Changing bioavailability of per- and polyfluoroalkyl substances (PFAS) to plant in biosolids amended soil through stabilization or mobilization

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Abstract

Biosolids containing per- and polyfluoroalkyl substances (PFAS) could contaminate the receiving environments once they are land applied. In this study, we evaluated the feasibility of controlling the bioavailability of PFAS in biosolids to timothy-grass through stabilization or mobilization approaches. Stabilization was accomplished by adding a sorbent (i.e. granular activated carbon (GAC), RemBind, biochar) to biosolids, while mobilization was achieved by adding a surfactant, sodium dodecyl sulphate (SDS), to biosolids. The results showed that the ΣPFAS concentration in grass shoots grown in biosolids amended soil treated by GAC or RemBind at 2% was only 2.77% and 3.35% of the ΣPFAS concentration detected in shoots grown in biosolids amended soil without a sorbent, respectively, indicating the effectiveness of GAC and RemBind for stabilizing PFAS and reduce their bioavailability. On the other hand, mobilization by adding SDS to biosolids at a dose range of 10 – 100 mg/kg significantly increased the plant uptake of ΣPFAS by 15.48% - 108.57%. Thus, mobilization by adding SDS could be a valuable approach for enhancing the PFAS removal if phytoremediation is applied. Moreover, higher rate of PFAS uptake took place after grass cutting was observed in this study. Thus, proper mowing and regrowth of timothy-grass could lead to efficient and cost-effective removal of PFAS from biosolids amended soil through phytoremediation and leave the site clean to be used for other purposes.

Keywords: Per- and polyfluoroalkyl substances (PFAS); timothy-grass; stabilization; sorbent; mobilization.

Introduction

Per- and polyfluoroalkyl substances (PFAS) have been used in food packaging materials, household items, stain-resistant furniture, nonstick cookware, outdoor gear, firefighting foam, etc. worldwide due to their water/oil repelling property and recalcitrant nature (Glüge et al., 2020; Meegoda et al., 2020). The broad applications of PFAS lead to their detection in municipal wastewater (Brusseau et al., 2020; Lenka et al., 2021). Unfortunately, the conventional physical, chemical and biological processes for wastewater treatment are found to be ineffective to remove or degrade PFAS, resulting in PFAS discharge in the effluent and accumulation in sewage sludge (Coggan et al., 2019; Sinclair and Kannan, 2006). For example, 14 perfluoroalkyl acids (PFAAs) with the number of carbons ranging from 3 to 14 were detected in the sewage sludge collected from 25 wastewater plants (WWTPs) in Shanghai, China, at concentrations up to 809 μg/kg (Yan et al., 2012). Another investigation showed that the concentrations of total perfluorinated carboxylic acids (PFCAs) and total perfluoroalkane sulfonic acids (PFSAs) in sewage sludge collected from 45 WWTPs in Switzerland were up to 233 μg/kg and 2,314 μg/kg, respectively (Alder and van der Voet, 2015). In the Czech Republic, 32 PFAS were detected in the sewage sludge from 43 WWTPs and the total PFAS concentrations ranged from 5.6 to 963.2 μg/kg (Semerád et al., 2020). If the screening levels (PFOA= 2.5 μg/kg and PFOS = 5.2 μg/kg), set by the state of Maine in the U.S. for soil beneficial use (Maine-DEP, 2021), become widely adopted, then most of the sludges generated worldwide may not be permitted for land application.

Sewage sludge can be turned to Class A or B biosolids and land-applied once it goes through certain type of pretreatment. Reports have shown that around 60% of biosolids in the U.S, mainly Class B biosolids, were land applied in the pass decades (Lu et al., 2012; Pepper et al., 2008; Robinson et al., 2012). According to U.S. EPA 40 CFR (Code of Federal Regulations)

Part 503, Standards for the Use or Disosal of Sewage Sludge, the recommended pretreatment technologies include composting, heat treatment, lime stabilization, and aerobic/anaerobic digestion (AD) (Peccia and Westerhoff, 2015; Wang et al., 2018). Unfortunately, these treatment techniques were not able to remove PFAS effectively. For example, Lakshminarasimman et al. (2021) found increasing ΣPFAS-F mass after treating sewage sludge through pelletization and alkaline stabilization. Li et al. (2021) noticed significantly higher concentrations of short-chain PFAAs (PFCAs: $C < 8$; PFSAs: $C < 6$) in sewage sludge after AD, indicating the transformation of PFAS precursors to more stable PFAAs. Thermal treatment (e.g., the CambiTHP process) and ultrasonication at 20 kHz were also reported to be ineffective to decrease PFAS concentrations in sewage sludge (Zhang and Liang, 2021a; Zhang et al., 2022b). Similarly, higher concentrations of some PFAAs after the thermal and ultrasonication treatments were observed, possibly due to conversion of PFAS precursors and desorption of PFAS from sewage sludge particles. Given the ubiquitous detection of PFAS in sewage sludge and biosolids, land application of these materials inevitably leads to contamination of the receiving environment. Although U.S. EPA does not have regulations regarding PFAS in biosolids for land application, regulatory actions are in progress. Current, the Maine Department of Environmental Protection (DEP) requires PFAS testing of all sludge material licensed for land application and the screening levels of PFBS, PFOS, and PFOA in solid waste for beneficial use, including sludge and biosolids, are 1,900 µg/kg, 5.2 µg/kg, and 2.5 µg/kg, respectively. It is known that plants can uptake and accumulate PFAS in roots and shoots, which then obviously leads to contamination of the food chain. (Costello and Lee, 2020; Ghisi et al., 2019; Lesmeister et al., 2021). Thus, it is urgent to identify approaches that can decrease bioavailability of PFAS in biosolids amened to soil.

Immobilization/stabilization approach that strives to hold pollutants in their original environment while restricts their transport to the surrounding locations has been demonstrated to be effective for remediating PFAS contaminated soil. Biochar, granular activated carbon (GAC), clay minerals, and some commercial products such as RemBind® and FLUORO-SORB® have been tested as sorbents to stabilize PFAS in soil and showed promising performance (Askeland et al., 2020; Hale et al., 2017; Hearon et al., 2021; Kabiri and McLaughlin, 2021; Lath et al., 2018; McDonough et al., 2021; Silvani et al., 2019; Sorengard et al., 2019; Sörengård et al., 2019; Zhang and Liang, 2022). The mechanisms of the enhanced sorption between PFAS and chosen sorbents during stabilization process include electrostatic attraction, hydrophobic interaction, surface complexation, hydrogen-bonding and physical entrapment (Alves et al., 2020; Xiao et al., 2011; Zhao et al., 2014; Zhu et al., 2021). Different from soil that is mainly composed of abiotic and inorganic materials, biosolids formed from biological wastewater treatment contain higher concentration of biotic and organic materials (Collivignarelli et al., 2019). It is unknown at this stage whether PFAS in biosolids can be stabilized by a sorbent.

Opposite to stabilization, a mobilization approach could be used to remove PFAS from soil though plant uptake (i.e., phytoremediation). Phytoremediation aims to use plants to extract and remove pollutants in the contaminated environments. Previously, we have demonstrated the feasibility of utilizing plants including *Juncus effusus* (soft rush), *Typha latifolia* (cattail), *Carex comosa* (longhair sedge), and *Lemna minor* (duckweed) to reduce PFAS concentrations in contaminated water and soil (Cao et al., 2020; Zhang et al., 2019a; Zhang et al., 2021b; Zhang et al., 2020; Zhang and Liang, 2021b; Zhang et al., 2019b). The PFAS removal by plants was found to be strongly and positively correlated with PFAS' water solubility or hydrophilicity (Zhang et al., 2021b). Theoretically, the PFAS' water solubility could be affected by the physicochemical properties of PFAS such as carbon chain length, functional groups, and octanol/water partition coefficient (K_{ow}) (Bolan et al., 2021; Sima and Jaffé, 2021; Zhang et al., 2021b). PFAS' hydrophilicity or apparent solubility could be enhanced by adding a mobilizing reagent, such as surfactant. In general, anionic surfactants are reported to enhance the solubility of PFAS in water and decrease PFAS sorption to soil, facilitating the mobilization of PFAS (Bolan et al., 2021; Guelfo and Higgins, 2013). However, it remains unknown whether adding surfactants to biosolids could increase the bioavailability of PFAS in biosolids amened soil and enhance the performance of phytoremediation.

To control the environmental and health risks led by PFAS in biosolids amended to soil, we hypothesized that (1) adding a sorbent to biosolids can decrease plant uptake of PFAS in biosolids amended soil, while (2) adding a surfactant to biosolids can increase plant uptake of PFAS. In this study, timothy-grass (*Phleum pratense*) was selected as the model plant. It is an abundant perennial grass native to Europe and was introduced to North America by early settlers. Currently, timothy-grass is a major source of hay and cattle fodder (Lacefield et al., 1980). Previous reports also demonstrated the capability of timothy-grass for phytoremediation of heavy metal contaminated soil (Balsamo et al., 2015; Jani et al., 2014). To test the hypothesis, the uptake of PFAS by timothy-grass grown in soil amended by biosolids that was treated by a sorbent (i.e., biochar, GAC, or RemBind®) or an anionic surfactant (i.e., sodium dodecyl sulphate (SDS) was investigated. The objective of this study was to evaluate the feasibility of controlling bioavailability of PFAS in the biosolids-soil-grass system through a stabilization or mobilization approach.

Materials and methods

1. Preparation of biosolids amended soil

The chemicals and reagents used in this study were listed in Table S1. The sandy loam soil used in this study (pH = 7.56) was collected from a local farm in Albany County, New York. The soil was passed through a 2-mm sieve before use. The contents of natural organic matter and total organic carbon of the sieved soil were 4.82 ± 0.12% and 2.44 ± 0.45%, respectively. The sieved soil was mixed with vermiculite in a 1:1 volume ratio for increasing water retention of the soil and improving soil aeration. Then, the processed soil for plant cultivation was distributed to 7-quart polypropylene containers (14.375"L x 8.25"W x 6"H, Fig. S1). Each container had 1.3 kg of dry soil.

The biosolids after anaerobic digestion of sludge were collected from a nearby wastewater treatment plant close to University at Albany. The total solid and total organic carbon of the biosolids were 24.16 ± 0.21% and 28.64 ± 0.28%, respectively. Ten PFAS, including perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), undecafluoro-2-methyl-3-oxahexanoic acid (GenX), perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnA), were added to and homogenized with the biosolids, reaching the target concentration of each PFAS at 300 µg/kg dry biosolids. The mixture was then stored at 4 °C for 7 days. Afterwards, the PFAS spiked biosolids were homogenized with one sorbent (biochar, GAC, or RemBind) at 0.2 or 2 wet wt.% or a surfactant, SDS, at 10, 50 or 100 mg/kg. The biochar (Biochar Supreme, LLC, Everson, WA) was derived from forest wood waste and contained over 85% of organic carbon with a specific surface area of ~800 m^2/g. The size of the GAC (Alfa Aesar, Ward Hill, MA) ranged from 425 µm to 850 µm with a specific surface area of ~650 m^2/g. The porosity and density of the GAC were around 0.78 and 2.26 g/cm^3, respectively. RemBind was gifted from AquaBlok Ltd as a distributor for RemBind® (Australia). After aging for 7 days, the biosolids with a sorbent or a surfactant were thoroughly mixed with the prepared soil at a ratio of 0.17 kg biosolids/kg of dry soil. This ratio is equivalent to 5 dry ton biosolids/acre of soil recommended by US EPA for biosolids land application. Based upon the TS of the biosolids and the spiked PFAS concentrations, there were 55.56 g of dry biosolids and 16.67 μg of each spiked PFAS in each container with 1.3 kg of dry soil. Two sets of controls were set up as well: (1) soil amended with the original biosolids without PFAS spiking, sorbents, or surfactants (non-spiked control); (2) soil amended with PFAS spiked biosolids without any sorbents or surfactants (spiked control). Three replicates were prepared for each control and treatment.

2. *Plant cultivation and analysis*

Timothy-grass seeds were purchased from OrOlam LLC (South Miami, FL). One gram of seeds was sown evenly in each tank containing the prepared biosolids amended soil. Enough water was then added to each tank to reach 100% of field capacity. The plants were cultivated in a greenhouse and watered every day. The timothy-grass started germinating on the 5th day after sowing. On Day-40 after seed germination, the above-ground shoots of the grass in each tank were cut and rinsed thoroughly using deionized water. Around 1 g of the plant tissues from each treatment was used for chlorophyll extraction and quantification. The remaining plant shoots were freeze-dried at -37 °C for 48 h and subject to PFAS extraction and analysis. The plants after cutting were kept under the same growth condition. On Day-80 after seed germination or day-40 after the first cut, the plant shoots were removed again and subject to chlorophyll and PFAS analysis. The soil in each tank was then kept under a fume hood for air drying. After 2 weeks, the dried soil was crushed into fine particles and thoroughly homogenized by using a glass rod. Representative soil samples were collected for PFAS analysis.

3. *Chlorophyll extraction and quantification*

On Day-40 and Day-80 after seed germination, 100 mg of fresh grass leaves were collected and used to quantify chlorophyll content. The leaf tissues were placed in a 15-mL glass tube with 10 mL of 90% acetone. After 48 hours, the chlorophyll concentration in the solvent was determined using a UV-Vis spectrophotometer (GENESYS 10S, Thermo Fisher Scientific, Waltham, MA, USA). The absorbance was read at 664 and 647 nm and used to calculate chlorophyll a and b and total chlorophyll content according to Equation 1 – 3 (Ritchie, 2006).

$$Chl_a\,(\mathrm{mg/L}) = 12.72\ \mathrm{OD}_{663} - 2.59\ \mathrm{OD}_{645} \quad (1)$$

$$Chl_b\,(\mathrm{mg/L}) = 22.9\ \mathrm{OD}_{645} - 4.67\ \mathrm{OD}_{663} \quad (2)$$

$$Chl_{total}\,(\mathrm{mg/L}) = Chl_a + Chl_b = 20.31\ \mathrm{OD}_{645} + 8.05\ \mathrm{OD}_{663} \quad (3)$$

4. *PFAS extraction and analysis*

PFAS in the freeze-dried timothy-grass shoots were extracted by MTBE-NaOH according to a previously developed method (Zhang et al., 2021b; Zhang et al., 2020; Zhang et al., 2019b). Before extraction, each sample for extraction was spiked with 10 ng of $^{13}C_2$-PFHxA as the surrogate for determining the extraction efficiency. EPA method 1312 (Synthetic Precipitation Leaching Procedure, SPLP) was performed on the biosolids amended soil after drying to determine the leachability of PFAS. Briefly, 20 mL of H_2SO_4/HNO_3 reagent (60/40 wt./wt.) at pH = 4.2 were added to the soil sample. The soil with the H_2SO_4/HNO_3 reagent was then vortexed for 30s and shaken on an end over end mixer overnight. After centrifugation at 3,500 g for 30 min, the supernatant was collected and subject to solid phase extraction (SPE) using HyperSep C18 cartridges (Thermo Scientific, Waltham, MA). The SPLP leachable PFAS on the C18 cartridges were eluted with 2 mL of methanol, followed by 2 mL of 0.1% NH_4OH amended methanol. The SPLP leachable PFAS in clean soil without biosolids amendment were analyzed as well. PFAS in the final plant extracts and SPLP leachates were quantified using a 1290 Infinity II LC system coupled with a 6470 Triple Quad Mass Spectrometer (LC-MS/MS, Agilent Technologies, Santa Clara, CA, USA). The detailed instrumental setup can be found in our previous report (Zhang and Liang, 2022).

5. *Data analysis*

The experimental data are presented as means ± the standard deviation of three replicates. One-way analysis of variance and Tukey's test for post hoc comparisons were performed with IBM SPSS Statistics 22. Statistical significance was defined as $p < 0.05$.

Results and discussion

1. *PFAS stabilization in biosolids-amended soil*

Timothy grass has a shallow and fibrous root system, making the root recovery difficult. Thus, the concentrations of PFAS in the roots were not determined. Our results indicated that the added sorbents/surfactant significantly affected the distribution of PFAS in the biosolids-soil-grass system. As shown in Fig. 1, for the soil amended with the biosolids but without PFAS addition (non-spiked control), PFHxA, PFHpA, PFOA, and PFOS at 24.70 ± 1.46, 1.96 ± 0.29, 3.89 ± 2.90, and 0.70 ± 0.05 μg/kg, respectively, was detected in shoots on Day-40. The presence of the same PFAS in grass shoots on Day-80 was also detected. The studied PFAS in the pristine soil without biosolids amendment were non-detectable. Thus, the source of these PFAS should be the biosolids used in this study. This biosolids is known to comprise PFAS as we previously reported (Zhang and Liang, 2021a). In the same biosolids, PFOS had the highest concentration of 10 µg/kg, while the concentration of PFHxA, PFHpA and PFOA was around 1 – 5 µg/kg.

Unsurprisingly, amending soil with biosolids spiked with PFAS at 300 µg/kg but without a sorbent or a surfactant (spiked control) resulted in significantly higher PFAS concentrations in grass shoots compared to the non-spiked control. All spiked PFAS except PFDA and PFUnA were taken up by timothy-grass. There was an obvious trend that the uptake of PFCAs and PFSAs in the shoots decreased with increasing carbon chain length. Among PFCAs, PFHxA had the highest concentration in shoots on Day-40 (217.23 ± 16.95 µg/kg), followed by PFHpA (90.66 ± 6.74 µg/kg), PFOA (21.73 ± 0.59 µg/kg), and PFNA (1.79 ± 0.44 µg/kg). When the carbon chain length was higher than 8, such as PFDA and PFUnA, the upward translocation was non-detectable. Similarly, PFBS at 23.61 ± 1.90 µg/kg had the highest concentration in shoots among PFSAs, followed by PFHxS (17.61 ± 0.88 µg/kg) and PFOS (1.88 ± 0.19 µg/kg). PFCAs had significantly higher concentrations than PFSAs in plants when their carbon chain length was the same. This is in line with numerous studies that have demonstrated the effect of PFAS chain length and functional group on plant uptake (Costello and Lee, 2020; Ghisi et al., 2019; Lesmeister et al., 2021; Zhang et al., 2019b).

With sorbents at different doses, the bioavailability of PFAS in the biosolids amended soil varied significantly. On Day-40, biochar at both doses did not show any PFAS stabilization effect when compared to the spiked controls. Instead, it notably increased the plant uptake of PFAS, especially at the lower dose of 0.2%. The total PFAS concentration (∑PFAS) in grass shoots grown in biosolids amended soil treated by biochar at 0.2% and 2% was 120.03% and 56.99% higher than that of the spiked control. Such enhancement of plant uptake of PFAS was also observed in soil with biosolids treated by 0.2% of GAC and RemBind, a sorbent containing activated carbon, aluminum oxyhydroxide, and clay minerals (Bräunig et al., 2021a).

Biochar has been investigated for PFAS stabilization in soil previously (Askeland et al., 2020; Silvani et al., 2019). Hydrophobic interaction and intra-particle diffusion were proposed to be the main mechanisms for PFAS adsorption onto biochar (Dalahmeh et al., 2019; Inyang and Dickenson, 2017). We also reported that biochar largely decreased the water and SPLP leachable PFAS with long carbon chains in soil (Zhang and Liang, 2022). However, in sewage sludge spiked with PFAS at 30 or 300 μg/kg, the same biochar at a dose of 2% showed a limited reduction of leachable PFAS (Zhang et al., 2022a). The different compositions and characteristics between soil and biosolids could explain the different performance of biochar in these two materials. Biochar is commonly used as a soil amendment to alter soil structure and increase soil porosity and water-holding capacity (Hardie et al., 2014). Adding biochar to soil could provide a more hospitable environment for soil microorganisms and facilitate plant uptake of air, water, and nutrients (Palansooriya et al., 2019), possibly leading to the increased uptake of PFAS by timothy-grass. Similar beneficial effect on plants was also observed for soil amended with GAC. Brennan et al. (2014) found that carbonaceous sorbent such as biochar and GAC at 3% in soil had a positive effect on the growth of maize in terms of chlorophyll content and plant biomass. Jakob et al. (2012) also reported that GAC amendment at 2% in soil increased growth rate of squash and carrot. There is no evidence in the literature showing the positive effects of RemBind on plants. RemBind at high dose (5 – 30%) was even reported to decrease the biomass of wheatgrass and the weight of earthworms in treated soil (Bräunig et al., 2021a; Bräunig et al., 2021b). The adverse effect of RemBind was proposed to be due to the sorption of essential nutrients in soil to this sorbent. The dose used in this study was based on the dry weight of biosolids. The overall weight percent of RemBind in the biosolids-amended soil at 0.2% was even lower. Thus, we speculated that GAC and RemBind at 0.2% in biosolids could also increase the water and nutrients uptake, leading to higher PFAS bioaccumulation in grass shoots compared to no-sorbent controls.

At the higher dose (i.e., 2%), GAC and RemBind significantly reduced the bioavailability of PFAS in biosolids-amended soil. The ∑PFAS concentration in grass shoots grown in biosolids amended soil treated by GAC or RemBind at 2% was only 2.77% and 3.35% of the ∑PFAS concentration detected in shoots in the spiked control, respectively. This is in line with our previous observation that GAC and RemBind at 2% had excellent performance regarding stabilizing PFAS in sludge (Zhang et al., 2022a). Strong stabilization performance of decreasing 99% of water and SPLP leachable PFAS by GAC and RemBind in soil was also reported (Zhang and Liang, 2022). Thus, it appears that, the effect of sorption of PFAS to GAC or RemBind surpassed the potential enhancement of plant uptake led by the amendment of a sorbent. Considering the high complexity of the tested system, it is difficult to pinpoint exactly the reasons or mechanisms controlling the results we observed. Further in-depth investigations on the effects of biochar/GAC/RemBind on plant physiology and biochemistry are needed to elucidate the mechanisms underlying the decreased or enhanced uptake of PFAS in the presence of carbon-based sorbents at different doses.

In this study, the timothy-grass was cut about 1 inch above the soil surface on Day-40 after germination. Afterwards, the grass was allowed to grow to a similar height on Day-80 and was subject to PFAS analysis again. For these Day-80 shoots, a similar PFAS uptake pattern was observed as those harvested on Day-40. Basically, compared to the spiked controls without a sorbent, GAC and RemBind at 0.2% and biochar at both 0.2% and 2% led to significant increase of plant uptake of PFAS, while GAC and RemBind at 2% reduced over 99% of PFAS concentration in grass shoots. It should be noted that the concentrations of all studied PFAS on Day-80 (orange bars in Fig. 1) were significantly higher than their counterparts on Day-40 (blue bars in Fig. 1), which hinted higher rate of PFAS uptake during the second 40 days than the first 40 days. This is in agreement with reports demonstrating that proper mowing could promote the water consumption and regrowth of grasses (Biran et al., 1981; Doležal et al., 2019; Klimeš and Klimešová, 2002). Similarly, the PFAS removal from biosolids amended soil by timothy-grass shoots during the second 40 days was significantly higher than that during the first 40 days (Fig. S2). After 80 days of cultivation, the total removal of ΣPFAS from biosolids amended soil, which was the sum of ΣPFAS removal on Day-40 and Day-80, in spiked control was 5.03 ± 0.68%. Treating biosolids with biochar at 0.2% led to the highest total removal of ΣPFAS by timothy-grass shoots, reaching 9.11 ± 0.85%. GAC and RemBind at 2% decreased the total removal of ΣPFAS to 0.14 ± 0.01% and 0.08 ± 0.02%, respectively. Overall, RemBind at 2% had the best stabilization performance after 80 days of plant growth, while biochar at 0.2% had the worst performance regarding PFAS stabilization.

To investigate the effects of PFAS exposure, stabilization treatment for biosolids, and mowing on the growth of timothy-grass, we quantified the contents of chlorophyll a and b, which are the main pigments used in photosynthesis, in grass shoots on Day-40 and Day-80 (Fig. 2). The results indicated that spiking PFAS at 300 μg/kg in biosolids had no significant effect on the chlorophyll content of timothy-grass. Although GAC and RemBind resulted in significantly higher total chlorophyll content compared to the non-spiked controls on Day-80, this effect was absent when compared to the spiked controls. Eliminating the potential effect from chlorophyll content, the increased uptake of PFAS after the first cutting could be due to: 1) established root systems as a result of the first 40-day growth; 2) roots adapted to the presence of and exposure to PFAS; and 3) PFAS already accumulated in the roots. Although the exact reason is unclear at this point, it leads to a sound hypothesis that repeated cutting of the shoots will result in faster uptake of PFAS in soil and eventually lead to significant removal of PFAS from target sites. This will be beneficial from the perspective of phytoremediation.

Besides the PFAS concentrations in grass shoots, the concentrations of acidic water (SPLP) leachable PFAS in soil were also determined. Fig. 3 showed the percentages of leachable PFAS in biosolids amended soil, which were calculated by dividing the mass of SPLP leachable PFAS by the total mass of spiked PFAS. Without a sorbent, almost 100% of leaching of PFHxA, PFHpA and PFOA was observed. Similar to PFAS uptake by the shoots, a chain length effect was visible: the shorter the carbon chain of PFAS, the higher the leachability. Expectedly, GAC and RemBind at 2% significantly reduced the leachability of all studied PFAS, except PFUnA, in the biosolids amended soil. Interestingly, biochar at 2% and RemBind at 0.2% also resulted in significantly lower acidic water leachable PFAS, while biochar and GAC at 0.2% had no significant effect on PFAS leaching compared to the spiked controls. Compared to PFAS concentrations in the shoots (Fig. 1), there was no positive correlation between acidic water leachable PFAS and the extent of shoot uptake for treatment with biochar at both doses and RemBind at the dose of 0.2%. Thus, results from SPLP at pH = 4.2 for sites east of the Mississippi River may not be able to predict bioavailable PFAS to the studied grass. At present, there are no EPA methods for studying PFAS leaching from soil. The SPLP is an established and widely adopted EPA method for investigating leaching of organic contaminants in soil. Whether this method is suitable for PFAS or not is unclear at this point. But we do recognize that the acidic water used in the leaching procedure may actually decrease PFAS leaching due to the acidic nature of PFAAs. But since the focus here was to evaluate effect of different sorbents toward stabilizing PFAS in soil amended by biosolids, the SPLP served our purpose well.

2. *PFAS mobilization in biosolids-amended soil*

Anionic surfactants could affect PFAS sorption in soil through partition-like interactions between nonionic portions of the surfactant and PFAS, leading to an increase in water solubility and a decrease in sorption (Kile and Chiou, 1989; Tao et al., 2006). This has been observed with PFOS in the presence of an anionic surfactant, sodium dodecylbenzene sulfonate (SDBS), at 50 mg/L in an aqueous solution (Pan et al., 2009). Adding such surfactant increased the solubility of PFOS, resulting in decreasing sorption of PFOS on sediment and increasing mobility of PFOS. Guelfo and Higgins (2013) also reported that SDS at 100 mg/L decreased the sorption of PFOS, PFNA, and PFDA in soil. Anionic surfactants may also form hemimicelles near a solid surface, compete for sorption sites with PFAS, and decrease the sorption of PFAS on soil (Guelfo and Higgins, 2013). Cationic surfactant, such as cetyltrimethylammonium bromide (CTAB), however, was shown to have enhancing effect on PFAS sorption. Pan et al. (2009) found that CTAB at a concentration up to 50 mg/L remarkably enhanced the sorption of PFOS to sediment and reduced the mobility of this PFAA. This cationic surfactant was assumed to form hemimicelles coupled with PFOS and sorb on the sediment, thus decreasing PFOS mobility.

In this study, although adding SDS to biosolids at a dose up to 100 mg/kg did not significantly increase acidic water leachable PFAS in the biosolids amended soil (Fig. S3), the plant uptake of PFAS was positively affected by such surfactant treatment (Fig. 4). On Day-40, only the highest dose of 100 mg/kg resulted in significantly higher uptake of PFAS by the shoots. The exceptions were PFOA and PFOS, for which no dose effect was observed. On Day-80, however, no dose effect was visible for PFSAs as all three doses led to significantly higher uptake of PFBS, PFHxS, and PFOS by the shoots. This was also true for GenX. Regarding PFCAs, the dose effect was specific to each individual PFAS. With respect to PFDA and PFUnA, SDS at ≤ 100 mg/kg did not lead to detection of these two long chain PFCAs in the shoots. The absence of these two PFCAs in the shoots and their low leachability by SPLP, especially for PFUnA, hinted that they may bind to biosolids and soil tightly. For ∑PFAS, all three doses led to significantly higher PFAS uptake by the shoots on Day-80. With SDS, similar effect of chain length and PFAS functional group on their uptake by the shoots as those revealed in Fig. 1 was observed. Similarly, the grass shoots accumulated more PFAS and achieved higher ΣPFAS removal during the second 40 days than those during the first 40 days (Fig. S2). After two cuts, the total removal of ΣPFAS, which was calculated by dividing the mass of ΣPFAS in plant shoots by the total mass of ΣPFAS spiked to the biosolids, in the biosolids amended soil treated by SDS at 10, 50, and 100 mg/kg was 8.90 ± 1.49%, 7.24 ± 0.41%, 9.16 ± 0.95%, respectively. Regarding individual PFAS, the uptake by shoots from soil followed this order: PFHxA (46.94 ± 6.37%) > PFHpA (18.66 ± 2.47%) > PFBS (11.46 ± 2.72) > GenX (9.91 ± 0.49%) > PFOA (3.79 ± 0.46%) ≈ PFHxS (3.45 ± 0.45%) > PFNA (0.52 ± 0.06%) > PFOS (0.21 ± 0.04) > PFDA (0%) = PFUnA (0%). The mobilization treatment with SDS did not change the chlorophyll content in timothy-grass either, although shoots exposed to SDS at 50 or 100 mg/kg had higher chlorophyll content compared to the non-spiked controls on Day-40 after mowing (Fig. 2). Overall, SDS significantly increased the bioavailability of PFAS to timothy grass in biosolids amended soil. In light of the increased uptake by shoots over time, it is reasonable to expect that this treatment could be used to improve the performance of phytoremediation at PFAS contaminated sites.

3. *Environmental implications*

In summary, this investigation revealed that PFAS in biosolids must be controlled. Without proper treatments, PFAS in the biosolids-soil-plant systems will be translocated to plant shoots, are mobile and easily leached by water although the extent of upward and downward movement depends significantly on plant species and individual PFAS's physicochemical properties. In this context, this study provided two strategies for controlling PFAS in biosolids destined for land application. One strategy is to amend the biosolids with either GAC or RemBind at 2% to stabilize PFAS in the biosolids-soil matrix. In this case, it is expected that PFAS will become immobilized and stable in the matrix. To demonstrate the practicality of this strategy, long-term field studies are needed in view of the possibility that PFAS can desorb from the sorbent and/or the biosolids/soil particles due to microbial degradation and the dynamic nature in the real world.

Another approach is to add SDS at 10-100 mg/kg to increase PFAS bioavailability to plants. In this scenario, the rationale is to allow PFAS to be uptaken by plants to the maximum extent. The harvested plants can then be handled by a hydrothermal liquefaction process for complete PFAS destruction (Zhang et al., 2021a; Zhang et al., 2020). Such an approach, however, could potentially lead to an uncontrolled leaching of PFAS to surrounding environments as PFAS are more mobile due to the presence of a surfactant. Therefore, the long-term effect of this strategy needs to be further evaluated in the field to justify its value.

Conclusions

This study demonstrated the feasibility of controlling the bioavailability of PFAS in biosolids through stabilization or mobilization. GAC and RemBind at a dose of 2% stabilized PFAS in the biosolids and significantly reduced the bioavailability of PFAS in the biosolids amended soil, while a lower dose of GAC and RemBind at 0.2% notably promoted the plant uptake of PFAS. Biochar enhanced the uptake of PFAS by timothy-grass as well regardless of dose. The mobilization approach through adding SDS significantly increased the bioavailability of PFAS to timothy-grass, offering a better remediation solution as PFAS are removed from a given site. Proper mowing and regrowth of timothy-grass could promote the uptake of PFAS from the biosolids amended soil, thus enhancing the performance of mobilization treatment.

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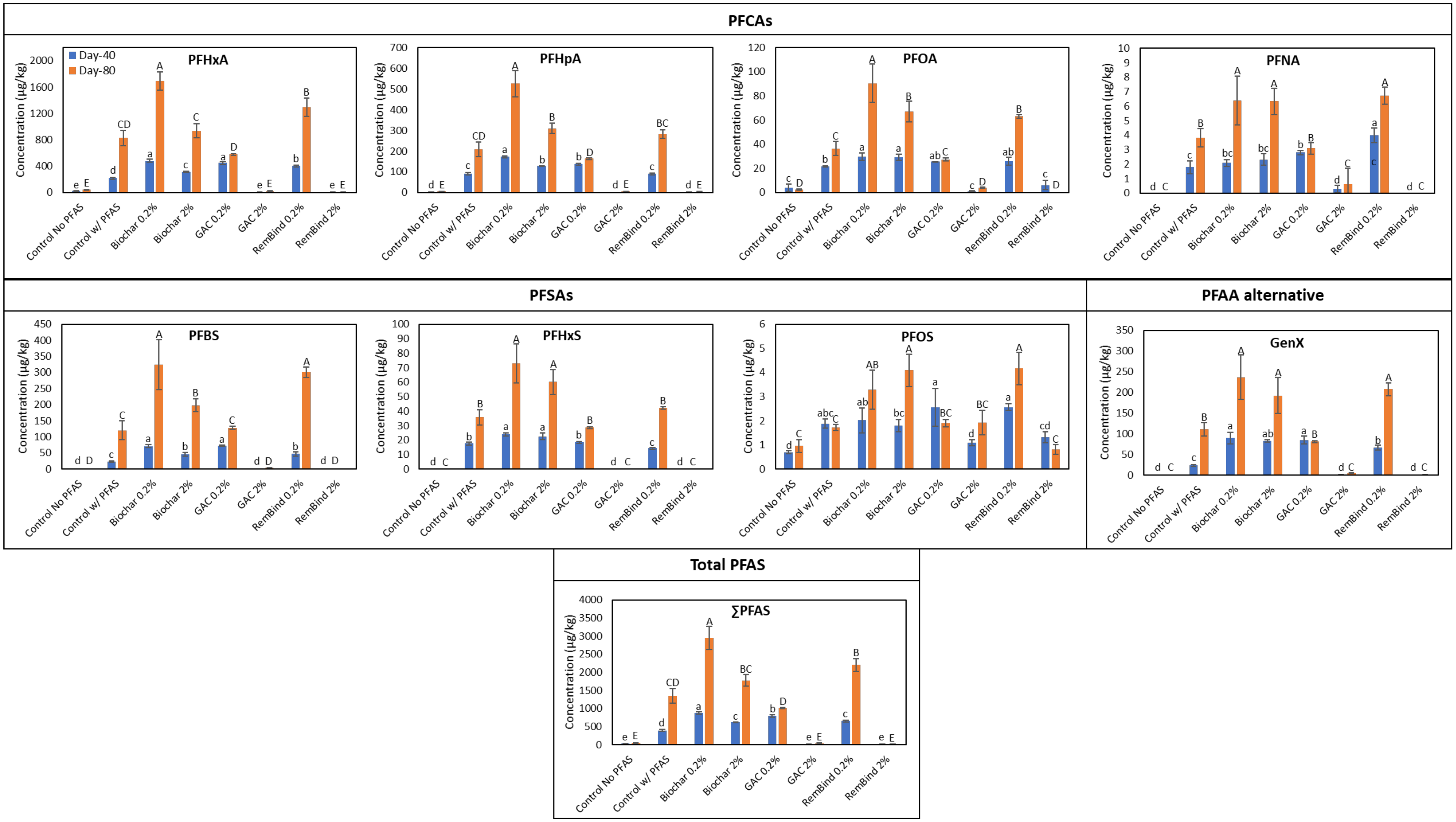


Fig. 1. Concentrations of PFAS in timothy-grass shoots grown in biosolids-amended soil treated by different sorbents on Day-40 and Day-80 (n=3). PFDA and PFUnA were not detected in timothy-grass shoots. Different letters in lower case and upper case represent significant differences among the treatments ($p < 0.05$).

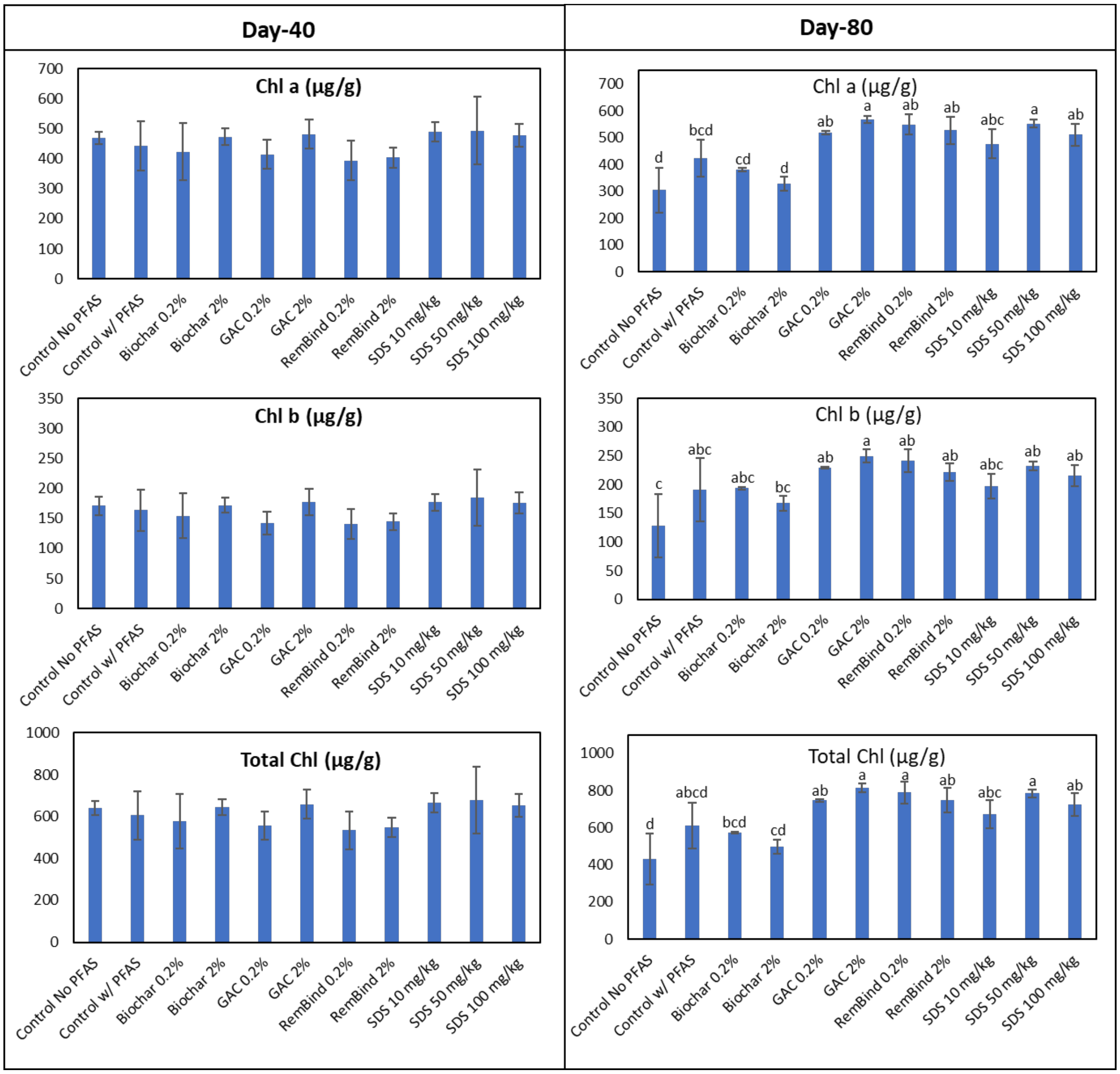


Fig. 2. Levels of chlorophyll a, chlorophyll b and total chlorophyll in timothy-grass shoots on Day-40 and Day-80 (n=3). Different letters represent significant differences among the treatments ($p < 0.05$).

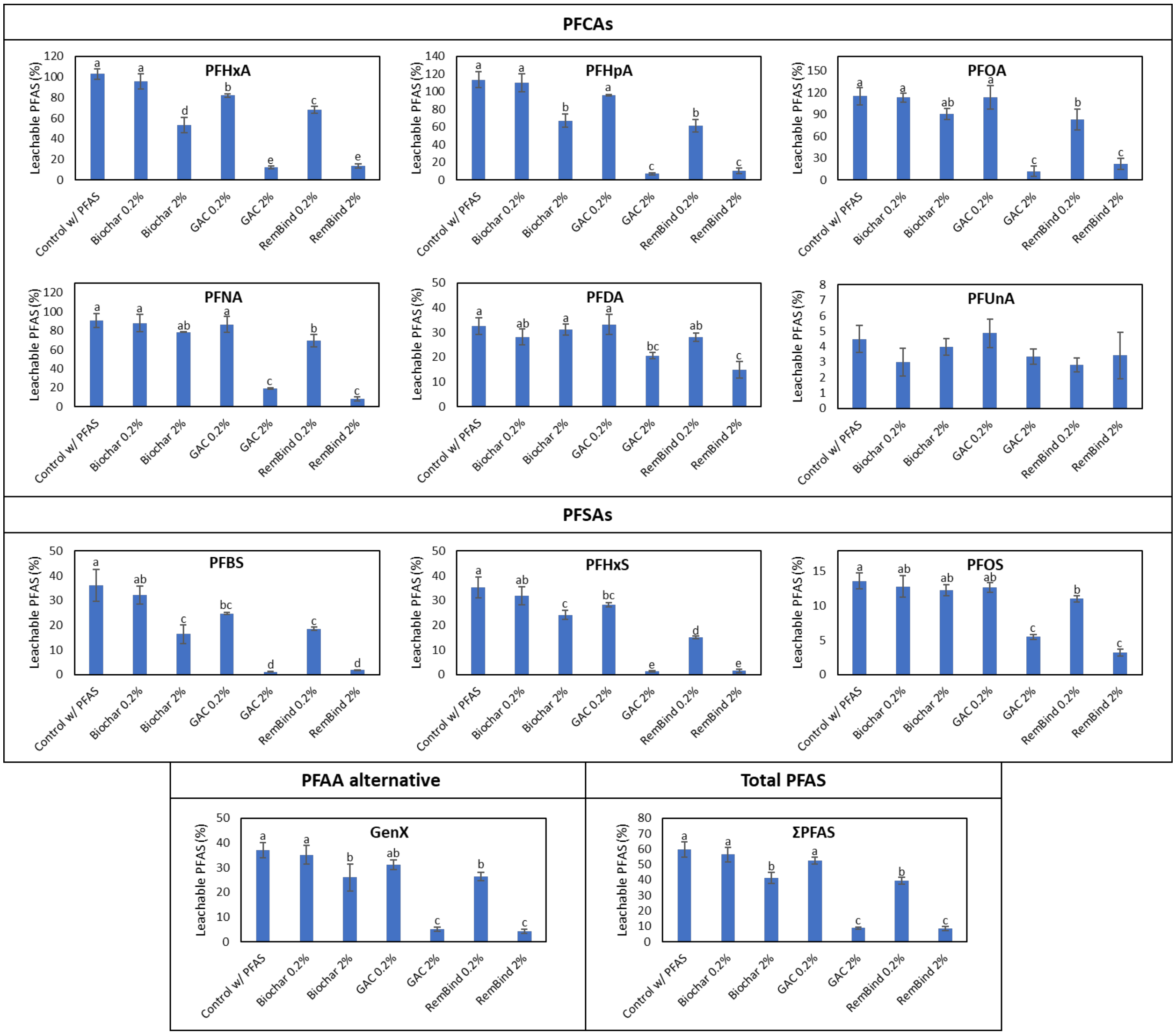


Fig. 3. Percentages of leachable PFAS in biosolids-amended soil treated by different sorbents on Day-80 (n=3). Different letters represent significant differences among the treatments ($p < 0.05$).

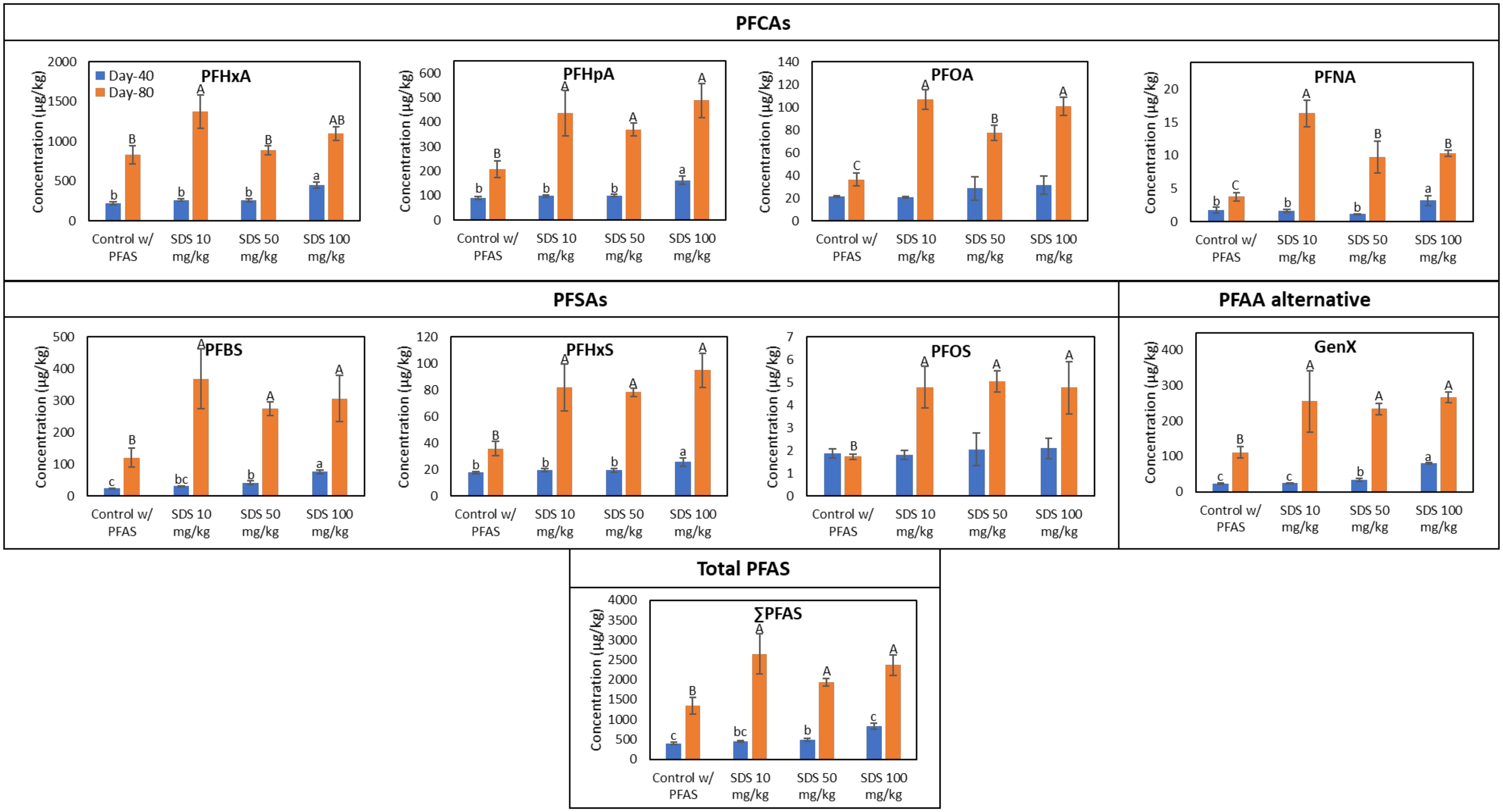
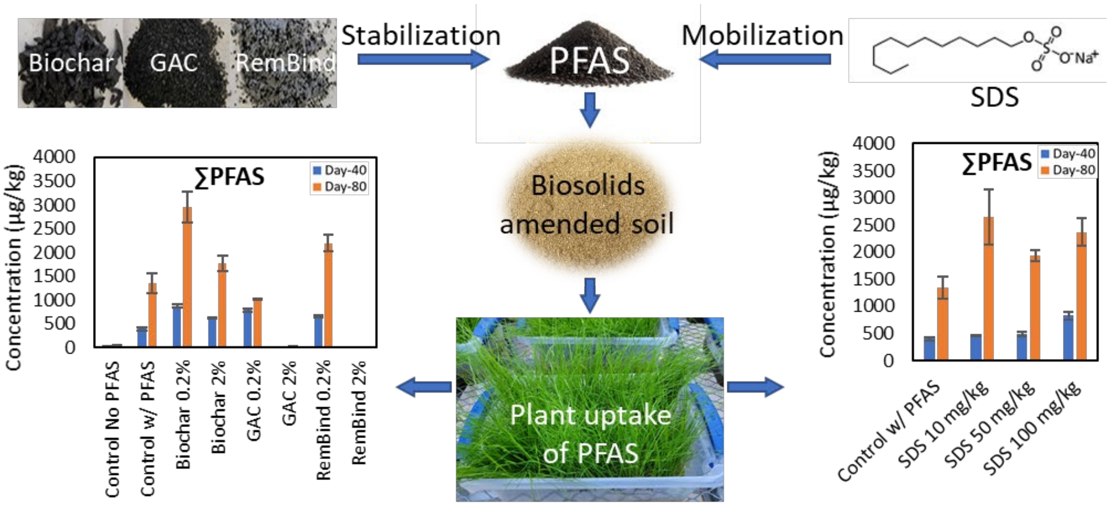


Fig. 4. Concentrations of PFAS in timothy-grass shoots grown in biosolids-amended soil treated by SDS on Day-40 and Day-80 (n=3). PFDA and PFUnA were not detected in timothy-grass shoots. Different letters in lower case and upper case represent significant differences among the treatments ($p < 0.05$).


Biochar
GAC
RemBind
Stabilization
PFAS
Mobilization
SDS
Biosolids amended soil
Plant uptake of PFAS
∑PFAS
Day-40
Day-80
Concentration (µg/kg)
4000
3500
3000
2500
2000
1500
1000
500
0
Control No PFAS
Control w/ PFAS
Biochar 0.2%
Biochar 2%
GAC 0.2%
GAC 2%
RemBind 0.2%
RemBind 2%
∑PFAS
Day-40
Day-80
Concentration (µg/kg)
4000
3500
3000
2500
2000
1500
1000
500
0
Control w/ PFAS
SDS 10 mg/kg
SDS 50 mg/kg
SDS 100 mg/kg