S.T. Yau High School Science Award

Research Report

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Title of Research Report

Phyto-Microbial Remediation of PFAS in Sewage Sludge: Links to Soil Properties and Biogeochemical Cycling with Constructed Wetland Applications

Date

2025/08/24

Phyto-Microbial Remediation of PFAS in Sewage Sludge: Links to Soil Properties and Biogeochemical Cycling with Constructed Wetland Applications

Qiuen Yu

Abstract

Per- and polyfluorinated alkyl substances (PFAS) in biosolids repurposed from wastewater treatment sewage sludge threaten food safety by entering agricultural soils and the food chain. Around 60% of U.S. sewage sludge is repurposed as biosolids, in which PFAS concentrations can reach hundreds of parts per billion (ppb), far surpassing EPA's parts per trillion limits of PFAS in drinking water. This research develops a cost-effective phyto-microbial system to treat PFAS-contaminated biosolids. For wetland soils spiked with 1 mg/kg of PFOA or PFBA, LC-MS results showed that chives in acidic conditions exhibited 3 times more PFOA uptake (60.76 µg/kg) than the chives in neutral conditions (18.92 µg/kg) over 14 days. Average liquid-phase phosphorus concentration in PFAS-amended acidic soils decreased from 19.40 mg/L to 7.40 mg/L over 14 days, which was a 76.2% greater decrease compared to the conditions without PFAS, suggesting synergistic effects between PFAS presence and plant phosphorus uptake. Additionally, alkaline phosphatase (*phoD*) gene expression, determined via qPCR, increased from $1.25*10^5$ copies/g to $2.28*10^5$ copies/g in PFAS-amended acidic soil over 7 days ($p = 0.042$). For the soil-based biosolid growth experiment involving two treatment stages with cilantro over 32 days, the average PFOA concentration post-treatment across all pH conditions was 95.8% lower compared to the no plant control, indicating high phytoremediation efficiency. In a third hydroponic phyto-microbial raw sludge experiment spanning 14 days, the chives in acidic conditions concentrated over twice the amount of PFOA in their roots (1139.5 µg/kg) than the chives in neutral conditions (547.65 µg/kg), $p = 6.6x10^{-5}$, corroborating how acidic pH may promote chive uptake of PFOA. Compared to PFOA, the chive roots concentrated higher amounts of PFBA—1897.9 µg/kg at pH 5.0 and 1960.6 µg/kg at pH 7.0. Potential PFOA degradation may have also occurred via microbial pathways, supported by fluoride production and the detection of PFAS intermediates in chive roots. This study offers important contributions to mitigating PFAS contamination with real-world constructed wetland applications.

Key Words: Per- and polyfluorinated alkyl substances (PFAS), biosolids, phyto-microbial remediation, wastewater treatment plants (WWTPs), sewage sludge, pH, phosphorus cycle, functional gene expression

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1 INTRODUCTION

1.1 Background

Per- and polyfluorinated alkyl substances (PFAS), commonly known as "forever chemicals," are a synthetic group of organofluorine contaminants that pose serious threats to human health and global ecosystems. Structure-wise, PFAS consist of a hydrophilic functional-group head linked to a hydrophobic fluorinated tail (Buck et al., 2011). Given how the carbon-fluorine (C-F) bond is one of the strongest bonds in organic chemistry, PFAS are extremely persistent and recalcitrant to degradation.

Because of their amphipathic nature and thermal stability, PFAS can be found in a variety of consumer products such as water-resistant clothing, food packaging, aqueous film-forming foams, and cleaning agents (CDC, 2024). Spanning over 12,000 different compounds, PFAS' high industrial utility comes with a heavy environmental cost (Morris, 2022). PFAS exposure can cause severe health effects including cancer, liver damage, and birth defects (Coulson, 2024; Fenton et al., 2020). Approximately 98% of Americans have ≥ 2ng/mL of PFAS in their blood, and between 1999-2018, 6.5 million deaths in the U.S. may be linked to PFAS exposure (ATSDR, 2024; Wen et al., 2022). Furthermore, PFAS adversely impacts the survival of aquatic and terrestrial species, while inducing cascading effects on ecosystem balance through distorting microbial community structures and impacting nutrient cycling (Evich et al., 2022; Oviedo-Vargas et al., 2025). As such, PFAS has increasingly become the focal point of public attention, news outlets, and government agencies (Tian et al., 2022).

PFAS contamination in biosolids repurposed from wastewater treatment plant (WWTP) sewage sludge proves especially alarming (Huang et al., 2022; Lenka et al., 2021). In the U.S., 98% of tested rivers in 19 states contain detectable PFAS, with elevated concentrations in 95% of the rivers bordering WWTPs and at 80% of sites downstream from biosolid-fertilized land (Waterkeeper Alliance, 2025). Upon entrance into agricultural soil, PFAS may migrate into crops, subsequently bioaccumulating and biomagnifying in the food chain (Huang et al., 2022; Lee et al., 2013; Stoiber et al., 2020). In the U.S., 2.39 million dry metric tons of sewage sludge is directed towards land applications annually (US EPA, 2016). Around 18% of total agricultural land is amended with biosolid fertilizers (US EPA, 2016). While the upcycling and repurposing of WWTP sewage sludge are sustainable for the environment, studies reveal that total PFAS concentrations in biosolids can range from 182 to 1650 parts per billion (ppb) (Thompson et al., 2023). According to EPA's 2025 sewage sludge risk assessment for perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS)—two legacy PFAS compounds, applying biosolids with more than 1 ppb of PFOA or PFOS may pose threats to human health (EPA, 2025). Thus, remediating PFAS in biosolids proves to be a pressing and crucial concern.

Traditional treatments methods for PFAS, such as thermal incineration and filtration using absorbents, remain expensive and unrealistic in alleviating widespread PFAS pollution in soils and sludge (Cantoni et al., 2021; Meegoda et al., 2022). Additionally, these methods often create harmful byproducts or are difficult to implement on a large scale due to time and cost

concerns (Cantoni et al., 2021; Meegoda et al., 2022). Thus, as of now, there is a lack of effective, systemized strategies to mitigate PFAS contamination in biosolids, which puts millions of lives at risk.

1.2 Study Focus

Considering the current challenges facing WWTP PFAS remediation, this research aims to explore and optimize sustainable, emerging phyto-microbial biotechnologies to treat biosolid PFAS contamination. Through multi-stage growth experiments, incubations, and result validation, this study develops a preprocessed biosolid product that can be safely applied on agricultural soils, which would preserve the sustainable practice of sewage sludge upcycling while simultaneously improving global food safety and ecosystem health.

Significant advancements have been made to last year's research, as illustrated in the flow chart below (**Figure 1**). Last year's work investigated how soil pH affects PFAS uptake by chives, with connections to phosphorus cycling and functional gene expressions. This year, the study was taken to the next level in terms of experimental conditions and real-world application. Specifically, a preprocessed biosolid product with significantly reduced PFAS levels was developed using two rounds of phytoremediation growth experiments. Additionally, a new phyto-microbial remediation system was set up with proven ability to concentrate and potentially degrade large amounts of PFAS in complex environmental matrices. If approved, a patent application will be filed. Moreover, analysis of a novel phyto-microbial-activated carbon PFAS treatment system is currently underway.

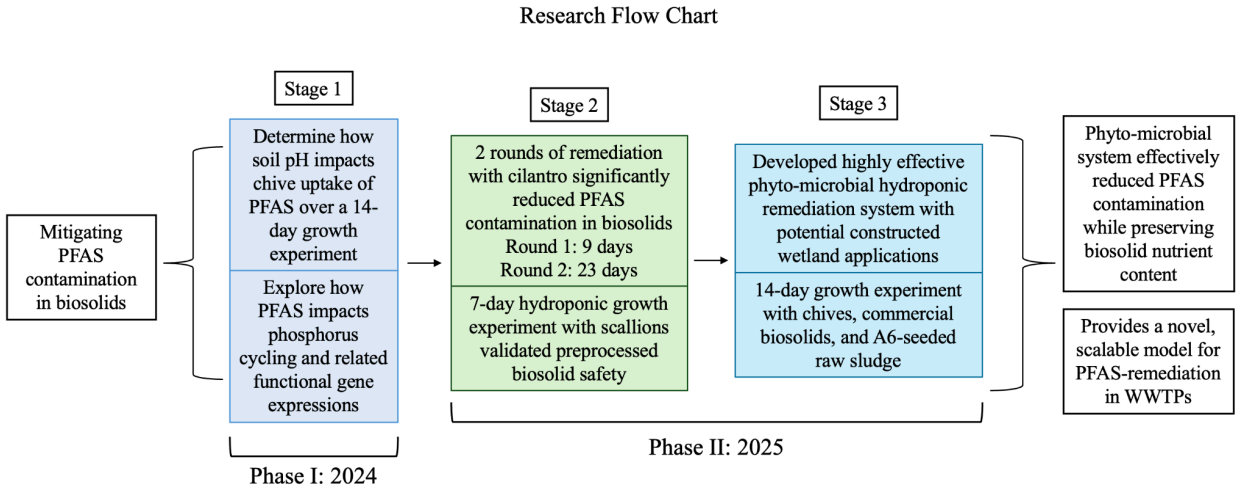


Figure 1: Research flowchart.

The research is divided into 3 stages, delving into plant-based PFAS treatment technologies, coupled with investigations into novel chemical-biological systems that combine the power of plants and microbes to remediate PFAS in biosolids. The 8-carbon chain legacy compound perfluorooctanoic acid (PFOA, $C_8HF_{15}O_2$) and its 4-carbon chain homologue

perfluorobutanoic acid (PFBA, $C_4HF_7O_2$) were selected for this study. Short-chain PFAS normally exhibit higher environmental mobility and lower bioaccumulation compared to their long-chain homologues, so PFBA is often used as a replacement compound for PFOA (Brendel et al., 2018; Zhao et al., 2016). Recent studies, however, revealed that short-chain PFAS may be just as toxic as long-chain PFAS, which necessitates comparative research between the environmental fate and transport of the two compounds (Solan et al., 2023).

In total, 3 different types of plants were used: *Allium schoenoprasum* (chives), *Coriandrum sativum* (cilantro), and *Allium fistulosum* (scallions). Chives were selected as phytoextractors due to their popularity and fast growth in both soil and hydroponic environments—maturing between 7 to 10 days after being cut. Furthermore, chives have been shown to effectively extract the heavy metal cadmium (Cd) in soils where concentrations reached 60 mg/kg (Eisazadeh et al., 2018). In another study, the bioaccumulation factor (BAF, [plant]/[soil]) of PFBA in chives was shown to be between 20.63 and 44.04, which attests to chives' phytoremediation potential of PFAS (Liu et al., 2019; Xu et al., 2024). Cilantro was chosen because it serves as a common agricultural crop and has been shown to effectively extract lead (Pb) from the environment (Garrett & Trott, 2019). Finally, scallions have a small leaf area and thin roots, characteristics that have been correlated with enhanced phytoextraction efficiencies (Liu et al., 2019; Xu et al., 2024). A study by Cho et al. (2008) also revealed that the stem of green onions (*Allium fistulosum*) were able to accumulate close to 225 mg/kg of Pb when chelating agents were amended. Additionally, scallions are common garden plants and widely cultivated on large agricultural plantations, thus serving as a good model plant for crops.

To study microbial degradation of PFAS in biosolids, a microbe-rich raw sludge slurry was amended to commercial biosolids in the Stage 3 growth experiment. The sludge had also been previously seeded with *Acidimicrobium* sp. strain A6 (A6), a microbe with a proven ability to degrade PFAS (Huang & Jaffé, 2019). Found naturally in acidic, iron-rich environments, the autotrophic bacterium A6 was first discovered in New Jersey's Assunpink wetlands by Huang and Jaffé (2015). A6 can reductively defluorinate PFAS compounds under anaerobic conditions during the Feammox process—ammonium oxidation to nitrite coupled with Fe(III) reduction to Fe(II), with stoichiometry shown in **Equation 1** below (Huang & Jaffé, 2015; Huang & Jaffé, 2019).

$$3Fe_2O_3 \bullet 0.5H_2O + 10H^+ + NH^{+4} \rightarrow 6Fe^{2+} + 8.5H_2O + NO^{-2} \quad (\textbf{Equation 1})$$

Specifically, A6 has been shown to degrade up to 60% of PFAS in 100-day incubations with PFOA and PFOS, supported by fluoride (F^-) buildup and the production of shorter-chain perfluorinated intermediates (Huang & Jaffé, 2019). The expression of a novel reductive dehalogenase gene (*rdhA*) in A6 was also discovered to be strongly correlated with F^- production (Jaffé et al, 2024). Considering A6's ability to thrive in the environments of WWTP digester tanks and wetland soils, A6 may potentially present a suitable option for bioremediating WWTP PFAS pollution (Huang et al., 2022).

The study involves the exploration of three distinct types of soils: 1) PFAS-spiked wetland soil amended with potting mix, 2) biosolids amended with filter cakes from a wastewater treatment plant (WWTP) 3) biosolid slurry amended with microbe-rich raw sludge. Results from this study can provide valuable insights in optimizing phytoremediation strategies of PFAS across a wide range of soil mediums.

In Stage 1, the impact of soil physicochemical properties—i.e. soil pH—on PFAS bioavailability and chive uptake was explored, with connections to phosphorus cycling and microbial functional gene expressions. Certain pH levels could potentially alter the chemical composition of root exudates and initiate shifts in microbial communities. Hence, studying functional gene changes of microbes could explain why pH levels may have influenced PFAS uptake by plants, as well as nutrient cycling at the air-soil-plant interfaces. Phytoremediation stands as a cost-effective, aesthetic, and efficient method for concentrating PFAS in complex environmental matrices, especially since many plants can thrive under environmentally relevant concentrations of PFAS (He et al., 2023; Mayakaduwage et al., 2022; Nason et al., 2024; Nassazzi et al., 2023; Greger & Landberg, 2024). For instance, He et al. (2023) found that certain weed species can remove up to 41% total weight PFAS from the soil and that plants with thin roots and small leaf areas possessed the best phytoextraction outcomes. Yet, the impacts of soil pH on plant uptake of PFAS remain largely unknown, as with how PFAS contamination impacts phosphorus cycling and expressions of genes such as the alkaline phosphatase gene (*phoD*) and the phosphate transporter gene (*pst1*). Phosphorus is essential to life, making up DNA, adenosine triphosphate (ATP), and other major biological molecules. Because phosphorus is oftentimes a limiting nutrient in nature, it serves as a major component in fertilizers. The relation between PFAS and the phosphorus cycle, however, have not been widely explored. Thus, this growth experiment is crucial to bridge the knowledge gaps and supplement valuable information regarding how to optimize phytoextraction efficiency.

In Stage 2, a new batch of growth experiments involving cilantro was set based on the results from Stage 1, with the goal of using phytoremediation to produce a usable, preprocessed fertilizer from PFAS-contaminated commercial biosolids. This treatment would in turn function as a small-scale proxy to in situ remediation of WWTP sludge. Ethylenediaminetetraacetic acid (EDTA), a chelating agent, was also amended to certain experimental conditions. EDTA increases the bioavailability of nutrients and can help plants withstand metal-contaminated soils; therefore, the study also tested whether EDTA presence would further promote phytoextractors' health and phytoremediation efficiency. For Stage 2, two rounds of growth experiments were set up in biosolids amended with PFOA, where the cilantro from Round 1 was replaced with a new batch of cilantro in Round 2 to increase phytoremediation potential and mimic natural crop cycles. Stage 2 research concluded with a hydroponic growth experiment, which aimed to validate the effectiveness of phytoremediation at reducing biosolid PFAS concentrations while affirming the feasibility and safety of utilizing the treated biosolids to promote plant growth. Scallions were grown in water-based environments with soils collected at the end of the Round 2 cilantro treatment, thereby modelling the spread of biosolid fertilizers on agricultural soils.

Through a new set of hydroponic growth experiments, Stage 3 research combined phytoremediation and biodegradation to target PFAS contamination in WWTP sludge. Chives were selected as the phytoextractor, and each growth cup was amended with commercial biosolids and A6-seeded sewage sludge. The effects of pH on phyto-microbial remediation efficiency were explored, along with trends in biogeochemical cycling through soil anion analysis.

2 MATERIALS AND METHODS

2.1 Experiment materials

The PFBA and PFOA used in this study were purchased from Sigma Aldrich and prepared in stock solutions prior to experiment-specific dilutions. EDTA was obtained from Sigma Aldrich. Sodium Hydroxide in Water (NaOH, 1.0 mol/L) was obtained from TCI America, and pH-indicator strips with test range 0-14 were purchased from MQuant® and Fisher Scientific™ for pH testing of liquid and soil mediums, respectively. All aqueous samples were extracted with a syringe (BD Plastipak™ 3mL Syringe Luer-Lok™ Tip) and needle (BD PrecisionGlide™ Needle) set, then passed through a 0.22 μm or 0.45 μm membrane filter (CELLTREAT® Scientific Products) for chemical analysis. The chives, cilantro, and scallions were freshly excavated from the Princeton Hulu Farm, with the roots kept intact. Prior to experimental usage, the chives were temporarily kept in small pots with All Purpose Garden Mix, while the cilantro and scallions were kept in hydroponic vases. Transparent plastic cups or 220 mL Falcon® Sterile Containers were utilized as pots for growth experiments. For Stage 1, Lambert Professional Organic All-Purpose Mix was used, along with soil obtained from New Jersey's Assunpink Wildlife Management Area. Stage 2 biosolids were purchased from Bloom® and amended with filter cakes from a local WWTP in New Jersey. The raw sludge, previously seeded with A6, originated from Stony Brook Regional Sewerage, New Jersey.

2.2 Stage 1: Wetland soil growth experiment and sampling

The soil incubation study lasted 14 days and included 16 experimental conditions (**Table 1**), covering setups with 1 mg/L of PFOA or PFBA, pH adjustments to 5.0 or 7.0, and rigorous sets of no PFAS and no plant controls.

Water medium	pH	
PFOA (around 1 ppm)	4.5-5.0	with chives
		no chives
	7.0	with chives
		no chives
PFBA (around 1 ppm)	4.5-5.0	with chives
		no chives
	7.0	with chives
		no chives
Deionized water x2	4.5-5.0	with chives
		no chives
	7.0	with chives
		no chives

Table 1: Experiment setup for the wetland soil incubation study.

For soil preparations, the All-Purpose Mix was blended with Assunpink soil in a 5:1 ratio (Huang & Jaffé, 2018). For liquid medium preparations, pH adjustments and PFAS additions were made to deionized water (DIW) in 500 mL Kimax® Kimble bottles. The pH range of 5.0 to 7.0 was chosen because it is environmentally relevant and tolerable for chives, while covering both acidic and neutral conditions.

Afterwards, 100 g of soil was weighed into each cup and amended with 250 mL of the appropriate liquid medium. The soil and liquid were then stirred with a spatula to ensure saturation of the soil particles. The cups were covered with double-layer parafilm and let sit overnight to allow PFAS-soil sorption processes take place before day 0 (d0) sampling.

Following d0 sampling, two chive plants with roots trimmed to approximately the same length were transplanted into the appropriate growth cups. The chives were cut close to the soil to facilitate the tracking of chive growth and ensure consistency between conditions. An environmental growth chamber was set up with a temperature of 25.0˚C and relative humidity of 50%. A four head LED growth light with 12/12 hour light/dark cycle provided an equal amount of lighting for all cups.

Sampling was performed at 3 time points: day 0 (d0), day 7 (d7), and day 14 (d14). The d0 samples were taken without replicates, as it was unlikely for different soil compositions to have emerged. The d0 soil levels were marked on the cups with a sharpie. After thorough stirring, 10g wet weight (ww) of soil was placed into Falcon™ 15 mL Conical Centrifuge Tubes.

Before sampling the d7 and d14 soils, ultrapure water was added to the cups to return the soil waterline to d0 levels, accounting for any evaporation that occurred. Because PFBA and PFOA are not volatile, no potential atmospheric losses of the compounds were considered during the analysis. The re-saturated soils were stirred to ensure homogeneity and allowed to sit for 3 hours before sampling. For d7 and d14 samples, 4 ± 0.050g ww of soil were taken in triplicate from the mid-depth front, mid-depth back, and deep-center positions, thus covering potential differences in the soil composition.

Due to the high levels of moisture in the soil, supernatant extraction for PFAS and PO_4^{3-} was first performed. Immediately after sampling, the Falcon tubes were centrifuged for 10 minutes at full speed. Afterwards, between 0.5 mL to 1.0 mL of supernatant was collected with a syringe and needle set and then passed through a 0.45 µm membrane filter for phosphate anion analysis in the IC. Subsequently, all samples with PFAS were diluted 10 times (900 µL ultrapure water and 100 µL filtrate) for LC-MS analysis. For d0 samples with PFBA and PFOA, two sets of supernatant were filtered from one Falcon tube to create duplicates for each condition. Between supernatant sampling, the contents in Falcon tubes were mixed by vigorous shaking and then re-centrifuged for 10 minutes. The d7 and d14 supernatants were sampled one per each triplicate tube. The remaining solid-phase samples were stored at -20˚C for subsequent solid-phase PFAS extraction and microbial analysis.

2.3 Stage 2: Soil-based biosolid growth experiment and sampling

The soil-based biosolid growth experiment featured six conditions displayed in **Table 2**. Round 1, Round 2, and hydroponic growth experiments lasted 9, 23, and 7 days, respectively.

PFAS	pH	EDTA	Plant
1 ppm PFOA	6.5-7.0 (DIW pH)	No	Yes
1 ppm PFOA	Acidic (3.5 in solution)	No	Yes
1 ppm PFOA	Basic (9.0 in solution)	No	Yes
1 ppm PFOA	6.5-7.0 (DIW pH)	Yes	Yes
no PFOA	6.5-7.0 (DIW pH)	No	Yes
1 ppm PFOA	6.5-7.0 (DIW pH)	Yes	No

Table 2: Experiment setup for the biosolid incubation study.

Bloom® biosolid and WWTP filter cake were crushed manually and homogenized in a 9:1 biosolid:filter cake ratio. Then, 200 g aliquots of the soil mixture were weighed into each growth cup. Concentrated PFOA, PFBA, 6N HCl, 1.0 NaOH, and/or 0.5 M EDTA were added to DIW for condition-specific liquid medium preparations. Afterwards, 50 mL of tailored liquid medium was amended to each growth cup, and cilantro was transplanted into the cups. All cups

were partially covered with parafilm to reduce evaporation-induced water loss and kept in a lab fume hood at 25˚C. Day 9 (d9) soil samples were taken in triplicates, with 3 x ~0.8 g ww soil samples preserved in a -4˚C freezer. Following the 9-day growth period, the cilantro was also extracted and stored in a deep freezer.

Subsequently, a new batch of cilantro was planted into the same cups, mimicking natural crop cycles. Roughly 50 mL of DIW was amended to all conditions to re-saturate the soil with water and promote cilantro growth. This time, the cups were placed in a growth chamber set to 30˚C and 50% relative humidity. A four head LED growth light with 16/8 light/dark cycle provided equal lighting for all cups. After a 23-day growth period, 3 x ~0.8 g ww were taken and preserved in a -4˚C freezer.

After two rounds of cilantro growth experiments in the biosolids, 20g of soil was taken from each of the 6 conditions and amended with 200 mL of DIW in new cups for a hydroponic growth experiment. Theoretically, the soils should have decreased PFAS content, and the hydroponic growth experiment would serve as a validation to safety of the preprocessed biosolids. With roots and shoots trimmed to similar lengths, scallions were placed into the cups, which were then partially covered with parafilm to reduce water evaporation. All cups were kept in the fume hood during the growth period. On d0, 1.0 mL of aqueous samples were taken in triplicates after letting the biosolid and DIW mixture settle. On d7, 1.0 mL of aqueous solution was extracted from each condition for chemical analysis. **Figure 2** provides a schematic overview of the Stage 2 experiment setup.

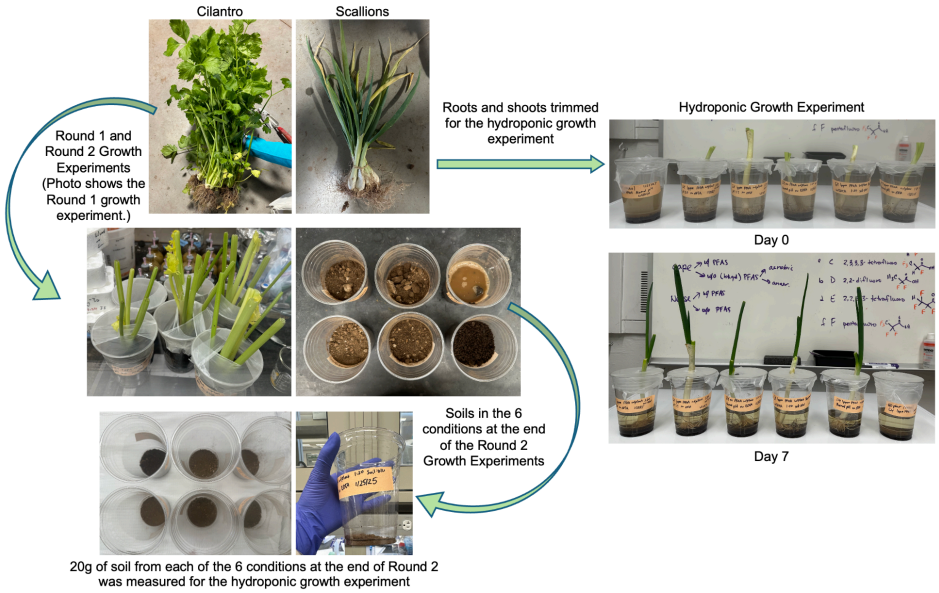


Figure 2: Experiment setup flowchart for Stage 2.

2.4 Stage 3: Hydroponic microbe-rich raw sludge growth experiment and sampling

The hydroponic-based, phyto-microbial raw sludge growth experiment had a duration of 14 days and included 6 conditions, displayed below (**Table 3**). Controls without PFAS and plants were included.

6 Conditions	
PFOA (1 ppm) with chives	pH 5.0
	pH 7.0
PFBA (1 ppm) with chives	pH 5.0
	pH 7.0
No plant control (1 ppm PFOA, pH 7.0)	
No PFAS control (pH 7.0) with chives	

Table 3: Experiment setup for the hydroponic-based raw sludge incubation study.

For soil preparations, Bloom® commercial biosolid and A6-seeded raw sludge were mixed in an approximate 5:2 ratio. The biosolid-sludge mixture was crushed and homogenized manually in Ziploc bags. Afterwards, 30 ± 0.5 g of the soil mixture was weighed into individual 220 mL Falcon® sterile containers, which were used as growth cups. For liquid medium preparations, a set amount of DIW was mixed with a pre-calculated volume of concentrated PFOA or PFBA stock solution to reach an approximate final concentration of 1 ppm PFOA or PFBA. Since the DIW's original pH was 5.0, a small amount of 1.0 M NaOH was added to create pH 7.0 liquid mediums. Subsequently, 150 mL of the tailored liquid was amended to each growth cup, which were then covered with double layer parafilm. The cups were placed in the laboratory fume hood overnight prior to d0 sampling in order to let preliminary PFAS-soil sorption processes take place. The d0 water level for each cup was marked with a Sharpie.

Aqueous and solid-phase d0 sampling was conducted for each growth cup. A syringe and needle set was used to extract the liquid, which was then passed through a 0.22 µm filter for chemical analysis. All aqueous-phase sampling was performed in triplicate per growth cup. Soil samples were extracted with a spatula and placed into Falcon tubes. Sample wet weights (ww) per growth cup ranged between 3 g and 4 g. The soil was immediately preserved in a -20˚C freezer after sampling to prevent further metabolic processes from taking place.

Following d0 sampling, DIW with pH 5.0 or 7.0 was amended to the growth cups to return the water to pre-sampling levels. Freshly excavated chives cut to similar heights and biomasses were weighed and placed into the cups, which were then covered with parafilm to minimize evaporation-induced water losses. After the chive transplant, the water levels were re-marked with a sharpie. The cups were placed in a fume hood at 25˚C, and a four head LED

growth light with 16/8 light/dark cycle provided equal lighting to all cups. A detailed growth cup diagram is shown in **Figure 3**.

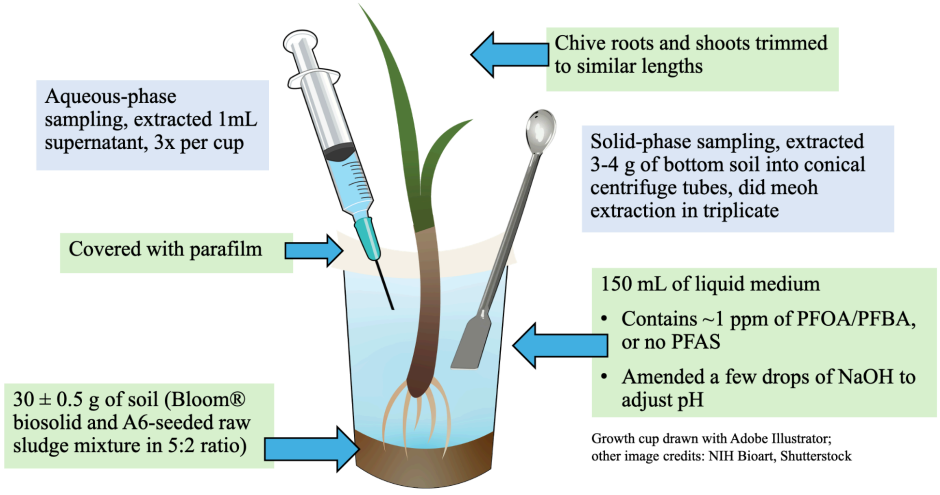


Figure 3: Hydroponic growth cup diagram.

Subsequent sampling occurred on d7 and d14. DIW with adjusted pH was amended to the cups to return the water to d0 levels, and the cups were allowed to sit for 2 hours prior to d7 and d14 aqueous and solid-phase sampling. On d14, the chives were extracted and preserved in a -20˚C freezer for subsequent PFAS analysis.

2.5 Chemical analysis

Anion (PO_4^{3-}, NO_3^-, SO_4^{2-}, F^-, and Cl^-) and cation (NH_4^+) analysis was performed using a Dionex™ Ion Chromatograph (LC3000) with an AS18 column (4 mm i.d. x 200 mm) for anions and a CS16 column (4 mm i.d. × 200 mm) for cations.

Quantitative PFOA and PFBA analysis was performed with liquid chromatography-mass spectrometry—LCMS-2050 Single Quadrupole Mass Spectrometer set to a negative-ion electrospray mode (SHIMADZU) with a Shimpak Velox C18 column (I.D. 2.1 mm, length 50 mm, particle size, SHIMADZU). The programming was based on the method used in Jaffé et al. (2023). The flow rate was set to 0.4 mL/min and the temperature was maintained at 40˚C. The mobile phase first consisted of 95% solvent A (5 mM ammonium acetate in LC-MS-grade water) and 5% solvent B (LC-MS-grade methanol), which was held for 0.5 minutes. Then, the solvent B concentration was increased to 95% over 12 minutes, held constant for 2 minutes, decreased to 10% over 1 minute, and held constant for 4 minutes.

Methanol extraction was conducted to quantify solid-phase PFOA and PFBA concentrations. The procedure followed the method described by Chiavola et al. (2020). First, the soil was amended with 50% methanol in a 1:1 volume ratio. After vortexing, the tubes were sonicated in a water bath for 15 minutes at 60˚C and later centrifuged at 4000 rpm for 15 minutes. The supernatant was subsequently extracted with a syringe and needle set and passed

through a 0.22 µm or 0.45 µm filter. If needed, the filtrant was diluted with ultrapure water for LC-MS analysis to improve the detection quality.

Plant extraction of PFAS was carried out for Stage 4 chives, adopting a procedure modified from Hearon et al. (2022). After the 14-day growth experiment, the chives were gently extracted, rinsed with DIW, and air dried. They were then separated into the root and shoot compartments and weighed. Subsequently, approximately 1.2 g ± 0.3 g of roots and shoots were cut into pieces < 5 mm in length, placed into conical centrifuge tubes, and amended with a 5 mL solution of 50% methanol and 1% NH_4OH (**Figure 4**).

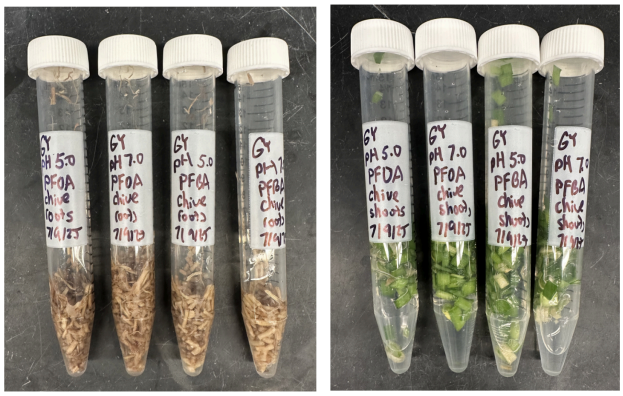


Figure 4: PFAS extraction from chive root (left) and shoot (right) in conical centrifuge tubes.

The samples were agitated on a rotational shaker at 200 rpm for 20 mins and centrifuged at full speed for 20 mins. The supernatant was then extracted in triplicate and passed through a 0.22 µm filter for PFOA and PFBA quantification on the LC-MS.

2.6 Microbial analysis: DNA extraction and qPCR

DNA extraction was performed for selected samples from Stage 1 and 4 experiments. The FastDNA® Spin Kit for Soil was used per the manufacturer's protocol, with 500 mg of soil weighed into each lysing tube and 100 µL of DES elution solution added in the final step to elute the DNA. Total bacteria numbers, alkaline phosphatase genes (*phoD*), phosphate transporter genes (*pst1*), and reductive dehalogenase genes (*rdhA*) were quantified using Real-time StepOnePlus qPCR system from Thermo Fisher Scientific, USA with the TaKaRa Biotechnology qPCR kit (Cat# RR820A) (Lowe et al., 2023; Pitt et al., 2010). Each qPCR well contained 18 µL of master mix (10 µL TB Green Premix Ex Taq II, 0.8 µL10 nM forward primer, 0.8 µL10 nM reverse primer, 0.4 µL ROX Reference Dye, and 6 µL DNAase-free H2O) and 2 µL of extracted DNA (Huang & Jaffé, 2019). The qPCR run method was initiated at 30s and maintained at 90˚C, which was followed by 40 cycles of 5s at 94˚C, 30s at 57˚C, and 30s at 70˚C (Huang & Jaffé, 2019). Primer sequences targeting *phoD* and *pst1* are based on those used in previous studies (Lowe et al., 2023; Pitt et al., 2010). Copies/g of total bacteria and the target genes were determined using their respective calibration curves obtained from previous qPCR runs for standards and graphed after $\log_{10}$ processing to obtain a linear regression (**Figure 5**). The CT runs were then converted to copies/g based on the standard curves.

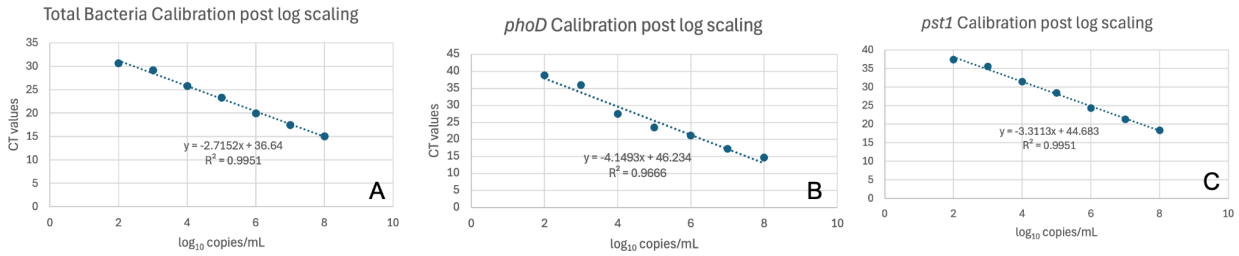


Figure 5: qPCR calibration curves with CT values and $\log_{10}$(copies/mL) showing the linear regression line, regression equation, and R^2. A) Calibration for total bacteria. B) Calibration for *phoD*. C) Calibration for *pst1*.

2.7 Data analysis

Type 2, two-tailed student's t-tests were performed for the data being compared using Microsoft Excel, and significance was accepted at $p < 0.05$. All error bars, unless otherwise specified, represent the standard deviation of the data.

3 RESULTS AND DISCUSSION

3.1 Stage 1: wetland soil growth experiment

3.1.1 Acidic pH enhances chive uptake of PFOA.

Over the 14-day growth period, all chives grew approximately the same amount and developed into mature chives (**Figure 6**). No visible growth inhibition of chives was observed in the cups amended with PFBA and PFOA. The lack of an observable negative impact of PFAS on chive growth was expected, as previous studies have documented the negligible visible impacts of PFAS on certain plants at environmentally relevant concentrations (He et al., 2023; Nassazzi et al., 2023).

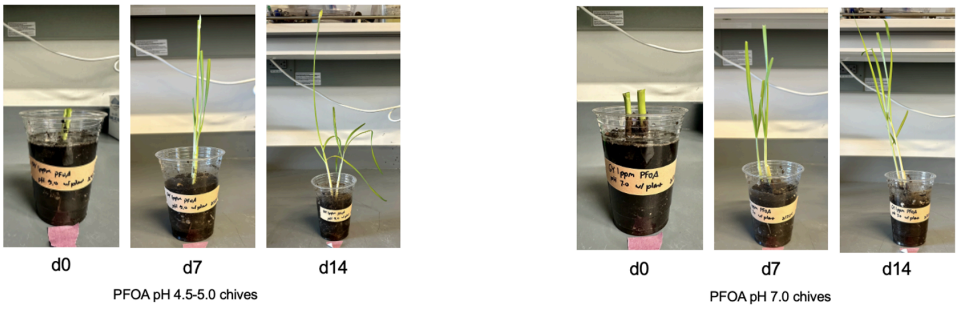


Figure 6: Images of chive growth over the 14-day period.

Figure 7A shows that between d0 and d14, pH 4.5-5.0 with chives experienced a 20.9% liquid-phase [PFOA] decrease from an average of 442 µg/L to 326 µg/L ($p = 0.014$), while the pH 7.0 with chives observed a 23.2% decrease from 426 µg/L to 327 µg/L ($p = 0.11$). There was no significant PFOA concentration difference in pH 4.5-5.0 no chives between d0 and d14 ($p =$

0.40), highlighting how the aqueous-phase PFOA decrease in pH 4.5-5.0 with chives could be attributed to uptake by chives rather than increased soil sorption over the growth period. Interestingly, for pH 7.0 no chives, a 11.9% reduction in aqueous-phase PFOA was observed, in which [PFOA] decreased from 340 ppb to 299 ppb ($p = 0.35$). To further confirm that the PFOA decrease in pH 4.5-5.0 with chives can be attributed to plant uptake rather than soil adsorption, coupled with the need to better understand the PFOA decrease in both pH 7.0 cups (with and without chives), methanol extraction for solid-phase PFOA was performed in triplicate and the results were graphed (**Figure 7B**).

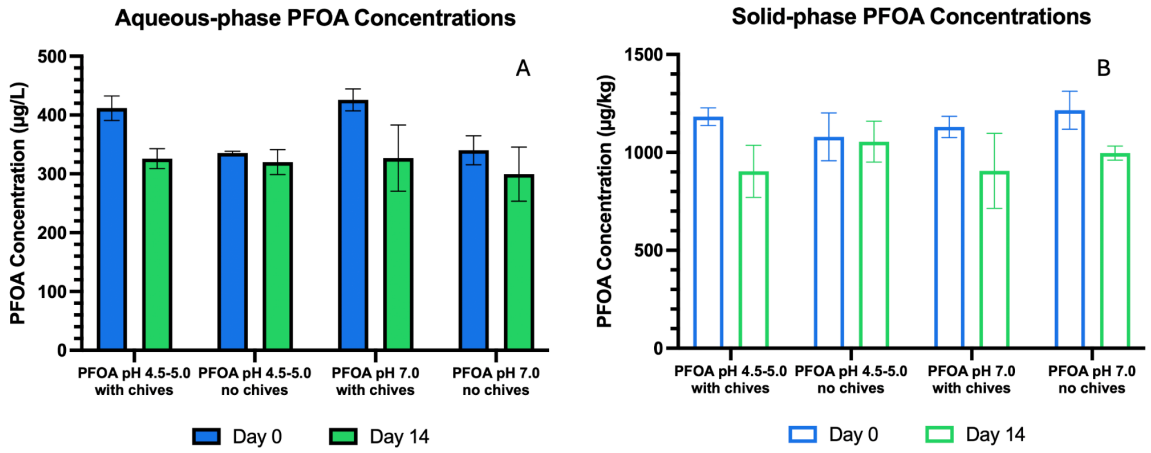


Figure 7: A) PFOA aqueous-phase concentrations in µg/L for d0 (blue) and d14 (green) for each condition. B) PFOA solid-phase concentrations in µg/kg for d0 (blue) and d14 (green) for each condition.

The solid-phase PFOA concentration decreased over the 14-day growth period under pH 4.5-5.0 with chives and remained stable in pH 4.5-5.0 no chives, strongly suggesting that the overall reduction in both the liquid- and solid-phase PFOA concentrations in pH 4.5-5.0 with chives may be attributed to chive uptake. Specifically, the decrease from 1,183 ppb to 904 ppb in [PFOA] under the pH 4.5-5.0 with chives condition was significant ($p = 0.026$). An overall downward trend was observed for the soil PFOA concentration in pH 7.0 with chives. Because no significant decrease in aqueous- or solid-phase PFOA concentration ($p = 0.11$ and 0.12, respectively) was observed for pH 7.0 with chives, and significant decreases in both aqueous- and solid-phase PFOA concentration ($p = 0.014$ and 0.026, respectively) were detected for pH 5.0 with chives over the 14-day growth period, it was concluded that chives showed enhanced PFAS uptake under acidic soil conditions. Intriguingly, while the PFOA concentration in pH 5.0 no chives remained stable over the growth duration, the pH 7.0 no chives condition exhibited a decrease in soil PFOA concentration in addition to the reduction detected in liquid PFOA concentration. One potential explanation is that soils are expected to show decreased adsorption of PFOA at higher pH levels, as more soil particles become deprotonated, which increases electrostatic repulsion between soil particles and PFOA (Wang et al., 2023). Yet, the decrease in

liquid-phase PFOA under pH 7.0 no chives between d0 and d14 is fascinating and requires further study, especially due to the unlikelihood for PFOA to become airborne, as PFOA has a vapor pressure of 3.16×10^{-1} mm Hg at 25˚C and a negligible volatility at pH > 2.5 (Johansson et al., 2017). **Figure 8** displays the total PFOA mass balance in the soil-water system.

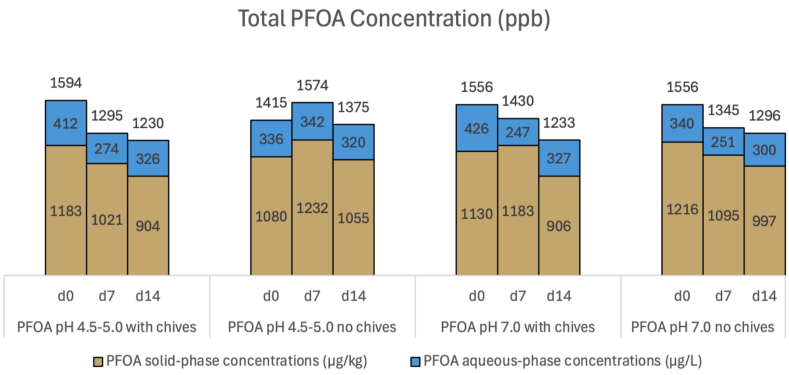


Figure 8: Total PFOA mass balance in soil (brown) and water (blue) on d0, d7, and d14.

In order to further confirm the finding that acidic conditions enhanced chive uptake of PFOA, plant extraction of PFOA was completed from the preserved chives grown in the acidic and neutral soils. Around 5 g of chive leaves was weighed from each chive and then cut into small pieces. Afterwards, the chive pieces were placed in conical Falcon tubes and amended with 6 mL of 50% methanol. The tubes were then placed on a 200 rpm shaker for ten minutes, and the liquid was subsequently extracted and filtered for LC-MS analysis. **Table 4** displays the LC-MS results from the plant extractions of PFAS. Because the PFOA concentration in the pH 4.5-5.0 chive leaves (60.76 µg/kg) was around 3 times higher than the PFOA concentration in the pH 7.0 chive leaves (18.92 µg/kg), the plant extraction results corroborate how chives exhibit an enhanced uptake of PFOA under acidic conditions.

Sample	[PFOA] µg/kg
PFOA 4.5-5.0 chives	60.76
PFOA 7.0 chives	18.92

Table 4: LC-MS results for PFOA concentration in chive leaves.

The enhanced uptake of PFAS in acidic soil conditions is noteworthy, as previous studies demonstrated that lower soil pH levels reduce PFAS mobility due to increased soil sorption, which appeared to not be the case in this study (Campos-Pereira et al., 2018). Many factors such as the specific type of soil used, the possible enhancement of certain rhizosphere microbial communities under lower soil pH levels, and organic acids exuded from the roots could have contributed to greater PFOA uptake by chives under an acidic pH (Xia et al., 2024; Xu et al.,

2022). The increased PFOA absorption by chives under a soil pH of 4.5-5.0 also contains important agricultural implications and field applications by indicating how neutral soils could potentially reduce PFAS bioaccumulation in edible crops.

3.1.2 Total PFBA concentrations decreased in conditions with chives.

Aqueous-phase PFBA concentrations appeared to increase over the 14-day growth period (**Figure 9A**), which differed from the trends in aqueous-phase PFOA concentrations observed in the study, as PFOA concentrations experienced an overall decrease from d0 to d14. In order to further delve into why aqueous-phase PFBA concentrations increased, solid-phase PFBA extractions were conducted, with the results shown in **Figure 9B**.

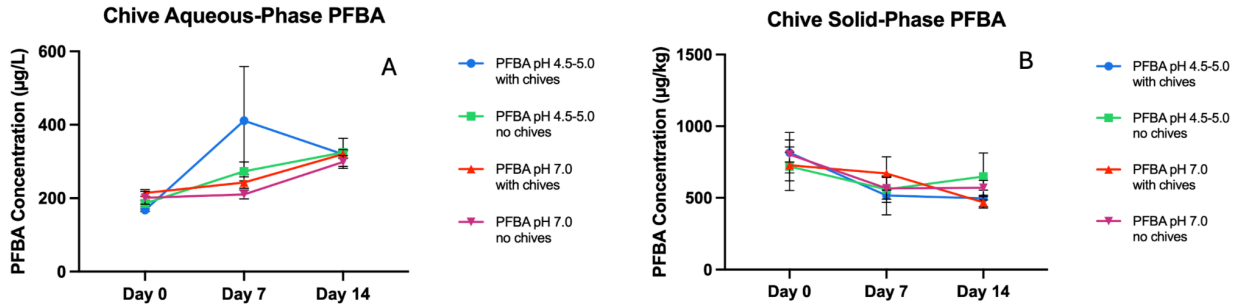


Figure 9: A) PFBA aqueous-phase concentrations. This figure shows PFBA in µg/L for d0 (blue) and d14 (green) for each condition. B) PFBA solid-phase concentrations. This figure shows PFBA in µg/kg for d0 (blue) and d14 (green) for each condition.

Based on **Figure 9B**, all the PFBA solid-phase concentrations exhibited a decrease over the 14-day growth period. The pH 4.5-5.0 with chives condition showcased the most rapid decrease in PFBA-concentration, as its d0 solid-phase PFBA level was the highest of all conditions, but its solid-phase PFBA concentration became the lowest on d7. Specifically, the pH 4.5-5.0 with chives condition exhibited a 36.6% decrease in average solid-phase PFBA concentration over the 7 days across the triplicates, although the decrease was not significant ($p = 0.0575$). On the other hand, the solid-phase PFBA concentration in the pH 7.0 with chives condition remained generally stable over the first 7 days and then decreased rapidly from d7 to d14. Since PFBA levels decreased more readily in the acidic conditions with chives compared to the neutral conditions, these results support the aforementioned finding that acidic conditions may potentially enhance phytoremediation efficiency. The decreases in the solid-phase PFBA concentration, however, may also be attributed to increases in the aqueous-phase PFBA concentration instead of plant uptake; thus, the total solid and aqueous-phase PFBA concentrations were graphed together to visualize the overall trends in PFBA levels across the 4 conditions (**Figure 10**).

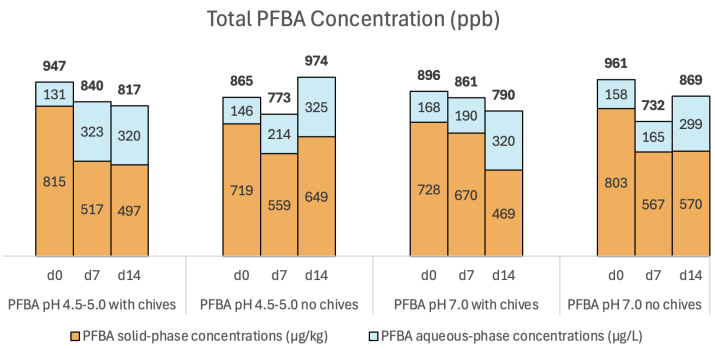


Figure 10: Total PFBA mass balance in soil (brown) and water (blue) on d0, d7, and d14.

Figure 10 highlights how the total PFBA concentration remained relatively stable across all 4 conditions over the 14-day growth period. While the small decreases in PFBA levels in the conditions with chives may be due to plant uptake, PFBA extraction in the chive roots and shoots would need to be conducted to affirm the results. The fluctuations in PFBA concentration in the acidic and neutral conditions without chives may be due to PFBA interactions with soil particles or microbes, and more studies would be needed to uncover the specific reasons behind these PFBA concentration fluctuations.

3.1.3 Phosphorus cycling and functional gene analysis

As for chive phosphorus uptake, all conditions with chives appeared to exhibit a greater % decrease in phosphate concentration from d0 to d14 (except DIW1 7.0, which had no data point because there was difficulty identifying its phosphate peak) (**Figure 11**). This trend is logical as chives are expected to uptake phosphorus for their growth and function. Both the presence of PFBA and PFOA under the pH 4.5-5.0 condition appeared to increase chive uptake of phosphorus over 14 days, as the average aqueous-phase phosphate concentrations exhibited a 76.2% greater decrease in the PFAS-amended acidic conditions compared to the conditions without PFAS. All pH 7.0 conditions with chives showcased similar phosphate uptake levels (relative to their d0 phosphate concentrations), highlighting how the presence of PFBA and PFOA had negligible effects on chive phosphorus uptake under neutral soil pH levels. This trend proves noteworthy as it suggests potential synergism between phosphorus and PFAS uptake by plants, especially since PFAS and nutrients enter plant roots through similar membrane pathways.

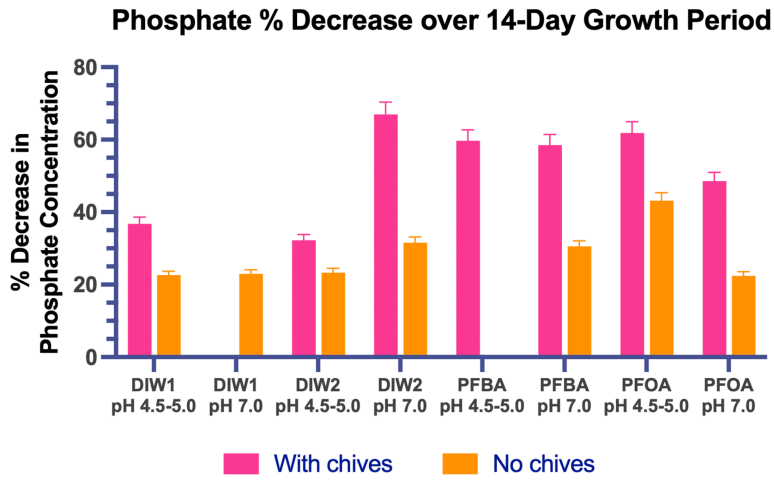


Figure 11: Percent decrease in phosphate concentrations over 14 days for conditions with chives (green) and without chives (pink). The % decrease was calculated with averages from the d0 and d14 phosphate concentrations, so the 5% error bars are based on the instrument analysis error.

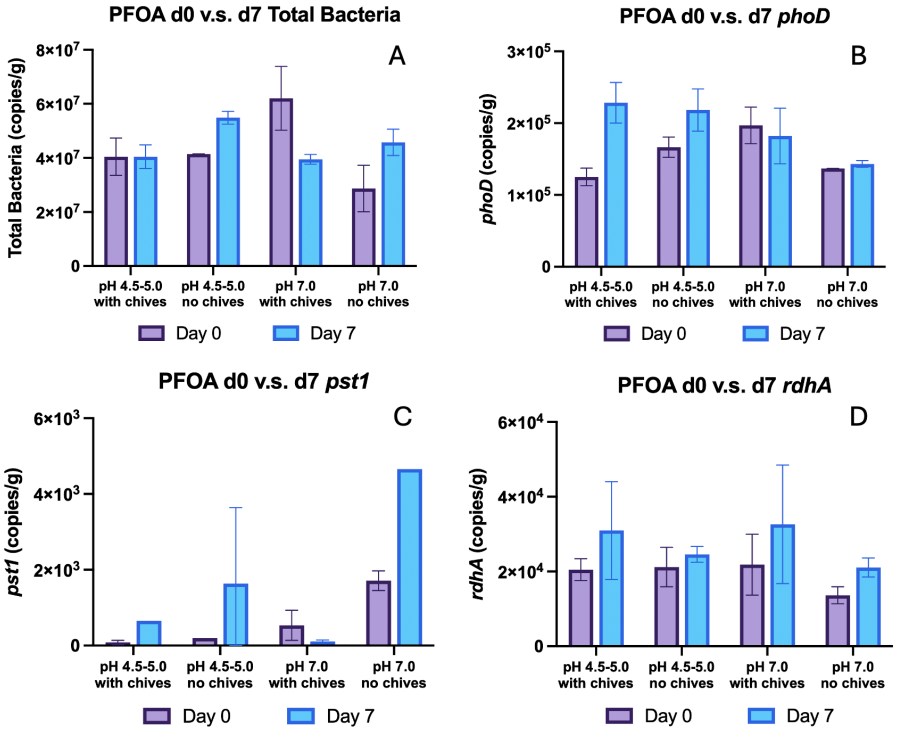


Figure 12: These figures show qPCR screening for various functional genes (copies/g) in conditions with PFOA for day 0 (blue) and day 7 (orange). A) total bacteria numbers. B) *phoD* numbers. C) *pst1* numbers. The d7 pH 4.5-5.0 with chives bar, the d0 pH 4.5-5.0 no chives bar, and the d7 pH 7.0 no chives bar do not have error bars due to undetermined CT values in one of their duplicate wells. D) *rdhA* numbers.

The pH 4.5-5.0 and pH 7.0 no chives conditions exhibited increases in total bacteria numbers, with the pH 4.5-5.0 increase being significant ($p = 0.015$) (**Figure 12A**). In addition, total bacteria numbers in pH 4.5-5.0 with chives remained stable and decreased in pH 7.0 with chives, demonstrating that chive roots likely did not enhance bacteria growth in soils contaminated by around 1 ppm of PFOA and possibly reduced total bacteria numbers under a neutral pH, which could affect the amount of PFOA uptake by chives. A study by Shittu et al. (2023) have shown that while certain microbes such as *Escherichia coli* and *Proteobacteria* can withstand and even thrive in PFAS-contaminated environments, other microbes, such as *Actinobacteria* and *Chloroflexi* are severely impacted by PFAS and would experience a decrease in population size. Additionally, microbial operational taxonomic units (OTUs) in acidic conditions reached a peak height at a soil pH of approximately 5.5, while microbial OTUs in alkaline conditions reached a peak height at a soil pH of approximately 8.3, indicating how potential rhizosphere acidification by the chives' root exudates from an initial pH of 4.5-5.0 and 7.0 could have factored into the decrease in total bacteria observed (Shi et al., 2021).

The number of *phoD* increased from $1.25*10^5$ copies/g to $2.28*10^5$ copies/g in the pH 4.5-5.0 conditions, with the increase being significant ($p = 0.042$) in pH 4.5-5.0 with chives at 82.5% compared to d0 (**Figure 12B**); however, the number of *phoD* maintained relatively stable in both pH 7.0 conditions. Previous research has shown that perfluorooctanesulfonic acid (PFOS) uptake by *Arabidopsis thaliana* may be intertwined with the plant's phosphorus uptake system (Kim et al., 2024). Specifically, PFOS appeared to increase gene expressions related to the plant's uptake and transport of phosphorus (Kim et al., 2024). While the study by Kim et al. (2024) focused on potential phosphorus-related plant-based mechanisms influencing the degree of PFOS absorption by *Arabidopsis*, soil microbes mediating the amount of phosphorus available may also play a major role in the uptake of both PFAS and phosphorus by plants. Since *phoD* encodes an alkaline phosphatase that catalyzes the hydrolysis of phosphate monoesters and releases inorganic phosphate, its increased numbers under pH 4.5-5.0 with chives could be correlated with enhanced PFOA uptake by plants exhibited in that condition (Liu et al., 2015; Sharma et al., 2013).

With the available data, *pst1* number increased in pH 4.5-5.0 with chives and decreased in pH 7.0 with chives (**Figure 12C**). No significance can be concluded due a lack of replicate data. This trend is counterintuitive, as *pst1* normally operates at a pH of 7 to 10 (Burut-Archanai et al., 2011). The increase in *phoD*, however, could potentially factor into the increase in *pst1* observed under pH 4.5-5.0 with chives, since with more inorganic phosphate bioavailable in the soil, bacteria might become more efficient at transporting the phosphate into their cells for metabolic purposes. The presence of PFAS under an acidic condition compounded with possible synergistic effects of the chives' organic root exudates could have initiated higher copies of *phoD* genes, which then induced greater numbers of bacterial *pst1* genes and chive absorption of PFAS.

As shown in **Figure 12D**, *rdhA* was present in all samples. The *rdhA* gene encodes the reductive dehalogenase enzyme, which is embedded in the cell membrane to drive the cleavage

of carbon-halogen bonds by functioning as electron acceptors in the electron transport chain (Wagner, 2013). Although increases in *rdhA* were observed over 7 days of the growth experiment, the increases were not significant. Yet, the presence of *rdhA* across all samples suggests potential defluorination activities in the soils. Further conclusions would need to be confirmed with additional qPCR and fluoride analysis data.

3.2 Stage 2: Soil-based biosolid growth experiment

3.2.1 Round 1 and Round 2 phytoremediation stages significantly reduced biosolid PFOA concentrations under all pH levels.

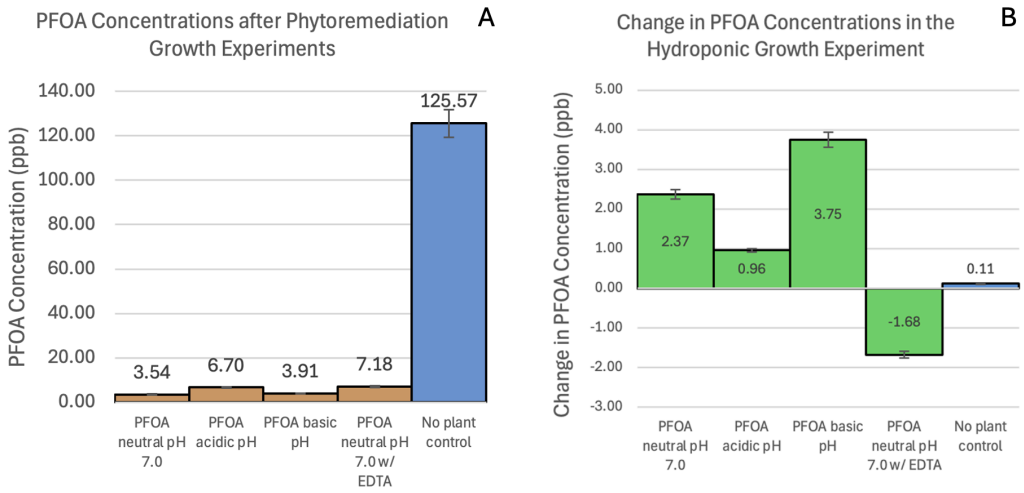


Figure 13: A) Aqueous-phase PFOA concentrations after a 11x dilution at the end of the Round 2 bioremediation stage with cilantro. B) Change in PFOA concentrations during the hydroponic growth experiment over 7 days. The 5% error bars for both graphs are based on the instrument analysis error.

Upon the conclusion of the Round 2 bioremediation experiment, 20 g of soil from each of the 6 conditions were amended with 200 mL of DIW to reach a 11x dilution for PFOA and nutrient analysis. The amount of PFOA remaining in the soil after the 2 rounds of phytoremediation growth periods would be a good indicator for cilantro's potential to uptake PFAS from biosolids.

As seen in **Figure 13A**, the PFOA concentration of the no plant control was significantly higher than the PFOA concentrations in all the other conditions with scallions. Specifically, the aqueous-phase PFOA concentration for the no plant control was 125 ppb, while that of the conditions with plants were more than an order of magnitude lower, with PFOA concentrations ranging between 3 and 7 ppb. The average diluted biosolid PFOA concentration of the 4 conditions was 95.8% lower compared to the no plant control. This large PFOA concentration difference between the no plant control and the conditions with plants highlights the

effectiveness of the Round 1 and Round 2 phytoremediation of PFOA in the biosolid mixtures, as the results show that the cilantro in Round 1 and Round 2 were able to absorb large quantities of PFOA in the soil. The presence of EDTA did not appear to promote or hinder the cilantro uptake of PFOA. The varying pH conditions of the biosolids also did not appear to greatly impact the phytoremediation efficiency of the cilantro, although more studies could be conducted to investigate this subject further.

For the hydroponic growth experiment, the PFOA concentration remained relatively stable throughout the 7-day growth period, as shown in **Figure 13B.** All changes in the PFOA aqueous-phase concentration were below 4 ppb in magnitude, which suggests how no obvious plant uptake of PFAS occurred. Since the scallions function as proxies for agricultural crops grown in biosolid-amended soils, the unlikelihood of the scallions' absorbing detectable amounts of PFOA highlight the safety of the pretreated biosolids, which is a major advancement in limiting plant uptake of PFAS and protecting food safety.

The proposed phytoremediation stages of PFOA, therefore, proved successful in greatly reducing the PFOA concentrations in biosolids, indicating how the biosolids may then be safely applied to agricultural soils where the crops would not bioaccumulate toxic levels PFAS in their tissues. The difference between the changes in PFOA under different pH conditions are too minor to conclude any specific pH-mediated trends, so a hydroponic-based biosolid growth experiment was conducted to further investigate pH-mediated chive uptake of PFAS, along with the potential for a novel, integrated phyto-microbial system to concentrate and degrade PFAS.

3.3 Stage 3: Hydroponic microbe-rich raw sludge growth experiment

3.3.1 PFOA total mass decreased significantly with potential microbial defluorination and degradation; chives showcased increased uptake of PFOA under acidic conditions; chives under both pH 5.0 and pH 7.0 exhibited higher uptake and translocation of PFBA compared to PFOA.

Over the 14-day growth experiment, aqueous-phase PFOA exhibited a consistent downward trend across all conditions, from approximately 1000 μg/L to 200 μg/L (**Figure 14A**). All the decreases are statistically significant ($p < 0.05$). Interestingly, aqueous-phase PFOA in the no plant control exhibited similar amounts of removal compared to the conditions with chives. Thus, methanol extraction for PFOA was performed to ascertain if any of the PFOA removal can be attributed to soil sorption.

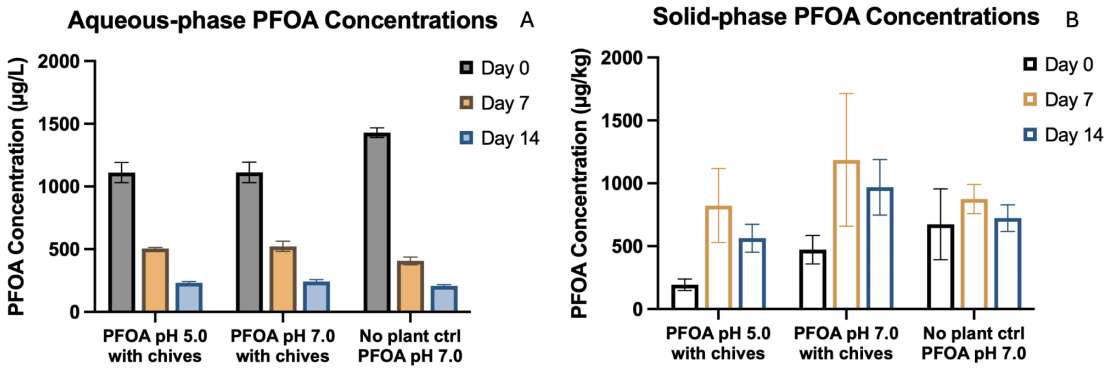


Figure 14: A) PFOA aqueous-phase concentrations in µg/L for d0 (grey), d7 (gold) and d14 (blue) for each condition. B) PFOA solid-phase concentrations in µg/kg d0 (grey), d7 (gold) and d14 (blue).

Solid-phase PFOA concentrations for all conditions exhibited increases between d0 and d7, which may account for the PFOA concentration decrease observed in the aqueous-phase (**Figure 14B**). Only the increase in the PFOA pH 5.0 with chives condition, however, was significant ($p = 0.02$). While part of the aqueous-phase PFOA decrease may be attributed to soil sorption, a preliminary mass balance showed that a major portion of the initial PFOA remained unaccounted for when taking into consideration both PFOA sorption to soil particles and aqueous-phase PFOA (**Figure 15**). Additionally, solid-phase PFOA concentrations exhibited decreases between d7 and d14, indicating that the reduction in aqueous-phase PFOA between d7 and d14 cannot be due to soil sorption processes or plant uptake alone. Interestingly, aqueous-phase and solid-phase PFOA trends for the no plant control were similar to those in the conditions with chives. This major decrease in total PFOA concentration across all conditions is intriguing, suggesting potential microbial-mediated PFAS degradation pathways taking place concurrently with plant uptake of PFAS.

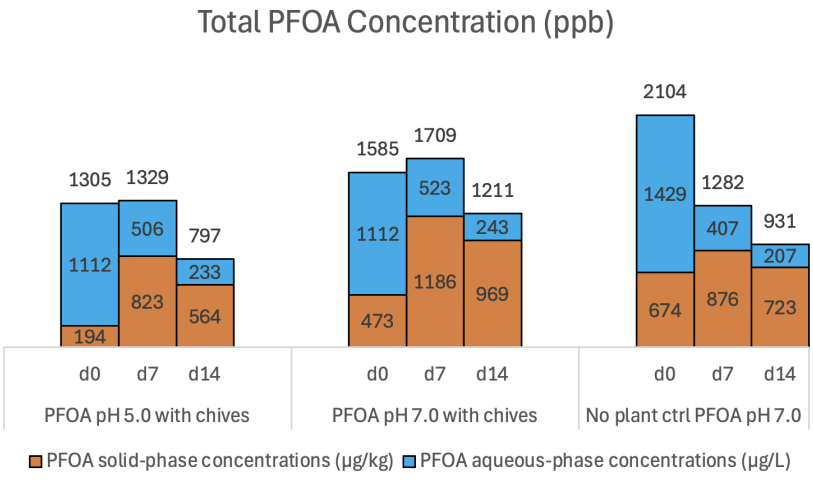


Figure 15: Total PFOA mass balance in soil (orange) and water (blue) on d0, d7, and d14.

The amount of PFOA accumulated in the chive roots under the pH 5.0 growth condition (1139.5 µg/kg) was more than twice the amount compared to the pH 7.0 growth condition (547.65 µg/kg), with $p = 6.6 \times 10^{-5}$ (**Figure 16A**). Similarly, the amount of PFOA accumulated in the chive leaves under the pH 5.0 growth condition (68.3 µg/kg) was also significantly higher than that accumulated in the leaves of chives under the pH 7.0 growth condition (34.0 µg/kg), with $p = 0.0016$. The enhanced uptake of PFOA by chives under acidic growth condition matches the conclusion for the wetland soil experiment, in which the chives grown in the acidic soil condition also appeared to exhibit increased uptake of PFOA compared to the chive grown in neutral soil condition. The translocation factor (TF) of PFAS was determined based on the total µg of PFAS accumulated in the shoots over the total µg of PFAS accumulated in the roots, which factored in the root and shoot weights of the chives. While the concentration of PFOA accumulated in the roots and shoots varied based on the pH of the growth cup, the TF of PFOA was similar for chives under both growth conditions: 0.077 for pH 5.0 chives and 0.068 for pH 7.0 chives.

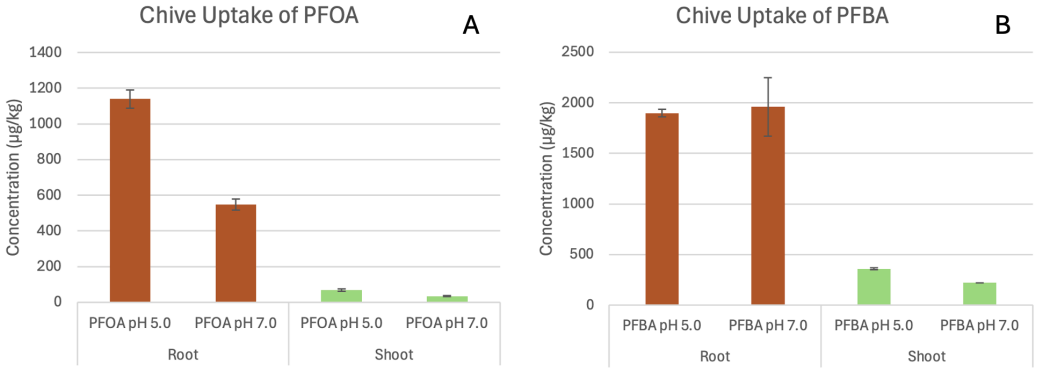


Figure 16: A) Chive root (red) and shoot (green) uptake of PFOA in µg/kg. B) Chive root (red) and shoot (green) uptake of PFBA in µg/kg.

Unlike the trends seen with PFOA, chive of take of PFBA in the roots did not differ significantly based on the pH of the amended liquid growth medium ($p = 0.73$) (**Figure 16B**). The difference between chive response to PFOA and PFBA could be explained by differing PFAS-soil interactions. Specifically, PFOA and PFBA may have varying impacts on soil microbial populations under different pH conditions, which could potentially impact nutrient cycling and subsequent PFAS uptake by plants. According to Nguyen et al. (2020), short-chain PFAS are also less sensitive to pH changes compared to long-chain PFAS, which may explain why the amount of PFBA uptake by chives was barely affected by the growth-condition pH.

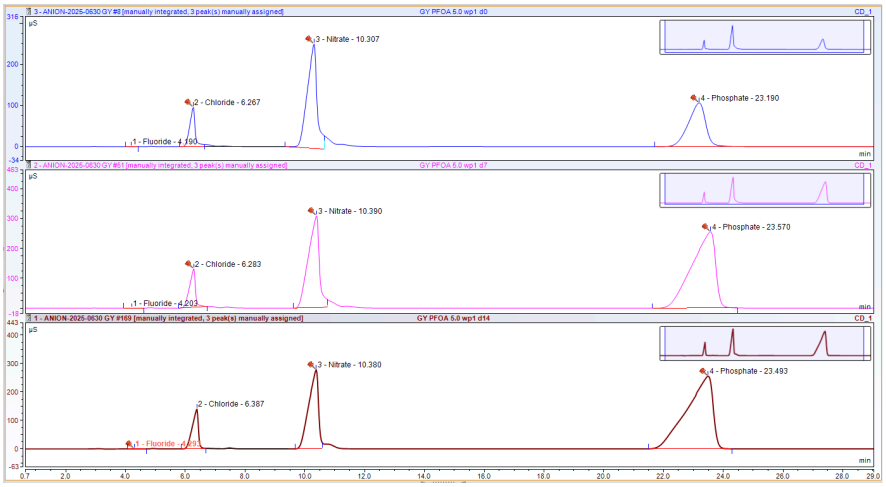
Compared to PFOA, the chive roots concentrated higher amounts of PFBA, at 1897.9 µg/kg for the pH 5.0 PFBA condition and 1960.6 for the pH 7.0 PFBA condition. Since PFBA has a shorter carbon chain length, it is more hydrophilic and mobile compared to PFOA, so the higher bioaccumulation of PFBA is logical (Costello & Lee, 2024; Zheng et al., 2023). Additionally, this trend corresponds to the observations made in previous studies involving plant

uptake of PFAS, in which short-chain PFAS are more likely to be absorbed by plants than long-chain PFAS because of their high environmental mobility (Costello & Lee, 2024). The translocation factors of PFBA under both pH conditions, 0.26 for pH 5.0 chives and 0.23 for pH 7.0 chives, were also higher than that of PFOA, which is in line with the findings of previous research (Sima & Jaffé, 2021; Zhang et al. 2019).

Notably, peaks for PFAS intermediates, i.e. PFBA, was detected in the roots of chives grown in conditions that had supposedly only been amended with PFOA (**Figure 17**), further supporting how potential microbe-mediated PFOA defluorination may have occurred and contributed to the total decrease seen in PFOA for the analyzed growth cups.

Figure 17: Mass spectrometry chromatogram for PFOA pH 7.0 chives. PFBA is selected as the target compound (m/z: 212.98[M-H]1-).

3.3.3 Biosolid and raw sludge mixture released nutrients; significant fluoride production detected over the growth period, suggesting potential microbe-mediated PFAS degradation.



Sample IC anion chromatograms with visible fluoride, chloride, nitrate, and phosphate peaks are shown in **Figure 18**, where the area under the phosphate peaks increased consistently from d0 to d14.

Figure 18: IC anion chromatograph with fluoride, chloride, nitrate, and phosphate peaks for the first aqueous-phase replicate sample for pH 5.0 with chives on d0 (top, blue), d7 (middle, pink), and d14 (bottom, red).

Since all hydroponic conditions had been amended with WWTP sewage sludge and commercial biosolids as the nutrient source, the phosphate and nitrate levels in the cups were relatively high compared to normal environmental concentrations, with phosphate levels exceeding 4000 mg/L and nitrate levels being around 1000 mg/L for most growth cups at the

termination of the experiment period (**Figure 19A and 19B**). Nevertheless, the chives exhibited healthy, normal development during the 14-day growth experiment, showcasing their tolerance and ability to thrive in matrices with a high nutrient content.

Aqueous-phase phosphate levels showcased significant increases between d0 and d7 across all conditions, with *p*-values ranging from $1.4x10^{-9}$ to 0.015 (**Figure 19A**). This trend may be explained by the biosolids' potential release of phosphate during the growth experiment, as biosolids have been known to release nutrients once applied to agricultural soils through microbe-mediated mineralization. Aqueous-phase phosphate levels also increased significantly between d7 and d14 across all conditions (*p*-values from $9.5x10^{-6}$ to 0.025), as the biosolids may still be releasing phosphate at a slower rate, albeit the increases were not as great in magnitude compared to that between d0 and d7.

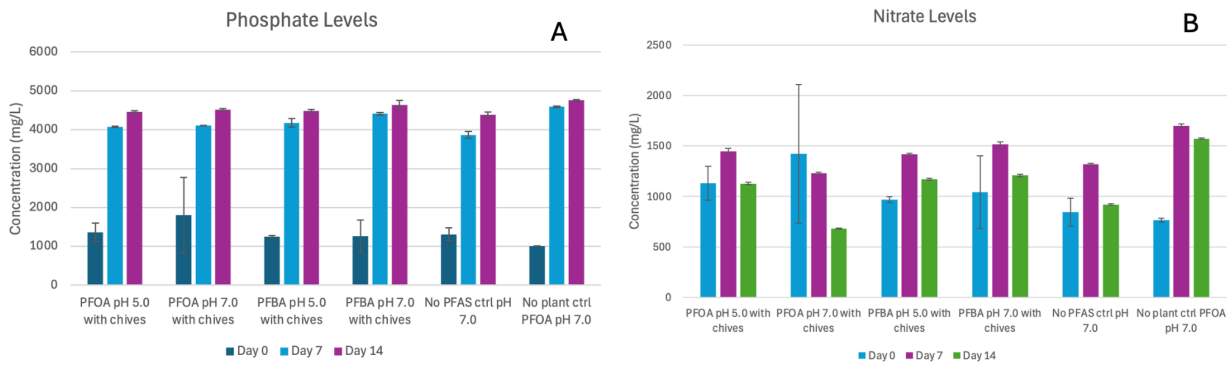


Figure 19: A) Phosphate levels (mg/L) in all hydroponic growth cups on d0, d7, and d14. B) Nitrate levels (mg/L) in all hydroponic growth cups on d0, d7, and d14.

Aqueous-phase nitrate levels exhibited increases between d0 and d7 in all conditions except PFOA pH 7.0 with chives, which had a large error bar around its d0 nitrate level (**Figure 19B**). Similar to the case with phosphate, this increase can be explained by the biosolid's release of nutrients as the experiment proceeded. Unlike the trend with phosphate, however, aqueous-phase nitrate levels decreased in all conditions between d7 and d14 (*p*-values from $5.4x10^{-8}$ to 0.00029), with the decrease for the no plant control being the smallest in magnitude. Thus, the reduction of nitrate could potentially be attributed to plant uptake, though other soil metabolic processes may not be ruled out.

Both fluoride and chloride concentrations showed consistent increases in all conditions across the 14-day growth experiment (**Figure 20A and B**). The increase in fluoride levels proves especially interesting, as the fluoride increase may potentially be caused by microbial defluorination and degradation of PFAS, especially since the raw sludge had been previously seeded with A6, which is known to reductively defluorinate PFAS and release fluoride. Although the no PFAS control also exhibited a fluoride buildup, all conditions technically contain a background PFOA concentration, as the raw sludge itself contains PFOA. The increase in fluoride also supports the decrease in total PFOA mass seen. Microbial and functional gene

analysis are currently underway to determine A6 and functional gene activities in the growth cups.

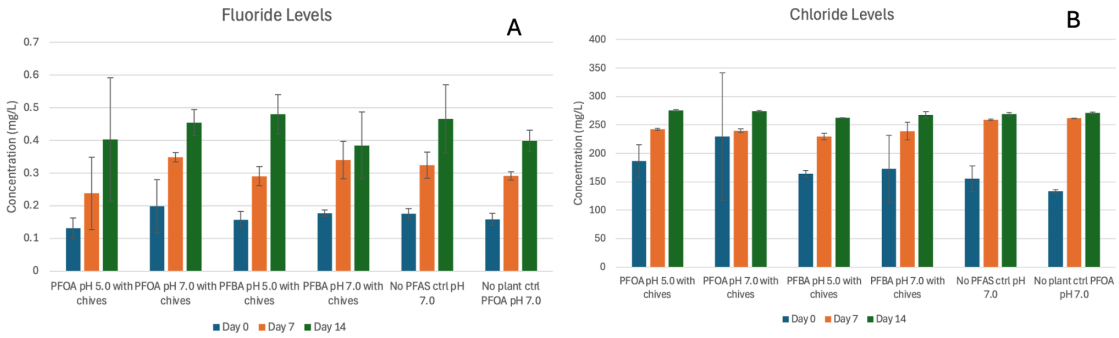


Figure 20: A) Fluoride levels (mg/L) in all hydroponic growth cups on d0, d7, and d14. B) Chloride levels (mg/L) in all hydroponic growth cups on d0, d7, and d14.

3.2.4 Real-world implications of results with constructed wetland applications

The promising results for phyto-microbial remediation to effectively reduce PFAS levels in biosolids contain real-world constructed wetland applications, with potential to significantly remediate PFAS contamination in sewage sludge.

Around 2 to 5 dry tonnes of biosolid can be applied on 1 acre of farmland, and approximately 20,000 ears of corn or 25,000 pounds of potatoes can be planted on an acre of agricultural soil (*Biosolids Technology Fact Sheet*, 2000; Brown, 2017; *How Much Food*, 2022). Coupled with the experimental data that two rounds of phytoremediation can potentially reduce the biosolid PFAS concentration by 95.8%, along with the assumption that the a biosolid has a total PFAS concentration of 100 ppb, phytoextractors such as cilantro would be able roughly extract 173,781 to 434,453 µg, or 0.17 to 0.4 kg, of PFAS within the span of 1 month and ensure the safety of over 20 thousand crops based on a proportional scaling from Stage 2 results. Additionally, assuming that 1 large cilantro bunch costs around $0.83 and can treat 500 g of biosolid, one round of remediation would only cost around $2,000 to $5,000 (*Fresh Cilantro*, n.d.). On the other hand, it could take between $900,000 and $67 million to remediate 1 kg of PFAS from soil using traditional chemical degradation methods (Jaworowski, 2024). Thus, phytoremediation can save immense costs and serve as an accessible solution to biosolid remediation, especially for communities disproportionately impacted by PFAS pollution.

4 Conclusions

This research hopes to inform ways to promote the phyto-microbial remediation of PFAS through mediating soil conditions, such as soil pH and bioavailable phosphate levels. This study also experimented with various soils, including wetland soils, commercial biosolids, and WWTP raw sludge. The lab-conditioned soil incubation research explored the optimal pH conditions for phytoremediation to occur, along with connections to phosphorus cycling and microbial functional gene expressions. The soil-based biosolid incubation research showcased how the

PFOA concentration in the biosolids were significantly reduced over two rounds of growth incubations with cilantro. The hydroponic-based, microbe-rich raw sludge growth experiment proved the high efficiency of a phyto-microbial system to remediate PFAS contamination in sewage sludge, allowing for cost-effective concentration and degradation of PFAS in complex environmental matrices. With potential applications to constructed wetlands and retention ponds, results from this research can help alleviate PFAS contamination in the environment as well as reducing the bioaccumulation of PFAS in crops, allowing for improved global food safety and human health.

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Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA is final and no appeal will be accepted.

(Signatures of full team below) 08/24/25

x Qiuen Yu

Name of team member: Qiuen Yu

x Shan Huang

Name of supervising teacher: Shan Huang

Declaration of Academic Integrity

The participating team declares that the paper submitted is comprised of original research and results obtained under the guidance of the instructor. To the team's best knowledge, the paper does not contain research results, published or not, from a person who is not a team member, except for the content listed in the references and the acknowledgment. If there is any misinformation, we are willing to take all the related responsibilities.

Names of team members: Qiuen Yu

Signatures of team members: Qiuen Yu

Name of the instructor: Shan Huang

Signature of the instructor: Shan Huang

Date: 08124125

Acknowledgement

I want to thank my instructor Dr. Shan Huang for her invaluable guidance in this research. I was able to discuss all the steps and phases of my research with her, from experimental setup to utilizing various analytical instruments. I am deeply grateful for her support and mentorship throughout the research process. Additionally, I want to thank Ph.D. student Chiara Smorada for teaching me how to use the IC and the LC-MS, along with overseeing some of my sampling operations to ensure my safety. Furthermore, I want to thank my chemistry teacher, Mrs. Mary Calvert, and Hutchins Science Program Director, Mr. Kenneth Mills, for supporting me on my scientific journey and training me to become a better laboratory researcher. Moreover, I am very grateful to have the opportunity to conduct this research at Prof. Peter Jaffe's lab at Princeton University while participating in the 2025 Princeton Laboratory Learning Program.