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Abstract

Strategies for remediation of per- and polyfluoroalkyl substances (PFAS) generally prioritise highly contaminated source areas. However, the mobility of PFAS in the environment often results in extensive low-level contamination of surface waters across broad areas. Constructed Floating Wetlands (CFWs) promote the growth of plants in buoyant structures where pollutants are assimilated into plant biomass. This study examined the hydroponic growth of *Juncus krausii*, *Baumea articulata* and *Phragmites australis* over a 28-day period for remediation of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) contaminated (0.2 µg/L to 30 µg/L) urban stormwater. With increasing PFOA and PFOS concentrations, accumulation in plant species increased although root and shoot distribution varied depending on PFAS functional group. Less PFOA than PFOS accumulated in plant roots (0.006 – 0.16 versus 0.008 – 0.68 µg/g), while more PFOA accumulated in the plant shoots (0.02 – 0.55 versus 0.01 – 0.16 µg/g) indicating translocation to upper plant portions. *Phragmites australis* accumulated the highest overall plant tissue concentrations of PFOA and PFOS. The NanoSIMS data demonstrated that PFAS associated with roots and shoots was absorbed and not just surface bound. These results illustrate that CFWs have the potential to be used to reduce PFAS contaminants in surface waters.

Keywords: Floating treatment wetland; Artificial floating island; PFAS uptake; Urban stormwater treatment; *Phragmites australis.*

1. Introduction

Water treatment through constructed wetlands (CWs) is a common practice in many countries [1, 2] and offers a potentially cost-effective treatment system for a range of water effluent types [3, 4]. CWs use a combination of planted vegetation, soil and microorganisms to remove pollutants from contaminated waters. These systems are mainly used for reducing nutrient concentrations in stormwater or wastewater effluent and for inhibiting eutrophication which results in oxygen depletion, odour generation and fish mortality [5]. However, CW treatment systems also reduce the concentration of many organic contaminants [6, 7], including pesticides, pharmaceuticals, personal care products and per-and polyfluoroalkyl substances (PFAS).

Recently, there has been significant interest in PFAS due to the potential health impacts on children and reproductive health [8]. In addition, contamination-impacted community residents may face many stressors, including pervasive uncertainty, future health worries, long-term impacts on day-to-day activities, financial uncertainty, and complex chronic social stressors [9, 10]. PFAS are a group of synthetic chemicals with broad commercial applications worldwide, including manufacturing and fire-fighting foams. PFAS substances, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) which are predominant in fire-fighting foams, are soluble in water with low adsorption potential and negligible volatility (e.g., PFOA has a low $LogK_{oc}$ of ~ 2 and high solubility in water of ~ 9.5 g/L at 25 °C) [11]. The presence of PFAS in the environment has emerged as a significant environmental and human health issue. Upon release to the environment, PFAS such as PFOA and PFOS (compounds with strong and highly stable carbon-fluorine bonds) are extremely persistent [12] and can accumulate in organisms, causing adverse health effects in humans and animals including immune system impairment [13].

Yi et al. [7] reported that CW treatment systems have the potential to remove PFAS (e.g. 60% of PFOA and 63% of PFOS) from surface waters (median concentrations in the inflow: 0.815 µg/L of PFOA and 0.142 µg/L of PFOS) due to a combination of sorption to soils and sediments and plant uptake (plant species: *Typha angustifolia* L.*, Chrysopogon zizanioides* L., Roberty and *Cyperus papyrus* L.; plant density: 4 plants per m^2). In pilot-scale CWs, Chen et al. [14] reported that both PFOA and PFOS were phytoextracted (11.6 – 5.6 µg/g and 0.046 – 0.026 µg/g, respectively) by aquatic plant species including *Hygrophila pogonocalyx* Hayata*, Ipomoea aquatica* Forssk*, Ludwigia (×) taiwanensis* Peng and *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch. Chen et al. [14] also reported that plants with

large root surface areas and fast root growth had higher PFOA and PFOS uptake rates. In mesocosm experiments, Pi et al. [15] found that PFOA and PFOS accumulated in the roots in preference to the shoots/leaves of aquatic plants (*Echinodorus horemanii* Rataj and *Eichhornia crassipes* (Mart.) Solms). Bioaccumulation factors (BAFs), which represent the ratio between PFAS concentrations in the roots or shoots to that in the aqueous solution at the beginning of the experiment (20 µg/L), were higher in the roots (40 – 50 L/kg and 202 – 236 L/kg, for PFOA and PFOS, respectively) than in the corresponding leaves (23 – 41 L/kg and 17 – 55 L/kg, for PFOA and PFOS, respectively) [15].

Mudumbi et al. [16] collected random samples from eleven commonly found riparian wetland plants and reeds [e.g. *Xanthium strumarium, Phragmites australis, Schoenoplectus corymbosus*]. Among these species, bioaccumulation of PFOA was typically higher in plants that grew closer to the water's edge. Bioaccumulation and translocation rates may also be influenced by the microstructure of the roots [17, 18] with thicker taproots allowing more bioaccumulation of PFAS compared to the finely branched root systems [19]. These previous studies indicate that selection of plants with higher PFAS affinity can enhance uptake and removal of PFAS. Further, to avoid breakthrough of PFAS contaminants, plants should be harvested and replanted regularly to have a sustainable plant uptake of PFAS [7].

The concept of CWs can be extended through the development of constructed floating wetlands (CFWs), which are a more recent innovation for both stormwater and wastewater treatment [20, 21]. CFWs promote the growth of plant species in buoyant structures, where pollutants are assimilated into the plant biomass. CFWs offer an alternative treatment approach to CWs [22] in that they can be readily retrofitted into existing water environments such as lakes (natural and urban), ponds, dams and retention basins for the treatment of urban surface runoff. The ability to retrofit within existing areas can often be problematic for conventional CW systems [22] (i.e., where plant root masses are anchored within underlying soils). In contrast, CFWs use a buoyant structure onto which vegetation is planted. Similar to hydroponic systems, the vegetation is not rooted in soil and this allows roots to grow freely in the water column. The large surface area of plant roots also provides a habitat for microorganisms (biofilms) which facilitates nutrient removal through phytodepuration [23] and the capture of suspended particles within the water [21, 24]. However, plant selection is a key factor influencing CFW design [5, 25, 26] and the ability of plants to thrive in the water and remove nutrients, minerals and other pollutants from the water source needs to be carefully considered.

While numerous studies have shown that PFAS may accumulate in riparian wetland plants [16], aquatic plants [15] and edible crops [27], to date there have been limited studies assessing the potential application in CFWs as a passive, low-cost remediation strategy. Therefore further research is required to investigate the PFAS removal efficiency by various wetland plant species [28]. This research study investigated the potential of three Australian native plant species, namely *Juncus krausii* Hochst., *Baumea articulata* (R.Br.) S.T. Blake and *Phragmites australis* (Cav.) Trin. ex Steud., for their ability to bioaccumulate and translocate PFOA and PFOS from stormwater. These species were chosen because they are adaptable to CFWs [29, 30] and have demonstrated ability to successfully remove nutrients and pollutants [31, 32].

2. Materials and Methods

2.1. Chemicals

PFOA (95% purity), PFOS-K salt (≥ 98% purity), analytical grade HCl (37%) and NaOH (≥ 97.0%, pellets) were sourced from Sigma-Aldrich (Australia) while methanol (Optima® LC/MS grade) was sourced from Fisher Chemical (Australia). Isotopically labelled $^{13}C_4$-PFOA, $^{13}C_8$-PFOS and $^{13}C_8$-PFOA were sourced from Wellington Laboratories (Canada).

2.2. Experiment design

Approximately 200 L of water was collected from a South Australian urban stormwater detention basin that had previously been reported to be impacted by runoff from a PFAS contaminated site. This water was used as the medium in all PFAS-plant uptake studies. Following collection, water quality parameters (pH, organic concentration measured as DOC, conductivity, dissolved oxygen (DO) and PFAS concentration) were assessed as detailed in the Supplementary Information (SI). Plants within this catchment include *Phragmites australis, Eleocharis sphacelata* R.Br., *Schoenoplectus validus* (Vahl) A. & D.Löve, *Baumea articulata* (R.Br.) S.T.Blake and *Typha orientalis* C. Presl.

Wetland species from the genus *Juncus,* such as *Juncus effusus* L., are among the most commonly used macrophytes selected for their demonstrated capacity of nutrient removal from both stormwater [33] and wastewater [34]. These monocotyledonous plants are typically found in wetland systems and are easily adaptable to CFWs, as are other dominant macrophytes such as *Phragmites* [29, 30]. Species from both these genera have demonstrated the ability to successfully remove nutrients and pollutants [31]. For these reasons, in this study,

three native species (*Juncus krausii*, *Baumea articulata* and *Phragmites australis*) were selected for an assessment of PFOA and PFOS accumulation from PFAS-impacted stormwater.

Juncus krausii, *Baumea articulata* and *Phraghmites australis* plants were sourced from State Flora (Belair National Park, South Australia, Australia). Soil attached to root surfaces was gently removed by rinsing plants with tap water followed by deionised water, with excess moisture removed by absorbent towel. The wet mass of plants was measured, with mean values of 20.6 ± 3.4 g, 28.3 ± 8.5 g and 33.3 ± 8.6 g for *Juncus krausii*, *Baumea articulata* and *Phragmites australis*, respectively.

The plants were transferred to 250-mL polypropylene (PP) bottles initially filled with 200 mL of 0.2 µm filtered stormwater. During the study period (up to 28 days), the PP bottles were topped up weekly with filtered stormwater to maintain the initial volume (200 mL). The PP bottles were covered with aluminium foil for adequate light blocking and air was supplied via an air bubbler (using 4 mm polypropylene tubing) for aeration and positive pressure to prevent contamination from airborne spores. Experiments were conducted in a plant growth control room maintained at 20 ± 0.5 °C during day-time and 15 ± 0.5 °C during night-time with a 12 h light photoperiod. The plants were acclimatised for two weeks to allow their root systems to recover from potential damage prior to the introduction of PFOA or PFOS. The plants were self-sustained in the bottles given their well-developed roots systems so floating accessories were not added in the bottles.

Initially, *Juncus krausii* was utilised to examine the effect of PFAS concentration on plant uptake and distribution with *Juncus krausii* exposed to PFAS for up to 14 days. Immediately before the beginning of the experiment, which is denoted *Trial 1 (T1)*, stock solutions of PFOA and PFOS were prepared by dissolving the pure chemicals with sterile ultrapure water (Merck Millipore) in methanol-washed volumetric glassware and then opportune aliquots of PFOA or PFOS solutions were spiked into filtered stormwater to achieve concentrations ranging from 0.2 µg/L to 30 µg/L (n = 3 per concentration) (see SI, **Table S2**). At days 1, 7 and 14, plants were harvested, rinsed with ultrapure water and divided into roots and shoots. Wet mass was recorded prior to sample freezing (-20 °C) and freeze drying using a Modulyo freeze dryer (ThermoFisher, Australia). Freeze dried material was used for the determination of PFOA and PFOS concentrations in roots and shoots. In addition, the PFOA and PFOS concentrations in the stormwater at the time of plant harvest were determined for mass balance purposes.

Following T1, which provided an assessment of timeframes associated with PFOA and PFOS accumulation in *Juncus krausii*, two other plant species (*Baumea articulata* and *Phragmites australis*) were assessed for their ability to remove PFAS from stormwater in comparison to *Juncus krausii*. In Trial 2 (T2), experimental parameters were refined from the initial *Juncus krausii* assessment whereby PFOA or PFOS was supplied at 10 µg/L and the exposure time was extended to 28 days (see SI, **Table S3**). Further assessment (Trial T3) investigated the effect of co-contaminants at elevated concentrations (30 µg/L of PFOA and PFOS) on PFAS accumulation and translocation in *Baumea articulata* and *Phragmites australis*. A concentration of 30 µg/L of PFOA and PFOS was chosen based on reported values in contaminated surface waters within Australia [35-38].

For all plant species, PFAS concentrations and exposure time points, three replicates were prepared and analyzed. In addition, control bottles were prepared consisting of plants grown in non-spiked filtered stormwater. All bottles were arranged in the growth chamber according to a complete randomised plot design.

2.3. Sample preparation

Freeze-dried plant material was finely ground using a sample grinder (IKA A11 basic, Australia) prior to PFAS extraction. The plant material (0.1 g) was spiked with 4 ng of isotopically labelled $^{13}C_4$-PFOA and $^{13}C_8$-PFOS before extraction according to Braeunig et al. [39]. Briefly, 1.5 mL of 200 mM NaOH (Sigma Aldrich, Australia) and 3.5 mL of methanol Optima ® LC/MS grade (Fisher Chemical, Australia) were added to samples after which they were vortexed and left overnight in the dark at 4°C in closed containers. Samples were then sonicated for 20 minutes in a benchtop ultrasonic water bath (Soniclean, Australia) with 120 W pulse swept power operating at 43 ± 2 kHz sweep bandwidth with 20 Hz pulses. Sonication was followed by neutralisation with 4 M HCl (~ 75 µL) and centrifugation for 20 minutes at 4000 RCF, at room temperature. Supernatants were transferred to PP tubes and a second extraction step was performed using methanol (1 mL). Extracts were pooled and reduced in volume at 40 ˚C on a Multivap solvent evaporator (Organomation, U.S.A.) using a gentle flow of nitrogen gas until samples reached approximately 1 mL. To remove interferences, extracts were cleaned up using 250 mg Bond Elut Carbon cartridges (Agilent, Australia) that were pre-conditioned with methanol; the filtrates from the cartridges were collected directly in PP HPLC vials. The empty supernatant tubes were also rinsed with 300 µL of methanol followed by another filtration with the carbon cartridges used for the same sample in order to minimise potential losses in both tubes and cartridges and maximise

recovery. All volumes of samples collected in the HPLC PP vials were finally reduced to 1 mL on the solvent evaporator. The overall average recoveries using this procedure were 89% and 114% for $^{13}C_4$-PFOA and $^{13}C_8$-PFOS, respectively. The recoveries for each tested batch are reported in the SI (**Table S4**). Waters samples collected at the start and end of the experiments were diluted with methanol (50:50) and spiked with 4 ng of isotopically labelled $^{13}C_4$-PFOA and $^{13}C_8$-PFOS before analysis.

2.4. Analytical determination of PFOA and PFOS

PFAS analysis was conducted using high performance liquid chromatography (Thermo Scientific UltiMate 3000 HPLC system) coupled to a tandem mass spectrometer (Thermo Altis Triple Quadrupole Mass Spectrometer) operating in negative electrospray ionisation mode and using multiple reaction monitoring (MRM).

Briefly, a 10 µL sample was introduced onto a Hypersil GOLD PFP column (100 x 2.1mm, 3 µm particle size; Thermo Scientific, Australia) held at a constant temperature of 40ºC, with a flow rate of 0.25 mL/min. Separation was achieved by gradient elution from the column. LC-MS grade methanol and 5 mM ammonium formate (prepared in ultrapure water) were used as mobile phases. Identification and confirmation of peaks were performed using retention times and comparing the ratios of MRM transitions between samples and calibration standards. Details on separation and detection conditions are described in the SI (**Tables S5 and S6**).

Concentrations of PFOA and PFOS in samples were quantified by isotope dilution. Eight calibration standards with PFOA and PFOS concentrations ranging from 0.1 to 100 µg/L were prepared in the same matrix as the samples, i.e. methanol for plant extracts, and 50:50 methanol:water for the stormwater samples which were diluted with methanol (50:50). Each standard also had 4 ng of isotopically labelled $^{13}C_4$-PFOA and $^{13}C_8$-PFOS – the same amount introduced to plants during extraction and preparation of the stormwater samples.

2.5. Analytical quality assurance and quality control

To prevent cross-contamination, all reusable labware and glassware were acid washed and methanol rinsed prior to use. For each batch of extractions, blanks, duplicates and fortified samples were included and treated in the same way as real samples. For each analytical batch, continuing calibration verification standards (CCV) and continuing calibration blanks (CCB) were included multiple times (approximately every 15 samples injected) to verify if the calibration was still suitable; results for the CCV within ± 2.5% of

its expected concentration were considered acceptable. Samples with concentrations outside the range of the calibration standards were diluted in methanol then reanalyzed. Instrumental limits of detection (LOD) and limits of quantitation (LOQ) were estimated to be 0.10 and 0.40 µg/L for PFOA and 0.25 and 0.75 µg/L for PFOS based on 3x and 10x the signal to noise.

2.6. Data and statistical analysis

Translocation factors (TF) at different harvest days (1, 4, 7, 14 and 28) were calculated according to Eq. 1. Bioaccumulation factors (BAF) for roots (BAF_{root}, Eq. 2), i.e. the ratio between PFAS concentration in the roots at time points throughout the exposure period and stormwater at the beginning of the experiment was calculated for each treatment. BAF values were also calculated for shoots (BAF_{shoot}, Eq. 2) and for whole plants ($BAF_{whole\ plant}$, Eq. 3) following the method previously reported by Zhang et al. [40].

$$TF = \frac{PFAS\ concentration\ in\ the\ shoots\ (\mu g/g)}{PFAS\ concentration\ in\ the\ roots\ (\mu g/g)} \quad (1)$$

$$BAF_{roots\ or\ shoots} = \frac{PFAS\ concentration\ in\ the\ roots\ or\ shoots\ (\mu g/kg)}{PFAS\ concentration\ in\ the\ \text{stormwater}\ (\mu g/L)} \quad (2)$$

$$BAF_{whole\ plant} = \frac{1}{m_{roots}+m_{shoots}}(BAF_{roots} \times m_{roots} + BAF_{shoots} \times m_{shoots}) \quad (3)$$

where, m_{roots} is the dry mass (g) of plant roots and m_{shoots} is the dry mass (g) of plant shoots.

Analysis of variance (ANOVA) was used to evaluate the effect of PFAS concentration on plant uptake and total PFAS removal. When a specific concentration was found to influence uptake or total PFAS removal, statistical differences within treatments were determined using the "Two-Sample Student's t test" comparison. Further, the same approach was used to assess changes in TF and BAF values over the study period among treatments. Calculations were performed using Minitab Software (Version 18.1.0) with *p*-values < 0.05 being considered as significant.

2.7. ^{13}C-PFOA and ^{13}C-PFOS labelling

In order to demonstrate that PFOA and PFOS is taken up by plant tissues (both root and shoot) and not just surface bound, high resolution mass spectrometry (NanoSIMS) analysis was conducted for labelled *J. krausii* plants. For this, after 7 days under simulated control conditions, aliquots of ^{13}C-PFOA or ^{13}C-PFOS were spiked into filtered stormwater to achieve 10 µg/L (n = 3 per concentration). *J. krausii* plants were then added to the PP

bottles and grown in a plant growth control room maintained at 20 ± 0.5 °C during day-time and 15 ± 0.5 °C during night-time with a 12 h light photoperiod for 28 days to allow ^{13}C-PFOA or ^{13}C-PFOS uptake. Further control bottles were prepared consisting of *J. krausii* grown in non-spiked filtered stormwater. At the end of the exposure period, plants were harvest with shoots, roots and water separated. Half of the shoot and root samples were oven-dried and weighed, and the other half chemically fixed with 2.5% glutaraldehyde and stored at -80 °C before further sample preparation for NanoSIMS analysis.

Plant tissue, individual root portions and shoots (both 5-10 mm) were rinsed in milli Q water. Samples were stored at 4°C for ~ one week before being dehydrated in a graded series of 30 min ethanol (20, 50, 70, 100 %) incubations. Dehydrated plant tissue was cut into smaller pieces (~2 mm) and resin embedded in a graded series of 'ultra-low viscosity embedding media (Polysciences, Pensylvania USA) as per manufacturers protocol for a 'hard' mix. Plant tissue was incubated in each solution (25, 50, 75, 100% resin in Ethanol) overnight before a final overnight incubation in 100% resin under low vacuum. Resin was then cured at 70°C for 24 hours. 350 nm sections were cut from resin impregnated tissue samples (Leica EM UC6 Ultramicrotome; Leica Microsystems, Wetzlar, Germany) using a 45-degree diamond knife (Diatom, Switzerland). Sections were mounted onto 5 mm^2 silicon wafers, dried and coated with 10nm Au.

2.8. NanoSIMS analysis

High resolution mass spectrometry analysis was performed on a NanoSIMS-50 ion microprobe (CAMECA, France) at The University of Western Australia using a 16 keV Cs^+ primary ion beam. The nanoSIMS was operated in multi-collection mode with trolleys/detectors positioned to simultaneously detect the negative secondary ions ^{17}F-, $^{12}C_2^-$,$^{13}C^{12}C^-$, $^{12}C^{14}N^-$, $^{31}P^-$. The mass spectrometer was tuned to high mass resolution of c. 10000 (CAMECA definition) to separate the $^{12}C^{13}C$ from the $^{12}C_2H$ peak on mass 25 allowing determination of $^{13}C/^{12}C$ ratios as well as $^{14}N^{12}C$ and ^{31}P and secondary electron imaging (for identification of cellular and sub-cellular structures). Prior to analysis, selected areas of interest were sputtered (Cs^+ implanted) by rastering a defocused primary ion beam (current density 7.8×10^{16}ions cm^{-2}) over a slightly larger area to allow samples to reach sputtering equilibrium. Generally, analysis was performed in a chained method to allow 'stitching together' of many smaller images (30 um2; 256 x 256 pixels) to create a single larger image of root or shoot sections. Images were processed and analysed using the OpenMIMS data analysis software plugin in ImageJ (http://www.nrims.hms.harvard.edu/software.php). Single

images were stitched together using nrrd mosaics script (available and described at https://github.com/BWHCNI/OpenMIMS/wiki/nrrd-Mosaics).

3. Results and Discussion

Stormwater used for PFAS experiments was collected from an urban stormwater detention basin which had the following water quality characteristics: DOC 4.35 ± 0.05 mg/L; pH 8.2 ± 0.1; TDS 248 ± 18 µS/cm; DO 9.1 ± 0.1 mg/L. The background PFAS concentration in the stormwater was low (below the drinking water trigger level of 0.07 µg/L) with only PFOS being detected above the level of reporting (see Table S1). This concentration was approximately 3-430 times lower than the PFOS exposure concentrations used in the plant uptake studies. The PFOS and PFOA concentrations in the roots and shoots of plants grown in non-spiked water (used as a control) were below the limit of reporting indicating that potential PFAS cross-contamination from the environment, chemical reagents, bottles and / or aeration systems did not occur.

3.1. PFOA and PFOS accumulation in *Juncus krausii*

To examine the effect of PFAS concentration on PFAS-plant accumulation, *Juncus krausii* was selected as the test species and was grown in stormwater spiked with PFOA or PFOS at concentrations ranging from 0.2 µg/L to 30 µg/L. PFOA and PFOS accumulation in roots and shoots was determined after 1, 7 and 14 days (**Figure 1**). For both PFOA and PFOS, root and shoot PFAS concentration increased with increasing source concentration in stormwater (p = 0.02; **Figure 1**). A positive correlation between PFOA and PFOS accumulation in plant tissue (root + shoot) and the initial stormwater concentrations was also observed in this study (**Figure 2a**). PFOA accumulation in shoots was significantly higher compared to PFOS at the same exposure concentrations (at C_0 = 30 µg/L: 0.55 ± 0.03 µg/g vs 0.10 ± 0.08 µg/g; at C_0 = 10 µg/L: 0.24 ± 0.03 µg/g vs 0.03 ± 0.01 µg/g; at C_0 = 2 µg/L: 0.03 ± 0.01 µg/g vs 0.03 ± 0.02 µg/g; at C_0 = 2 µg/L: 0.004 ± 0.0 µg/g vs 0.01 ± 0.01 µg/g; p = 0.03). In contrast, PFOA accumulated in *Juncus krausii* roots at significantly lower concentrations compared to PFOS for the same corresponding treatment (at C_0 = 30 µg/L: 0.16 ± 0.08 µg/g vs 0.56 ± 0.07 µg/g; at C_0 = 10 µg/L: 0.11 ± 0.11 µg/g vs 0.19 ± 0.08 µg/g; at C_0 = 2 µg/L: 0.01 ± 0.01 µg/g vs 0.02 ± 0.01 µg/g; at C_0 = 2 µg/L: 0.006 ± 0.002 µg/g vs 0.008 ± 0.004 µg/g; p = 0.04).

At the end of the exposure time, at exposure concentrations of 0.2 µg/L and 2 µg/L, the overall plant tissue accumulations of PFOA (0.004 and 0.025 µg PFOA/g, respectively)

were lower than those of PFOS (0.01 and 0.032 µg PFOS/g, respectively) for corresponding treatments. However, in water spiked with PFAS concentrations of 10 µg/L and 30 µg/L, the overall plant tissue accumulations of PFOA (0.24 and 0.55 µg PFOA/g, respectively) were larger than those of PFOS (0.03 and 0.1 µg PFOS/g, respectively) for corresponding treatments, **Figure 2a**. A linear correlation between PFOA and PFOS accumulation in plant tissue and the exposure time was observed and uptake rates (µg/g-d) were also found to be higher (but not significantly, $p = 0.27$) for PFOA compounds compared to those of PFOS at exposure concentrations of 30 µg/L (0.051 vs 0.046) and 10 µg/L (0.026 vs 0.016).

TF ratios at the end of the exposure time of 14 days were calculated and the values are presented in **Figure 2b**. At exposure concentrations of 0.2 µg/L and 2 µg/L, no significant differences were found for the TF values for PFOA and PFOS (at $C_0 = 0.2$ µg/L: 0.64 vs 1.16; at $C_0 = 2$ µg/L: 1.74 vs 1.65; $p = 0.72$). In contrast, at exposure concentrations of 10 µg/L and 30 µg/L, the TF values for PFOA were significantly higher than those for PFOS (at $C_0 = 10$ µg/L: 2.11 vs 0.14; at $C_0 = 30$ µg/L: 3.47 vs 0.23; $p = 0.06$). Furthermore, for PFOA-spiked waters, the TF values increased with increasing initial contaminant levels ($p = 0.02$) while for PFOS-spiked waters, no such correlation was found ($p = 0.73$), as shown in **Figure 2b**. Zhang et al. [28] and Pi et al. [15] also reported that PFOS was largely accumulated in the roots with limited upward translocation. Zhang et al. [28] reported similar TF values (TF: < 0.4 for PFOS and ~2 for PFOA after 21 days from exposure) for *Juncus effusus* grown hydroponically in nutrient solution spiked with 250 µg/L and 4,300 µg/L of PFOA and PFOS, respectively. Zhang et al. [40] also reported similar TF values for PFOS (TF: < 0.5) but lower TF values for PFOA (TF: < 0.5) for *Juncus effusus* grown in soil using nutrient solution spiked with 50 µg/L and 4,300 µg/L of PFOA and PFOS, respectively. It has been reported previously that relatively higher hydrophobicity and lipophilicity compounds (such as PFOS) might have greater interactions with biological macromolecules in plant roots, resulting in their limited upward translocation during transpiration processes [28, 41]. These TF values indicate an effectiveness in translocating PFOA from *Juncus krausii* roots to shoots, which may suggest a potential phytoremediation ability for this compound in this plant species. Although these data indicate limited upward translocation of PFOS, the entire plant can be harvested and replanted regularly in a CFW system, which provides a mechanism for sustainable plant uptake of PFOS, without breakthrough should uptake capacity be exhausted.

Calculated BAF_{shoot} and BAF_{root} values are reported in the SI (**Table S7)**, while whole plant values ($BAF_{whole\ plant}$) are shown in **Figure S1**. A decreasing trend of BAFs for roots,

shoots and whole plants with increasing PFAS concentrations was observed (BAF_{root}: from 30.7 to 5.3 L/kg for PFOA and from 41.5 to 14.0 L/kg for PFOS at exposure concentrations of 0.2 to 30 µg/L; BAF_{shoot}: 19.5 – 12.2 L/kg (PFOA) and 48.1 – 3.2 L/kg (PFOS); $BAF_{whole\ plant}$: 24.2 – 9.6 L/kg (PFOA) and 45.5 – 7.5 L/kg (PFOS), as shown in **Table S7**. These observations agree with previously reported findings [40] where a decreasing trend of BAFs with increasing PFAS concentration was also observed for *Juncus effusus* growth in soil at three different PFAS concentrations (PFOS: 4.2, 4,300 and 43,000 µg/L; PFOA: 0.405, 250 and 2,500 µg/L).

In this study, BAF values increased over the experimental period for both PFOA and PFOS spiked at all four concentrations ($BAF_{whole\ plant}$ rate, L/kg.d: +0.93 and +0.60 at C_0 = 0.2 µg/L; +0.67 and +0.93 at C_0 = 2.0 µg/L; +1.47 and +0.65 at C_0 = 10.0 µg/L; +0.67 and +0.52 at C_0 = 2.0 µg/L). BAF_{root} values were significantly lower for PFOA (5.3 – 30.7 L/kg) than corresponding values for PFOS (9.8 – 41.5 L/kg, p = 0.04). Similar to TF values, for stormwater spiked with 10 µg/L and 30 µg/L of PFOA, BAF_{shoot} values were significantly higher compared to stormwater spiked with PFOS (ratio: 8.89 and 3.77, p = 0.09). However, for stormwater spiked with 0.2 µg/L and 2 µg/L, no significant difference was observed for PFOA and PFOS BAF_{shoot} values (p = 0.77).

3.2. Comparison of PFOS and PFOA plant uptake for different native species

The initial *Juncus krausii* experiments determined that both PFOA and PFOS may accumulate in the plant when exposed to a range of PFAS concentrations. However, differences in TF and BAF were observed depending on the functional group. A concentration of 10 µg/L was chosen for the assessment of other plant species (*Baumea articulata* and *Phragmites australis*) as the differentiation between plant behaviour to translocate and accumulate PFOA and PFOS was more significant at this concentration. Furthermore, 10 µg/L represents the average PFOS concentration detected in contaminated surface waters within Australia [35-38].

PFOA and PFOS accumulation in roots and shoots was determined over a 28-day exposure period, as shown in the SI (**Figure S2)**. A trend was observed where increasing shoot uptake of both PFOA and PFOS was associated with increasing exposure time for all species, which is similar to the findings reported by Zhang et al. [28] for *Juncus effusus* and by Zhang et al. [42] for *Carex comosa* where exposure time also positively affected plant uptake of PFAS compounds.

For all three plant species, PFOA accumulated in plant roots at significantly lower concentrations than PFOS, while PFOA accumulated in plant shoots at significantly higher concentrations than PFOS. At the end of the exposure (D28) and for all plants under consideration, PFOA accumulated in shoot tissue (µg PFOA/g shoot) was high compared to corresponding values in the root tissue (*Baumea articulata*: 0.08 ± 0.01 vs 0.04 ± 0.02; *Phragmites australis*: 0.16 ± 0.03 vs 0.05 ± 0.02; *Juncus krausii*: 0.06 ± 0.01 vs 0.02 ± 0.01, **Figure 3a**). In contrast, PFOS accumulated in shoot tissue (µg PFOS/g shoot) was generally low compared to corresponding values in the root tissue (*Baumea articulata*: 0.07 ± 0.01 vs 0.11 ± 0.03; *Phragmites australis*: 0.07 ± 0.04 vs 0.27 ± 0.06; *Juncus krausii*: 0.05 ± 0.02 vs 0.06 ± 0.00, **Figure 3a)**.

Furthermore, to demonstrate that PFOA and PFOS was taken up by plant tissues (both root and shoot) and not just surface bound, NanoSIMS analysis was conducted using ^{13}C labelled PFOS and PFOA and *Juncus krausii*. Images from the NanoSIMS clearly demonstrates the presence of added ^{13}C (derived from PFOA or PFOS) within plant tissue, both root and shoot, albeit at low enrichment ($^{13}C/^{12}C$: 0.013, **Figure 4**) while data from unlabeled tissue (**Figure S3, SI**) indicates homogenous $^{13}C/^{12}C$ across all tissue types and natural abundance values (0.011).

Isotope ratio images enabled visualization of the in-situ flow of ^{13}C -PFOA and ^{13}C-PFOS through *Juncus krausii* root and shoots (**Figure 4**). After 7 days of the initial labelling, the apoplastic pathway of the epidermidis, cortex and phloem root cells were significantly enriched in ^{13}C -PFOA and ^{13}C-PFOS compared to the symplastic pathway (**Figure 4**). Higher ^{13}C -PFOA and ^{13}C-PFOS enrichment was visible in the apoplastic pathway of the shoot and to a lesser extent in the cytoplasm. Higher ^{13}C -PFOA and ^{13}C-PFOS was commonly located at intersections of more than two cells (**Figure 4**), and to a lesser extent in the symplastic areas of the cortex. The ^{13}C-PFOS taken up from the water solution was detected in both cells and cell walls of the shoot (symplast and apoplast) and was observed in the epidermidis and cortex (apoplast only) (**Figure 4**). There was a quantitative difference in the ^{13}C -PFOS and accumulation in the leaves and roots, with the roots being less enriched in the cortex symplast. ^{13}C-PFAS enrichment was higher in the apoplast than in the symplast of the shoot and was observed in the root epidermidis and cortex at higher concentration than the ^{13}C-PFAS (**Figure 4**).

The highest overall plant tissue accumulation of PFOA and PFOS was found for *Phragmites australis* species (0.21 ± 0.02 µg/g and 0.33 ± 0.04 µg/g) followed by *Baumea*

articulata (0.13 ± 0.00 µg/g and 0.18 ± 0.01 µg/g) and then *Juncus krausii* (0.09 ± 0.01 µg/g and 0.11 ± 0.01 µg/g). PFOA and PFOS uptake (µg/g) was significantly lower than values reported by Chen et al. [14] for (between 5.6 to 11.6 for PFOA and between 26 to 46 for PFOS) for four aquatic plants i.e. *Hygrophila pogonocalyx* Hayata*, Ipomoea aquatic* Forssk*, Ludwigia (×) taiwanensis* and *Eleocharis dulcis* species. This may be attributed to the high concentrations (C_0 = 5,000 µg/L) that were used in their study. García-Valcárcel et al. [43] also reported higher overall accumulation of PFOA (~ 2 - 3.2 µg/g) and PFOS (~ 2 - 3 µg/g) in grass (*Bromus diandrus*) tissues grown in nutrient solution but at higher contaminant concentrations (500 and 1,000 µg/L).

For all species under consideration and similar to the outcomes from Trial 1, PFOA TF values were also found to be significantly higher compared to PFOS TF values (mean at D28: 1.93 vs 0.63 for *Baumea articulata*; 3.29 vs 0.26 for *Phragmites australis*; 2.65 vs 0.84 for *Juncus krausii* p = 0.001), **Figure 5**. The TF values also increased with increasing exposure times (ΔTF/Δtime for PFOA: +0.059, +0.116 and +0.087; ΔTF/Δtime for PFOS: +0.012, +0.006 and +0.031 for *Baumea articulata*, *Phragmites australis* and *Juncus krausii*, respectively, as shown in **Figure 5b**) and a plateau was only observed for PFOS TF values for *Juncus krausii* at the end of the exposure time. For PFOA, the highest TF value was for *Phragmites australis* (mean at D28: 3.29) followed by *Juncus krausii* (2.65) then *Baumea articulata* (1.93) while for PFOS, the highest TF value was for *Juncus krausii* (0.84) followed by *Baumea articulata* (0.63) and *Phragmites australis* (0.26). Poor translocation of PFOS can be attributed to the fact that these plants have hollow stems (helophytes), or that they have large aerenchyma with piths evolved into pith cavities. As a result, the cross-sectional area of the stem is reduced and this results in fewer acropetal translocation routes following aboveground uptake of large chain compounds [17]. However, the plant roots can be readily harvested from a CFW system and this provides a potential mechanism for sustainable plant uptake of PFOS.

Values for BAF_{shoot}, BAF_{root} and $BAF_{whole\ plant}$ were calculated over a 28-day exposure period and are presented in the SI (**Table S8**). $BAF_{whole\ plant}$ values at the end of the exposure time (D28) are shown in **Figure 3c**. The highest BAF_{root} values were for *Phragmites australis* species (5.0 L/kg for PFOA and 26.5 L/kg for PFOS) followed by *Baumea articulata* (4.4 L/kg and 11.3 L/kg) and then *Juncus krausii* (2.4 L/kg and 5.8 L/kg). A similar trend was found for BAF_{shoot} and $BAF_{whole\ plant}$ for both PFOA and PFOS, where higher values were observed for *Phragmites australis* (BAF_{shoot}: 16.4 and 6.9; $BAF_{whole\ plant}$: 11.8 and 14.8)

compared to *Baumea articulata* (BAF_{shoot}: 8.5 and 7.2; $BAF_{whole\ plant}$: 7.2 and 8.5) and *Juncus krausii* (BAF_{shoot}: 6.3 and 4.8; $BAF_{whole\ plant}$: 4.6 L/kg and 5.2 L/kg). For all three plant species, $BAF_{whole\ plant}$ values for PFOA were lower compared to corresponding values for PFOS (mean values for *Phragmites australis*: 11.8 vs 14.8 ($p = 0.02$); for *Baumea articulata*: 7.2 vs 8.5 ($p = 0.04$); for *Juncus krausii*: 4.6 vs 5.2 L/kg, ($p = 0.04$)), **Figure 3c**. A similar finding was also reported by Pi et al. [15] with PFOA $BAF_{whole\ plant}$ values were lower compared to values for PFOS for both *Echinodorus horemanii* (43 vs 86) and *Eichhornia crassipes* (27 vs 90) grown in nutrient solution spiked with 20 µg/L of PFOA and PFOS after 14 days from exposure.

The overall percentage removal values for both PFOA and PFOS by the three species under consideration at the end of the exposure time are presented in **Figure 3b**. The highest overall PFOA and PFOS removal efficacy was found for *Phragmites australis* species (mean: 53% and 42%) followed by *Baumea articulata* (29% and 24%) and then *Juncus krausii* (5% and 5%).

3.3. Assessment of PFOA and PFOS accumulation in plant tissues when exposed to high initial concentrations

The two plant species exhibiting the highest overall PFOA and PFOS removal efficacy (i.e. *Phragmites australis* and *Baumea articulata,* as shown in **Section 3.2**) were tested further under extreme conditions (Trial 3) where plants were grown hydroponically in water spiked with 30 µg/L of PFOA and 30 µg/L of PFOS. The 30 µg/L is equivalent to the sum of PFAS compounds that have been detected in surface waters [35-38].

The concentrations of PFOA and PFOS accumulated in root and shoot tissues were measured and the results are shown in **Figure 6**. The TF values over a 28-day exposure period are shown in **Figure 7a.** BAF_{shoot}, BAF_{root} and $BAF_{whole\ plant}$ at the end of the exposure time (D28) are also shown in **Figure 7b** while these values over a 28-day exposure period are presented in the SI (**Table S10**). As observed in Trial 2 (experiment conducted at an exposure concentration of 10 µg/L), the increasing trend of shoot uptake with increasing exposure time ($p = 0.01$) was also found for both plant species. PFOA was found to be accumulated in both *Phragmites australis* and *Baumea articulata* roots at significantly lower concentrations than PFOS (*Baumea articulata*: $p = 0.004$; *Phragmites australis*: $p = 0.001$). Consistent with data obtained at an exposure concentration of 10 µg/L, the overall plant tissue accumulation of PFOS was found to be higher for *Phragmites australis* species (0.62 ± 0.12 µg/g) compared to *Baumea articulata* (0.24 ± 0.05 µg/g), $p = 0.001$. In contrast, no significant difference was

observed for PFOA values (0.38 ± 0.15 µg/g vs 0.32 ± 0.1, $p = 0.72$). Similar to the findings for *Juncus krausii* (Trial 1), a positive correlation between PFOA and PFOS accumulation in plant tissue and the initial exposure concentrations was also observed (*Baumea articulata*: 0.32 vs 0.13 µg/g for PFOA and 0.24 vs 0.18 µg/g for PFOS at C_0 = 10 and 30 µg/L, respectively; *Phragmites australis*: 0.38 vs 0.21 µg/g and 0.62 vs 0.33 µg/g), as shown in **Figure 3** and **Figure 6**.

As for the previous trials (Trials 1 and 2), TF values increased with increasing exposure time (ΔTF/Δtime for PFOA: +0.11 and +0.09; ΔTF/Δtime for PFOS: +0.016 and +0.01 for *Baumea articulata* and *Phragmites australis*, respectively) and a plateau was also not observed at the end of the exposure time, as shown in **Figure 7a**. In addition, PFOA TF values were significantly ($p = 0.001$) higher than those of PFOS (mean at D28: 3.38 vs 0.68 for *Baumea articulata*; 2.76 vs 0.40 for *Phragmites australis*). At the end of the exposure time, TF values were found to be higher for *Baumea articulata* species compared to the corresponding values for *Phragmites australis* (PFOA: 3.38 vs 2.76; PFOS: 0.68 vs 0.40).

Similar to Trial 2 (i.e. C_0 = 10 µg/L), PFOS BAF values were higher for *Phragmites australis* compared to the corresponding values for *Baumea articulata* (BAF_{root}: 15.4 vs 4.8 L/kg; BAF_{shoot}: 5.4 vs 3.3 L/kg; $BAF_{whole\ plant}$: 10.7 vs 3.8 L/kg). PFOS BAF values were found to be lower at C_0 = 30 µg/L compared to the corresponding treatment at C_0 = 10 µg/L (BAF_{root}: 4.8 vs 11.3 L/kg for *Baumea articulata* and 15.4 vs 26.5 L/kg for *Phragmites australis*; BAF_{shoot}: 3.3 vs 7.2 L/kg for *Baumea articulata* and 5.4 vs 6.9 L/kg for *Phragmites australis*; $BAF_{whole\ plant}$: 3.8 vs 8.5 L/kg for *Baumea articulata* and 10.7 vs 14.8 L/kg for *Phragmites australis*), as shown in **Figure 7b** and **Figure 3c.** PFOA BAF values followed the same trend with values being lower at C_0 = 30 µg/L compared to the corresponding treatment at C_0 = 10 µg/L (BAF_{root}: 2.4 vs 4.4 L/kg for *Baumea articulata* and 4.5 vs 5.0 L/kg for *Phragmites australis*; BAF_{shoot}: 8.2 vs 8.5 L/kg for *Baumea articulata* and 8.3 vs 16.4 L/kg for *Phragmites australis*; $BAF_{whole\ plant}$: 6.1 vs 7.2 L/kg for *Baumea articulata* and 5.9 vs 11.8 L/kg for *Phragmites australis*), as shown in **Figure 7a** and **Figure 3c**. These data indicate that the BAFs for roots, shoots and whole plants decrease with increasing PFAS concentrations, which is similar to the findings for *Juncus krausii* (Trial 1, **Figure S1**).

As observed in Trial 2, the overall PFOS removal efficacies were also found to be higher for *Phragmites australis* (mean: 27%) compared to *Baumea articulata* (9.5%). In contrast, no such distinction was apparent for the PFOA removal efficacies (15.2% vs 16%). The overall removal efficacy was found to decrease with increases in PFAS concentration in

stormwater (*Baumea articulata*: 24.3%, 16.0%, 28.7% and 9.5%; *Phragmites australis*: 42.3%, 15.2%, 53.2% and 26.9% for water spiked with 10 µg/L of PFOA, 30 µg/L of PFOA, 10 µg/L of PFOS and 30 µg/L of PFOS, respectively), which is similar to the findings for *Juncus krausii* (Trial 1).

It has been reported that the uptake process of PFAS is initiated with adsorption onto the root surface followed by transportation to the root epidermal cells and then radial transportation to the cortex where vascular bundles are present in diverse forms [17]. The plants differentially allow the bioaccumulation of PFAS mass in their tissues and this role is crucial for PFAS remediation of contaminated waters [27]. Several wetland species have previously been studied and their efficiency for PFAS removal has been reported [15, 28, 40, 42]. However, a direct comparison between the efficiency for PFAS removal observed in the present study to those values reported previously is difficult because the experimental conditions are different. These differences include plant media and water (soil, nutrient solution, wastewater vs stormwater) as well as different initial contaminant types and concentrations.

Although long-chain PFAS compounds can accumulate in the roots and shoots of plants, as described above, it has been reported that long-chain PFAS compounds are removed largely by sorption processes [17]. Consequently, additional measures such as the inclusion of removable sorptive materials could be an additional means of removing PFAS from solution [7] i.e. PFOA will be taking up in the plant while PFOS could be adsorbed by the bedding layer. Some CFWs include interchangeable plant baskets which can be pre-established with removable sorptive materials such as granular activated carbon or biochar. The buoyant structures of CFWs can also include aeration systems that can increase aerobic microbial actions resulting in improved degradation of PFAS in the presence of molecular oxygen [17]. Zhang and Liang [44] reported that aeration significantly improves the removal by duckweed of PFAS compounds such as PFOA and PFOS.

Furthermore, management of harvested PFAS-contaminated plant material is required. Management strategies for harvested PFAS-contaminated plant material includes pyrolysis to produce PFAS-free biochar materials. Thermal desorption of PFAS from the waste followed by destruction will reduce the total amount of the compound requiring destruction since only the off-gases are destroyed instead of the entire waste material itself. The resultant biochar, which would otherwise enter the waste stream, can be then utilized to improve urban water quality.

4. Conclusion

This study demonstrated the ability of three wetland species (*Phragmites australis, Baumea articulata* and *Juncus krausii*) to uptake, bioaccumulate and translocate long-chain PFAS compounds (i.e. PFOA and PFOS) from contaminated stormwater (level: 0.2 µg/L to 30 µg/L). A trend was observed where increasing shoot uptake of both PFOA and PFOS was associated with increasing exposure time for all three plant species and increasing concentration of these chemicals in stormwater. However, bioaccumulation factors decreased with increasing PFAS concentrations. Both the translocation factors (TF) and bioaccumulation factors increased with longer exposure times.

For all three plant species, PFOA accumulated in plant roots at significantly lower concentrations than PFOS, while PFOA accumulated in plant shoots at significantly higher concentrations than PFOS. The PFOA TF values were also found to be significantly higher compared to PFOS. The TF values indicate the plants' effectiveness in translocating PFOA from roots to shoots but only limited upward translocation of PFOS was observed. However, plant roots can be readily harvested and replanted regularly from a CFW system, which provides a mechanism for sustainable plant uptake of PFOS, without breakthrough should uptake capacity be exhausted.

The highest overall PFOA and PFOS removal efficacies were found to be for *Phragmites australis* followed by *Baumea articulata and* then *Juncus krausii*. However, for all plants under consideration, the overall removal efficacy was found to decrease with increases in PFAS concentration in stormwater. The NanoSIMS data clearly demonstrate the presence of PFOA and PFOS within plant tissue, both root and shoot but not on external surfaces. These results show that CFWs planted with native plant species can be used to reduce long-chain PFAS contaminants in surface waters.

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Figures

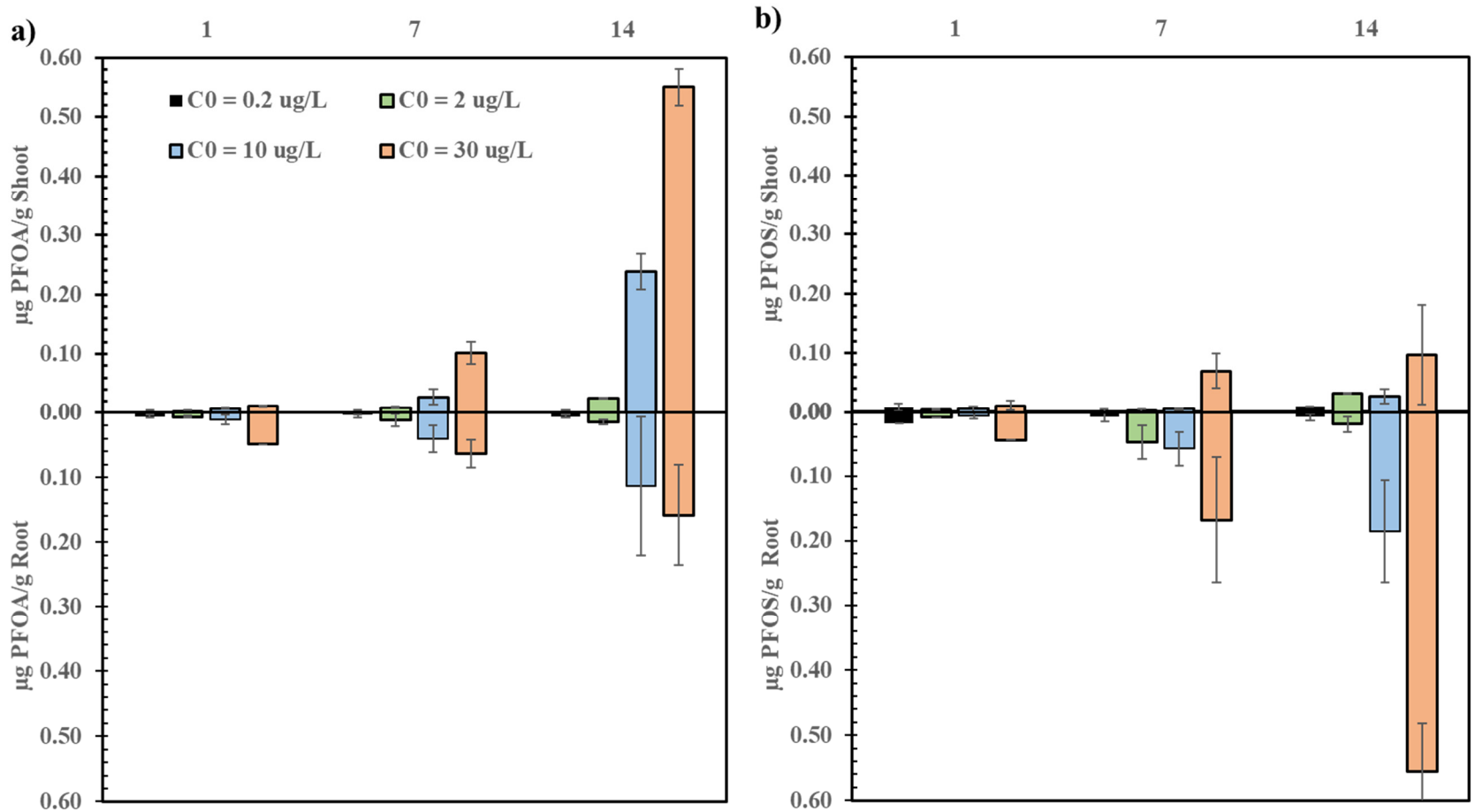
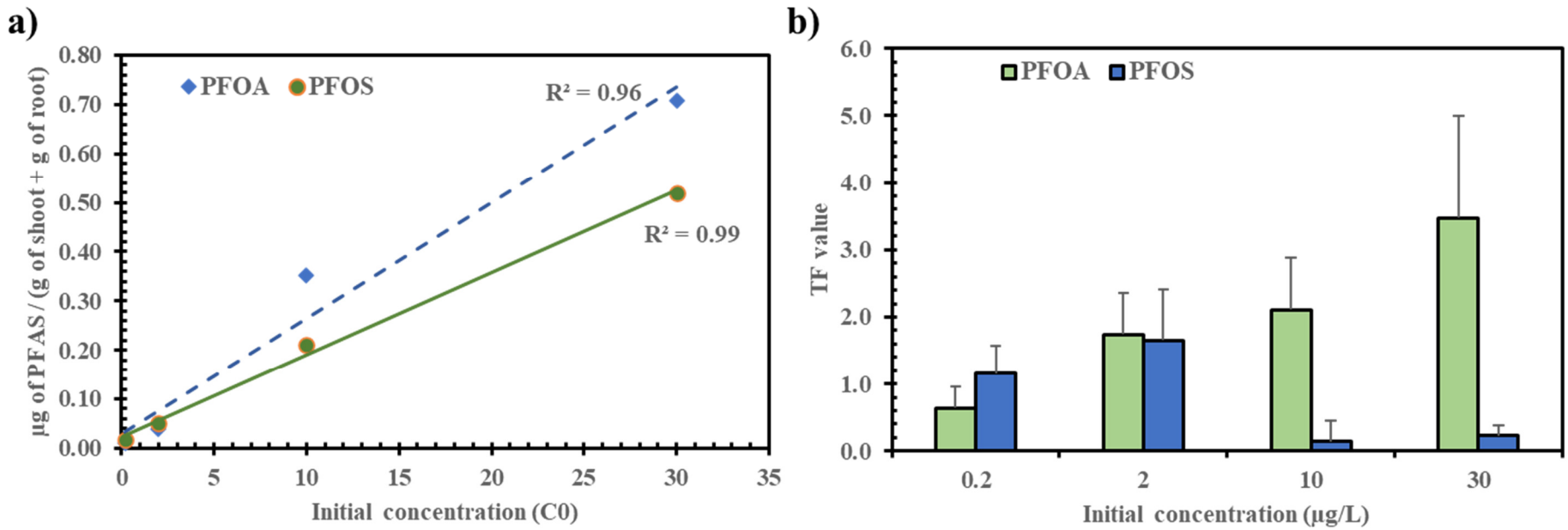


Figure 1. Concentration of a) PFOA and b) PFOS in *Juncus krausii* shoots and roots after 1, 7 and 14 days of exposure to 0.2, 2, 10 and 30 µg/L of PFOA or PFOS in stormwater. Error bars represent the standard deviation (n= 3).



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703 **Figure 2**. PFOS and PFOA accumulation in plant biomass (roots + shoots) (a) and *Juncus krausii* translocation factors after 14 days exposure to
704 PFOA- or PFOS-spiked stormwater (b).

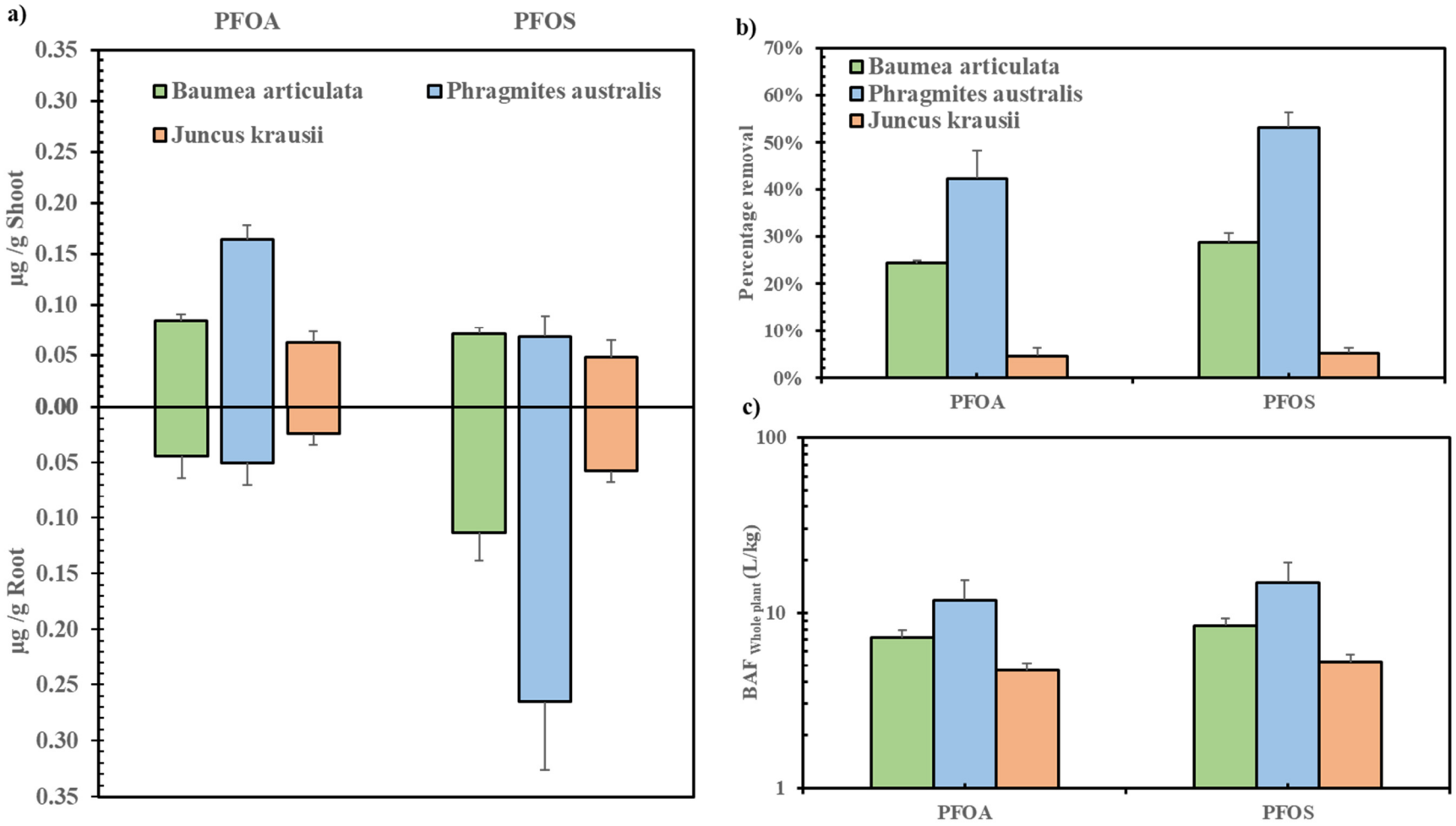


Figure 3. Concentration of PFOS and PFOA in shoots and roots of *Baumea articulata*, *Phragmites australis* and *Juncus krausii* (a), percentage removal (%) (b) and $BAF_{whole\ plant}$ values for both PFOS and PFOA (c) at the end of the exposure period for water spiked with 10 µg/L of PFOA and 10 µg/L of PFOS. Error bars represent the standard deviation (n= 3).

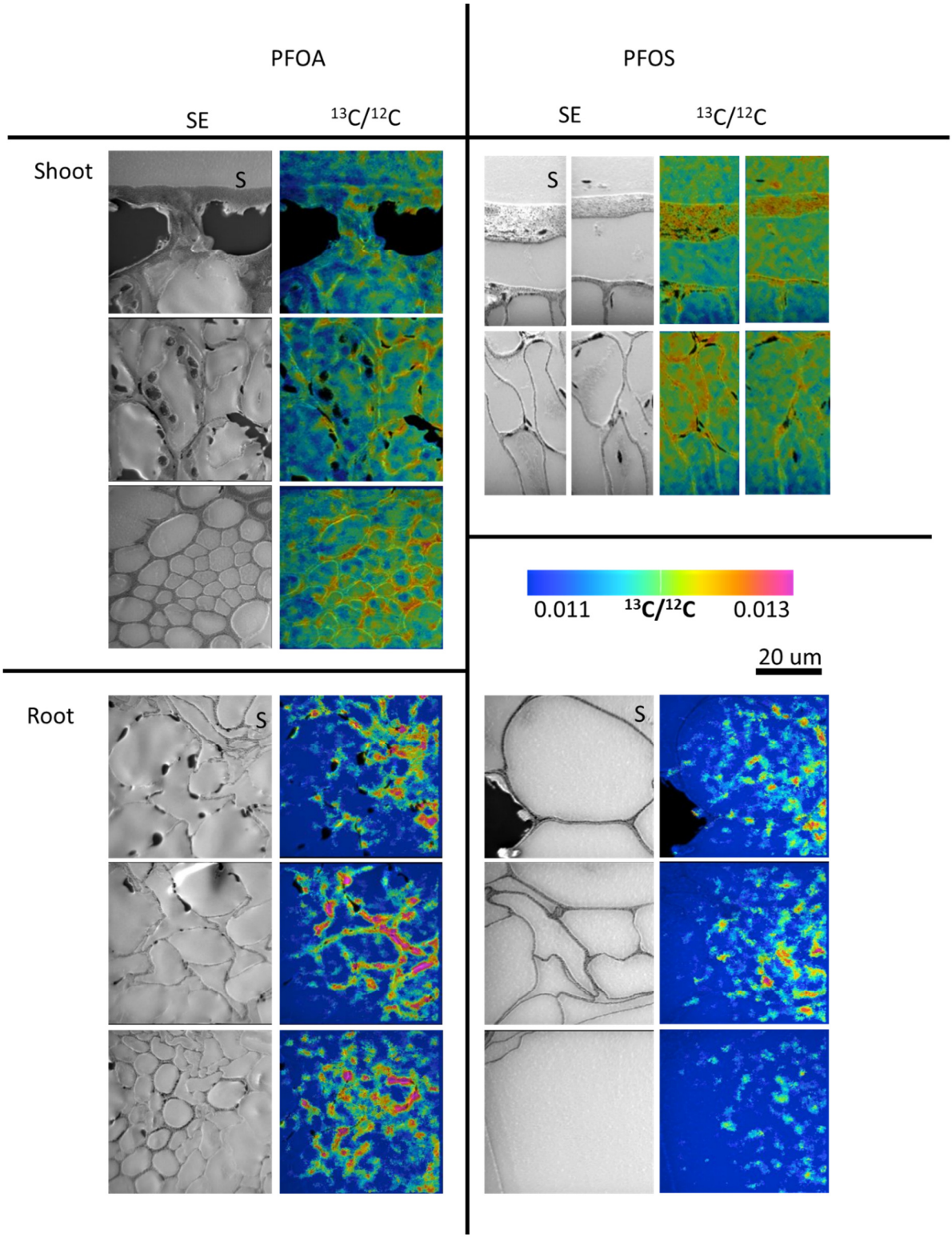
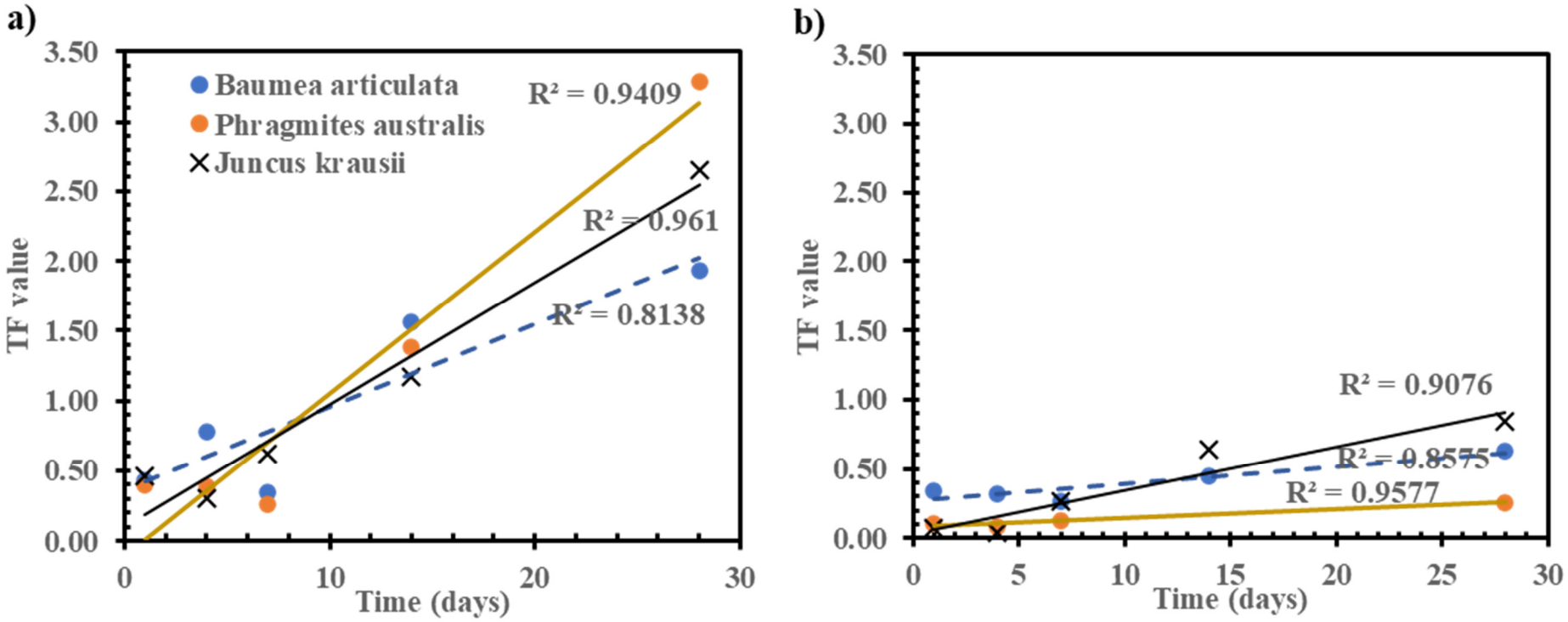


Figure 4. Distribution of ^{13}C enrichment (proxy for ^{13}C-PFOA or ^{13}C-PFOS respectively) in the shoots (top) and roots (bottom) of *Juncus krausii* after 28 days incubation. Each of the four sample types are represented by a combined secondary electron micrograph to show structures of interest and a $^{13}C/^{12}C$ overlaid hue saturated intensity image (HSI) of the same area indicating where ^{13}C enrichment is present. For each sample type, the outer surface (S)

716 of the tissue is at top with inner tissue below. Larger black areas on samples are indicative of
717 sample tears and should be ignored.



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719
720 **Figure 5.** TF values for PFOA (a) and PFOS (b) of *Baumea articulata*, *Phragmites australis* and *Juncus krausii* during the study period for water
721 spiked with PFOA and PFOS (10 µg/L each).

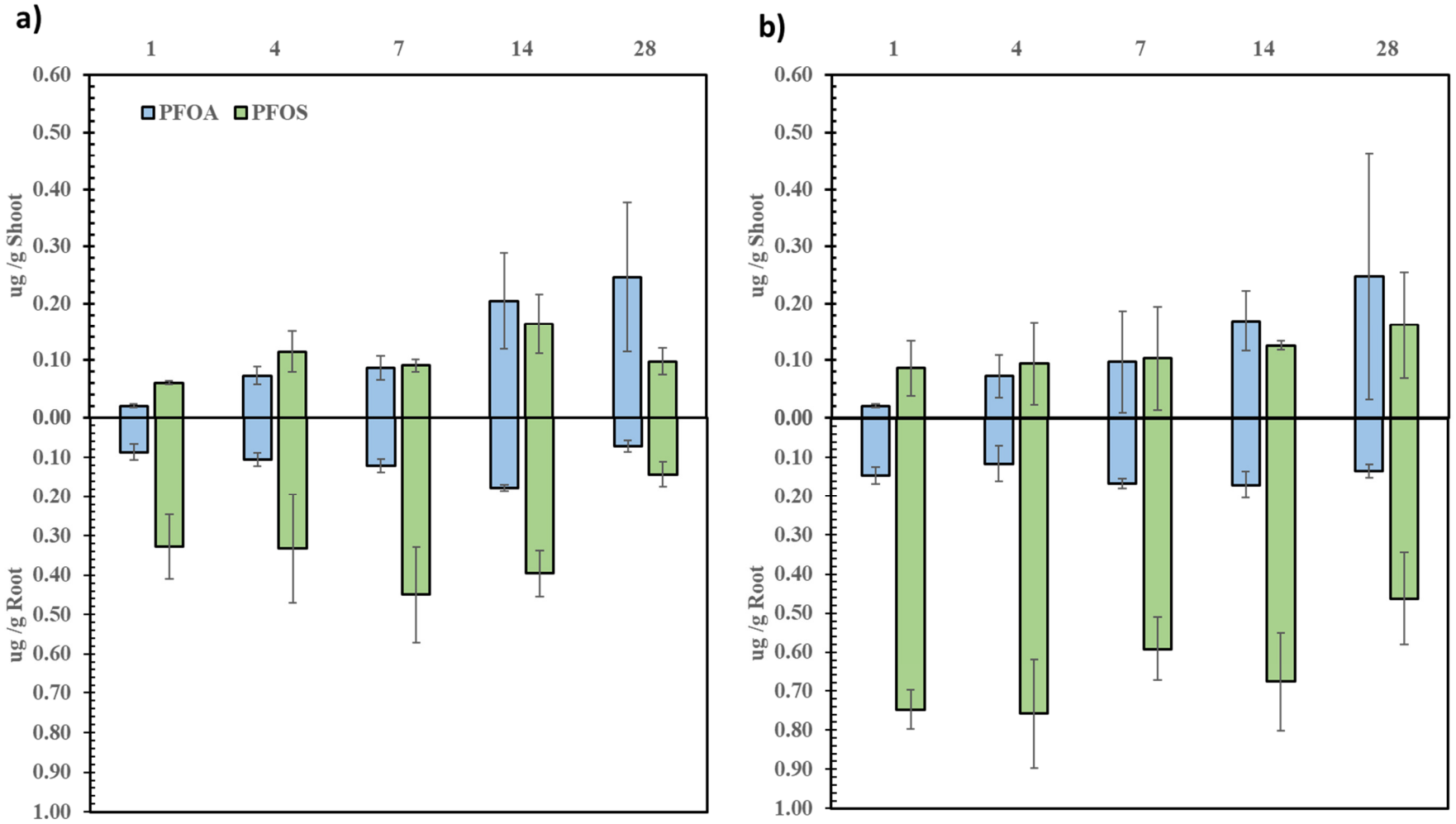

Figure 6. Concentrations of PFOA and PFOS in the shoots and roots of *Baumea articulata* (a) and *Phragmites australis* (b) at harvest days since exposure for water spiked with both PFOA and PFOS (30 µg/L each). Error bars represent the standard deviation (n= 3).

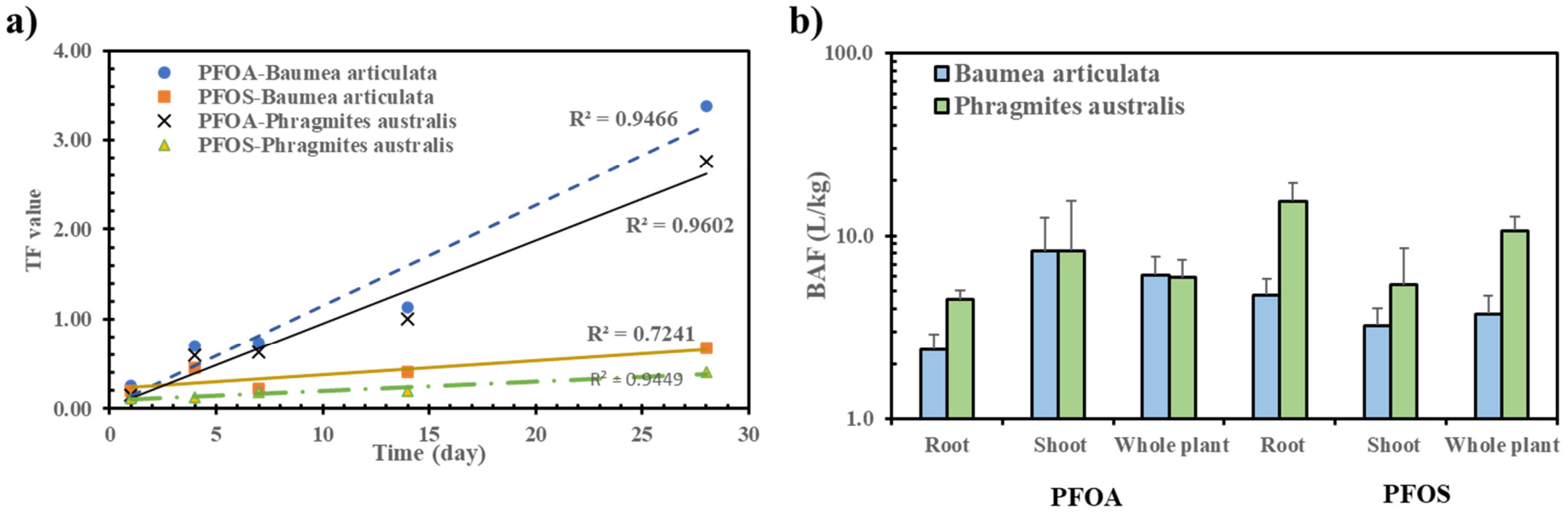

Figure 7. TF values (a) and BAF values (b) for *Baumea articulata* and *Phragmites australis* exposed to PFOA and PFOS for 28 days. Error bars represent the standard deviation (n= 3).