Stable Isotope Probing (SIP) of Rhizosphere Bacteria in 6:2 Fluorotelomer Sulfonic Acid (6:2 FTSA)-Contaminated Soil

Kung-Hui (Bella) Chu, P. E.

Professor, Zachry Department of Civil & Environmental Engineering

Texas A&M University, College Station, TX

kchu@civil.tamu.edu ; https://chulab.engr.tamu.edu

Presentation to

2023 Battelle Bioremediation Symposium at Austin, Texas

May 9, 2023

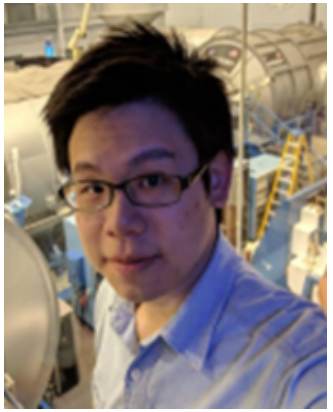
CHU LABORATORY

Research Team

Dr. Kung-Hui (Bella) Chu

Civil and Environmental Eng
Texas A&M University

Jason Yang, former Ph.D. Student



Dr. Libo Shan

Institute for Plant Genomics and Biotechnology
Texas A&M University

Supported by

National Science Foundation

TiCER Texas A&M University

TEXAS A&M UNIVERSITY

Per- and Polyfluoroalkyl Substances (PFAS)

- PFAS are a group > 3,000 man-made fluorinated chemicals as of 2018

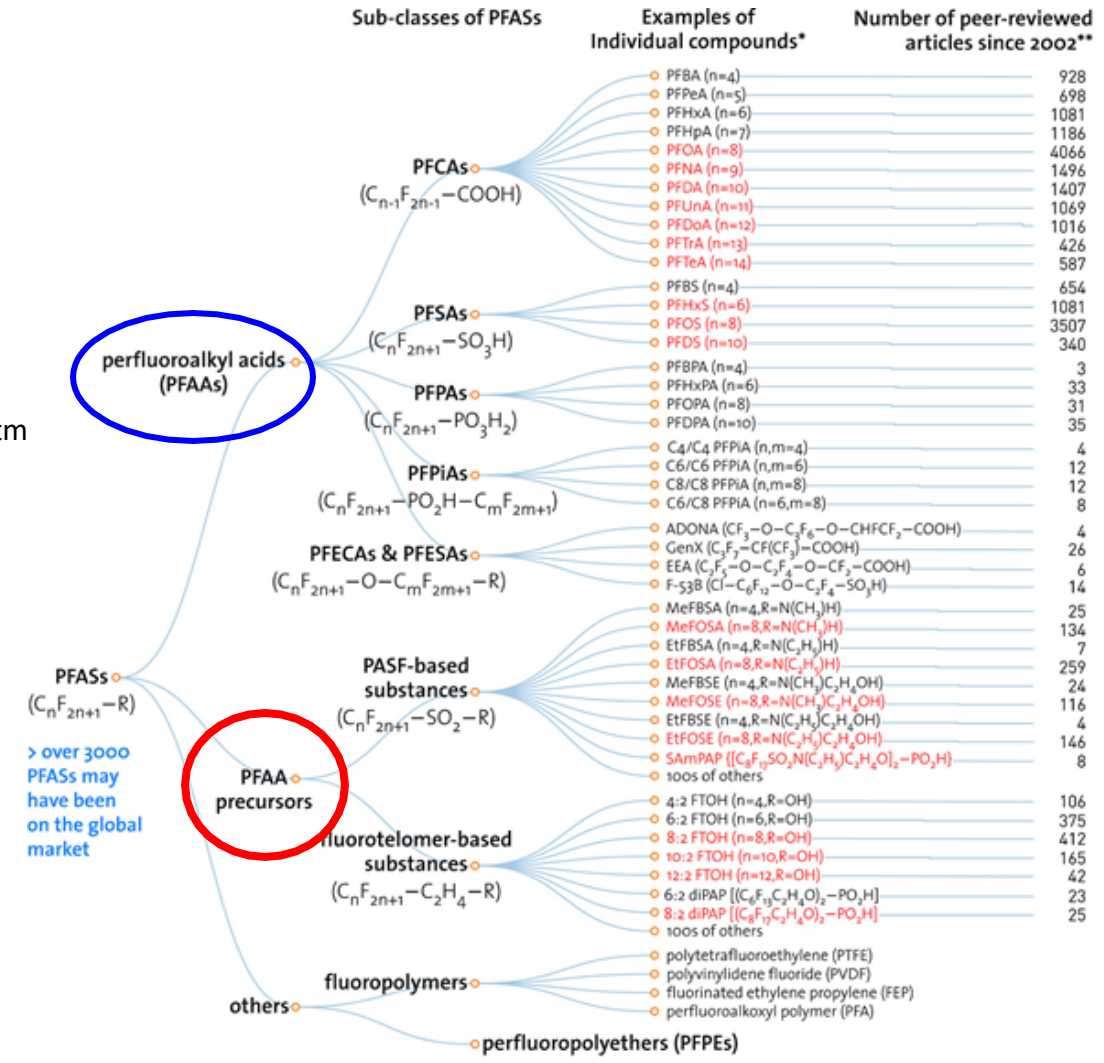
  (10.1021/acs.est.6b04806)

- > 9000 PFAS has been identified (source: CDC)

(https://www.cdc.gov/niosh/topics/pfas/default.htm

Precursors

Various chain-length PFAS

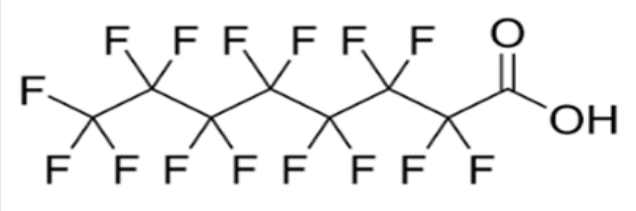


Per- and Polyfluoroalkyl Substances (PFAS)

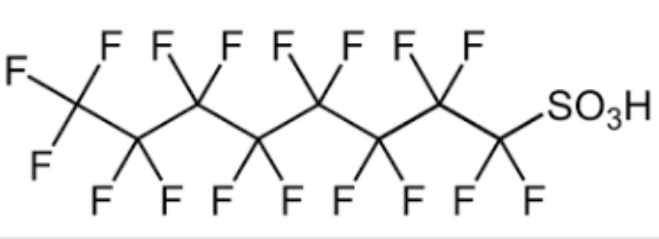
- **Man-made fluorinated chemicals**
- **Unique property**
  - Thermally stable and chemically inert
  - High surface activity
  - Water- and oil-repellent

Per – fully fluorinated
Poly – partially fluorinated

8:2 FTOH
Fluorotelomer alcohol



PFOA
Perfluorooctanoic acid



PFOS
Perfluorooctanesulfonic acid

6:2 Fluorotelomer Sulfonic Acid (6:2 FTSA)

- **1H,1H,2H,2H-Perfluorooctanesulfonic acid**

  Molecular weight = 428.17 g/mol

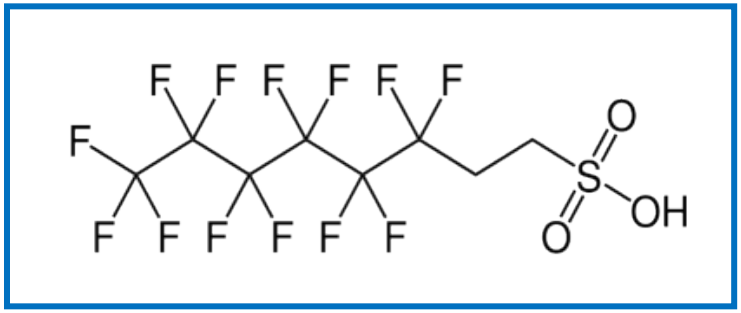
  log water solubility (mol/L, 25 ^{o}C)= -2.51

  log K_{OC} (pH 7, 25 C)= 1.0

  log K_{OW}= 4.44

  Density = 1.68 g/mL

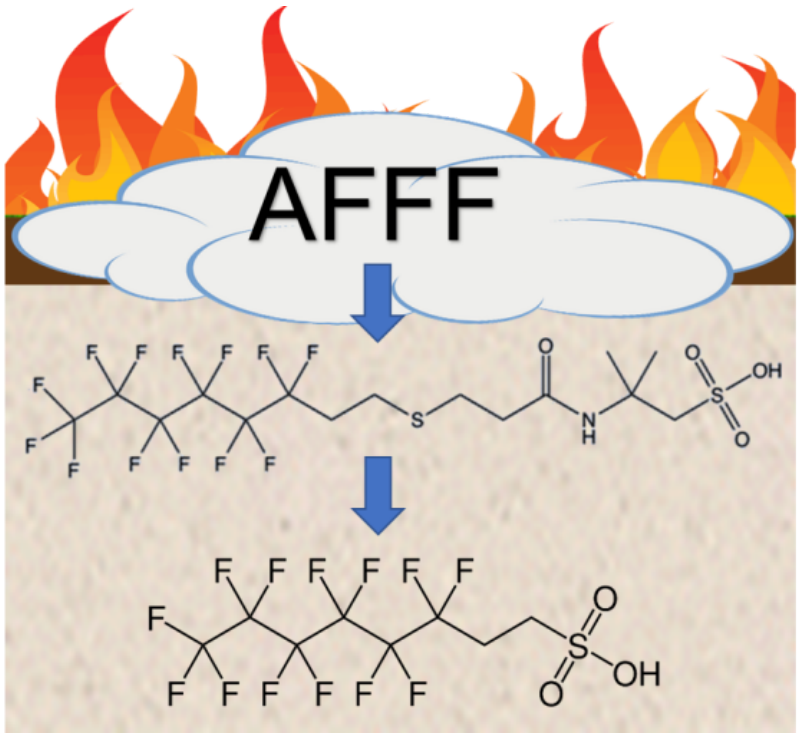
  Boiling point = 219 - 272 ^{o}C



- **Resistant to heat, acids, and bases**
- **Alternative to perfluorooctane sulfonate (PFOS)**
- **Persistent, bio-accumulative, toxic**

Occurrence, Source, and Fate

- **Fluorotelomer sulfonates**
  - as ingredients in aqueous film-forming foams (AFFFs)
  - as transformation metabolites from precursors in AFFFs
- **Known Precursors to fluorotelomer sulfonates**
  - fluorotelomer thioether amido sulfonate (FtTAoS)
  - fluorotelomer sulfonamide betaines (such as 6:2 FTBA)



- **6:2 FTSA found in high levels in AFFF-impacted soils and groundwater**
  - AFFF-impacted soil: 612-2,101 µg/kg
- **6:2 FTSA is also detected in**
  - River, groundwater and wastewater: 1.6-37.9 ng/L
  - Landfill leachate: 582 ng/L

Biodegradation of 6:2 FTSA

Degradation of 6:2 FTSA in activated sludge

- Formation of similar biodegradation metabolites as those observed during 6:2 fluorotelomer alcohols (6:2 FTOH) biodegradation.
- Slow biotransformation. ~ 63.7% remaining on day 90
- Half life: ~ 2 years

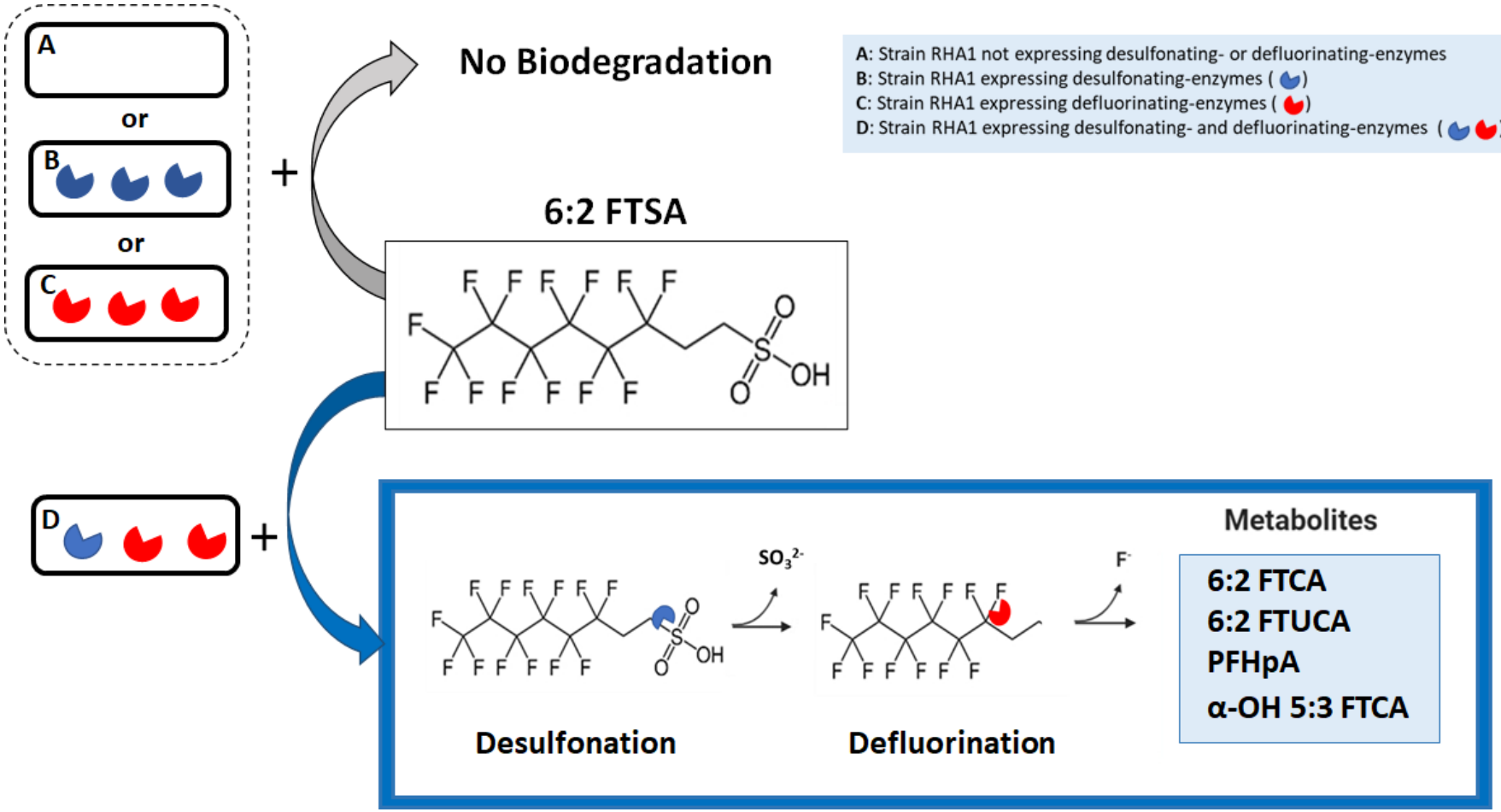
Aerobic River Sediments

- Rapidly biotransformed.
- Half life: ~ 5 days

Anaerobic River Sediments

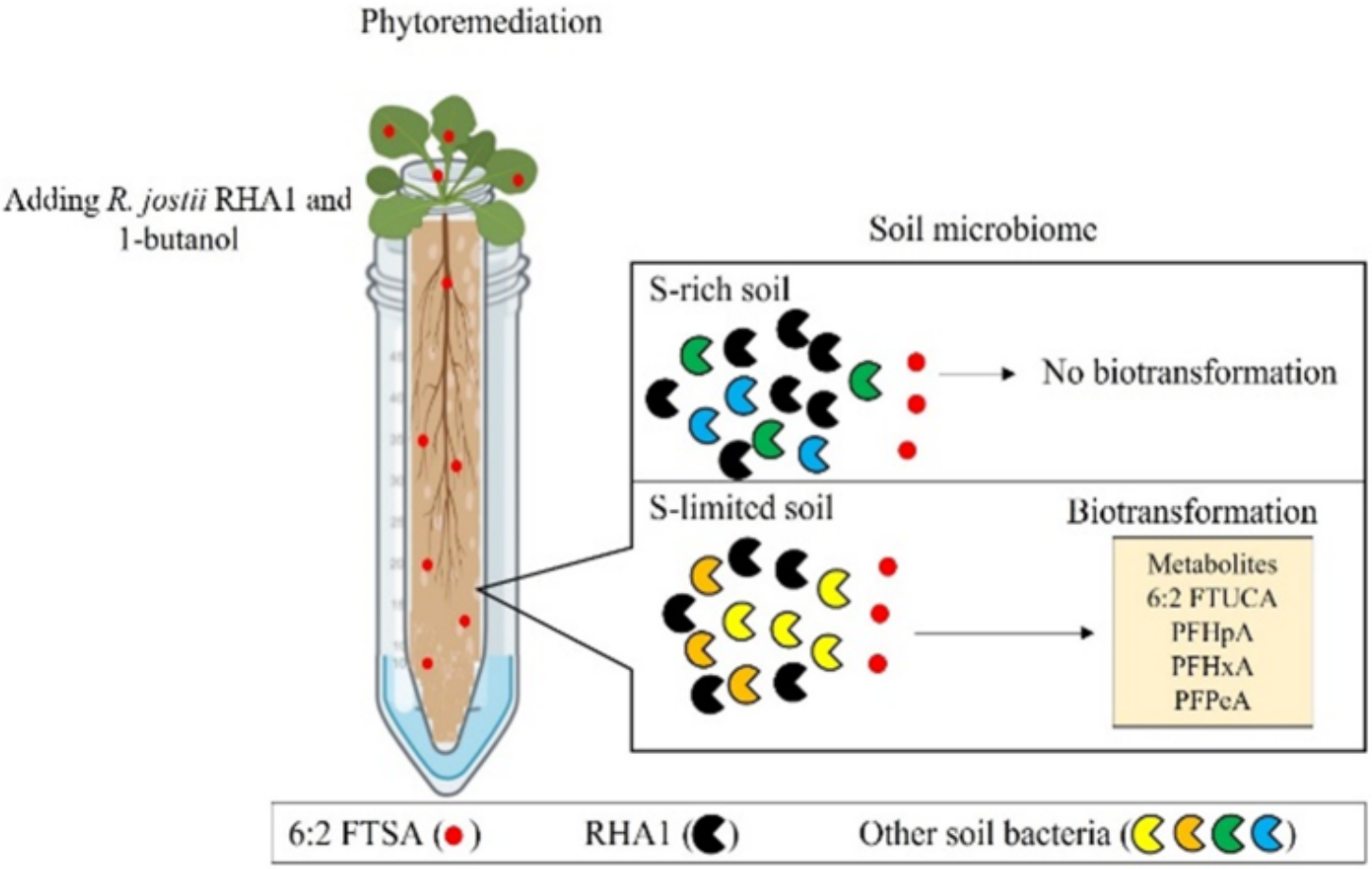
- No evidence of biodegradation or biotransformation over 100 days

Carbon and sulfur sources affected biodegradation of 6:2 FTSA by *Rhodococcus jostii* RHA1



Source: DOI: 10.1016/j.jhazmat.2021.127052

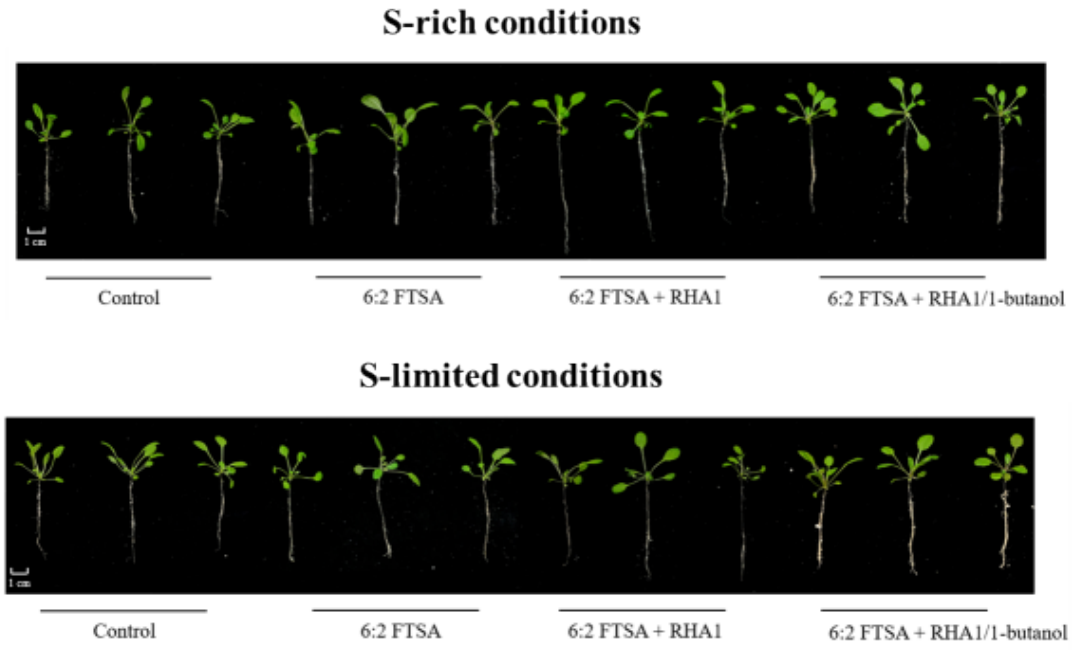
Fate and transformation of 6:2 FTSA affected by plant, nutrient, bioaugmentation & soil microbiome interactions



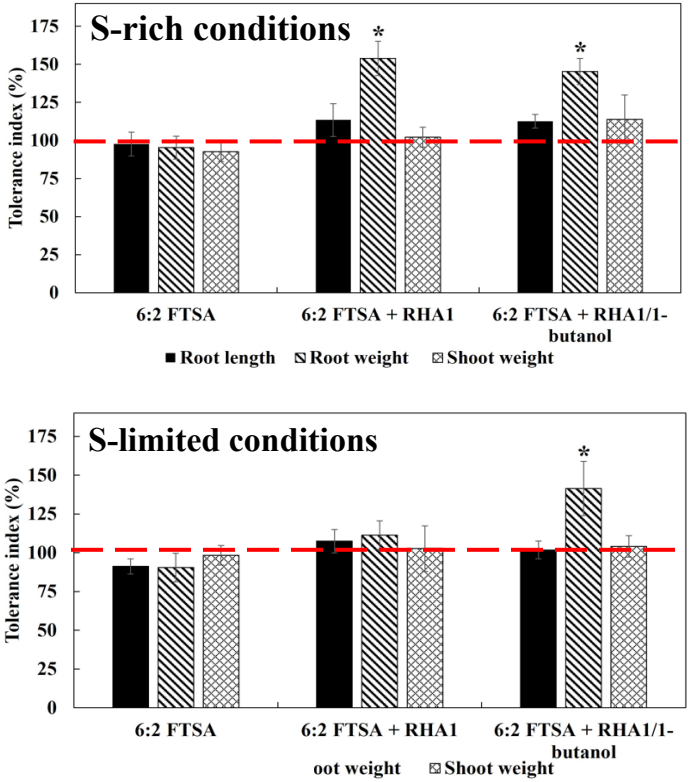
Source: https://doi.org/10.1021/acs.est.2c01867

6:2 FTSA had no effects on plant growth while RHA1 promoted the growth

No plant growth effect by 6:2 FTSA

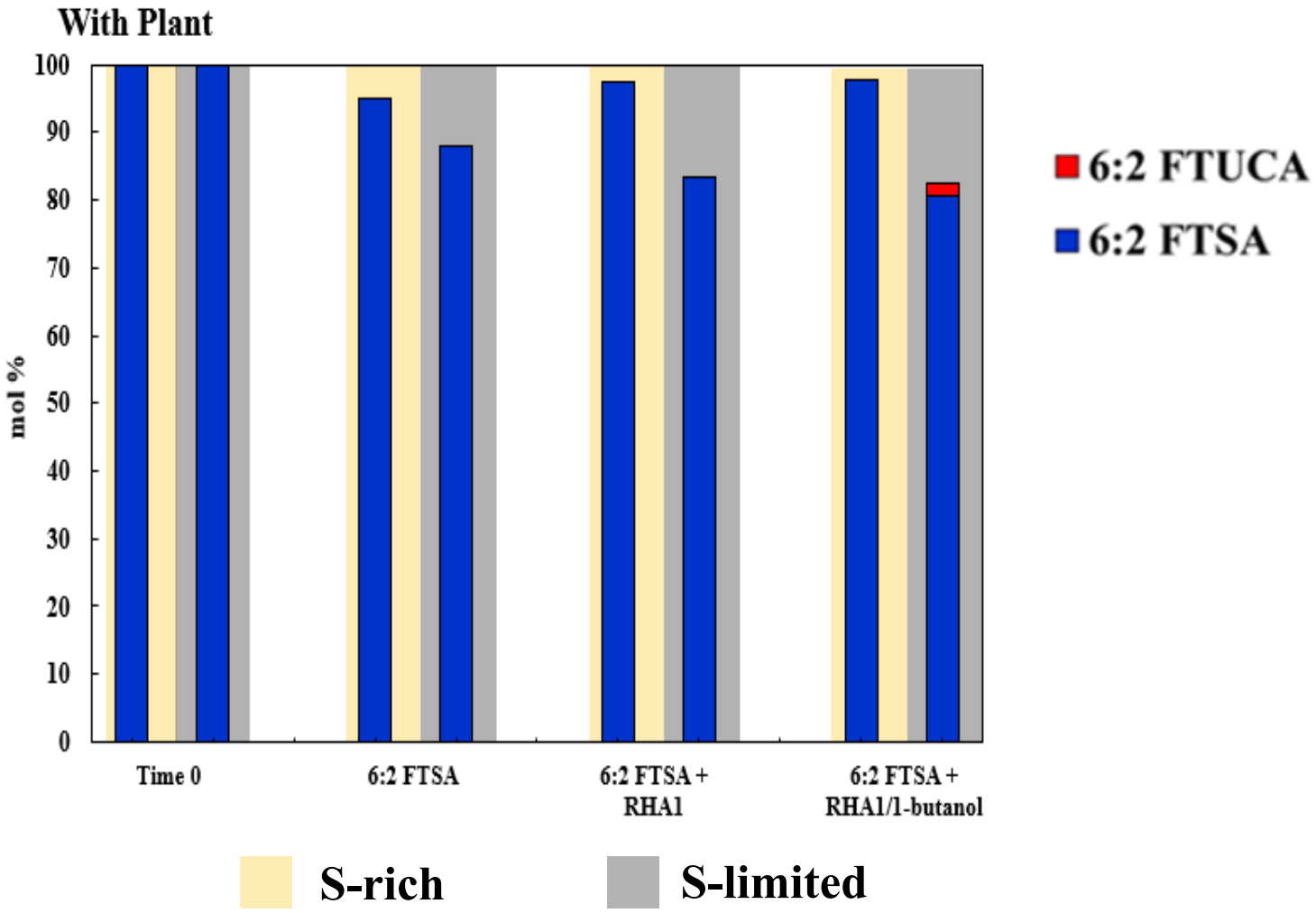


RHA1 promoted plant growth



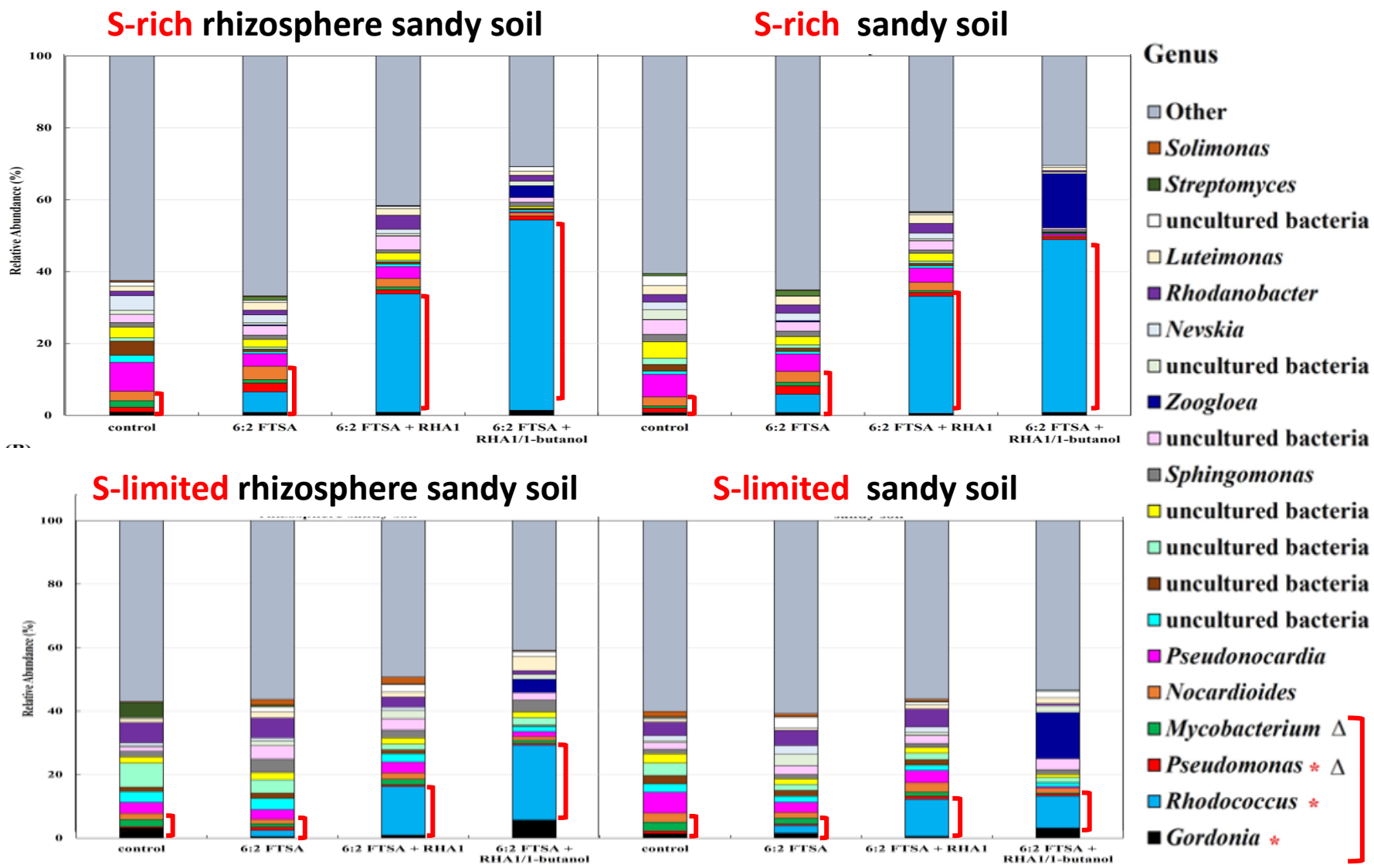
$$Tolerance\ Index\ (TI) = \frac{mean\ phenotype\ measurement\ of\ treatment}{mean\ phenotype\ measurement\ of\ control}$$

Degradation of 6:2 FTSA in the rhizosphere of plants amended with RHA1 and 1-butanol under S-limited conditions



$$Molar\ yield = 100\% \times \frac{Mass\ of\ PFAS\ species\ dected\ in\ soil\ (mole)}{Initial\ mass\ of\ 6{:}2\ FTSA\ spiked\ in\ soil(mole)}$$

Rhodococcus is one of the dominant species in RHA1- spiked treatments

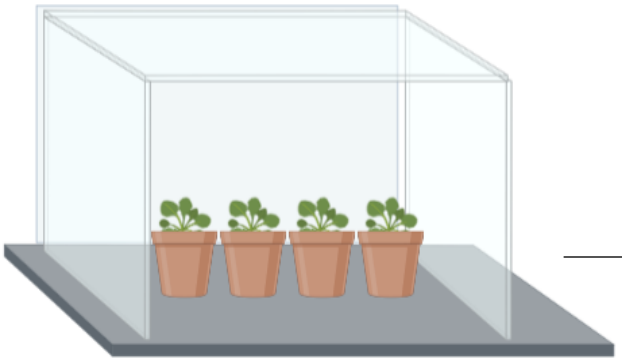


Q1: Which rhizosphere microbes were supported by the root exudate?

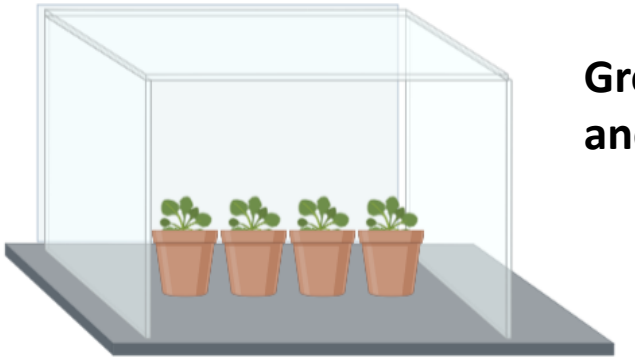
Q2: Was root-exudate-supported microbial community affected by 6:2 FTSA, carbon sources, and bioaugmentation?

Experimental Design

Grow the plant for 20 days



S-limited sandy soil



S-limited sandy soil

Grow the plant for another 10 days

Control (no RHA1, no 6:2 FTSA)
RHA1
6:2 FTSA
6:2 FTSA+RHA1
6:2 FTSA + RHA1 +1-butanol
6:2 FTSA + n-octane
6:2 FTSA + 1-butanol
6:2 FTSA + DGBE

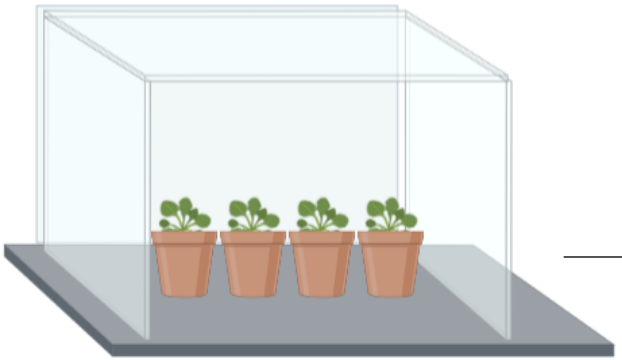
- Purged the chamber with N_2, then spiked O_2 to 21% and $^{13}CO_2$ to 500ppmv.
- Incubated with light intensity of 100 µE m−2 s−1 for 16 hours.

* Initial 6:2 FTSA concentration = 1.5 mg/kg.

Experimental Design (conti.)

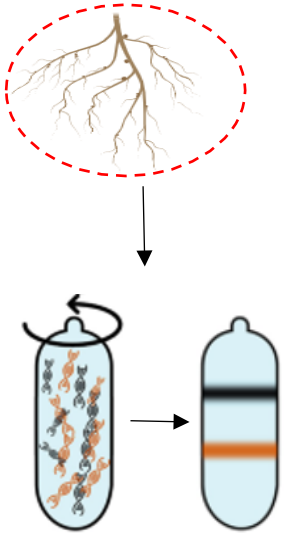
$^{13}CO_2$

(30 days total)



S-limited sandy soil

Control (no RHA1, no 6:2 FTSA)
RHA1
6:2 FTSA
6:2 FTSA+RHA1
6:2 FTSA + RHA1 +1-butanol
6:2 FTSA + n-octane
6:2 FTSA + 1-butanol
6:2 FTSA + DGBE

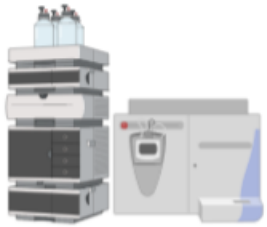


Soil DNA fractionation

C^{12} DNA

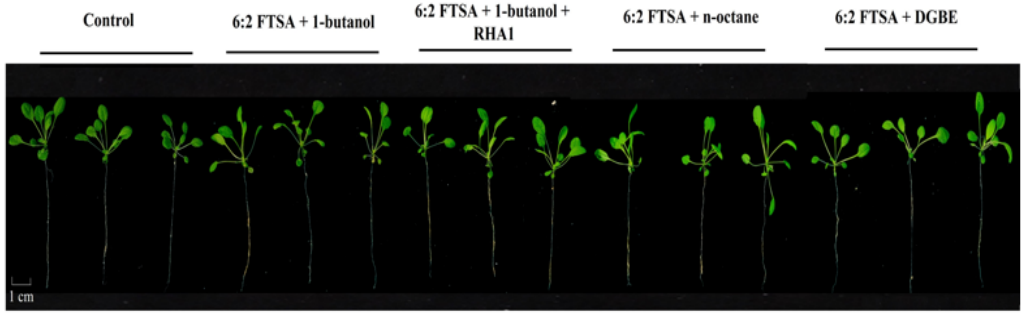
C^{13} DNA

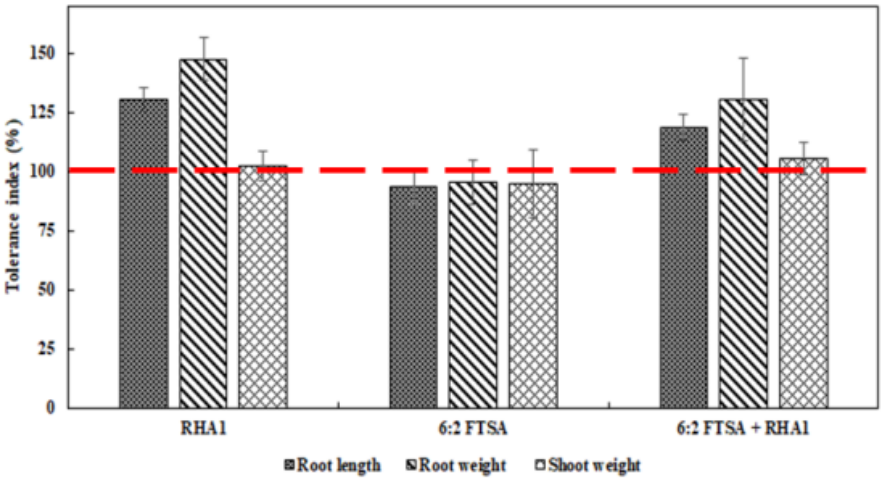
Phenotype:
Root length & Biomass

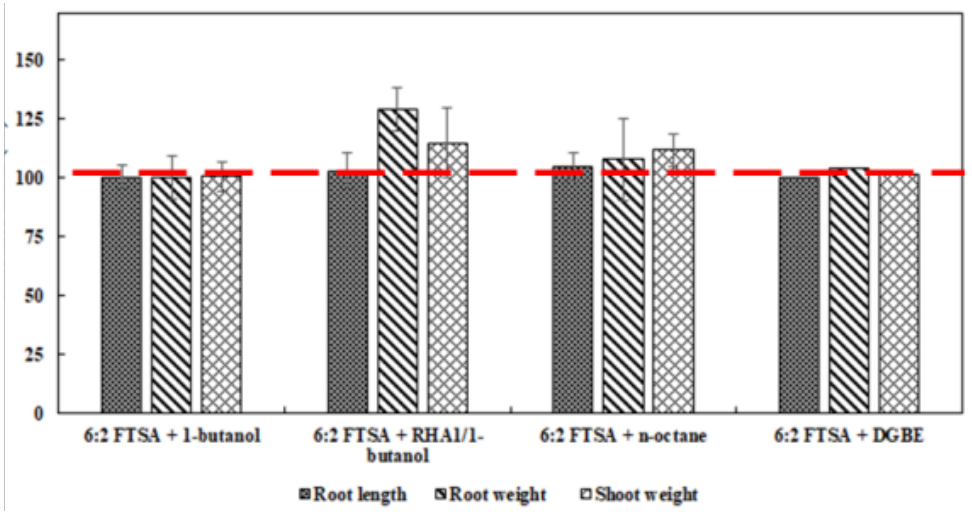


PFAS analysis

Microbial community analysis

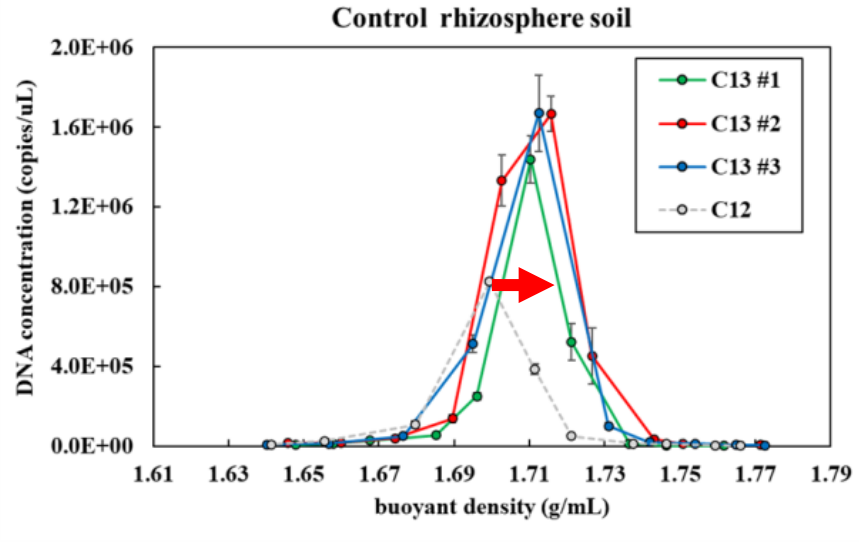


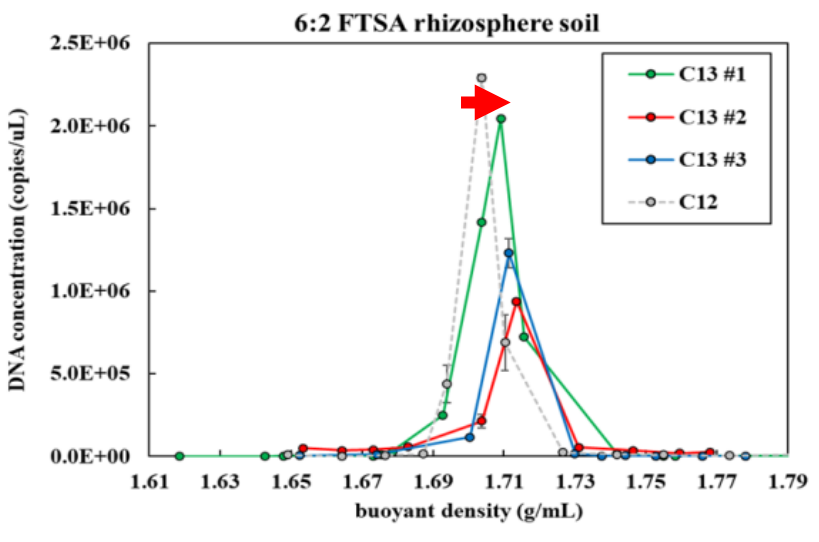


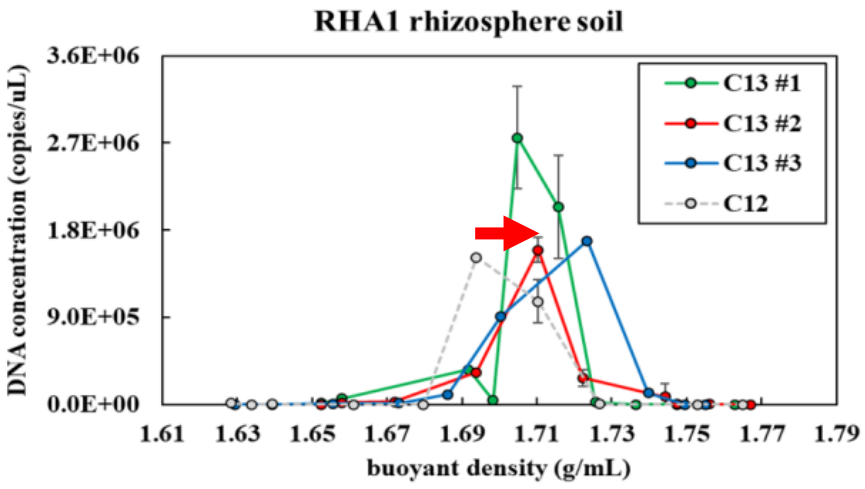


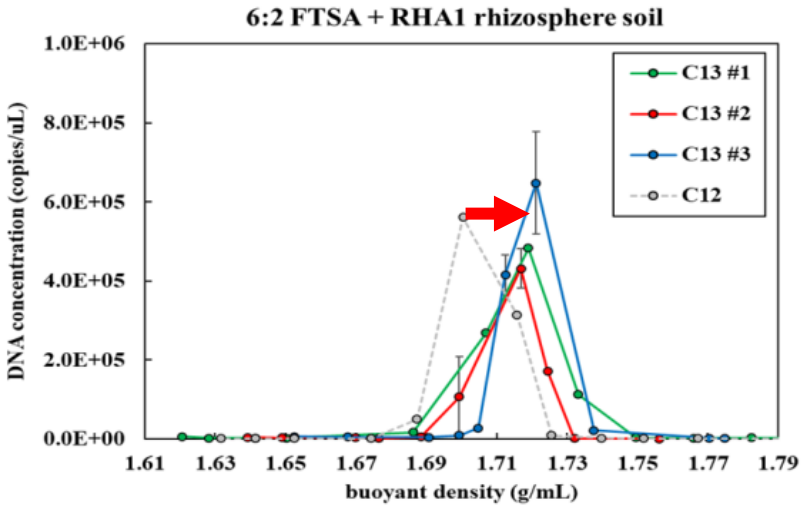
- 6:2 FTSA had no effects on plant growth
- RHA1 promoted plant growth
- Butanol and DGBE did not affect plant growth, but addition of octane promoted plant growth slightly.
- Approximately 15 % of spiked 6:2 FTSA was removed from the soil.

Peaks of the ^{13}C-DNA shifted toward right

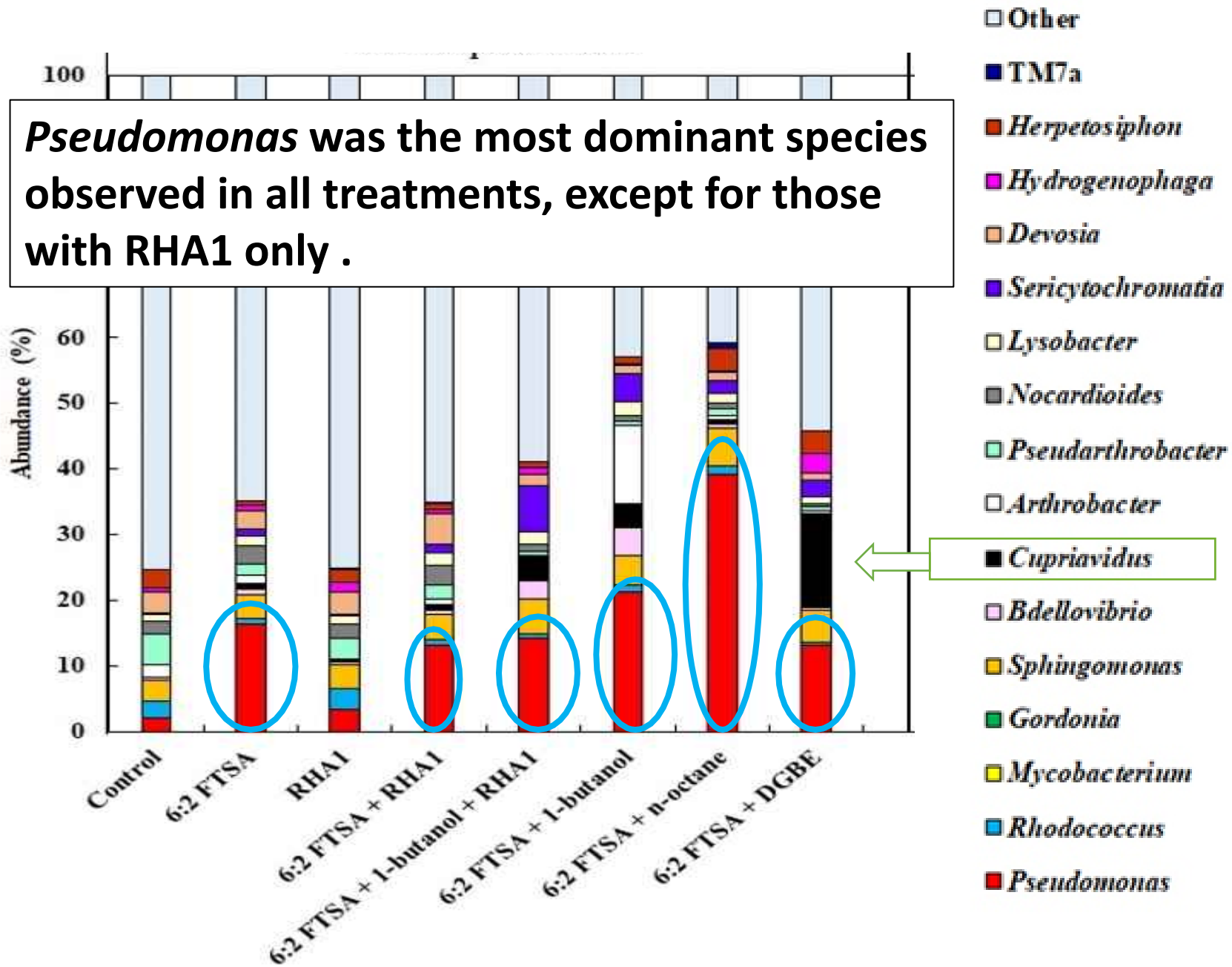




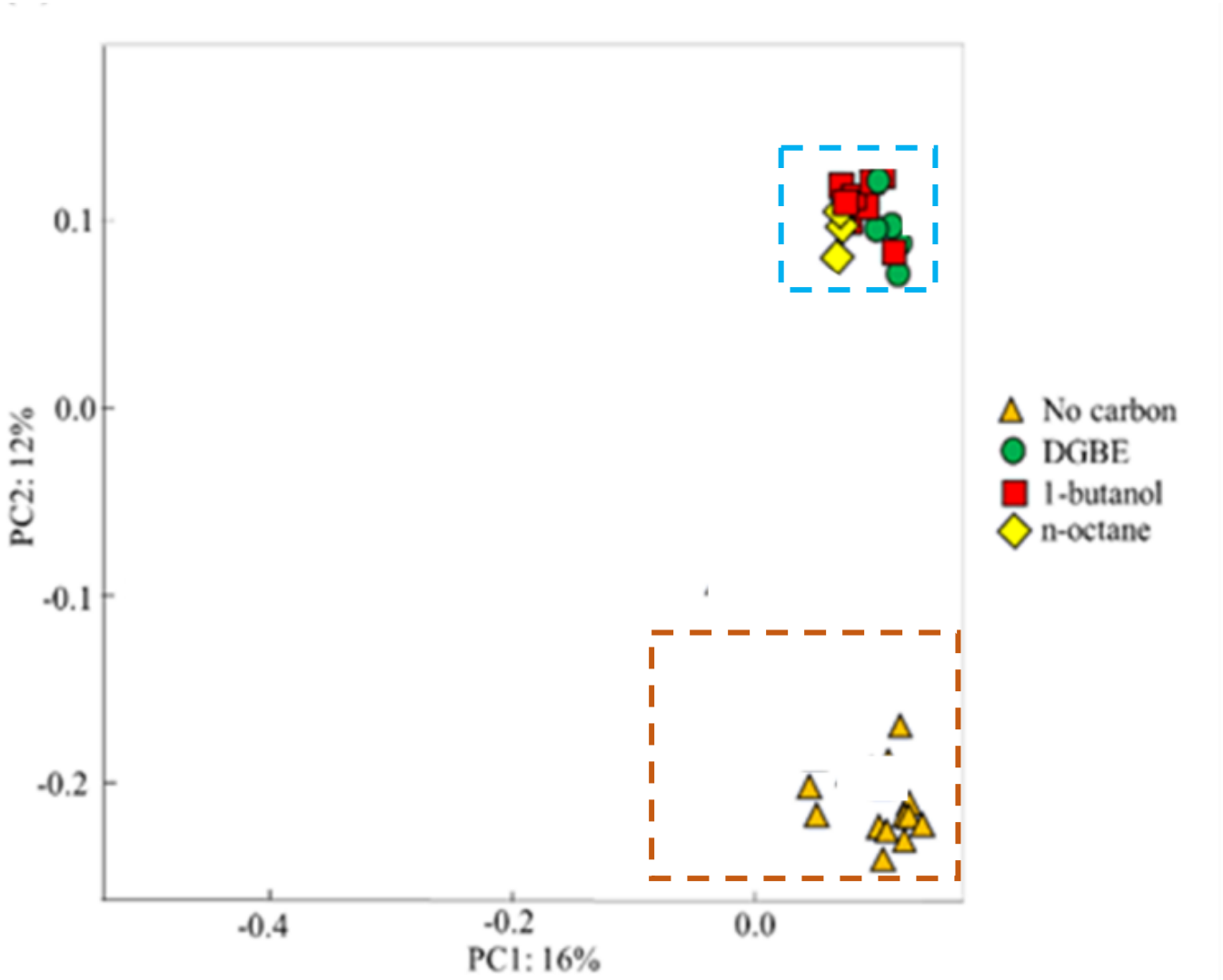




Microbial communities assimilated ^{13}C-labeled root exudate



Shifts of rhizosphere microbiomes that assimilated ^{13}C-labled root exudate: Effects of C Sources



Take Home Messages

- Amendment of 6:2 FTSA-degrading culture, strain RHA1, can promote plant growth.
- 13C stable isotope probing suggested that Pseudomonas was the most predominant active rhizosphere bacteria while the spiked RHA1 was less competitive in rhizosphere zone compared to bulk soil.
- Carbon sources, such as 1-butanol, octane, and DGBE, are an important driver caused the composition of shifting in the microbial community, in spike of the presence of root exudates.

Thank You