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: 28 April 2026

Presented by: Chris Cohu, Ph.D.
CEO and Lead Scientist
Intrinsyx Environmental
Chris@intrinsyxenvironmental.com

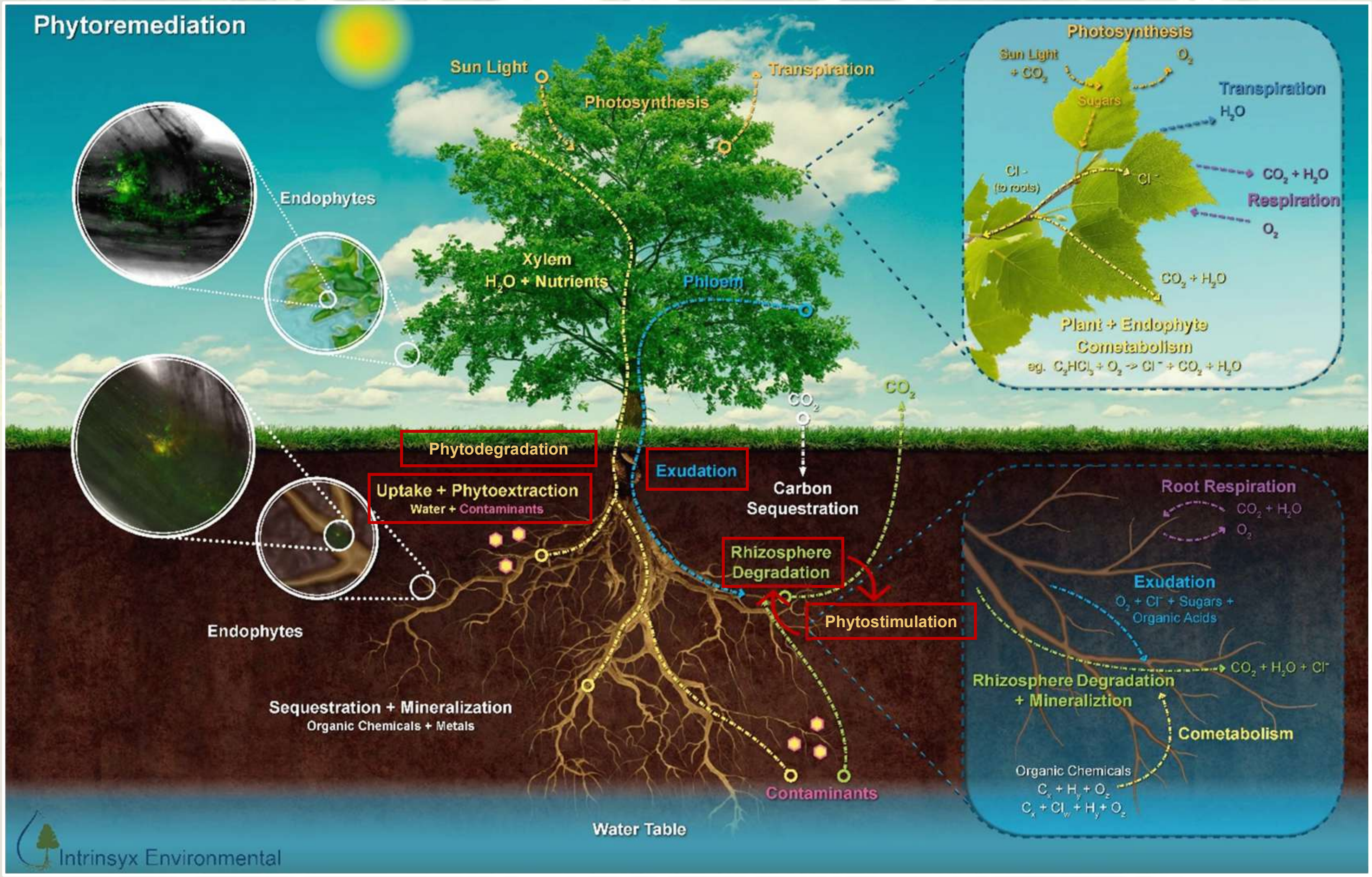
Presented to:
AIT Austrian Institute of Technology GmbH

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

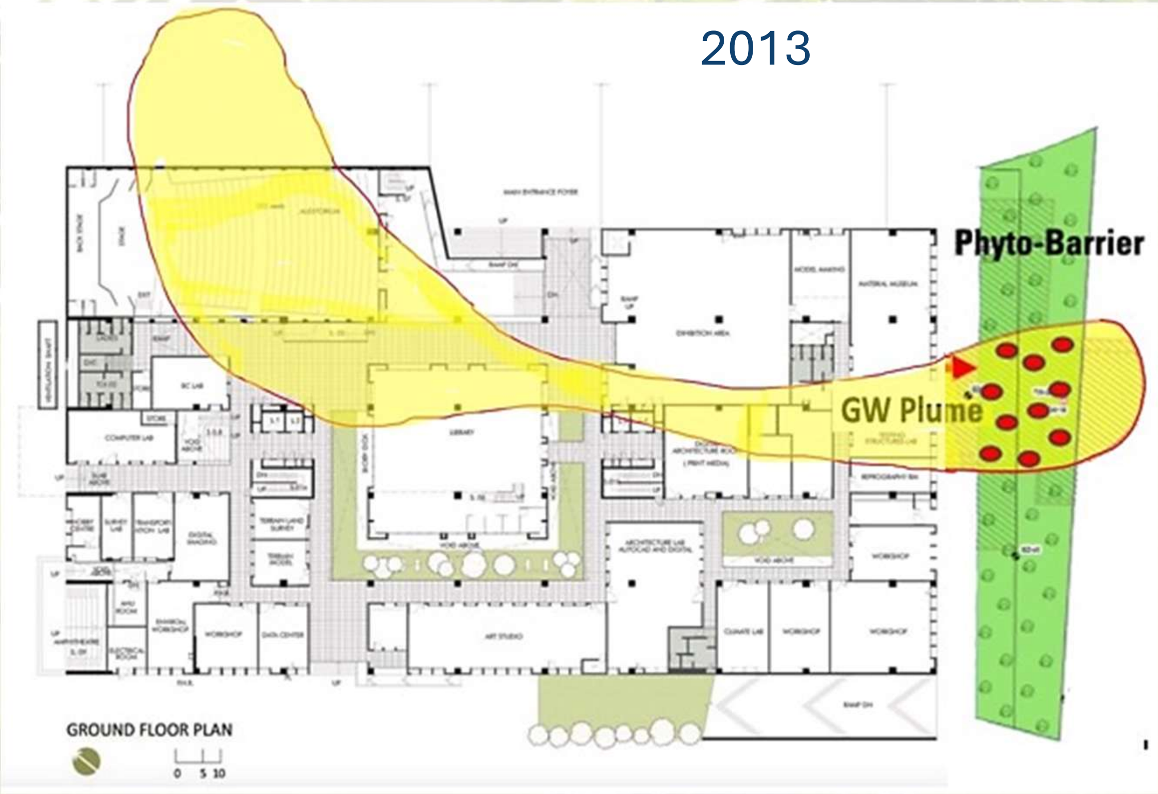
Phytoremediation Overview

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



Intrinsyx Environmental

Previous Limitations of Phytoremediation



Trees Dead
Trees Alive

Accumulation of VOCs
= Phytotoxicity
= Decreased Growth
and Tree Mortality

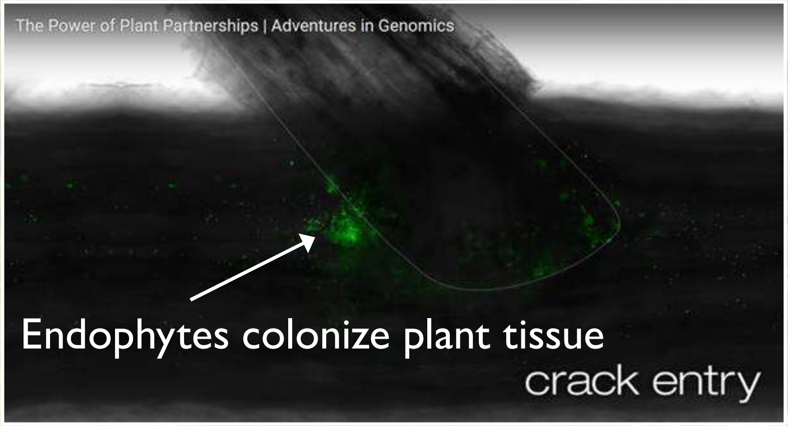
How do we solve this?

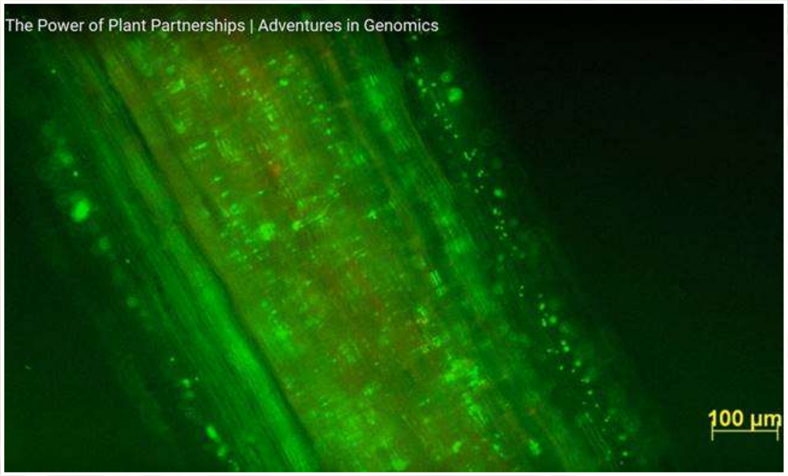
Intrinsyx Environmental

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

Endophyte-Assisted Phytoremediation

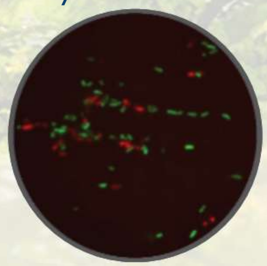
Endophyte – microorganisms living **inside** (“***endo***”) of **plants** (“***phyte***”)

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***

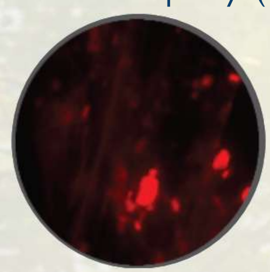
BioTREEt-HC

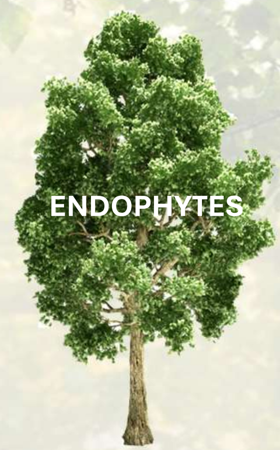
Hydrocarbons



BioTREEt-PCB

Polychlorinated biphenyl (PCB)



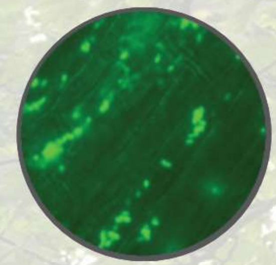


BioTREEt-Restore

Enhance Growth and Nutrients, Salts and Drought Tolerance

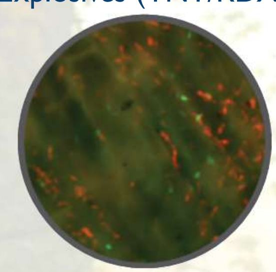
BioTREEt-CVOC

Chlorinated VOCS



BioTREEt-RDX

Explosives (TNT/RDX)



Case Study
TCE Groundwater Plume
MEW Superfund

NASA Ames Pilot Study using Endophytes

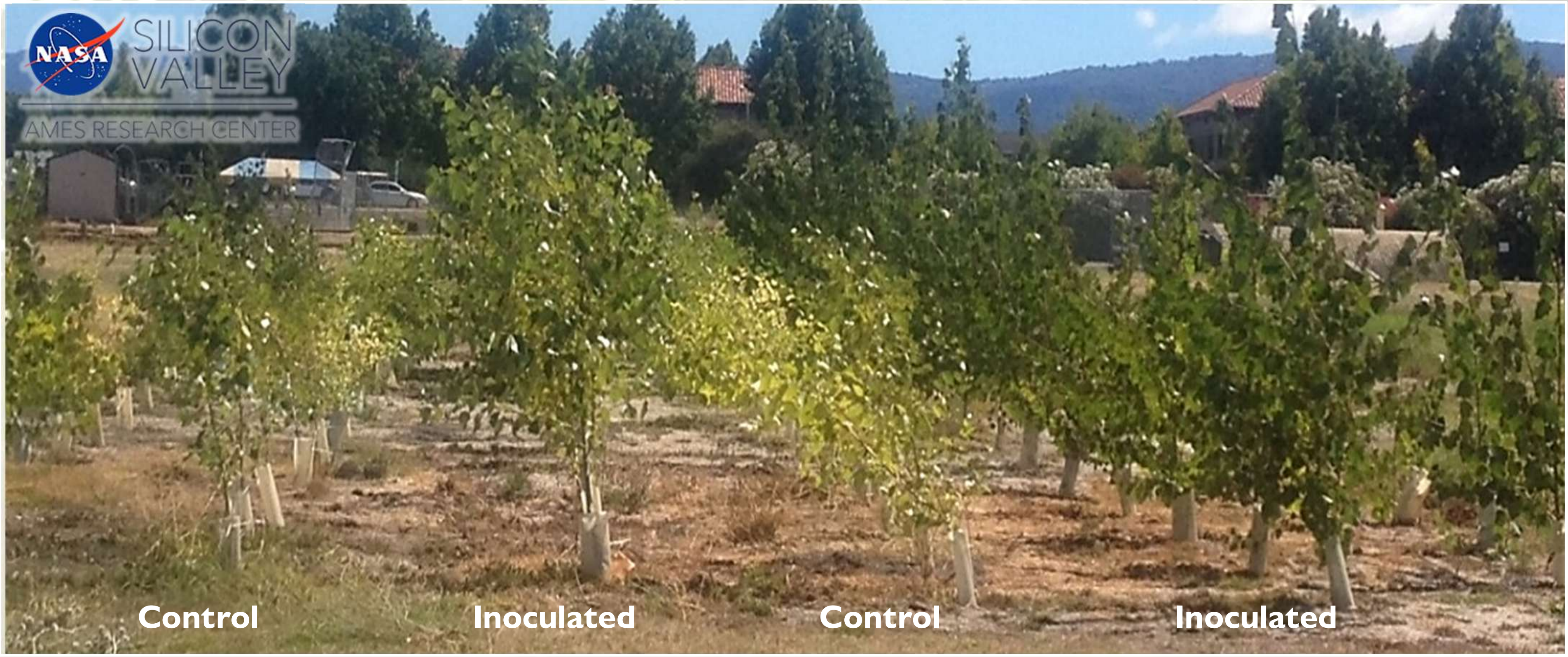
- Middlefield-Ellis-Whisman (MEW) Superfund site
  - Former site of microchip manufacturing
- Trichloroethylene (TCE, a cVOC) in groundwater
- Migrated onto NASA property
- Phytoremediation using hybrid poplars

Initial planting (April 2014)

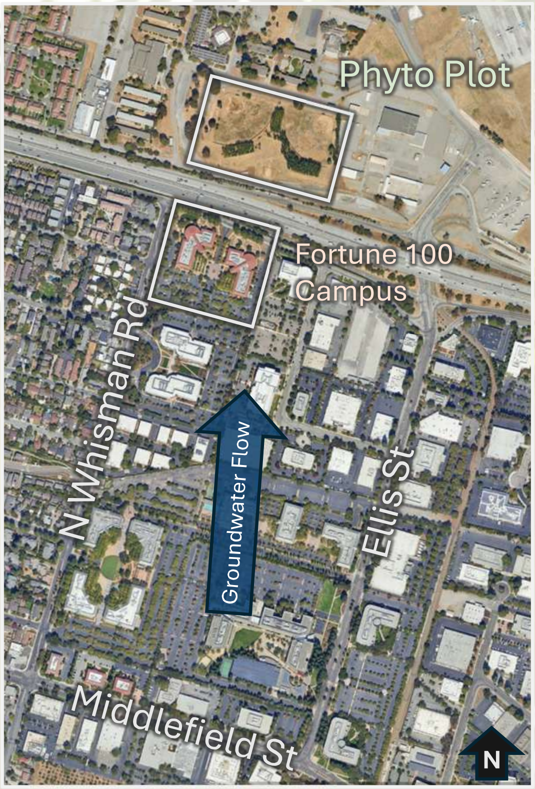
SILICON VALLEY
AMES RESEARCH CENTER

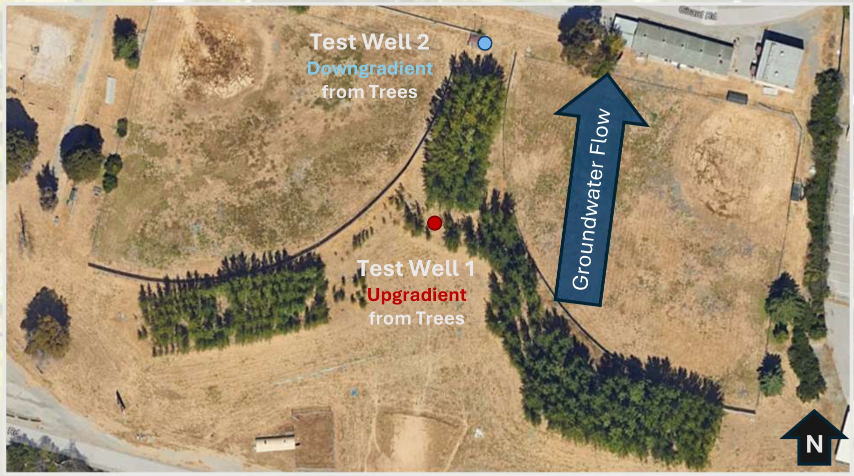
Tree growth after 12 months

Endophyte-Enhanced TCE Tolerance Growth



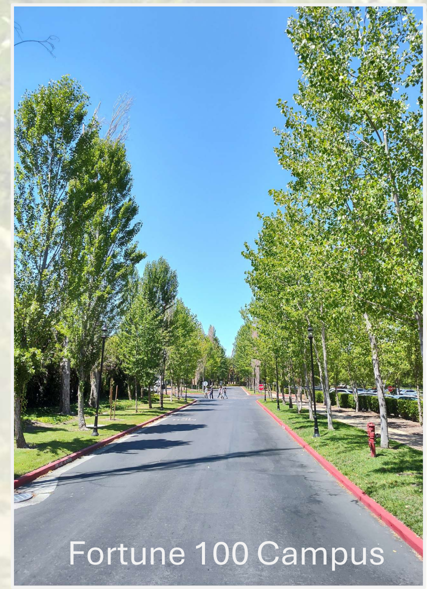
NASA Ames Pilot Study





	September 28 2016 Test Well		November 21 2016 Test Well		June 27 2017 Test Well		July 17 2018 Test Well		December 4 2024 Test Well		Detection Limit	Reporting Limit
	1	2	1	2	1	2	1	2	1	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

SILICON VALLEY
AMES RESEARCH CENTER



Intrinsyx Environmental

NASA Ames Pilot Study

September 2021

SILICON VALLEY

AMES RESEARCH CENTER

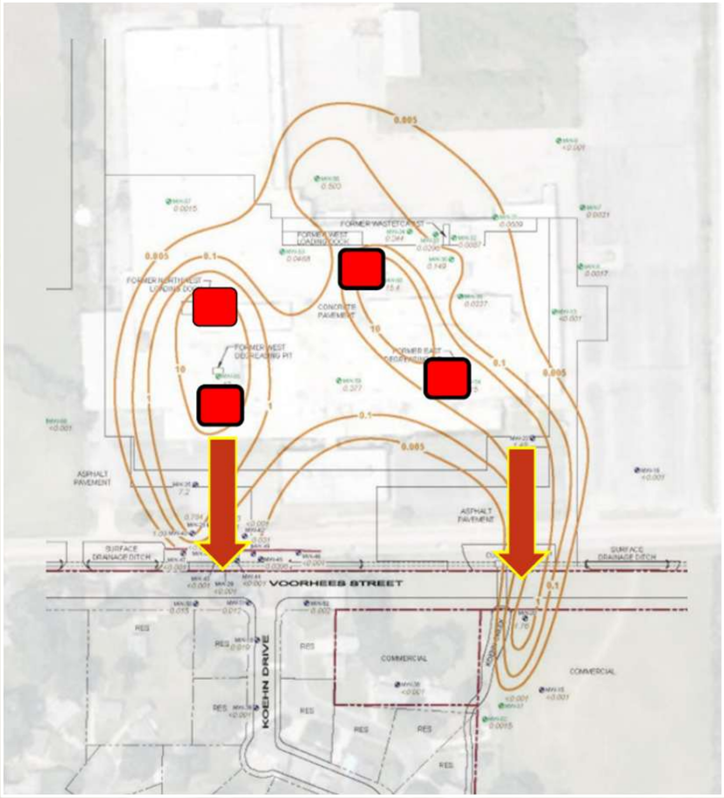
- Effectively cut off TCE plume migrating onto base
- EPA Approval to expand remediation efforts – incorporating phytoremediation into any redevelopment plans

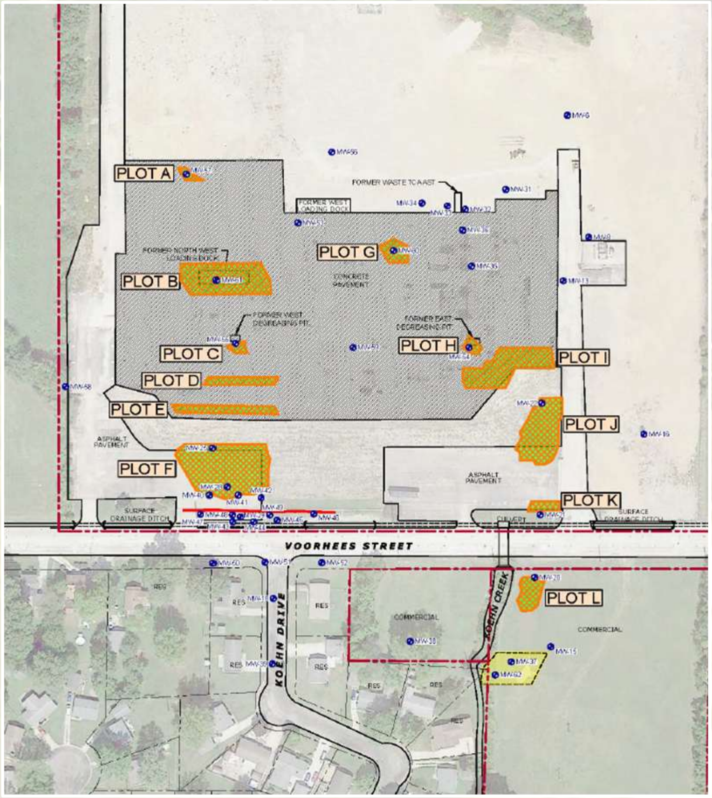
Case Study
TCE Groundwater Plume
East Illinois

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

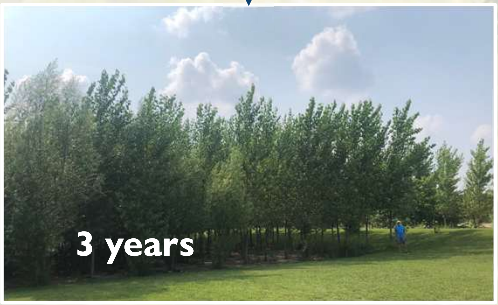




RAMBOLL

Intrinsyx Environmental

Case Study – East Illinois



Intrinsyx Environmental

Case Study – East Illinois

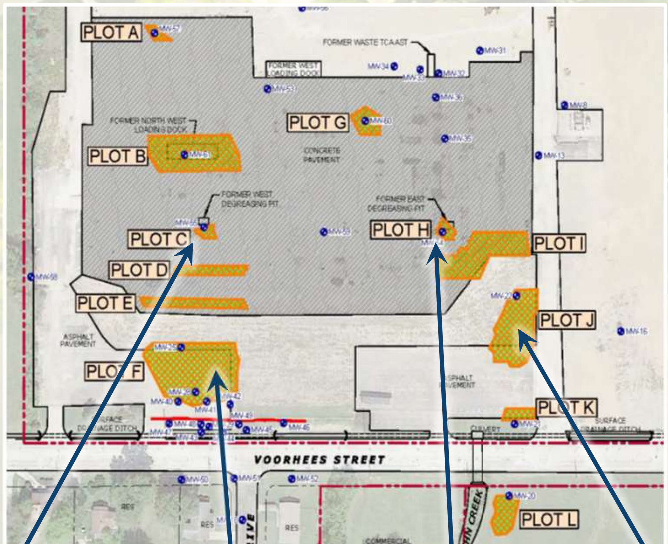
Results

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

Broad acceptance:

- Client
- Illinois EPA
- City Government
- Local Residents

Total VOCs Reductions (mg/L)

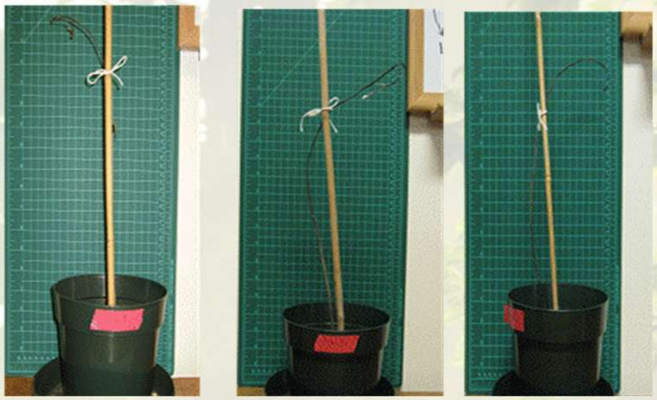


Plot C	Plot F	Plot H	Plot J
Degreasing Pit	Downgradient	Degreasing Pit	Downgradient
81 to 4.4	21.9 to 0.74	11.6 to 0.45	22 to 2.5
95%	**97%**	**96%**	**89%**

Case Study
Petroleum Hydrocarbons
Dos Palos, CA

Case Study – Dos Palos, CA

Scientific Basis for Endophytes on Petroleum Hydrocarbon Sites



Control

Inoculated

Environmental Science & Technology

Article

pubs.acs.org/est

Degradation, Phytoprotection and Phytoremediation of Phenanthrene by Endophyte *Pseudomonas putida*, PD1

Zareen Khan, David Roman, Trent Kintz, May delas Alas, Raymond Yap, and Sharon Doty*

School of Environmental and Forest Sciences, College of the Environment, University of Washington, Seattle 98195-2100, United States

Supporting Information

School of Environmental and Forest Sciences

UNIVERSITY *of* WASHINGTON

College of the Environment

Control Inoculated

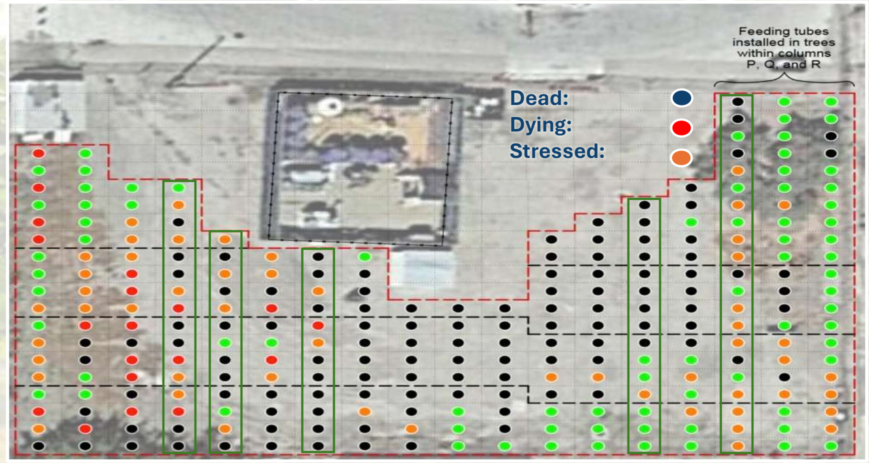
- 40% removal of phenanthrene (PAH) from soil by willow and grasses when inoculated, compared to un-inoculated controls
- BTEX, gasoline and diesel degradation also discovered

Intrinsyx Environmental

Case Study – Dos Palos, CA

<u>Background</u>

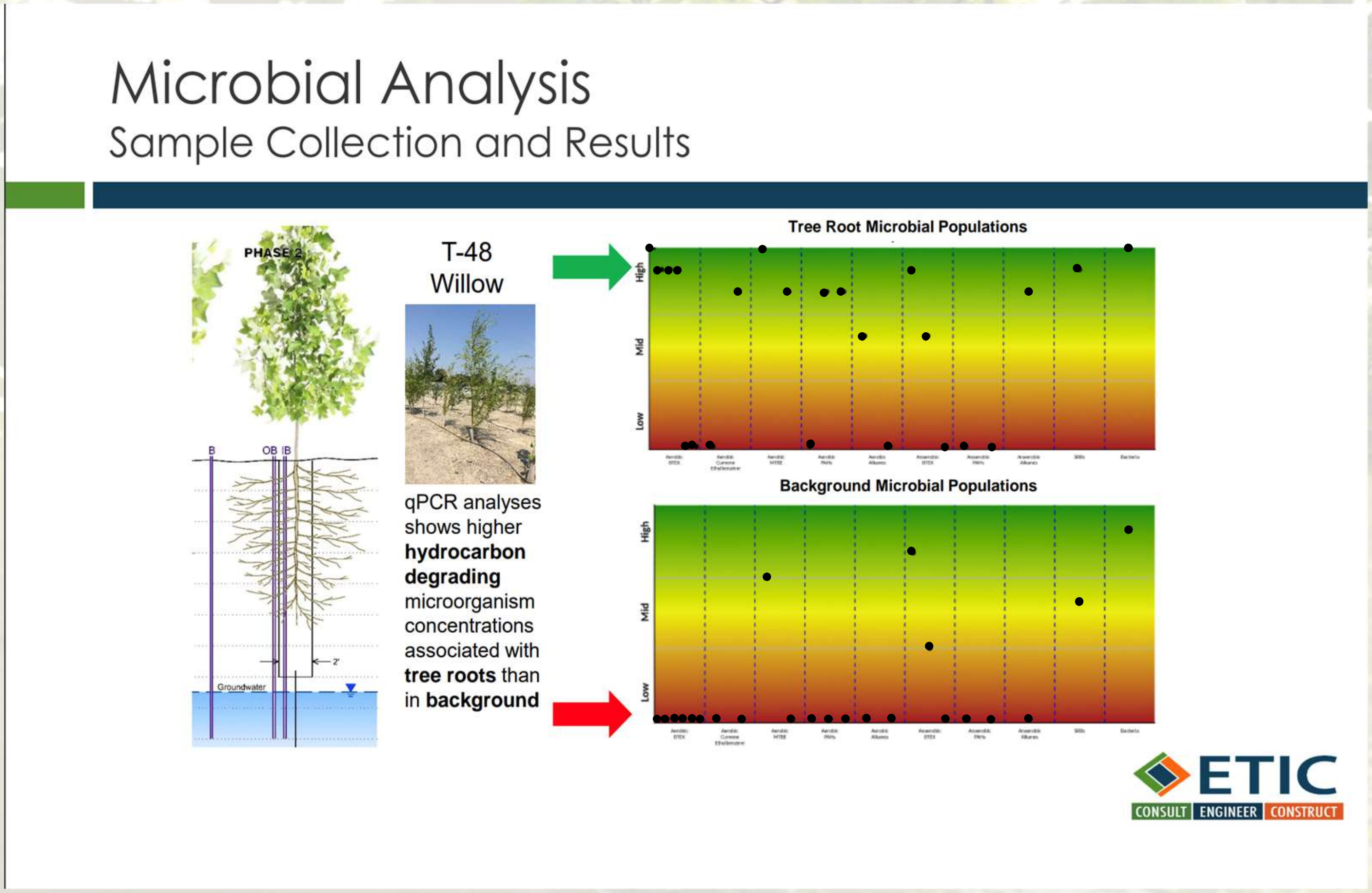
- Former fuel transfer station (GRO/DRO)
- Multiple feet of LNAPL
- High concentrations of gas and diesel range compounds at or near solubility limits
- High O&M for SVE and P&T systems



Species Biodiversity on the Dos Palos Site

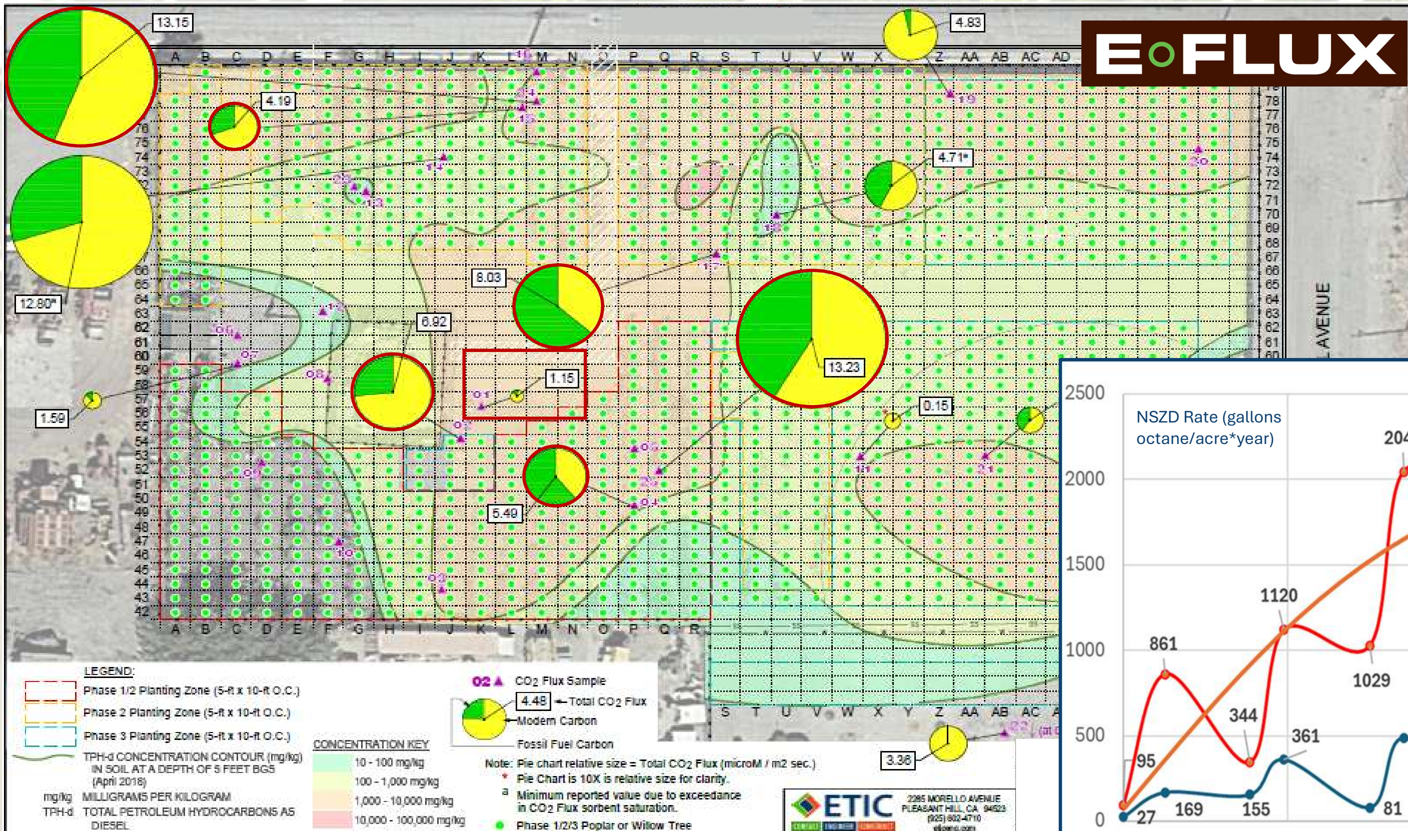
Trees	Grasses	Bacterial Endophyte	Fungi
Salix lucida willow	Salt grass, *Distichlis spicata*	*Pseudomonas alloputida*	White rot mycelium- *Phanerochaete chrysosporium*
RRR poplar	Alkali milk-vetch *Astragalus tener*	*Sphingobium salicis*	*Pleurotus ostreatus*
	American vetch, *Vicia americana*	*Rhizophagus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

Tree Root Microbial Analysis versus Background Soils



- 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

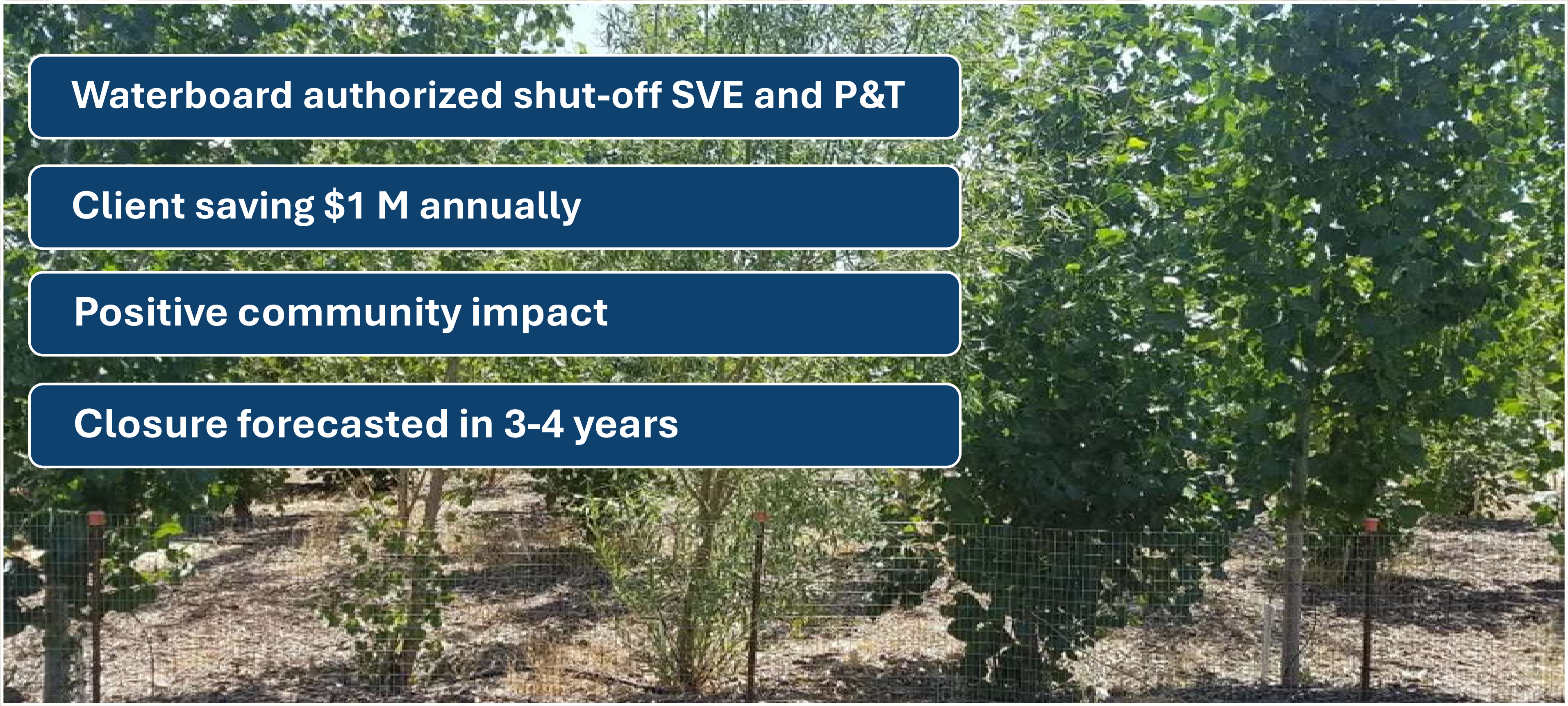


Natural Source Zone Depletion (NSZD)

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

Intrinsyx Environmental

Case Study – Dos Palos, CA

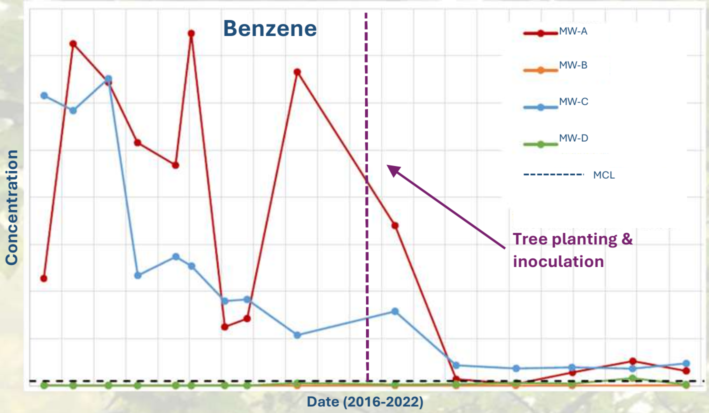


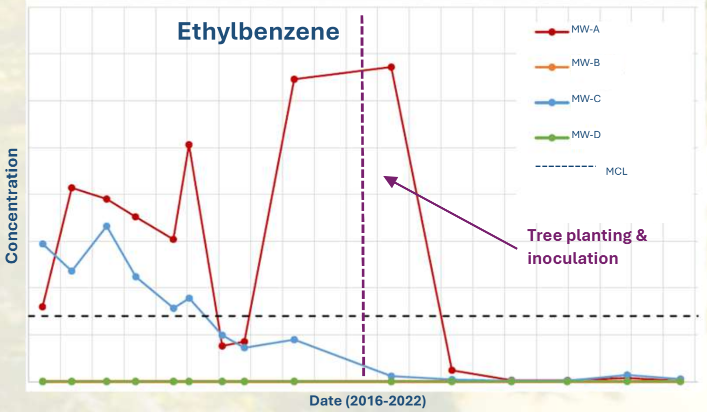
- Waterboard authorized shut-off SVE and P&T
- Client saving $1 M annually
- Positive community impact
- Closure forecasted in 3-4 years

Case Study – Coastal TX

<u>Background</u>

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





Intrinsyx Environmental

Additional Phytoremediation

&

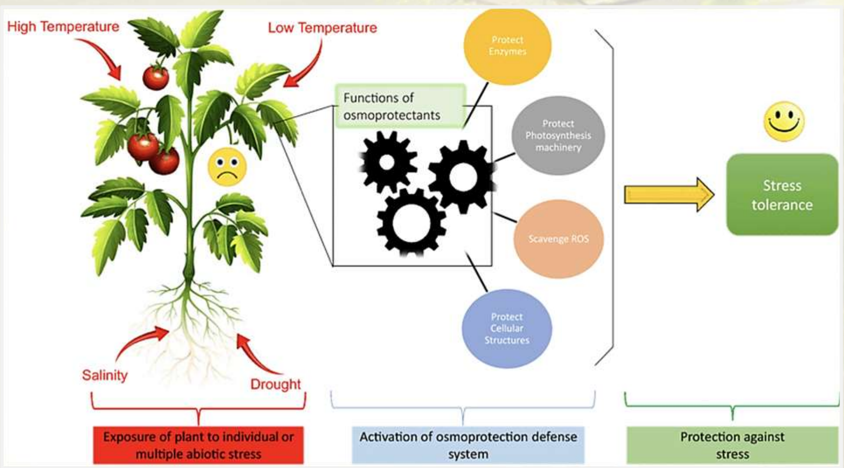
Eco-restoration Benefits

Phytoremediation & Ecorestoration Benefits

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

Ectoine

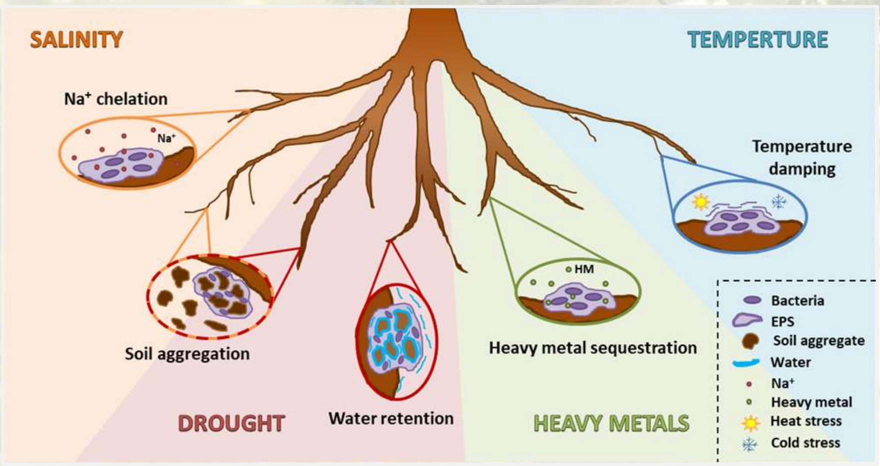
- Acts as osmolyte
  - Osmolyte = organic compounds that influence the properties of biological fluids
- Protective against stressors such as drought, salinity, temperature, and heavy metals



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.

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



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

Intrinsyx Environmental

Phytoremediation & Ecorestoration Benefits

Drought Tolerance

Inoculated Control

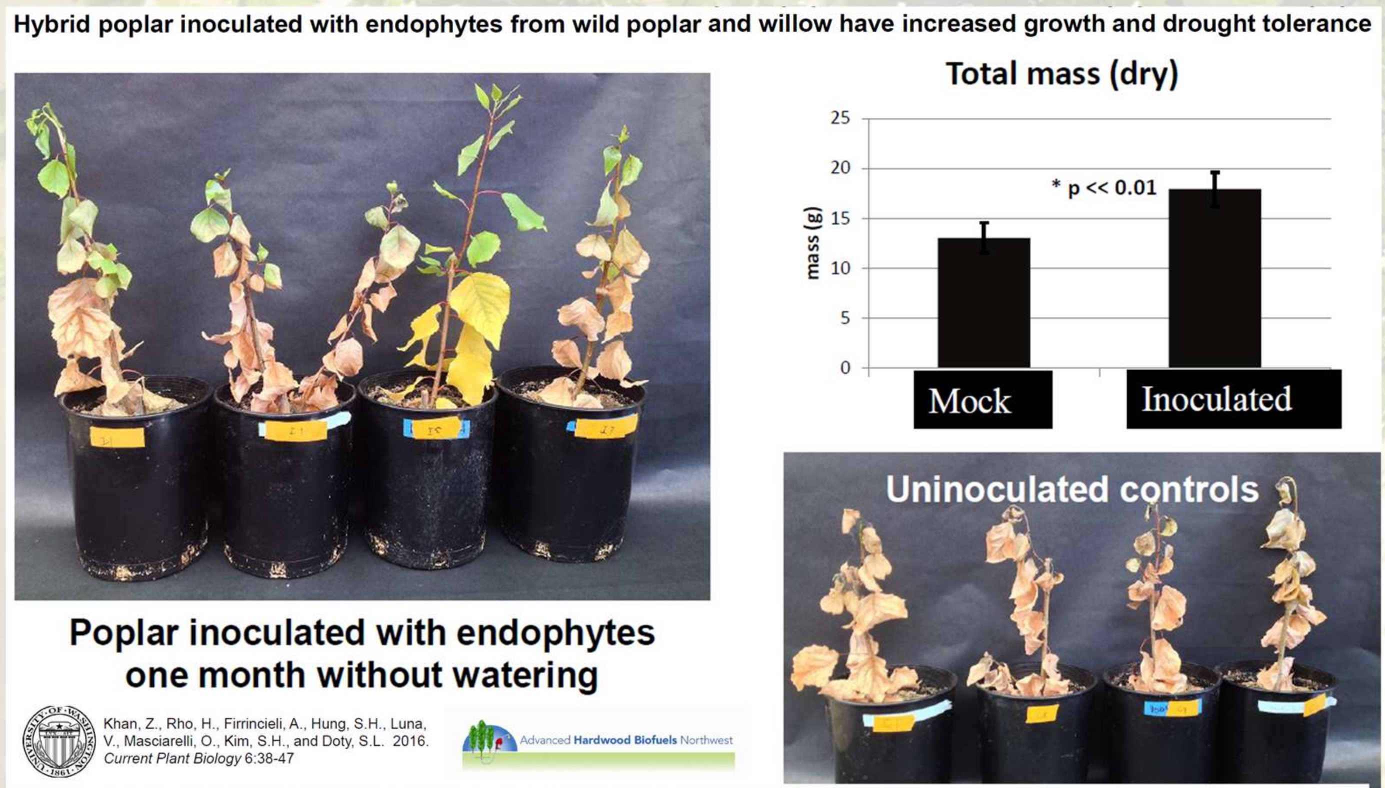
Inoculated Control

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

Phytoremediation & Ecorestoration Benefits

Drought Tolerance

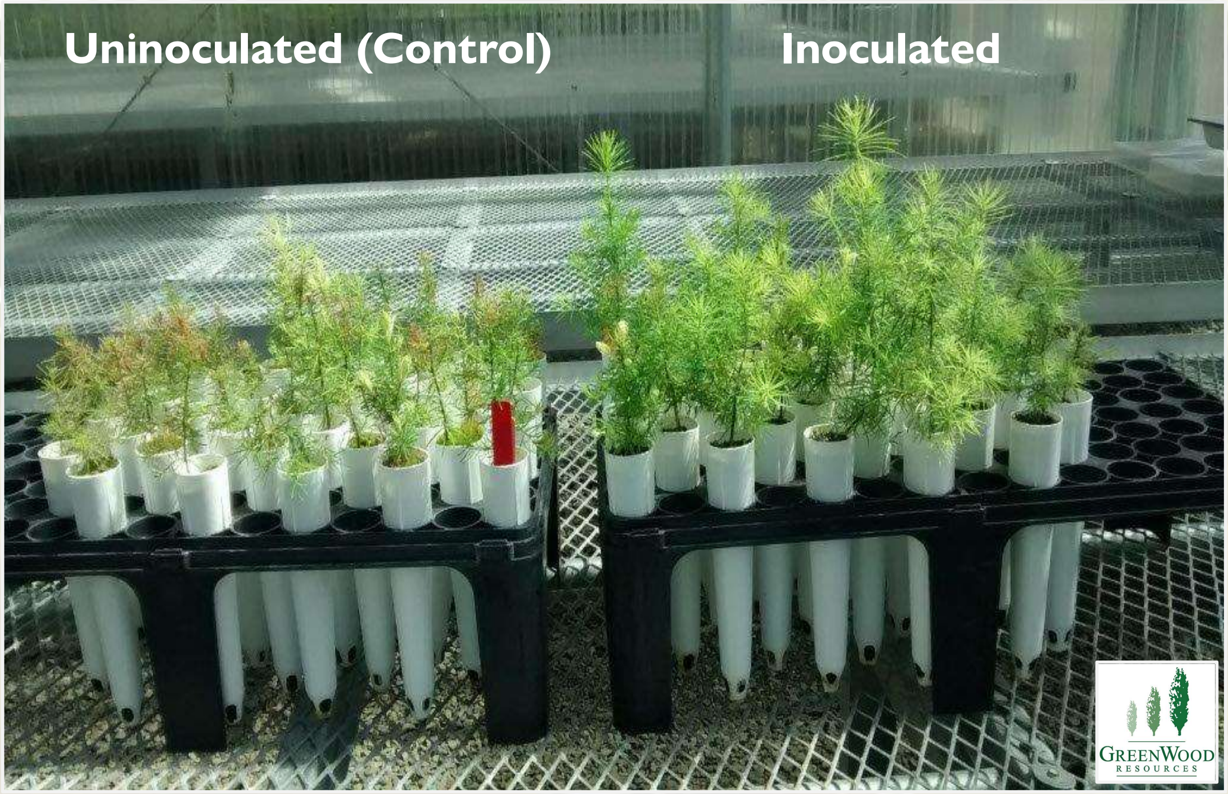
University of Washington Study on Water Use Efficiency (WUE) and Drought Tolerance



Intrinsyx Environmental

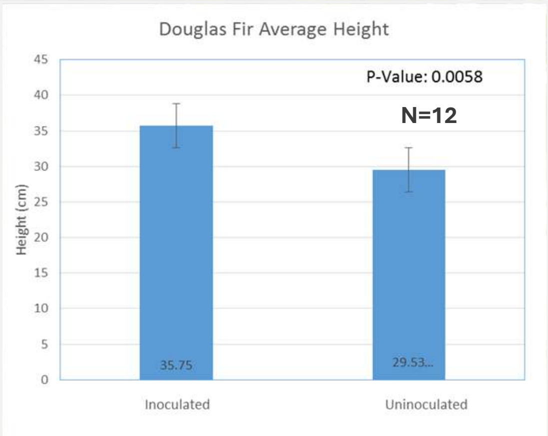
Phytoremediation & Ecorestoration Benefits

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

- Continental US
- Alaska
- Puerto Rico
- South Africa
- Austria

Developing Business

- Canada
- Africa
- EU
- Middle East
- Australia

Intrinsyx Environmental

Partners and Collaborators

Clients

AECOM
TETRA TECH
BURNS McDONNELL
Stantec
wsp GOLDER
ETIC CONSULT ENGINEER CONSTRUCT
JACOBS
ERM
RAMBOLL
APEX
KJ Kennedy Jenks
Trihydro
RSK

USDA
FOREST SERVICE U S DEPARTMENT OF AGRICULTURE
USGS science for a changing world
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NASA SILICON VALLEY AMES RESEARCH CENTER

UNIVERSITY OF WASHINGTON 1861
ecolotree
MISSOURI S&T
STATE
Floating Islands West LLC

Partners

GreenSoil Group
EARTHMASTER environmental consulting
LEACHATE MANAGEMENT SPECIALISTS
THE REMEDIATORS INCORPORATED
LIFE GREEN GROUP
Arbor Day Foundation

Brownfields 2025 Sustainable Communities Start Here
BATTELLE It can be done
NICOLA
International Phytotechnology Society
AEHS Foundation
REMTECH Europe

Contact Us
Visit our website

Christopher Cohu, Ph.D.

CEO and Lead Scientist

chris@intrinsyx.com