Soil Treatability Study

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Bioremediation Study
FINAL RESULTS

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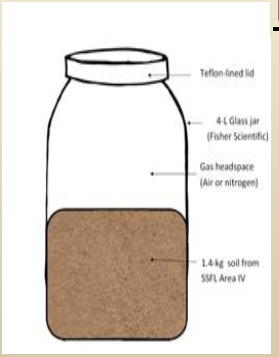
Bioremediation Study: Two Parts

Objective: Determine the potential for microbial biodegradation of contaminants of interest (COIs) in SSFL Area IV soils

Part 1: Analysis of microbial communities in the field

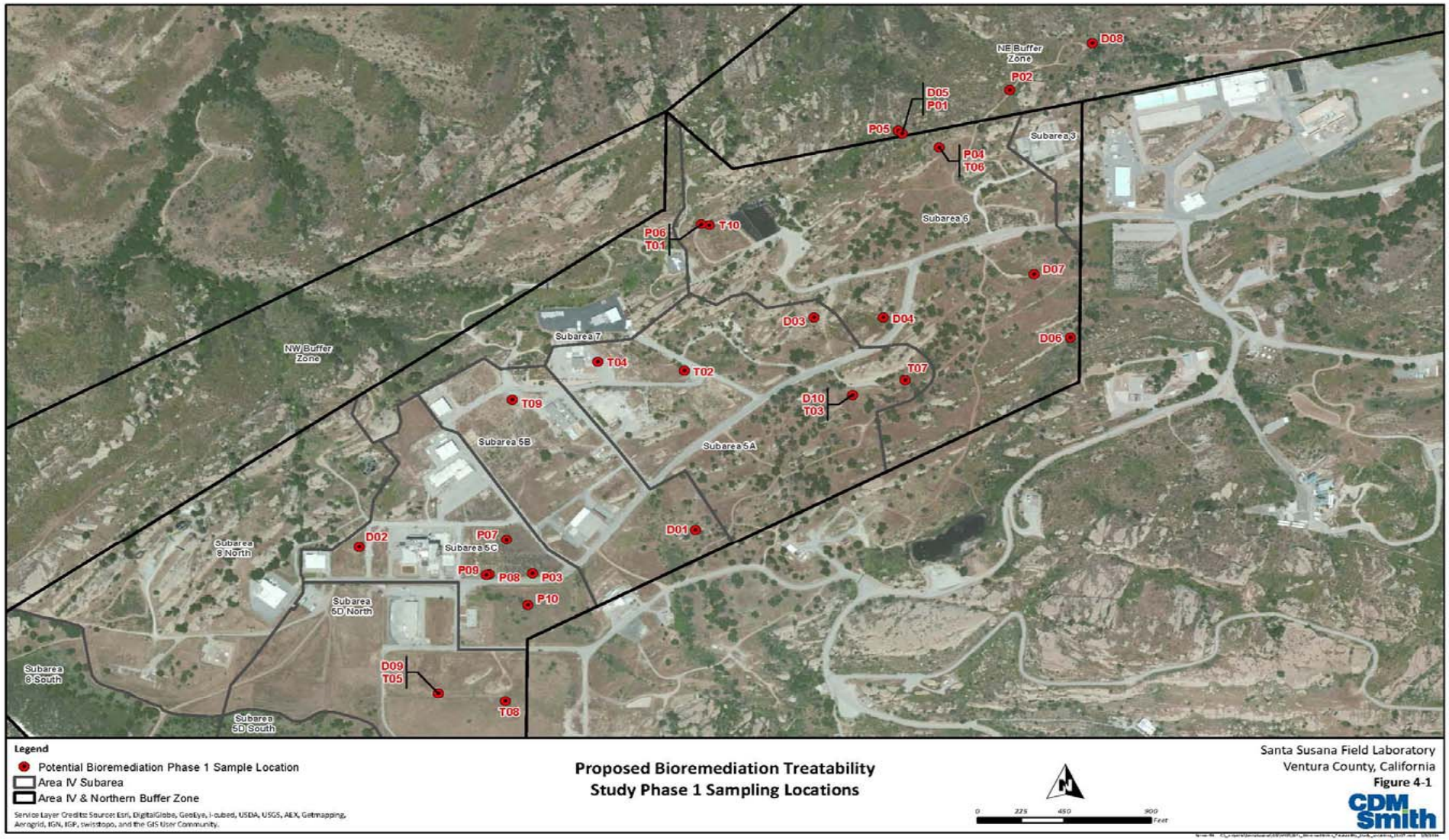
- Identify potential chemical-degrading microorganisms in Area IV soils
  - Culturing of microorganisms from Area IV soils & sequencing
  - DNA analyses
    - Quantitative polymerase chain reaction (qPCR)
    - Terminal restriction fragment analysis (TRFLP)



Part 2: Laboratory microcosms using soil from Area IV

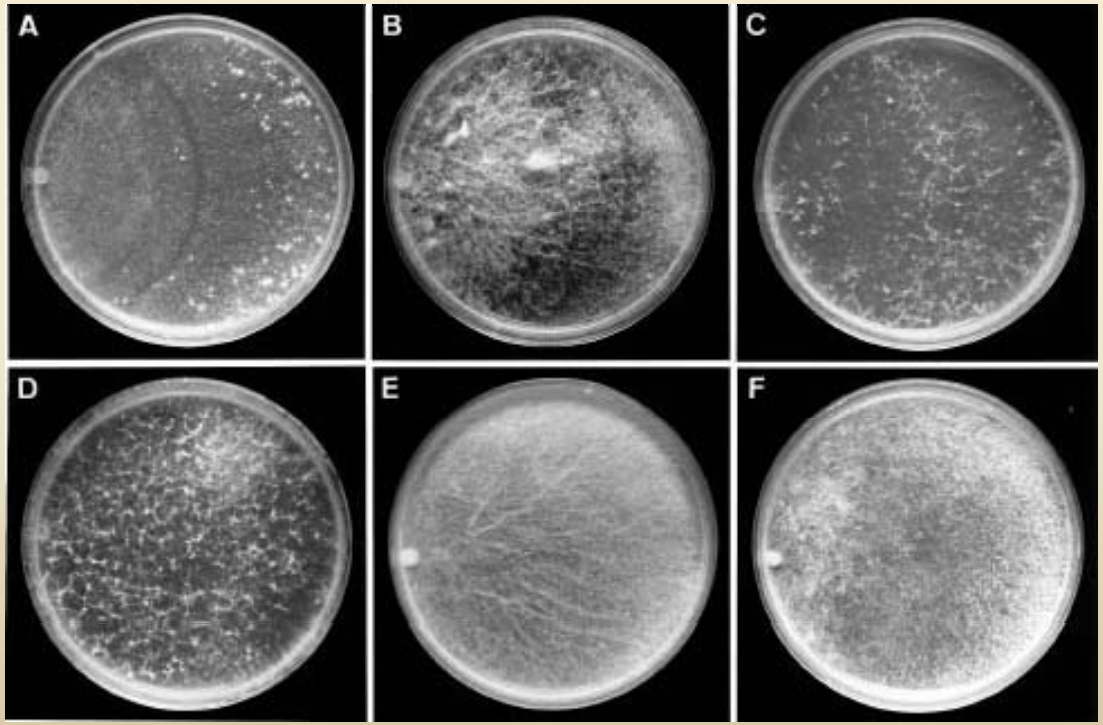
- Incubate in the laboratory under controlled conditions
- Measure biodegradation rates under natural attenuation conditions
- Estimate efficacy of biostimulation and bioaugmentation

Part 1 Field Study: Soil samples collected from 30 locations in SSFL Area IV



Isolated pure-cultures of soil bacteria and fungi from Area IV soils

- Grown on COI model compounds with no other carbon source
- Sequenced 16s DNA
- Compared to known degraders of Area IV chemicals



e.g. *Phanerochaete chrysosporium*, White-Rot Fungi PCB degrader

Results of Culturing

- 21 unique organisms were identified growing on plates enriched with the COIs, including 14 bacteria and 7 fungi
- Fungi isolated include 3 strains of the fungi *Phanerochaete chrysosporium*
- Bacteria isolated include*, Arthrobacter, Streptomyces*, *Micromonospora,* and *Variovorax,* and 6 strains of *Pseudomonas*
- 10 of the bacteria and 3 of the fungi isolated are known degraders of the COIs or come from a genus that contains known degraders

	Petroleum Hydrocarbons	**Polyaromatic hydrocarbons (PAHs)**	**Polychlorinated biphenyls (PCBs)**	**Dioxins**
Bacteria Isolated	**10**	**8**	**9**	**9**
Fungi Isolated	**2**	**2**	**2**	**3**

qPCR Analysis:

Quantitative polymerase chain reaction assay for specific microbial species or genes

Assay performed by Microbial Insights, Inc.

Collect Soil Samples (Sample D03 and composite sample)

→

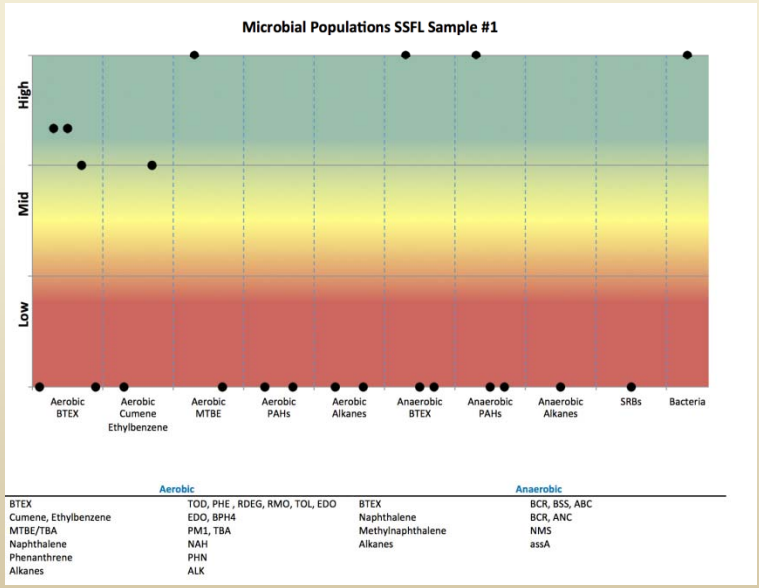
Extract DNA

→

Amplify DNA using PCR with specific primers

↓

Quantify DNA

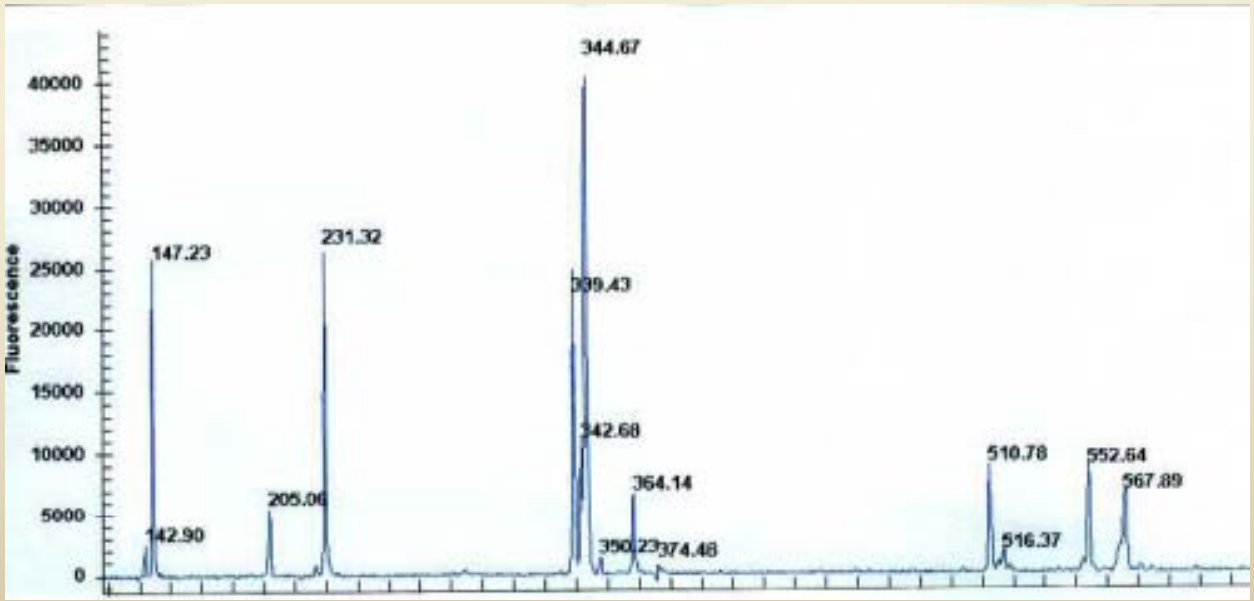


qPCR Findings

- Six genes associated with aerobic biodegradation of benzene, toluene, ethylbenzene and xylenes (BTEX) were detected, 4 of which were detected in both samples
- No anaerobic target genes were detected in either soil sample with the exception of benzoyl coenzyme A in both samples
- No aerobic or anaerobic PAH biodegradation targets were detected
- A very small amount ($2.3x10^4$ cells per g) of *Dehalococcoides* was found in the fresh soil sample (this bacterial species is known to dechlorinate PCBs and dioxins)

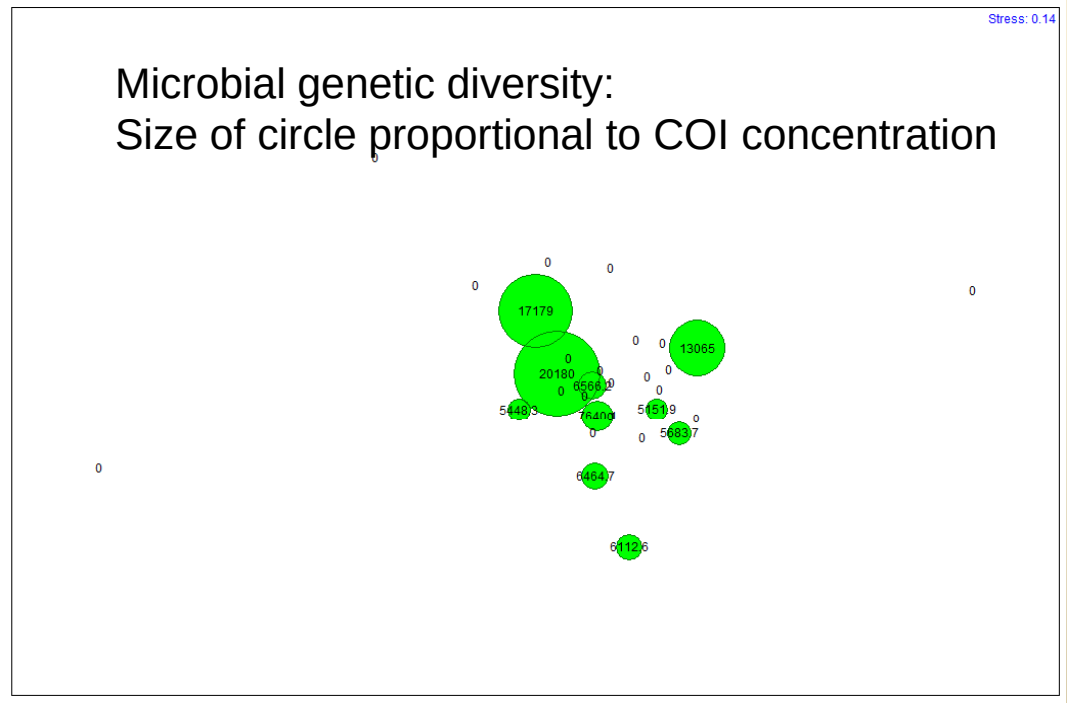
Microbial diversity of soil microorganisms: Terminal Restriction Fragment Length Polymorphism (TRFLP) Analysis

- Each peak represents a genetic sequence
- Provides the relative abundance of specific genetic sequences
- Gives a good indication of the microbial population diversity in a sample
- Attempted to correlate these genetic sequences to COI concentrations to identify sequences associated with potential microbial degraders



Findings from TRFLP Assays

- Soil samples exhibited good microbial diversity
- No significant correlations between specific TRFLP patterns and specific COIs



Conclusions from Field Microbial Assays

- Culturing and qPCR assays indicated the presence of many strains of bacteria and fungi which are known to be capable of biodegrading the COIs.
- These assays do not provide an indication of biodegradation rates in SSFL soils: Microcosm experiments are necessary for this determination.
- Anaerobic conditions were not observed in site soils
  - Bacterially-mediated reductive dechlorination of PCBs and chlorinated dioxins unlikely
  - Fungi, such as *Phanerochaete chrysosporium,* are capable of degrading these COIs under aerobic conditions

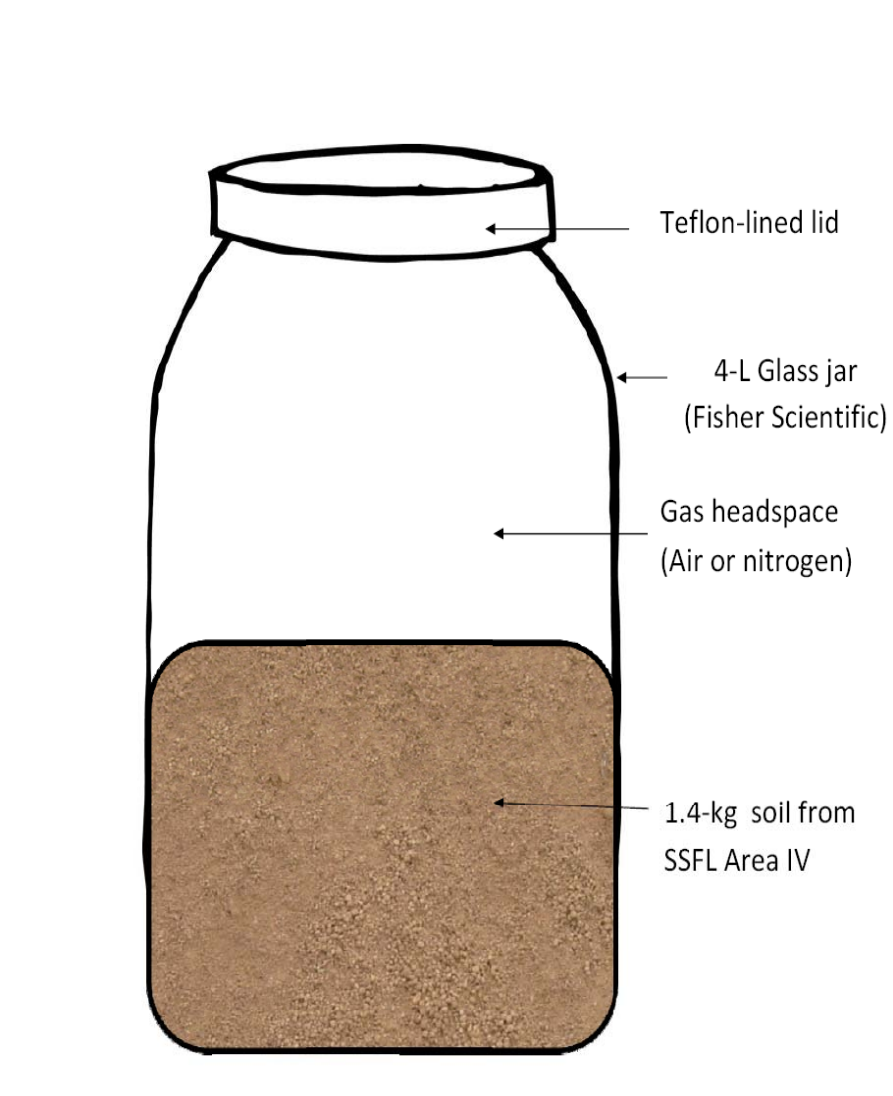
Part 2: Bioremediation Microcosms

- Soil collected from three locations and processed for use in controlled laboratory microcosms

Bioremediation Study: Soil Collection

Bioremediation Microcosm Experimental Design

- Place soil from SSFL Area IV into 1-gallon glass jars
- Incubate under conditions simulating conditions at SSFL
  - Aerobic
  - 27 ± 3°C
  - Dark
  - 12-16% moisture
- Measure COI concentrations in soil after 0, 4 and 8 months



Microcosm Amendments and Controls

	Microcosm Type
Un-amended	Sterilized (Soil A)
	Unsterilized: Soils A, B and C
Amended (Soil A)	Fertilizer
	Rice hulls
	Rice hulls + fertilizer + *P. chrysosporium* fungi
	Soya lecithin
	Combination

Five replicates for each treatment (45 total)

Microcosm Soil Amendments

Microcosm ID	Abbreviation	Collection Location	Amendments	Amount Amendment Added	Sterilized (Control)	Sample Collection
A1	NUTR	5C_DG-516	Nutrient solution	2.0 g/L KH2PO4, 0.3 g/L $MgSO_4 \cdot 7H_2O$ 0.4 g/L CaCl2H2O	No	0, 4, 8 months
A2	SOLE	5C_DG-516	Soya lecithin	1.5% w/w	No	0, 4, 8 months
A3	RICE	5C_DG-516	Rice hulls	10% w/w	No	0, 4, 8 months
A4	AUGM	5C_DG-516	Nutrient solution *P. chrysosporium* Rice hulls	2.0 g/L KH2PO4, 0.3 g/L $MgSO_4 \cdot 7H_2O$ 0.4 g/L $CaCl_2H_2O$ Malt extract *P. chrysosporium* 10% w/w rice hulls	No	0, 4, 8 months
A5	COMB	5C_DG-516	Nutrient solution *P. chrysosporium* Soya lecithin Rice hulls	2.0 g/L KH2PO4 0.3 g/L $MgSO_4 \cdot 7H_2O$ 0.4 g/L $CaCl_2H_2O$ Malt extract *P. chrysosporium* 1.5% w/w soya lecithin 10% w/w rice hulls	No	0, 4, 8 months
A6	UNAA	5C_DG-516	None	None	No	0, 4, 8 months
A7	STER	5C_DG-516	Gamma irradiation	25 kilograys	Yes	0 and 8 months

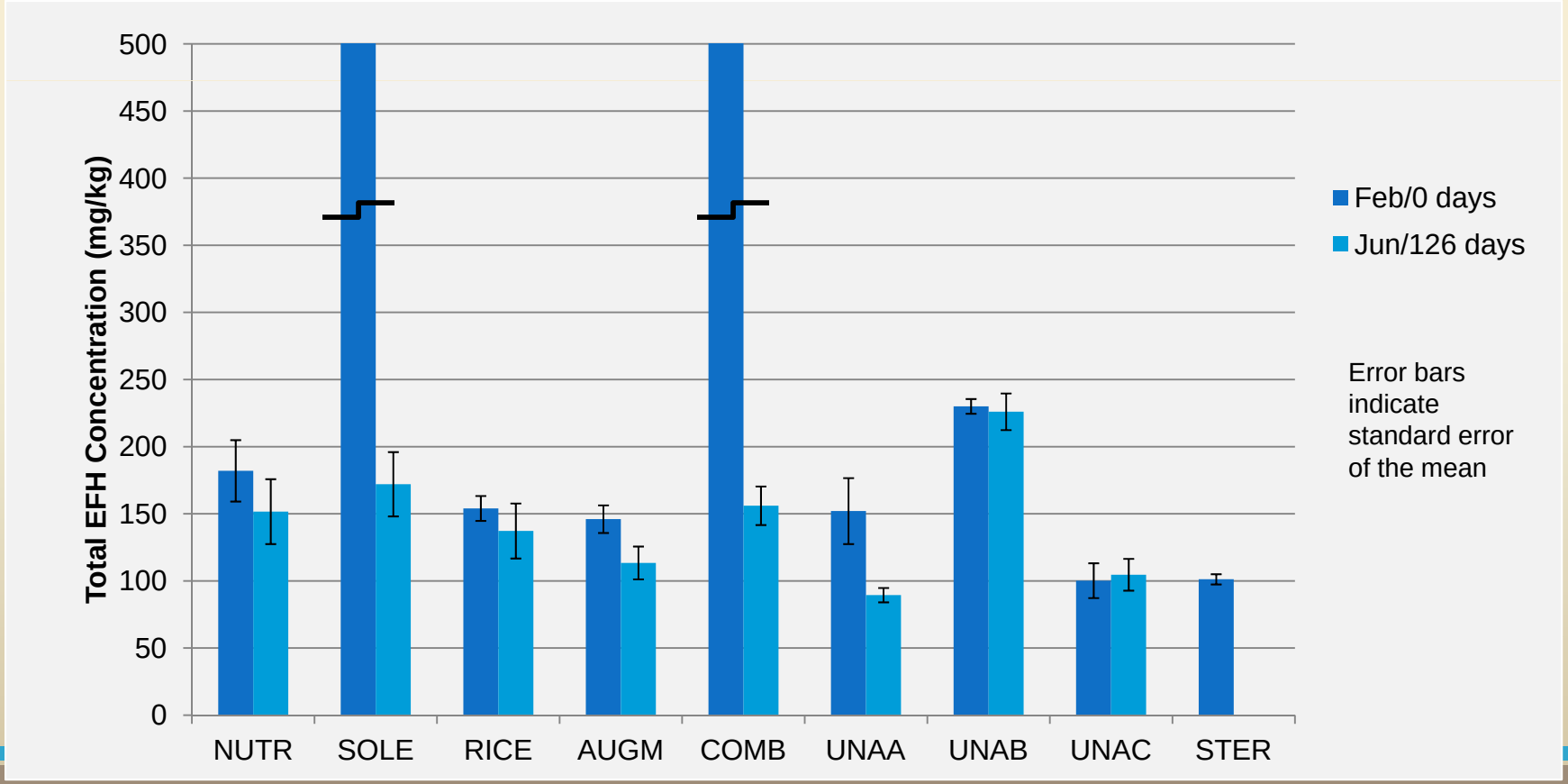
Microcosm Soil Amendments, Cont'd.

Microcosm ID	Abbreviation	Collection Location	Amendments	Amount Amendment Added	Sterilized	Sample Collection
A6	UNAA	5C_DG-516	None (Unamended)	None	No	0, 4, and 8 months
B6	UNAB	PUBS 1044	None (Unamended)	None	No	0, 4, and 8 months
C6	UNAC	5C_DG-755	None (Unamended)	None	No	0, 4, and 8 months


A2D1

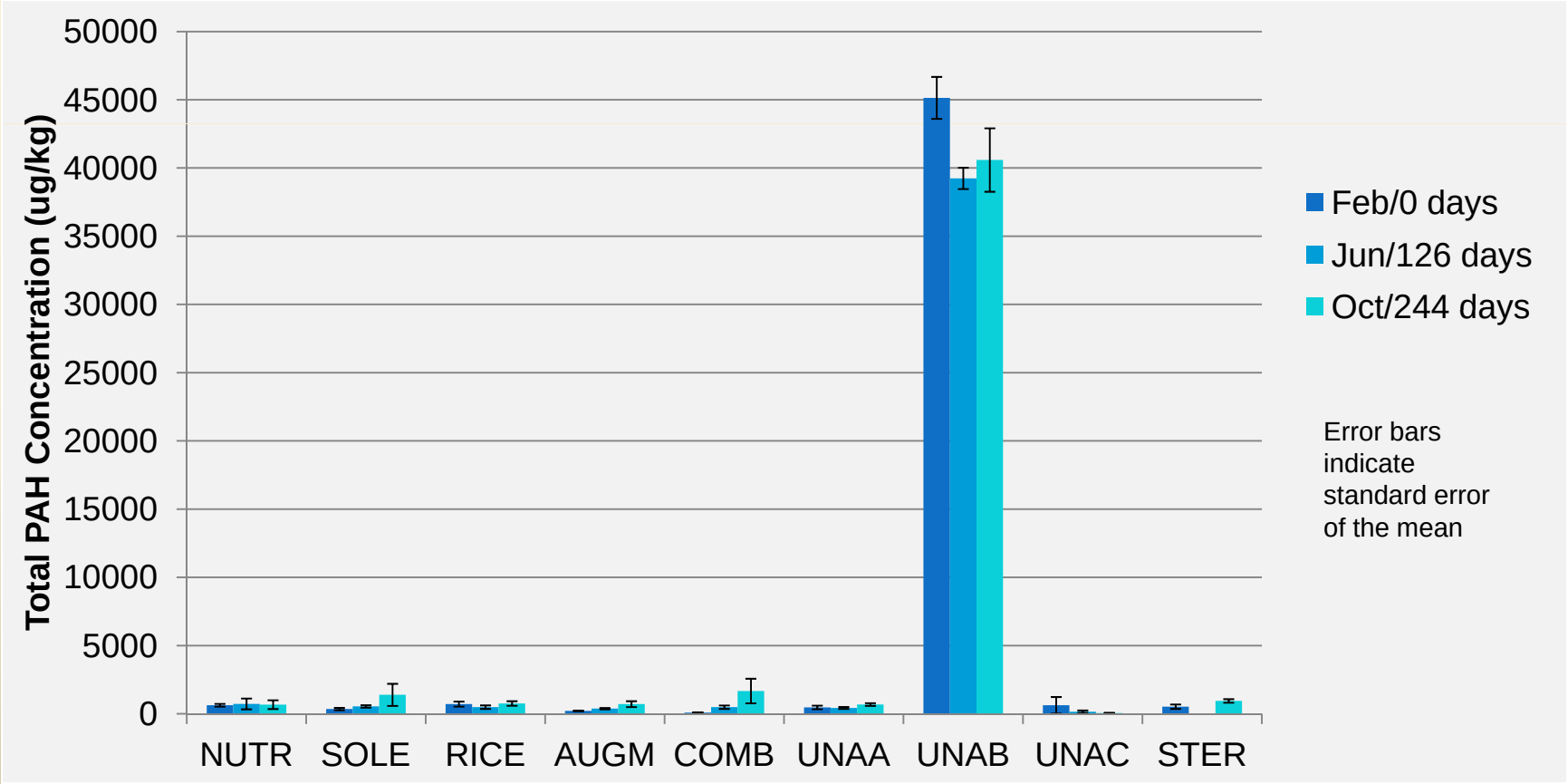
Microcosm Results: Extractable Fuel Hydrocarbons (EFH)

- Soy lecithin caused high EFH readings
- EFH decreased in 126 days:
  - 40% reduction for Soil A
  - No significant change for Soils B & C
- EFH measurement at 244 days in progress/ under investigation



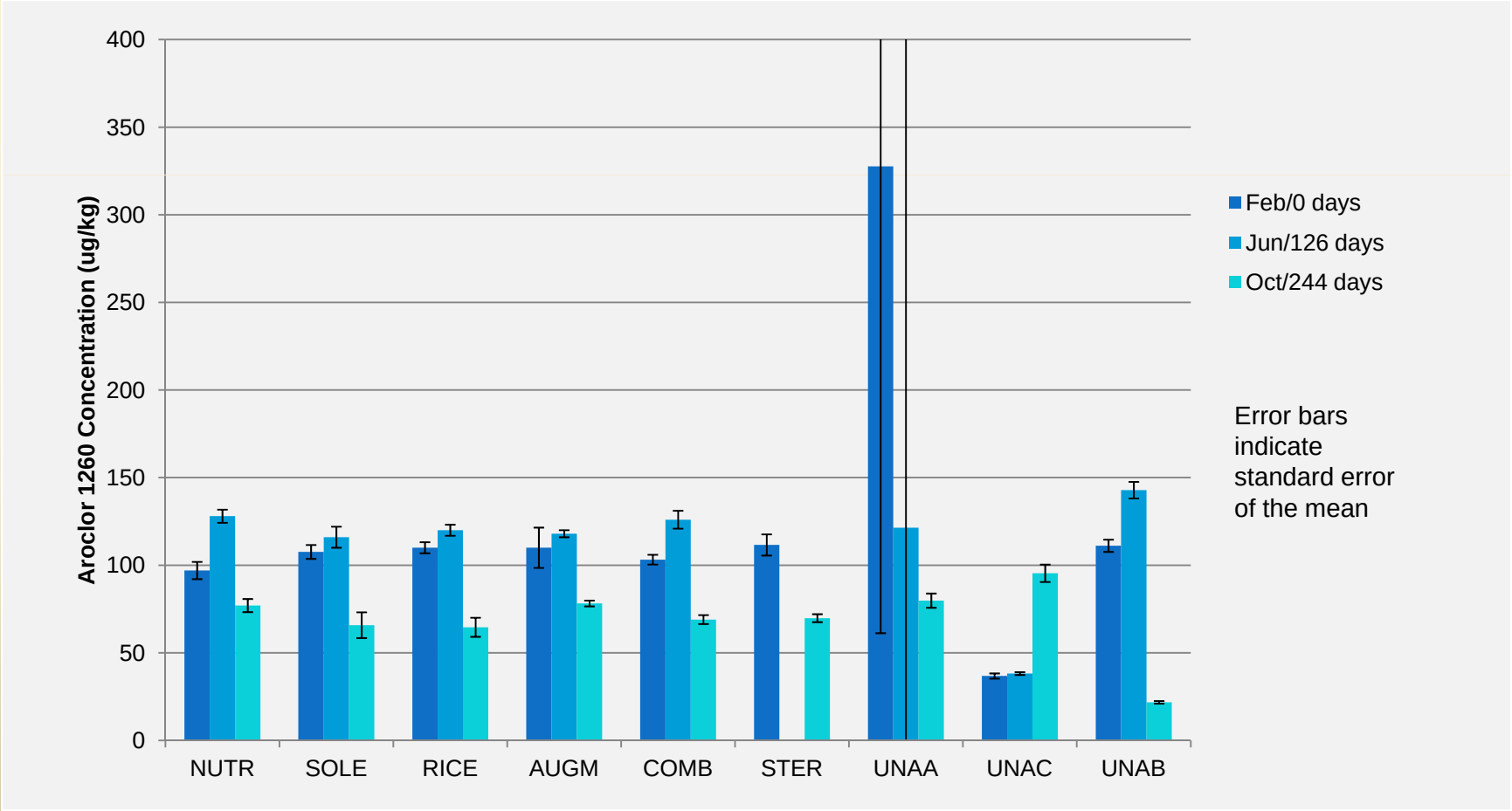
Microcosm Results: Polyaromatic Hydrocarbons (PAH)

- Little or no PAH biodegradation observed
- Remaining PAHs mostly 4-6 aromatic rings
- Lack of degradation may be due to low bioavailability



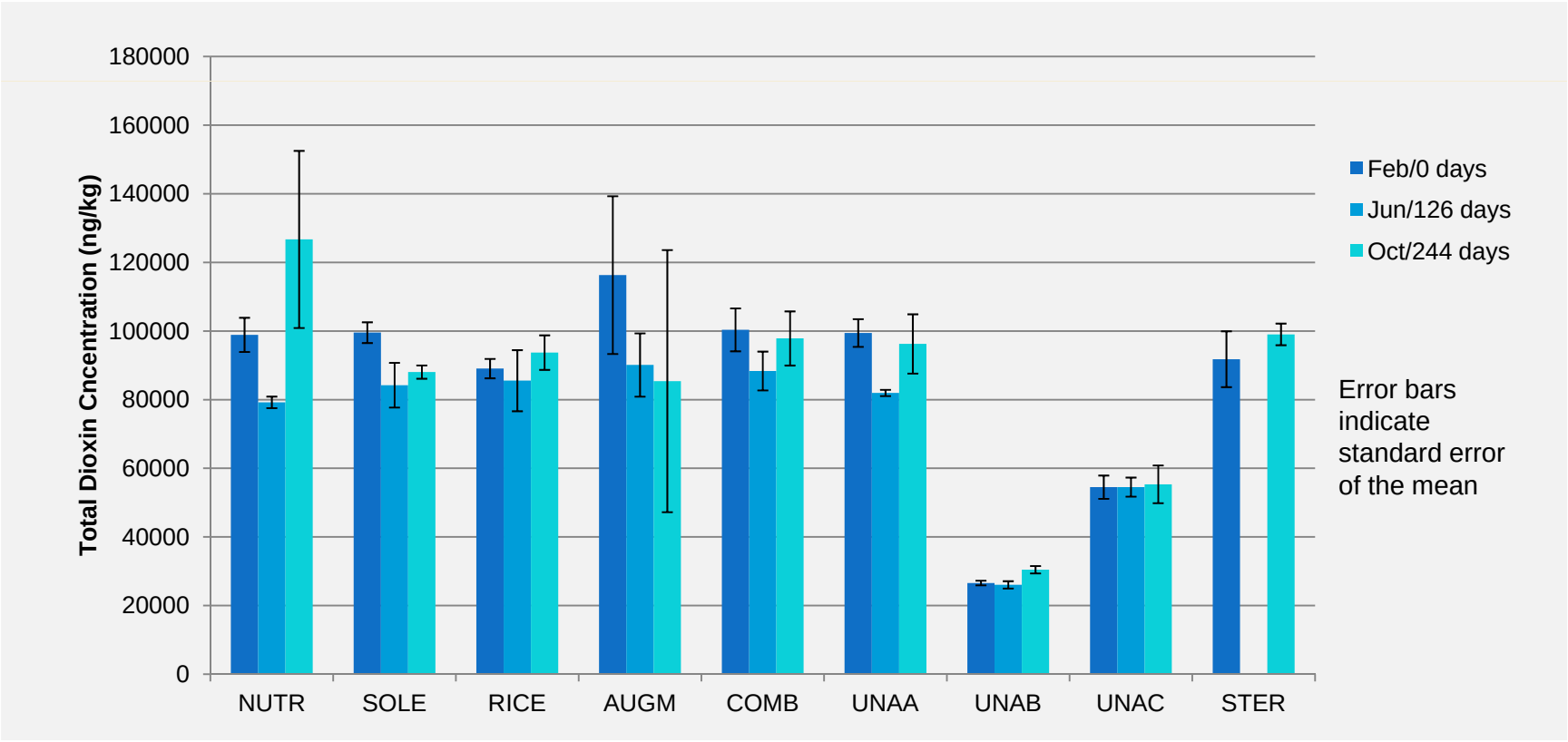
Microcosm Results: Polychlorinated Biphenyls (PCBs)

- PCB soil concentrations (Aroclor 1260) decreased
- Sterile control PCB concentration also decreased
- PCB adsorption onto glass is a possibility



Microcosm Results: Chlorinated Dioxins/Furans

- Bioaugmented sample (fungi): Decrease in dioxin concentrations observed
- Other treatments: Dioxin concentration stayed the same or increased (due to variability of soil concentrations)

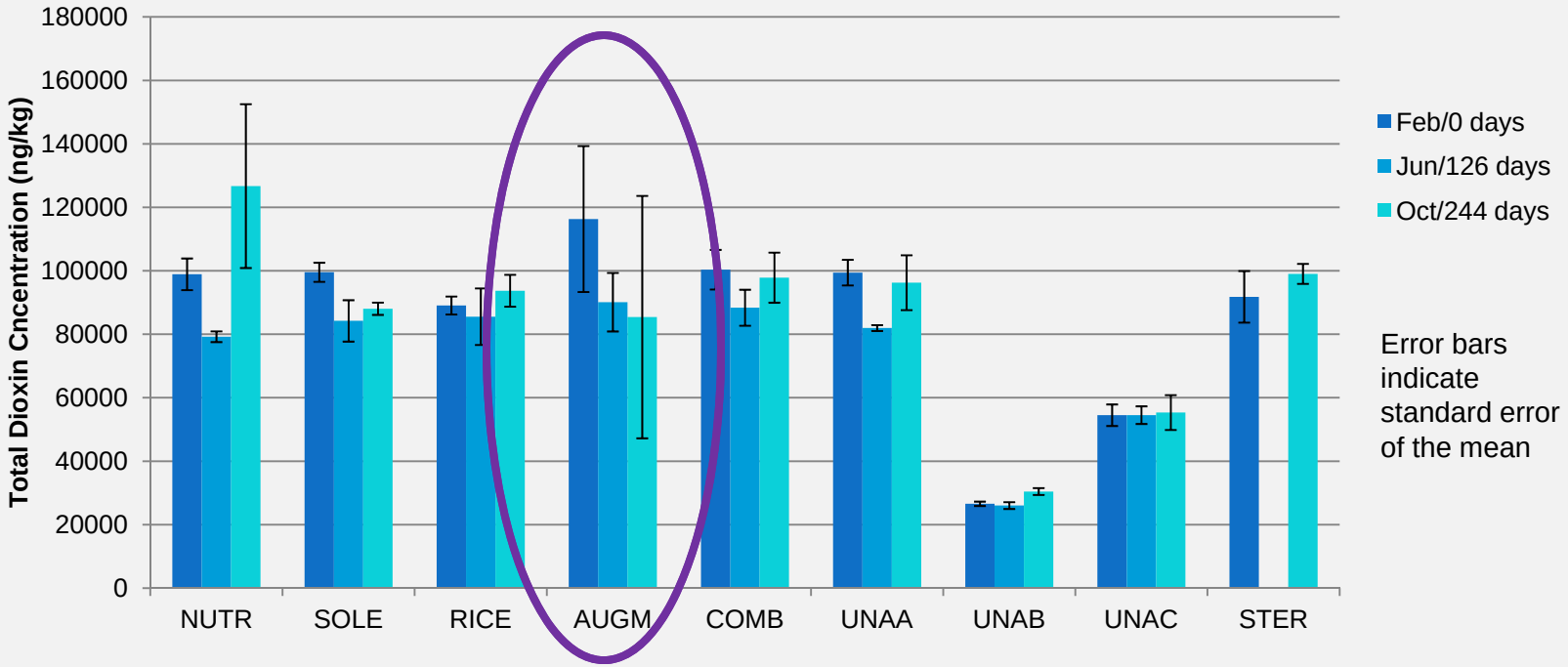


Microcosm Results: Biostimulation

- Fertilizer Addition:
  - Little or no effect of fertilizer (N&P) addition on biodegradation rates of any of the COIs
  - Either adequate fertilizer is already available, or biodegradation stalled for some other reason
- Bulking Agent Addition
  - Rice hulls did not improve biodegradation rates
  - Soil is already aerobic, so aeration provided by rice hulls was not helpful
- Surfactant Addition:
  - No observable effect of surfactant on biodegradation of most of the COIs
  - Slight improvement for chlorinated dioxins (but not statistically significant)

Microcosm Results: Effect of Bioaugmentation with Fungi

- No observable effect of bioaugmentation with the white-rot fungi *Phanerochaete chrysosporium* on biodegradation of most of the COIs
- Bioaugmentation appeared to increase biodegradation of dioxins
  - However, bioaugmentation in combination with other additives did not improve dioxin biodegradation



Conclusions

- Field microbial assays indicate the presence of bacteria and fungi in SSFL Area IV soils which are capable of biodegrading the COIs.
- Laboratory microcosm experiments suggest slow biodegradation rates for all of the COIs
  - Slight decreases in COI concentrations observed over 8 months
  - Decreases were not statistically significant at 95% confidence
  - Additional incubation time may be necessary
- Biostimulation with fertilizer and bulking was not effective.
- Biostimulation with surfactant had limited effect.
- Bioaugmentation with the white-rot fungi *Phanerochaete chrysosporium* is somewhat promising for dioxins.

More Conclusions

- COIs at SSFL appear to be highly weathered – most of the readily biodegradable compounds have already biodegraded or volatilized, and remaining compounds are likely to be sequestered in the soil, reducing their bioavailability.
- Bioremediation could be considered for areas with low contaminant concentrations.
- Field trials could be used for future testing of biostimulation and/or bioaugmentation, although these approaches were not very promising in the laboratory microcosm experiment.

Questions?