Environmental Science & Technology

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Fate and Transformation of 6:2 Fluorotelomer Sulfonic Acid Affected by Plant, Nutrient, Bioaugmentation, and Soil Microbiome Interactions

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Cite This: https://doi.org/10.1021/acs.est.2c01867

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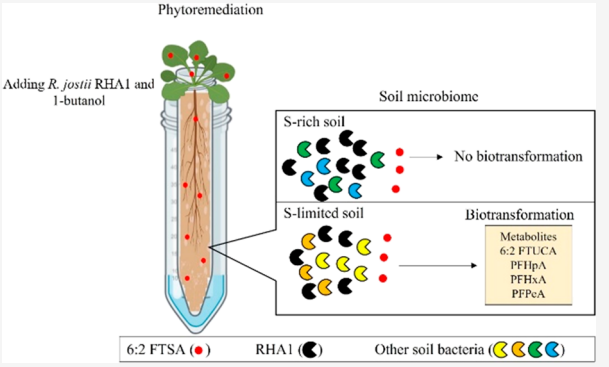
ABSTRACT: 6:2 Fluorotelomer sulfonic acid (6:2 FTSA) is a dominant per- and poly-fluoroalkyl substance (PFAS) in aqueous film-forming foam (AFFF)-impacted soil. While its biotransformation mechanisms have been studied, the complex effects from plants, nutrients, and soil microbiome interactions on the fate and removal of 6:2 FTSA are poorly understood. This study systematically investigated the potential of phytoremediation for 6:2 FTSA by*Arabidopsis thaliana*coupled with bioaugmentation of*Rhodococcus jostii*RHA1 (designated as RHA1 hereafter) under different nutrient and microbiome conditions. Hyperaccumulation of 6:2 FTSA, defined as tissue/soil concentration > 10 and high translocation factor > 3, was observed in plants. However, biotransformation of 6:2 FTSA only occurred under sulfur-limited conditions. Spiking RHA1 not only enhanced the biotransformation of 6:2 FTSA in soil but also promoted plant growth. Soil microbiome analysis uncovered *Rhodococcus* as one of the dominant species in all RHA1-spiked soil. Different nutrients such as sulfur and carbon, bioaugmentation, and amendment of 6:2 FTSA caused significant changes in - microbial community structure. This study revealed the synergistic effects of phytoremediation and bioaugmentation on 6:2 FTSA removal. and highlighted that the fate of 6:2 FTSA was highly influced by the complex interactions of plants, nutrients, and soil microbiome.



KEYWORDS: *6:2 FTSA, phytoremediation, bioaugmentation, biotransformation, Rhodococcus jostii RHA1, Arabidopsis thaliana*

1. INTRODUCTION

6:2 Fluorotelomer sulfonic acid (6:2 FTSA), which has been directly used in chromium plating industries to reduce the mist formation and a processing aid in emulsion polymerization of fluoropolymers,[1] is a key ingredient in aqueous film-forming foams (AFFFs).[2,3] Applications of AFFFs in firefighting and training have been considered as a significant source of per- and poly-fluoroalkyl substances (PFAS) in the environmental matrix.[2−6] Not surprisingly, high concentrations of 6:2 FTSA were usually detected in the AFFF-impacted soil due to the direct release from AFFF or the transformation of 6:2 FTSA precursors.[7] More than 2 mg/kg of 6:2 FTSA was detected in soil samples collected from Holloman Air Force Base, New Mexico.[8] In some cases, the concentration of 6:2 FTSA is even higher than those long-chain perfluoroalkyl substances. For example, the concentrations of 6:2 FTSA in soil samples collected from the monitor well (MW01005) of Patrick Air Force Base, Florida was 0.778 mg/kg, which is higher than the concentration of perfluorooctanoic acid (PFOA)(0.003 mg/kg) and perfluorooctanesulfonic acid (PFOS)(0.578 mg/kg).[9]

Many physical and chemical techniques such as UV irradiation,[10] electrochemical oxidation,[11] and advance oxidation[12] have been developed to remove 6:2 FTSA. However, the effectiveness of phytoremediation toward 6:2 FTSA is not fully explored for PFAS-contaminated soil. The knowledge of PFAS uptake and accumulation, phytotoxicity, and tolerance to PFAS by plants is growing but remains limited. The removal of perfluoroalkyl substances, i.e., perfluorobutanoic acid (PFBA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS),PFOS, and PFOA, by different plant species has been intensively studied.[13−19] The uptake of different perfluoroalkyl substance precursors (i.e., *N*-ethyl perfluorooctane sulfonamide (EtFOSA), fluorotelomer alcohol (FTOH) polyfluoroalkyl phosphate diesters (PAPs), and perfluoroalkyl phosphinic acids (PFPiAs)/phosphonic acids (PFPAs)) has also been reported.[20−23] However, knowledge of the uptake of long-chain PFAS alternatives (i.e., 6:2 FTSA) is relatively limited. *Arabidopsis*

Received: March 15, 2022
Revised: June 28, 2022
Accepted: June 30, 2022

thaliana was chosen as the model plant in this study due to its relatively short life cycle and well-studied genetic traits. Although a high bioconcentration factor (BCF) and translocation factor (TF) of PFOA and its alternative, GenX, were reported,[15] the removal efficiency of *A. thaliana* toward 6:2 FTSA is still poorly understood.

Microbial-assisted phytoremediation (i.e., bioaugmenting contaminant degraders during phytoremediation) has been shown to promote plant health, improve biodegradation, and thus enhance overall phytoremediation efficiency.[24−27] Our previous study showed that *Rhodococcus jostii* RHA1 (designated as RHA1 hereafter) can express alkanesulfonate monooxygenase to desulfonate 6:2 FTSA and many defluorinating enzymes such as alkane monooxygenase, haloacid dehalogenase, and cytochrome P450 to defluorinate 6:2 FTSA.[28] Furthermore, approximately 99% of 6:2 FTSA was biotransformed by 1-butanol- and ethanol-grown RHA1 cultures under S-free conditions within 64 hours. The formation of four major metabolites, namely, 6:2 fluorotelomer carboxylic acid (6:2 FTCA), 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), α-hydroxy-5:3 saturated fluorotelomer carboxylate (α-OH 5:3 FTCA), and perfluoroheptanoic acid (PFHpA), were confirmed, and one metabolite, 6:2 fluorotelomer unsaturated sulfonic acid (6:2 FTUSA), was tentatively detected. While the toxicity of all metabolites of 6:2 FTSA compared to its parent compound (i.e., 6:2 FTSA) is not unclear, the biotransformation activity of 6:2 FTSA by RHA1 has suggested itself as a promising bioaugmenting agent for phytoremediation of 6:2 FTSA-contaminated soil.

While 6:2 FTSA biotransformation in different environmental media and by pure cultures has been reported,[29−33] little is known about the fate and transformation of 6:2 FTSA in the plant−soil environment under different nutrient conditions, particularly the sulfur availability. Sulfur is a key nutrient for plant growth and soil microbes, and biotransformation of 6:2 FTSA was observed only under sulfur-limited conditions.[28,31] Mostly, little is known about the potential of microbial-assisted phytoremediation for 6:2 FTSA-contaminated soil. To fill these knowledge gaps, the objective of this study is to investigate the fate and biotransformation of 6:2 FTSA under the influence of plants, nutrient availability (specifically sulfur), bioaugmentation, and soil microbiome. Specifically, an environment-related concentration of 6:2 FTSA (1.5 mg/kg)[7,8] was used to assess the growth of *A. thaliana* seedlings and their uptake of 6:2 FTSA. RHA1, the known 6:2 FTSA degrader, and 1-butanol, the carbon source for RHA1 and inducer for defluorinating alkane monooxygenase and butane monooxygenase, were used to assess the feasibility of bioaugmentation for 6:2 FTSA biotransformation in the soil. Biotransformation of 6:2 FTSA in soil and plants was determined. In addition, the bulk soil and rhizosphere soil microbial communities with different treatments were characterized to determine the role of soil microbes associated with 6:2 FTSA biotransformation.

2. MATERIALS AND METHODS

2.1. Chemicals, Soil, Bacterial Strains, and Plants. Information on the chemicals used in this study is available in the Supporting Information. Sandy soil was prepared by mixing 95% of sand (Pavestone, USA) and 5% of perlite (Scotts Miracle-Gro, USA). The pH, conductivity, and major nutrients, including sulfate concentration (11 mg/kg), of the sandy soil are listed in Table S1. To create sulfur-limited (S-limited) or sulfur-rich (S-rich) conditions, S-free or S-rich Hoagland solution[15] was added into the sandy soil, respectively. The components of S-free and S-rich Hoagland solutions are available in the Supporting Information. To minimize the sorption of 6:2 FTSA, 15 mL and 50 mL polypropylene tubes were used as pots and reservoirs, respectively. See the Supporting Information and Figure S1 for details.

A. thaliana ecotype Col-0 was chosen as the model plant in this study. The procedures of seed surface sterilization and growth conditions were followed as described previously[15] (see details in the Supporting Information). *R. jostii* RHA1 is capable of using 6:2 FTSA as a sole S-source[28] and thus was selected as a model bioaugmenting agent. RHA1 was precultured in R2A medium at 30 °C at 150 rpm on a shaker (KS 260 basic, IKA, USA) to reach an optical density (OD_{600}) of 1 for experimental use. The plant-promoting ability of RHA1 was assessed using an amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity assay[34] and indole-3-acetic acid (IAA) production assay[35] (see the Supporting Information).

2.2. Soil Treatments with 6:2 FTSA, RHA1, 1-Butanol, and/or Plants. 6:2 FTSA stock solution (1 g/L) was spiked into the soil to make the final concentration 1.5 mg/kg. Clean sandy soils without 6:2 FTSA were used as controls. To investigate the effects of bioaugmentation on the 6:2 FTSA biotransformation, 150 μL of R2A-pregrown RHA1 culture was applied to the soil. To understand the effects of the inducer of defluorinating enzymes, another parallel set was spiked with RHA1 culture (150 μL) and of 1-butanol (15 mg).[28] To understand the effects of plants on 6:2 FTSA biodegradation, one surface-sterilized seed was placed on each of the treatments (see details in Table S2). Each treatment was conducted in triplicate. After 25 days of incubation, the plant biomass (roots and shoots) and root length were measured.[15] The plant tolerance index (TI) to the different treatments was calculated. See details in the Supporting Information.

2.3. Analysis of 6:2 FTSA and Its Transformation Metabolites. Extraction of 6:2 FTSA and its transformation products from plant and soil is described in the Supporting Information. The extract was analyzed as described previously.[36] Briefly, the extract (10 μL) was injected into an ultrahigh-performance liquid chromatography (HPLC) (Vanquish, Thermo Scientific, USA)/triple-quadrupole mass spectrometer (Altis, Thermo Scientific, Waltham, MA, USA). The analytes were separated by a Hypersil Gold column (3 mm × 50 mm, 5 μm) (Thermo Scientific, USA). The column was maintained at 30 °C, and the separation was achieved using an 8 min solvent gradient method at a flow rate of 0.5 mL min^{-1}. Sample acquisition and analysis were performed using TraceFinder 3.3 (Thermo Scientific, USA). The bioconcentration factor (BCF) and translocation factor (TF) of 6:2 FTSA were then calculated to assess the uptake, accumulation, and mobility of 6:2 FTSA in plants grown in different treatments (see the Supporting Information).

2.4. 16S rRNA Gene Amplicon Sequencing for Microbial Community Analysis. Genomic DNA of soil was extracted using a FastDNA spin kit for soil following the manufacturer's instructions (MP biomedicals, USA). The extracted DNA were used to amplify 16S rRNA gene amplicons using a pair of primers (16S Forward: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′; 16S Reverse: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′), targeting the V3 and V4 regions. The DNA libraries were prepared using a Nextera

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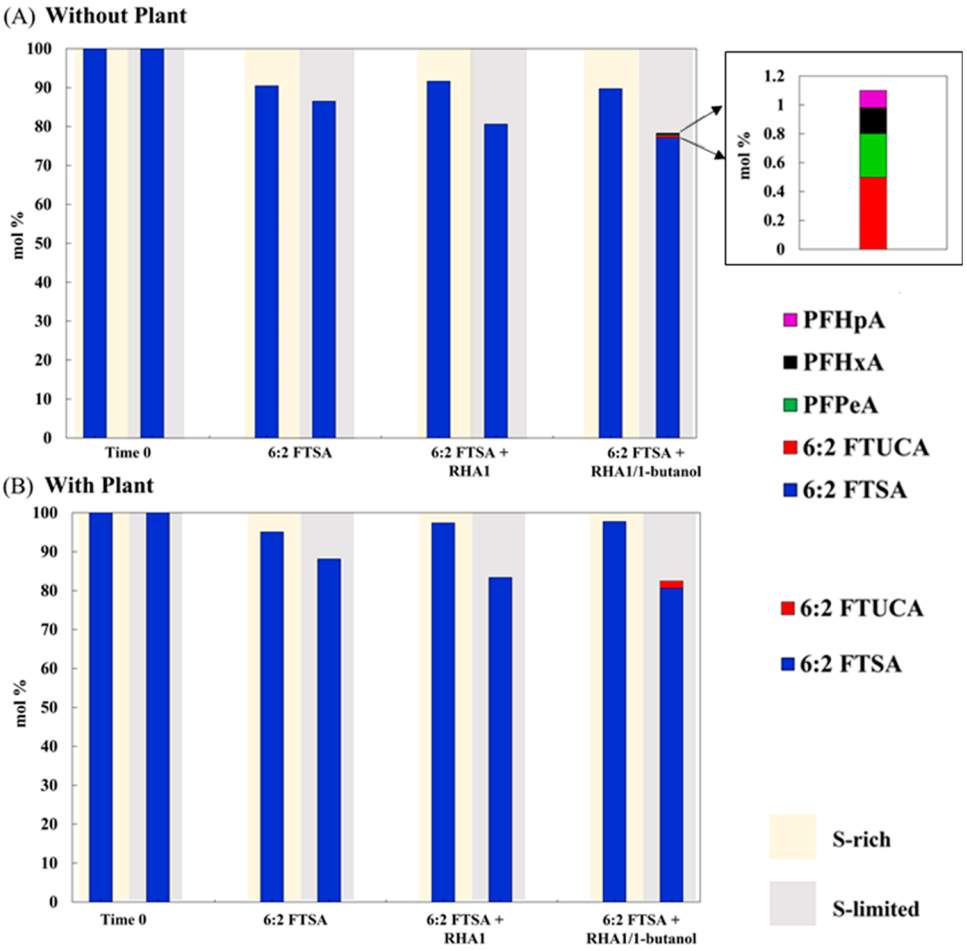

Figure 1. Sulfur, RHA1, and 1-butanol affected the biotransformation of 6:2 FTSA in sandy soil and rhizosphere sandy soil. The molar yields of 6:2 FTSA and its transformation products in sandy soil (A) and rhizosphere sandy soil (B) after 25 days of incubation. The sandy soil and rhizosphere sandy soil were amended with 6:2 FTSA, or 6:2 FTSA with RHA1, or 6:2 FTSA with RHA1 and 1-butanol. The molar yields were calculated based on total moles of recovered 6:2 FTSA and biotransformation products from sandy soil versus 6:2 FTSA applied at day 0. Each treatment was performed in triplicate (n = 3).

XT library prep kit (Illumina, USA). A Qubit high-sensitivity (HS) dsDNA assay and TapeStation D1000 HS system (Agilent, USA) were used to determine the prepared libraries' concentration and average fragment size. The library was sequenced in the Illumina MiSeq platform by using a 300 by 300 cycle v3 sequencing kit (Illumina, USA). The raw reads were merged, denoised, and filtered using qiime2-2020.2 software. Operational Taxonomic Units (OTUs) were assigned based on 97% similarity. Taxonomy annotation was conducted using the SILVA 132 ribosomal RNA (rRNA) database. The relative abundance of each genus was calculated by using R studio software. Shannon index (alpha diversity) was calculated using qiime2-2020.2 to assess the impact of different treatments on the richness and evenness of the community. Bray−Curtis dissimilarity and principal coordinates analysis (PCoA) were conducted to identify the difference in microbial composition among treatments. Bray−Curtis dissimilarity was calculated by using qiime2-2020.2. The PcoA was conducted in R studio. The profiles of the desulfonating and defluorinating genes presented in the soil microbiome were predicted by Tax4Fun2 based on 16S rRNA gene as described in the previous study.[37] The Venn diagram was analyzed and plotted by Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to understand the unique species in treatments.

2.5. Statistical Analyses. The F-test was conducted first to check the equality of variance before using Student's t-test. The Student's t-test was then used to evaluate a significant difference between treatment groups and controls. All the statistical analyses were performed using Excel (Microsoft, USA).

3. RESULTS AND DISCUSSION

3.1. Fate and Transformation of 6:2 FTSA in Sandy Soil and Rhizosphere Sandy Soil. After 25 days of incubation, 6:2 FTSA in sulfur-rich and sulfur-limited sandy soil accounted for 90.5 and 86.5% compared to the initial spike of 6:2 FTSA on day 0 (Figure 1A and Table S3), respectively. When RHA1 was amended, approximately 91.6% of 6:2 FTSA remained in the S-rich sandy soil while 80.6% of 6:2 FTSA remained in the S-limited sandy soil. No transformation products were observed under these conditions, regardless of the sulfur availability. When both RHA1 and 1-butanol were amended, a similar level of 6:2 FTSA (89.7%) to S-rich sandy soil was observed. Mostly, in the S-limited sandy soil with RHA1 and 1-butanol amendment, a significant decrease ($p < 0.05$) in 6:2 FTSA was found compared to those in 6:2 FTSA-spiked soil with about 77.1% of 6:2 FTSA remaining, and four stable transformation products were detected. These metabolites were 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), perfluor-

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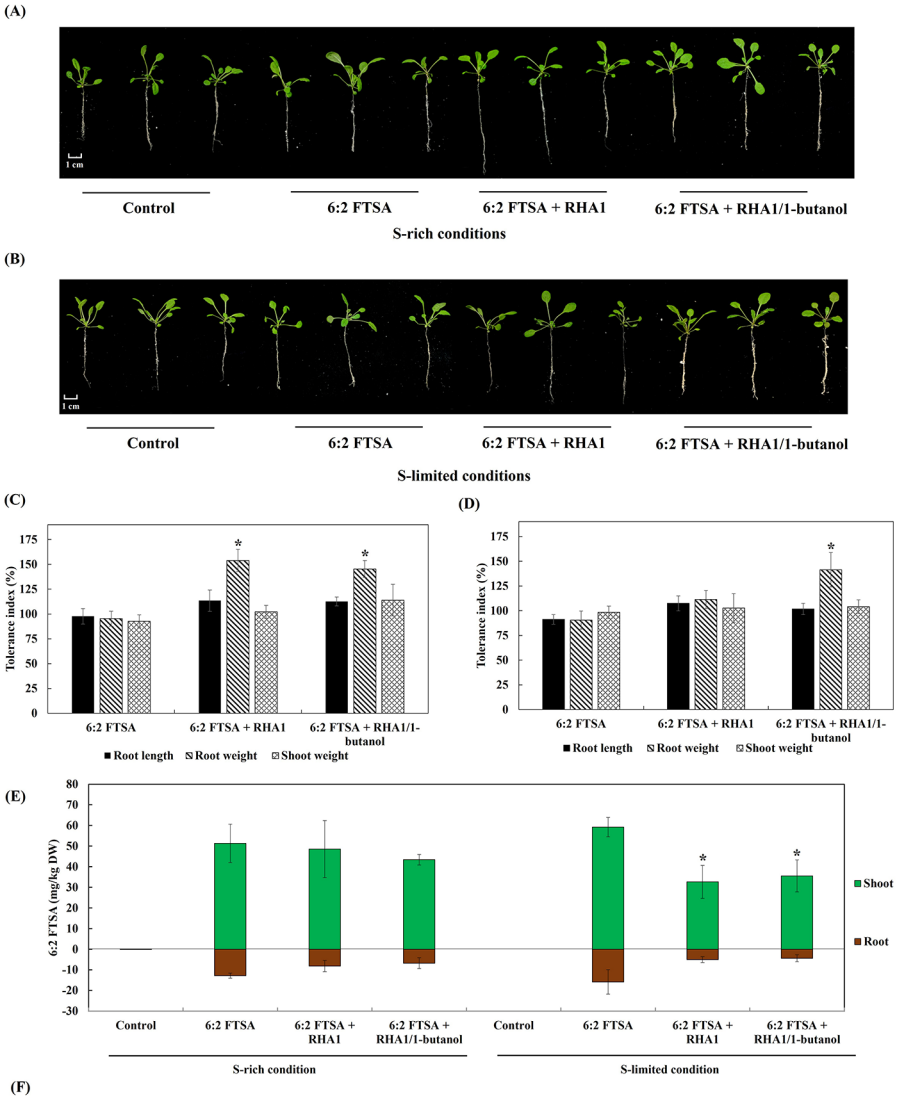

(F)

	S-rich condition			S-limited condition		
	6:2 FTSA	6:2 FTSA + RHA1	6:2 FTSA + RHA1/1-butanol	6:2 FTSA	6:2 FTSA + RHA1	6:2 FTSA + RHA1/1-butanol
Bioconcentration factor[a]	28.4	27.4	24.9	33.1	18.4	16.1
Translocation factor[b]	4.0	5.9	6.4	3.7	6.5	8.1
Plant biomass[c] (mg DW)	6.2 ± 1.4	8.0 ± 2.0	7.7 ± 0.7	6.0 ± 1.5	8.4 ± 2.1	7.2 ± 1.7
Uptake[d] (ng)	240.8 ± 25.5	346.2 ± 56.7	286.3 ± 8.1	289.5 ± 13.9	239.5 ± 44.2	202.2 ± 24.7

a. Bioconcentration factor (BF) = concentration of 6:2 FTSA in tissue/ concentration of 6:2 FTSA in soil
b. Translocation factor (TF) = concentration of 6:2 FTSA in shoot/ concentration of 6:2 FTSA in root
c. Plant biomass = shoot DW+ root DW.
d. 15000 ng of 6:2 FTSA was added initially in soil.

Figure 2. The growth of*A. thaliana*was affected by sulfur and RHA1 but not 6:2 FTSA. Hyperaccumulation, high translocation, and bioconcentration factor of 6:2 FTSA were observed in*A. thaliana*. Phenotypic comparison of three seedlings of*A. thaliana*grown on S-rich (A) and S-limited (B) soil with different treatments. The tolerance indexes (TI) of*A. thaliana*grown on different treatments under S-rich conditions (C) and S-limited conditions (D) were calculated using changes in shoot weight, root weight, and root length. 6:2 FTSA concentrations in the shoots and the roots of*A. thaliana* grown on different treatments (E). Bioaccumulation factor, translocation factor, plant mass, and 6:2 FTSA uptake by*A. thaliana*(F). Each treatment was performed in triplicate ($n = 3$). The bars represented the standard deviation of the triplicates. The asterisk indicates a significant difference ($p < 0.05$).

opentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA), which accounted for 0.5, 0.3, 0.2, and 0.1% of the initial applied 6:2 FTSA, respectively (Figure 1A). These results suggested that the sulfur availability in soil is an important factor determining the fate and biotransformation of 6:2 FTSA and that bioaugmentation of RHA1 with 1-butanol can promote the biotransformation of 6:2 FTSA in the S-limited sandy soil.

In S-rich rhizosphere sandy soil, regardless of the treatments, 6:2 FTSA remained high in rhizosphere sandy soil, ranging from 95.1 to 97.7% of those on day 0 (Figure 1B and Table S3). However, in S-limited rhizosphere sandy soil, the molar yield of 6:2 FTSA was 88.1% for those that received only 6:2 FTSA followed by 83.4% for those that received RHA1 amendment. The 6:2 FTSA concentration significantly decreased ($p < 0.05$) to 80.0% for those that received RHA1 and 1-butanol compared to those in 6:2 FTSA-spiked soil. Additionally, one metabolite

https://doi.org/10.1021/acs.est.2c01867
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6:2 FTUCA, accounting for 1.8% of the initial 6:2 FTSA spiked on day 0, was detected in the S-limited rhizosphere sandy soil that received RHA1 and 1-butanol amendment. Similar to the results shown in Figure 1A, bioaugmentation of RHA1 and amendment of 1-butanol into S-limited rhizosphere sandy soil promoted 6:2 FTSA biodegradation.

The spiked 1-butanol served as the carbon source and the enzyme inducer of butane monooxygenase or alkane monooxygenase, which increased the co-metabolic biotransformation of 6:2 FTSA by RHA1.[28] Similar to the previous study, 6:2 FTUCA and PFHpA are the two major metabolites observed in RHA1/1-butanol spiked treatment; however, no 6:2 FTCA was identified in this study. Interestingly, two metabolites—PFHxA and PFPeA—were detected in S-limited sandy soil in this study but were not detected in the previous study using 1-butanol-grown RHA1,[28] suggesting the presence of other 6:2 FTSA degraders in soil microbiome and different 6:2 FTSA transformation pathways. In addition, more 6:2 FTSA biotransformation and diverse metabolites were observed in S-limited sandy soil compared to those in S-limited rhizosphere sandy soil, implying that the interactions of plant and soil microbes, possibly due to competition for the available S-source derived from 6:2 FTSA, slightly impacted the degree of defluorination of 6:2 FTSA.

3.2. Phytotoxicity of 6:2 FTSA in Plants Grown under Different Conditions. Sulfur is an essential nutrient to plant growth. The symptoms of severe sulfur deficiency in plants include growth retardation and development of reddish color on the shoot. Under the condition we used in this study,*A. thaliana*seedlings grown in the S-limited soils showed a morphology similar to those grown in S-rich soils (i.e., control samples in Figure 2A,B). In addition, the average root length and shoot weight of*A. thaliana*grown in the S-limited sandy soil (control) for 25 days were similar to those grown in the S-rich sandy soil (control) (Figure S2). However, the root weight in the S-limited sandy soil was about 46.1% lower than that in the S-rich sandy soil (Figure S2). Regardless of the sulfur content in the sandy soil, exposure to 1.5 mg/kg of 6:2 FTSA for 25 days had no significant impacts on the development of*A. thaliana*in terms of phenotype, root length, and shoot weight (i.e., 6:2 FTSA only in Figure 2A−D).

Interestingly, when RHA1 was amended, a positive trend in phenotype was observed in plants grown in both S-rich and S-limited sandy soils. An increased root length (7.5%), root weight (11.3%), and shoot weight (2.7%) were observed under S-limited conditions. Stronger positive impacts on the root length (13.5%) and root weight (54%, $p < 0.05$) than on the shoot weight (2.2%) were observed under the S-rich conditions (i.e., 6:2 FTSA + RAH1 in Figure 2C,D). Such positive impacts were also observed in plants grown in S-rich and S-limited soil that received amendments of 6:2 FTSA, RHA1, and 1-butanol. The results suggested that RHA1 might be a plant growth-promoting bacterium.

The plant-promoting effects and the possible mechanisms induced by RHA1 were further explored. Two assays were performed to assess if RHA1 (i) can produce indole-3-acetic acid (IAA), a significant plant auxin (i.e., a plant hormone) that regulates plant growth and development[35] and (ii) has a deaminase activity to degrade aminocyclopropane-1-carboxylic acid (ACC), a stress (ethylene) precursor of the plant.[34] While RHA1 did not have ACC deaminase activity (Figure S3A), RHA1 produced high concentrations of IAA when spiked with the IAA precursor, tryptophan (Figure S3B). Tryptophan is naturally present in root exudates of*A. thaliana*[38] or can be potentially derived from protein hydrolysis of dead cells in the soil.[39] The assay results suggested that RHA1 promoted plant growth by producing plant auxin IAA. This finding explained the observations of a higher biomass and root length in*A. thaliana* grown on the RHA1-spiked soil (Figure 2 and Figure S2).

Phytotoxicity of legacy long-chain PFAS (PFOA and PFOS) to different plant species has been intensively investigated.[15,40−43] However, knowledge about the phytotoxicity of their alternatives is limited. Inhibition of shoot and root growth was observed in*A. thaliana* and*Nicotiana benthamiana*grown on $^{1}/_{2}$ MS agar spiked with 20 mg/L GenX, an alternative of PFOA, while no growth defects were found when these two species were exposed to 5 mg/L GenX.[15] Zhang and Liang reported that 6:2 FTSA (200 μg/L) had no effects on the growth of hydroponic-grown *Lemna minor*.[44] In this study, exposure to 1.5 mg/kg of 6:2 FTSA did not cause growth and development inhibition of -*A. thaliana* seedlings grown on S-rich or S-limited sandy soil over 25 days. Our result indicated that-*A. thaliana*could resist the environment-related concentration of 6:2 FTSA and implied that native species similar to *A. thaliana*might be further screened for their suitability for the phytoremediation of 6:2 FTSA.

Both negative and positive impacts of *Rhodococcus* strains on the growth of different plant species have been reported.[45−47] *Rhodococcus fascians*is a plant pathogen that can infect a wide range of plant species and interfere with their development and growth.[45] *R. fascians*causes leafy gulls and retarded growth. Infected seedlings of*A. thaliana* resulted in bunches of shoots, whereas vacuum infiltrated infection causes the formation of numerous shoots and malformed leaves.[45] On the other hand, some *Rhodococcus* species can promote plant growth. A higher plant biomass, height, and germination rate was observed in *Pisum sativum* grown in soil with inoculation of *Rhodococcus erythropolis* MtCC 7905.[46] In our study, there is no malformation or growth inhibition of*A. thaliana* when exposed to RHA1 over 25 days (Figure 1A,B), indicating that RHA1 is not a plant pathogen. Bioaugmentation of three*R. jostii*species (3B12, 2B23, and 2B27) has also been shown to increase the biomass and root length of*A. thaliana*.[47]

3.3. Uptake of 6:2 FTSA in Plants Grown under Different Conditions. 6:2 FTSA accumulated at different levels in the roots and the shoots of*A. thaliana*(Figure 2E). Significantly higher 6:2 FTSA concentrations ($p < 0.05$) were observed in the shoots (32.7−59.1 mg/kg) than in the roots (4.3−15.8 mg/kg) of*A. thaliana*. The translocation factors of 6:2 FTSA between different sulfur and bioaugmentation treatments were similar, ranging from 3.8 to 8.1 (Figure 2F).

Under the S-rich conditions, no significant difference in 6:2 FTSA concentrations was observed in the shoot (43.4−51.3 mg/kg) or root (6.8−12.8 mg/kg) between each treatment (Figure 2E). The bioconcentration factor was 24.9−28.4 (Figure 2F). Interestingly, under S-limited conditions, higher 6:2 FTSA concentrations were observed in the shoot (45.5 mg/kg) and root (12.2 mg/kg) of*A. thaliana*grown on 6:2 FTSA soil compared to the shoot (25.1−27.3 mg/kg) and root (3.4−3.9 mg/kg) of*A. thaliana*grown on 6:2 FTSA soil with RHA1 or RHA1 and 1-butanol spiked treatment. The bioaccumulation factors were 33.1 and 16.1−18.4 in the absence and presence of RHA1, respectively. Hyperaccumulation of 6:2 FTSA, defined as the ratio of the concentration of 6:2 FTSA in tissue/the concentration of 6:2 FTSA in soil >10,[13] was found in*A. thaliana*grown in every treatment. The total uptake of 6:2 FTSA

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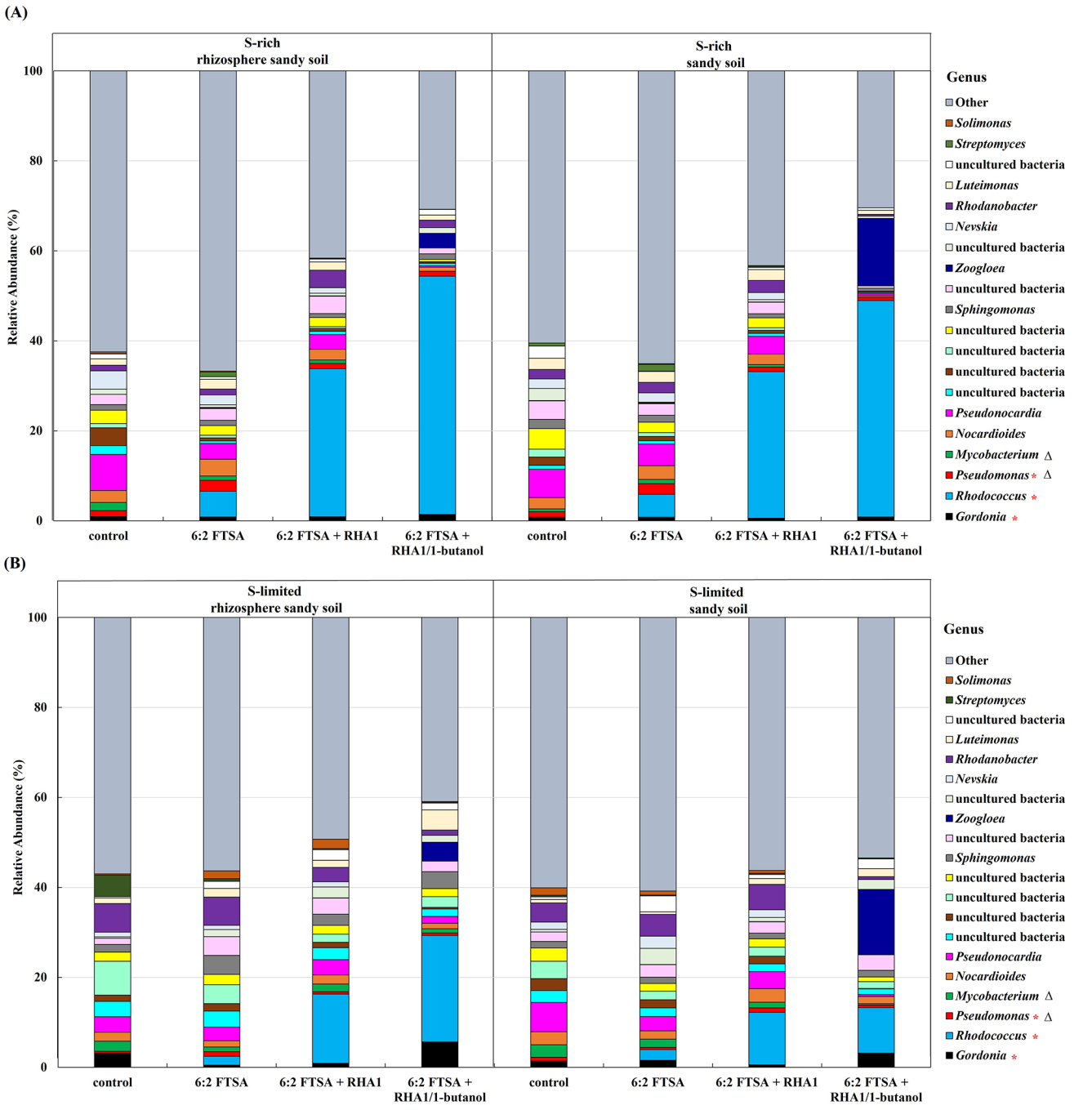

Figure 3. Spiked RHA1 becomes one of the dominant species in the microbial community. Relative abundance of the microbial community at the genus level in S-rich (A) and S-limited (B) sandy soil and rhizosphere sandy soil that received different treatments: 6:2 FTSA, 6:2 FTSA + RHA1, and 6:2 FTS + RHA1 + 1-butanol. The 20 taxa with the highest abundance were shown, while the other taxa were the sum and represented as "other". The red star indicates known 6:2 FTSA degraders,[31,34] while the black triangle indicates known 6:2 FTOH degraders.[49]

by *A. thaliana* grown in different treatments was 202.2−346.2 ng, which accounted for 1.3−2.3% removal efficiency.

Most research has been focused on the uptake and translocation of long- and short-chain perfluoroalkyl substances by plants,[13−19] with little attention on the uptake and translocation of precursors like 6:2 FTSA. A recent study reported accumulation of 6:2 FTSA in hydroponic-grown *Lemna minor*,[44] ranging from 60 to 1300 μg/kg of 6:2 FTSA in plant tissue when exposed for 14 days to 10 and 200 μg/L of 6:2 FTSA. The ratio of tissue/soil concentration for 6:2 FTSA was around 6 for *L. minor*, which does not pass the threshold of hyperaccumulation (i.e., tissue/soil concentration ≥ 10).[13] The total removal efficiency ranged from 0.3 to 0.6%,[44] much lower than those observed in this study (1.3−2.3%). Additionally, in this study, a high TF and BCF of 6:2 FTSA were observed in *A. thaliana* grown in sandy soil containing 1.5 mg/kg of 6:2 FTSA over 25 days. Along with our previous study reporting hyperaccumulation of GenX and PFOA by *A. thaliana*,[15] *A. thaliana* also hyperaccumulated 6:2 FTSA.

3.4. Recovery and Distribution of 6:2 FTSA in Sandy Soils with and without Plants That Received Different Treatments. The mass balance of 6:2 FTSA in both sandy soil

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

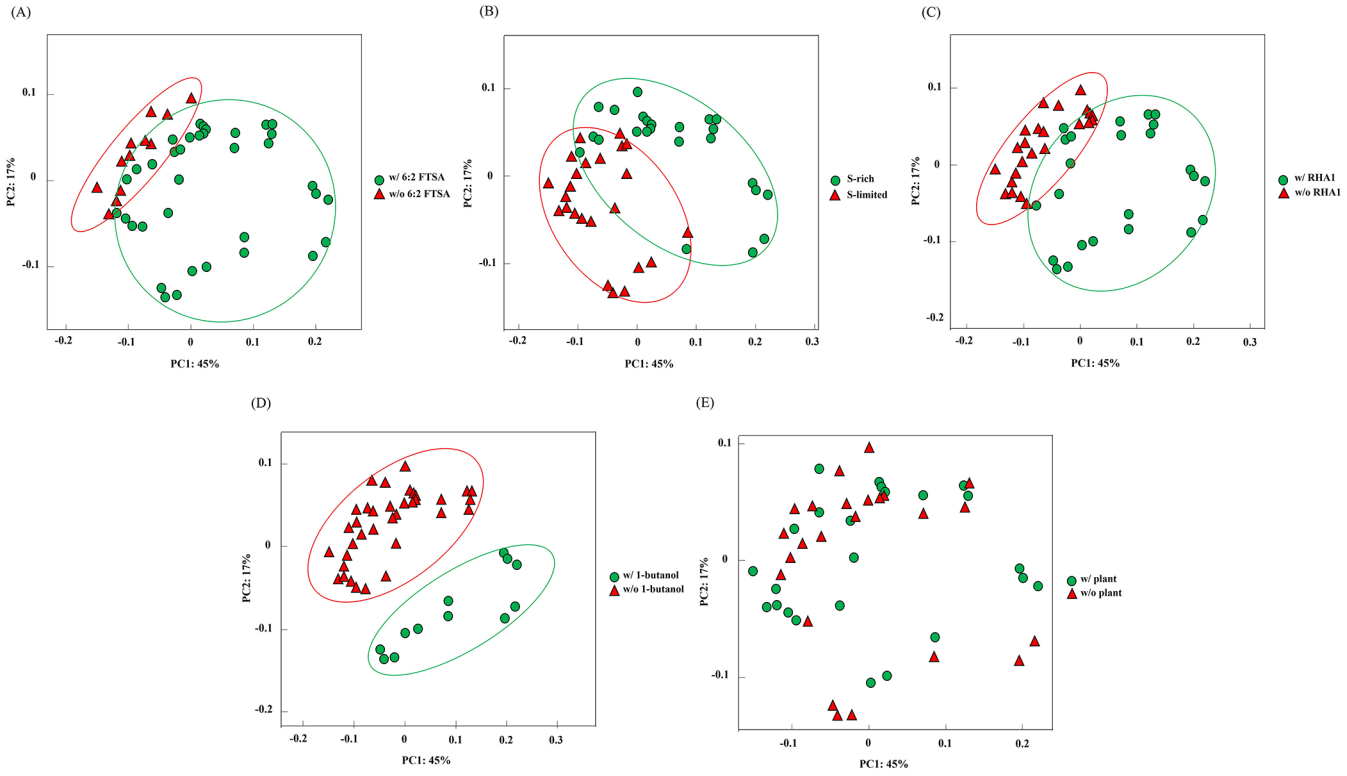

Figure 4. The composition of the microbial community is affected by 6:2 FTSA, sulfur, RHA1, and 1-butanol but not by plants. Principal coordinates analysis (PCoA) using the unweighted UniFrac distance of the microbial community. The data was highlighted based on with 6:2 FTSA and without 6:2 FTSA spiked treatments. (A), S-rich and S-limited conditions (B), with RHA1 and without RHA1 spiked treatments (C), with 1-butanol and without 1-butanol spiked treatments (D), and with plant and without plant (E).

and/or plant with other treatments was attempted and is shown in Figure S4. Under S-rich conditions, a high recovery of 6:2 FTSA from both plant and soil was observed under S-rich conditions, ranging from 89.7 to 99.1%. Most initial spiked 6:2 FTSA remained in sandy soil (89.7−97.1%). Approximately 1.6−2.3% of 6:2 FTSA was accumulated in plants under plant-grown conditions.

Under S-limited conditions, 6:2 FTSA and its metabolites were included in assessing the mass balance of 6:2 FTSA, showing a slightly lower range from 78.3 to 90%. Compared to those detected under S-rich conditions, much less 6:2 FTSA (77.1−88.1%) remained in the sandy soil. A slightly lower uptake of 6:2 FTSA by the plant (about 1.3−1.9% of 6:2 FTSA) in S-limited sandy soil than in S-rich sandy soil (1.6−2.3% of 6:2 FTSA) was observed. Combining 6:2 FTSA and its metabolites accounted for 1.2−1.8% recovery in S-limited sandy soil that received RHA1 and 1-butanol, the lower mass balance observed in S-limited sandy soil might be due to (i) formation of volatile products during the biotransformation, which were not captured in this experimental setup or (ii) formation of unknown transformation products, which were not in the targeted analysis used in this study. The results suggested that non-targeted PFAS (volatile and non-volatile) analysis is needed for seeking a better mass balance of 6:2 FTSA.

3.5. Diversity of Soil Microbiome. Figure S5 shows the relative abundance of the microbial community at the phylum level of S-rich and S-limited sandy soil and rhizosphere sandy soils with different treatments: 6:2 FTSA only, 6:2 FTSA + RHA1, and 6:2 FTSA + RHA1 + 1-butanol. After 25 days of receiving either S-free or S-rich Hoagland solution, *Proteobacteria* and *Actinobacteria* were the two dominant phyla in the microbiome of S-limited and S-rich sandy soil and rhizosphere sandy soil. The same dominant phyla with different abundances, *Proteobacteria* (32−65%) and *Actinobacteria* (19−58%), were observed in microbial communities of all samples (Figure S5). Figure 3 shows the relative abundance of the microbial community at the genus level of S-rich and S-limited sandy soil and rhizosphere sandy soil with different treatments.

Shannon index was used to assess the richness and evenness of the community in response to different treatments. A higher Shannon index suggests a higher diversity. Regardless of sulfur availability, Shannon indexes increased in 6:2 FTSA-spiked sandy soil and rhizosphere sandy soil (Figure S6A,B). However, amending RHA1 alone or together with 1-butanol significantly decreased the Shannon index, in part, due to (1) the enrichment of certain bacterial species that can directly use 1-butanol and (2) the spiked RHA1 outcompete the other indigenous bacteria in the microbial community.

Bray−Curtis dissimilarity was also used to determine the similarity and contrast of the microbial community structure between the two samples. Under S-rich conditions, significant differences of the microbial community were observed in 6:2 FTSA, 6:2 FTSA + RHA1, and 6:2 FTSA + RHA1/1-butanol treatment when compared individually to the control in rhizosphere sandy soil (i.e., no exposure to 6:2 FTSA) (Figure S7A). However, except for those soil amended with 6:2 FTSA, RHA1, and 1-butanol, the distance of the microbial community of control was very close to 6:2 FTSA and 6:2 FTSA + RHA1 spiked treatment in sandy soil (Figure S7B). Similar trends were observed under S-limited conditions (Figure S7C,D).

To identify unique and overlapping bacteria in response to different treatments, Venn plots were constructed and showed

https://doi.org/10.1021/acs.est.2c01867
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the number of bacteria at the genus level that was shared or unique among different treatments (Figure S8). In the control soils (i.e., no exposure to 6:2 FTSA), they were 179−193 genera in total; 209−264 genera in 6:2 FTSA-spiked soil; 188−219 genera in 6:2 FTSA and RHA1-spiked soil; and 84−170 genera in 6:2 FTSA, RHA1, and 1-butanol-spiked soil.

A smaller number of genera (57 (18.5%)) was shared among the four treatments (controls, 6:2 FTSA only, 6:2 FTSA + RHA1, and 6:2 FTSA + RHA1 + 1-butanol) of S-rich rhizosphere sandy soil when compared to that (93 (30.8%)) shared among four groups in S-limited rhizosphere sandy soil (Figure S8A,B). A similar trend, 84 (26.3%) versus 94 (30.8%), was observed for S-rich and S-limited sandy soil, respectively (Figure S8C,D).

The number of unique genera in each treatment was also identified. In the control groups of S-rich and S-limited sandy soil and rhizosphere sandy soils, 18−26 (5.9−8.4%) unique genera disappeared upon receiving 6:2 FTSA treatment. In the meantime, when 6:2 FTSA was amended, higher numbers of unique genera appeared in S-rich sandy soil (44, 13.7%) and S-rich rhizosphere sandy soil (37, 12%) than those in S-limited sandy soil (26, 8.9%) and S-limited rhizosphere sandy soil (14, 4.6%). When 6:2 FTSA and RHA1 were both introduced, 19−28 unique genera ranging from 5.9 to 9.2% responded to the treatment in S-rich and S-limited sandy soil. Relating to the two treatments that resulted in 6:2 FTSA biodegradation and metabolite production (Figure 1), 19 unique genera (6.5%) for S-limited rhizosphere sandy soil and 30 unique genera (9.8%) for S-limited sandy soil were observed. These unique genera are of particular interest as they might be the key players involved in 6:2 FTSA biodegradation under bioaugmented-phytoremediation and bioaugmentation of the sandy soils under S-limited conditions. Among these genera, two bacterial families, *Blastocatellaceae* and *Burkholderiaceae*, were found in this treatment at the highest frequency under S-limited conditions.

3.6. Dynamic of the Soil Microbiome. Soil microbiome of sandy soil and rhizosphere sandy soil changed in response to the available sulfur source, the amendment of RHA1, and the amendment of RHA1 and 1-butanol to different degrees. The dynamic was illustrated in principal coordinates analysis (PCoA) using the unweighted UniFrac distance of microbial community for tested conditions. As shown in Figure 4A, in the absence of 6:2 FTSA, the microbial community clustered closely compared to that in the presence of 6:2 FTSA. Similar cluster patterns were shown in sandy soil that received a high sulfur-containing Hoagland solution, amended with RHA1 or 1-butanol (Figure 4B−D). However, the presence or absence of plants in sandy soil did not cause clustering (Figure 4E). These results indicated that sulfur, 6:2 FTSA, RHA1, and 1-butanol were important drivers that caused significant shifts in microbial community composition, while the composition was not affected significantly in the presence of plants. Another set of PCoA plots also showed the same trend that the structure of the microbial community shifts due to 6:2 FTSA, RHA1, or 1-butanol amendment (Figure S9).

3.7. Abundance of Precursor Degraders. Several pure strains belonging to *Actinobacteria* are known to degrade precursors such as 6:2 FTSA and fluorotelomer alcohols (FTOHs).[28,31] They were detected at different levels in the sandy soil and rhizosphere sandy soil with different treatments (Figure 3). While the low abundance of *Rhodococcus* (0.1−0.4%) was present in the control soil (without exposure to 6:2 FTSA and bioaugmentation), a higher abundance of *Rhodococcus* (2−6%) was detected in 6:2 FTSA-amended sandy soil, suggesting that *Rhodococcus* is indigenous species and the amendment of 6:2 FTSA into the sandy soil enriched 6:2 FTSA degraders such as *Rhodococcus*. The increased abundance of *Rhodococcus* might explain the observations of the slight degradation of 6:2 FTSA (12%) in the S-limited sandy soil that received 6:2 FTSA (Figure 1B).

Rhodococcus was one of the dominant species in S-limited and S-rich RHA1-spiked sandy soil, accounting for 10.5−15.4% and 29.9−29.3%, respectively (Figure 3). When 1-butanol was amended along with RHA1, the population of *Rhodococcus* increased to 11.4−27.4% in S-limited sandy soil and to 48.3−52.9% in S-rich sandy soil. The results suggested that the spiked RHA1 could survive and outcompete the other indigenous bacteria in the microbial community. Our previous study had shown that RHA1 could use 6:2 FTSA as a sole S-source and 1-butanol as a carbon source and defluorination enzyme inducer under S-limited conditions.[28] As such, the high abundance of *Rhodococcus* in S-limited sandy soil that received RHA1 or RHA1 and 1-butanol might be linked to the observation of biotransformation of 6:2 FTSA and transformation products in these two treatments (Figure 1). However, despite the fact that *Rhodococcus* was the dominant species (about 50% of the community) in S-rich sandy soil that received RHA1 or RHA1 and 1-butanol, little or no biotransformation of 6:2 FTSA was observed (Figure 1A). Again, the results supported that the sulfur content in soil is an important factor controlling 6:2 FTSA biotransformation.

Two reported 6:2 FTOH degraders *Pseudomonas* from *Proteobacteria* and *Mycobacterium* from *Actinobacteria*[48] were detected at low abundance in most samples (Figure 3). The abundance of *Pseudomonas* in all soil samples ranged from 0.37 to 2.45%. About 0.3−2.74% of *Mycobacterium* was detected in all samples except those amended with RHA1 and 1-butanol. In addition, a 6:2 FTSA degrader *Gordonia* from *Actinobacteria*[31] was detected in all samples (0.48−5.65%). Interestingly, the abundance of *Gordonia* also increased in sandy soil that received RHA1 and 1-butanol, regardless of the sulfur content (Figure 3), suggesting that the addition of 1-butanol promotes the growth of not only RHA1 but also *Gordonia*. Also, a high abundance of *Zoogloea* was observed in samples that received 1-butanol. However, it was unclear if *Zoogloea* played a role in 6:2 FTSA biotransformation or they were simply co-occurrent with other known precursor degraders.

3.8. Predicted Functional Features. Within a microbial community, diverse and multiple species can perform similar catabolic functions that affect the microbial community structure over time or in response to stimuli. To evaluate the functional capacity of the soil microbiome in sandy soil and rhizosphere sandy soil that received different treatments, Tax4Fun2 was used to predict functional features based on the 16S rRNA gene sequencing data.[49−53] Our previous study reported desulfonation and defluorination enzymes involved in 6:2 FTSA biodegradation by RHA1.[28] So, the analysis was focused on these known desulfonation and defluorination enzymes. The profiles and abundance of the desulfonating and defluorinating genes encoding the desulfonating enzyme, alkanesulfonate monooxygenase, and defluorinating enzymes, haloacetate monooxygenase, alkane monooxygenase, and cytochrome P450, in the microbial community were predicted, as shown in Figure S10. A slightly higher abundance of defluorinating and desulfonating genes (0.1%−0.13% in total) was observed in 6:2 FTSA-spiked soil than the control (0.1%−

https://doi.org/10.1021/acs.est.2c01867
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0.11% in total) under both S-limited and S-rich conditions. In RHA1- and RHA1/1-butanol-spiked soil, an increasing trend of these genes was observed under both S-limited and S-rich conditions (0.13−0.21% in total). The high abundance of the desulfonating and defluorinating genes found in RHA1/1-butanol treatment explains why the higher biotransformation and more transformation products were observed under S-limited conditions. However, even though the highest abundance of desulfonating and defluorinating genes was observed in RHA1/1-butanol treatment under S-rich conditions, low biotransformation of 6:2 FTSA and no transformation products observed again support our statement that the sulfur content in soil play an important role in the biotransformation of 6:2 FTSA. Note that in this study, the functional features predicted by the Tax4fun2 program are based on 16S rRNA gene sequences. Thus, future studies using whole-genome sequencing-based approaches are needed to validate the predicted functional profiles and to elucidate other genes that might be involved in 6:2 FTSA biotransformation.

3.9. Implications. In this study, we observed no significant impacts of growth and development of*A. thaliana* seedlings grown in sandy soil with the environmental relevant concentration (1.5 mg/kg) of 6:2 FTSA.[7,8] The high translocation factor and hyperaccumulation of 6:2 FTSA observed in this study implied the potential of screening species similar to*A. thaliana*for phytoremediation of 6:2 FTSA-impacted soil. The low biotransformation of 6:2 FTSA observed in the S-rich soil confirmed that the sulfur content of soil plays an important role in the bioremediation of 6:2 FTSA, and an effective means is needed to remove sulfur availability in the soil to enhance 6:2 FTSA biotransformation. Despite the fact that no increase in biotransformation of 6:2 FTSA was observed in RHA1- or RHA1/1-butanol spiked treatment under S-rich conditions, the promotion of plant growth and total uptake of 6:2 FTSA was found in RHA1-amended treatments, suggesting the potential of bioaugmented phytoremediation of 6:2 FTSA. On the other hand, the observations of (i) biotransformation of 6:2 FTSA and its transformation products and (ii) the domination of *Rhodococcus* in the microbial community under S-limited conditions indicate that the bioaugmentation and enzyme inducer spiking can serve as effective ways to degrade 6:2 FTSA.

A slightly low biotransformation of 6:2 FTSA observed in rhizosphere sandy soil compared to those in the sandy soil was surprising. Studies have reported that the root exudate secreted out from plant roots can facilitate the microbial activity in the rhizosphere, increase the abundance of functional genes and degraders, and promote the biodegradation of contaminants such as polycyclic aromatic hydrocarbons (PAHs).[54,55] As the compositions of root exudate are complex, which usually contain organic acid, amino acid, sugar, enzymes, and secondary metabolites,[38] a possible explanation for this phenomenon is that those sulfur-containing amino acids (cysteine and methionine) or secondary metabolites present in root exudates potentially serve as the sulfur source to RHA1 or other possible indigenous 6:2 FTSA degraders and simultaneously repress the expression of desulfonating genes and thus hinder the biotransformation of 6:2 FTSA.[28] As the profile of root exudates is species-dependent and varies under different growth conditions, more studies are needed to confirm and understand the effects of root exudate on PFAS bioremediation. Overall, these data enhance our understanding on phytoremediation and bioremediation of 6:2 FTSA, which can be used to design the large-scale bioremediation of 6:2 FTSA from AFFF-impacted soil in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c01867.

The supporting information contains materials and methods, 3 table, and 10 figures. (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the National Science Foundation (award number 1914707) and partially by the National Institute for Environmental Health Science Center P30 ES029067 and Strategic Environmental Research and Development Program (ER22-3124).

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https://doi.org/10.1021/acs.est.2c01867
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Supporting Information

for

Fate and Transformation of 6:2 Fluorotelomer Sulfonic Acid (6:2 FTSA) Affected by Plant, Nutrients, Bioaugmentation, and Soil Microbiome Interactions

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This supporting information includes text describing methods, 3 tables, and 10 figures.

Methods

- Chemicals
- Sulfur rich (S-rich) and Sulfur free (S-free) Hoagland solution
- Pot preparation
- Seed sterilization
- Aminocyclopropane-1-carboxylic acid (ACC) deaminase activity
- Indole-3-acetic acid (IAA) production assay
- Extraction of 6:2 FTSA and its transformation products
- Tolerance index (TI), Bioconcentration factor (BCF) and Translocation factor (TF)

Tables

Table S1. The pH, conductivity, and major nutrients composition of the sandy soil used in this study.

Table S2. Treatments and experimental setup. One surfaced-sterilized *Arabidopsis thaliana* were grown in each rhizosphere soil. The concentration of 6:2 FTSA were 1.5 mg/L.

RHA1 culture 150 μL (OD_{600} = 1) were added to bioaugmentation treatment. Pure 1-butanol 15 mg were spiked to the corresponding treatment.

Table S3. The mass and molar yields of 6:2 FTSA and its transformation products in sandy soil and rhizosphere sandy soil after 25 days incubation. Each treatment was performed in triplicate (n=3). The errors represented the standard deviation of the triplicates.

Figures

Figure S1. Experimental setup of the pots (15- mL polypropylene tubes) and the reservoirs (50-mL polypropylene tubes) used for the growth of *A. thaliana*. Five holes were poked at the bottom of the pot. 10 mL of corresponding Hoagland solution was added to the reservoirs every 7 days.

Figure S2. The root length, root weight and shoot weight of *A. thaliana* grown in S-rich (A-C) and S-limited (D-F) sandy soils with different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTSA+RHA1+1-butanol. Each treatment was performed in triplicate (n=3). The bars represented the standard deviation of the triplicates.

Figure S3. ACC deaminase activity (A) and IAA production assay (B) of RHA1 cell. Each assay was performed in duplicate (n=2). The bars represented the range of the duplicates.

Figure S4. The mass balance of 6:2 FTSA under S-rich (A) and S-limited conditions (B). Each treatment was performed in triplicate (n=3).

Figure S5. Relative abundance of the microbial community at phylum level in S-rich (A) and S-limited (B) sandy soil and rhizosphere sandy soil with different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTS+RHA1+1-butanol. Each treatment was performed in triplicate (n=3).

Figure S6. The Shannon index of the soil microbial communities in S-rich (A) and S-limited (B) sandy soil and rhizosphere sandy soil receiving different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTS+RHA1+1-butanol. The asterisk indicates a significant difference ($p < 0.05$) compared to control.

Figure S7. Pairwise comparison Bray-Curtis dissimilarity of the soil microbial communities between control and different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTS+RHA1+1-butanol in S-rich rhizosphere sandy soil (A), S-rich sandy soil (B), S-limited rhizosphere sandy soil (C) and S-limited sandy soil (D). The asterisk indicates a significant difference ($p < 0.05$) compared to control.

Figure S8. Venn diagrams of the microbial community showing the degree of unique and overlap bacterial genera in S-rich rhizosphere sandy soil (A), S-limited rhizosphere sandy soil (B), S-rich sandy soil (C), and S-limited sandy soil (D). As shown in (D), 30 unique genera were identified in this treatment and their family were shown as possible 6:2 FTSA degraders in the corresponding samples. Each treatment was performed in triplicate (n=3).

Figure S9. Principal Coordinates Analysis (PCoA) using unweighted UniFrac distance of the microbial community among different treatments. S-rich rhizosphere sandy soil (A), S-rich sandy soil (B), S-limited rhizosphere sandy soil (C), and S-limited sandy soil (D). Each treatment was performed in triplicate (n=3).

Figure S10. Abundance of defluorinating and deslufonating gene in the microbial community in S-rich (A) and S-limited (B) rhizosphere sandy soil and sandy soil. Each treatment was performed in triplicate (n=3). The bars represented the standard deviation of the triplicates.

Materials and Methods

- **Chemicals**

1H, 1H, 2H, 2H-perfluorooctanesulfonic acid (6:2 FTSA, CAS# 27619-97-2, 98% pure), 1H, 1H, 1H-perfluoroheptan-2-one (5:2 ketone, CAS# 2708-07-8, 97% pure), 4,4,5,5,6,6,7,7,7-nonafluoroheptanoic acid (4:3 FTCA, CAS# 80705-13-1, 97% pure) and 2H,2H,3H,3H-perfluorooctanoic acid (5:3 FTCA, CAS# 914637-49-3, 97% pure) were obtained from Synquest Laboratories (Alachua, FL, USA). 2-perfluorohexyl ethanoic acid (6:2 FTCA, CAS# 53826-12-3, 98% pure), 2H-perfluoro-2-octenoic acid (6:2 FTUCA CAS# 70887-88-6, 98% pure), 1-perfluoropentyl ethanol (5:2 sFTOH, CAS# 914637-05-1, 98% pure) were purchased from Wellington Laboratories (Guelph, Canada). Perfluorobutyric acid (PFBA, CAS# 375-22-4, 98% pure), perfluoropentanoic acid (PFPeA, CAS# 2706-90-3, 97% pure), perfluorohexanoic acid (PFHxA, CAS# 307-24-4, 97% pure), perfluoroheptanoic acid (PFHpA, CAS# 375-85-9, 99% pure) and the graphitized non-porous carbon powder Supelclean™ ENVI-Carb™ were obtained from Sigma- Aldrich (St. Louis, MO, USA). ACS reagent grade dichloromethane (DCM) was obtained from Acros Organics (Pittsburgh, PA, USA). HPLC grade methanol (MeOH) and trace metal grade ammonium hydroxide (20%), and 1-butanol (99.4% pure) were obtained from Fisher Scientific (Pittsburgh, PA, USA). A stock solution of 6:2 FTSA (1 g/L) was prepared in ethanol.

- **Sulfur rich (S-rich) and Sulfur free (S-free) Hoagland solution**

Sulfate was the sole sulfur source in the S-rich Hoagland solution [1] consisting of EDTA-FeNa (36.7 mg/L), $Ca(NO_3)_2 \cdot 4H_2O$ (590.4 mg/L), $NH_4H_2PO_4$ (57.5 mg/L), $MgSO_4 \cdot 7H_2O$ (246.5 mg/L), KNO_3 (252.5 mg/L) and trace minerals: H_3BO_3 (1.24 mg/L), $ZnSO_4 \cdot 7H_2O$ (0.29 mg/L), $CuSO_4 \cdot 5H_2O$ (0.04 mg/L), $MnSO_4 \cdot H_2O$ (0.17 mg/L), and $(NH_4)_6Mo_7O_{24}$ (6.18 mg/L). For S-free Hoagland solution, it was prepared as follows: of EDTA-FeNa (36.7 mg/L), $Ca(NO_3)_2 \cdot 4H_2O$ (590.4 mg/L), $NH_4H_2PO_4$ (57.5 mg/L), $MgCl_2 \cdot 6H_2O$ (203.3 mg/L), KNO_3 (252.5 mg/L) and trace mineral: H_3BO_3 (1.24 mg/L) $ZnCl_2$ (0.14 mg/L) $CuCl_2 \cdot 2H_2O$ (0.05 mg/L) $MnCl_2 \cdot 4H_2O$ (6.18 mg/L), and $(NH_4)_6Mo_7O_{24}$ (6.18 mg/L).

- **Pot preparation**

Five holes were created at the bottom of each pot (i.e., the 15-mL tube) for drainage. Fifteen grams of soil were added to each pot which was then wrapped with aluminum foil to avoid light exposure. To provide adequate soil moisture, ten milliliters of corresponding Hoagland solution were added weekly to the reservoirs (the solution volume was determined in the lab).

- **Seed surface-sterilization and growth condition**

Seeds of *A. thaliana* ecotype Col-0 were surface-sterilized by soaking in 70% ethanol for 5 min and 50% bleach for 10 min, followed by rinsing with sterile deionized-water for five times and then were placed at 4°C in dark for 24 hours to synchronize seedling emergence rates [1]. Plants were grown with a 16/8 hour light/dark period with a light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$ at 23 ± 1°C.

- **Aminocyclopropane-1-carboxylic acid (ACC) deaminase activity**

The ACC deaminase activity of RHA1 were conducted by following the previous study [2]. Briefly, RHA1 cells were prepared by growing in R2A medium to optical density (OD_{600}) 0.9 before harvesting by centrifugation, washed and resuspend in nitrogen-free medium. ACC (3 mM), as an inducer for ACC deaminase, was then added to the cell suspension which was then incubated at 30 ºC at 150 rpm overnight. Cell in nitrogen free medium contained nitrate and glucose were served as negative control. The ACC-induced cells were then collected by centrifugation, washed and responded in Tris-HCl buffer for experimental use. The assay was conducted by incubating 0.2 µL of cell and 20 µL ACC stock solution (0.5 M) at 30 ºC for 15 min. The production of α-ketobutyrate were then measured by spectrophotometer at OD_{540} as described in the study [2].

- **Indole-3-acetic acid (IAA) production assay**

The IAA production assay were conducted as described in the previous study [3]. Briefly, RHA1 cells were first grown in R2A medium to optical density (OD_{600}) 0.9. Cell were then sub-cultured in 10 mL minimal salt medium containing 1 mL tryptophan (50 mM) and glucose (10%) stock solution. RHA1 culture contained only glucose was prepared as negative control. Cell were incubated at 30 ºC at 150 rpm for 48 hr. To measure the IAA production, 1.5 mL culture were centrifuge at 13000 rpm for 5 minutes. One milliliter of supernatant was added to 2 ml $FeCl_3$-H_2SO_4 reagent as described in the study. Reaction was conducted at room temperature for 25 minutes. The concentration of IAA was measured by spectrophotometer at OD_{530}.

- **Extraction of 6:2 FTSA and its transformation products**

Samples of soil, roots, and shoots were freeze-dried for 24 hours before stored at -20 ºC for later chemical analysis. The 6:2 FTSA and its transformation products in the soil and plant biomass was extracted as described by Chen et al 2019 [1]. Briefly, a mixture of 50:50 (v/v) of DCM and MeOH with 1% ammonia hydroxide (v/v) was prepared as extraction solvent. Samples were extracted in a 15-mL polypropylene tube containing 5 mL of the extraction solvent. After vortexed for 30 sec, the tube was shaken on a shaker (KS 260 basic, IKA®, USA) at 200 rpm at 37°C for 1 hr. The supernatant was then collected by centrifugation at 1500 rpm for 5 min (Sorvall™ Legend™ XTR, Thermo scientific, USA). The extraction process was repeated twice. The extracts were pooled, followed by drying under a gentle nitrogen stream. The dried extract was reconstituted with 1 mL of MeOH, and then cleaned up by 50 mg of ENVI-Carb. Based on spike-recovery tests, the average recovery of this extraction process was 98.2% and 89.1%, respectively for soil and plant biomass.

- **Tolerance index (TI), Bioconcentration factor (BCF) and Translocation factor (TF)**

After 25 days, the plant biomass (roots and shoots) and root length were measured. Tolerance index (TI) was used to determined effects of different treatments on plants growth. It is defined as the ratio of phenotypes (biomass or root length) of plants grown in different treatments (Eq.1) [1].

$$Tolerance\ Index\ (TI) = \frac{mean\ phenotype\ measurement\ of\ treatment}{mean\ phenotype\ measurement\ of\ control} \quad \text{Eq.1}$$

Two indices, bioconcentration factor (BCF), and translocation factor (TF), were used to assess the uptake and mobility of 6:2 FTSA in plants grown in different treatment. The tendency of uptake of 6:2 FTSA can be assessed by bioconcentration factor defined as the ratio of the concentration of 6:2 FTSA in plants over the concentration of 6:2 FTSA in soil (Eq. 2) [1]. Translocation factor (TF) explains the mobility of 6:2 FTSA moving from the root system to the shoot system of the plant which was calculated by dividing the concentration of 6:2 FTSA in shoot by the concentration of 6:2 FTSA in root (Eq.3) [1].

$$Bioconcentration\ factor\ (BCF)\ \frac{\textbf{chemical concentration in plant biomass}}{\textbf{Initial 6:2 FTSA concentration in soil}} \quad \text{Eq.2}$$

$$Translocation\ factor\ (TF)\ = \frac{\textbf{6:2 FTSA concentration in shoot}}{\textbf{6:2 FTSA concentration in root}} \quad \text{Eq.3}$$

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Table S1. The pH, conductivity, and major nutrients composition of the sandy soil used in this study.

pH	7.2	Ca^{2+}	225 mg/kg
Conductivity	193 µmho/cm	Mg^{2+}	32 mg/kg
NO_3^-	32 mg/kg	SO_4^{2-}	11 mg/kg
PO_4^{2-}	10 mg/kg	Na^+	40 mg/kg
K^+	23 mg/kg		

Table S2. Treatments and Experimental setup. One surfaced-sterilized *Arabidopsis thaliana* was grown in each rhizosphere soil. The concentration of 6:2 FTSA were 1.5 mg/L. RHA1 culture (150 µL of OD_{600} = 1) was added to bioaugmentation treatment. Pure 1-butanol (15 mg) was spiked to the corresponding treatment.

	Plant	PFAS	Bioaugmentation	Carbon source
S-limited sandy soil				
Control				
6:2 FTSA		6:2 FTSA		
6:2 FTSA + RHA1		6:2 FTSA	RHA1	
6:2 FTSA + RHA1		6:2 FTSA	RHA1	1-butanol
S-limited rhizosphere soil				
Control	*A. thaliana*			
6:2 FTSA	*A. thaliana*	6:2 FTSA		
6:2 FTSA + RHA1	*A. thaliana*	6:2 FTSA	RHA1	
6:2 FTSA + RHA1	*A. thaliana*	6:2 FTSA	RHA1	1-butanol
S-rich sandy soil				
Control				
6:2 FTSA		6:2 FTSA		
6:2 FTSA + RHA1		6:2 FTSA	RHA1	
6:2 FTSA + RHA1		6:2 FTSA	RHA1	1-butanol
S-rich rhizosphere soil				
Control	*A. thaliana*			
6:2 FTSA	*A. thaliana*	6:2 FTSA		
6:2 FTSA + RHA1	*A. thaliana*	6:2 FTSA	RHA1	
6:2 FTSA + RHA1	*A. thaliana*	6:2 FTSA	RHA1	1-butanol

Table S3. The mass and molar yields of 6:2 FTSA and its transformation products in sandy soil and rhizosphere sandy soil after 25 days incubation. Each treatment was performed in triplicate (n=3). The errors represented the standard deviation of the triplicates.

	6:2 FTSA		6:2 FTUCA		PFHpA		PFHxA		PFPeA	
	Mass (nmole)	Mol %	Mass (nmole)	Mol %	Mass (nmole)	Mol %	Mass (nmole)	Mol %	Mass (nmole)	Mol %
Time 0	47.30 ± 2.06	100	0	0	0	0	0	0	0	0
S-limited Rhizosphere soil										
Control	0	0	0	0	0	0	0	0	0	0
6:2 FTSA	41.67 ± 0.18	88.1	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1	39.44 ± 2.01	83.4	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1/1-butanol	38.13 ± 0.55	80.6	0.86	1.8	0	0	0	0	0	0
S-limited Sandy soil										
Control	0	0	0	0	0	0	0	0	0	0
6:2 FTSA	40.92 ± 1.36	86.5	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1	38.13 ± 1.89	80.6	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1/1-butanol	36.50 ± 1.50	77.1	0.24[a]	0.5	0.14 [a]	0.3	0.10 [a]	0.2	0.05 [a]	0.1
S-rich Rhizosphere soil										
Control	0	0	0	0	0	0	0	0	0	0
6:2 FTSA	44.98 ± 0.49	95.1	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1	46.07 ± 0.70	97.4	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1/1-butanol	46.22 ± 1.66	97.7	0	0	0	0	0	0	0	0
S-rich Sandy soil										
Control	0	0	0	0	0	0	0	0	0	0
6:2 FTSA	42.79 ± 0.76	90.5	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1	43.34 ± 1.66	91.6	0	0	0	0	0	0	0	0
6:2 FTSA + RHA1/1-butanol	42.43 ± 2.50	89.7	0	0	0	0	0	0	0	0

a. The errors are smaller than 0.01 nmole

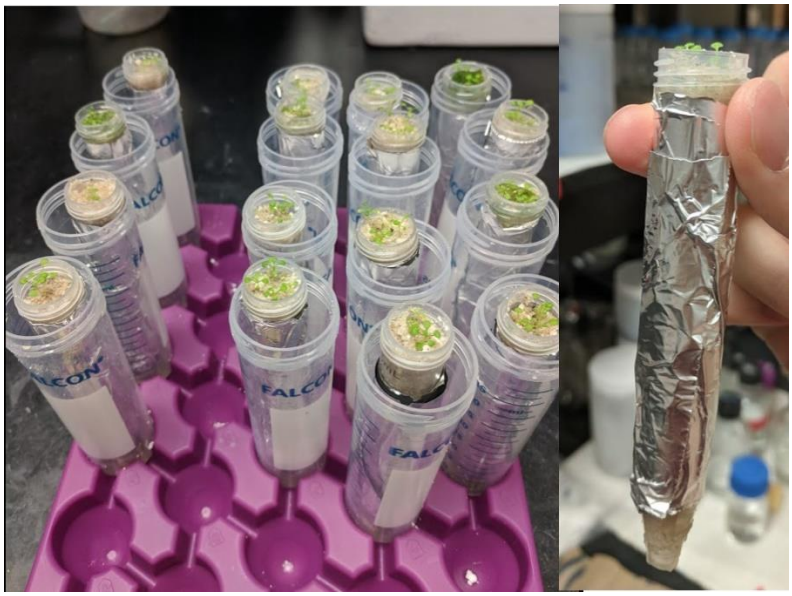


Figure S1. Experimental setup of the pots (15- mL polypropylene tubes) and the reservoirs (50-mL polypropylene tubes) used for the growth of *A. thaliana*. Five holes were poked at the bottom of the pot. 10 mL of corresponding Hoagland solution was added to the reservoirs every 7 days.

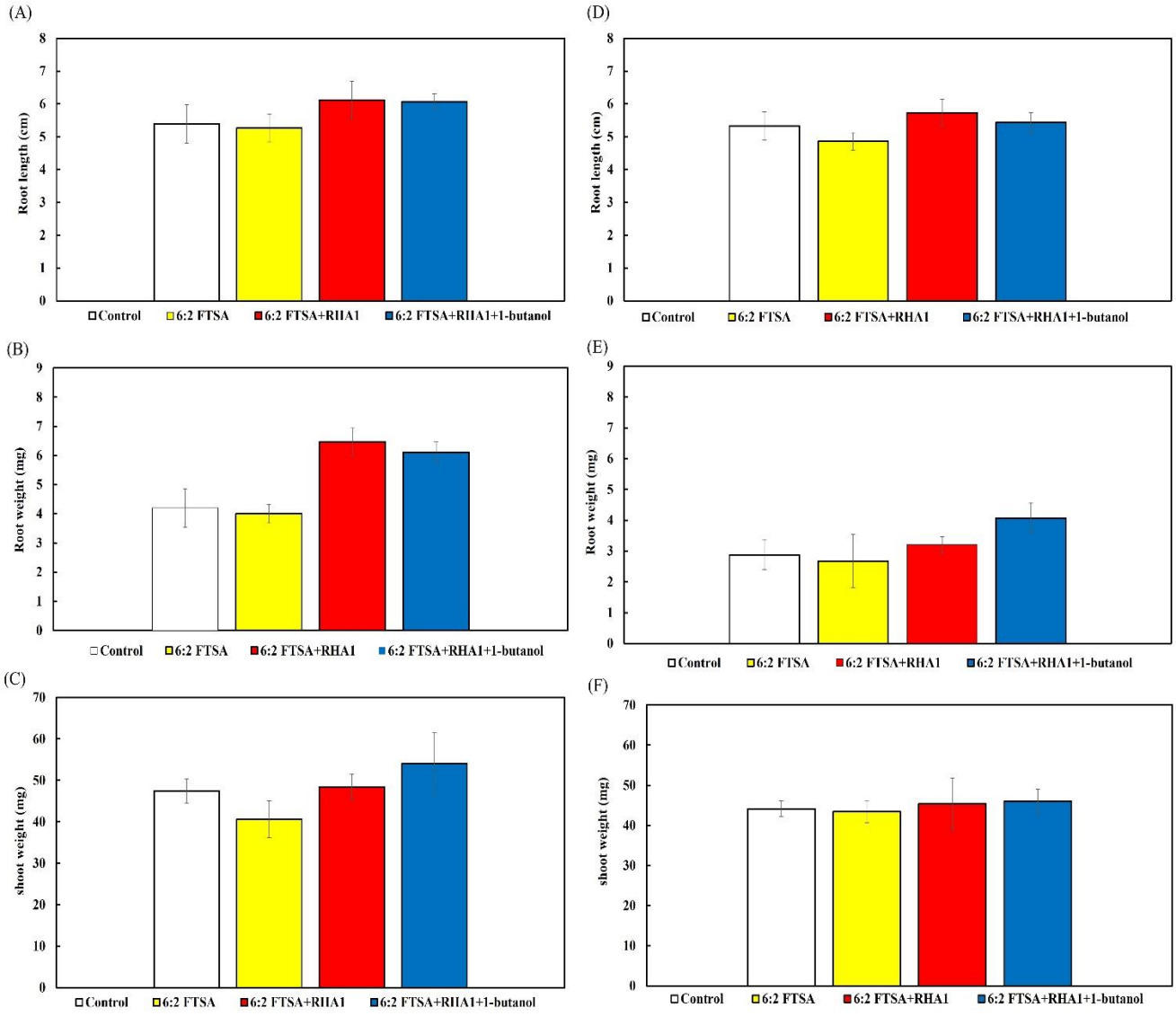


Figure S2. The root length, root weight and shoot weight of *A. thaliana* grown in S-rich (A-C) and S-limited (D-F) sandy soils with different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTSA+RHA1+1-butanol. Each treatment was performed in triplicate (n=3). The bars represented the standard deviation of the triplicates.

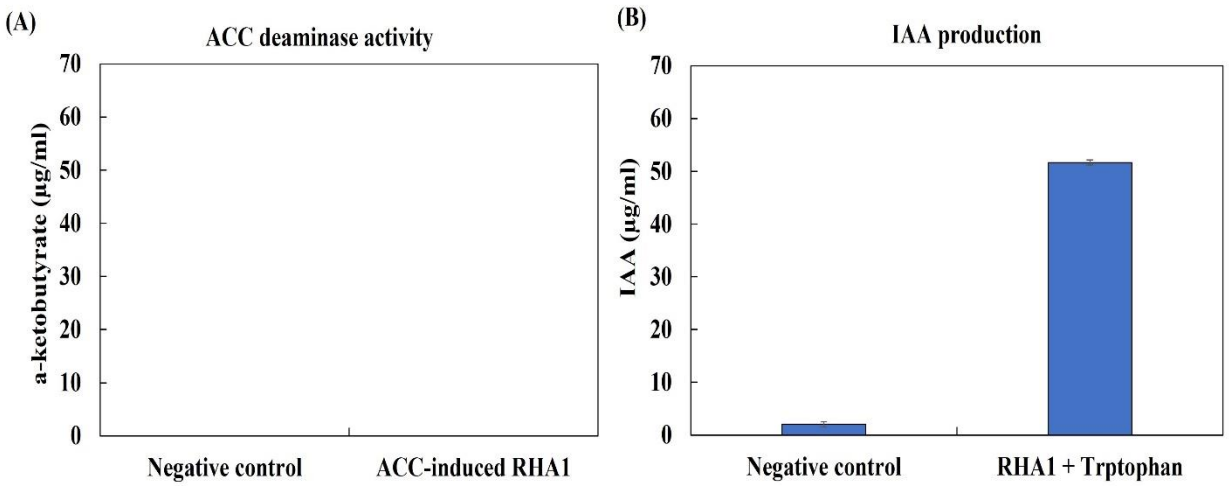


Figure S3. ACC deaminase activity (A) and IAA production assay (B) of RHA1 cell. Each assay was performed in duplicate (n=2). The bars represented the range of the duplicates.

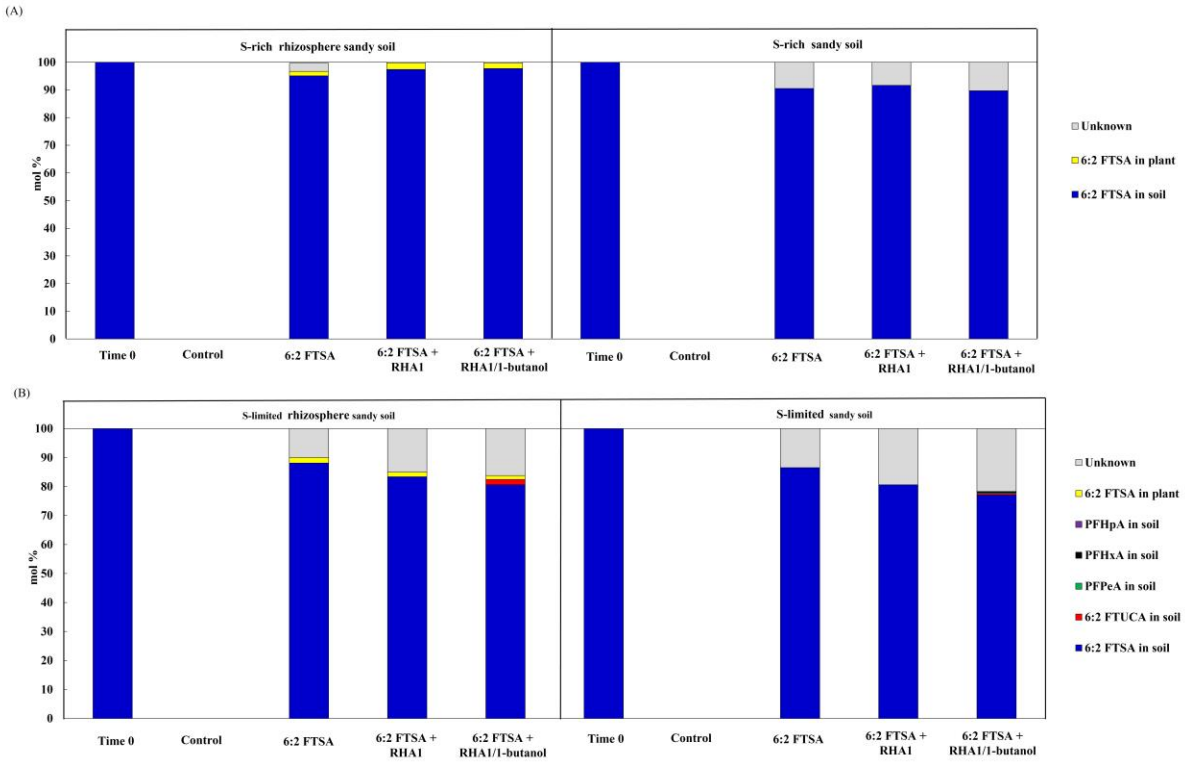
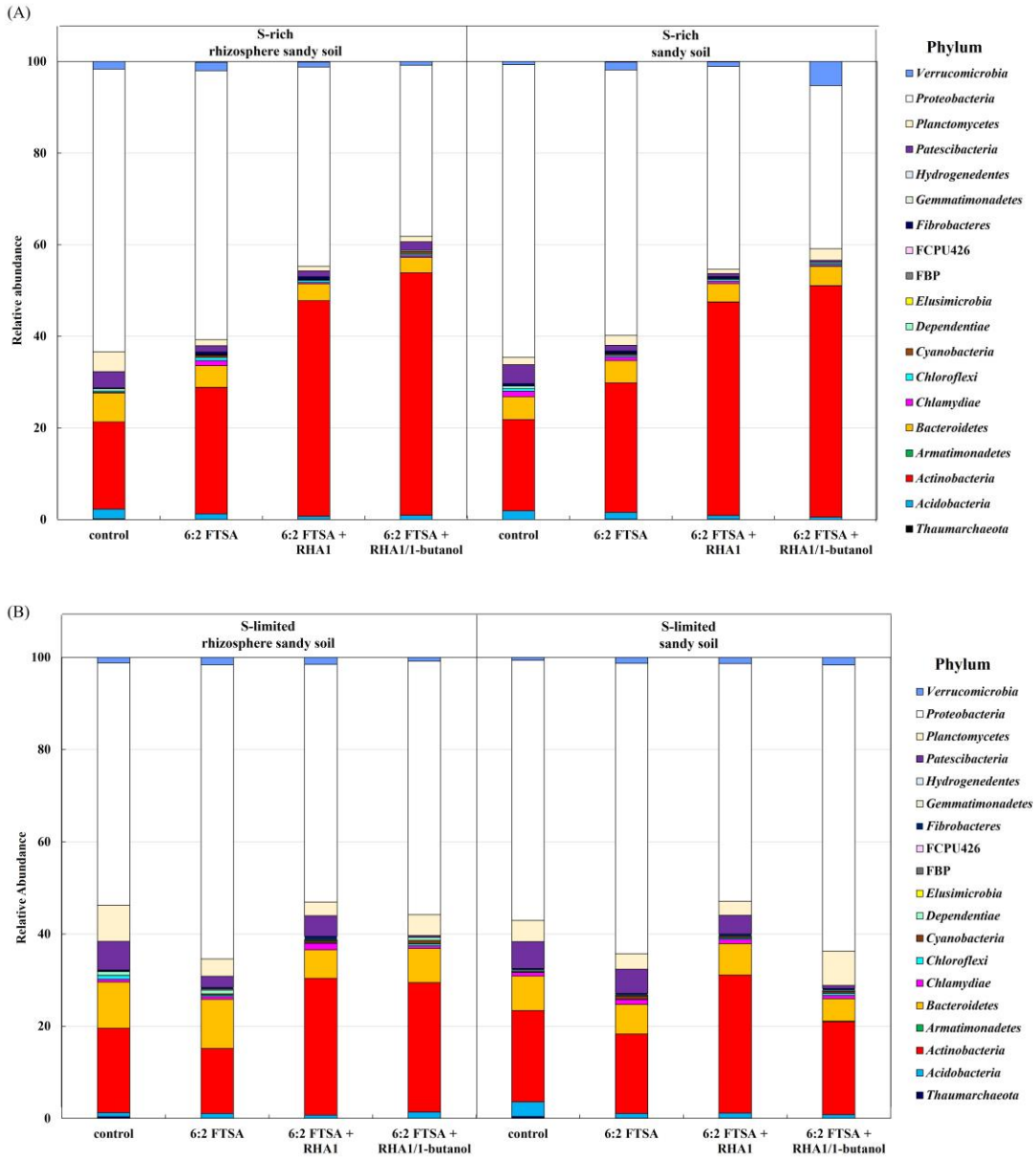


Figure S4. The mass balance of 6:2 FTSA under S-rich (A) and S-limited conditions (B). Each treatment was performed in triplicate (n=3).



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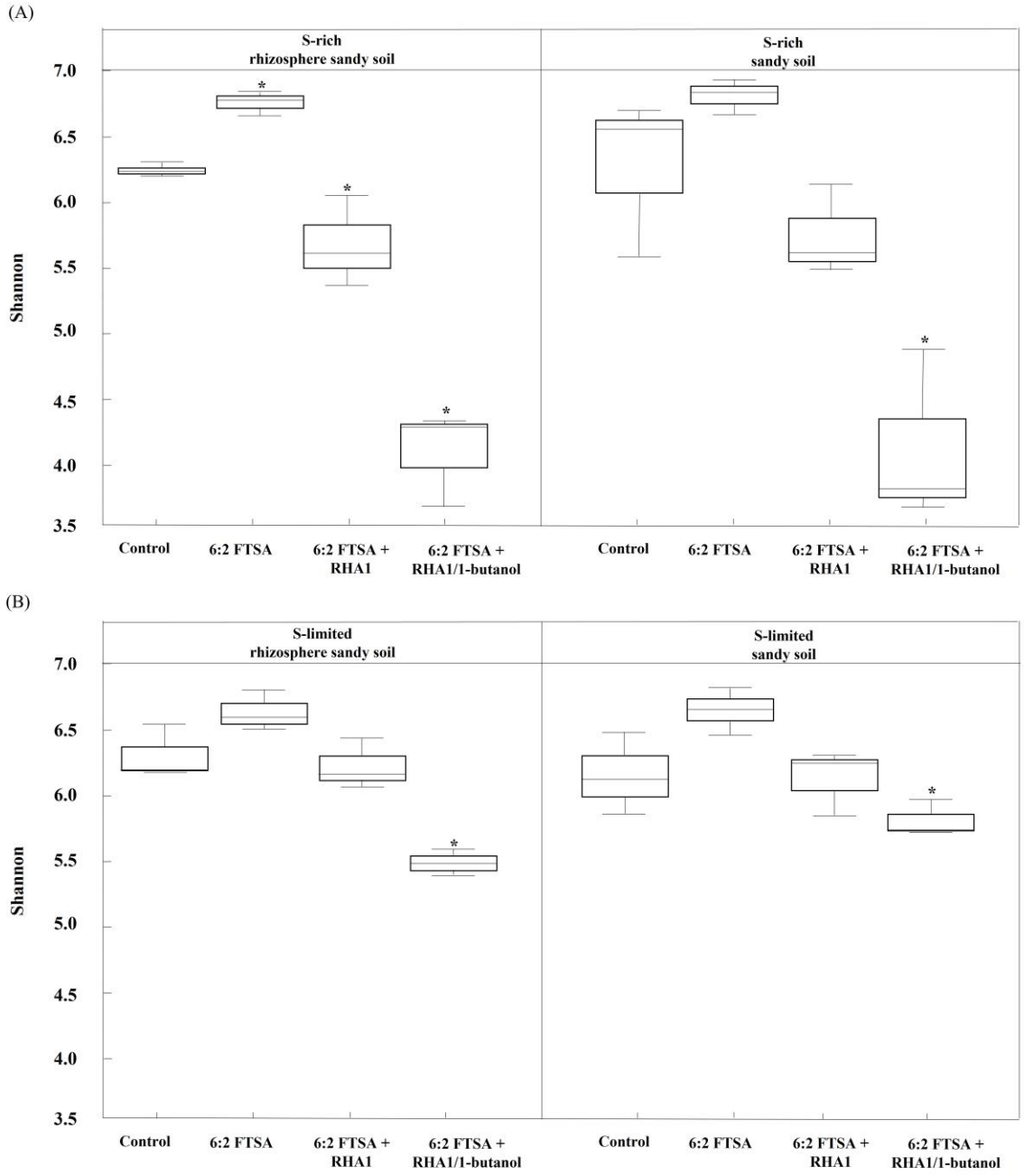

Figure S6. The Shannon index of the soil microbial communities in S-rich (A) and S-limited (B) sandy soil and rhizosphere sandy soil receiving different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTS+RHA1+1-butanol. The asterisk indicates a significant difference ($p < 0.05$) compared to control.

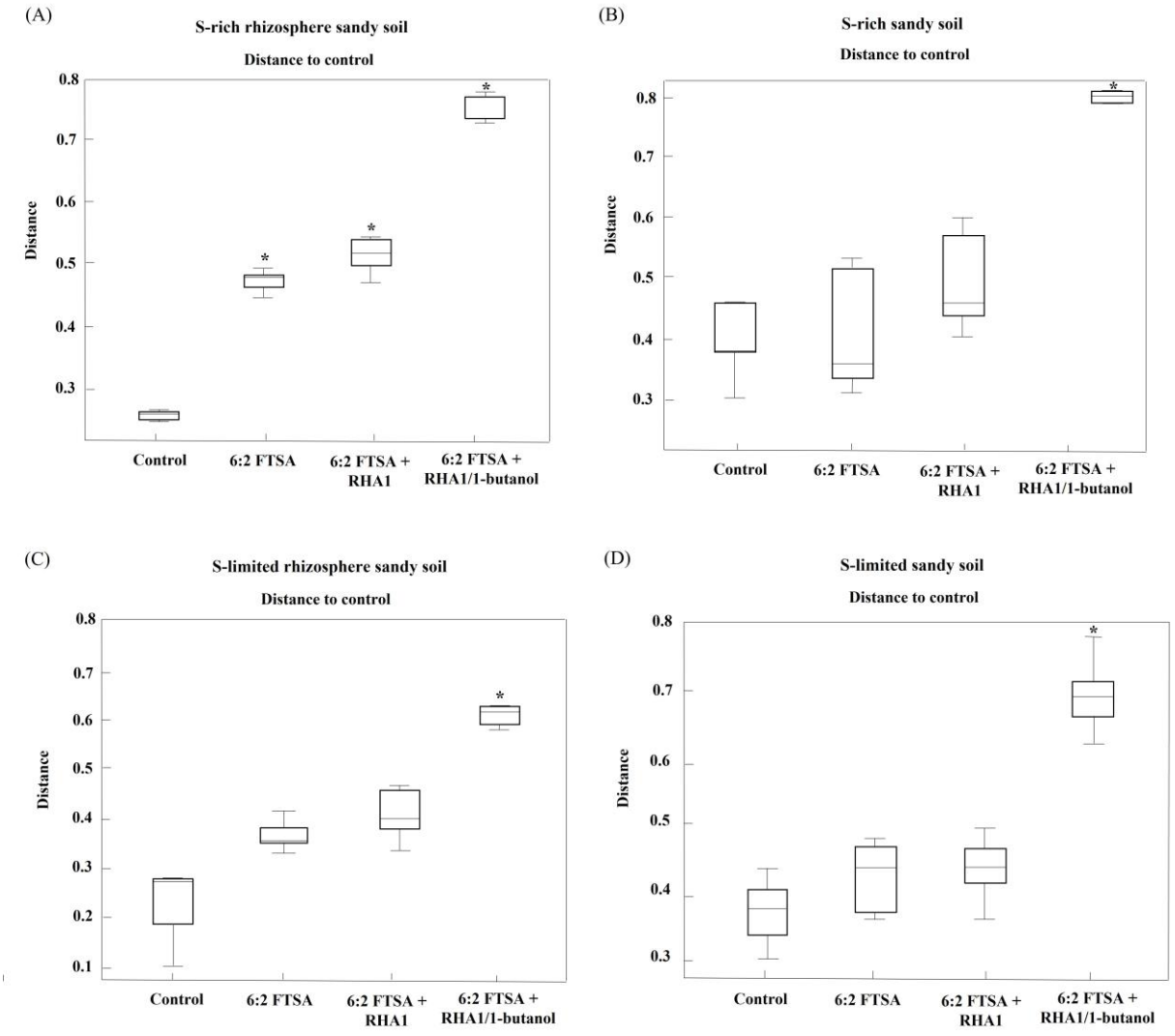

Figure S7. Pairwise comparison Bray-Curtis dissimilarity of the soil microbial communities between control and different treatments: 6:2 FTSA, 6:2 FTSA+ RHA1, and 6:2 FTS+RHA1+1-butanol under S-rich rhizosphere sandy soil (A), S-rich sandy soil (B), S-limited rhizosphere sandy soil (C) and S-limited sandy soil (D). The asterisk indicates a significant difference ($p < 0.05$) compared to control.

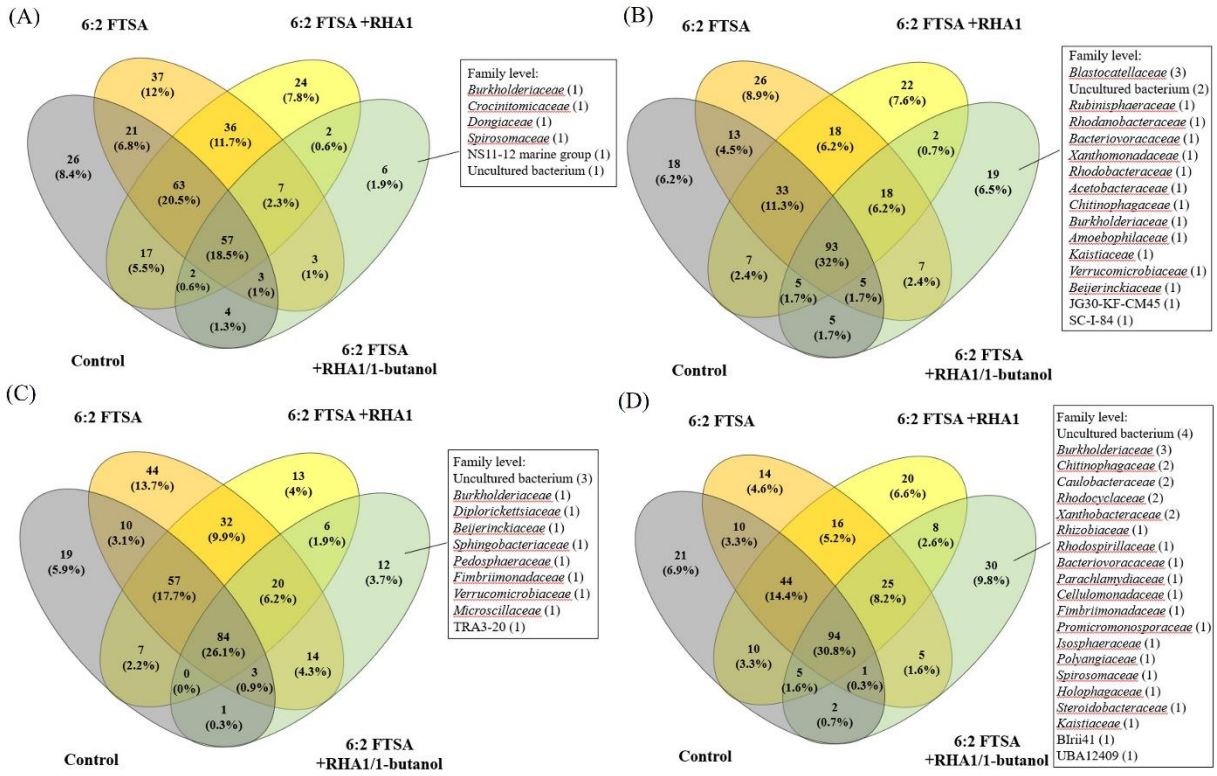

Figure S8. Venn diagrams of the microbial community showing the degree of unique and overlap bacterial genera in S-rich rhizosphere sandy soil (A), S-limited rhizosphere sandy soil (B), S-rich sandy soil (C), and S-limited sandy soil (D). As shown in (D), 30 unique genera were identified in this treatment and their family were shown as possible 6:2 FTSA degraders in the corresponding samples. Each treatment was performed in triplicate (n=3).

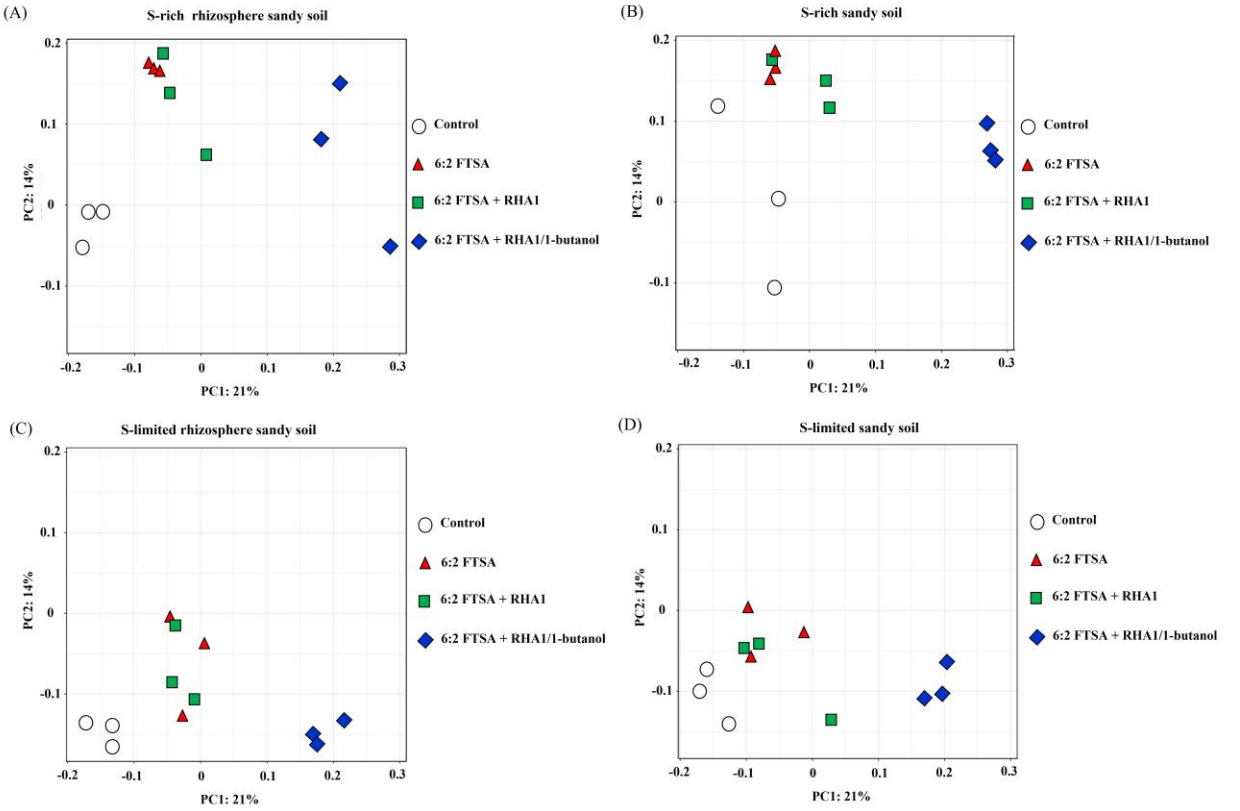

Figure S9. Principal Coordinates Analysis (PCoA) using unweighted UniFrac distance of the microbial community among different treatments. S-rich rhizosphere sandy soil (A), S-rich sandy soil (B), S-limited rhizosphere sandy soil (C), and S-limited sandy soil (D). Each treatment was performed in triplicate (n=3).

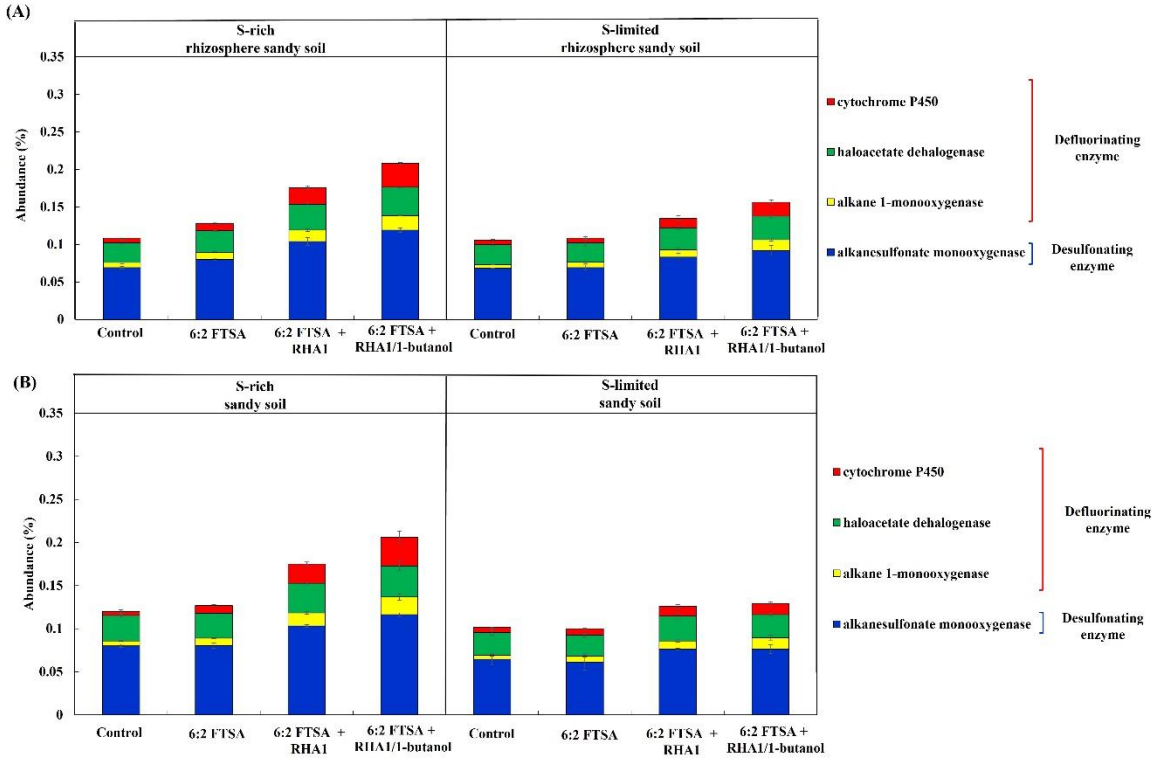

Figure S10. Abundance of defluorinating and deslufonating gene in the microbial community in S-rich (A) and S-limited (B) rhizosphere sandy soil and sandy soil. Each treatment was performed in triplicate (n=3). The bars represented the standard deviation of the triplicates.