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RESEARCH ARTICLE

# Insight into the uptake and translocation of per- and polyfluoroalkyl substances in hydroponically grown lettuce

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## Abstract

The prevalence of per- and polyfluoroalkyl substances (PFASs) in agricultural soils has raised concerns regarding the health risks associated with the consumption of PFAS-contaminated agricultural products. The present study investigated the uptake and translocation of nine PFASs in lettuce using a hydroponic setting. During the uptake experiments, long-chain PFASs (≥C8) exhibited greater accumulations in lettuce roots, while short-chain PFASs (≤C7) manifested preferential transport to the shoots. The average root concentration factors of PFASs were positively correlated with their log $K_{ow}$ values. A significantly negative relationship was found between the average translocation factors of PFASs and their molecular volume. Sorption of long-chain PFASs by lettuce roots was enhanced after heating the roots to increase the cell membrane permeability. The accumulation of perfluorododecanoic acid increased significantly in shoots of lettuce plants without roots as compared to whole lettuce plants. Results of the present study indicate that sorption to root surface tissues and efficiency in passing through the root Casparian strip are two important factors that affect the uptake and distribution of PFASs within plants.

**Keywords** Plant uptake · Translocation · PFASs · Hydroponics · Sorption · Transport pathway

## Introduction

Per- and polyfluoroalkyl substances (PFASs) are a large group of artificial persistent organic compounds that have aroused increasing concern owing to their ubiquitous environmental occurrence, resistance to degradation, and ability to bioaccumulate. PFASs are amphiphilic chemicals with a hydrophilic head functional group (e.g., carboxylate and sulfonate), and an aliphatic $–CF_2$ tail chain, and thus have many unique physical and chemical characteristics, including water and oil repellency, surfactant properties, and thermal stability (Buck et al. 2011; Lindstrom et al. 2011b). This makes these compounds very useful in the manufacture of a wide range of industrial and consumer products, such as aqueous firefighting film foam, pesticides, carpets, clothing, paint, packaging paper, lubricant, and stain-resistant cookware (Backe et al. 2013; Buck et al. 2011; Kannan 2011; Lindstrom et al. 2011b). The extensive application of PFASs has resulted in their ubiquitous presence in water, soil, and air worldwide (Codling et al. 2018; Dalahmeh et al. 2018; Taniyasu et al. 2003). The strong covalent bond (~460 kJ/mol) between C and F makes PFASs extremely resistant to degradation; therefore, these chemicals can persist for extended periods of time in the environment following the initial contamination event. Long-term exposure to these chemicals can lead to high levels of bioaccumulation in animals and humans (Biegel et al. 2001; Frisbee et al. 2010; Gebbink et al. 2009; Giesy and Kannan 2001; Kannan et al. 2004; Kannan et al. 2001; Kannan et al. 2005; Lau et al. 2006). Once bioaccumulated, PFASs can interact with blood proteins and induce several pathological responses, including hepatotoxicity, developmental toxicity, immunotoxicity, neurotoxicity, tumor induction, and endocrine disorder (ASTDR 2018; DeWitt 2015; Lindstrom et al. 2011b).

Agricultural soils are important environmental reservoirs for PFASs. The major pathways for PFASs to enter agricultural soils are through land application of biosolids,

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irrigation with reclaimed water or contaminated freshwater, industrial emissions, leakage from landfills, and pesticide application (Wang et al. 2020). For instance, the total amount of PFASs introduced annually into agricultural soils via land application of biosolids in America is estimated to be 1.4–2.1 tons (Venkatesan and Halden 2013). From 2004 to 2015, approximately 150 tons of perfluorooctane sulfonamide and 20 tons of perfluorooctanesulfonic acid (PFOS) were discharged into farmlands in Brazil due to the application of sulfluramid, a widely used pesticide with N-ethyl perfluorooctane sulfonamide as the main active ingredient (Lofstedt Gilljam et al. 2016). In some highly contaminated areas, soil concentrations of PFASs can reach mg/kg levels (Braunig et al. 2019; Jin et al. 2015). Such prevalence of PFASs in soils makes agricultural crops susceptible to contamination (Jiao et al. 2020). Furthermore, it has been demonstrated that crops can absorb PFASs from contaminated soils or water via roots and translocate them to edible plant parts (Blaine et al. 2013, 2014a; Jiao et al. 2020; Wang et al. 2020). The presence of PFASs has been detected in a wide spectrum of vegetables and grain crops including lettuce, tomato, maize, pumpkin, wheat, pea, broccoli, carrot, onion, pepper, potato, oat, cucumber, barley, canola, celery, radish, strawberry, cabbage, zucchini, soybean, alfalfa, and spinach (Bizkarguenaga et al. 2016; Blaine et al. 2014a, b; Lee et al. 2014; Lindstrom et al. 2011a; Navarro et al. 2017; Wang et al. 2020). Considering that food consumption represents a major route for the entry of PFASs into livestock and humans (Perez et al. 2014; van Asselt et al. 2011), it is crucial to elucidate the uptake and accumulation of PFASs in edible crops, which constitute the most important food source.

Although thousands of papers have been published on PFASs in the environment over the past decades, fewer than one hundred have focused upon the uptake of PFASs in dietary plants (Buck et al. 2011; Wang et al. 2020; Xiao 2017). The majority of these initial research efforts have focused on finding evidence relating to the transport of PFASs from external growth media to plant roots and other parts, or evaluating the effects of PFAS properties, plant physiology, and environmental factors on the uptake behavior (Blaine et al. 2013; Felizeter et al. 2014; Krippner et al. 2014; Lan et al. 2018; Lechner and Knapp 2011; Wang et al. 2020; Xiang et al. 2018). However, relatively few studies have attempted to unravel the mechanisms that determine the movement of PFASs within plants and some of the available results appeared to be contradictory. For instance, by using relevant inhibitors, Wen et al. (2013) reported that the uptake of PFOS by maize roots was a passive process that was independent of cellular metabolic energy, whereas in the study of Zhang et al. (2019), root uptake of PFOS was considered an energy-dependent active process. Additionally, the application of root inhibitors can affect other physiological processes, like stomatal movement, photosynthesis, and hydraulic conductivity, which may translate to consequences on PFAS uptake (Zelitch 1965; Zhang and Tyerman 1991). Further studies are needed to clarify the driving biological and physicochemical factors that affect the uptake and transport of PFASs in plants.

Compared with the soil-based field or pot studies, hydroponic settings can eliminate the interferences caused by soil components and thus have been widely used to explore the uptake mechanisms of xenobiotic pollutants by plant roots (Li et al. 2018; Wang et al. 2020). Previous hydroponic studies have shown that the uptake and acropetal transport of PFASs in plants can vary significantly among congeners with different carbon chain lengths and head functional groups. Krippner et al. (2014) observed that the uptake of perfluoroalkyl carboxylic acids (PFCAs) by roots of hydroponic maize decreased as carbon chain lengths increased from C4 (perfluorobutanoic acid (PFBA)) to C7 (perfluoroheptanoic acid (PFHpA)) and then increased with carbon chain lengths from C7 to C10 (perfluorodecanoic acid (PFDA)). Felizeter et al. (2012) reported a similar U-shape trend, decreasing from C4 (PFBA) to C6 (perfluorohexanoic acid (PFHxA)) and then increasing from C6 to C12 (perfluorododecanoic acid (PFDoA)), in the uptake of PFCAs by hydroponic lettuce. However, in other hydroponic studies with wheat, tomato, cabbage, and zucchini, root accumulation of PFCAs and perfluoroalkyl sulfonic acids (PFSAs) was found to increase with their carbon chain lengths (Zhao et al. 2018). The inconsistence in the reported results calls for further factorial research to achieve a mechanistic understanding.

Xenobiotic uptake by plants is considered to be a predominantly passive process, with the majority of contaminants entering plant roots from the soil solution via diffusion (Collins et al. 2011; Kumar and Gupta 2016; Trapp et al. 1994). Once a compound has entered the roots, it can either diffuse between cells through the relatively permeable cell wall (the apoplastic pathway), or pass through the plasma membrane and travel in the intracellular space (the symplastic pathway). Water flow is the primary carrier for the transport of compounds in plants (Chuang et al. 2019; Kumar and Gupta 2016). The movement of solutes into the vascular cylinder via the apoplastic pathway can be retarded or inhibited by the Casparian strip, located at the root endodermis. Only compounds that can cross the selective plasma membrane can enter the xylem for upward translocation. Although the Casparian strip has been studied by plant biologists since its discovery in 1865 (Caspary 1865; Nagahashi et al. 1974), its impact on the uptake and translocation of xenobiotic contaminants such as PFASs has rarely been investigated.

This study investigated the uptake and translocation of nine PFASs, including six PFCAs and three PFSAs in lettuce using a hydroponic setting. The sorption of PFASs by

excised live and dead lettuce roots was compared to infer their transport pathways in lettuce roots and evaluate the effect of PFAS affinity to roots on their distribution in different plant parts. The effect of the Casparian strip on the upward transport of PFDoA was elucidated by comparing the difference in shoot accumulation of long-chain PFASs between whole lettuce plants and those without roots.

## Materials and methods

### Chemicals

Nine PFASs, perfluoropentanoic acid (PFPeA, 97%), perfluoroheptanoic acid (PFHpA, 99%), perfluorooctanoic acid (PFOA, 96%), perfluorononanoic acid (PFNA, 98%), perfluorodecanoic acid (PFDA, 98%), perfluorododecanoic acid (PFDoA, 95%), perfluorobutanesulfonic acid (PFBS, 99%), perfluorohexanesulfonic acid (PFHxS, 98%), and perfluorooctanesulfonic acid (PFOS, 98%), were purchased from Sigma Aldrich (St. Louis, MO, USA). Detailed information regarding the physicochemical properties of the selected PFASs is presented in Table 1. The stock standard solution of PFASs was prepared in methanol as a mixture of all homologues and stored at 4 °C in a refrigerator. The concentration of each PFAS homologue in the stock solution was 20 mg/L.

HPLC-grade methanol and ammonium hydroxide ($NH_4OH$) were obtained from CNW Technologies (GmbH, Düsseldorf, Germany). Methyl *tert*-butyl ether (MTBE) and tetrabutylammonium hydrogen sulfate (TBAHS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade sodium carbonate ($Na_2CO_3$), sodium bicarbonate ($NaHCO_3$), and sodium hydroxide (NaOH) were purchased from Aladdin Chemistry (Shanghai, China). Hoagland nutrient solution was provided by the Haibo Bio-Technology Company (Qingdao, China). The 15-mL polypropylene (PP) tubes with screw caps were acquired from Fisher Scientific (Shanghai, China). Oasis Wax SPE cartridges (150 mg, 6 mL) were purchased from Waters (Milford, MA, USA), and the Supelclean graphitized carbon (ENVI-Carb) used for cleanup was purchased from Supelco (Bellefonte, PA, USA). Milli-Q water was generated using a water purification system (Burlington, MA, USA) and used throughout the study.

### Plant culture and exposure experiments

Lettuce (*Lactuca sativa* L.) seeds were germinated on moist tissue paper in the dark for 5–7 days. The lettuce seedlings were transferred to a sterile plastic container with half-strength Hoagland's nutrient solution and cultivated in a plant incubator (temperature 18 ± 1 °C, photoperiod 16 h per day, light intensity 150 μmol/$m^2$/s). Following 28 days of growth, uniform-sized lettuce seedlings were selected for uptake experiments. The selected lettuce seedlings were 9.0–11.5 g in fresh biomass and 30–40 cm in height. A single lettuce plant was transferred to a polypropylene Erlenmeyer flask containing 200 mL of nutrient solution (pH 5.6–6.0) spiked with the PFAS standard stock solution to achieve an initial concentration of 10 μg/L for each homologue. The concentration of methanol in the growth medium was 0.5‰ (v/v). All flasks were completely wrapped with aluminum foil to prevent algal growth. The weight of each flask was recorded every 12 h to measure the water loss (mostly due to plant transpiration), and fresh nutrient solution (free of PFASs) was added to the flask to maintain the

**Table 1** Physicochemical properties of the target analytes

| Compounds | Acronym | Formula | Molecular mass[a] (g/mol) | Molecular volume[a, b] ($nm^3$) | Water solubility[a] (mol/L) | Log $K_{ow}$ [a] |
|---|---|---|---|---|---|---|
| Perfluoropentanoic acid | PFPeA | $C_5HF_9O_2$ | 264.05 | 0.19 | 4.54E-04[c] | 1.98[c] |
| Perfluoroheptanoic acid | PFHpA | $C_7HF_{13}O_2$ | 364.06 | 0.35 | 3.24E-01[c] | 4.49[d] |
| Perfluorooctanoic acid | PFOA | $C_8HF_{15}O_2$ | 414.07 | 0.39 | 1.56E-02[c] | 3.6[c] |
| Perfluorononanoic acid | PFNA | $C_9HF_{17}O_2$ | 464.08 | 0.44 | 1.00E-03[d] | 6.18[d] |
| Perfluorodecanoic acid | PFDA | $C_{10}HF_{19}O_2$ | 514.09 | 0.48 | 2.94E-03[d] | 6.89[d] |
| Perfluorododecanoic acid | PFDoA | $C_{12}HF_{23}O_2$ | 614.1 | 0.58 | 2.15E-05[d] | 8.39[d] |
| Perfluorobutanesulfonic acid | PFBS | $C_4HF_9O_3S$ | 300.09 | 0.27 | 1.70E-03[c] | 1.98[d] |
| Perfluorohexanesulfonic acid | PFHxS | $C_6HF_{13}O_3S$ | 400.11 | 0.36 | 6.08E-04[c] | 2.2[d] |
| Perfluorooctanesulfonic acid | PFOS | $C_8HF_{17}O_3S$ | 500.13 | 0.45 | 2.79E-04[b] | 4.05[d] |

[a]Acquired from USEPA website: https://comptox.epa.gov/dashboard/chemical_lists/pfasmaster

[b]Calculated from molar volume

[c]Experimental average values

[d]Predicted average values

solution at its original volume. The solution pH was also checked and adjusted to its initial range using 0.1 M potassium bicarbonate or citric acid buffer solution. Triplicate flasks were randomly sacrificed to collect the lettuce samples at time intervals of 24, 48, 72, 96, and 144 h. The sampled plants were thoroughly rinsed with Milli-Q water and wiped dry with tissue paper. Subsequently, the lettuce plants were separated into roots and shoots, then lyophilized and homogenized to obtain tissue powders for analyte extraction. Experimental controls included the plant-free control (nutrient solution with the same concentrations of PFASs, but no lettuce plants) and the PFAS-free control (with lettuce plants, but no PFASs added).

### Sorption of PFASs by live and dead lettuce roots

Heating can kill root cells and increase membrane permeability, and thus comparing the sorptive characteristics of chemicals by live and heat-treated roots could provide insight into the uptake pathways of chemicals in roots (Huve et al. 2011; Su and Zhu 2007). Fresh roots of lettuce plants from the PFAS-free control were rinsed with Milli-Q water, dried superficially, and then excised at the basal node. Approximately 1 g of fresh lettuce roots was immediately weighed into a clean conical flask, tightly sealed with a glass stopper, and then heated at 105 °C for 40 min (Su and Zhu 2007). Care was taken during this course to minimize exposure of the roots to the open air. The heat-treated roots (dead roots) and a comparable amount (~ 1 g) of fresh roots (live roots) were individually placed in polypropylene centrifuge tubes containing 50 mL of half-strength Hoagland's nutrient solution spiked with the PFAS standard stock solution to achieve an initial concentration of 10 µg/L for each homologue. The centrifuge tubes were shaken end-over-end at 60 rpm for 72 h at 18 ± 1 °C in the dark. Control solutions containing the same concentration of PFASs, but no lettuce roots, were processed simultaneously. All samples were prepared in triplicate. The concentrations of PFASs in the control solutions after shaking were set as the initial aqueous concentrations of PFASs in the root-containing samples. The sorbed concentrations of PFASs by lettuce roots were acquired from the difference between their initial and final aqueous concentrations. The distribution coefficient ($K_d$) of a PFAS was calculated as the ratio of its root-sorbed concentration to its final aqueous concentration after 72 h of shaking.

### Uptake of PFDoA by whole lettuce plants and lettuce shoots

To investigate the effects of the root Casparian strip on upward translocation of long-chain PFASs, PFDoA (C12) was selected in the following uptake experiment. Lettuce plants from the PFASs-free control were divided into two groups. Roots of lettuce from one group were removed from the basal node (shoot-only treatment), whereas the other group retained the roots (whole lettuce plants). Whole lettuce plants and lettuce shoots from the shoot-only treatment were individually transferred to an Erlenmeyer flask containing 10 µg/L of PFDoA in 200 mL of nutrient solution. The roots of the whole lettuce plants were fully submerged in the nutrient solution. For the shoot-only treatment, ~ 1 cm of the lettuce shoots (incised bottom) was soaked in the solution. Following exposure for 72 h, the plant samples were removed, rinsed with Milli-Q water, and blotted dry with tissue paper. The roots and shoots of the whole lettuce were separated. All plant tissues, including roots and shoots from the whole lettuce plants and shoots from the shoot-only treatment, were freeze-dried prior to analyte extraction. Concentrations of PFDoA in plant tissues were measured.

### Extraction and analysis

Extraction of PFASs from plant tissues was performed according to the method described by Wen et al. (2014), with some modifications. In brief, 0.01–0.02 g of plant powders were weighed into a 15-mL PP centrifuge tube. Following the addition of 2 mL of 0.4 M NaOH, the centrifuge tube was vortexed for 2 min and then retained in the refrigerator for 8 h. Two milliliters of 0.25 M TBAHS and 4 mL of 0.25 M $Na_2CO_3$/$NaHCO_3$ buffer (pH ~ 10) were added to each tube and mixed via vortexing. Subsequently, 5 mL of MTBE was added to the mixture, vortexed for 2 min, and sonicated for 30 min. After the sample was centrifuged at 5000 rpm for 10 min, the supernatant (MTBE phase) was transferred to a new 15-mL PP centrifuge tube. The extraction process was repeated twice with 5 mL × 2 MTBE. The supernatants collected from the three extraction cycles were combined and evaporated to dryness under a gentle stream of nitrogen at room temperature. One milliliter of methanol was added to the tube to redissolve the extracts. The sample was diluted with 9 mL of Milli-Q water and subsequently passed through an Oasis Wax SPE cartridge (containing 10 mg of ENVI-Carb) that had been preconditioned with 5 mL of methanol and 5 mL of Milli-Q water. Target analytes retained in the cartridge were eluted with 4 mL of methanol and then 4 mL of 0.1% ammonia methanol (ammonia/methanol, v/v). The eluate was concentrated to 1 mL under gentle nitrogen flow, vortexed for 30 s, and then passed through a 0.22-µm nylon mesh filter connected to a polypropylene syringe. Nutrient solution samples were directly filtered through the 0.22-µm syringe filter. The filtered sample solutions were transferred into glass autosampler vials and stored at 4 °C until further analysis.

Analysis of PFASs was performed using an Agilent 1260 Infinity high-performance liquid chromatography

(Agilent Technologies, CA, USA) connected to an AB Sciex 4000 triple quadrupole mass spectrometer (Applied Biosystems, MA, USA). A Gemini C18 110A column (50 × 2.0 mm, 5 µm, Phenomenex, CA, USA) was used for the chromatographic separation of the target analytes. The temperature of the column oven was maintained at 40 °C. The binary mobile phase included (A) 20 mM ammonium acetate in Milli-Q water and (B) acetonitrile, and was set at a flow rate of 0.6 mL/min. A 10-µL aliquot of the extract was injected for analysis. The mass spectrometer was operated in electrospray negative ionization mode. Identification and quantification of the target analytes was performed in the scheduled multiple reaction monitoring (MRM) mode. High-purity nitrogen (> 99.99%) at a flow rate of 600 L/h and a manipulating temperature of 350 °C was used as the desolvation gas. The ion source spray voltage was − 4500 V. The curtain gas pressure was 35 psi. The entrance potential was − 10 V and the collision cell exit potential − 15 V. The optimized parameters for the parent and product ions of the target analytes are listed in Table S1.

### Quality assurance and quality control measures

Qualitative and quantitative analyses of the target analytes in the samples were based on the retention time, MRM ion transitions, and ratios of ion abundance. Procedural blanks, solvent blanks, and matrix spikes were included in every batch of analytical samples. To correct matrix effects, external matrix-matched standard calibrations were employed for quantification of the analytes. Calibration was performed using a 7-point curve for each analyte. All the fitted lines had $R^2$ values > 0.99. The method detection limits (MDLs) for the studied analytes were determined by spiking a trace amount of PFASs (0.5 µg/L in nutrient solution or 0.5 ng/g in plant tissues) into the blank samples and calculated as three times signal-to-noise ratio (S/N). The matrix extraction recoveries and MDLs of the PFASs are summarized in Table S2.

#### Statistical analysis

Data were processed using Microsoft Office Excel 2010 and SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Illustrations were made using SigmaPlot 10.0 (Systat Software, Inc., UK). Results are presented as mean ± standard deviation. Concentrations of PFASs in plant tissues were calculated on dry weight (dw) bases. Statistical difference between experimental treatments was analyzed using one-way analysis of variance (ANOVA). A $p$-value < 0.05 indicates a statistically significant difference.

## Results and discussion

### Uptake of PFASs by lettuce roots

The concentrations of PFASs in the roots and shoots of lettuce were plotted against exposure time (Fig. 1). All the studied PFASs could be taken up by lettuce roots and translocated to the aboveground portions. Long-chain PFASs (≥ C8) exhibited greater accumulation in lettuce roots than short-chain homologues (≤ C7). Following 144 h of exposure, the concentrations of PFASs in lettuce roots were in the range of 25.1–298.8 ng/g dw. PFDoA had the largest root concentrations (112.4–298.8 ng/g dw) along the assay, followed by PFDA (89.7–167.8 ng/g dw) and PFOS (89.5–147.5 ng/g dw), whereas PFBS exhibited the least accumulation in lettuce roots with a concentration range of 9.6–27.8 ng/g dw. Root concentrations of PFASs generally increased with exposure time, although some congeners, such as PFOA, PFDA, PFBS, and PFOS, exhibited fluctuations in their root concentrations at specific time intervals. The possible reasons for these exceptions could be measurement errors of the analytes, different upward translocation, and differences in plant growth and physiological properties.

The accumulation of PFASs in lettuce roots was evaluated using the root concentration factor (RCF), which is defined as the ratio of the PFAS concentration in the roots to that in the nutrient solution. The RCF values of PFASs generally increased with exposure time (Table S3). The average RCF values of PFCAs and PFSAs were in the ranges of 3.1–40.4 and 2.6–17.9 mL/g, respectively, both of which increased with the carbon chain lengths of PFASs (Fig. 2(A) and Table S3). Similar relationships between RCF and PFAS carbon chain length have also been observed in previous studies (Felizeter et al. 2012, 2014; Zhao et al. 2018). Long-chain PFASs are typically more hydrophobic (Table 1), which confers a higher sorption affinity to the lipophilic solids of plant roots (Felizeter et al. 2012, 2014; Krippner et al. 2014). A significant positive correlation was found between the RCF values of PFASs and their log $K_{ow}$ values (Fig. 2(B)). The stronger sorption of long-chain PFASs to root extracellular surface tissues before and after they crossed the Casparian strip could be an important reason for their higher accumulation in lettuce roots (Felizeter et al. 2012, 2014). Additionally, due to their large molecular size (Table 1), long-chain PFASs transported in the apoplastic pathway are more likely to be filtered out by the Casparian strip (Felizeter et al. 2012, 2014; Krippner et al. 2014; Wang et al. 2020), which tends to accumulate in plant roots.

Compared to PFCAs, PFSAs with comparable perfluorocarbon chain lengths demonstrated higher

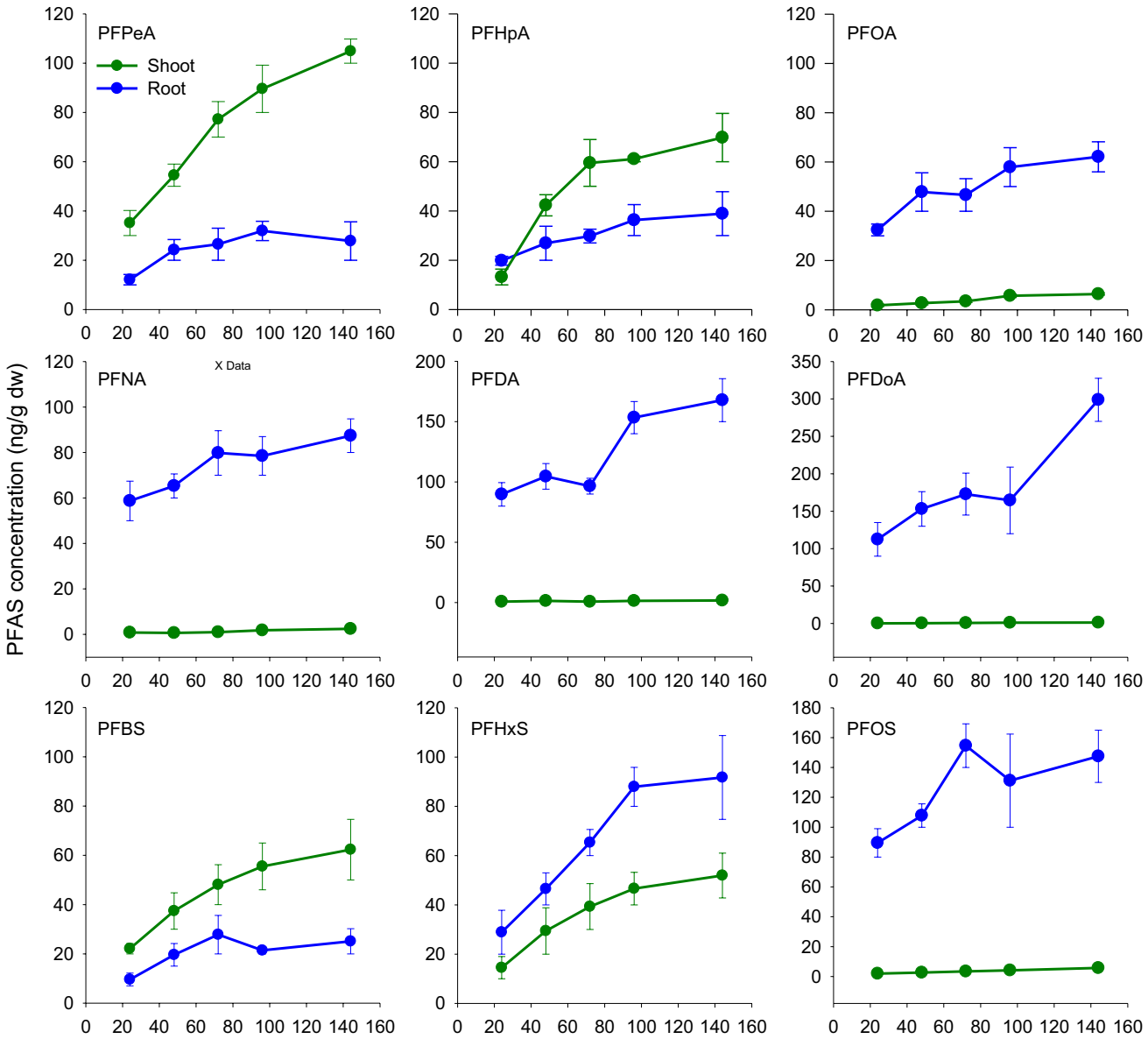



**Fig. 1** Concentrations of PFASs in lettuce roots and shoots as a function of exposure time. The error bars represent the standard deviations (n = 3)

accumulation in lettuce roots (Fig. 2(A)). For instance, the RCF of PFOS was calculated to be 17.9 mL/g, which was 1.7 times greater than PFNA. This could be partly attributed to the higher sorption affinity of perfluorosulfonates relative to perfluorocarboxylate analogs (Higgins and Luthy 2006). Additionally, the sulfonate moiety is larger than the carboxylate moiety, as result of which the apoplastic movement of PFSAs is more effectively retarded by the Casparian strip, leading to their relatively higher root accumulation as compared to PFCAs. Whether other mechanisms such as specific interactions between plant components and these moieties contributed to this observed difference remains unclear.

## Upward translocation of PFASs to lettuce shoots

The shoot concentrations of PFASs increased with exposure time (Fig. 1). The shoot concentration factors (SCFs) of PFASs, which were calculated as the ratio of PFAS concentrations in the shoots to that in the nutrient solution, also exhibited an overall upward trend with time (Table S4). Plant uptake and translocation of xenobiotic contaminants are believed to be predominantly passive processes, which are primarily driven by plant transpiration (Kumar and Gupta 2016; Lechner and Knapp 2011; Trapp et al. 1994). The amount of water transpired via lettuce leaves increased with time, leading to increased translocation of PFASs

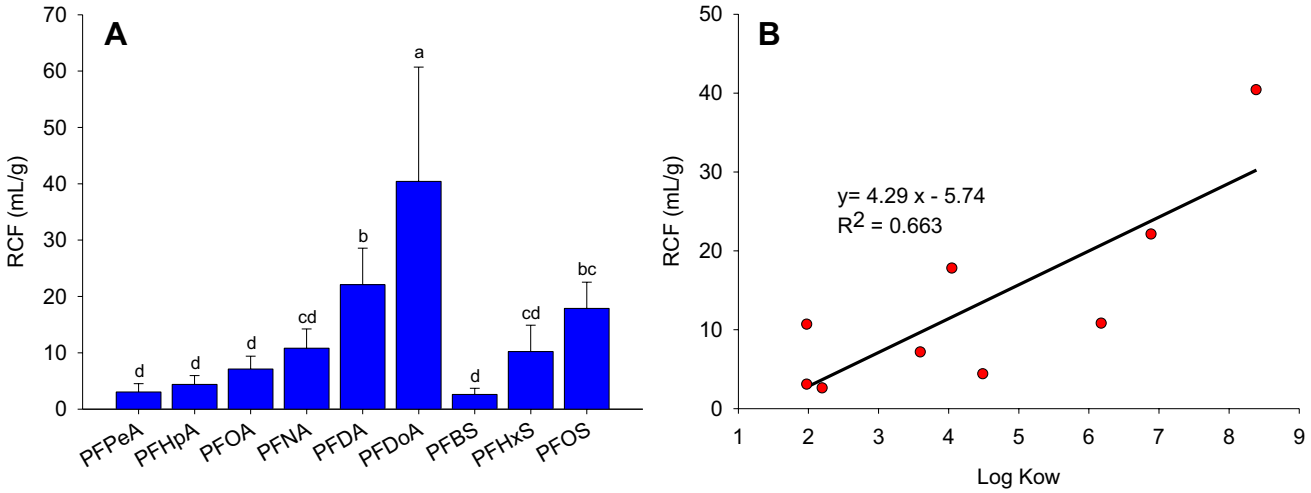



**Fig. 2** Average root concentration factors (RCFs) of PFASs in lettuce during the 144-h uptake experiment (**A**), and relationship between RCFs and log $K_{ow}$ of PFASs (**B**). The error bars in (A) denote the standard deviations ($n=5$)

from roots to shoots. Additionally, PFASs are low-volatile chemicals and hardly degrade in plant tissues (Buck et al. 2011; Jiao et al. 2020; Mei et al. 2021). These could be the major reasons for the increasing accumulation of PFASs in lettuce shoots with exposure time. During the 144-h uptake experiment, PFPeA exhibited the highest accumulation (35.1–104.9 ng/g dw) in lettuce shoots, followed by PFHpA (13.2–69.8 ng/g dw), PFBS (22.1–62.3 ng/g dw), and PFHxS (14.5–51.9 ng/g dw). Long-chain PFASs (≥ C8), such as PFOA, PFNA, PFDA, PFDoA, and PFOS, were less transferable in lettuce plants, with the shoot concentrations less than 10 ng/g dw throughout the entire experimental period. By contrast with RCFs, the SCF values of PFCAs and PFSAs generally decreased with the carbon chain lengths (Table S4). This suggests that although the long-chain PFASs were concentrated by the roots at higher levels via sorption, they were less available for upward transport to the shoots.

The translocation factor (TF) was calculated by dividing the shoot concentrations of PFASs by their root concentrations to evaluate the transferability of PFASs from roots to shoots (Miller et al. 2016). The TF of PFASs varied with exposure time with the mean values in the range of 0.004 to 2.93 (Fig. 3(A) and Table S5). For both PFCAs and PFSAs, the TF values in lettuce plants decreased with increasing carbon chain length. Similar trends have been reported in previous studies. In a hydroponic study with maize, Krippner et al. (2014) found that the shoot to root ratios of PFASs (i.e., TF) became smaller as the carbon chain increased. Felizeter et al. (2012) investigated the uptake behavior of

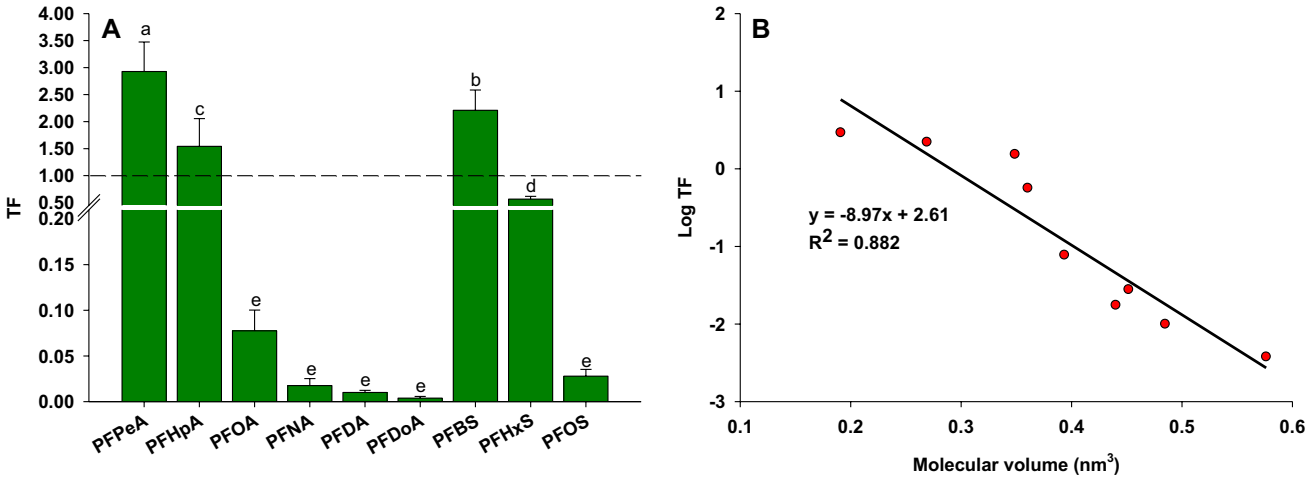



**Fig. 3** Average values of translocation factors (TF) for PFASs in lettuce during the 144-h exposure environment (**A**), and relationship between log TF values and molecular volumes of PFASs (**B**). The error bars in (A) denote the standard deviations ($n=5$)

PFASs in hydroponically grown lettuce and observed an exponential decrease in the TF values with increasing carbon chain length for both PFCAs and PFSAs. The negative relationship between TF and carbon chain length of PFASs has also been reported in soil-based pot studies. For wheat grown in soils spiked with PFASs, the accumulation potential of PFASs in wheat shoots was found to decrease with increasing carbon chain length and no significant difference in TF values was observed between treatments with different spiking levels (Lan et al. 2018).

The average TF values of PFPeA, PFHpA, and PFBS were 2.93 ± 0.55, 1.54 ± 0.51, and 2.21 ± 0.38, respectively, all higher than 1.0 (Fig. 3(A) and Table S5), suggesting that these three PFASs were readily transported to the shoots. The lower sorption affinity to root surface and higher efficiency in crossing the Casparian strip could be the interpretations for their higher transferability in lettuce plants. For the other PFASs, the TF values followed the order PFHxS (0.57) > PFOA (0.078) > PFOS (0.028) > PFNA (0.018) > PFDA (0.010) > PFDoA (0.004), all of which were below 1.0 (Fig. 3(A) and Table S5). A significant negative correlation was found between log TF values and the molecular volumes of PFASs (Fig. 3(B)) ($R^2 = 0.88$, $p < 0.01$). As the molecular volumes of the studied PFASs are strongly correlated with their molecular masses ($R^2 = 0.98$, $p < 0.01$) (Fig. S1), the negative relationship between log TF and PFAS molecular volume suggests decreased transferability of PFASs with their carbon chain lengths. Compounds must cross a range of biological layers, including the epidermis, cortex, endodermis, and pericycle before entering the vascular cylinder for upward transport (Kumar and Gupta 2016; Miller et al. 2016). Short-chain PFASs have smaller molecular volumes and thus can pass through these layers more rapidly than those with longer carbon chains (Kvesitadze et al. 2015; Zhang et al. 2017). Additionally, compared to the long-chain homologues, short-chain PFASs were less sorptive to the root structural tissues, which was another possible reason for their higher TF values. The relatively smaller molecular volumes and lower sorption to root tissues of PFCAs may also be partly responsible for their higher TF values as compared to the perfluorosulfonate analogs with equal carbon chain lengths. Furthermore, the high water solubility of short-chain PFASs facilitates their movement with transpiration flow, the major carrier of solutes for upward transport. Therefore, short-chain PFASs demonstrated relatively higher accumulation in lettuce shoots.

## Sorption of PFASs to root tissues

Figure 4 shows the $K_d$ values for the sorption of PFASs by live and dead lettuce roots, which were in the ranges of 15.1–548.9 and 14.0–679.3 L/kg fresh weight (fw), respectively. For a given PFAS, no significant difference (one-way ANOVA, $p > 0.05$) in $K_d$ values was observed between the live and dead roots. The $K_d$ values of both PFCAs and PFSAs increased with their carbon chain lengths. Muller et al. (2016) also observed a linear relationship between sorption affinity of PFASs on dead roots and their carbon chain length. Partitioning into the root organic components was an important mechanism for PFAS accumulation in the lettuce roots, as PFASs with longer carbon chains are typically more hydrophobic (Table 1). The stronger sorption affinities of long-chain PFASs to both live and dead roots further confirmed our previous statement that sorption could largely account for the higher accumulation of long-chain PFASs in lettuce roots.

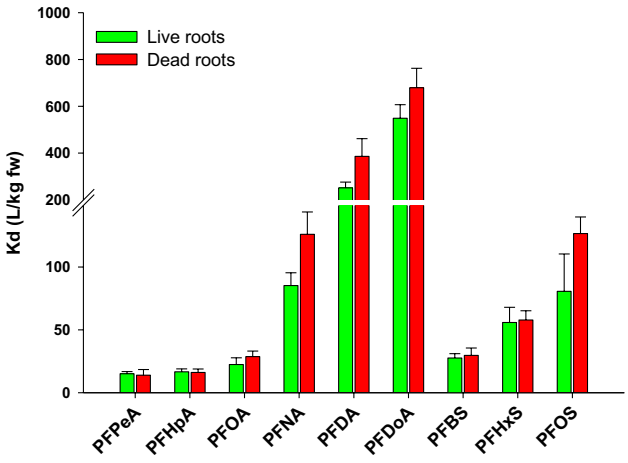



**Fig. 4** The distribution coefficients ($K_d$) for sorption of PFASs by live and dead lettuce roots. The error bars represent the standard deviations ($n = 3$)

For short-chain PFASs (≤C7), such as PFPeA, PFHpA, PFBS, and PFHxS, their $K_d$ values for sorption by live lettuce roots were 15.1 ± 1.7, 16.6 ± 2.3, 27.6 ± 3.5, and 55.8 ± 12.3 L/kg fw, respectively. They were close to those measured in dead roots, i.e., 14.0 ± 4.5, 16.1 ± 2.7, 29.7 ± 5.8, and 57.9 ± 7.3 L/kg fw for PFPeA, PFHpA, PFBS, and PFHxS, respectively. As the carbon chain length increased, the respective $K_d$ values for PFAS sorption by dead roots surpassed those of live roots. The $K_d$ values for the sorption of PFOA, PFNA, PFDA, PFDoA, and PFOS by dead roots were 28.7 ± 4.4, 125.9 ± 17.8, 386.2 ± 75.7, 679.3 ± 82.9, and 126.6 ± 13.2 L/kg fw, respectively, which were 1.3 to 1.6 times that of fresh roots. Heating fresh roots can increase the permeability of the root cell membrane several times (Su and Zhu 2007). Owing to their higher solubility and smaller molecular volume, short-chain PFASs can enter root cells more easily probably via pathways like anion or water channels (Muller et al. 2016; Wen et al. 2013). Heating of roots may have limited effects on the transport of short-chain PFASs into root cells. Therefore, the sorption affinities of short-chain PFASs to live and dead lettuce

roots were comparable. Long-chain PFASs (≥ C8), on the contrary, were less efficient at passing through the cell membrane of live lettuce roots because of their large molecular volumes. The increase in cell membrane penetrability via heating led to an increasing amount of long-chain PFASs entering the symplast and binding with intracellular substrates. This could be a possible reason for the higher $K_d$ values of long-chain PFASs in dead roots than in live roots.

### Effect of the Casparian strip on upward translocation

The Casparian strip is an impermeable barrier between root endodermis cells, which blocks diffusion and directs the flow of water and solutes into cells through the selective plasma membrane and cytoplasm (Kumar and Gupta 2016; Sicbaldi et al. 1997). Once inside the roots, transport of xenobiotic contaminants to the root vascular cylinder via the apoplastic pathway and upward movement in the plant via the transpiration stream can be retarded or even inhibited by the Casparian strip (Collins et al. 2011; Kumar and Gupta 2016). The uptake and accumulation of PFDoA in shoots of lettuce plants with and without roots were compared to further elucidate the effect of the Casparian strip on the upward transport of long-chain PFASs in lettuce (Fig. S2). After 72 h of exposure, the shoot concentration of PFDoA in the shoot-only treatment (lettuce without roots) was 19.5 ± 6.8 ng/g dw, whereas a minimal amount of PFDoA was detected in shoots of the whole lettuce plants. This indicates that PFDoA entered lettuce roots, presumably mainly via the apoplastic pathway, and the Casparian strip could block the movement of PFDoA from the extracellular space into the root xylem for upward translocation. The lower sorption of PFDoA on live lettuce roots relative to that on dead roots also indicates that the movement of PFDoA occurred predominantly in the extracellular space or via the apoplastic pathway. These explanations may also apply to other long-chain PFASs, such as PFOA, PFNA, PFDA, PFDoA, and PFOS, whose TF values were far below 1.0 (Fig. 3(A)).

## Conclusion

This study investigated the uptake and translocation of nine PFASs with different carbon chain lengths in hydroponically grown lettuce. The results revealed that lettuce could take up PFASs via roots and translocate them to the shoot. Long-chain PFASs (≥ C8) exhibited higher accumulation in lettuce roots predominantly via sorption to root surfaces. Short-chain PFASs (≤ C7) were more transferable to the shoots, as evidenced by their higher TF values. Hydrophobicity and molecular volume are important factors that affect the distribution of PFASs in lettuce. Compared to the long-chain homologues, the shorter-chain PFASs could enter root cells more easily. The Casparian strip in lettuce roots could function as an effective barrier to the upward translocation of PFASs, especially the long-chain homologues. The results of this study can extend the current understanding of the uptake and transport of PFASs in edible plants and provide useful information for evaluating the risks of PFAS contamination in agricultural systems.

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**Data availability** All data generated or analyzed during this study are included in this manuscript.

## Declarations

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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