



Intrinsyx Environmental

*Combining nature with engineered
solutions for sustainable environmental
remediation*

Trees and Microbes as Sustainable Nature-Based Treatment on Hydrocarbons and Mixed Waste Sites

Date: 16 October

Presented by: Chris Cohu, Ph.D.
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Intrinsyx Environmental
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Presented to:
Remediation Technologies
Symposium 2025

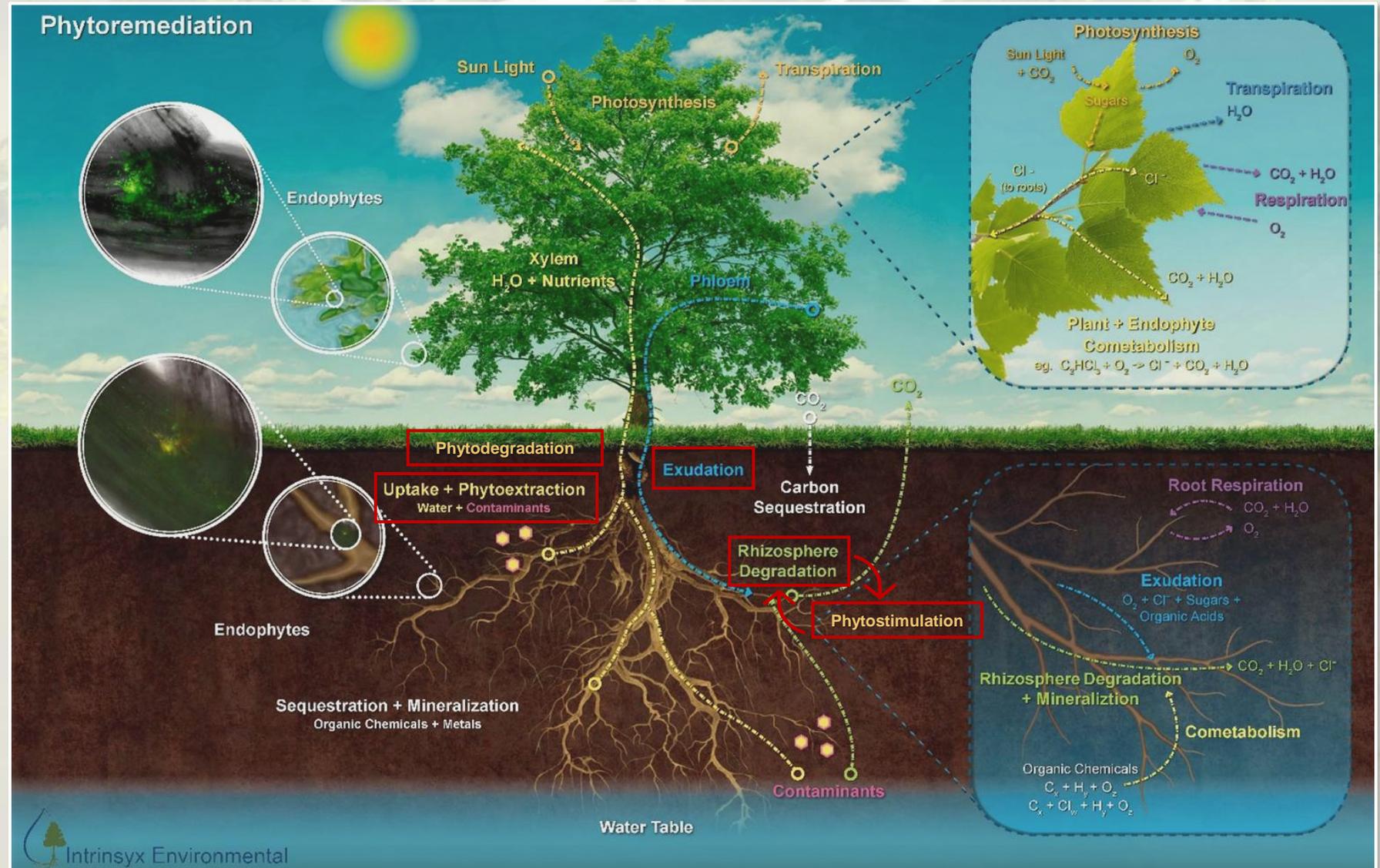


Agenda

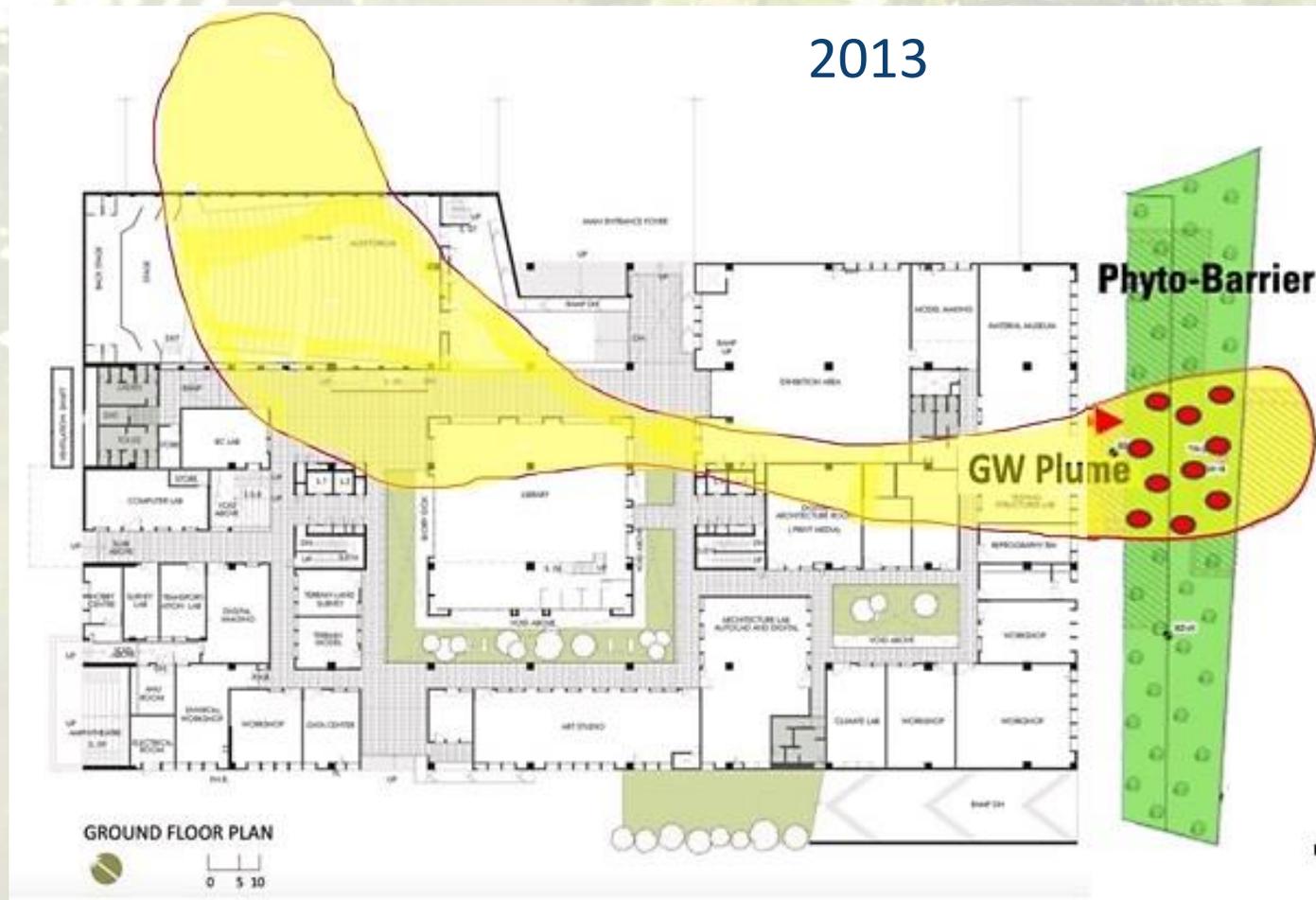
- Phytoremediation Science
- Endophytes and Benefits for Phytoremediation
 - Case Study - TCE (MEW Superfund Site)
 - Case Study - Petroleum Hydrocarbon (Dos Palos Site)
 - Endophyte Applications for Metals and Restoration

Phytoremediation Overview

- Bioremediation technology
- Uses plants, trees, & microbes
- Can remediate:
 - Soil
 - Sediments
 - Groundwater
 - Surface water
 - Wastewater
 - Leachate



Previous Limitations of Phytoremediation



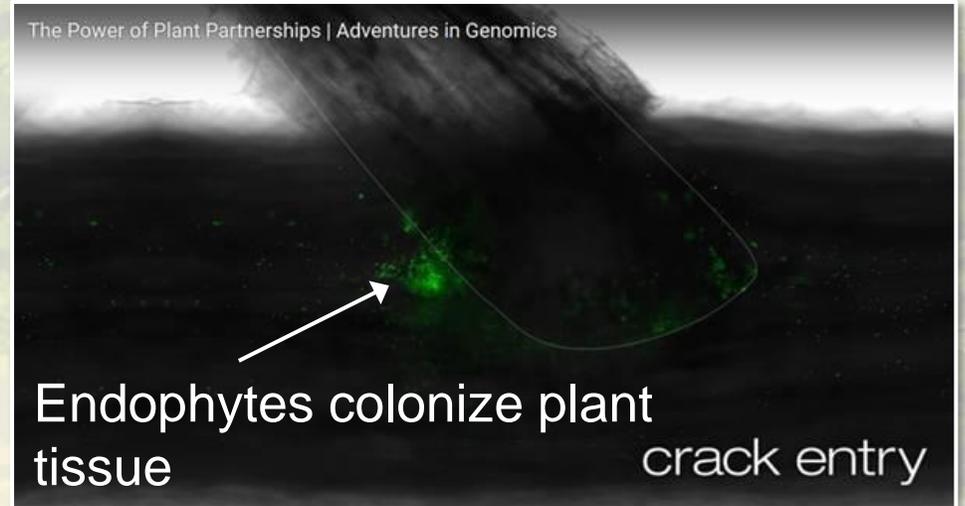
Accumulation of VOCs =
Phytotoxicity =
Decreased Growth and
Tree Mortality

How do we solve this?

Phytoremediation using Endophytes

Microorganisms living inside (“endo”) of plants (“phyte”)

- Endophytes are bacteria and fungi living naturally in the environment
- They create symbiotic relationships with plant hosts
- Our goal is to understand these symbiotic relationships and utilize them to make phytoremediation successful in the harshest of environmental conditions!





Professor Sharon Doty

Prof Sharon Doty (University of Washington)

- 3 decades studying unique microbes in plants adapted to harsh environments
- Research collaboration with IE Chief Science Officer Dr. John Freeman
- Discovered highly characterized strains published in dozens of scientific journals
- Intrinsyx is exclusive licensee of Dr. Doty's microbial collection

Explore genomic discoveries

Videos highlighting recent breakthroughs and the scientists behind them



Sharon L. Doty

The Power
of Plant
Partnerships

Endophyte-Assisted Phytoremediation

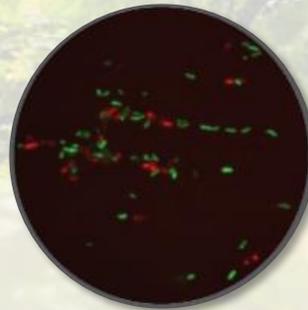
Endophyte – microorganisms living inside (“*endo*”) of plants (“*phyte*”)

Endophyte strains in plants **adapted to contaminated environments**

- Enhanced **resilience/tolerance to environmental contamination**
- Enhanced **resilience to other stressors** (salts, drought, heat, poor soil quality)
- Enhanced **extraction (organics and inorganics) and degradation (organics)**
- Enhanced **plant growth and nutrient uptake**

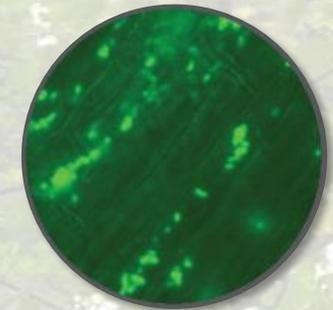
BioTREEt-HC

Hydrocarbons



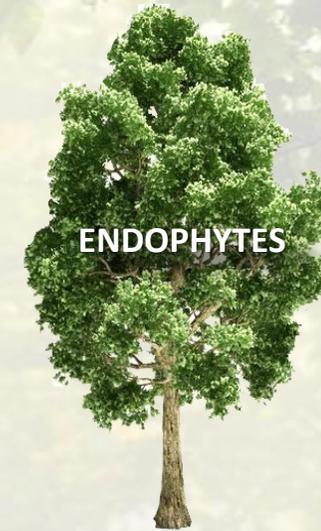
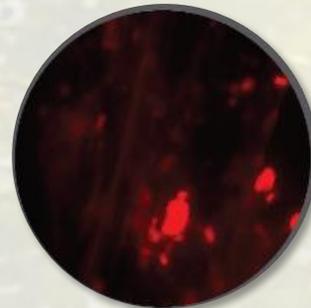
BioTREEt-CVOC

Chlorinated VOCS



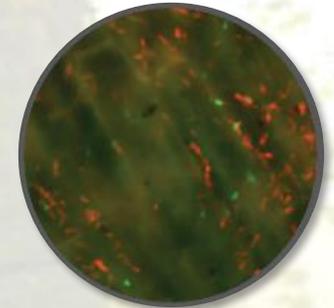
BioTREEt-PCB

Polychlorinated biphenyl (PCB)



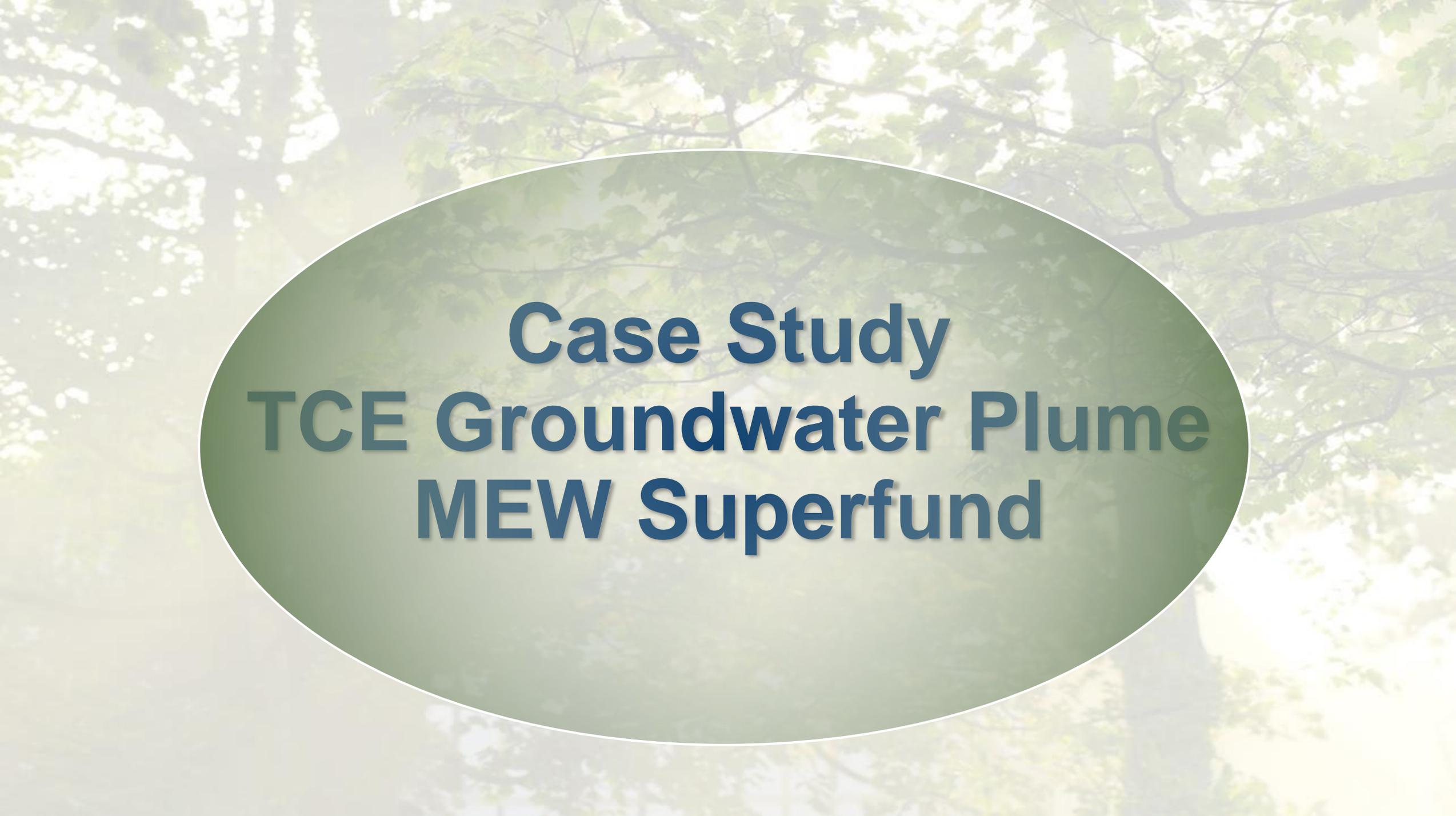
BioTREEt-RDX

Explosives (TNT/RDX)



BioTREEt-Restore

Enhance Growth and Nutrients,
Salts and Drought Tolerance



Case Study
TCE Groundwater Plume
MEW Superfund

NASA Ames Pilot Study using Endophytes

- Middlefield-Ellis-Whisman (MEW) Superfund site
 - Former site of microchip manufacturing
- Trichloroethylene (TCE, a cVOC) in groundwater
- Migrated onto NASA property
- Phytoremediation using hybrid poplars



Tree growth after 12 months

Endophyte-Enhanced TCE Tolerance Growth



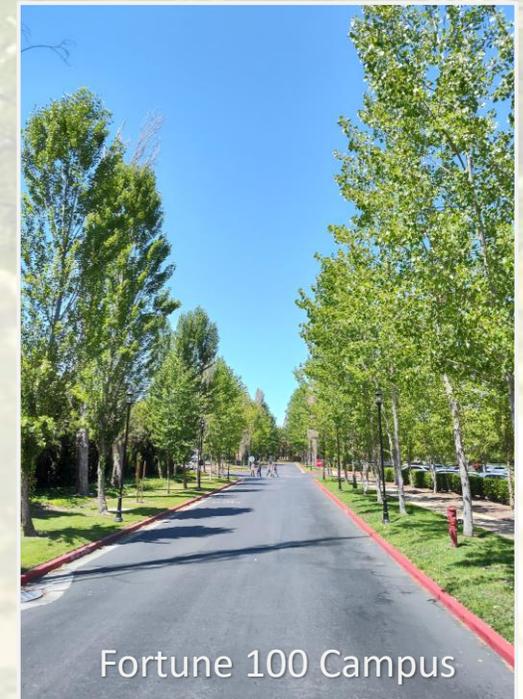
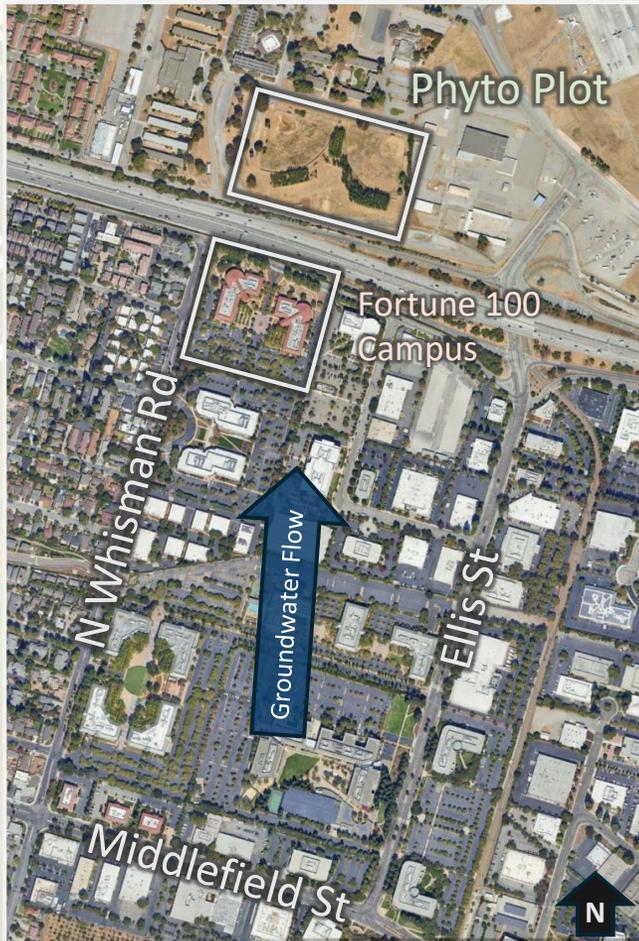
Control

Inoculated

Control

Inoculated

NASA Ames Pilot Study



	September 28 2016		November 21 2016		June 27 2017		July 17 2018		December 4 2024		Detection Limit	Reporting Limit
	Test Well 1	Test Well 2	Test Well 1	Test Well 2	Test Well 1	Test Well 2	Test Well 1	Test Well 2	Test Well 1	Test Well 2		
	µg/L											
Trichloroethene (TCE)	280	ND	300	ND	260	ND	130	ND	300	ND	0.85	5
trans-1,2-Dichloroethene (tDCE)	1.7	ND	3.1	ND	2.2	ND	2	ND	5.3	ND	0.15	0.5
cis-1,2-Dichloroethene (cDCE)	140	ND	160	ND	120	ND	90	ND	150	13	0.085	0.5
1,1-Dichloroethene (1,1-DCE)	7.6	ND	6.8	ND	5.8	ND	4.6	ND	4.6	ND	0.18	0.5
1,1,1-Trichloroethane (1,1,1-TCA)	<0.18	ND	2.4	ND	2.4	ND	0.7	ND	0.76	ND	0.11	0.5
1,1-Dichloroethane (1,1-DCA)	7.9	0.64	0.9	0.58	8.1	ND	6	0.48	9.1	1.4	0.11	0.5
Vinyl chloride (VC)	0.45	ND	0.77	ND	<0.5	ND	0.5	ND	0.87	ND	0.12	0.5
Tetrachloroethene (PCE)	0.5	ND	0.88	ND	0.86	ND	<0.12	ND	1.3	ND	0.13	0.5
1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113)	1.5	ND	1.5	ND	2.2	ND	2.4	ND	<0.15	ND	0.15	0.5

NASA Ames Pilot Study

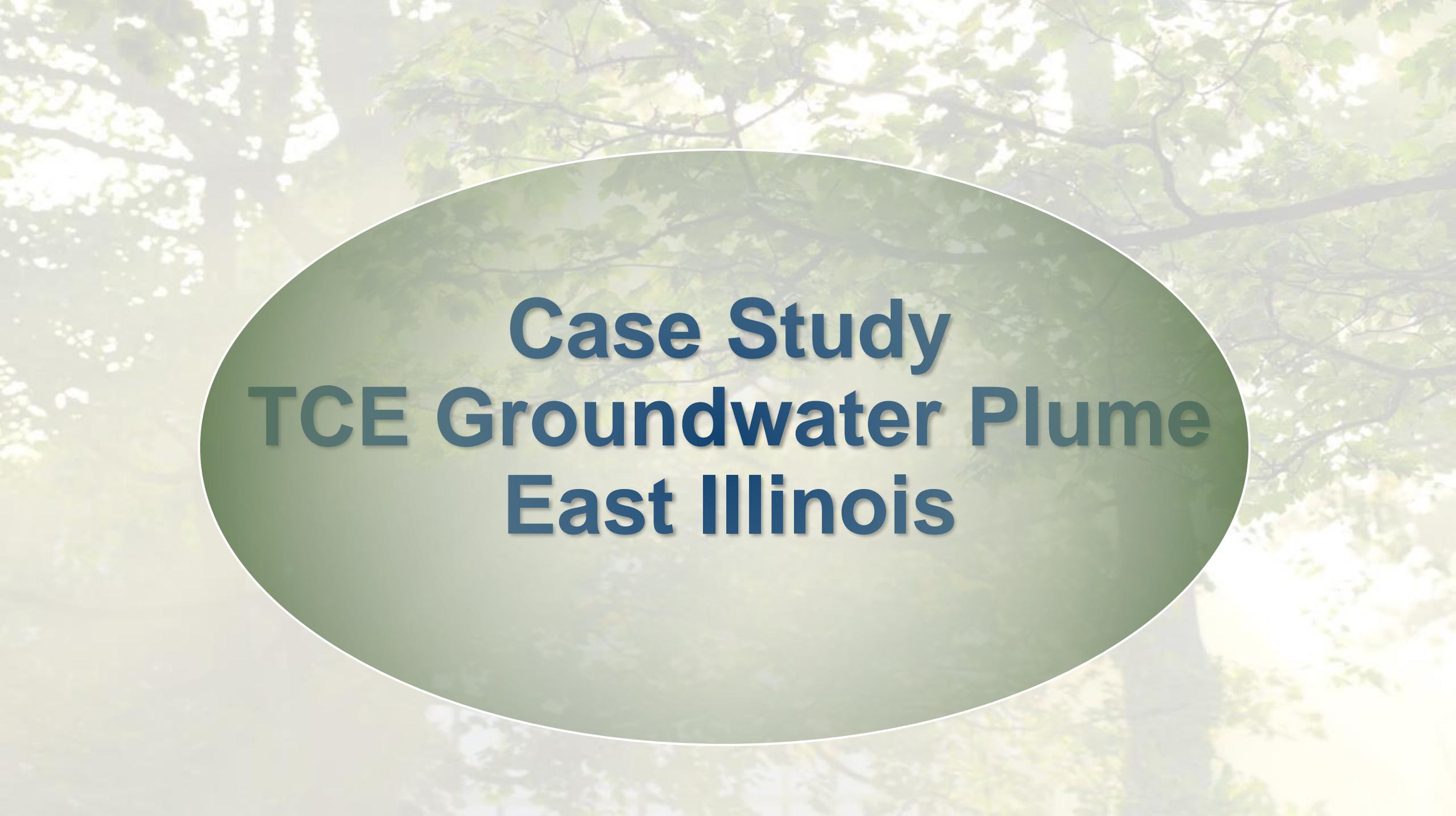


September 2021



AMES RESEARCH CENTER

- Effectively cut off TCE plume migrating onto base
- EPA Approval to expand remediation efforts – incorporating phytoremediation into any redevelopment plans



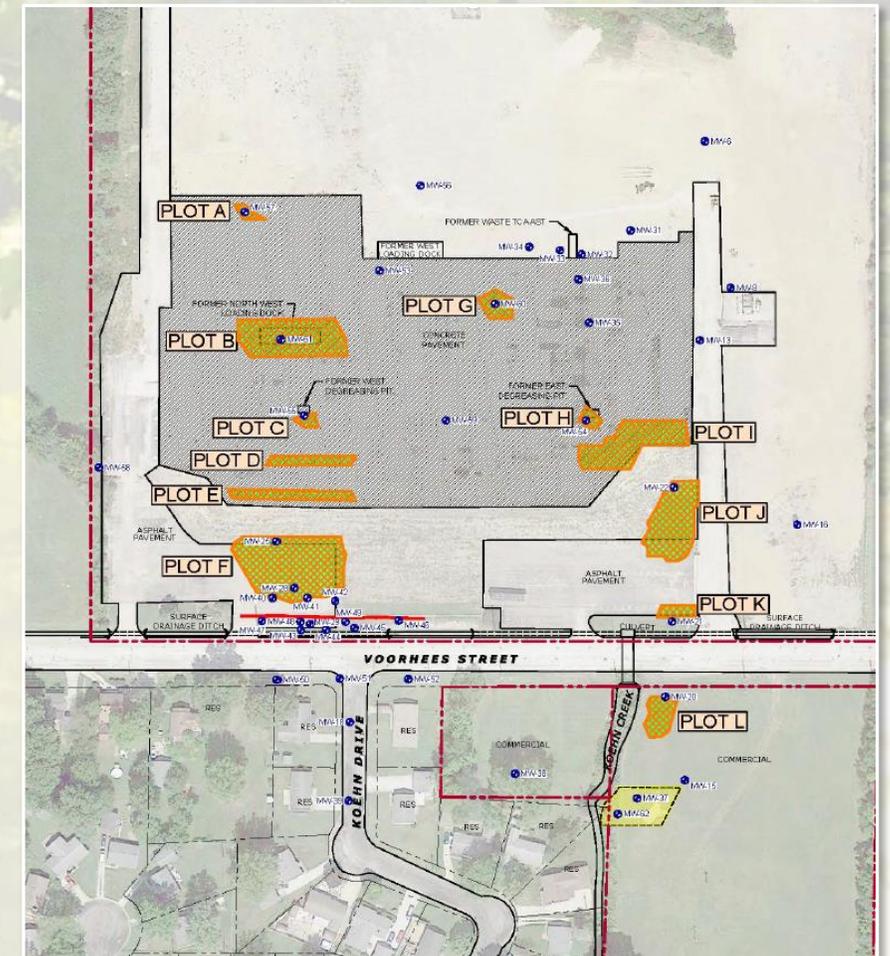
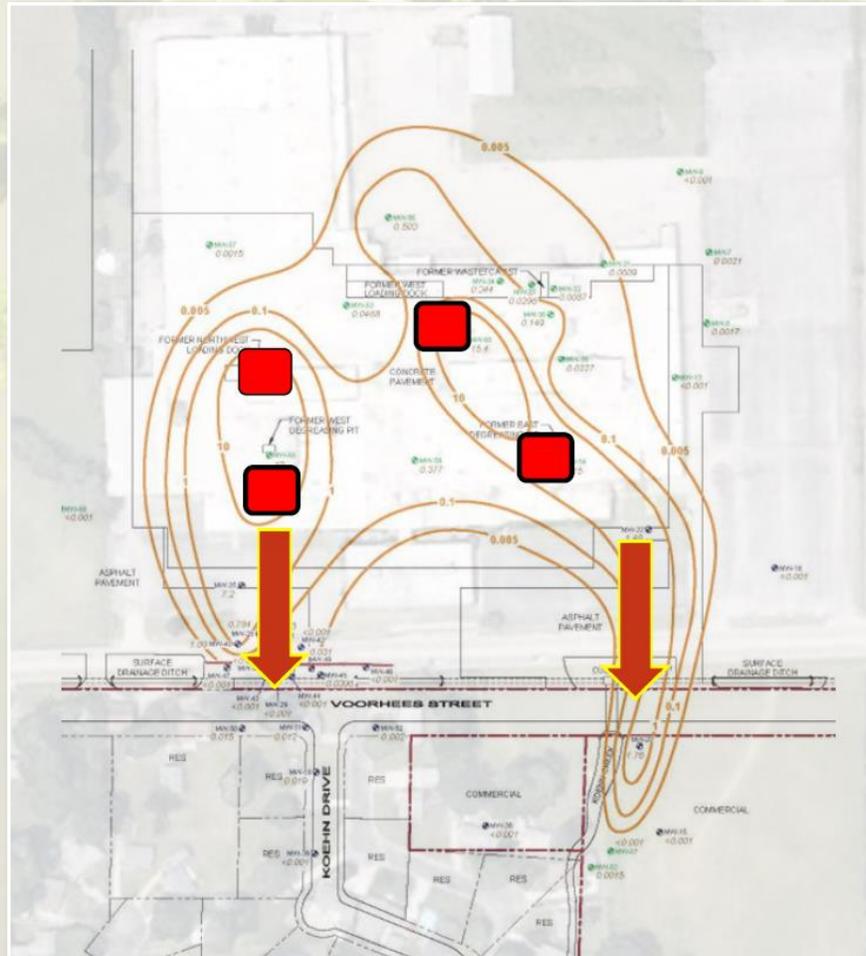
Case Study
TCE Groundwater Plume
East Illinois

Case Study – East Illinois

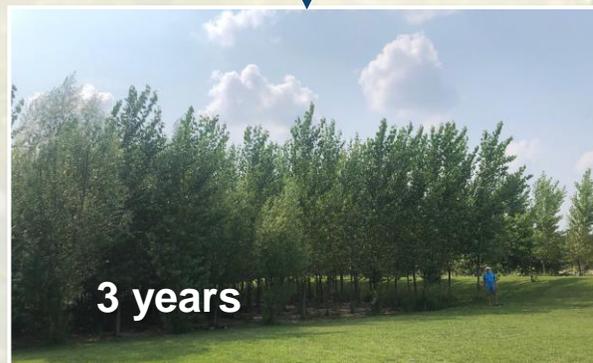
Background

- Refrigerant manufacturer
- CVOC plume under concrete (*TCE 81 mg/L*)
- Phyto installed through concrete
- Objectives
 1. Hydraulic control
 2. Contaminant reduction

RAMBOLL



Case Study – East Illinois



Case Study – East Illinois

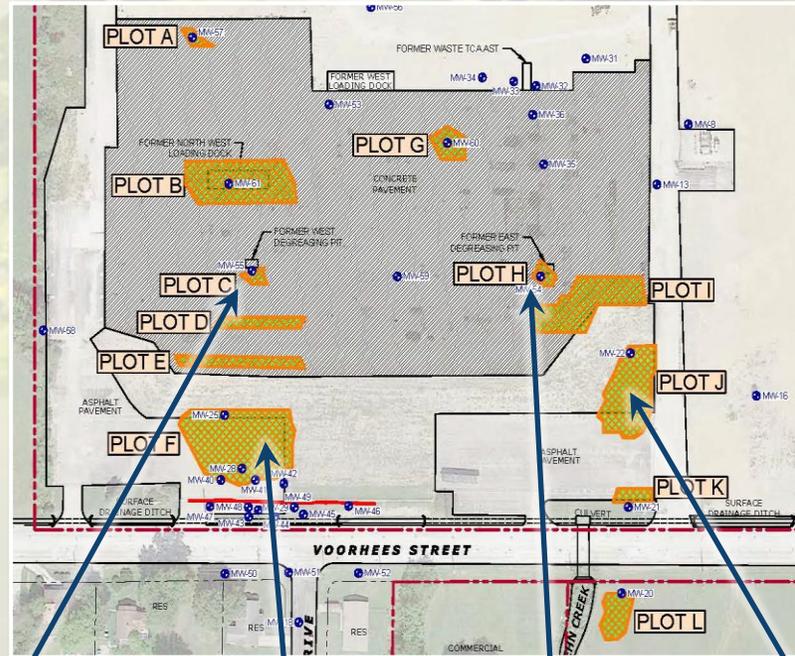
Results

- 100% tree survival
- 3-4 yrs objectives met
- Closure

Broad acceptance:

- Client
- Illinois EPA
- City Government
- Local Residents

Total VOCs Reductions (mg/L)



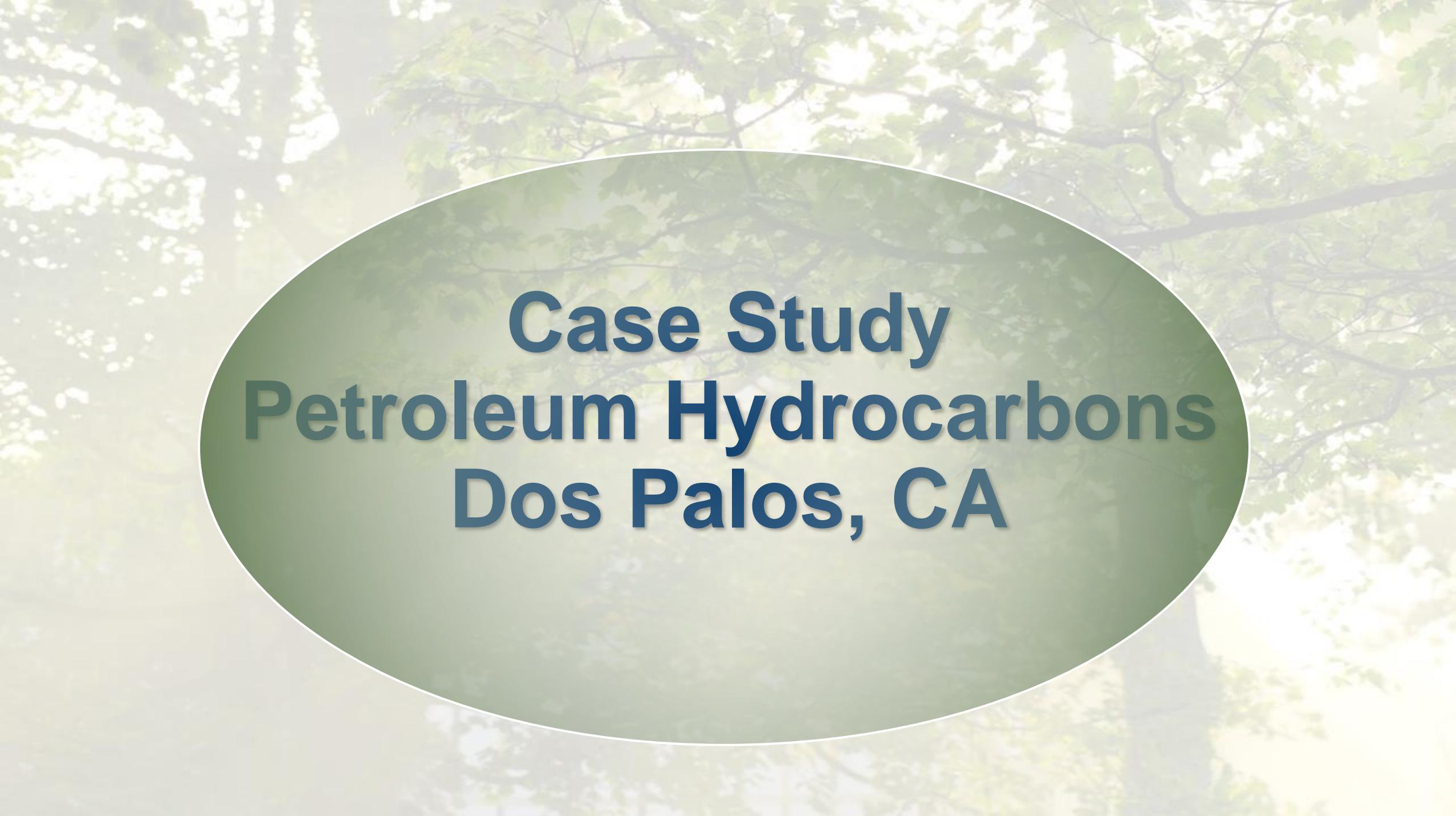
Plot C
Degreasing Pit
81 to 4.4
95%

Plot F
Downgradient
21.9 to 0.74
97%

Plot H
Degreasing Pit
11.6 to 0.45
96%

Plot J
Downgradient
22 to 2.5
89%





Case Study
Petroleum Hydrocarbons
Dos Palos, CA

Case Study – Dos Palos, CA

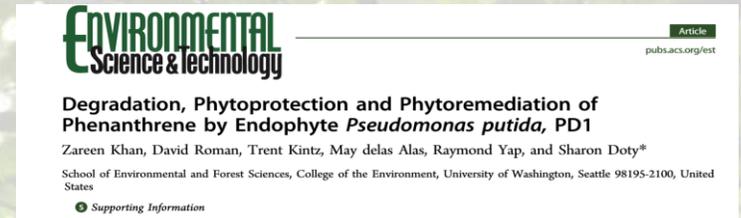
Scientific Basis for Endophytes on Petroleum Hydrocarbon Sites



Control

Inoculated

- 40% removal of phenanthrene (PAH) from soil by willow and grasses when inoculated, compared to un-inoculated controls
- BTEX, gasoline and diesel degradation also discovered



**School of Environmental
and Forest Sciences**

UNIVERSITY of WASHINGTON

College of the Environment



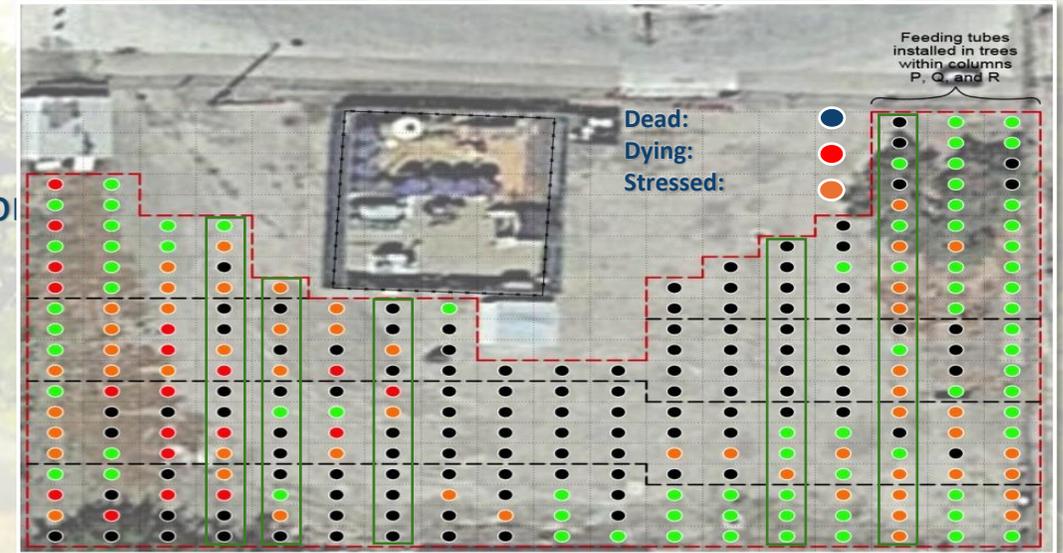
Control

Inoculated

Case Study – Dos Palos, CA

Background

- Former fuel transfer station (GRO/DRO)
- Multiple feet of LNAPL
- High concentrations of gas and diesel range compounds at or near solubility limits
- High O&M for SVE and P&T systems



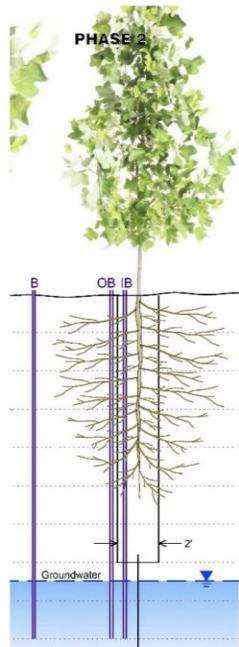
Species Biodiversity on the Dos Palos Site



Trees	Grasses	Bacterial Endophyte	Fungi
<i>Salix lucida</i> willow	Salt grass, <i>Distichlis spicata</i>	<i>Pseudomonas</i> <i>alloputida</i>	White rot mycelium- <i>Phanerochaete</i> <i>chrysosporium</i>
RRR poplar	Alkali milk-vetch <i>Astragalus tener</i>	<i>Sphingobium salicis</i>	<i>Pleurotus</i> <i>ostreatus</i>
	American vetch, <i>Vicia americana</i>	<i>Rhizophagus</i> <i>intradices</i>	<i>Trametes</i> <i>versicolor</i>
	Joaquin milk-vetch <i>Astragalus</i> <i>asymmetricus</i>		<i>Irpex lacteus</i>
	Fiddleneck, <i>Amsinckia tessellata</i>		Mycorrhizae- <i>Rhizophagus</i> <i>intradices</i>
	Desert bells, <i>Phacelia</i> <i>campanularia</i>		

Tree Root Microbial Analysis versus Background Soils

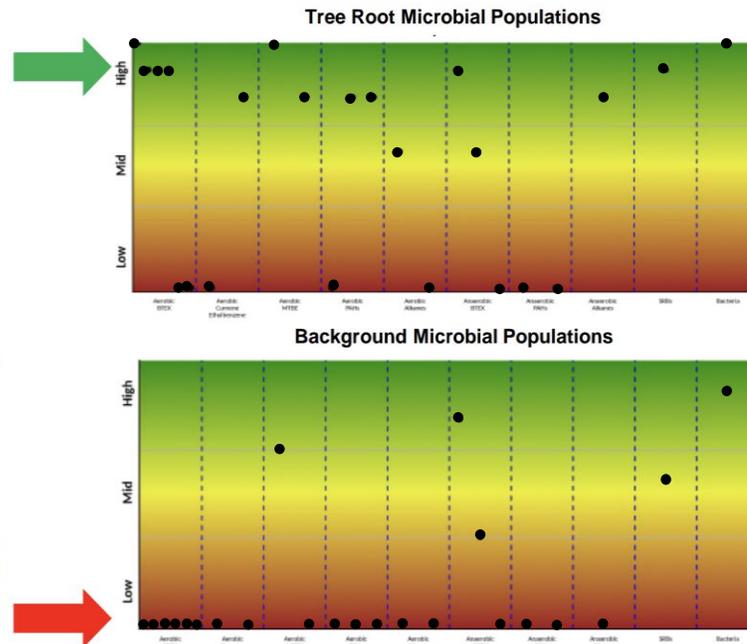
Microbial Analysis Sample Collection and Results



T-48
Willow

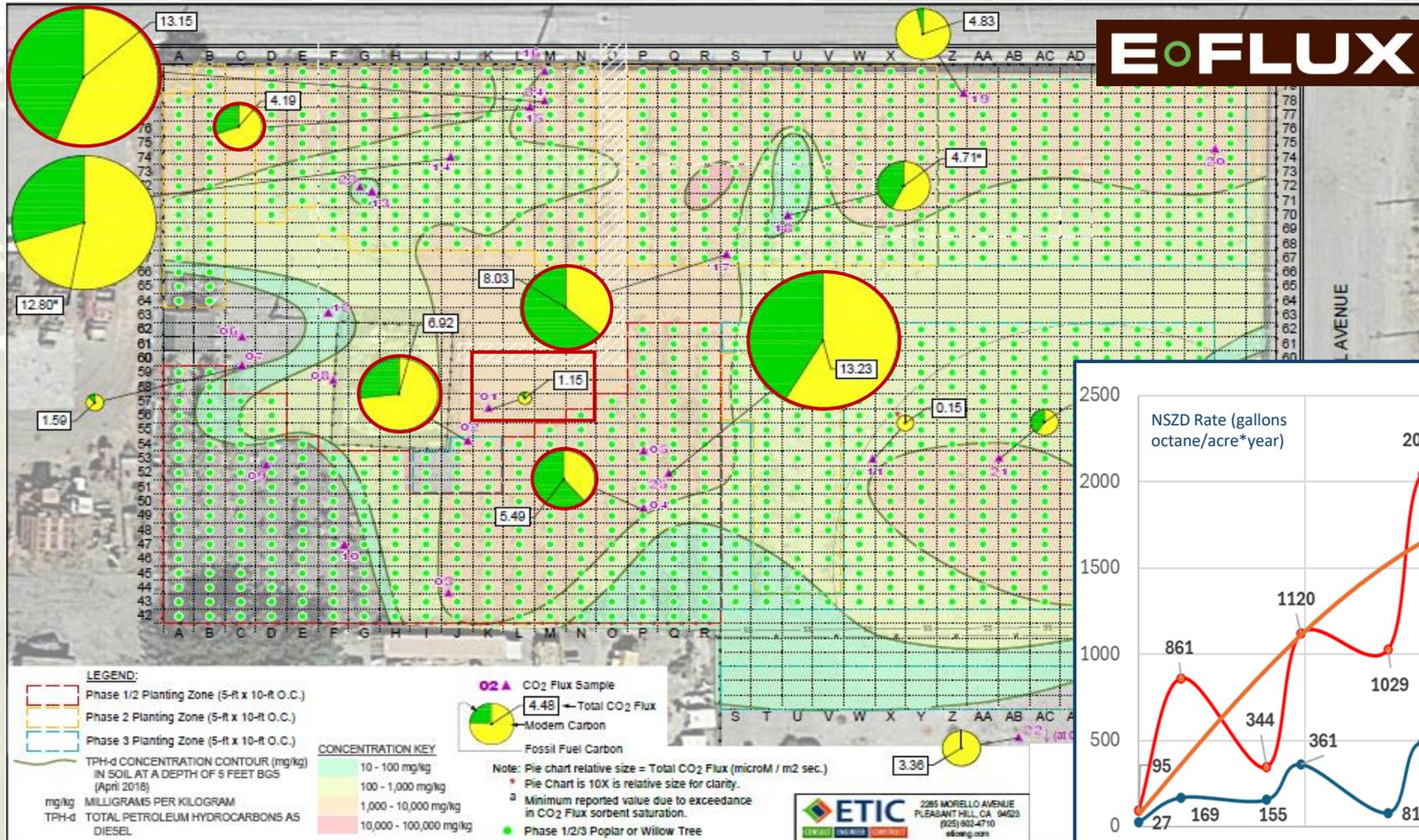


qPCR analyses shows higher **hydrocarbon degrading** microorganism concentrations associated with **tree roots** than in **background**



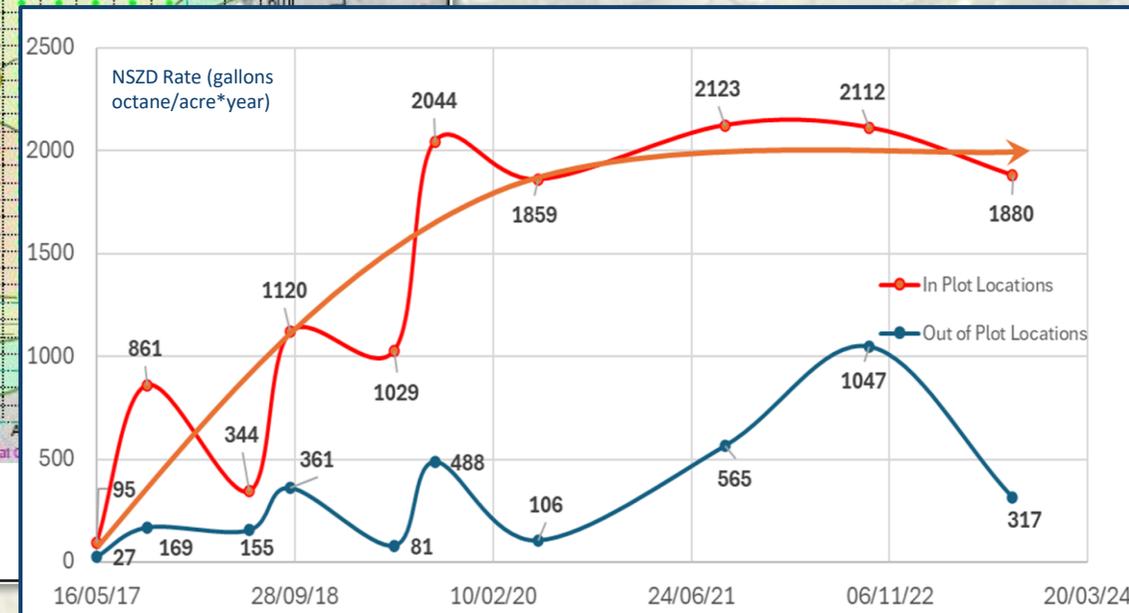
- Threefold increase in biodiversity near tree roots (number of dots present)
- Increased functional diversity and hydrocarbon degrading bacteria near tree roots (number of x-axis bacterial groups being detected).

Case Study – Dos Palos, CA



Natural Source Zone Depletion (NSZD)

Occurs through the combined action of natural processes that reduce the mass of LNAPL in the subsurface.



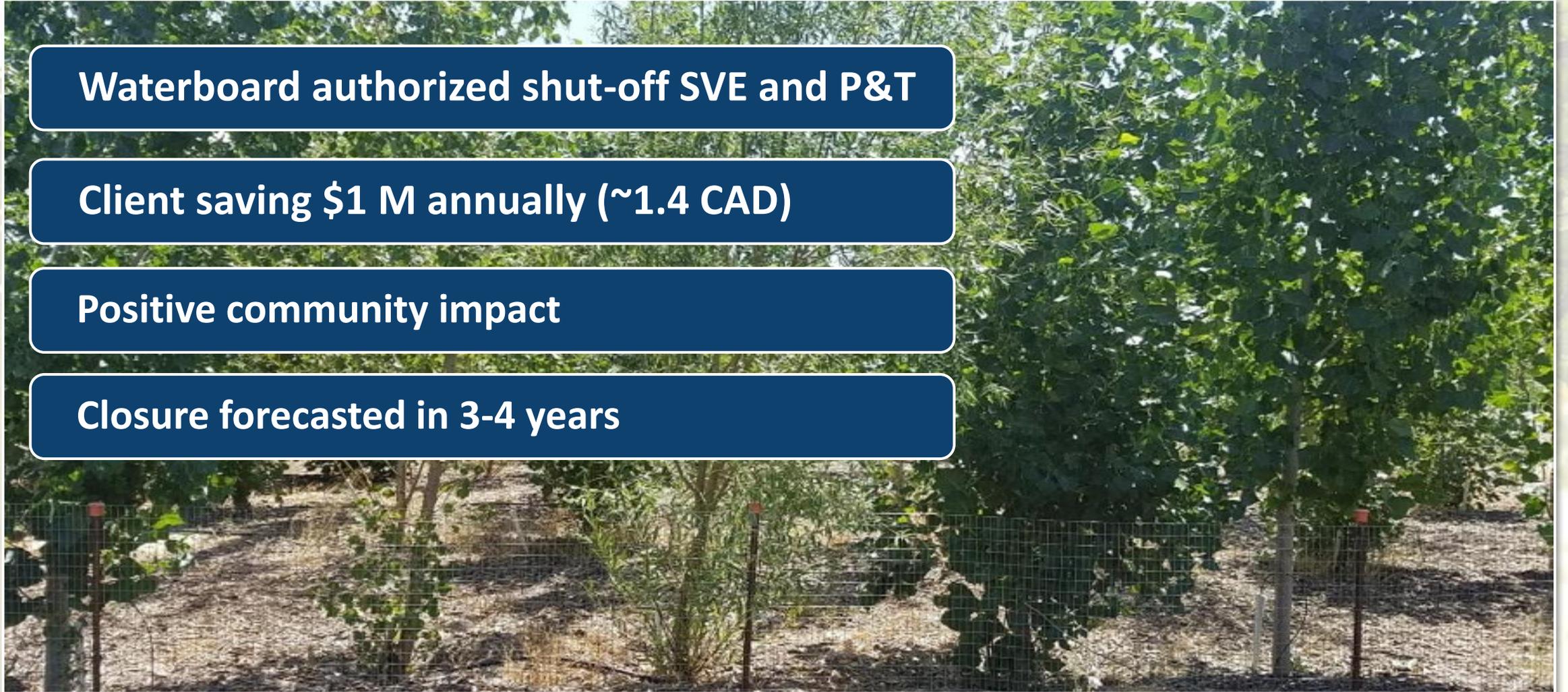
Case Study – Dos Palos, CA

Waterboard authorized shut-off SVE and P&T

Client saving \$1 M annually (~1.4 CAD)

Positive community impact

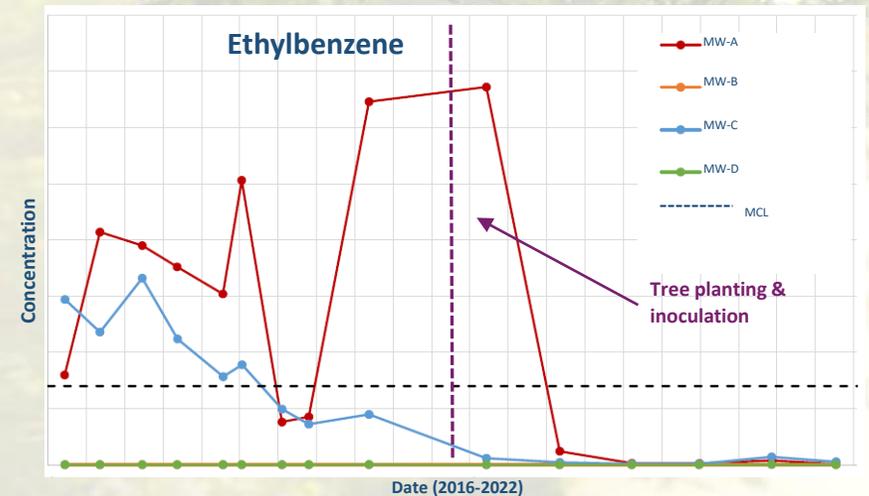
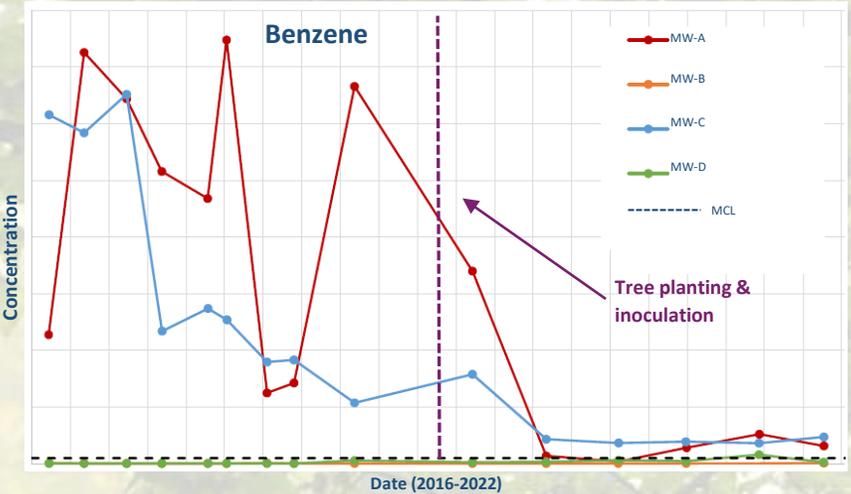
Closure forecasted in 3-4 years



Case Study – Coastal TX

Background

- Brownfield site
- Hydrocarbons (TPH, BTEX, PAH) → BioTREEt-HC inoculation
- Barren site → thriving plants
- Significant reduction in COC concentrations



**Additional
Phytoremediation
&
Eco-restoration Benefits**

Phytoremediation & Eco restoration Benefits

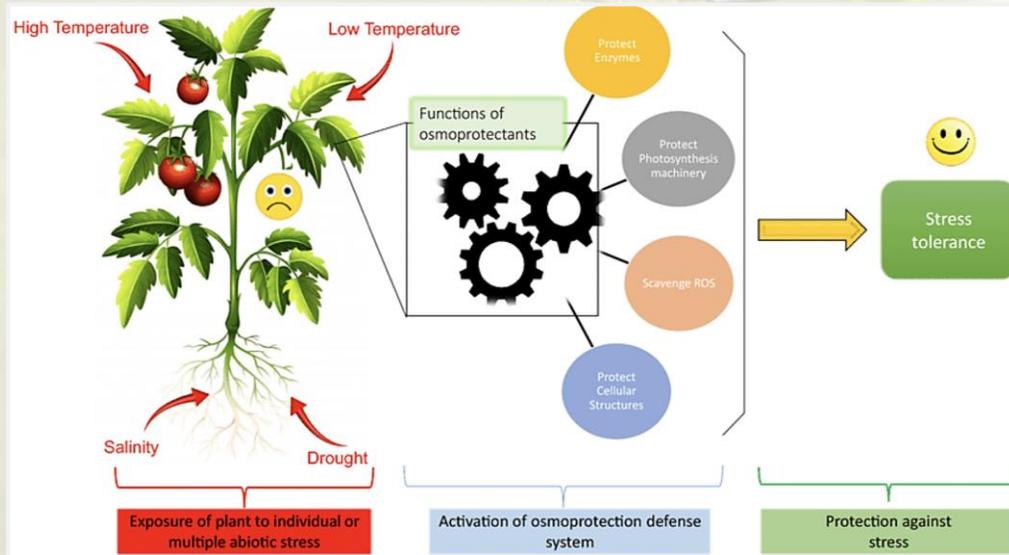
Endophytes Increase Metals Uptake, Nutrient Content, and Salt and Drought Tolerance

Ectoine

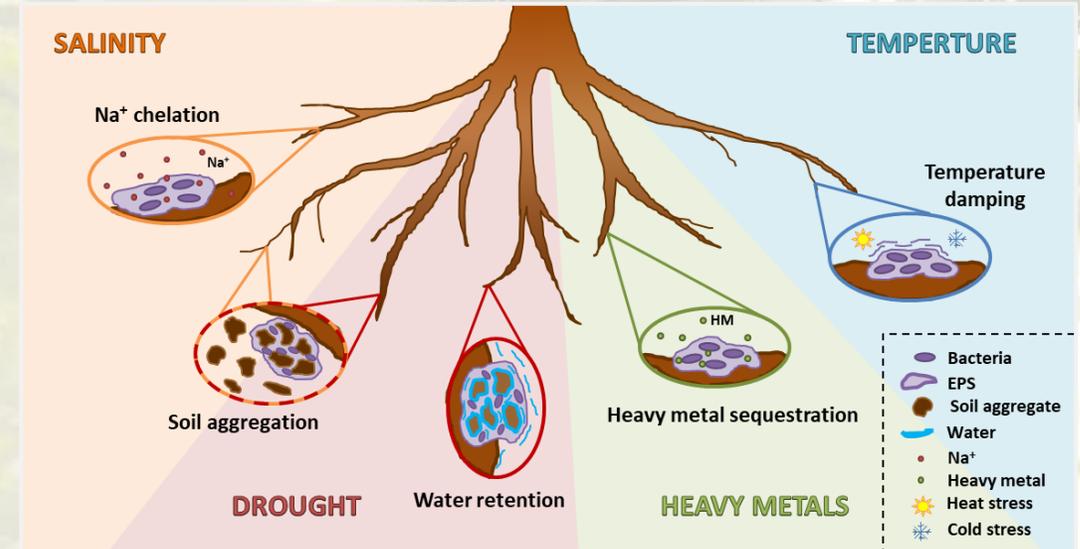
- Acts as osmolyte
 - Osmolyte = organic compounds that influence the properties of biological fluids
- Protective against stressors such as drought, salinity, temperature, and heavy metals

Exopolysaccharides (EPS)

- Increase water permeability
- Increase nutrient uptake
- Increase soil stability & fertility
- Increase plant biomass (increased root & shoot length + leaf surface area)
- Increase chlorophyll content



Zulfiqar, Faisal & Aisha, Nudrat & Ashraf, Muhammad. (2019). Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon. *Planta*. 251. 10.1007/s00425-019-03293-1.



Morcillo, R.J.L.; Manzanera, M. The Effects of Plant-Associated Bacterial Exopolysaccharides on Plant Abiotic Stress Tolerance. *Metabolites* 2021, 11, 337. <https://doi.org/10.3390/metabo11060337>

Phytoremediation & Ecorestoration Benefits

Drought Tolerance

Inoculated

Control



Inoculated

Control



Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. *ISRN Agronomy*

Phytoremediation & Ecorestoration Benefits

Drought Tolerance

University of
Washington Study
on Water Use
Efficiency (WUE)
and Drought
Tolerance

Hybrid poplar inoculated with endophytes from wild poplar and willow have increased growth and drought tolerance



Poplar inoculated with endophytes
one month without watering

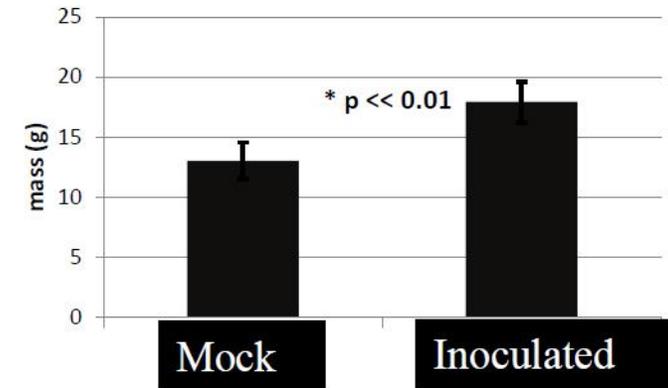


Khan, Z., Rho, H., Firrincieli, A., Hung, S.H., Luna, V., Masciarelli, O., Kim, S.H., and Doty, S.L. 2016. *Current Plant Biology* 6:38-47



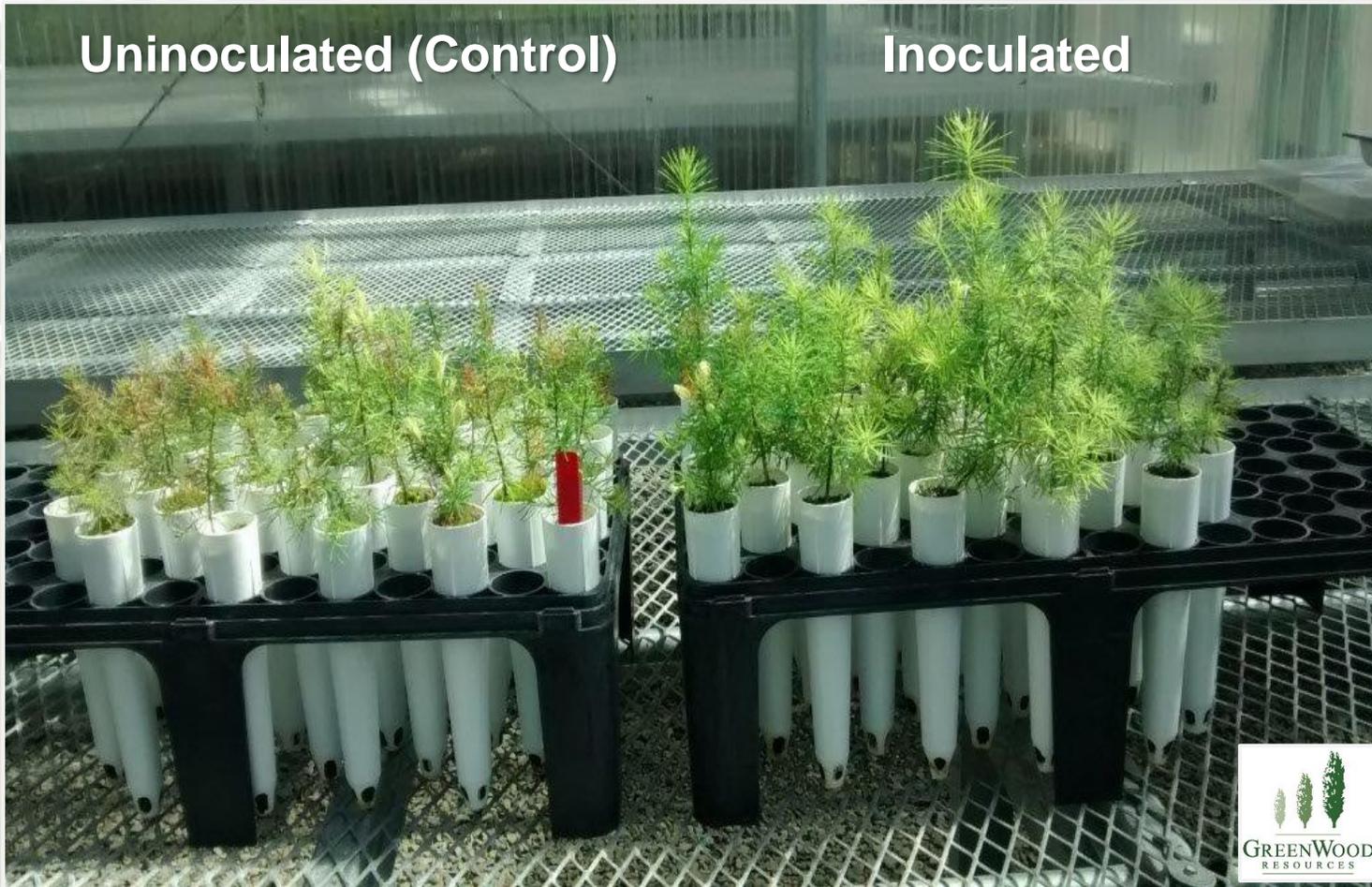
Advanced Hardwood Biofuels Northwest

Total mass (dry)



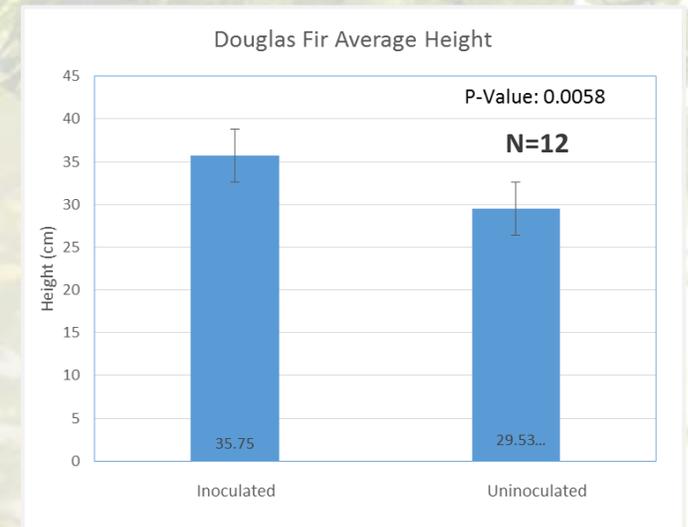
Phytoremediation & Ecorestoration Benefits

3rd Party Forestry Trials - Douglas Fir (*Pseudotsuga menziesii*)



Zareen Khan

Increased growth of Douglas fir in nutrient-poor soil in response to endophyte consortium from poplar & willow



Khan, Z., Ramos, D. Ettl, G., Kim, S.H., and Doty, S.L. 2015 *Forests* 6:3582-3593

Phytoremediation & Ecorestoration Benefits

Endophyte Restoration Solutions

Solution:

Land restoration using plants, trees, and eco- endophytes

Revegetation: Eco-endophytes are plant, tree, and soil microbes that protect young seedlings from harsh post-fire conditions, including chemicals released from fires, heat, drought, and low nutrients



Survival, Establishment, and Drought Tolerance: Eco-endophytes provide faster establishment and growth in depleted soil—providing nutrients, structure, and retaining water for soil microbiome recovery, survival and drought tolerance

Erosion Control: Inoculated trees and grasses show enhanced root growth and rapidly establish soil cover. Side-by-side tests show increased biomass compared to uninoculated species

Our Projects

Current Projects

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- Puerto Rico
- South Africa
- Austria

Developing Business

- Canada
- Africa
- EU
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It can be done

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