

TECHNICAL REPORT

Special Section: PFAS in Agroecosystems—Sources, Impacts, and Opportunities for Mitigating Risks to Human and Ecological Health

Effects of drinking water treatment residual amendments to biosolids on plant uptake of per- and polyfluoroalkyl substances

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Abstract

Drinking water treatment residuals (DWTRs), solid by-products of drinking water treatment, are dominated by calcium (Ca), iron (Fe), or aluminum (Al), depending on the coagulant used. DWTRs are often landfilled, but current research is exploring options for beneficial reuse. Previous studies have shown that Al- and Fe-rich materials have potential to reduce the mobility of per- and polyfluoroalkyl substances (PFAS). Here, we investigated how amending biosolids with 5% wt/wt DWTRs affected plant bioavailable PFAS in two different simulated scenarios: (1) agricultural scenario with *Solanum lycopersicum* (tomato) grown in soil amended with an agronomically relevant rate of DWTR-amended biosolids (0.9% w/w, resulting in 0.045% w/w DWTR in the biosolids-amended soil) and (2) mine reclamation scenario examining PFAS uptake by *Lolium perenne* (perennial ryegrass) grown in soil that received DWTR-amended biosolids amendment at a rate consistent with the mine remediation (13% w/w, resulting in 0.65% w/w DWTR in the biosolids-amended soil). Amending biosolids with Ca-DWTR significantly reduced perfluorobutanoic acid (PFBA) uptake in ryegrass and perfluorohexanoic acid uptake in tomatoes, possibly due to DWTR-induced pH elevation, while Fe-DWTR amendment reduced PFBA bioaccumulation in ryegrass. The Al-DWTR did not induce a significant reduction in accumulated PFAS compared to controls. Although the reasons for this finding are unclear, the relatively low PFAS concentrations in the biosolids and relatively high Al content in the biosolids and soil may be partially responsible.

Abbreviations: DWTR, drinking water treatment residuals; ICP-MS, inductively-coupled plasma mass spectrometer; PFAS, per- and polyfluorinated alkyl substances; PFBA, perfluorobutanoic acid; PFCAs, perfluoroalkyl carboxylic acids; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexanesulphonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFPeA, perfluoropentanoic acid; PFSA, perfluoroalkyl sulfonates; WHC, water holding capacity.

1 | INTRODUCTION

Per- and polyfluorinated alkyl substances (PFAS) are a family of chemicals that has received increased attention due to their potential human health risks and prevalence in the environment (Buck et al., 2011). First manufactured in the 1940s, PFAS have been used in non-stick cooking implements, packaging, waxes, paints, and aqueous firefighting foams (Vo et al., 2020). Characteristic of PFAS is the highly thermally and chemically stable carbon-fluorine bond, resulting in PFAS tails being hydrophobic (though PFAS heads are hydrophilic, the hydrophobic tail dominates molecular behavior) and resistant to natural degradation processes, therefore making them highly persistent in the environment (Buck et al., 2011; USEPA, 2014).

Studies have yielded evidence supporting linkages between high concentrations of certain PFAS and human health impacts including heightened cholesterol concentrations (due to perfluorooctanoic acid [PFOA], perfluorooctane sulfonate [PFOS], perfluorononanoic acid [PFNA], and perfluorodecanoic acid; Dunder et al., 2022; Jain & Ducatman, 2018), alterations in liver enzymes (due to PFOA, PFOS, and perfluorohexane sulfonate [PFHxS]; Sheng et al., 2018), high blood pressure in pregnant women (due to PFOA and PFOS; Birukov et al., 2021), minor reductions in newborn birth weights (due to PFOA and PFOS; Fei et al., 2007; Govarts et al., 2018), and in particular, reduced vaccine response in children (Grandjean, 2012; Grandjean, Heilmann, Weihe, Nielsen, Mogensen, & Budtz-Jorgensen, 2017; Grandjean, Heilmann, Weihe, Nielsen, Mogensen, Timmermann et al., 2017; Timmermann et al., 2020). In June 2022, the EPA's lifetime health advisories for four PFAS chemicals were updated to 0.004 ng L⁻¹ (for PFOA), 0.02 ng L⁻¹ (for PFOS), 0.01 ng L⁻¹ (for GenX), and 2 µg L⁻¹ (for perfluorobutane sulfonate [PFBS]) based on the results of child vaccine response studies (USEPA, 2022). Finally, in early 2023, the USEPA proposed maximum contaminants levels (MCLs) for six PFAS (PFOA, PFOS, PFBS, PFNA, PFHxS, and GenX) and in drinking water (USEPA, 2023b).

Biosolids are a solid product of the waste treatment process that have separated from the liquid portion of influent waste and chemically and physically treated (e.g., composting and heat treatment) to reduce pathogens and vector attraction (USEPA, 2023a). Land application of biosolids is a highly beneficial reuse of this material that which is otherwise often incinerated or landfilled. In addition to delivering nutrients, biosolids improve soil quality by enhancing water retention and replenishing soil organic matter (Nicholson et al., 2018). However, biosolids land application has been identified by the USEPA as a potential pathway by which PFAS enter the environment (Armstrong et al., 2016; Gallen et al., 2018; Helmer et al., 2022; Pepper et al., 2021; Stahl et al., 2018). The latter, coupled with aggressive drinking or groundwater regulations

Core Ideas

- Plant uptake of per- and polyfluorinated alkyl substances (PFAS) from biosolids-amended soil is unaffected by amendment of biosolids with Al-DWTR or Fe-DWTR (where DWTR is drinking water treatment residuals).
- Amending biosolids with Ca-DWTRs reduced perfluorobutanoic acid (PFBA) accumulation in ryegrass, likely due to pH modification.
- Amending biosolids with Al/Fe DWTRs may be more effective in reducing PFAS uptake in low Al/Fe high OM media.

on PFAS concentrations, has increased liability concerns, thus threatening the beneficial practice of land applying biosolids.

Some materials that are rich in aluminum (Al), iron (Fe), or calcium (Ca), such as alumina, boehmite, hematite, and clays, have shown an affinity to sorb PFAS, thus the potential to decrease PFAS mobility. Most of this research has focused on a few long-chain PFAS, namely, PFOA and PFOS (Hearon et al., 2022; Hellsing et al., 2016; Wang et al., 2012; Wang & Shih, 2011; Willemsen & Bourg, 2021). Particular attention has been paid to Al-rich compounds (Hellsing et al., 2016; Wang et al., 2012; Wang & Shih, 2011; Z. Zhang et al., 2021). PFAS sorption is affected by PFAS headgroup and perfluoroalkyl chain length. For example, PFAS with carboxyl heads are able to form inner-sphere complexes with Fe-carboxylates, whereas sulfonate PFAS can only form outer-sphere complexes with these surfaces (Du et al., 2014). However, the importance of headgroup is inversely correlated with PFAS hydrophobicity, which increases with increasing fluorocarbon chain length, thus as PFAS chain length decreases, non-hydrophobic partitioning becomes more important. Non-hydrophobic partitioning of PFAS is driven largely by electrostatic forces, particularly in acidic conditions, as PFAS (many of which exist as anions at environmentally relevant pH values) are attracted to the positive surface charge of variable charge Fe/Al minerals (Buck et al., 2011; Wang & Shih, 2011). One notable plant uptake study found that the Al-rich mineral montmorillonite (added to a soil/compost growth media at 2% wt/wt) decreased uptake of PFOA and PFOS in cucumber sprouts by ~51% and ~50%, respectively (Hearon et al., 2022).

Drinking water treatment residuals (DWTRs) are by-products of drinking water treatment. DWTR composition is dominated by coagulants, most often composed of Al hydroxides, Fe sulfates, or Fe chlorides, that are added to water to remove particulates and organic matter. Calcium hydroxide is also used in situations when the water requires only minimal

clarification (Ackah et al., 2018). Two million tons of DWTRs are generated every day in the United States alone, and as a result, significant research has been undertaken investigating beneficial reuses of these materials (Prakash & Sengupta, 2003; Turner et al., 2019). Currently, the most significant beneficial reuses for DWTRs are as a component in construction materials and land application for immobilization of contaminants or excess nutrients (Turner et al., 2019).

One recent study notably demonstrated the capabilities of Al-DWTRs to irreversibly sorb PFOA and PFOS in a bench-scale setting with the Al-DWTR sorbing ~92% of PFOA and ~98% of PFOS, and subsequent desorption steps showed sorption was almost irreversible (Z. Zhang et al., 2021). To the best of our knowledge, no similar studies have investigated the feasibility of using Fe and Ca-DWTRs as a way to reduce PFAS availability, and no studies have investigated the effects of adding various DWTRs to biosolids on plant uptake of PFAS.

Our objective in this study was to evaluate the efficacy of Ca, Fe, and Al-DWTR biosolids amendments in reducing the phytoavailability of PFAS in biosolids-amended soil. To address this objective, plant uptake studies were conducted under two different scenarios: (1) agricultural, where plant uptake was examined in tomato plants (*Solanum lycopersicum*) in soil amended with an agronomically relevant rate of biosolids, and (2) mine reclamation, where plant uptake was examined in ryegrass (*Lolium perenne*) grown in soil amended with biosolids at a rate appropriate for mine reclamation. We hypothesized that the Al-DWTR will be most effective in achieving these objectives due to electrostatic attractions between anionic PFAS forms and positively charged Al surfaces.

2 | MATERIALS AND METHODS

2.1 | Soil, DWTRs, and Biosolids

Soil (Candler sand; a deep, excessively drained Entisol common in Florida) was collected from the University of Florida/IFAS Plant Science Research and Education Unit located in Citra, FL (29.4106, -82.1640). DWTRs were collected from three drinking water treatment facilities in Florida using different coagulation approaches to obtain one sample each of Al-DWTR, Fe-DWTR, and Ca-DWTR. DWTRs and soil samples were air-dried and sieved to <2 mm. Class A biosolids were obtained from a large population-serving water resource facility that uses anaerobic wastewater digestion and sludge composting processes. These biosolids were produced in 2007 after an extended residence time (18 months) in the lagoons prior to composting, followed by long-term storage in a

warehouse. Biosolids were received at the University of Florida in 2021 and were subsequently freeze-dried (to remove moisture from condensation, etc.) and sieved to <2 mm.

A variety of physiochemical properties of the soil, biosolids, and DWTRs were characterized. Organic matter was determined by loss on ignition at 450°C (Mylavarapu & Moon, 2007), and pH was determined using EPA method 9045D (USEPA, 2004). Nitric-acid leachable Al and Fe contents (described as “total” Al and Fe hereafter) were determined via EPA method 3051A and subsequent analysis via an inductively coupled plasma mass spectrometer (ICP-MS; NexIon 300; Perkin Elmer; USEPA, 2007). Oxalate extractable Al and Fe were determined by acid ammonium oxalate solution extraction (Loeppert & Inskeep, 1996). Mehlich-3 (M3) extractable P and Ca of the soil and biosolids were determined via M3 extraction (Helmke & Sparks, 1996) and subsequent analysis via ICP-MS. All samples were run in triplicate.

Biosolids were extracted as described in Gravesen et al. (2023) (see Supporting Information for extraction details; Gravesen et al., 2023). Total PFAS content in biosolids extractions was characterized using a Shimadzu 8040 ultra-high-performance liquid chromatographic triple quadrupole mass spectrometer system (uPLC-MS/MS) in negative electrospray ionization (ESI) mode with multiple reaction monitoring (MRM) mode. The analytical column was Kinetex 5 µm EVO C18 (Phenomenex, 100 Å, 73 LC Column 100 × 2.1 mm) with a guard filter (Phenomenex, KrudKatcher ULTRA HPLC InLine Filter, 2.0 µm Depth Filter × 0.004 in ID) all maintained at 40°C, and a delay column (Restek, PFAS Delay Column, 5 µm, 50 × 2.1 mm) was employed prior to the guard and analytical columns for removal of potential contamination from the mobile phase system (see Table S1 for analysis details, including limits of quantitation [LOQ]). A gradient mobile phase run at 0.35 mL min⁻¹ consisted of 20 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B) with the following gradient: initially 10% B, increased to 50% in the first 0.5 min, 99% at 8 min and maintained for 5 min, decreased back to 10% in 0.5 min, and maintained for 20 min.

2.2 | Plant uptake experiment

Ryegrass and tomato plants were used to evaluate the effects of DWTRs on plant uptake of PFAS. Ryegrass and tomato (both of which are recommended test organisms in the USEPA Ecological Test Guidelines [USEPA, 2012]) were selected as plants representative of land reclamation and agronomic settings, respectively. Seeds were purchased from Great Basin Seeds and Johnny Seeds, respectively. The plant study

methodology was highly informed by the EPA's protocol for plant uptake and translocation tests (USEPA, 2012).

Biosolids were amended with one of three DWTRs at a rate of 50 g DWTR per kilogram of biosolids. This rate was selected based on preliminary screening of DWTR effects on ^{14}C radiolabeled PFOA retention (see Supporting Information for experimental details), where significant effects on PFOA retention were observed at this amendment rate. A control treatment of unamended biosolids was also included. After mixing, DWTR-treated biosolids were amended to soils at rates of 9 g biosolids per kilogram of soil (agricultural scenario; resulting in 0.45 g DWTR per kilogram of biosolids-amended soil) and 130 g biosolids per kilogram of soil (mine reclamation scenario; resulting in 6.5 g DWTR per kilogram of biosolids-amended soil), after which the growth media were homogenized. The agricultural scenario rate was based on guidance for nitrogen application to tomato crops: 200 pounds acre $^{-1}$ (0.224 Mg ha $^{-1}$) is the recommendation, but 150% of that recommendation may be applied (Hochmuth & Hanlon, 2000). As the biosolids were found to contain 3% total nitrogen, this is approximately equivalent to a 9 g kg $^{-1}$ biosolids application rate. The mine reclamation rate was based on the maximum rate recommended for mine reclamation of 100 pounds acre $^{-1}$ (224.2 Mg ha $^{-1}$; USEPA, 2000).

Four-inch pots were lined with polypropylene bags and filled with ~300 of biosolids-amended soil, brought to 60% water holding capacity (WHC), and planted with either three tomato seeds (agricultural) or ~30 ryegrass seeds (mine reclamation). Each treatment included six replicates. Pots were arranged randomly in a growth chamber set to a light cycle of 18 h on, 6 h off. A light meter placed at the top of the pots registered 17,500 lux. Plants were kept at 60% WHC through tri-weekly waterings with deionized water on Mondays and Fridays and 20% strength Hoagland's solution (Hoagland & Arnon, 1950) on Wednesdays. Tomato sprouts were culled to one seedling per pot once 50% of the total seeds had germinated. Tomato plants were harvested after 6 weeks, ryegrass after 5 weeks. Those endpoints were determined based on literature finding that 6 weeks was the point at which uptake of PFAS by ryegrass significantly decelerated (Wen et al., 2018) and the generation of sufficient tomato tissue for analysis, which the EPA protocol defines as an acceptable endpoint (USEPA, 2012). At harvest, ryegrass was clipped off above the soil line and tomato leaves were clipped off. Each plant's tissue was stored in a separate centrifuge tube. Plant matter was frozen, freeze-dried, chopped into small pieces, and sieved to <1 mm.

2.3 | Plant tissue sampling and analysis

PFAS standards (MPFAC-24ES, PFAC30PAR, FTA-MXA, and MFTA-MXA) were purchased from Wellington Laboratories (Guelph). An internal standard (40 μL of 125 ng mL $^{-1}$ mixture of Wellington PFAS standards) was added to each sample. Plant tissue was extracted for total PFAS content determination using an Optima-grade tetrahydrofuran and water 3:1 mixture using the method described in Hoover et al. (2017). The supernatants were concentrated under nitrogen and brought up to volume with HPLC-grade methanol and ultrapure water, then kept at 4°C prior to shipment to Purdue University for analysis. PFAS content was characterized using the uPLC-MS/MS as described previously.

2.4 | Statistical analysis

Statistical analysis of data was conducted using ANOVA tests to determine significance. The p -value used to determine significance was 0.05. Significant differences were examined between treatment groups (i.e., examining differences between the Ca, Fe, and Al DWTRs), and Tukey's honest significant difference (Tukey's HSD) test was used to do pairwise means comparisons when treatment(s) produced significant effects. One outlier was identified via the Grubbs test and excluded.

3 | RESULTS

3.1 | Growth media physiochemical characterizations

The physicochemical characteristics of the soils, biosolids, and DWTRs are summarized in Table 1. Each of the three DWTRs was dominated by their nominal constituent element (i.e., Al, Fe, or Ca). The Al-DWTR (24%) and Fe-DWTRs (43%) also contained a significant amount of organic matter, whereas the Ca-DWTR contained very little Al or Fe (3%). The pH varied substantially between the three DWTRs, from alkaline (Ca-DWTR, pH 9.7) to the Al-DWTR circumneutral (Al-DWTR, pH 6.9) to acidic (Fe-DWTR, pH 4.4). The biosolids used here contained relatively high total Fe and Al (20.7 and 20.8 g kg $^{-1}$, respectively) compared to the national median of 14.2 g kg $^{-1}$ for total Fe and 11 g kg $^{-1}$ for total Al (USEPA, 2005).

TABLE 1 Summary of physiochemical characteristics of the soil, biosolids, and drinking water treatment residuals (DWTRs).

Property mean $\pm$ SD	Soil	Biosolids	Al-DWTR	Fe-DWTR	Ca-DWTR
pH	6.5 $\pm$ 0.1	6.2 $\pm$ 0.0	6.9 $\pm$ 0.1	4.4 $\pm$ 0.0	9.7 $\pm$ 0.0
Organic matter (% mass)	0.9 $\pm$ 0.0	32.6 $\pm$ 0.5	24.3 $\pm$ 1.1	43.0 $\pm$ 0.2	2.6 $\pm$ 0.2
M3 extractable P (mg kg ⁻¹)	92.9 $\pm$ 6.5	967 $\pm$ 7.1	2720 $\pm$ 371	3520 $\pm$ 46.0	59.4 $\pm$ 9.8
M3 extractable Ca (mg kg ⁻¹)	29.5 $\pm$ 4.5	8730 $\pm$ 227	5100 $\pm$ 617	3350 $\pm$ 141	361,000 $\pm$ 4550
Total Al (g kg ⁻¹)	1.5 $\pm$ 0.2	20.8 $\pm$ 1.8	90.9 $\pm$ 12.9	1.5 $\pm$ 0.3	2.4 $\pm$ 0.1
Total Fe (g kg ⁻¹)	0.5 $\pm$ 0.1	20.7 $\pm$ 1.7	3.7 $\pm$ 0.5	272 $\pm$ 4.0	0.4 $\pm$ 0.0
Oxalate extractable Al (mg kg ⁻¹)	494 $\pm$ 33	17,000 $\pm$ 150	72,300 $\pm$ 7490	712 $\pm$ 107	1900 $\pm$ 36
Oxalate extractable Fe (mg kg ⁻¹)	343 $\pm$ 19	16,500 $\pm$ 485	1.1 $\pm$ 0.1	54.4 $\pm$ 5.5	0.2 $\pm$ 0.0

Abbreviations: M3, Mehlich-3; SD, standard deviation.

3.2 | PFAS in biosolids

The biosolids contained a wide variety of PFAS, including perfluoroalkyl carboxylic acids (PFCAs, C4-C14), perfluoroalkyl sulfonates (PFSAs, C4-C10), perfluoroalkyl sulfonamidoacetic acids, perfluorosulfonamides, and fluorinated telomers (Table 2). PFCAs concentrations ranged from 74 perfluorotetradecanoic acid to 789 (PFOA) ng g⁻¹. PFSAs concentrations were low (single digit concentrations) for the odd number chain lengths (C5, C7, and C9) compared to C4, C6, and C8, which ranged from 43.1 ng g⁻¹ to 1925 ng g⁻¹ with the highest PFSA being PFOS. Concentrations measured for perfluoroalkyl sulfonamidoacetic acids, perfluorosulfonamides, fluorinated telomer sulfonates, and saturated and unsaturated fluorotelomer carboxylic acids ranged from 560 to 953 ng g⁻¹, 6.4 to 48.3 ng g⁻¹, 48.3 - 263 ng g⁻¹, and 28.8 to 235 ng g⁻¹, respectively.

3.3 | Plant tissue PFAS content

The majority of the PFAS detected within the biosolids were not detected within exposed plant tissues. Among all the biosolids-originated PFAS, three PFCAs (perfluorobutanoic acid [PFBA], perfluoropentanoic acid [PFPeA], and perfluorohexanoic acid [PFHxA]) were detected in all plant samples from both species (Figures 1 and 2). Of these three, PFBA was most concentrated in both plant types followed by PFHxA in the tomato plant tissues (Figure 1) and PFPeA in the ryegrass (Figure 2). Also, almost all tomato plant tissues also had measurable PFOA concentrations as well (Figure 1). PFAS concentrations were approximately two-to four-fold higher in tomato plant tissues compared to ryegrass. In the tomato study (Figure 1), DWTR treatments did not result in statistically significant reductions, when compared to control, in the accumulation of any of the PFAS tested here. In the ryegrass experiment, however, both the Ca- and Fe-DWTR treatments resulted in significantly lower PFBA compared to control (Figure 2).

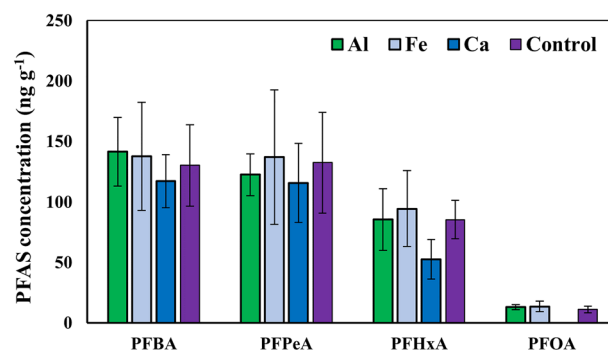


FIGURE 1 Mean per- and polyfluorinated alkyl substances (PFAS) concentrations (ng g⁻¹) detected in the leaves of tomatoes grown in biosolids-applied soil amended with Al-DWTR, Fe-DWTR, and Ca-DWTRs (where DWTR is drinking water treatment residuals) and non-amended controls (biosolids only). Error bars indicate standard deviation. Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA), and Perfluorooctanoic acid (PFOA).

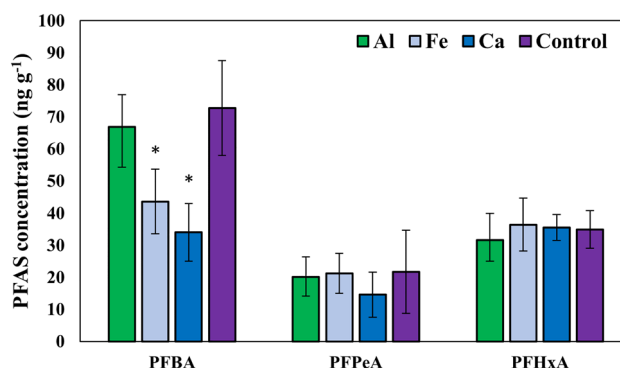


FIGURE 2 Mean per- and polyfluorinated alkyl substances (PFAS) concentrations (ng g⁻¹) detected in ryegrass grown in biosolids-applied soil amended with Al-DWTR, Fe-DWTR, and Ca-DWTRs (where DWTR is drinking water treatment residuals) and non-amended controls (biosolids only). * A result that is statistically significantly different from the control at $\alpha = 0.05$. Error bars indicate standard deviation. Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPeA), and Perfluorohexanoic acid (PFHxA).

TABLE 2 Concentrations of per- and polyfluorinated alkyl substances (PFAS) in biosolids.

Abbreviation	PFAS Name	Formula	Biosolids mean $\pm$ SD (ng g ⁻¹)
PFBA	Perfluorobutanoic acid	C ₄ HF ₇ O ₂	90.6 $\pm$ 21.1
PFPeA	Perfluoropentanoic acid	C ₅ HF ₉ O ₂	121.3 $\pm$ 6.8
PFHxA	Perfluorohexanoic acid	C ₆ HF ₁₁ O ₂	271.6 $\pm$ 15.5
PFHpA	Perfluoroheptanoic acid	C ₇ HF ₁₃ O ₂	187.3 $\pm$ 16.6
PFOA	Perfluorooctanoic acid	C ₈ HF ₁₅ O ₂	789.4 $\pm$ 61.6
PFNA	Perfluorononanoic acid	C ₉ HF ₁₇ O ₂	334.3 $\pm$ 28.2
PFDA	Perfluorodecanoic acid	C ₁₀ HF ₁₉ O ₂	430.2 $\pm$ 42.5
PFUdA	Perfluoroundecanoic acid	C ₁₁ HF ₂₁ O ₂	298.0 $\pm$ 11.8
PFDoA	Perfluorododecanoic acid	C ₁₂ HF ₂₃ O ₂	143.1 $\pm$ 17.9
PFTTrDA	Perfluorotridecanoic acid	C ₁₃ HF ₂₅ O ₂	148.0 $\pm$ 18.3
PFTeDA	Perfluorotetradecanoic acid	C ₁₄ HF ₂₇ O ₂	74.1 $\pm$ 1.0
L-PFBS	Perfluorobutanesulfonic acid	C ₄ HF ₉ O ₃ S	152.6 $\pm$ 13.6
L-PFPeS	Perfluoropentane sulfonic acid, linear	C ₅ HF ₁₁ O ₃ S	1.26 $\pm$ 0.04
PFHxS	Perfluorohexanesulphonic acid	C ₆ HF ₁₃ O ₃ S	43.1 $\pm$ 0.5
L-PFHpS	Perfluoroheptanesulfonic acid, linear	C ₇ HF ₁₅ O ₃ S	5.5 $\pm$ 1.4
PFOS	Perfluorooctanesulfonic acid	C ₈ HF ₁₇ O ₃ S	1924.9 $\pm$ 131.3
L-PFNS	Perfluorononanesulfonic acid	C ₉ HF ₁₉ O ₃ S	4.0 $\pm$ 0.7
L-PFDS	Perfluorodecane sulfonic acid	C ₁₀ HF ₂₁ O ₃ S	168.7 $\pm$ 7.7
N-MeFOSAA	N-Methylperfluorooctane sulfonamidoacetic acid	C ₁₁ H ₆ F ₁₇ NO ₄ S	952.6 $\pm$ 127.2
N-EtFOSAA	N-Ethylperfluorooctanesulfonamidoacetic acid	C ₁₂ H ₈ F ₁₇ NO ₄ S	560.1 $\pm$ 40.5
FBSA	Perfluorobutane sulfonamide	C ₄ H ₂ F ₉ NO ₂ S	34.2 $\pm$ 11.0
FHxSA	Perfluorohexane sulfonamide	C ₆ H ₂ F ₁₃ NO ₂ S	6.4 $\pm$ 2.5
FOSA	Perfluorooctanesulfonamide	C ₈ H ₂ F ₁₇ NO ₂ S	48.3 $\pm$ 8.1
6:2 FTS	6:2 Fluorotelomer sulfonate	C ₈ H ₅ F ₁₃ O ₃ S	145.6 $\pm$ 7.2
8:2 FTS	8:2 Fluorotelomer sulfonic acid	C ₁₀ H ₅ F ₁₇ O ₃ S	262.9 $\pm$ 27.7
5:3 FTCA	5:3 Fluorotelomer carboxylic acid	C ₈ H ₅ F ₁₁ O ₂	45.8 $\pm$ 12.1
7:3 FTCA	7:3 Fluorotelomer carboxylic acid	C ₁₀ H ₅ F ₁₅ O ₂	235.1 $\pm$ 42.1
8:2 FTUCA	8:2 Fluorotelomer unsaturated carboxylic acid	C ₁₀ H ₂ F ₁₆ O ₂	28.8 $\pm$ 10.6
10:2 FTUCA	10:2 Fluorotelomer unsaturated carboxylic acid	C ₁₂ H ₂ F ₂₀ O ₂	32.3 $\pm$ 16.4
6:2 diPAP	6:2 Fluorotelomer phosphate diester	C ₁₆ H ₈ F ₂₆ PO ₄ Na	29.3 $\pm$ 18.5

Abbreviation: SD, standard deviation.

4 | DISCUSSION

Despite the abundance of PFAS species identified in the biosolids, only three PFAS were detected in all plant samples (PFBA, PFPeA, and PFHxA), all of which are short-chain PFCAs. This finding is consistent with previously published reports indicating that short-chain PFCAs bioaccumulate in plants at higher concentrations than longer chain PFASs (Ahrens & Bundschuh, 2014; Blaine et al., 2013, 2014; Felizeter et al., 2012; Knight et al., 2021; Stahl et al., 2018) and that longer chain PFAS are more likely to strongly sorb to soil solids, becoming less bioavailable (Brusseau, 2023; Sorengard et al., 2020). PFBA specifically has consistently been found to bioaccumulate in plant shoots at higher rates than other widely studied PFAS (Blaine et al., 2013, 2014; Felizeter et al., 2012; Scher et al., 2018). Higher uptake of

PFBA is consistent with having a short carbon chain length and lower hydrophobicity relative to larger PFAS, and that plant uptake has been found to be inversely correlated with PFAS hydrophobicity (Felizeter et al., 2012). Short-chain, less-hydrophobic PFAS such as PFBA may pass more readily through the Casparian strip, a hydrophobic barrier in plant roots that helps control the uptake of water and solutes, resulting in greater bioaccumulation (L. Zhang et al., 2019). It has also been suggested that uptake of longer-chain PFAS may be an energy-dependent active process, whereas short-chain PFAS may also bioaccumulate via passive transport mechanisms such as through aquaporins and anion channels (L. Zhang et al., 2019).

Tomato leaves accumulated 2 to 4-fold more PFAS than the ryegrass, despite being grown in media containing a lower amount of biosolids or PFAS. Other work in this area has

reported that ryegrass (and other monocotyledonous species such as maize and wheat) accumulate relatively low amounts of PFOA and PFOS compared to dicotyledonous species such as tomatoes (Wen et al., 2016). This is potentially the result of shoot protein content, as monocots such as ryegrass (7.6% shoot protein content) and maize (6.4%) have significantly less shoot tissue protein content than dicots such as soybean (16%), radish (13%), lettuce (20%), and mung bean (29%). PFAS have been found to preferentially bioaccumulate in proteins, and there is evidence that PFAS uptake by plants is largely an active process reliant on transporter proteins (Wen et al., 2016; L. Zhang et al., 2019). While relatively little data are available to evaluate the correlation between shoot protein content and short-chain PFAS bioaccumulation, the greater amount of accumulation measured in the tomatoes compared to the ryegrass reported here is consistent with protein content playing a key role (assuming that the ryegrass tissue analyzed here is low in protein compared to the tomato tissue, consistent with published data). Another potential consideration regarding variations in PFAS uptake between species is variations in transpiration volume between species, as plants transpiring more and pulling more water from the soil would seemingly be able to take up more PFAS than a plant transpiring less. While a link between PFAS and transpiration volume was not investigated here, it is unlikely that differences in hydraulic conductivity or transpiration volume between species are related to the large difference in uptake between species observed here, as the plants were grown for similar portions of their life cycles in the same moisture or humidity conditions (environmental factors and leaf surface area are drivers of plant transpiration volume).

The Al-DWTR, which we had hypothesized would be most effective in reducing plant uptake of PFAS, did not significantly reduce bioaccumulation. Our hypothesis was primarily based on existing evidence that Al-DWTRs were effective in irreversibly sorbing PFAS in a lab-scale study (Zhang et al., 2021) and our own ^{14}C PFOA sorption or desorption study, where we saw ^{14}C PFOA retention approximately double in biosolids treated with Al, Fe, or Ca DWTRs (Table S2; see Supporting Information for experimental details). While the reasons for the disconnect between the results of these plant exposures and our batch sorption/desorption results/previously published research are unclear, we hypothesize that the characteristics of the PFAS tested as well as the media used here were influential. As previously noted, phytoaccumulation of PFAS is greatest for short-chain carboxylates, particularly PFBA (Blaine et al., 2013, 2014; Felizeter et al., 2012). It is possible that short-chain PFCAs are not as strongly influenced by the presence of high Al phases as long-chain PFAS like the ^{14}C PFOA used in our sorption or desorption experiments. Furthermore, our media contained a mix of both soil and biosolids that were relatively high in Al and Fe content. The relatively low concentrations

of PFAS coupled with the relatively high concentrations of Al and Fe in the media may have resulted in a muted response to Fe- and Al-based amendments. Supporting this speculation are results from another ^{14}C PFOA partitioning study conducted using biosolids that had substantially lower Al and Fe content but much higher organic matter (Table S2). For the latter biosolids, retention of ^{14}C PFOA after three desorption steps was 1.86 and 2.53 times greater with Al-DWTR and Fe-DWTR amendments, respectively, than observed with the DWTR-amended biosolids used in the current plant-uptake study (Table S2).

The Ca-DWTR treatment resulted in a statistically significant reduction in tissue PFBA in ryegrass compared to the control. The reason that Ca-DWTR reduced PFAS bioavailability is unknown, but possibly the result of Ca-DWTR-induced change to overall soil pH. application of Ca-DWTRs raised the pH of the growth media by ~ 0.6 units, a significant increase in alkalinity versus the control treatments. However, increases in pH are correlated to decreases in PFAS retention, and consequently we would expect a slight increase in PFAS uptake by plants as pH increases (Liu et al., 2021; Lyu & Brusseau, 2020; Wang & Shih, 2011; Z. Zhang et al., 2021). However, an increase in pH has a concomitant decrease in available positive charges on variable charge Al and Fe oxides. It may be possible that the higher pH and resulting increase in PFAS mobility allowed the PFAS to migrate through the growing media to the bottom of the exposure pots, below the root zones of the plants used here. This is consistent with the Ca-DWTR only yielding treatment effects in the ryegrass, which has a shallower root system than tomatoes.

The Fe-DWTR also resulted in less uptake of PFBA into ryegrass as compared to the control. This could have been attributed to the material's elevated organic matter content (this DWTR was $\sim 43\%$ organic matter—almost double that of the Al-DWTR and ~ 20 times as much as the Ca-DWTR). High organic matter content has been identified as one variable (in addition to variables such as oxalate extractable Al and Fe content, which represent weakly crystalline Al and Fe mineral phases) linked to PFAS retention and could be responsible for this DWTR's success in reducing PFBA bioaccumulation (Blaine et al., 2013, 2014; Higgins & Luthy, 2006; Wen et al., 2014).

5 | CONCLUSIONS

Under the conditions studied here, which include realistic amendment rates and biosolids PFAS concentrations, amending biosolids with both Al-DWTR and Fe-DWTRs resulted in no significant reductions in PFAS uptake in either ryegrass (mine reclamation scenario) or tomato (agronomic scenario). While these results suggest that adding DWTRs to biosolids would result in only a limited reduction in PFAS

bioavailability, the biosolids used had relatively high Al and Fe content and low organic matter, which may have contributed to the lack of response to the Al-DWTR and Fe-DWTR amendments. Therefore, additional research with biosolids of varying characteristics is warranted to better understand under what conditions addition of DWTRs may yield beneficial outcomes.

AUTHOR CONTRIBUTIONS

Emma Broadbent: Investigation; methodology; writing—original draft. **Caleb Gravesen:** Methodology; formal analysis; writing—review and editing. **Youn Jeong Choi:** Formal analysis; methodology; writing—review and editing. **Linda Lee:** Conceptualization; supervision; methodology; formal analysis; writing—review and editing; funding acquisition. **Patrick C. Wilson:** Methodology; writing—review and editing. **Jonathan D. Judy:** Conceptualization; supervision; investigation; methodology; writing—original draft; funding acquisition.

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[Correction added on 05 September 2024, after first online publication: Acknowledgment is amended by adding a disclaimer.]

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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