combinations resulted in decreased BCFs of PFBA into tomato fruits ($F = 13.2$, $p = 0.04$).

4. Discussion

4.1. Species-level BCFs were lower than expected

BCFs reported by this study were lower than BCFs reported on a dry weight basis by other groups who conducted experiments in hydroponic and field conditions. For example, lettuce in this study yielded 5.7 times lower PFOA leaf BCFs than an average of previous pot studies reported by Costello and Lee (2020), and 6.1 times lower PFBA leaf BCFs than a study reported in municipal soil (Blaine et al., 2013). Likewise, transfer of tall fescue PFOS was 2.3 times lower than grasses, including tall fescue, reported by Yoo et al. (2011), and 1.3 times lower than the first cutting of grasses in a field dominated by tall fescue as reported by Simones et al. (2024). The same was true of tomato fruit, where this study reported lower fruit BCFs of PFBS, PFOA and PFOS than studies collected by Costello and Lee (2020) including Blaine et al. (2013). Tomato fruit BCFs of PFBA reported by our study exceeded the upper limit of the BCF ranges reported by other studies (Battisti et al., 2024; Blaine et al., 2013; Costello & Lee, 2020).

The lower-than-expected BCFs found in our study (except in tomato fruits) could be a result of treating concentrations below the PQLs, analogous to limits of quantitation (LOQs), as non-detects. Some studies preserve the uncertainty introduced by the laboratory by keeping the LOQs, which may result in comparatively fewer zeros in the data and higher mean concentrations (Battisti et al., 2024; Costello and Lee, 2020). This highlights a need for consistency among the treatment of non-detects across studies, especially for long-chain compounds which are often found in lower concentrations but have higher levels of adverse health impacts (Fenton et al., 2021). Another possible explanation could be the high concentrations of PFAS spiked into the soil. Bounds on the potential uptake of PFAS by a single plant are understudied, leading to uncertainty about the point at which soil concentration of PFAS becomes too high and irrelevant to demonstrate a meaningful relationship between the soil concentrations and plant concentrations through BCFs.

Rather than comparing specific BCFs, a more meaningful approach may be understanding which crops take up the most PFAS on a compound-by-compound basis and which take up the least. This would be more widely applicable to the range of conditions observed in contaminated agroecosystems. From our findings, the mean BCFs of PFBA among edible portions rank: Fruit vegetables > grasses > leafy vegetables (Table 1), however, BCFs of PFBS, PFOA and PFOS follow a different ranking: Grasses > leafy vegetables > fruit vegetables. In a review of the literature, leafy greens generally have higher BCFs than fruits and grains (Costello and Lee, 2020). Similarly, Liu et al., (2019) ranked BCFs of edible crop portions regardless of compound (in descending order) as follows: Leafy vegetables > fruit vegetables > grasses (Liu et al., 2019). These do not align with our findings, highlighting the necessity to report rankings by compound(s) to avoid overlooking the strong role played by the array of chemical characteristics of the PFAS family. However, our study did align with other reports of tomato fruits showing elevated levels of PFBA, while showing minimal or non-detectable PFBS, PFOA and PFOS (Battisti et al. 2024).

Other potential explanations for discrepancies in BCFs reported here versus other studies pertain to the use of potting media. Many uptake studies are conducted in hydroponic systems, or in municipal or industrial field soils. The use of potting mix in this study introduced characteristics that may have altered the bioavailability of PFAS to crops. Potting mix has larger particle sizes compared to most field soils. In a study utilizing a gradient of soil grain sizes, it was found that finer soil particles have greater capacity for PFAS sorption (Hubert et al., 2023). Based on this, it would be expected that larger particles such as those found in potting mix have less sorption potential than field soils with smaller particles, though we in fact saw the opposite for most compounds, except for PFBA. This introduces questions as to what other factors may be at play. One primary factor may be organic matter. Organic matter is more abundant in potting mix than field soil, generally, and has been linked to higher sorption of most compounds, except for PFBA, which is too soluble to be heavily influenced by organic matter (Hubert et al., 2023; Mei et al., 2021). Other potential factors could include differences of soil microbial communities, cultivar-specific uptake differences including root exudates, crop spacing, presence of a plant neighbor, limitation on growth due to container boundaries, and differences in bioavailability of spiked compounds in different growth media, many of which are sparsely documented in published methods or supplementary material (Bizkarguenaga et al., 2016; Lesmeister et al., 2021; Mei et al., 2021). It is also possible that there is variation that should be expected, and that these results fall within that range of expected variation, although this will only become clear as more studies are completed.

4.2. Crop compartment BCFs: Short-chain move aboveground, long-chain stay in roots

This study found that aboveground crop compartments consistently had higher short-chain PFAS BCFs compared to root compartments, except for PFBS in tomato (Fig. 2). Several studies align with our findings on this, while some report higher short-chain concentrations in the roots compared to the aboveground compartments, attributing this to root entry being the primary pathway of PFAS uptake (Blaine et al., 2014; Lesmeister et al., 2021). Compound distribution is relatively understood in the context of long-chain PFAS, for which the Casparian strip is thought to selectively restrict transport of compounds, more effectively preventing long-chain PFAS from reaching aboveground compartments (Lesmeister et al., 2021). Meanwhile, roots may be transporting rather than accumulating short-chain PFAS, leading to highly bioavailable short-chain compounds being moved out of the roots. Further, root BCFs in this study could be lower than other studies due to differences across studies in root tissue cleaning protocols. In this study, methods involved thorough cleaning of the roots and removing potting mix particles, which are also less likely to stick to roots than clay particles found in field soils (Supplementary Material Video S1). Other studies have documented concerns of root washing protocols and soil particle presence on crops driving elevated root BCFs, which may help explain why belowground BCFs in our study were lower than expected (Eun et al., 2020).

Root macrostructures including root surface area and fine root area have also been correlated with root BCFs (Costello & Lee, 2020; Lesmeister et al., 2021; Qian et al., 2023). Because tall fescue had the largest belowground biomass and most observed fine roots, it was expected that root BCFs would be highest in this species. However, tall fescue had the lowest root BCFs for all compounds, which is at odds with prior findings connecting root macrostructure and PFAS concentrations in ferns (Qian et al., 2023; Zhi et al., 2022). Moving forward, statements about the relationship between root macrostructures and root BCFs might be limitedly applied to closely related species with similar aboveground physiology.

Leaf area has also been found to correlate with BCFs (Zhi et al., 2022). Based on this, it was expected that lettuce would have had the highest leaf BCFs of all crop compartments, but instead it was found that tomato leaf BCFs exceeded those of lettuce and tall fescue leaves. This suggests that other factors may play a stronger role, at which point we assert the potential role of increased growing days as a driver of leaf BCFs. This is meaningful when thinking about crop selection, as long-season crops may be apt to accumulate more PFAS within a cropping cycle.

Additionally, physiological mechanisms such as the cambium barrier, a form of secondary growth in eudicots, present in tomato, have been hypothesized to shape the BCFs of this species (Lesmeister et al., 2021). The cambium serves as a barrier to non-selective apoplastic transport (Lesmeister et al., 2021). The present study reports elevated levels of PFBA in fruit portions, while significantly lower concentrations of other compounds, confirming that PFBA is highly mobile, and better able to overcome crop physiological barriers better than other compounds.

4.3. Chain length mediates BCFs more than functional group

Several studies have investigated chain-length dependencies of PFAS transfer across crop compartments and growth media, and most have found shorter chain compounds to be associated with higher BCFs (Bizkarguenaga et al., 2016; Blaine et al., 2014; Navarro et al., 2017; Wen et al., 2013; Zhao et al., 2018). Findings from this study corroborate literature reporting on chain-length-dependent uptake, where short-chain PFBA and PFBS showed significantly higher BCFs than long-chain PFOA and PFOS in most cases. One reason for this is that long-chain PFAS are more likely to stay bound to soil particles (Campos Pereira et al., 2018; Mejia-Avendaño et al., 2020).

When compounds are not sorbed to soil and are bioavailable to the plant, this study found that leaf portions had statistically higher BCFs of short-chain compounds than long-chain compounds, consistent with results reported elsewhere (Felizeter et al., 2012; Krippner et al., 2014; Navarro et al., 2017). This is thought to be a result of the selective transport taking place in the symplastic and transmembrane pathways of the root cortex which often filters out long-chain compounds due to larger molecular size and greater hydrophobicity (Weber & Miller, 1989).

In addition to chain length, the functional group was also linked to compartment-specific BCF differences. A comparison between uptake of PFCA and PFSA functional groups found that tomato roots had significantly higher root BCFs of PFCAs than PFSAs, but no statistically significant relationships between PFAS functional groups were observed in lettuce or tall fescue root BCFs. In aboveground portions, PFCAs were associated with higher BCFs of lettuce leaves and tomato stems, but otherwise there were no significant differences detected between functional groups. However, our results showed that BCFs of PFCAs exceeded PFSAs in every crop compartment besides all tall fescue roots (Table 2). This finding is aligned with the literature which states that PFCAs are often more bioavailable than PFSAs (Costello and Lee, 2020).

4.4. Intercropping: Alters uptake, often not favorably

The influence of crop neighbors on plant physiological processes including growth, water use, and heavy metal uptake have been well documented (Bybee-Finley et al., 2018; Wan and Lei, 2018; Wang et al., 2021). Our study only found crop neighbor interactions to be relevant for phytomanagement of highly mobile compounds such as PFBA into tomato fruit, while being less relevant for larger molecules with lower water solubility and greater tendencies to sorb to soil. Intercropping was considered as a phytomanagement option of interest due to success reported in heavy metal contaminated agroecosystems (An et al., 2011; Wang and Lei, 2018). However, the results of this study reinforce the importance of PFAS chemical structure, given that only PFBA uptake responded significantly to biotic plant-plant interactions.

Root BCFs of all three species were impacted by intercropping

(Fig. 3), raising questions about the viability of this cropping strategy for root crops. In root crops, the edible portion might be more directly influenced by crop neighbor interactions. Altered uptake of intercropped plantings is potentially influenced by whether intercropped root pairings interact or occupy different belowground rooting spaces (depth and width), which also suggests that crop spacing may have an impact on PFAS uptake.

Tomato with lettuce was the only intercropping treatment that successfully reduced the uptake of PFBA into the edible portion of tomato fruit. In an agroecosystem with high concentrations of PFBA contamination, it might be advised to plant lettuce alongside tomatoes if tomato fruits are the crop of choice. However, it should be noted that if lettuce is grown alongside tomato as a method of reducing PFBA in tomato fruit, the lettuce should not be consumed. It is necessary to integrate information on human health impacts of PFBA with outcomes of intercropping on reduced PFBA in edible portions to evaluate whether phytomanagement strategies that only reduce highly mobile, often less harmful, compounds would be worthwhile.

5. Conclusions

While BCFs reported in this study collectively agreed with trends observed in other studies, differences in mean values of BCFs within species, compartments and compounds sparks questions about the amount of variation that could be expected in the transfer of PFAS from soil to crop. Specifically, root BCFs across species were lower than expected and fruit BCFs of tomatoes were higher than expected for PFBA. It has been proposed that spiked soil offers greater bioavailability of PFAS, but the inconsistent directionality of BCFs across crop compartments indicate differences between our study and other studies are not solely attributable to the spiking of the potting media.

The association between chain length and BCFs aligned closely with other studies that found the two to be inversely related. Chain length proved to be more influential than functional groups on BCFs. As a result, more research should be done to understand the relationship between chain length and uptake across a gradient of contaminant concentration to test whether an inverse relationship exists between BCF and chain length across soil concentrations.

Lastly, results of crop pairings highlight that crop neighbors can both facilitate and interfere with uptake of PFAS, although this study did not attempt to answer how or why these results were observed. Most notably, tomato and lettuce pairings lowered PFBA BCFs of tomato fruits. Apart from tomato fruits, intercropping was largely unsuccessful for decreasing uptake into aboveground portions. Additional research could explore crop pairings further, specifically to address the phytomanagement of fields contaminated with short-chain PFAS, or the ability of intercropping to alter uptake into root crops. If conducted, these studies should consider crop mutualisms as well as give deeper thought to cropping combinations that align with culturally and economically beneficial practices likely to be implemented by growers. The findings of this study emphasize the need for continued research into the complex interactions affecting PFAS uptake by crops, particularly the roles of chain length, functional group, soil type and concentration, and crop pairings, to better inform future agricultural practices and contamination management strategies.

Data accessibility statement

The authors confirm that data supporting these findings are within the article.

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CRediT authorship contribution statement

Alexandra E. Scearce: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Jean D. MacRae:** Writing – review & editing, Methodology, Conceptualization. **Caleb P. Goossen:** Writing – review & editing, Investigation, Conceptualization. **Yong-Jiang Zhang:** Writing – review & editing, Resources. **Kylie P. Holt:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Rachel E. Schattman:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2025.100629.

Data availability

Data will be made available on request.

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