

Abstract

Phytoextraction, also called phytoaccumulation, is the uptake and translocation of contaminants in the soil, or other media, by plant roots into the aboveground portions of the plants. Based on the favorable results of our previous investigation published in the International Journal of Phytoremediation (Huff, et al. 2020) that established the proof of concept, Nutter & Associates, Inc. (NAI) and Brown University have implemented a new phytoremediation investigation to demonstrate the viability of our phytoextraction methodology for PFAS-contaminated sites by conducting an updated grow house investigation using the most effective perfluorooctane sulfonate (PFOS) accumulating plant species identified in our earlier studies. The plants are being grown in PFOS-impacted soils acquired from five DoW facilities employing a statistics-based randomized block design. The soils represent five different soil orders: Ultisol, Andisol, Aridisol, Inceptisol, Mollisol. Successful accumulation during the investigation will be defined by achieving PFAS shoot to soil (or *shoot + thatch* to soil) concentration ratios that are >1 and which show extrapolated PFOS accumulation rates meeting one-half reduction of the root zone PFOS soil concentrations within a 10-year time horizon. Additionally, phytoaccumulation will be assessed by evaluating the ratio of shoot + thatch to soil PFAS concentrations in solution in equilibrium with the soil.

Problem Statement

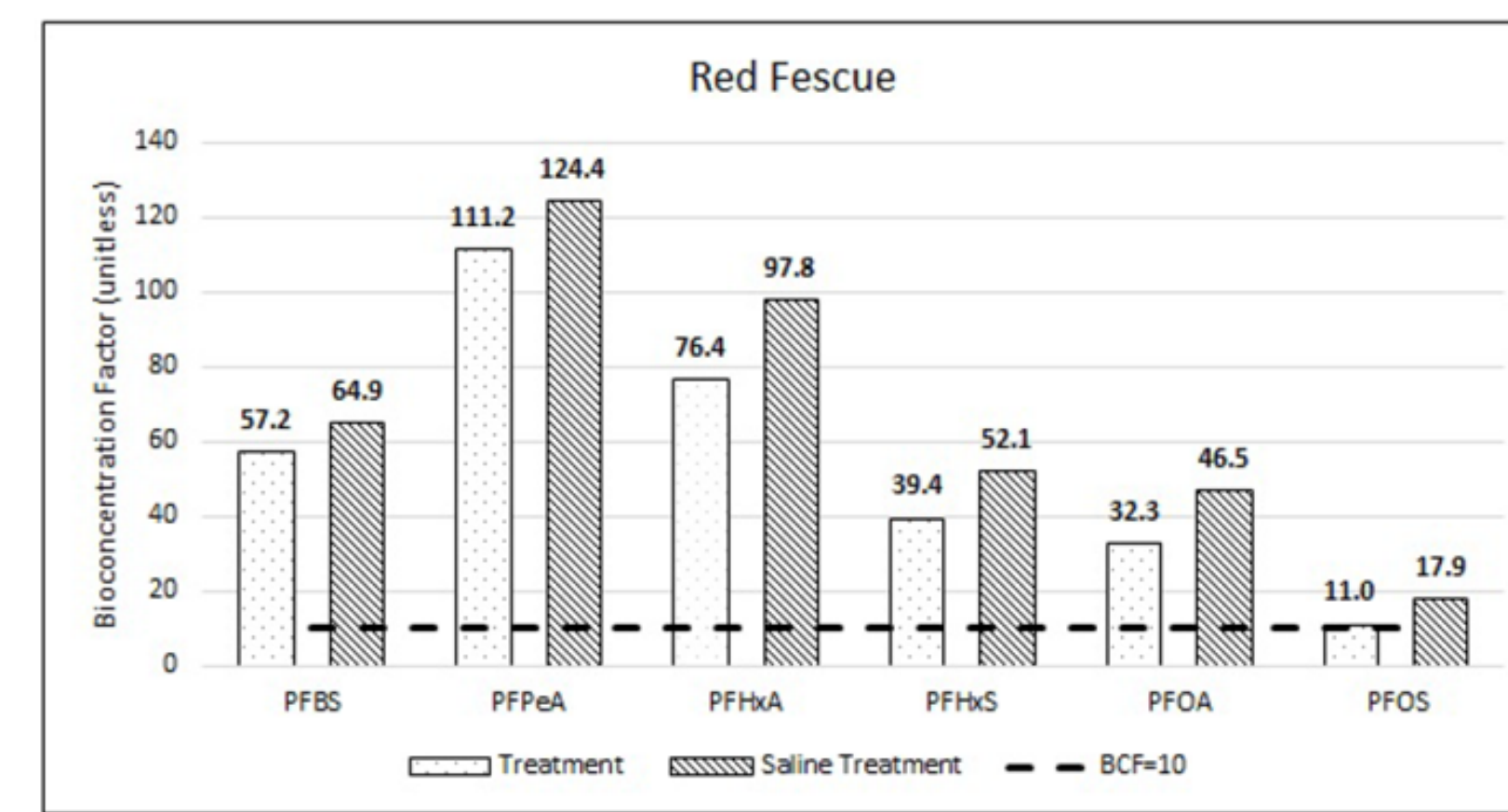
Current cleanup approaches for PFAS-impacted soil and sediment consists of expensive and energy consumptive approaches. As the federal hazardous substance designation of PFOS and PFOA became effective on July 8, 2024, there is a pressing need for a viable phytoremediation cleanup methodology to address PFAS-impacted soil and other media.

The Phytoremediation Opportunity

The EPA National Risk Management Research Laboratory has stated that phytoremediation approach can be 50-80% cheaper than other established alternatives. Thus, our phytoextraction methodology and process stands to be more economically viable than other remedial options and it will meet core elements outlined in the ASTM E2893 Standard Guide for Greener Cleanups that include minimizing greenhouse gas emissions, air pollutants, use of materials, generation of waste, disturbance to land and ecosystems, and noise and light disturbance.

Background

Our previous investigation established proof-of-concept for the use of phytoremediation to remediate PFAS-contaminated media via phytoextraction and the results of our investigation were published in the International Journal of Phytoremediation (Huff et al., 2020). Our investigation was sufficient basis for the US Patent Office to grant three Utility Patents for our methodology (U.S. Patents Nos. 10835940, 11000886, 11161160). A key metric in our study was Bioconcentration Factor (BCF), which is the ratio of contaminant concentration in plant tissue to the contaminant concentration in the soil. A BCF that is > 10 is indicative of strong viability for a plant to be effective in a phytoextraction application. Red fescue (*Festuca rubra*) grass performed best across all PFAS compounds tested in our previous study.



New Investigation

Our initial investigation was based on plants grown in a sand support matrix. Thus, the viability of our phytoextraction technique needs to be vetted to assess whether adequate phytoaccumulation can be achieved with actual PFAS-contaminated soils (e.g., finer textures, mixed mineralogy, greater organic matter, long-term absorption and complexation of PFAS within the soil, and greater biologic activity) found at DoW installations.

An innovation associated with our initial investigation was the use of a saline irrigant containing gypsum and magnesium sulfate dissolved in deionized water. Increases in PFOA and PFOS accumulations were observed in plants grown in soils receiving the saline irrigant; the increases ranged from 44% to 344% greater than contaminated treatments that did not receive salinity amendments (Huff, et al. 2020). Salinity treatments will be further assessed as part of this investigation.

With the initial investigation it was noted that the highest PFOS accumulating plant, *Festuca rubra*, developed a thick (5-10 centimeter) thatch on top of the mineral soil surface (Figures 1-4). This thatch is a combination of dead organic debris, living roots, crowns, and stems of grass (Christians, 2004). As shown in the photographs (Figures 1-4), the *Festuca rubra* thatch was thick enough to be harvested with the grass leaves (shoots), thus accelerating extraction from soil. Analysis of the PFAS concentrations within the thatch was not performed with our initial investigation, but thatch analysis is planned as part of the current investigation to determine PFOS extraction rates when shoot and thatch harvesting are combined.

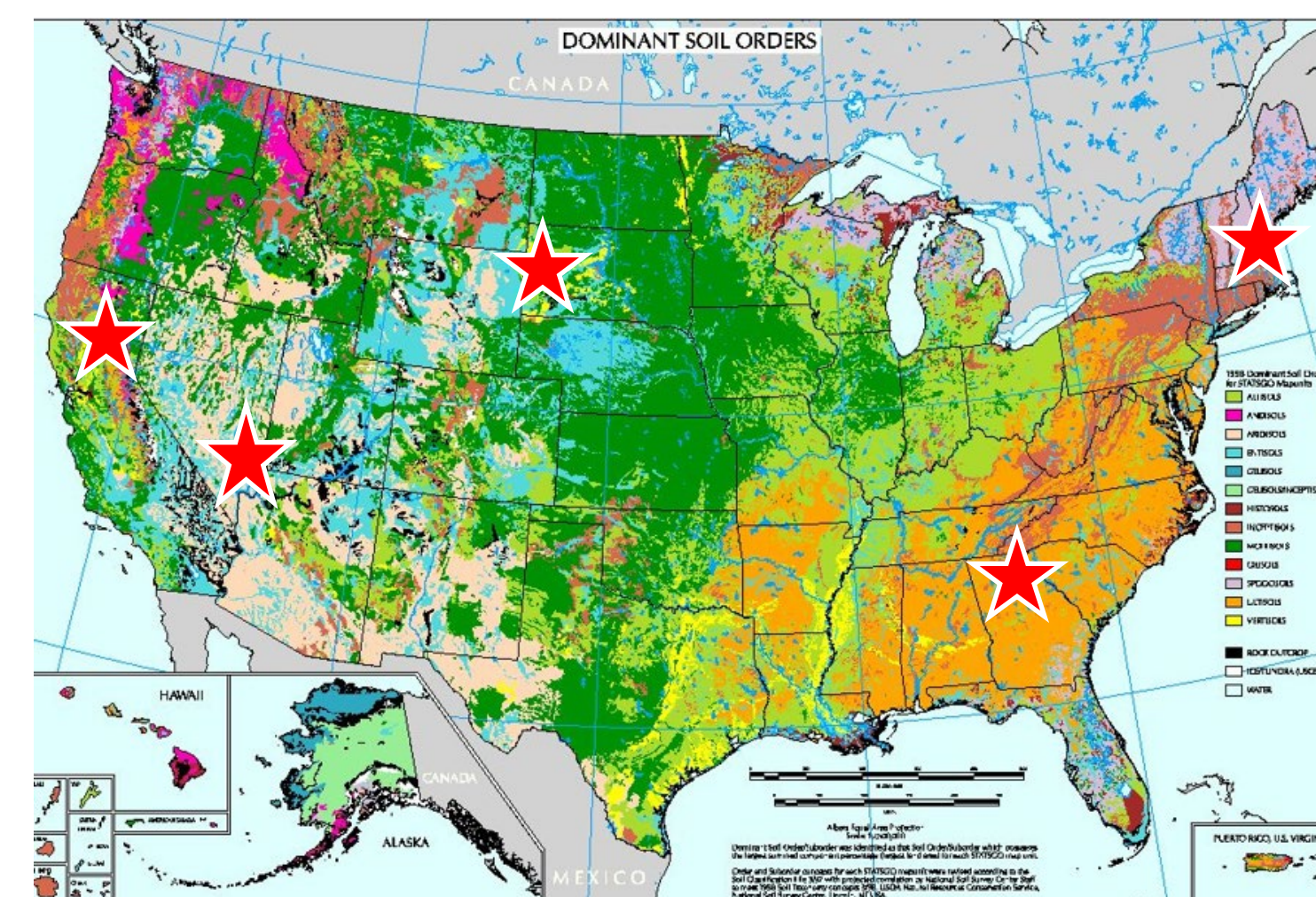
The translocation factor (TF) is a bioaccumulation metric that is defined as the ratio of the shoot concentration (C_{shoot}) to the root concentration (C_{root}) for a particular constituent:

$$TF = C_{shoot} / C_{root}$$

Numerous studies have shown root concentrations typically exceed the shoot concentrations in phytoextraction. Bei Wen et al. (2016) found that the PFOS TF ranged from 0.055 to 0.016 among the seven species they tested. Thus, root PFOS concentrations are generally expected to be substantially larger than the shoot concentrations.

Study Design

1. Randomized block design grow house investigation building on previous research (Huff et al., 2020)
2. Use the most effective PFOS-accumulating plant species, *Festuca rubra* (red fescue)
3. Plants are growing in PFAS-impacted soils acquired from five DoW facilities: including Dobbins ARB in Marietta, GA and others encompassing multiple soil types (e.g., Ultisols, Mollisols, Aridisols, Andisols, Inceptisols)
 - Dobbins Air Reserve Base, Marietta, Georgia
 - Pease Air Force Base, New Hampshire
 - Ellsworth Air Force Base, South Dakota
 - Beale Air Force Base, California
 - Creech Air Force, Nevada
4. Investigate using a statistics-based, randomized block design with six replications and include evaluation soil amendments shown to improve PFOS accumulation (e.g., salinity amendment)
5. Perform lab analysis to determine PFOS uptake levels in shoots and thatch, and evaluate rates of PFOS accumulation in various soil types



★ denotes Air Force base soil collection location



Figure 1. *Festuca rubra* after initial transplanting.

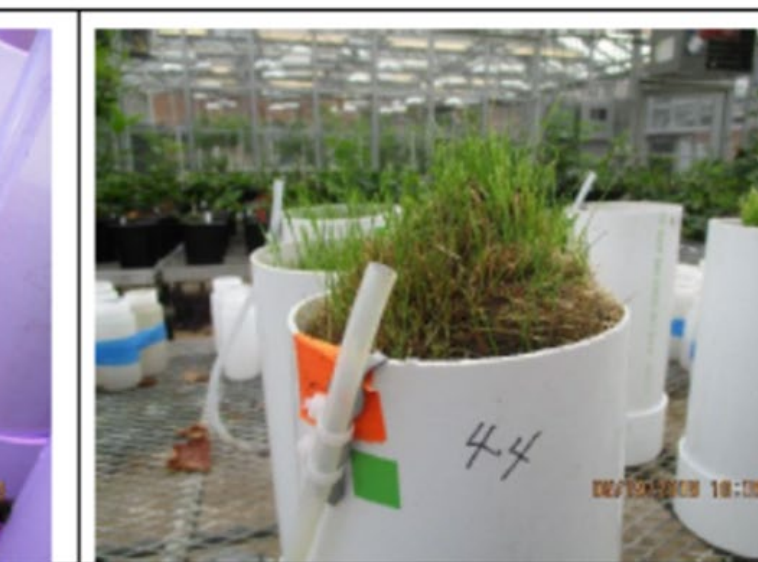


Figure 2. *Festuca rubra* at study completion, note the depth and height of the thatch.



Figure 3. Thatch easily removed from sand support media at the test completion.



Figure 4. Thatch removed at test completion; with no evident sand from the support media.

Progression of red fescue growth from transplant to harvest.



Excessive Thatch. Photo credit: Dr. Kevin Mathias, University of Maryland Extension

Site Location	Sample ID	ng/g		meq/100g		S.U.		%Base		mg/kg		% TOC		
		PFOA	PFOS	CEC	AEC	pH	Saturation****	ESP****	SAR****	Soluble Salts	Soil Texture**	Org Matter	TOC	
New Hampshire	08-40730	40.1	808	24	9.21	0.069	4.63	7.58	6.72	0.56	3267	Sandy Loam	5.52	2.88
New Hampshire	401-40440	7.35	292	8	8.77	0.044	4.37	12.34	3.89	0.35	3384	Sandy Loam	6.81	3.54
Nevada	CST-38-SB03-TW03	0.666	31.0	2	12.70	0.011	8.30	100.00	0.77	0.50	3188	Sandy Loam	3.54	2.23
Nevada	TDST-SR02-TW02	0.425	4.22	0	24.23	0.057	7.93	100.00	9.60	2.41	6443	Sandy Clay Loam	2.10	1.01
California	03P0025B	7.40	338	34	17.21	0.038	6.75	100.00	1.75	0.32	2559	Sandy Clay Loam	3.27	1.59
California	03P0085B	13.9	733	51	7.82	0.052	6.30	87.39	0.70	0.38	2652	Sandy Clay Loam	2.58	1.35
South Dakota	SB170127	2.87	122	4	18.66	0.001	8.01	100.00	0.24	0.25	2542	Clay Loam	3.37	1.70
South Dakota	DP0132	33.5	998	65	19.43	0.025	7.67	100.00	0.18	0.21	2501	Sandy Clay	4.53	2.39
Georgia	SS02P-DPTS07SS	5.31	347	10	9.43	0.018	5.65	61.64	1.46	0.32	2567	Loam	8.63	3.52
Georgia	SS004P-DPTS07SS	3.68	158	5	8.13	0.018	5.21	54.85	2.06	0.37	2683	Sandy Clay Loam	6.12	2.68
Georgia	SS007P-DPTS21SS	0.754	9.77	0	4.99	0.033	5.36	58.40	2.16	0.30	2747	Sandy Clay Loam	5.96	2.77
Georgia	CONTROL (5/9/25)	0.407	1.47	0	8.27	0.032	4.82	47.04	2.58	0.79	3563	Sandy Loam	4.73	2.80

**Concentration in Soil Solution in Equilibrium with the Soil

***USDA Soil Texture

****Saturated Paste

*****Ammonium Acetate at pH 7

• Agronomic soil test data used to characterize baseline fertility and inform pH adjustment and salinity rates

Treatment (grams per kg of soil)	EC μ S/cm													
	Oypsm	MgSO4 7H2O	08-40730	401-40440	CST-38-SB03-TW03	TDST-SR02-TW02	03P0025B	03P0085B	SB170127	DP0132	SS02P-DPTS07-SS	SS004P-DPTS07-SS	SS007P-DPTS21-SS	Control
5	5	3913	4015	4376	11530	4615	4516	4225	3790	3089	3144	3795	4020	
5	10	6071	4819	5927	6129	5833	5767	4844	3500	3740	4554	5252		
10	5	4035	3938	4441	11760	4563	4706	4431	3773	3056	3133	3807	4080	
10	10	5193	4742	5992	11140	6077	6023	5973	4927	3467	3729	4566	5312	

Creech AFB – (Aridisol) TDST-SR02-TW02 baseline EC 11.2 dS/m.

5 grams of Gypsum = 58.25 meq Ca
7 grams of Epsom = 56.84 meq Mg
ratio = 1.024806

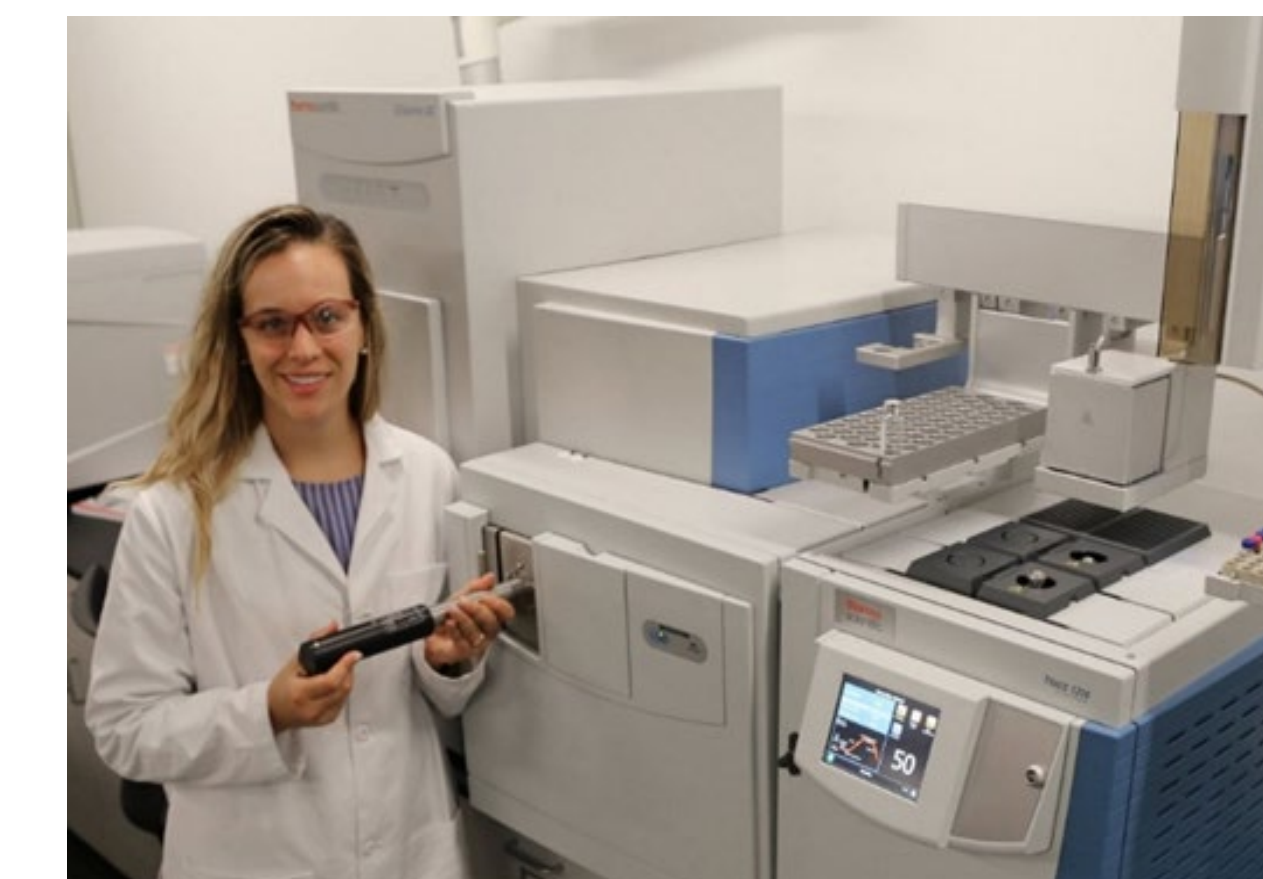


Laboratory Analysis Methodology - Pennell Lab – Brown University School of Engineering

Analysis of soil, plant tissue, and soil solution water are being conducted by Kurt Pennell, Ph.D. at Brown University School of Engineering, using state of the art liquid chromatography-mass spectrometry (LC-MS/MS) in accordance with the SERDP and ESTCP memo: Requirements for SERDP and ESTCP Projects Addressing PFAS-related Issues.



Waters uHPLC Xevo TQ-S



Thermo GC QExactive Orbitrap



Thermo LC QExactive HFX Orbitrap

- Targeted analysis of 40 PFAS using Waters uHPLC-MS/MS system (EPA Method 1633)
- PFAS method verified by an ELAP-certified laboratory (Battelle Memorial, Norwood, MA)
- Non-targeted analysis using high resolution liquid- and gas-chromatograph Thermo Orbitrap mass spectrometers to detect unknown PFAS and/or reaction products
- Metrohm combustion ion chromatograph (CIC) for TF and TOF analysis
- Plant tissue PFAS method published (Huff et al., 2020, Inter. J. Phytoremediation)

Objectives

The three primary objectives of the investigation are:

- **Goal 1** - achieve and scientifically document adequate phytoaccumulation via phytoextraction by *Festuca rubra* from natural soils collected from five different regions within the US that have been contaminated from past PFAS releases in a greenhouse setting,
- **Goal 2** - confirm and provide greater scientific basis proving specific management techniques (use of a saline irrigant treatment, tillage, synthetic nitrogen fertilizer) will significantly increase the rate of PFAS phytoaccumulation compared to control treatments, and
- **Goal 3** - achieve and scientifically document significantly greater PFAS phytoaccumulation through propagating thatch along with stems and leaves (shoots) of *Festuca rubra*.

References

Bei Wen, Yali Wu, Hongna Zhang, Yu Liu, Xiaoyu Hu, Honglin Huang, Shuzhen Zhang, 2016. The roles of protein and lipid in the accumulation and distribution of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in plants grown in biosolids-amended soils. Environmental Pollution 2016, 216, 682-688. <https://doi.org/10.1016/j.envpol.2016.06.032>

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Huff, D. K., Morris, L. A., Sutter, L., Costanza, J., and Pennell, K. D. 2020. Accumulation of six PFAS compounds by woody and herbaceous plants: potential for phytoextraction. Int. J. Phytoremediation 22, 1538-1550. doi: 10.1080/15226514.2020.1786004

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