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Characterization of per- and polyfluoroalkyl substances (PFAS) in willow and poplar and the impact of soil amendments on accumulation rates

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ABSTRACT

Phytoremediation technologies have the potential to be cost-effective solutions for managing per- and polyfluoroalkyl substances (PFAS). In this greenhouse study, we assessed the uptake of PFAS using two plant species commonly used for phytoremediation, *Salix miyabeana* (willow) and *Populus trichocarpa* (poplar). We also assessed the impact of a commercially available growth phytohormone (naphthalene acetic acid (NAA)) and a microbial amendment on plant growth and PFAS uptake. Overall, uptake was observed, depending on perfluorocarbon chain length and functional group. After 90 days, the uptake of individual PFAS in plants grown in PFAS contaminated soil ranged from 0.02 % to 35 % dry weight (dw) for willow and 0.4–29 % for poplar. Within plants, short chain PFAS (i.e., C_{4-7} perfluoroalkyl carboxylates (PFCA) and C_4 perfluoroalkyl sulfonates (PFSA)) primarily accumulated in aboveground biomass, whereas longer chained homologues (C_{8-14} PFCA, C_{6-8} PFSA) primarily accumulated in the roots. For hormone and microbial amendments, there were no statistically significant trends for both willow and poplar ($p > 0.05$). Interestingly, the microbial community composition did not shift based on PFAS exposure but did shift based on plant-species. The PFAS mass balance for willow and poplar after 90 days approached 100 % ($p > 0.05$) for all PFAS except PFBA, PFPeA, PFOS, and FOSA. These results suggest that while willow and poplar have the potential to extract short chain PFAS from soil, phytoremediation may be more effective at stabilizing PFAS within a given area (i.e., providing hydraulic control) than extracting.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) and perfluoroalkyl acids (PFAAs) are anthropogenically produced chemicals that are prevalent and persistent in the environment (Gluge et al., 2020; Cousins et al., 2020). They consist of carbon-carbon and carbon-fluorine (C-F) bonds and have been used in various industrial and consumer applications, including aqueous film-forming foams (AFFFs), non-stick coatings, water and stain repellents, and food packaging (Krafft and Riess, 2015). Areas in which PFAS-based products are utilized or disposed of (e.g., firefighting training areas, landfills) can serve as potential long-term sources of PFAS, as adsorbed material can continue to leach from these sources into the environment (Dauchy et al., 2017; Masoner et al., 2020). Their presence and persistence in the environment, and their potential uptake into some living organisms, have raised concerns over their potential effects on ecosystem and human health (De Silva et al., 2021; Sinclair et al., 2020). Over the last decade, regulatory criteria, or thresholds for PFAS in soil, groundwater, and drinking water sources, have become lower in many jurisdictions around the globe, sometimes as low as parts per trillion or parts per quadrillion concentration ranges (Scheringer et al., 2022). The concerns around legacy PFAS sites, and the need to meet evolving regulatory requirements, have provided a case for action towards simple, cost-effective, remediation and mitigation techniques.

Various PFAS remediation strategies are being explored today, including adsorption with activated carbon, destructive treatment using advanced oxidation processes, and physical soil excavation (Sorengard et al., 2021; Merino et al., 2016; Bolan et al., 2021). However, the diffuse nature of PFAS in the environment, the complex technological requirements, and the costs and limitations of scale present hindrances to their broader application (Mahinroosta and Senevirathna, 2020). Phytoremediation is a treatment technique which can help mitigate the risks of a contaminated site by making use of the natural biological processes associated with plants to remove (i.e., phytoextraction), degrade (i.e., phytodegradation), or stabilize (e.g. hydraulic control, phytostabilization) a particular constituent of concern (COC) (Chakravarty et al., 2017). These approaches have been utilized, with varying levels of success (Vangronsveld et al., 2009), to address a wide range of issues, including metals and organic contamination, and as mechanism for plume control (i.e., hydraulic control) (Pilon-Smits, 2005).

The uptake of PFAS by plants has previously been examined across various plant species (Gobelius et al., 2017; Huff et al., 2020; Nassazzi et al., 2023; He et al., 2023; Sharma et al., 2020; Würth et al., 2023). *Salix spp.* (willow) and *Populus spp.* (poplar) are pragmatic choices for PFAS remediation as these species are already used for plume control, as well as COC degradation and stabilization; they also have rapid growth rates, relatively high biomass, high evapotranspiration rates, and good adaptability across a range of soil conditions (Zalesny Jr et al., 2019). Recent work has begun to characterize the movement of PFAS in willow and poplar to better understand the role these plants can play in PFAS remediation and risk mitigation (Sharma et al., 2020), with a particular focus on phytoextraction (Kavusi et al., 2023; Mayakaduwage et al., 2022; Ghisi et al., 2019). PFAS, specifically PFAAs, accumulate within a plant based on chain length, with longer chain lengths concentrating in the roots, and shorter chain lengths concentrating in the stems and leaves (Gobelius et al., 2017). However, most of the studies mentioned have performed relatively short-term experiments on PFAS uptake (Zhao et al., 2018; Zhang et al., 2019; Wen et al., 2013), making longer-term studies, ones which reflect field conditions, needed to better inform potential deployment. Previous studies have shown that PFAS can influence the microorganisms diversity in terrestrial and aquatic ecosystems and there is a potential that the microbial community can enhance the uptake of PFAS by plants by promoting availability and mobility of PFAS and plant growth (Liu et al., 2022; Arslan and Gamal El-Din, 2021; Huang et al., 2024; Li et al., 2024). For example, the Sphingomonadaceae and Rhizobiaceae microbial communities have shown to contribute to phytoremediation potential in terrestrial ecosystems (Liu et al., 2022). Furthermore, PFAS have shown to effect phytohormone signaling pathways in plants and potentially the uptake of PFAS (Zhang et al., 2022). Moreover, while efforts to improve phytoremediation removal efficiency using microbes and phytohormones have been tested for heavy metals and other constituents of concern (Ye et al., 2014, Hadi et al., 2021, Zhang and Liang, 2020, Nassazzi et al., 2023), more research is needed to understand the role of microbial community and hormones on the uptake of PFAS to improve phytoremediation strategies.

In this study, we characterized the uptake and accumulation of PFAS by *Salix miyabeana* (willow) and *Populus trichocarpa* (poplar) over several months. The specific objectives were to i) quantify PFAS concentration in different plant tissues (roots, stems, leaves) and the associated soil and water, ii) measure temporal changes in PFAS uptake in plants up to 210 days, iii) investigate the effect of microbial inoculation and a phytohormone (naphthalene acetic acid) on their uptake, and iv) assess the impact of the microbial community composition on the PFAS uptake by plants.

2. Materials and methods

2.1. Chemicals and materials

Target PFAS ($n = 15$) is comprised of C_4-C_{14} perfluoroalkyl carboxylates (PFCA) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA), C_4, C_6, C_8 perfluoroalkyl sulfonates (PFSA) (PFBS, PFHxS, PFOS), and perfluorooctanesulfonamide (FOSA) (see Table S1 in Supporting Information (SI)). Mass labelled internal standards (IS) were used for quantification and included $^{13}C_3$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_2$-PFTeDA, $^{13}C_3$-PFHxS, $^{13}C_8$-PFOS, and $^{13}C_8$-FOSA. Native standards and ISs (purity ≥99 %) were obtained from Wellington laboratories (Sweden).

2.2. Plant cultivation

Two plant species, willow and poplar, were grown in a greenhouse at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. Willow cuttings were obtained from a local grower near Örebro, Sweden. Poplar was obtained as 50 cm long bare-root seedlings from RomLösa Plantskola AB, Helsingborg, Sweden. It was assured that all selected initial plants were in good and consistent condition. The planting material was cleaned by dipping them in water with 10 % hydrogen peroxide and then rinsing them with ultra-pure water. Cleaned poplar seedlings were grown in 4 kg wet weight (ww) of pot soil as recommended by the provider. Willow cuttings (30 cm long each) were soaked in 200 mL tap water and left in darkness for 21 days to promote rooting. After 21 days, the rooted willow cuttings were transplanted into a 2 kg ww pot soil. PFAS spiked potting soil and one plant per pot was used. Potting soil (S-jord garden soil, Hasselfors, Sweden) was spiked to achieve a final concentration of 500 μg absolute for each PFAS ($n = 15$). After thoroughly mixing, the pots were covered with a plastic lid and stored in the greenhouse to age for one month. The plants were grown under the following conditions: temperature of 22°C during day and 18°C at night, light/dark at 16 hour/8 hour intervals, light intensity of 150 micromoles and a humidity of 50–60 % (for details see SI).

2.3. Experimental set-up

The setup included two plants (willow and poplar) and treatments of i) PFAS spiked soil without the addition of hormones or microbes, ii) with the addition of microbes, and iii) with the addition of a hormone, in triplicates for 90 days (Fig. 1). Additionally, the uptake of PFAS in willow plants was investigated in iv) PFAS-spiked soil over a seven-month duration (210 days), however, this experiment could only be performed with willow due to a lack of sufficiently healthy poplar seedlings. Experimental blanks were included in triplicates for quality control, including plants grown on non-PFAS spiked soil, non-PFAS spiked soil without plants, and PFAS spiked soil without plants. To study the effect of hormones on PFAS uptake, both the willow and poplar were soaked in a 2 mg L^{-1} naphthalene acetic acid solution for 12 hours to hasten rooting before being planted in pots. For treatments amended with microbes, a commercially available bacterial amendment (Tarantula®, Advanced Nutrients (for details see SI)) and a commercial-available arbuscular mycorrhizae-containing amendment (Mykos®, Xtreme Gardening (*Ryzophagus intraradices*)) were applied to both plant species to assess the effect on PFAS uptake over 90 days according to the manufactures instructions (for details see SI). Commercially available products have been tested to ensure that these products are suitable to be used and directly implemented as part of phytoremediation strategy in the field. The bacterial amendment was added to water and used to irrigate the plants for two weeks from the time of transplanting. The arbuscular mycorrhizae-containing amendment was added to the planting hole when transplanting the rooted cuttings into PFAS spiked pots. At harvest, samples of different plant tissue (leaves, stem, roots) and soil samples were collected for both chemical and microbial analysis.

2.4. PFAS analysis

After harvesting, the plants were weighed, and the number of shoots, plant height and biomass weight of the shoots and roots were recorded. Plant tissues were classified into leaves, stems, and roots. Soil and water samples were collected as well. Plant and soil samples were prepared and extracted using a validated method (Nassazzi et al., 2022). Water samples were also extracted using a validated method, as described previously (Gobelius et al., 2017, for details see SI).

Instrumental analysis was performed using ultra-high-pressure liquid-chromatography (SCIEX ExionLC AC system) coupled with tandem mass spectrometry (SCIEX Triple Quad™ 3500) (UHPLC-MS/MS), as described previously (Nassazzi et al., 2022). The column oven was set to 40 °C, and 10 μL of the sample was injected into Phenomenex Kinetex C18 (30 ×2.1 mm, 1.7 μm) precolumn coupled to a Phenomenex Gemini C18 (50 mm×2 mm, 3 μm) analytical column for chromatographic separation. MilliQ water with 10 mM ammonium acetate and methanol was used as mobile phase.

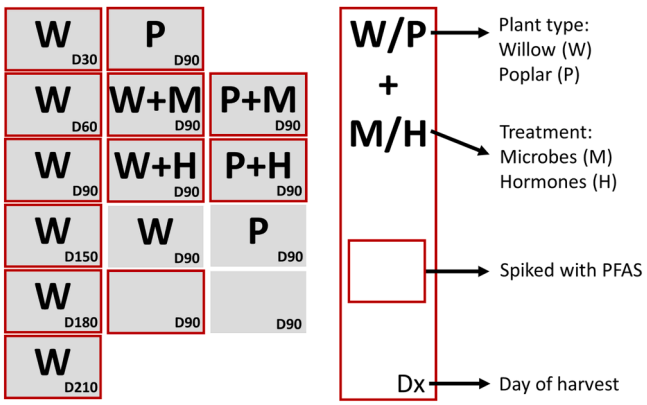

Fig. 1. Schematic diagram of the experimental setup of the plant pot experiments in the greenhouse (all in triplicate).

2.5. Quality assurance and control for PFAS analysis

The performance of the method was assessed based on blanks, method detection limits (MDLs) and recoveries. The blanks ranged from 0.28 to 2.6 ng g^{-1} dry weight (dw) for individual PFAS for all plant tissue and 0.01–1.4 ng g^{-1} dw for individual PFAS for soil samples. The MDL ranged from 0.055 to 4.8 ng g^{-1} dw for individual PFAS for all plant tissue, 0.15–4.5 ng g^{-1} dw for individual PFAS for soil and 0.05 ng L^{-1} for individual PFAS for water samples. The recoveries were an average of ~70 % for individual PFAS for analyzed samples (for details see Table S3 in SI).

2.6. Metagenomic and molecular analysis of the microbial communities

The soil bacterial community was assessed across the duration of the experiment. As a control, samples were analyzed from PFAS-free soil at the start of the experiment (S0), and after 90 days from pots without plants (S90). Soil samples were also collected after 90 days from pots grown with poplar (P90) and willow trees (W90). PFAS-spiked (+) soils were sampled 48 hours after transplanting for both willow ($W0^+$) and poplar ($P0^+$) and after 90 days without ($W90^+$ and $P90^+$) or with microbial supplement ($W90^+$_M and $P90^+$_M). Three replicates were analyzed from all samples except for Day 0 control soil (S0), which consisted of four replicates. Details on DNA extraction, amplification, and sequencing and/or quantification of bacterial and fungal organisms can be found in Supplementary Information.

2.7. Calculations and statistics

Various plant concentration factors (Nassazzi et al., 2023) represent the plant's ability to accumulate contaminants from soil; these were determined as follows:

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

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

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

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

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

Removal efficiency (r) within the experimental system was calculated as:

$$r = \frac{Cp\ Mp}{Csi\ Ms} \times 100 \quad (5)$$

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

For statistical analysis, log-linear regression analysis using individual values was carried out between concentration factors (LCF, SCF, RCF) and the perfluorocarbon chain length (Bewick et al., 2003). Student's t-test was performed to compare PFAS concentrations and composition for different treatments (Whitley and Ball, 2002). A multivariate comparison was performed on phyla data without S0 to assess the effect of time on the soil microbial community in PFAS-spiked soil with willow and poplar. A hierarchical clustering technique, based on the Ward's minimum variance method using the squared Euclidean distance, was used to cluster soils with similar bacterial community composition (Ward, 1963).

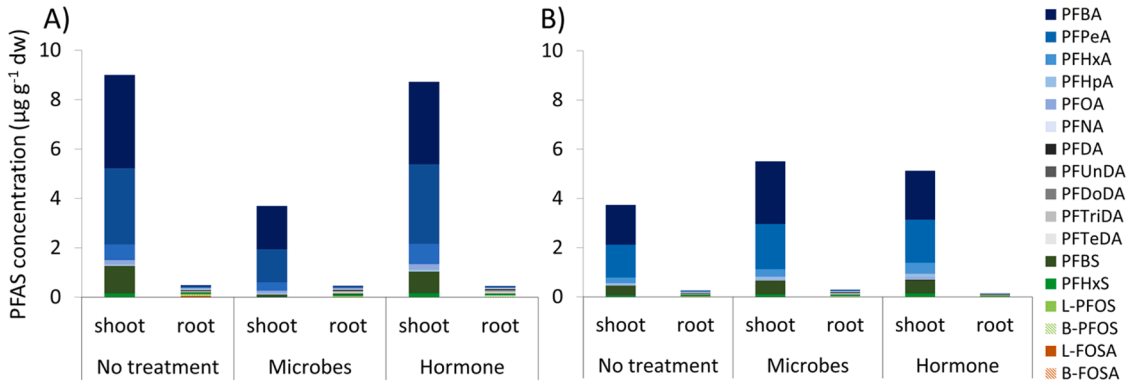

Fig. 2. PFAS concentration and distribution in shoot (sum of leaves and stem) and roots in A) willow and B) poplar under different experimental conditions after 30 days of exposure. For details and standard deviations see Table S4 in SI.

3. Results and discussion

3.1. PFAS concentration in willow and poplar

The concentration of the 15 spiked PFAS in willow and poplar, with or without the addition of microbes or a phytohormone (NAA),

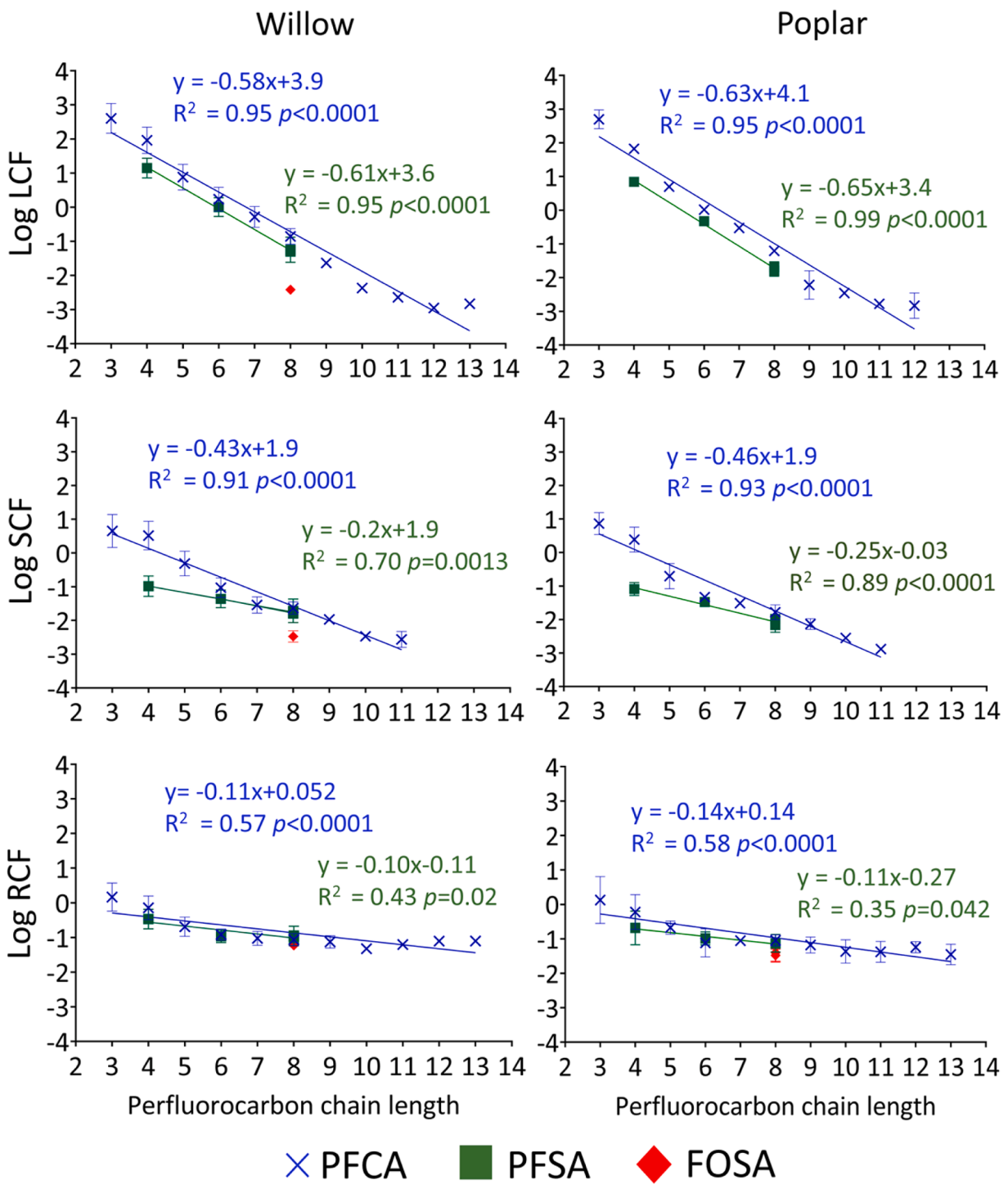

Fig. 3. The relationship between leaf concentration factor (LCF), stem concentration factor (SCF), root concentration factor (RCF) and perfluorocarbon chain length for willow and poplar plants grown on PFAS-spiked soil. For details see Table S6 in SI.

was monitored for 90 days after exposure (Fig. 2, Figures S1 and S2, and Table S4 in SI). For both species, ∑PFAS concentrations were significantly higher in the leaves compared to stems and roots for both plant species ($p < 0.05$, Student's t test) and were dominated by short chain PFAS (i.e., $C_{4–7}$ PFCA (85 % of ∑PFAS) and C_4 PFSA (13 % of ∑PFAS)). The higher concentrations of PFAS in leaves compared to stems and roots suggests that PFAS preferably accumulate in vegetative structures of plants, which could be partly due to enrichment from the greater transpiration stream (Nassazzi et al., 2023). Based on physical observations (e.g., growth), willow and poplar did not appear to be adversely affected by relatively high PFAS concentrations, even after seven months.

A similar PFAS composition was observed for the leaves and stem dominated by $C_{4–7}$ PFCA (85 % and 89 %, respectively, of ∑PFAS) and PFSA (14 % and 7.6 %, respectively) for willow, and $C_{4–7}$ PFCA (87 % and 84 %, respectively) and PFSA (12 % and 10 %, respectively) for poplar. In contrast, roots were observed to have mostly long-chain $C_{8–14}$ PFCA (37 % of ∑PFAS) and PFSA (31 %) for willow and $C_{8–14}$ PFCA (40 % of ∑PFAS) and PFSA (35 %) for poplar. FOSA was mainly detected in the roots, albeit with a low contribution in willow and poplar (1.6 % and 0.82 %, respectively). This partitioning of PFAS in plant tissue is consistent with data presented previously in willow (Huff et al., 2020) and other species (Würth et al., 2023; Ghisi et al., 2019; Gobelius et al., 2017).

The ∑PFAS concentration in willow was, on average, 19 ± 8.5 μg g^{-1} dw in the leaves, 0.48 ± 0.20 μg g^{-1} dw in the stem and 0.43 ± 0.12 μg g^{-1} dw in the roots, whereas the ∑PFAS concentration in poplar was, on average, 7.5 ± 3.0 μg g^{-1} dw in the leaves, 0.20 ± 0.08 μg g^{-1} dw in the stem and 0.26 ± 0.10 μg g^{-1} dw in the roots for individual PFAS after a 3 month exposure. These results are consistent with previous reports on PFAS plant compartment distributions, in both greenhouse experiments and field studies, for willow (Huff et al., 2020) and other plant species (Blaine et al., 2014; Krippner et al., 2015; Wen et al., 2014).

There was no significant difference in PFAS tissue concentrations relative to each species control in response to the microbial or hormone amendments ($p > 0.05$, Student's t test). In willow treated with the microbial amendment, individual PFAS concentrations were 50 % lower than the shoots treated with hormonal amendment or receiving no amendment (Fig. 2). For poplar, the ∑PFAS concentrations increased by 23 % and 36 % in shoots following treatment with either microbes or a hormone, respectively; however, the increase was not significant and there was no significant trend for individual PFAS ($p > 0.05$, Student's t test). The application of phytohormones and microbes has previously been observed to increase the phytoextraction potential of heavy metals (Israr and Sahi, 2008; Mench et al., 2009; Hąc-Wydro et al., 2017; Lu et al., 2020). However, we did not observe a similar increase with PFAS in willow or poplar and future studies are needed to assess if and how microbial communities and other soil amendments can influence the uptake of PFAS and if this effect truly varies across species (Bao et al., 2018; Cai et al., 2020; Xu et al., 2022, 2023).

3.2. Bioaccumulation factors

LCF, SCF, and RCF values for willow and poplar are shown in Fig. 3 and Tables S5 and S6 in SI. The LCF ranged from 0.0011 (PFTriDA) to 566 (PFBA), SCF from 0.0023 (FOSA) to 6.8 (PFBA), and RCF from 0.048 (PFUnDA) to 1.9 (PFBA) for willow (Table S5 in SI). Similarly, LCF ranged from 0.0011 (PFDoDA) to 580 (PFBA), SCF from 0.00088 (PFDoDA) to 8.9 (PFBA), and RCF from 0.035 (L-FOSA) to 2.3 (PFBA) for poplar (Table S6 in SI). No significant difference was observed between PFAS accumulation in the tissues of willow and poplar, which implies a similar PFAS accumulation potential for both plant species ($p > 0.05$, Student's t test).

The log transformed LCF, SCF, and RCF values had a significant negative linear correlation with perfluorocarbon chain length for PFCA and PFSA for both plant species ($p < 0.05$) (Fig. 3). The dependence of PFAS accumulation on perfluorocarbon chain length was strongest for the leaves (LCF slope −0.58 for willow and −0.63 for poplar) and weakest for the roots (RCF slope −0.11 for willow and −0.14 for poplar). Comparing the same perfluorocarbon chain length, bioaccumulation was stronger for PFCA compared to PFSA. For the C_8 perfluorocarbon chain length, bioaccumulation was: FOSA<PFOS<PFNA. These trends have previously been reported for other relationships (Blaine et al., 2013; Felizeter et al., 2012; Nassazzi et al., 2023; Sharma et al., 2020). Linear concentration factor relationships, as in this study, have been observed in previous studies (Zhao et al., 2018; Felizeter et al., 2014; Zhang et al., 2019;

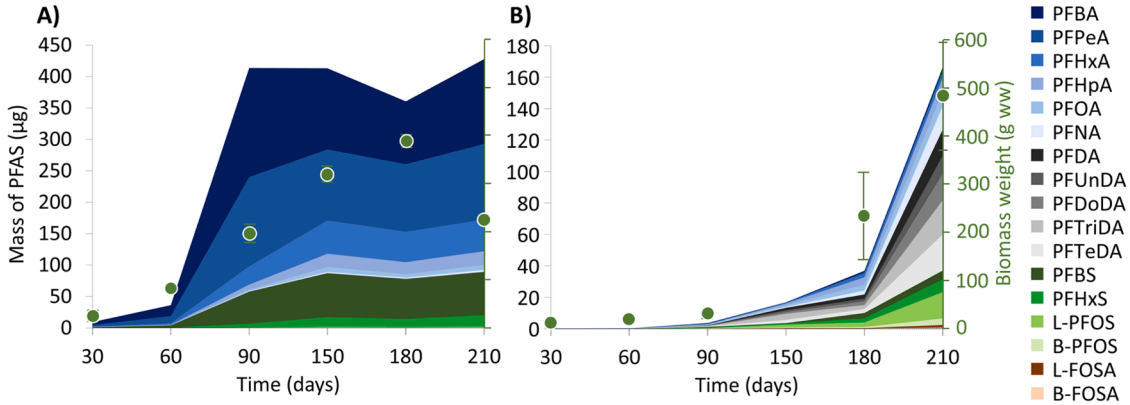

Fig. 4. Average mass PFAS uptake for each willow plant (n = 3), and the distribution of PFAS in A) shoots (the sum of leaves and stem) and B) root grown on PFAS-spiked soil over time and average biomass weight (g ww; greeb dots) (error bars represent the standard deviation of triplicates). For details see Table S7 in SI.

Nassazzi et al., 2023). However, a few previous studies have shown the lowest RCF at C_7 PFCA (PFHpA) with a U-shaped relationship (Nassazzi et al., 2023; Felizeter et al., 2012), typcially at higher PFAS concentrations (> 100 ng L^{-1}) (Felizeter et al., 2012).

3.3. Uptake of PFAS over time

Mass uptake in the shoot (sum of leaves, and stem) and roots for 15 PFAS was monitored in willow with PFAS spiked soil (500 μg for each individual PFAS) over 210 days and showed distinct patterns and trends in the uptake of individual PFAS over time (Fig. 4; Table S7 in SI). After 30 days of exposure, mass uptake was relatively low in the shoot (∑PFAS = 9.6 ± 3.5 μg absolute) and root (∑PFAS = 0.28 ± 0.044 μg absolute). In the shoots, PFBA (50 % of ∑PFAS), PFPeA (27 %) and PFBS (9.8 %) were dominant, whereas in roots, PFPeA (23 %), PFBA (19 %), PFBS (15 %) were dominant after 30 days. After 60 days of exposure, ∑PFAS uptake increased by a factor of ~4 in shoots (∑PFAS = 36 ± 16 μg absolute), whereas the ∑PFAS uptake in roots (∑PFAS = 0.27 ± 0.12 μg absolute) was relatively stable. By day 90 of exposure, PFAS accumulation increased by a factor of > 40 in shoots (∑PFAS = 414 ± 222 μg absolute) in comparison to the recorded mass PFAS uptake after 30 days. PFPeA, PFBS, and PFHxA showed the most pronounced increase, with factors of ~36, ~54, and ~48, respectively. Between 90 and 210 days of exposure, the ∑PFAS uptake in the shoots levelled off and reached a 428 ± 93 μg absolute amount after 210 days. The dominant PFAS in the shoots after 210 days were PFBA (32 % of ∑PFAS), PFPeA (28 %) and PFBS (16 %). In contrast, the ∑PFAS uptake in the roots increased by a factor of > 40, from 3.7 ± 2.1 μg absolute to 166 ± 84 μg absolute for ∑PFAS between 90 and 210 days of exposure. The dominant PFAS in the roots after 210 days shifted to the long-chained PFAS including PFTeDA (14 % of ∑PFAS), PFTriDA (13 %), PFDA, PFDoDA, L-PFOS (all 10 %) compared to day 30 of exposure.

The increase in PFAS accumulation over time could be attributed to the increase in plant biomass. Overall, there was a 17-fold increase in total willow biomass after 210 days (Figure S3 in SI). However, biomass growth differed between roots and shoots. Shoot biomass plateaued around 150 days, ultimately settling at 227 ± 5.8 g ww after 210 days, while the root biomass increased throughout the duration of the experiment (483 ± 112 g ww after 210 days; Figure S3 in SI). Despite the plateau in shoot biomass after 90 days, PFAS uptake continued to increase over time (Fig. 4), from 60 to 90 days in the shoots and after 150 days in the roots of the willow. It is unclear if the discrepancy in roots and shoot biomass growth patterns and associated PFAS accumulation are a result of PFAS exposure or simply an artifact of a limited pot size (Poorter et al., 2012). Roots are known to adapt to soil conditions (Karlova et al., 2021), and certain nutrient deficiencies can trigger a physiological response to increase root biomass (Lopez et al., 2023). Anecdotally, we observed that the plants were root bound in the pots at the end of the experiment (210 days). The space, soil, and potential nutrient limitations created by our experimental design may have limited overall aboveground biomass (Mcconnaughay, Bazzaz, 1991) and might explain why PFAS accumulation plateaued in the shoots. Since PFAS uptake and biomass are positively correlated (Fig. 4; *Arabidopsis spp.*, Müller et al., 2016), it is important to discern how plants will respond over extended periods of time. Future long-term growth experiments should consider biomass production in the experimental design and aim for an understanding of how the long-term mobilization of PFAS into plant material will impact the application and management of phytoremediation strategies in the field. While it is currently unclear if total biomass, growth rate, or a combination of both are key factors driving the uptake of PFAS, these results highlight the importance of plant growth as a critical parameter for any given phytoremediation strategy (He et al., 2023, Nassazzi et al., 2023).

Based on the total spiked mass of PFAS (500 μg for each individual PFAS), the overall removal of ∑PFAS was 7.9 ± 2.4 % in willow after 210 days, with the majority in the shoots (5.7 ± 1.2 %) relative to the roots (2.2 ± 1.1 %). This represents an overall ∑PFAS removal of 0.048 ± 0.011 % per day and 1.1 ± 0.34 % per month in willow. For individual PFAS, the highest total removal (the sum of the shoot and root) was achieved for the short chained PFAS, PFBA (35 ± 7.2 %), followed by PFPeA (29 ± 4.4 %), PFBS (18 ± 3.7 %), and PFHxA (12 ± 1.5 %) in willow after a 210-day exposure time. Distinct differences were observed in the removal of PFAS in the shoots dominated by short chain PFAS, PFBA (27 ± 7.2 %), PFPeA (24 ± 4.3 %) and PFBS (14 ± 3.5 %), and roots dominated by long

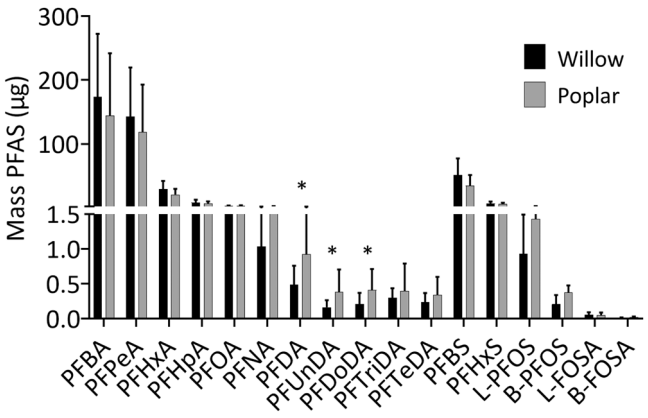

Fig. 5. Average mass PFAS uptake (μg; the sum of leaves, stem, root) in willow and poplar after 90 days of PFAS exposure (n = 3). * indicates significant difference ($p < 0.05$, Student's t test).

chain PFAS, PFTeDA (4.6 ± 0.32 %), PFTriDA (4.4 ± 0.48 %), and ∑PFOS (4.2 ± 0.32 %).

When comparing the PFAS mass uptake between plant species at 90 days of exposure, both plants demonstrated a similar ability to accumulate PFAS, 417 ± 224 μg and 338 ± 212 μg absolute (sum of leaf, stem, root) for willow and poplar, respectively (Fig. 5). Based on the total spiked mass of PFAS (500 μg for each individual PFAS), the overall removal of ∑PFAS was not significantly different between willow, with 5.6 ± 3.0 % (5.5 ± 3.0 % in shoots and 0.049 ± 0.028 % in roots), and poplar, with 4.5 ± 2.8 % (4.4 ± 2.8 % in shoots and 0.12 ± 0.052 % in roots), after 90 days ($p > 0.05$, Student's t test). The difference in root ∑PFAS between the two species could be attributed, at least in part, to the differences in root mass; after 90 days, root mass was three times higher in poplar (99 ± 48 g ww) than in willow (32 ± 10 g ww). For individual PFAS, the mass of PFDA, PFUnDA and PFDoDA was significantly higher for poplar compared to willow ($p < 0.05$, Student's t test, Fig. 5), which could be related to PFAS species specific uptake. In willow, the total removal of individual PFAS was dominated by short chain PFAS, PFBA (35 ± 20 %), PFPeA (28 ± 15 %) and PFBS (10 ± 5.1 %), in the shoots, and by PFSA, ∑PFOS (0.14 ± 0.065 %) and PFBS (0.099 ± 0.064 %) in the roots after a 90-day exposure time. Similarly, the total removal of individual PFAS in poplar was dominated by short chain PFAS, PFBA (29 ± 20 %), PFPeA (23 ± 15 %) and PFBS (6.7 ± 3.4 %), in the shoots, and by ∑PFOS (0.29 ± 0.13 %), PFPeA (0.22 ± 0.21 %), and PFBS (0.20 ± 0.15 %) in the roots after a 90-day exposure time.

3.4. Microbial communities in PFAS-spiked and PFAS-free greenhouse soil

Microbial communities can play a crucial role in PFAS bioavailability and degradation, potentially affecting PFAS uptake by plants (Liu et al., 2022). Thus the microbial soil communities in the root rhizospheres of willow and poplar were assessed for their potential role in the observed differences in ∑PFAS uptake over 90 days of exposure as well as determine the relative levels of the supplemented microbes. The control soil on Day 0 (S0) showed a distinct microbial profile from all other treatment conditions (Fig. 6D; Table S8 in SI), indicating that PFAS and transplantation results in a change in the microbial composition within the first 90 days. While this result is consistent with previous research (Bao et al., 2018; Cai et al., 2020; Xu et al., 2022, 2023), the microbial community compositions

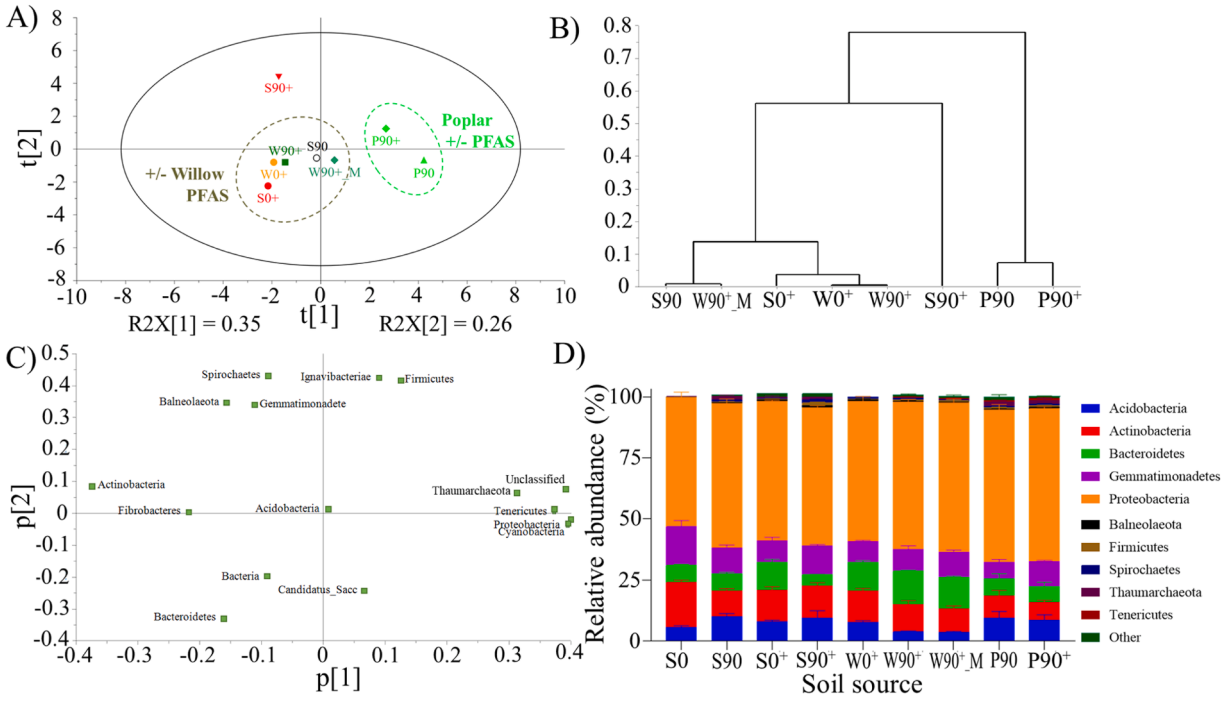

Fig. 6. Multivariate analysis of the bacterial communities in PFAS-contaminated and uncontaminated greenhouse soils. A) Principal component analysis score plot represents the variations in the sample explained by two components at 61 %. The control (S0) was highly distinct from all other groups and obscured any changes in the community composition among treatment groups, therefore S0 was excluded from the multivariate analysis to reveal these subtle changes. B) A loading plot showing the distribution of phyla in the different sample groups. C) Hierarchical clustering representing the dissimilarity between soil samples based on the taxonomic composition and respective relative abundance at the species level calculated using the Ward's method (n = 3). Distances are scaled. The height of the linkages between clusters corresponds to the scaled Ward distance (n = 3). D) Bacterial community composition of different soil samples at the phyla level. 'Other' includes Candidatus_Saccharibacteria, Cyanobacteria, Fibrobacteres, Ignavibacteriae, unclassified, and bacteria resolved at the kingdom level. The samples are: S0, control soil without plants or PFAS sampled at day 2 (Day 0), and S90, after 90 days in the greenhouse; $S0^+$, soil treated with PFAS spiked (+) sampled day 2, and $S90^+$, after 90 days (day = 90); P90, soil with poplar and without PFAS and $P90^+$, with PFAS sampled after 90 days; $W0^+$, soil with PFAS transplanted with willow sampled after day 2, and $W90^+$, after 90 days; $W90^+$_M, soil addition with microbial treatment and PFAS transplanted with willow sampled after 90 days.

tended to group by tree species irrespective of PFAS treatment, with the exception of W90$^+$ which was most similar to the control soil S90.

Multivariate analysis and hierarchical clustering revealed subtle but significant compositional differences among the exposure groups after excluding S0 from the analysis, and this identified three distinct clusters (Fig. 6A-6C). Principal component analysis showed low variability in community composition across groups, suggesting a minimal impact of PFAS and transplantation on the microbial communities (Fig. 6A). Three clusters were identified, based on the dissimilarities in relative abundance of individual phyla (Fig. 6B), specifically, PFAS-free soil (S90) clustered with W90$^+$_M and W90$^+$, while P90 clustered with P90$^+$. In contrast, S90$^+$ did not cluster with any other group, highlighting its distinct microbial composition. This pattern indicates that microbial community composition was influenced by exposure duration (i.e., 90 days), plant species (i.e., popular or willow) and PFAS. Interestingly, PFAS-free soil (with or without plants) shared a similar community structure with soil spiked with PFAS and transplanted with plants, suggesting a subtle effect of PFAS on microbial composition (Xu et al., 2022; Huang et al., 2024).

A comparison of the relative abundance of specific phyla between different conditions showed that differences among clusters were caused by changes in certain phyla influenced mainly by plant type but also PFAS exposure and time. At the start of the experiment, there was no significant difference in the relative abundance of the overall microbial composition between S0 and willow transplanted spiked soil (Fig. 6D). However, the relative abundance of Actinobacteria decreased after PFAS-spiking and willow transplantation from 18 % in S0 to 13 % in S0$^+$ and W0$^+$, whereas Bacteroidetes increased after willow transplantation in PFAS-spiked soil (S0: 7 %, W0$^+$: 12 %) ($p < 0.05$, Welch *t*-test) (Table S9 in SI). Spirochaetes significantly increased in abundance in W0$^+$ relative to S0$^+$ ($p < 0.05$) (Fig. 6D, Table S8 and S9 in SI). Balneolaeota and 'other' phyla were not detected in S0. Xu et al. (2022) have also shown that the overall microbial community did not change in the first 30 days after PFOA and PFOS exposure even though there were phylum-specific changes.

After 90 days, the relative abundance of Actinobacteria decreased in spiked soil with poplar (P90$^+$) (7.1 %) compared to soil without plants, S90$^+$ (13 %) and S90 (11 %) ($p < 0.05$), while Bacteroidetes increased in W90$^+$ (13 %) compared with S90$^+$ (4.6 %) and S90 (6.9 %) ($p < 0.05$) (Fig. 6D, Table S8 and S9 in SI). Over the 90 days, Acidobacteria increased in relative abundance from 5.7 % in S0 to 10 % in S90 but was lower in W90$^+$ ($p < 0.05$). Proteobacteria remained the most abundant phyla after 90-day exposure in all soil conditions, with P90$^+$ having the highest relative abundance (62.5 %). Its relative abundance is significantly higher compared to S0 (53 %), S0$^+$ (57.1 %) and S90$^+$ (56.5 %). Furthermore, the addition of microbes to soil growing willow (W90$^+$_M) did not significantly change the soil bacterial community compared to willow grown without microbial amendments (W90$^+$) ($r = 0.96$, 95 % CI:0.95–0.97).

Based on amplicon sequencing of the 16S rRNA gene, bacterial supplements were below the detection level at 0.1 % reads in samples collected at 90 days. The mycorrhizae were detected at an abundance in the order of 10^2 gene copies/g using ddPCR (Figure S4 in SI), and the abundance was observed to be consistent between samples collected at 0 and 90 days. Comparable concentrations of PFAS were measured in soil and plant biomass for plants grown with and without microbial amendment, suggesting no or limited influence of the microbial amendment tested under these conditions on PFAS removal from soil and into biomass. This may reflect insufficient exposure time for microbial adaptation or suboptimal amendment conditions in our study. Prior research indicates that short-term PFAS exposure disrupts microbial diversity and metabolic processes, affecting nutrient cycling and plant-microbe interactions (Cai et al., 2020; Xu et al., 2022).

Overall, these results suggest that willow or poplar influence the soil microbiome composition more than the presence of PFAS or the addition of microbial amendments (Foulon et al., 2016; Tardif et al., 2016). Based on these results, the addition and/or approach used for microbial amendments herein did not alter the removal of PFAS from soil by the willow and poplar, nor were there any negative effects on the microbial community. Further studies are required to evaluate microbial processes that could facilitate greater removal of PFAS from soil or groundwater in phytoremediation (e.g., by increasing plant growth rate).

3.5. Mass balance

A mass balance was performed for PFAS in the shoot (leaves + stem), roots, soil and leachate during the pot experiments for willow (Fig. 7) and poplar (Figure S5 in SI). After 30 days, the overall mass balance for ∑PFAS was 82 ± 15 % for willow with most PFAS

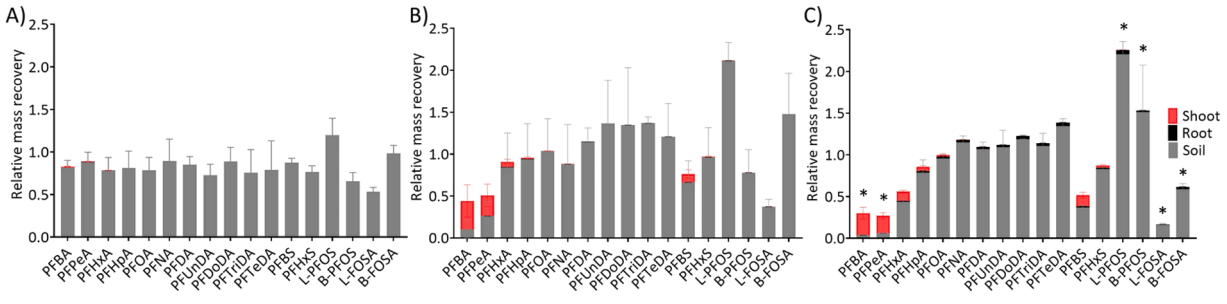

Fig. 7. Mass PFAS recovery for individual PFAS in willow after: A) 30 days, B) 90 days, and C) 210 days of PFAS exposure. Error bars represent the standard deviation for the replicates (n = 3). * indicates significant difference ($p < 0.05$, Student's t test).

found in the soil (82 ± 15 %) and a small percentage in shoots (0.12 ± 0.047 %) and roots (0.0038 ± 0.000059 %). After 60 days, the overall mass balance for ∑PFAS was 101 ± 23 % for willow with most PFAS in the soil (95 ± 20 % for ∑PFAS) and a small percentage in shoots (5.6 ± 3.0 % for ∑PFAS; mostly short chain PFAS PFBA (3.4 ± 1.9 %) and PFPeA (2.5 ± 1.3 %)) and roots (0.050 ± 0.028 % for ∑PFAS). Overall, the relative mass recovery at the 30-day and 90-day points of the experiment were not statistically different from the 100 % for individual PFAS ($p > 0.05$, Student's t test).

By the end of the 210-day point of the experiment, the overall mass balance for ∑PFAS was 94 ± 3.2 % for willow with most PFAS in the soil (85 ± 1.8 % for ∑PFAS) and a smaller portion in shoots (5.7 ± 3.0 % for ∑PFAS, mostly short chain PFAS, PFBA (26 ± 7.0 %), PFPeA (21 ± 3.8 %) and PFBS (13 ± 3.5 %)) and roots (2.2 ± 0.16 % for ∑PFAS, mostly long chain PFAS, L-PFOS (4.8 ± 0.33), PFTeDA (4.1 ± 0.29), and PFDoDA (3.8 ± 0.54)). However, we found significant low overall recovery for PFBA (30 ± 8.2 %), PFPeA (27 ± 4.4 %) ($p < 0.05$, Student's t test), and low but not significant overall recovery for PFBS (52 ± 6.6 %) and PFHxA (56 ± 6.3 %), which might be attributed to leaching of water through the perforations at the bottom of the pots during watering. A methanol rinse of the plant saucers (dishes placed under the pots to trap any excess water after irrigation) showed PFAS concentrations with 1.9–23 ng absolute for ∑PFAS after 30 days and 2.5–920 ng absolute for ∑PFAS after 210 days. However, only part of the pot leachate water was collected on the plant saucers, which makes a quantitative assessment impossible. This result is consistent with findings from a previous lysimeter experiment investigating PFAS uptake in cereals (e.g., wheat, rye, canola and barley) which also observed rapid PFAS loss through leaching (Stahl et al., 2013). On the other hand, PFOS mass recovery was significantly higher with 226 ± 16 % and 153 ± 57 % for L-PFOS and B-PFOS, respectively, whereas FOSA mass recovery was significant lower (17 ± 2.0 % and 62 ± 7.1 % for L-FOSA and B-FOSA, respectively) after 210 days ($p < 0.05$, Student's t test). The possible loss of FOSA during the experiment could be due to the transformation to PFOS (Zhang et al., 2017), which was observed to increase during the experiment period. Another explanation could be adsorption to the pots or an aging effect contributing to the formation of non-extract residues (NER) that could not be extracted by the used methods (Zhu et al., 2021).

After 90 days of poplar exposure to PFAS, the overall mass balance for ∑PFAS was 110 ± 40 % with most PFAS in the soil (107 ± 38 % for ∑PFAS) and a smaller portion in shoots (3.3 ± 2.1 %) and roots (0.072 ± 0.025 %) (Figure S5 in SI). A significantly lower overall mass balance was observed for PFBA (34 ± 23 %) and PFPeA (47 ± 27 %) ($p < 0.05$, Student's t test), which can be partially attributed to the leaching of water through the perforations at the bottom of the pots during watering, as observed for willow experiments. Furthermore, L-PFOS mass recovery was significant higher at 175 ± 36 %, whereas L-FOSA mass recovery was significant lower (46 ± 23 %) after 90 days ($p < 0.05$, Student's t test), which could be due to the transformation of FOSA to PFOS, as observed for willow (Zhang et al., 2017), or sorption to the pots or NER (Zhu et al., 2021).

3.6. Implications for field phytoremediation applications and management

In this study, the assessment of PFAS concentrations in willow and poplar, and the associated soil demonstrated the relative potential of these species for phytoremediation. Phytoextraction of PFAS has received attention as a potential mechanism for mitigating risks around legacy PFAS sites (Kavusi et al., 2023; Mayakaduwage et al., 2022). In our study, the overall uptake of ∑PFAS in willow was 5.6 ± 3.0 % (5.5 ± 3.0 % in shoots and 0.049 ± 0.028 % in roots) after 90 days and 9.2 ± 2.4 % (5.7 ± 1.2 % in shoots and 2.2 ± 1.1 % in roots) after 210 days. In poplar, the overall removal of ∑PFAS was slightly lower, at 4.5 ± 2.8 % (4.4 ± 2.8 % in shoots and 0.12 ± 0.052 % in roots) after 90 days of exposure. However, we also observed that an estimated 27–56 % of short chain PFAS (PFBA, PFPeA, PFHxA, PFBS) for willow and 34–47 % of short chain PFAS (PFBA, PFPeA) for poplar leached out of the system. While the relatively high amount of leached PFAS may be an artifact of the study's irrigation schedule, it does highlight the mobility of short-chain PFAS. These aspects, in conjunction with long-chain PFAS accumulation in belowground biomass, limit the potential utility of using plants solely for PFAS extraction. Despite these challenges, there are several possible approaches that could incorporate a phytoremediation approach for PFAS risk mitigation. These include utilizing plants as hydraulic pumps and barriers (Danielescu et al., 2020) to limit the vertical movement of PFAS through the vadose zone or laterally through the saturated zone (i.e., providing hydraulic control). There may also be value in exploring a combination of hydraulic control strategies with adsorption remediation techniques (e.g., activated carbon; Shih et al., 2024).

The temporal aspects of this study confirmed that short-chain PFAS accumulated in the aboveground biomass, while the more hydrophobic long-chain PFAS accumulated in the belowground biomass. Significant PFAS accumulation did not occur in the shoots until 60 days after planting and was only partly associated with increased biomass; this delay in accumulation could be due to the time needed for belowground biomass to develop and become established before meaningful accumulation can occur aboveground. On the other hand, root PFAS concentrations closely mirrored root biomass in that there was a delay in substantial increases until after 90 days. While our experimental conditions prevented us from clearly discerning the possible drivers of these patterns, understanding how plants respond to PFAS accumulation has important implications for the implementation of a given phytoremediation strategy, particularly if different PFAS compounds (e.g., short-chain or long-chain) accumulate in different compartments (e.g. roots vs leaves), trigger physiological shifts in growth patterns (e.g., promoting or inhibiting root growth) and the impact of microbes or hormones on the development of the roots, stems, and leaves which might affect the PFAS concentration in different parts of the plant. Furthermore, the relative efficacy of a phytoremediation-based management approach may vary within a growing season and over time, and the PFAS accumulation patterns observed in this greenhouse study may be different than those found when a tree is established (i.e., the second year after planting) and not physically restricted (i.e., "pot-bound"). Future studies looking to assess the efficacy of phytoremediation to manage PFAS should consider multi-year experimental designs to understand potential shifts in PFAS root, including shoot ratios and how PFAS moves within the plant-soil-water matrix. The collective observations described herein demonstrate the intentionality warranted when designing and monitoring phytoremediation strategies (e.g., phytoextraction vs. phytostablization vs.

phytohydraulics) for PFAS in soil and/or groundwater, such as targeted PFAS properties (e.g., long-chain vs. short-chain), growth limitations of plants, and temporal differences in leaf, stem, and root uptake.

CRediT authorship contribution statement

Nassazzi Winnie: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Ahrens Lutz:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Jass Jana:** Writing – review & editing, Funding acquisition, Conceptualization. **Lai Foon Yin:** Writing – review & editing. **Key Trent A:** Writing – review & editing, Conceptualization. **Jaffe Benjamin D:** Writing – review & editing, Conceptualization. **Tapase Savita:** Writing – review & editing, Methodology, Investigation, Data curation. **Guo Chao:** Writing – review & editing, Methodology, Investigation, Data curation. **Bezabhe Yared H.:** Writing – review & editing, Methodology, Investigation, Data curation.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104048.

Data availability

Data will be made available on request.

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