Reducing Per-and Polyfluoroalkyl Substances (PFAS) Water Contamination with Mycorrhizal Hydroponics Plants

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Per- and polyfluoroalkyl substances (PFAS), known as "forever chemicals", are one of the most common and dangerous water pollutants, having carcinogenic effects and causing 382,000 global deaths annually. Current methods to purify PFAS-contaminated water can cost millions of dollars and require existing infrastructure, making them difficult to implement in low-income and rural areas without industrial treatment plants. Hydroponics plants colonized by beneficial mycorrhizal fungi present an affordable and sustainable solution to purifying PFAS-contaminated water. In this study, mycorrhizal-inoculated basil and lettuce plants were cultivated in hydroponics systems under controlled conditions. Root samples were stained and analyzed under a light microscope to confirm mycorrhizal presence. PFAS was added to the systems and an LC/QQQ-MS instrument was used to measure the reduction in PFAS concentrations over 72 hours. Results showed that mycorrhizal plants removed 71.1% of PFAS in a water system compared to 59.9% by non-mycorrhizal plants, and a t-test (p-value=0.00367) was used to prove statistical significance. Relative health of plants was measured through root length, with results revealing that mycorrhizal plant roots were 2.8 inches longer on average than non-mycorrhizal roots. Further analysis revealed a direct relationship between plant root length and PFAS purification, indicating the suitability of species with naturally longer roots for real-world phytoremediation applications, such as at stormwater detention ponds. This study provided a proof-of-concept of the effectiveness of mycorrhizal hydroponics plants in reducing PFAS contamination in water systems, presenting applications as an inexpensive and large-scale purification system.

Keywords: Perfluoroalkyl and polyfluoroalkyl substances, hydroponics, mycorrhizal fungi, water contamination, stormwater detention ponds, pollution, environment

Abbreviation	Description
PFAS	Perfluoroalkyl and polyfluoroalkyl substances
PFNA	Perfluorononanoic acid
DWC	Deep water culture
PFBA	Perfluorobutanoic acid
AMF	Arbuscular mycorrhizal fungi
LC/QQQ-MS	Liquid chromatography-triple quadrupole mass spectrometry

Table 1 Abbreviations and Acronyms

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) pose a major threat to the environment and human health. This group of man-made chemicals have been utilized in various industrial applications and consumer products, including cleaning products, paint, and various cosmetics. Their frequent use has often led to contamination of water sources and drinking water systems around the country and world due to stormwater runoff. PFAS molecules consist of a chain of carbon atoms fully or partially fluorinated, rendering them resistant to natural degradation processes[1]. This resilience contributes to their widespread presence in various environmental matrices, including air, water, soil, and living organisms. Military personnel is exposed to dangerous levels of PFAS more than any other segment of the population due to the presence of PFAS in aqueous film-forming foam (AFFF), which is readily used on military bases[2]. This presents major issues, as concentrations above 1 ppb in the human bloodstream have been associated with an elevated risk of kidney cancer and fatal complications in pregnant women3. Among the multitude of PFAS compounds, long-chain perfluorononanoic acid (PFNA, $C_9HF_{17}O_2$) and short-chain perfluorobutanoic acid (PFBA, $C_4HF_7O_2$) have been the most extensively produced, and consequently, are the most prevalent in the environment[3]. In addressing the challenges posed by PFAS, various remediation technologies have been explored. Conventional methods, including adsorption, photocatalysis, and electrochemical oxidation have shown effectiveness in removing PFAS from contaminated sites[4]. However, these methods often come with high energy requirements and operational costs, making them less feasible for widespread application, especially in resource-limited settings, such as rural and low-income communities[5]. For instance, Lake

Erie, the provider of drinking water for over 11 million people, has recently experienced extreme PFNA and PFBA contamination, costing its border states more than $65 million to remove annually[6]. Amidst these challenges, phytoremediation emerges as a promising, sustainable, and cost-effective alternative. This green technology leverages the natural ability of plants to absorb, accumulate, and sometimes degrade pollutants from the environment[7]. Recent studies have increasingly focused on the role of terrestrial plants in removing PFAS in soil[8]. However, there remains a significant gap in understanding the potential of hydroponics plants, which are plants grown in water rather than soil, for PFAS remediation of water systems[9]. PFAS removal using hydroponics plants rather than terrestrial plants could be a major step forward in developing effective phytoremediation infrastructure, as hydroponic systems can be established in a variety of settings, including urban areas and regions with poor soil quality. Hydroponically grown plants require less space compared to traditional soil-based agriculture, making them suitable for large-scale filtration operations in space-constrained conditions[10]. Furthermore, some studies have identified mutualistic arbuscular mycorrhiza fungi (AMF) as improving PFAS remediation in soil[11], however, to the best of our knowledge, no comparable study has been done for phytoremediation by hydroponics plants. The colonization of plant roots by mycorrhizal fungi could result in an improvement of PFAS phytoremediation from water systems due to their capability to extend plant roots, leading to the uptake of additional nutrients. This study aims to bridge these gaps in knowledge by exploring the role of hydroponics plants in absorbing PFAS from water systems. Furthermore, it investigates the applicability and effectiveness of cultivating mycorrhizal fungi in deep water culture (DWC) hydroponic systems to remove PFAS from contaminated water. Additionally, to ensure that both short and long-chain PFAS are able to be remediated by hydroponics plants, the uptake of both PFNA and PFBA was studied. This is important because other filtration methods, such as activated carbon, have been found to be able to absorb long-chain PFAS better than short-chain, often leading to increased levels of short-chain PFAS in drinking water systems[12]. Also, lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) plants were chosen to be studied due to their high growth rates in hydroponics systems, low costs, and a large range of environments where they are native[13]. Two plant species were intentionally experimented with instead of just one to decrease the probability that any PFAS uptake observed is attributed to the unique genetic makeup of that species, so only that species of plant is able to remove PFAS in the hydroponics systems. Overall, in this study, it was hypothesized that PFAS uptake could occur in hydroponics plants to a statistically significant level and that mycorrhizal colonization of plant roots would improve the uptake capacity of the plants. This hypothesis was tested by cultivating both lettuce and basil in DWC hydroponic systems, with and without the addition of arbuscular mycorrhizal fungi (AMF), to assess their capacity to uptake PFNA and PFBA from contaminated water. Thus, there were 8 experimental groups tested for PFAS uptake in this experiment: 1) lettuce, mycorrhizae, and PFNA 2) basil, mycorrhizae, and PFNA 3) lettuce, no mycorrhizae, and PFNA 4) basil, no mycorrhizae, and PFNA 5) lettuce, mycorrhizae, and PFBA 6) basil, mycorrhizae, and PFBA 7) lettuce, no mycorrhizae, and PFBA 8) basil, no mycorrhizae, and PFBA. Two separate control groups were also set up with no plants and spiked levels of PFNA and PFBA, respectively.

The study involved detailed monitoring and analysis of PFAS concentrations in the water before and after exposure to the plants in the eight experimental groups above, allowing for a precise measurement of the uptake efficiency. The outcomes of this research could pave the way for developing innovative, sustainable, and cost-effective solutions for PFAS contamination, particularly in water systems, offering significant benefits for environmental protection and public health, especially in areas heavily impacted by PFAS pollution.

Material & Method

Germination Setup

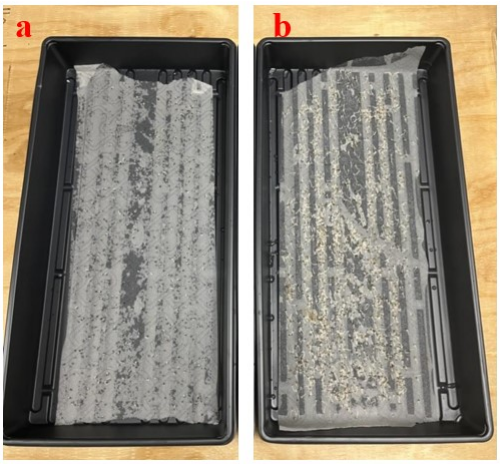

Fig. 1 a) Germinated basil seeds on grow trays b) Germinated lettuce seeds on grow trays. To set up for germination, 200 genovese basil seeds (Sow Right Seeds, Greenwood, MI) and 200 buttercrunch lettuce seeds (RDR Seeds, Lindsay, Canada) were uniformly dispersed on top of slightly damp paper towels on two respective grow trays (Figure 1). These grow trays were then covered with a humidity dome with the purpose of trapping moisture inside the container. The seeds were then exposed to a 2000W grow light with a full light spectrum (King LED, KING2000) set on a 12-hour on, 12-hour off cycle on the "germination" light setting. After five days, the sprouts were transferred to the DWC system.

Hydroponics Deep Water Culture (DWC) Setup

Fig. 2 DWC hydroponics system in controlled grow room. The left four buckets contain lettuce plants and the right four buckets contain basil plants. The four buckets in the front (two lettuce, and two basil) contain mycorrhizal fungi.

To set up the DWC hydroponics system, eight holes with a radius of 1.5 inches were cut in the lids of each 15 L bucket, and net pots with a radius of 1.75 inches were added to each hole. Then, approximately a cup of clay hydrostone was added to each net pot to act as a water-absorbing substrate for the plants (completes a similar job to soil). Next, 13.5 L of distilled water and 0.5 L of liquid fertilizer (General Hydroponics) with a nitrogen-phosphorus-potassium (NPK) ratio of 4-1-4 were mixed to form a Hoagland hydroponics growth solution. A solution with high levels of nitrogen and potassium but low levels of phosphorus was used because it has been found in literature that this particular combination improves the chances of mycorrhizal colonization of plant roots in hydroponics systems[14]. The pH of this solution was then adjusted to approximately 6.1 using an HCl and CaCO3 buffer, as recommended by literature for optimal lettuce and basil growth. The buckets were sterilized with bleach to remove any bacteria or contaminants before adding 14 L of this solution to each bucket. Then, for the groups requiring mycorrhizal fungi, 5 mL of liquid *Glomus intraradices* mycorrhizae (Plant Success, Gloucester City, NJ) was added directly to the container. This specific species of mycorrhizae was chosen as it has been found to have very high rates of colonization in hydroponics systems[15]. Next, two grow lights set on a 16-hour on-8 hour off cycle were hung above four buckets each. This extended period of light was used to accelerate plant growth through high photosynthesis levels. Additionally, 8 air tubes connected to a 40W 70 L/min air pump (Vivosun) were fed into the 8 buckets to provide sufficient oxygen to the roots, avoiding the development of root rot. Furthermore, reflective aluminum foil was wrapped around each of the buckets to prevent the algae growth within the container. The growing room (Figure 2) was kept in controlled conditions at room temperature, 50% humidity, and complete darkness other than the grow lights. Finally, the sprouts were transferred from the germination trays to the DWC system (one sprout was placed in each net pot). The plants were grown for 40 days before completing the PFAS uptake experiment.

Mycorrhizal Root Microscopy

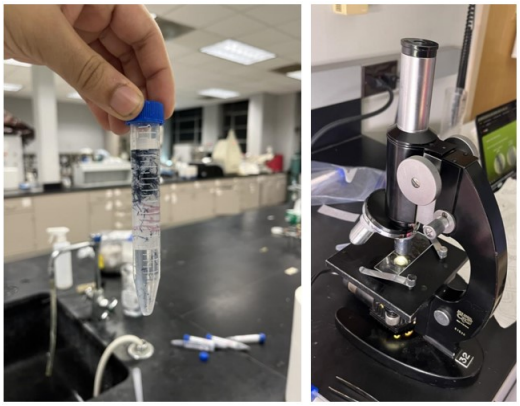

Fig. 3 a) Ink-vinegar stained plant roots in glycerol solution b) Microscope setup. After 30 days of growing the plants in the DWC system, 2-inch sections of two randomly chosen plants from each bucket were cut to be analyzed for mycorrhizal colonization. Then, a well-established mycorrhizal ink-vinegar staining procedure[16] was completed involving the storage of the root sections in 15 mL of 50% glycerol, 25% black ink, and 25% household vinegar for 3 days (Figure 3a). The purpose of this staining was to make any mycorrhizal vesicles, hyphae, or arbuscular sections in the roots more apparent. After the staining, the sections were placed on glass slides and analyzed under the 43x objective lens of a light microscope (Beck Kassel, Double Nosepiece) (Figure 3b), and the roots of mycorrhizal and non-mycorrhizal groups were compared.

PFAS Uptake Measurement

Fig. 4 a) Spiking containers with PFAS chemicals b) Collecting water samples with personal protective equipment (PPE) worn at all times c) Storage of 15 mL water samples to be subjected to PFAS concentration measurement.

To complete the PFAS uptake experimentation, the plants were transferred to a regulated BSL-2 New Jersey Institute of Technology (NJIT) laboratory in accordance with PFAS safety standards. The DWC setup described previously was recreated, including the growth solution, room conditions, and grow light setup. To prepare for uptake sampling, 5 mg of PFNA was added to each of the four plastic buckets – 2 basil groups and 2 lettuce

groups. 5 mg of PFBA was added to each of the remaining four plastic buckets – 2 basil groups and 2 lettuce groups (Figure 4a). This resulted in an initial PFAS concentration of around 1000 ppb in each bucket.

15 mL samples of each of the 8 buckets were taken after 0, 8, 16, 24, 32, 40, 48, 56, 64, and 72 hours (Figure 4b, 4c). The PFAS concentration of these samples was then determined using an LC/QQQ-MS, as described below, to analyze PFAS uptake by the plants over time.

LC/QQQ-MS Analysis

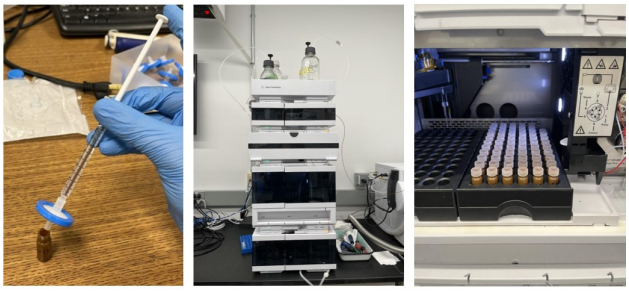

Fig. 5 a) Transferring diluted sample to 2 mL tubes with syringe and filter b) Agilent LC/QQQ-MS at NJIT laboratory c) Concentration analysis of samples by LC/QQQ-MS.

To measure the concentrations of the collected phytoremediation samples, the samples first had to be diluted because the Agilent LC/QQQ-MS (6475 Triple Quadrupole LC/MS) can only accurately read PFAS concentrations up to 50 ppb. For the dilution, 0.5 mL of each sample was added to 9.5 mL of methanol in a 15 mL centrifuge tube. Then, 2 mL of the diluted samples were transferred to the LC/QQQ-MS 2 mL tubes through a 3 µm syringe filter (Figure 5a). The samples were then loaded into the Agilent ZORBAX Eclipse Plus C18 column of the LC/QQQ-MS for separation and analysis (Figure 5b, 5c). The column ensured the effective separation of PFAS from other potential contaminants, such as fertilizer chemicals.

PFAS quantification was performed using mass spectrometry (MS) in multiple reaction monitoring (MRM) mode, which allows for the selective detection and quantification of specific PFAS compounds while minimizing background interference. The LC/QQQ-MS was prepared with 1 L of DI water and 1 L of solvent delivery. The system was calibrated to default pressure, pump, and sampling parameters to ensure accurate measurements [17].

Kinetics Linearization

$$f(x) = \ln(y) \tag{1}$$

$$g(x) = \frac{1}{y} \tag{2}$$

$$t_{0.5} = \frac{1}{k[A]_0} \tag{3}$$

$$N(t) = N_0 \left(\frac{1}{2}\right)^{\frac{t}{t_{0.5}}} \tag{4}$$

Equation 1 describes the linearization of data from an uptake experiment assuming first-order kinetics, where $f(x)$ is the new output of the linearized graph and y is the percent of initial PFAS concentration at a given time after starting the experiment. Equation 2 describes the linearization of data from an uptake experiment assuming second-order kinetics, where $g(x)$ is the new output of the linearized graph and y is the percent of initial PFAS concentration at a given time. Equation 3 describes the formula for determining half-life in hours of a reaction modeled by second-order kinetics, where k is the slope of the line and $[A]_0$ is the initial concentration in mol/L. Equation 4 describes the formula for determining the quantity of a substance remaining when the removal rate can be modeled by a half-life equation. N_0 is the initial quantity of the substance, t is the time elapsed (hours), and $t1/2$ is the half-life (hours) of the substance.

Results

Mycorrhizal Colonization

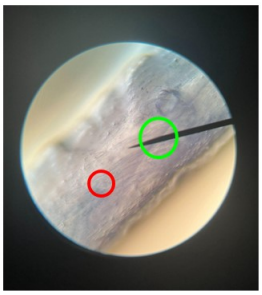

Fig. 6 Microscopic image of plant root with mycorrhizal colonization. An example vesicle is circled in green and an example hyphae strand is circled in red.

Mycorrhizal colonization is evident through the microscopic images taken 30 days after plant growth began (Figure 6). The vesicles and hyphae which are characteristic of mycorrhizal fungi, are made clear. This image provides sufficient evidence that mycorrhizae have successfully colonized the roots of the intended groups. With this finding, the main experiment comparing the effects of mycorrhizae on PFAS uptake could proceed. Furthermore, the easily visible mycorrhizae anatomy validates the ink-vinegar staining method described in the methodology section as an effective staining procedure.

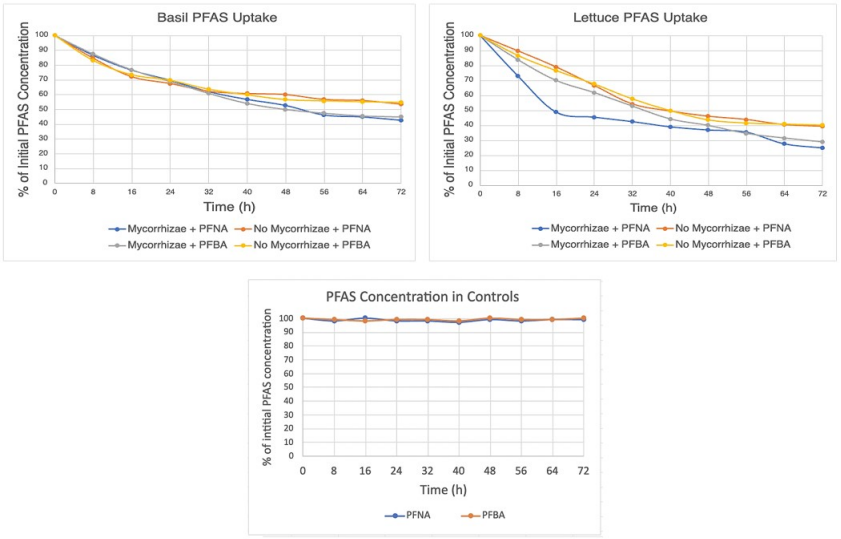

Fig. 7 a) Concentration of PFAS in water compared to initial concentration, modeling the removal of PFAS from the water by basil groups over 72 hours b) Concentration of PFAS in water compared to initial concentration, modeling the removal of PFAS from the water by lettuce groups over 72 hours c) Concentration of PFAS in water compared to initial concentration, modeling the removal of PFAS from the water with no plants over 72 hours

PFAS Uptake

In the completion of the main PFAS uptake experiment, it can be seen that every plant group, regardless of the plant species, presence of mycorrhizae, or type of PFAS, successfully removed PFAS from the water, although to varying degrees (Figure 7a, 7b). In comparison, there were relatively constant concentrations of PFAS in the PFNA and PFBA control groups with no plants over the 72 hour period (Figure 7c). This shows that observed reductions in PFAS concentrations are due to uptake by the plants rather than other processes such as adsorption to container walls or degradation. This provides a proof-of-concept that even household plants, with no specific genetic modifications, can be effective phytoremediators of PFAS from water systems. This finding in itself is notable, as no other study in literature has provided a fundamental example in controlled conditions of removal of PFAS by hydroponically-grown plants. Thus, this experiment lays the groundwork for future studies to be done on implementing phytoremediation infrastructure into current water filtration processes.

Mycorrhizal Effect on PFAS Uptake

By comparing each plant group to its identical counterpart except for the presence of mycorrhizae (for example, comparing the basil group with mycorrhizae removing PFNA to the basil group with no mycorrhizae removing PFNA), it was observed that the presence of mycorrhizae consistently improves PFAS absorption by a margin of at least 10% (Figure 8). In other words, a group where the plants were colonized with mycorrhizae removed 10% more PFAS over 72 hours than an identical group without mycorrhizal infection. To conclude the probability of this event occurring by chance, descriptive statistics, including the mean, and the range of values in the 95% confidence level were found based on the percent differences between final concentrations of PFAS in mycorrhizal uptake studies and their identical non-mycorrhizal counterparts (Figure 9). For example, one point in Figure 9a is found by subtracting the final concentration of the mycorrhizal group in Figure 9a from the non-mycorrhizal group in the same figure. There were four replicates for this experimental group, indicated by the four data points in Figure 9a. Because the 95% confidence interval when modeling these differences does not intersect with 0 (can be thought of as no quantitative difference in PFAS uptake by mycorrhizal and non-mycorrhizal groups), it can be concluded that there is a statistically significant difference ($p = 0.05$ confidence level) between mycorrhizal and non-mycorrhizal groups. In other words, based on the results, mycorrhizal colonization of plant roots statistically improves PFAS absorption from water systems. This finding provides evidence to answer the original research question of this study, which was determining the effect of mycorrhizal presence on PFAS removal from water. This is a notable result, as it encourages future PFAS phytoremediation systems to utilize mycorrhizal fungi as a means of increasing the efficiency of the filtration process. However, one area not considered in this study is the correlation between the extent of mycorrhizal colonization (e.g., percentage of root length colonized) and the uptake of PFAS by a plant. This should be explored in future studies to provide additional evidence of

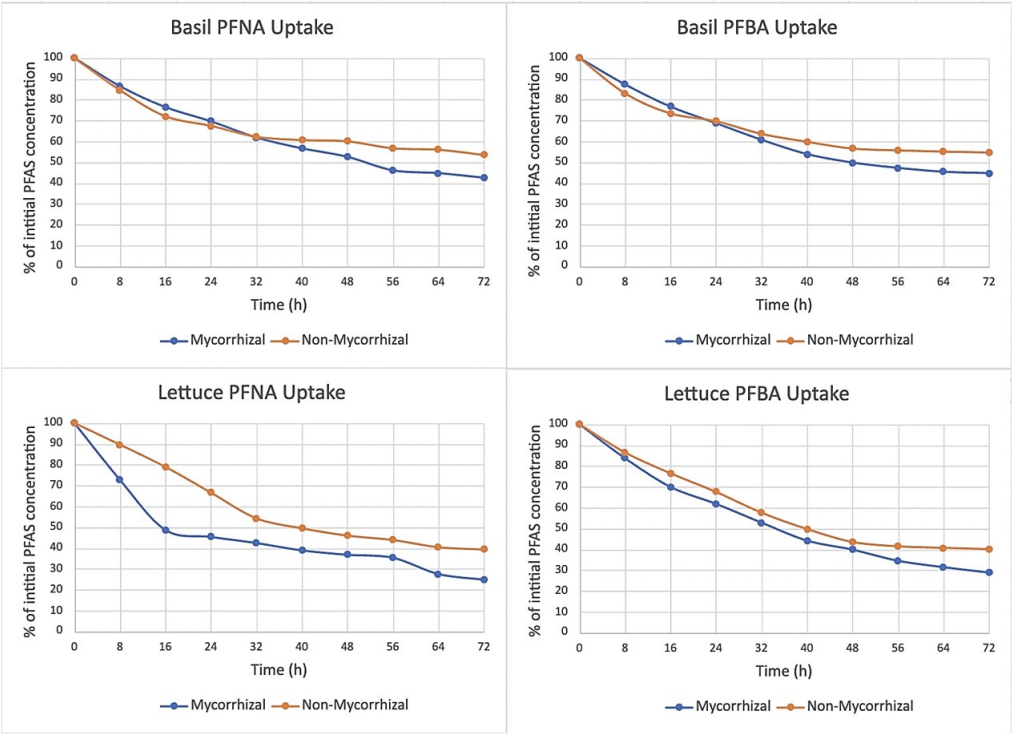

Fig. 8 a) PFNA removal from water by mycorrhizal and non-mycorrhizal basil groups b) PFBA removal from water by mycorrhizal and non-mycorrhizal basil groups c) PFNA removal from water by mycorrhizal and non-mycorrhizal lettuce groups d) PFBA removal from water by mycorrhizal and non-mycorrhizal lettuce groups

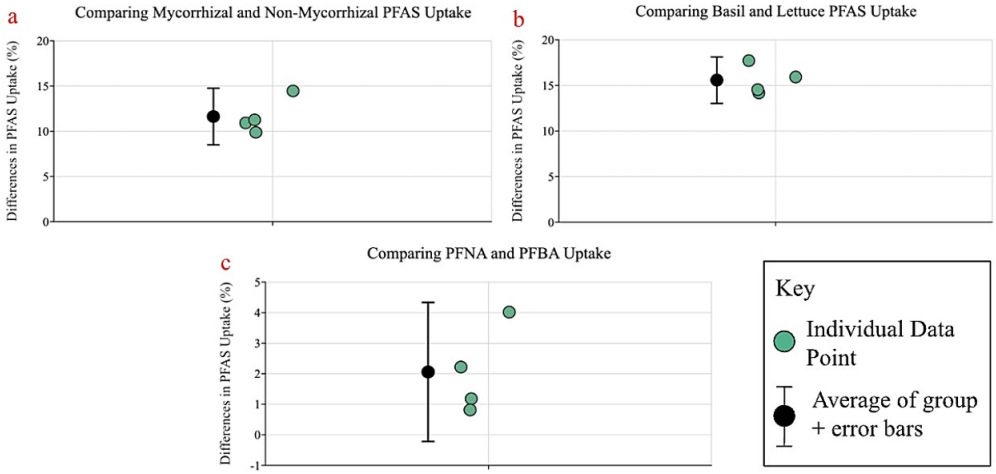

Fig. 9 a) Comparing PFAS uptake of mycorrhizal and non-mycorrhizal groups (mycorrhizal - non-mycorrhizal) (n=4) b) Comparing PFAS uptake of lettuce and basil groups (lettuce - basil) (n=4) c) Comparing PFNA and PFBA uptake (PFNA - PFBA) (n=4). Error bars above and below show 95% confidence level.

mycorrhizae enhancing phytoremediation of PFAS.

Plant Species Effect on PFAS Uptake

While both plant species experimented with in this study, basil and lettuce, were seen to be able to reduce PFAS water contamination (Figure 9), it was observed that the lettuce groups consistently outperformed their identical basil counterparts in the final percent of PFAS compared to the initial concentration by a margin of at least 14% every time. To determine the probability of this event occurring by random chance, the statistical tests used in the prior subsection (determining the effect of my-

corrhizal presence on PFAS uptake) were replicated (Figure 9b). There were four replicates for this experimental group, indicated by the four data points in Figure 9b. Because the 95% confidence error bars when modeling these differences do not intersect with 0 (can be thought of as no quantitative difference in PFAS uptake by identical basil and lettuce groups), it can be concluded that there is a statistically significant (p =0.05 confidence level) difference between basil and lettuce groups. In other words, based on the results, lettuce plants are statistically more effective than basil plants in removing PFAS from water systems. This is a notable result, as it makes necessary further optimization studies to determine the most efficient plant species at the removal of PFAS to be implemented in future phytoremediation infrastructure, since from this study, it can be seen that there are differences between species.

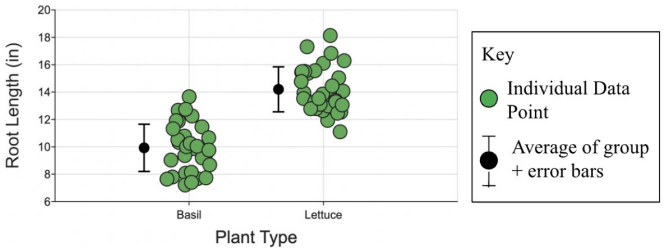

Fig. 10 Comparing root length of basil and lettuce plants (n=32). All error bars above and below show 1 standard deviation.

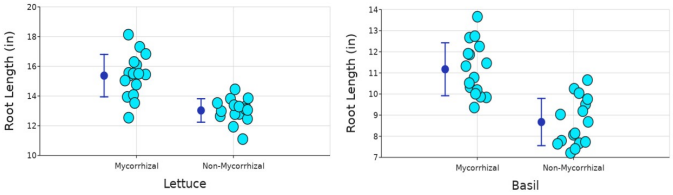

Fig. 11 a) Comparing root length of mycorrhizal and non-mycorrhizal lettuce plants (n=16) b) Comparing root length of mycorrhizal and non-mycorrhizal basil plants (n=16). All error bars above and below show 1 standard deviation.

PFAS Chain Length Effect on PFAS Uptake

Simply by comparing the final concentrations of identical groups except for the type of PFAS, either PFNA (long-chain PFAS) or PFBA (short-chain PFAS), it cannot be conclusively determined that one type is better at being absorbed than another, as in some cases, the group with PFNA ends with a lower final concentration and in other cases, the corresponding group with PFBA ends with a lower concentration. To determine the probability of this event occurring by random chance, the statistical tests used in the prior two sections (determining the effect of mycorrhizal presence and plant species on PFAS uptake) were replicated (Figure 9c). There were four replicates for this experimental group, indicated by the four data points in Figure 9c. Because the 95% confidence error bars when modeling these differences do intersect with 0, it can be concluded that there is no statistically significant (p =0.05 confidence level) difference between PFNA and PFBA groups. In other words, based on the results, both PFBA and PFNA are likely to be equally able to be absorbed by the plants. This is a notable result, as a downside of current PFAS water filtration systems, such as activated carbon, is that they are often not able to remove short-chain PFAS as well as long-chain PFAS, resulting in higher than recommended levels of short-chain PFAS in water systems. The lack of uptake difference between chain lengths suggests that the mechanism of uptake may be related to the acidic headgroup that the PFAS have in common, rather than their chains, however, further study is required to confirm this. Nonetheless, because these hydroponically grown plants can remove PFAS types at roughly the same rate, regardless of their chain length (based on the results of this study), further evidence is provided of phytoremediation systems as effective filtration methods over current systems.

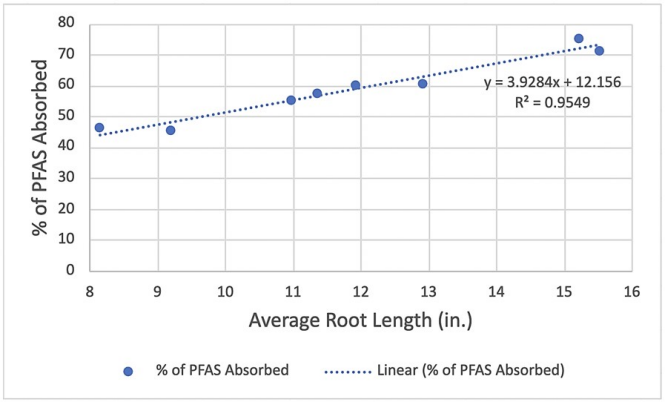

Fig. 12 Relationship between the average root length of each plant group in the study and the percent of PFAS absorbed during the 72-hour uptake. Each individual data point in the graph shows the average root length for a single plant sample. For example, one data point is the mycorrhizal basil group with PFNA, which has an average root length of 11.365 inches and after 72 hours, absorbed 57.4% of PFAS from the system.

Plant Root Length

Plant root length was measured directly prior to the start of the PFAS uptake experiment. The purpose of doing this was both as an indicator of plant health, since a larger root system has been found to correlate with a healthier plant[18], as well as giving information about how mycorrhizal presence and plant species affects root length, potentially providing some insight on why these factors affect PFAS uptake. After an independent t-test was conducted comparing the root lengths of all basil plants in

the study to all lettuce plants in the study, it can be concluded that the average root length of the lettuce plants was greater than that of the basil plants to the 99% confidence level (p =0.01) (Figure 10). This finding is relevant because it forces the comparison of average root lengths of lettuce mycorrhizal groups to lettuce non-mycorrhizal groups and basil mycorrhizal groups to basil non-mycorrhizal groups instead of simply comparing mycorrhizal to non-mycorrhizal groups with no distinction about plant species since if the latter was carried out, the results would be affected by the type of plant species instead of isolating the impact of mycorrhizal presence. Furthermore, when the two t-tests were completed comparing mycorrhizal and non-mycorrhizal groups within the respective plant species, for both tests, it was found that the average root length of the plants colonized by mycorrhizal fungi was greater than that of the plants with no mycorrhizal presence to the 99% confidence level (p =0.01) (Figure 11). This finding aligns with previous literature about the effect of mycorrhizae on root length in soil-based growth studies[19]. When comparing these results with those of the PFAS uptake experiment, a striking discovery can be made: groups with longer average root lengths appear to absorb higher amounts of PFAS from the water. In other words, lettuce and mycorrhizal groups had both greater average root lengths as well as a higher capacity for PFAS removal than their basil and non-mycorrhizal counterparts. To statistically support this correlation, the PFAS absorption by plant groups was plotted against the average root length of plants in the group and a linear regression was completed (Figure 12). The high coefficient of determination value (R^2) indicates that a linear model is very effective in characterizing the relationship between root length and PFAS uptake. The regression line conclusively depicts a direct relationship between root length and PFAS uptake, meaning that as root length increases, PFAS absorption from water also increases. This finding is notable as it provides a potential mechanism of how the PFAS in the water is being removed by the plants: the greater root surface area leads to more contact between the roots and PFAS chemicals, allowing the contaminants to be removed at greater rates from water. The proposed mechanism for PFAS absorption is supported by the fact that plants carry out this exact mechanism when absorbing nutrients since greater root surface area has previously been associated with greater nutrient uptake[20]. Furthermore, it provides an explanation to why mycorrhizae increase PFAS removal by plants, as the function of mycorrhizal fungi is colonizing and extending plant roots (through the addition of vesicles, hyphae, and arbuscular sections), giving the plant access to more nutrients, and for the purposes of this study, PFAS chemicals. In a similar fashion, it explains why lettuce plants, with their greater average root lengths, were more successful in uptaking PFAS than basil plants with their shorter average roots lengths. Additionally, this new mechanism of the removal of PFAS encourages plants with very long roots, such as tomatoes, switchgrass, and heath aster[21], to be further experimented with, as it is likely that their rates of PFAS removal would be even higher than those of lettuce and basil in this study. Thus, these plants could be highly useful in PFAS phytoremediation infrastructure.

Kinetics Reaction Order

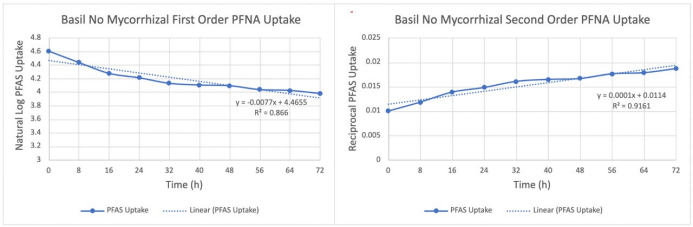

Fig. 13 a) Linearization of PFAS uptake for basil, no mycorrhizal, PFNA group assuming first-order kinetics b) Linearization of PFAS uptake for basil, no mycorrhizal, PFNA group assuming second-order kinetics. Lines of best fit and R^2 values are shown on the graphs.

Chemical processes can be modeled and classified by the relationship between the concentrations of species (contaminants) and the rate of the process. In this study, proper classification of phytoremediation into one of the three orders (zero, first, and second) is useful in predicting the time it will take to remove a certain amount of PFAS from a water system using plants. Immediately, it is apparent that zero-order kinetics is not the correct model because the PFAS removal process is not linear, rather, there is a great deal of uptake in the first few hours, and then uptake slowly levels off. However, linearization of the data is required to determine whether first or second-order kinetics is a more appropriate model for the uptake process, and the equations for this linearization are described in the methodology. Linearized data assuming first and second-order kinetics can be seen in Figure 13a and Figure 13b, respectively. It should be noted that the "better" kinetics model to describe the uptake data would be one where a line best represents the linearized data, and the R^2 value gives a quantitative measurement for this attribute (the higher the R^2 value, the "better" the kinetics model is). After determining the R^2 values for both kinetics orders for each plant group (Table 1), it can be determined that second-order kinetics more appropriately represents the PFAS uptake data, as in every case, its R^2 value is higher after linearization than the linearized data if assuming first-order kinetics. This aligns with definitions of first and second-order kinetics as first-order kinetics reactions generally level off at very low concentrations while second-order reactions level off at slightly higher concentrations (as the PFAS uptake data from this study does). With this conclusion in mind, the linearized equations for each plant group, assuming second-order kinetics, were determined (Table 2, Column B). Using these equations, the half-lives, or the amount of time it would take a particular

Group	Linearized First-Order Kinetics R^2	Linearized Second-Order Kinetics R^2
Basil + Mycorrhizae + PFNA	0.9823	0.9945
Basil + No Mycorrhizae + PFNA	0.866	0.9161
Basil + Mycorrhizae + PFBA	0.9555	0.9796
Basil + No Mycorrhizae + PFBA	0.8779	0.9195
Lettuce + Mycorrhizae + PFNA	0.8994	0.9455
Lettuce + No Mycorrhizae + PFNA	0.9537	0.9807
Lettuce + Mycorrhizae + PFBA	0.9907	0.9922
Lettuce + No Mycorrhizae + PFBA	0.9515	0.9674

Table 2 Experimental groups with their associated R^2 values after linearization, assuming first-order kinetics (column 2) and second-order kinetics (column 3).

Group	Second-Order Equation	Half-Life (h)
Basil + Mycorrhizae + PFNA	$y = 0.0002x + 0.01$	51.662
Basil + No Mycorrhizae + PFNA	$y = 0.0001x + 0.0114$	90.760
Basil + Mycorrhizae + PFBA	$y = 0.0002x + 0.0103$	54.0645
Basil + No Mycorrhizae + PFBA	$y = 0.0001x + 0.0114$	87.879
Lettuce + Mycorrhizae + PFNA	$y = 0.0004x + 0.0115$	27.117
Lettuce + No Mycorrhizae + PFNA	$y = 0.0002x + 0.0099$	43.009
Lettuce + Mycorrhizae + PFBA	$y = 0.0004x + 0.0088$	28.397
Lettuce + No Mycorrhizae + PFBA	$y = 0.0002x + 0.01$	42.863

Table 3 Experimental groups with their associated linear equations (column 2) and half-lives (column 3), assuming second-order kinetics.

plant group to remove half of the remaining PFAS in the solution, were determined using second-order reaction equations (described in the methodology section) (Table 2, Column C). From these results, it should be noted that lettuce plants with mycorrhizal colonization had the lowest half-life (27.117 h), meaning these plants would be able to reduce PFNA contamination at the fastest rate out of any of the groups tested. Using the standard half-life equation (described in the methodology), it would take just 117.2 hours for 95% of PFNA from a 1000 ppb solution (which is used in this study) to be removed by the lettuce mycorrhizal plants. This figure is significantly less than similar PFAS phytoremediation studies completed in literature, which have found that removing 95% of PFAS in a solution would take almost 360 hours[22]. This stark difference indicates the great benefit mycorrhizal presence has on the PFAS filtration process, as that is the key change between this study and other studies in literature. Overall, the identification of hydroponics phytoremediation of PFAS as a second-order kinetics reaction makes it possible to calculate how long it will take to remove any amount of PFAS from a water system, a useful tool in designing plant-based filtration systems and advocating for the efficiency of these forms of green infrastructure.

Discussion

Application and Economic Analysis

The major application of using mycorrhizal hydroponics plants for PFAS remediation comes in the form of managing stormwater detention ponds. Stormwater detention ponds are designed to collect and contain runoff water, especially during heavy rainfall, to prevent flooding and downstream erosion. These ponds are often found in urban areas, industrial parks, and near highways, where they accumulate a variety of pollutants, including PFAS, from surface runoff. After a few days, the water from these ponds then goes through a long, expensive filtration procedure to remove contaminants and return the water to drinking water systems. This process currently does not take advantage of a natural filtration system that would cost significantly less and be far more convenient: plants around stormwater detention ponds. As shown by this study, plants, even without mycorrhizal colonization, can be successful in removing more than 60% of PFAS from a system (Figure 8c, 8d), and this removal rate is shown to increase with mycorrhizal colonization as well as selection of plant species with naturally longer roots. Furthermore, it is estimated that it would cost as low as $1,200 to disperse lettuce seeds in bulk around the 925-foot perimeter of the currently barren Stormwater Runoff Pond in Glassboro, NJ[23,24]. This amount would also be a one-time cost if native NJ plants, such as milkweed plants, were planted instead of lettuce plants, as

these would return every year without additional planting. Additionally, this $1,200 figure is a small fraction of the $600,000 annual budget Gloucester County currently spends on traditional water contamination filtration techniques, which as described earlier, are still not entirely useful in decreasing contamination to non-hazardous levels[25]. This provides another incentive for utilizing phytoremediation of PFAS at the beginning of the filtration process at stormwater detention ponds, as it is the most efficient stage to do so because of the second-order kinetics characterization of phytoremediation – immediately after rainfall is the point where PFAS concentration in water systems is the highest, and because the half-lives of second-order reactions increase when concentrations increase, a great deal of PFAS can be removed by plants in a short amount of time at stormwater detention centers. As a result, less filtration will be required by industrial water treatment plants, potentially resulting in a major reduction of energy costs, as water treatment plants can spend up to $10 million a year reducing contamination from the initial concentrations of the stormwater detention pond to safe levels[26]. Overall, hydroponics plants can be easily and inexpensively integrated into current PFAS filtration processes through direct plantings around stormwater detention ponds, presenting a particular benefit to small and rural towns by dramatically cutting the costs of industrial water treatment plants. This application could then be further expanded to clean contaminated water in developing countries, presenting global benefits as an effective PFAS filtration system.

Future Directions

A few potential future directions of this study have been made readily apparent, including a more in-depth analysis of a broader range of plant species with long average root lengths, for example, Sweet Flag (Acorus calamus) or American waterweed (Elodea canadensis). Similar to this study, those experiments can track how PFAS removal changes between these species. Going along with this, it should be tested how well mycorrhizal fungi, which has been concluded in this study to improve PFAS uptake in hydroponics systems, colonize the roots of species with naturally longer root lengths. One of the main limitations of this study was that it was conducted in a controlled environment, so with real-world conditions such as weather and other biological agents coming into play, the effectiveness of plants in removing contaminants from water might be affected. To combat this, it should be further studied how well hydroponics plants infected with mycorrhizae remove contaminants in real-world stormwater detention ponds. Also, more research should be done on the eventual fate of PFAS within plants, as some preliminary studies in literature have found that PFAS chemicals are readily broken down in plants into less harmful compounds through metabolic pathways, but it is suggested that more evidence is necessary to make a final conclusion[27]. Overall, more research needs to be done into the factors affecting PFAS uptake by hydroponically grown plants and mechanisms of uptake before phytoremediation can be fully applied in real-world scenarios. However, for many of these studies, this experiment has laid the groundwork through the plant growth system methodology, PFAS uptake experimentation, and identification of the kinetics order of hydroponics phytoremediation.

Conclusion

This study investigated the uptake of two types of PFAS, short-chain PFBA and long-chain PFNA, by hydroponically grown lettuce and basil plants. The results revealed that hydroponics plants successfully removed more than 70This study also showed that through linearization tests the uptake of PFAS by plants could be modeled by a second-order kinetics reaction, allowing the estimation of the amount of time it would take hydroponics plants to uptake PFAS in real-world environments. The methodology used in this study also lays the groundwork for future research, as the setup of the hydroponics plant growth system and PFAS phytoremediation experimentation as well as the mycorrhizal staining technique can be applied in future phytoremediation studies. Furthermore, the discussion provided evidence that planting hydroponics plants at stormwater detention ponds is a green and affordable method of integrating phytoremediation into current PFAS water filtration systems, specifically in rural and low-income communities where access to energy-expensive filtration infrastructure is not readily available. Overall, the success of this project in demonstrating the effectiveness of hydroponics plants in PFAS phytoremediation opens new avenues for environmentally friendly and accessible water treatment options. It sets the stage for further research and development in this field, potentially revolutionizing the way we approach water purification and environmental restoration.

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