Endophyte Assisted Phytoremediation of TCE and Derivatives Using Hybrid Poplar Trees

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Collaborators

Intrinsyx
Space Age Biotechnologies for Earth and Beyond
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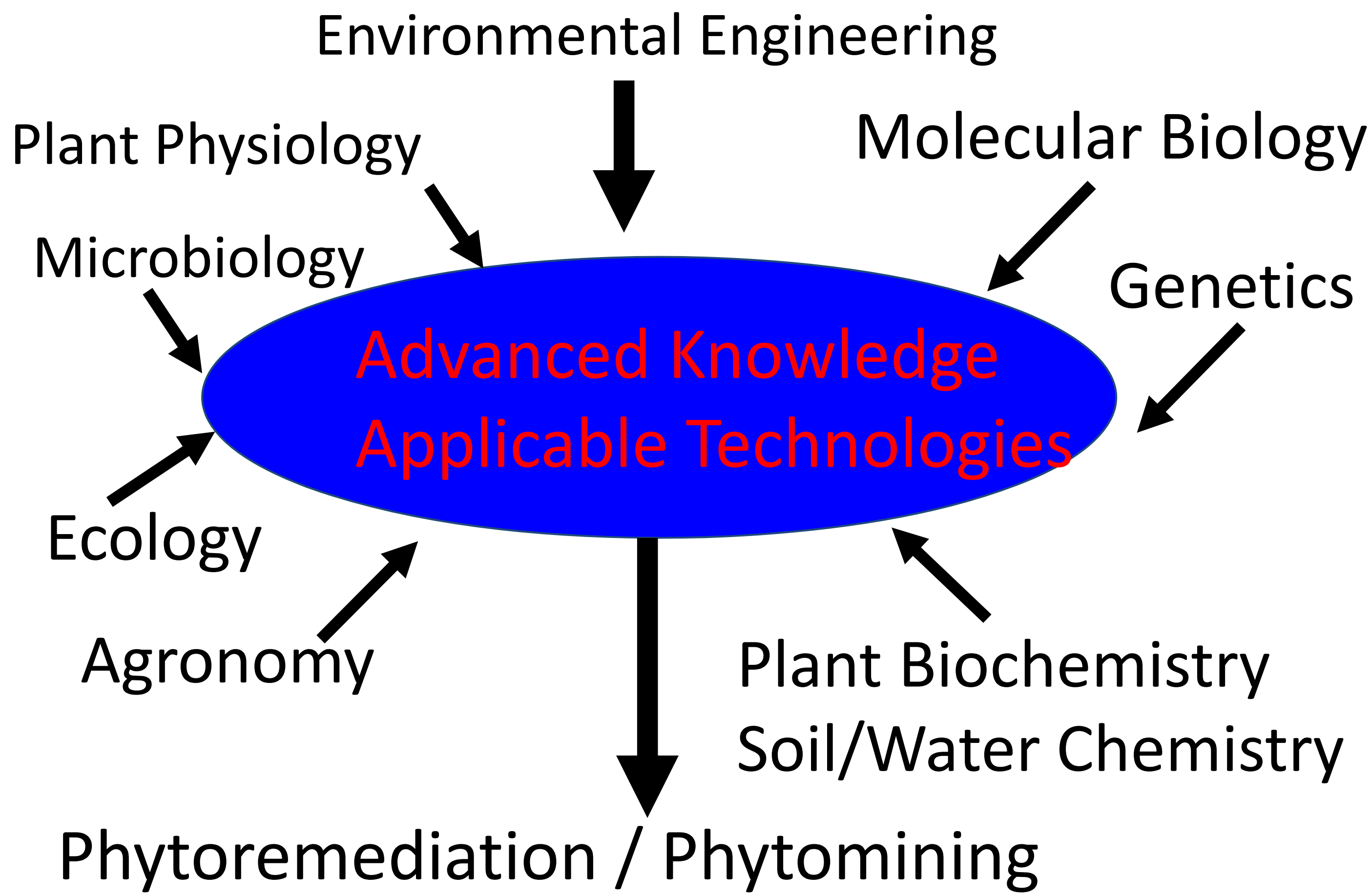
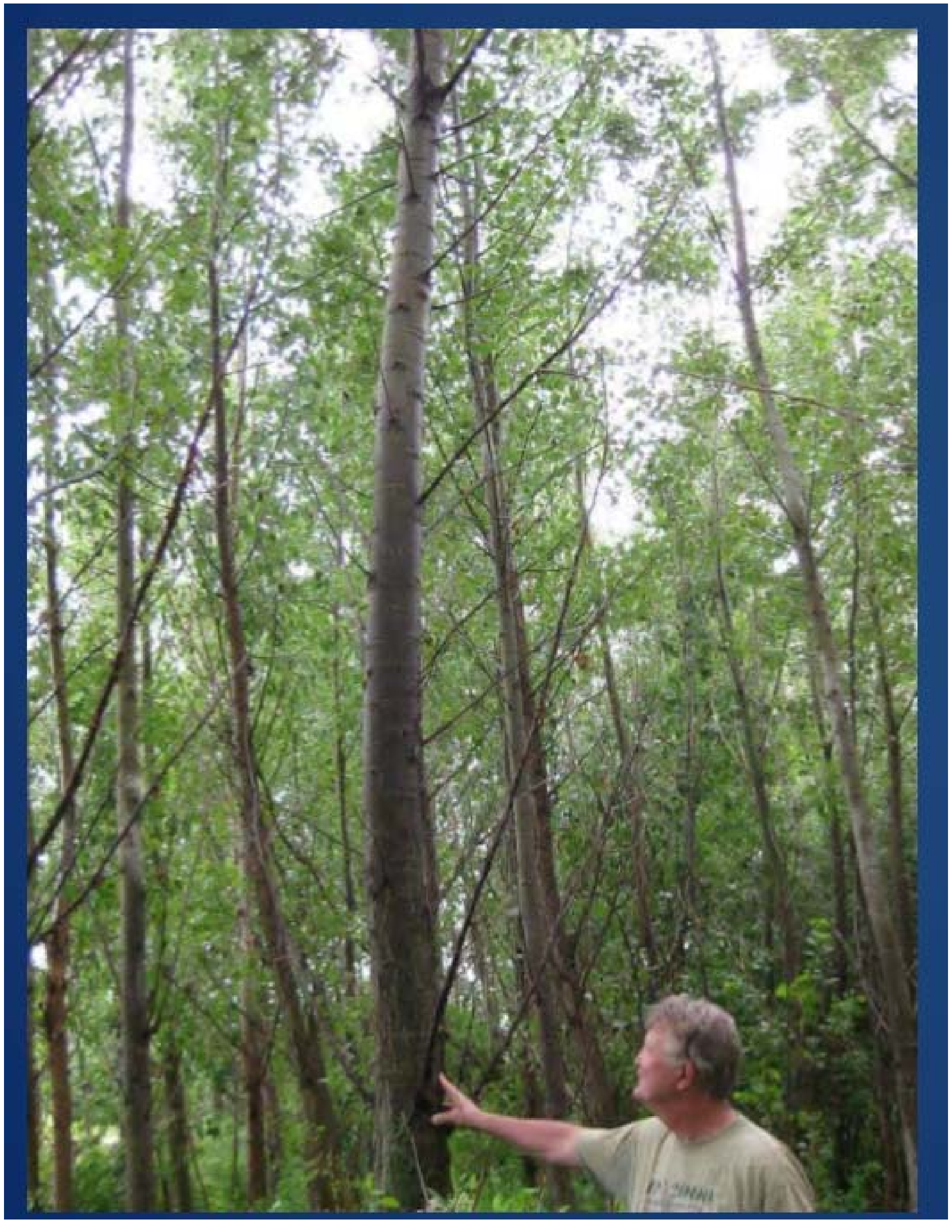
School of Environmental and Forest Sciences
UNIVERSITY *of* WASHINGTON
College of the Environment

Multidisciplinary Team Based Supportive Approach to Projects



Phyto-remediation:
Using plants and their associated microbes to clean up pollution

Poplar and willow can grow in extreme environments



Poplar is a Tree of Choice for Groundwater Phytoremediation
Deep root up to 30 feet down
Can be deep planted down to 8 ft bare trees.
15 feet down using tree wells.

Hybrid poplar
High rate of Hydrological Conductivity

Single mature tree can use >3000 gallons per year >50 gallons per day

EPA guidance
www.clu-in.org

https://clu-in.org/products/intern/phytotce.htm

Using Poplar, Cottonwood and Willow endophytes
“Screened the tree micro-biome for an Endophyte that can degrade TCE”

- *Rhizobium tropici*

- *Burkholderia vietnamiensis*
- *Herbaspirillum*
- *Pseudomonas graminis*
- *Rahnella sp.*
- *Acinetobacter sp.*
- *Enterobacter* sp.
- *Sphingomonas* sp.
- *Rhodotorula graminis*

Screened for endophytes of poplar that degrade trichloroethylene (TCE), one of the most common pollutants at SuperFund sites

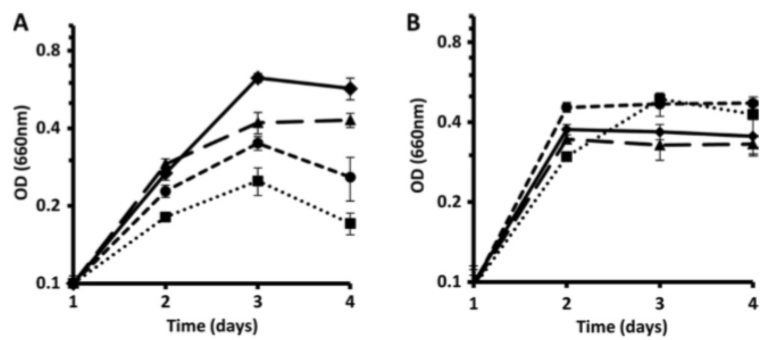
AEM
Journals.ASM.org

Biodegradation of Trichloroethylene by an Endophyte of Hybrid Poplar

Jun Won Kang, Zareen Khan, and Sharon L. Doty
School of Environmental and Forest Sciences, College of the Environment, University of Washington, Seattle, Washington, USA

strain PDN3, showed high tolerance to trichloroethylene (TCE). Without the addition of inducers, such as toluene or phenol, PDN3 rapidly reduced TCE levels in medium from 72.4 μM to 30.1 μM in 24 h with a concurrent release of 127 μM chloride ion, and nearly 80% of TCE (55.3 μM) was dechlorinated by PDN3 in 5 days with 166 μM chloride ion production, suggesting TCE degradation.

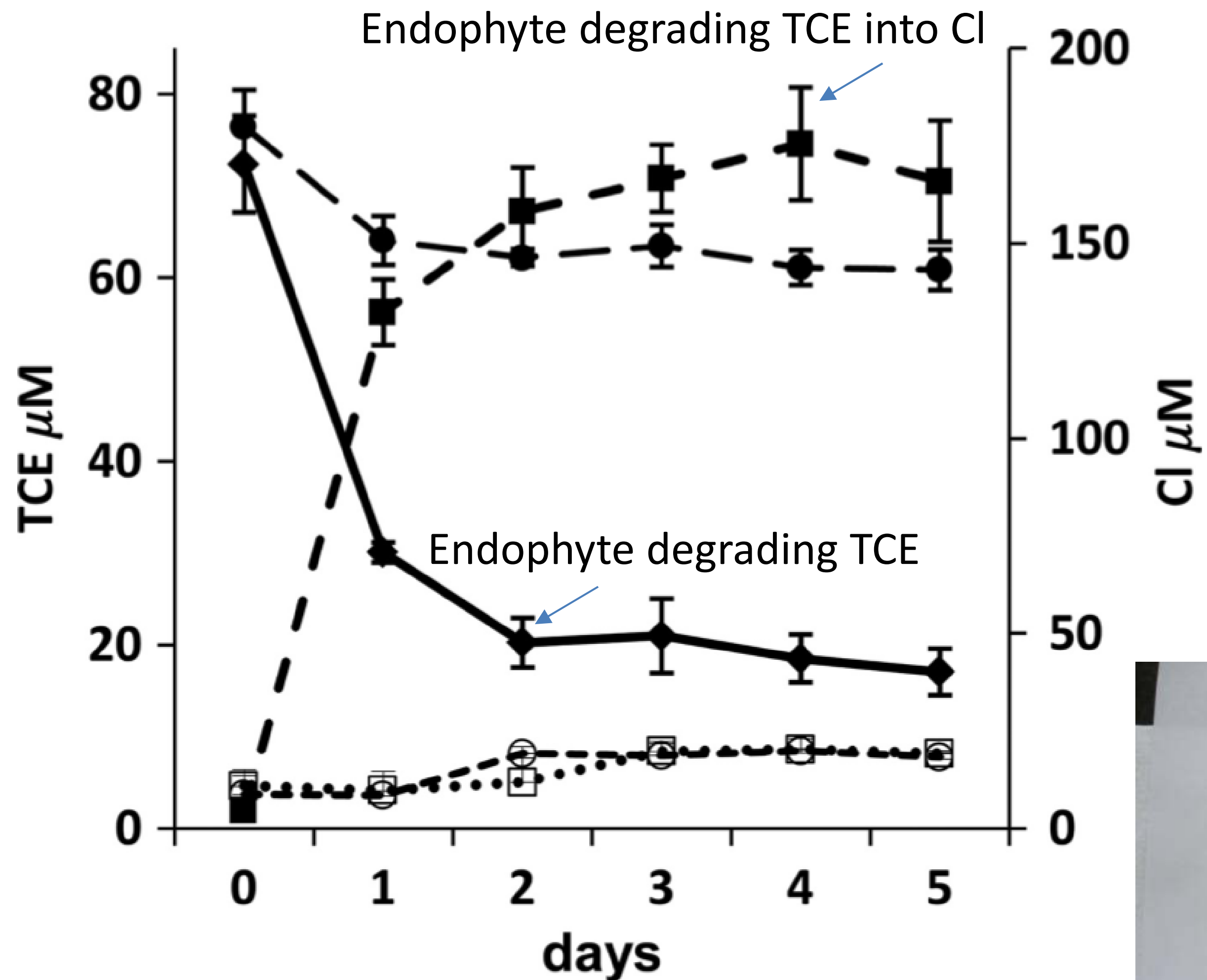
Trichloroethylene (TCE) is a common environmental contaminant (16). High levels of TCE have the potential to cause liver damage and malfunctions in the central nervous system, and it is considered a likely human carcinogen (13). More than 54% of Superfund sites in the United States are contaminated with TCE (19), and hence, its removal has become a priority for many contaminated sites all over the industrialized world. With the discovery of a number of soil microorganisms that are capable of degrading organic pollutants, microbial bioremediation is increasingly being considered a reasonable and effective method for removing environmental contaminants

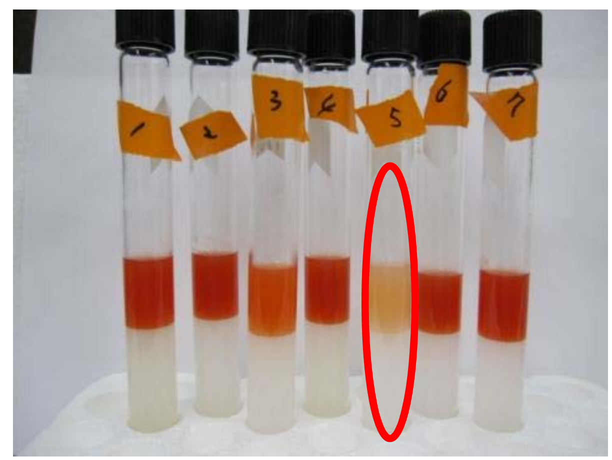


Applied and Environmental Microbiology 2012, 78(9): p. 3504–3507

Identified 1 Poplar Endophyte ,

Showed enhanced TCE Degradation and Chloride production in liquid and solid media

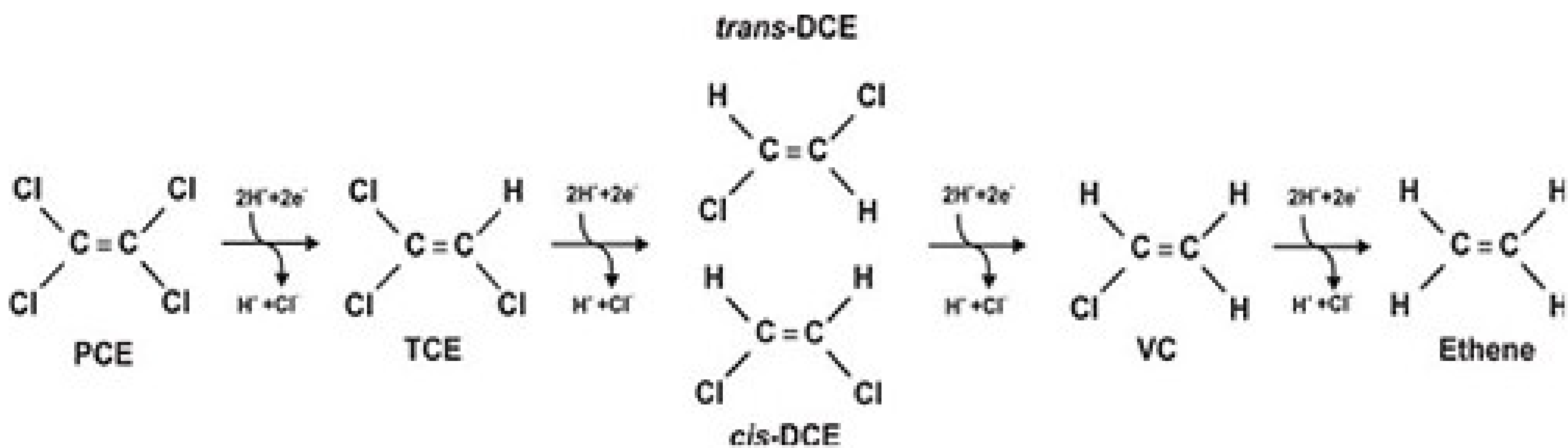




Endophyte Showed TCE Degradation Using Color indicator for TCE in agarose media

Applied and Environmental Microbiology 2012, 78(9): p. 3504–3507

Common Sequential Reductive Dechlorination Pathways:
The most common **anaerobic** dechlorination pathway is the degradation of tetrachloroethene (PCE) to ethene. In the sequential transformation of the chlorinated ethenes shown below, chlorine is replaced using hydrogen as an electron donor.

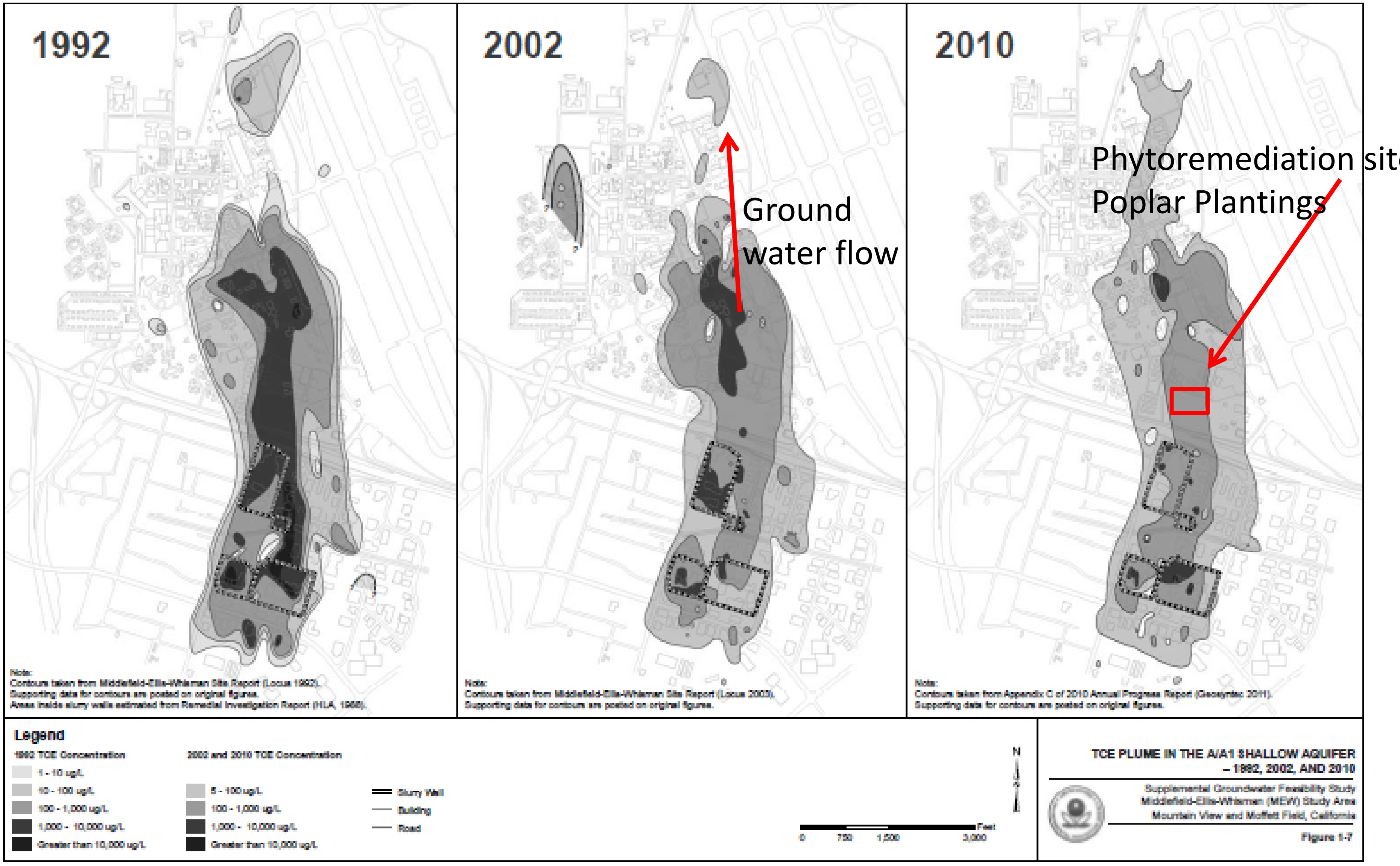


1. The facultative anaerobes Enterobacter sp. strain MS-1 and Pantoea agglomerans have so far been reported to reductively dechlorinate perchloroethylene (PCE), thereby converting it to dichloroethene (DCE). This occurs only under strictly anoxic conditions (14,29) and likely through action of a monooxygenase gene.

2. Attempts to amplify monooxygenase genes using conserved PCR primer sets were unsuccessful, suggesting that PDN3 uses a not-yet-discovered pathway for TCE degradation.

3. Since PDN3 grew to a higher optical density in medium containing TCE than in the same medium without TCE, these results suggest that PDN3 used TCE as an additional carbon source.

Applied and Environmental Microbiology 2012, 78(9): p. 3504–3507


1992
2002
2010
Ground
water flow
Phytoremediation sit
Poplar Plantings
Note:
Contours taken from Middlefield-Ellis-Whisman Site Report (Locus 1992).
Supporting data for contours are posted on original figures.
Areas inside slurry walls estimated from Remedial Investigation Report (HLA, 1988).
Note:
Contours taken from Middlefield-Ellis-Whisman Site Report (Locus 2003).
Supporting data for contours are posted on original figures.
Note:
Contours taken from Appendix C of 2010 Annual Progress Report (Geosyntec 2011).
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Legend
1992 TCE Concentration
1 - 10 ug/L
10 - 100 ug/L
100 - 1,000 ug/L
1,000 - 10,000 ug/L
Greater than 10,000 ug/L
2002 and 2010 TCE Concentration
5 - 100 ug/L
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1,000 - 10,000 ug/L
Greater than 10,000 ug/L
Slurry Wall
Building
Road
0
750
1,500
3,000
Feet
N
TCE PLUME IN THE A/A1 SHALLOW AQUIFER – 1992, 2002, AND 2010
Supplemental Groundwater Feasibility Study
Middlefield-Ellis-Whisman (MEW) Study Area
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Figure 1-7

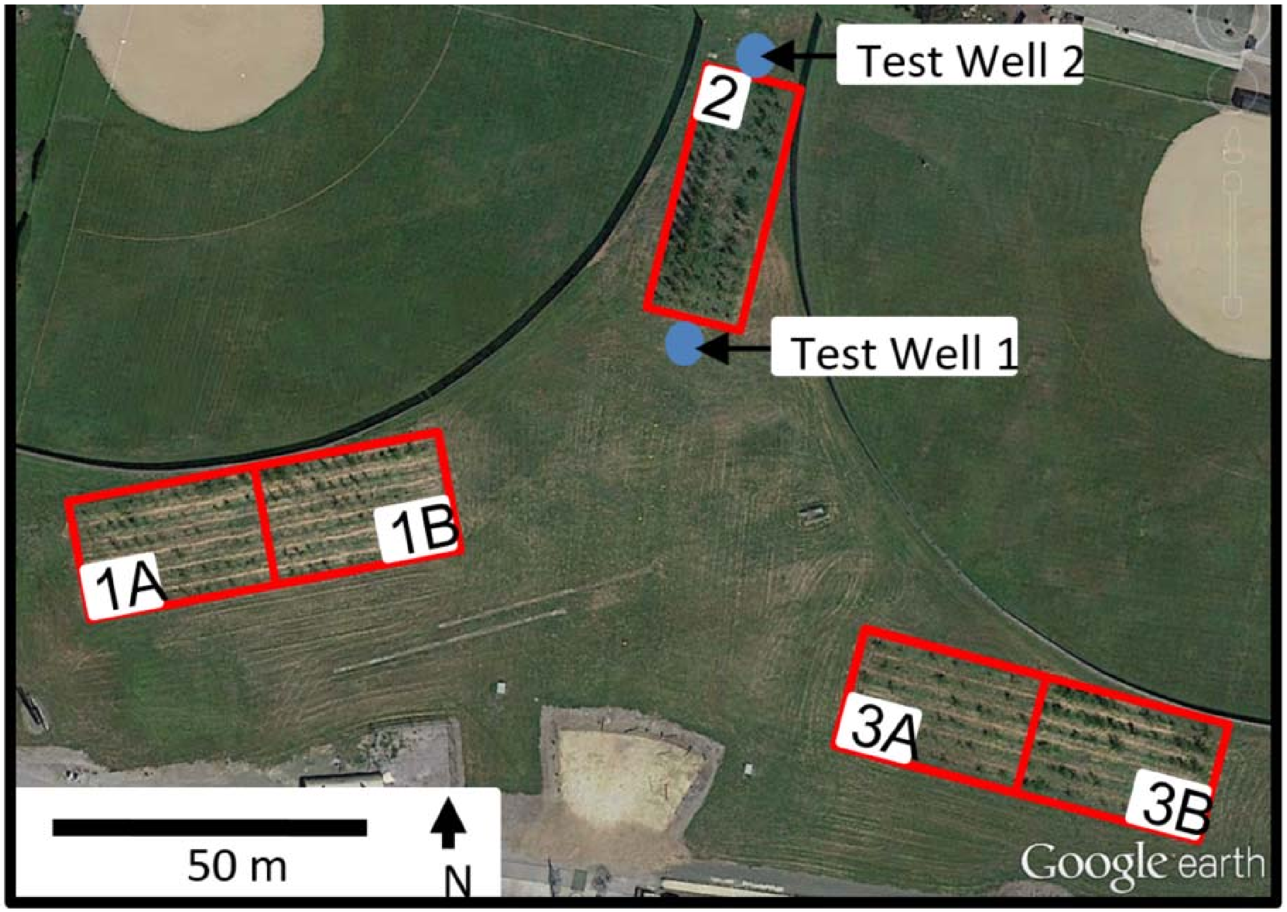
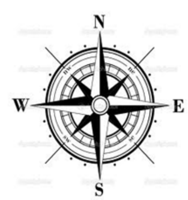
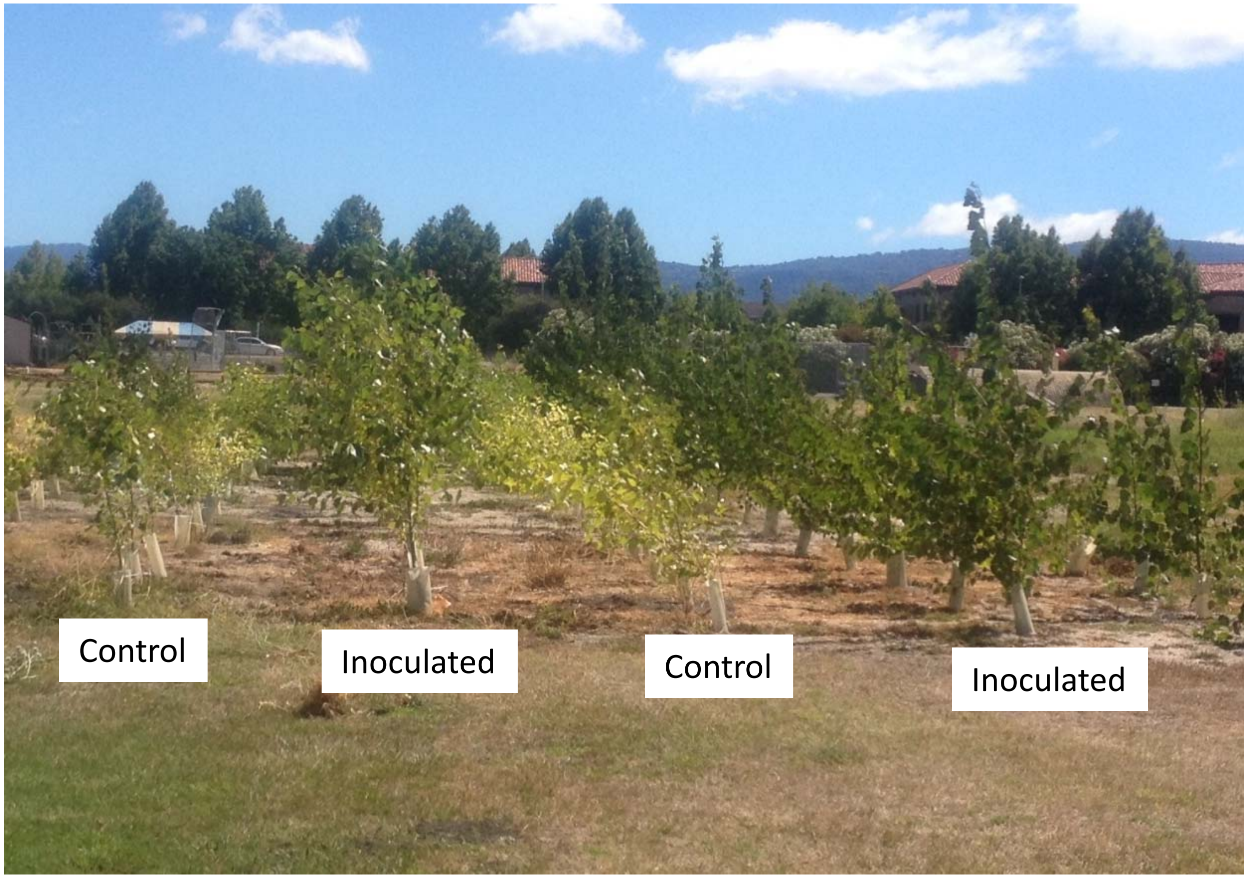


Figure 1. NASA-AMES Hybrid Poplar Test Plot Locations

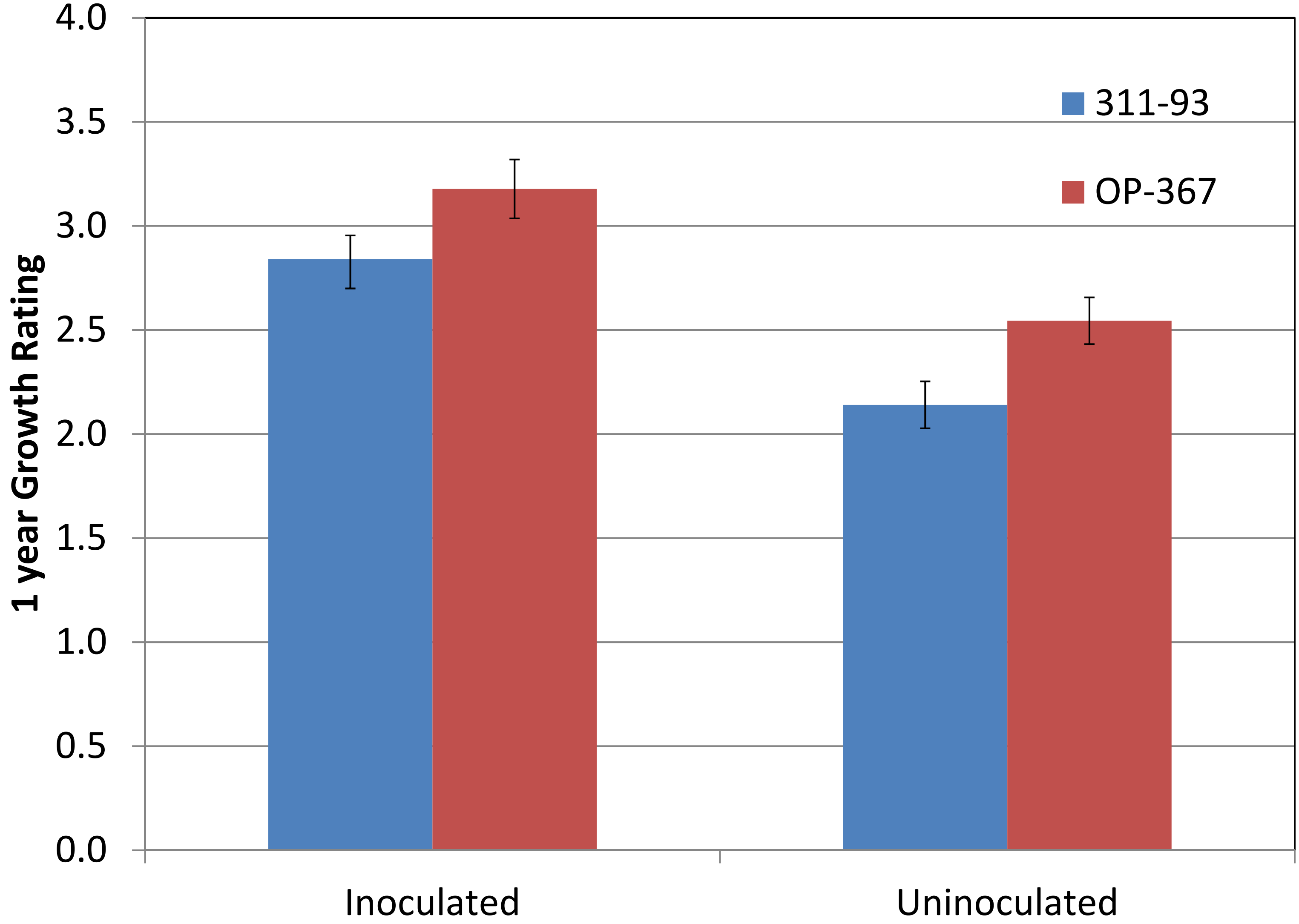


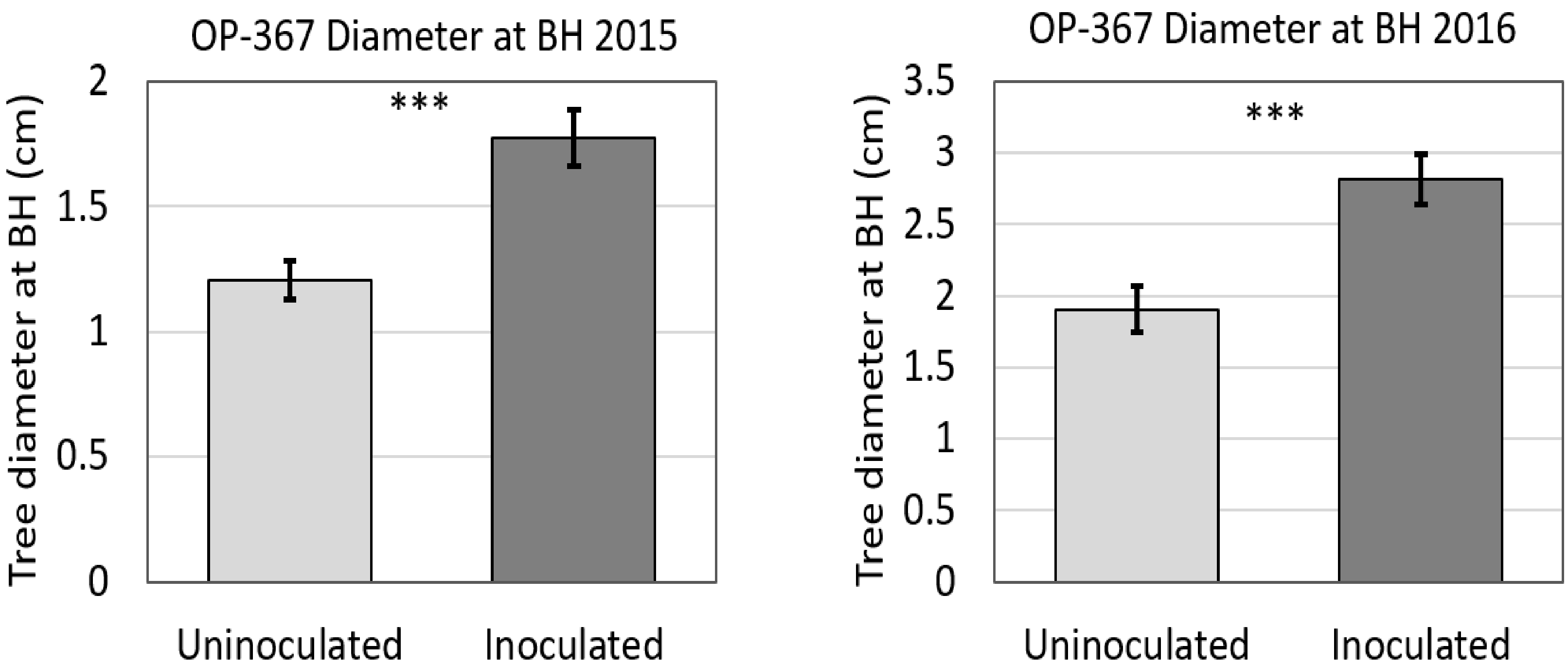
Test plots at Moffett Field after planting sp 2014.

TCE Tolerance Phenotype F 2014

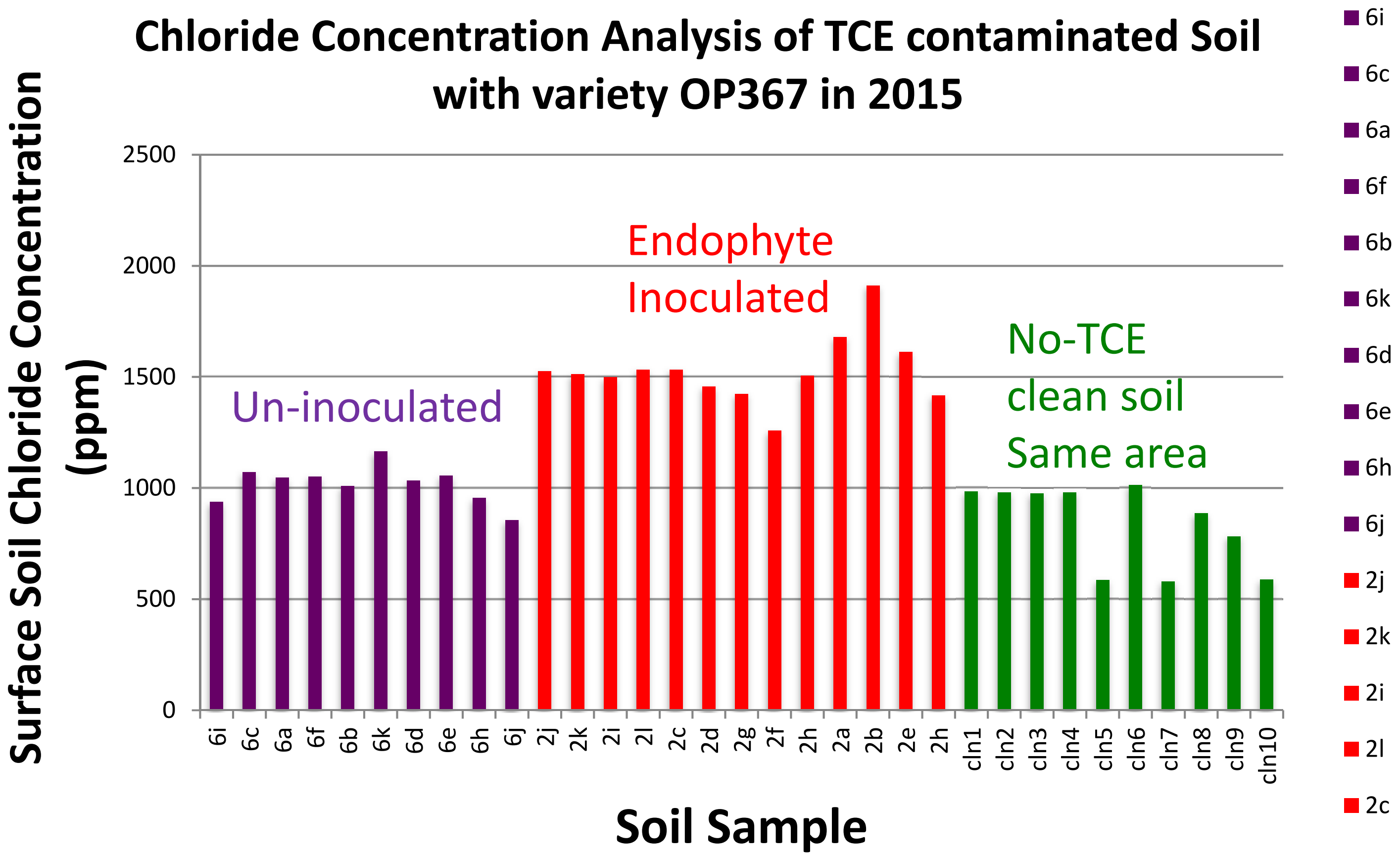


Growth Benefits/TCE tolerance from Endophyte Inoculation 2014





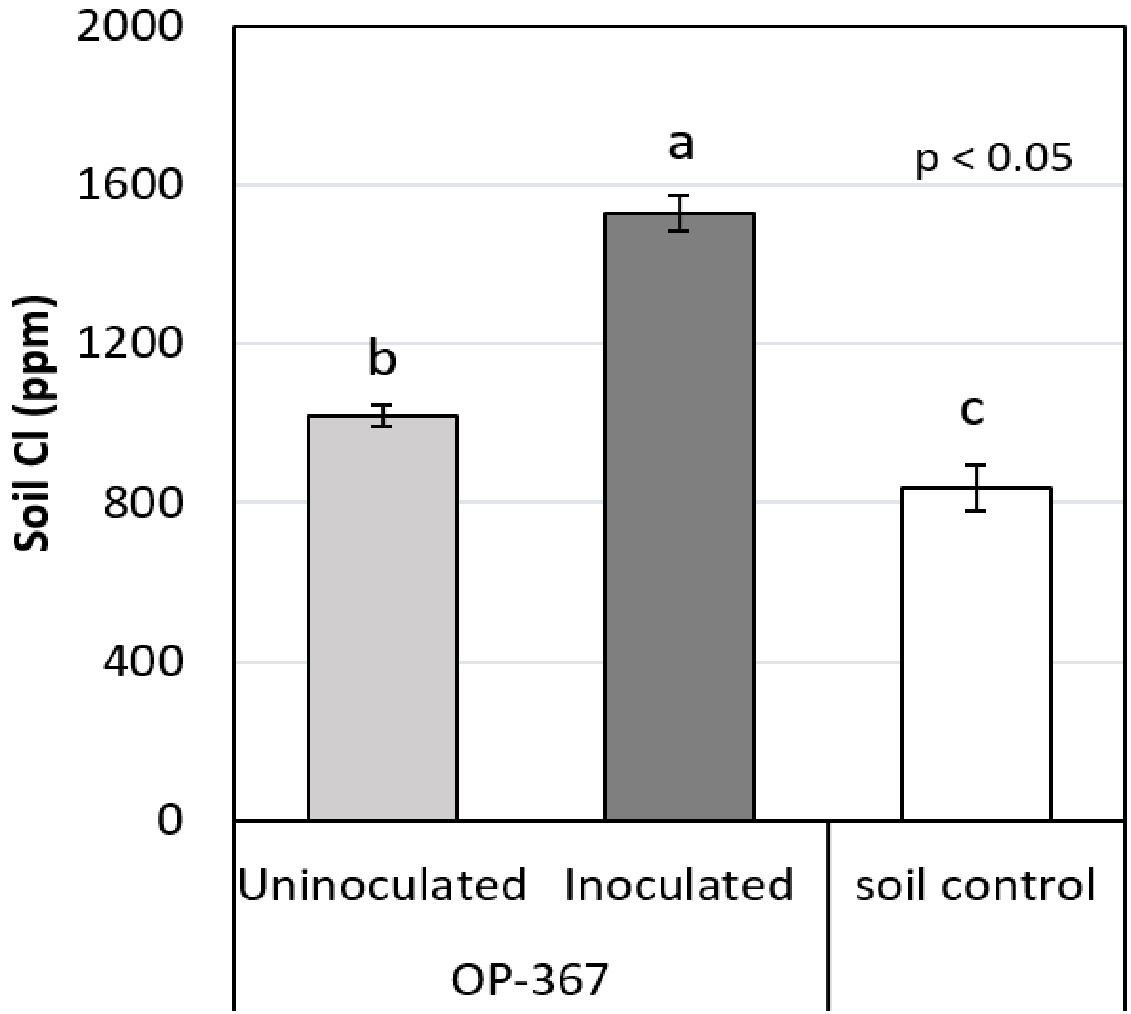
Tree trunk diameter at breast height in (cm)



<u>The TCE is being degraded and exuded by roots in upper soil zone as Chloride</u>

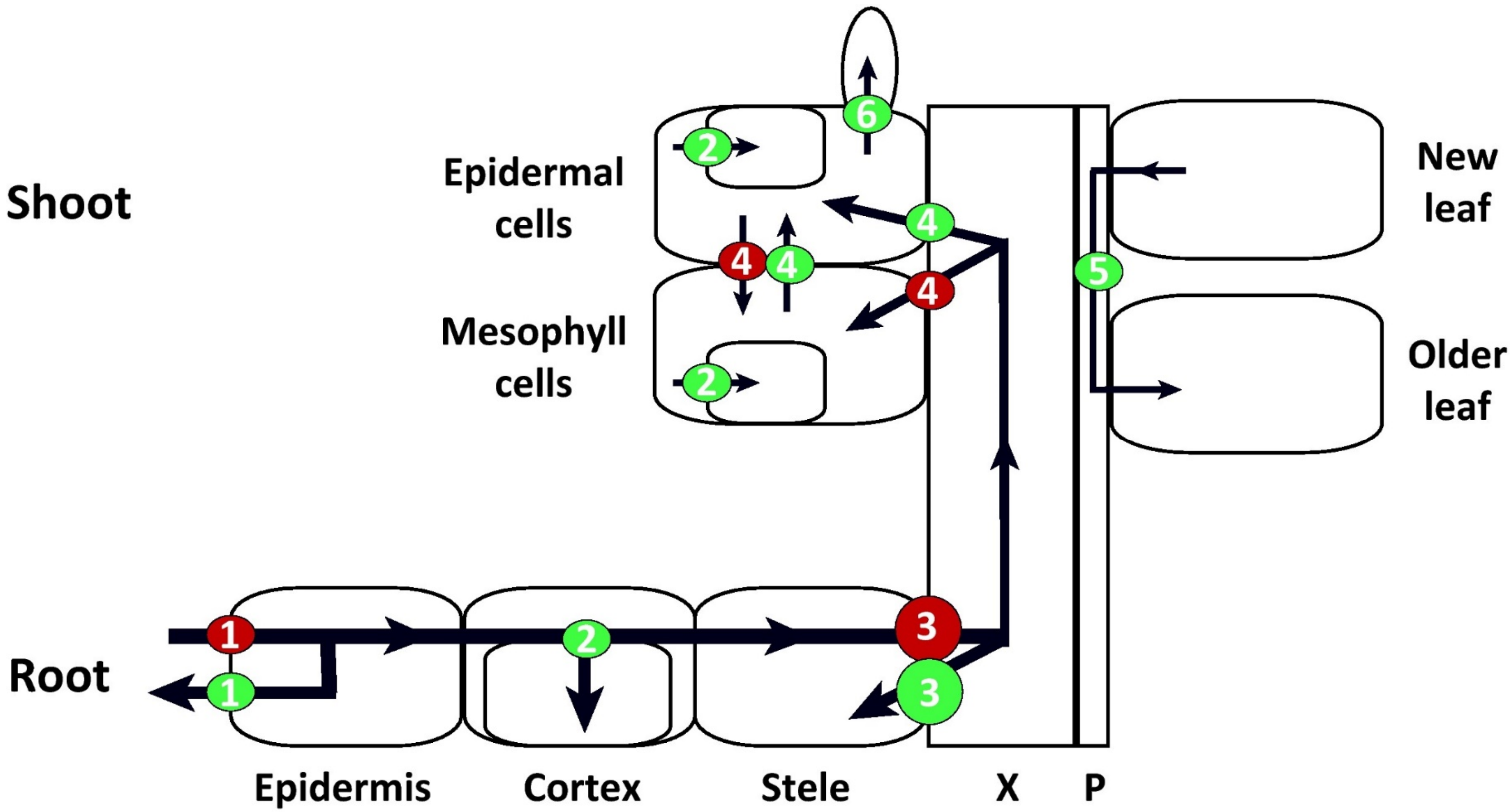
RESULTS

The results from the chloride analysis are demonstrated in the graph. On average, there was ~44% more chloride found in surface soil that was taken from two year old trees that were originally inoculated with the native endophyte in the TCE contaminated site. Soil samples were also taken from the local site that was not contaminated with TCE. The average chloride concentration of soil taken from un-inoculated plants was 1018.8 ppm while the average concentration of soil from inoculated trees was 1528.9 ppm. The average chloride concentration of soil taken from a clean site was 981.5 ppm. Chloride concentrations can range from 0.4 to 3000ppm, depending on the site's proximity to salt water. Given that the NASA Ames Research Center sits next to the San Francisco Bay, it is not unreasonable to have a relatively high chloride concentration as a baseline.



Average Soil chloride levels in tree associated rhizosphere (Cl^- ppm) 2015

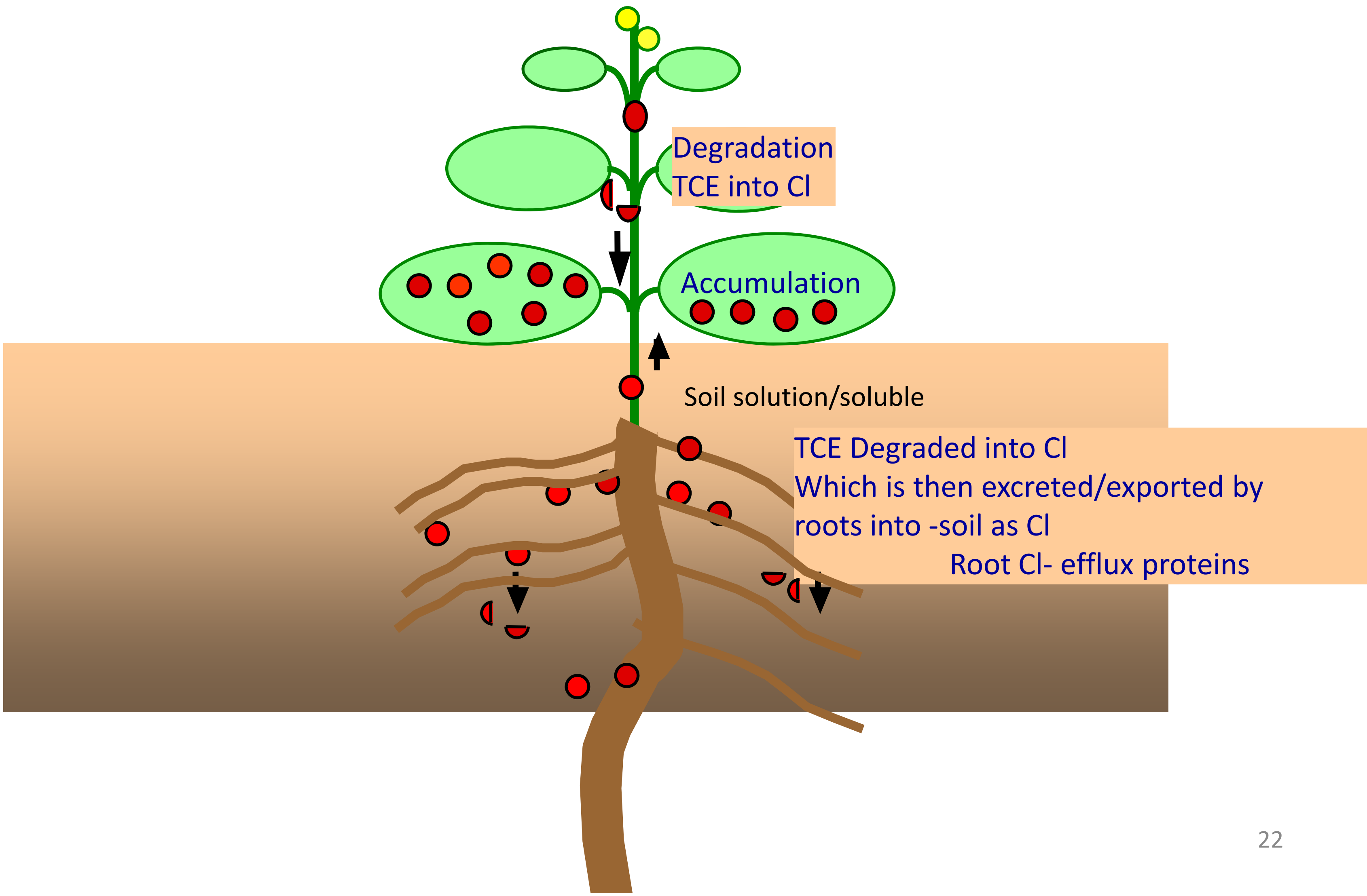
Mechanisms Contributing to Cl⁻ Exclusion from the Leaf Cytosol Thereby Leading to Cl⁻ Tolerance.



Trends in Plant Science

Trends in Plant Science 2017 22, 236-248DOI: (10.1016/j.tplants.2016.12.004)

Overview of TCE Phytoremediation Mechanisms

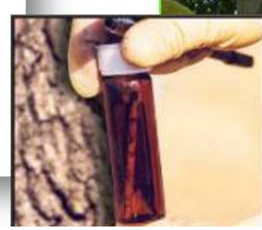


Test Well 2

Fall 2016

Tree Coring

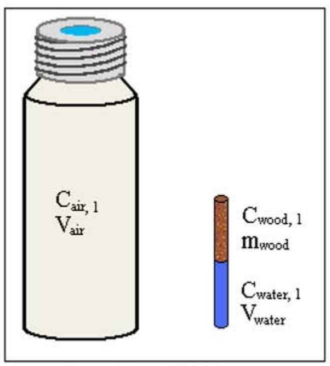
- 0.5 cm diameter core collected with a Haglöf increment Borer sample
- Place into 20 mL headspace vial
- Equilibrate, >24 hr
- Test Via SPME Headspace and Gas Chromatography



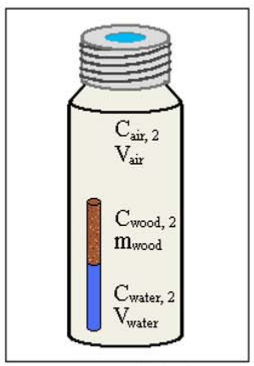
In vitro Solid-Phase Microextraction (SPME)

Equilibrated cores are analyzed with 100-µm PDMS fiber (Supelco, Bellefonte, PA), with an extraction time of 5 min, followed by a desorption time of 3 min.

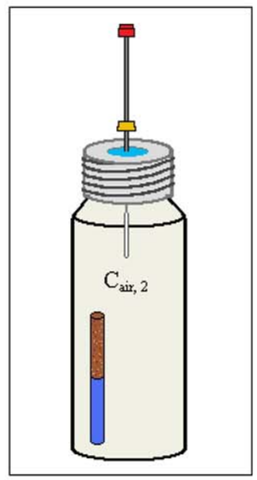
The SPME fibers were desorbed into an Agilent 7890 GC – with detection by µ-ECD. SPME sampling with a CombiPAL SPME auto sampler (CTC Analytics).



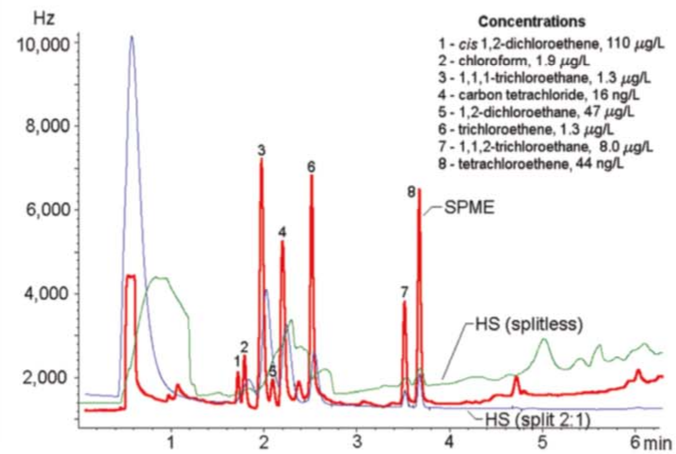
Pre-Equilibrium



Post-Equilibrium

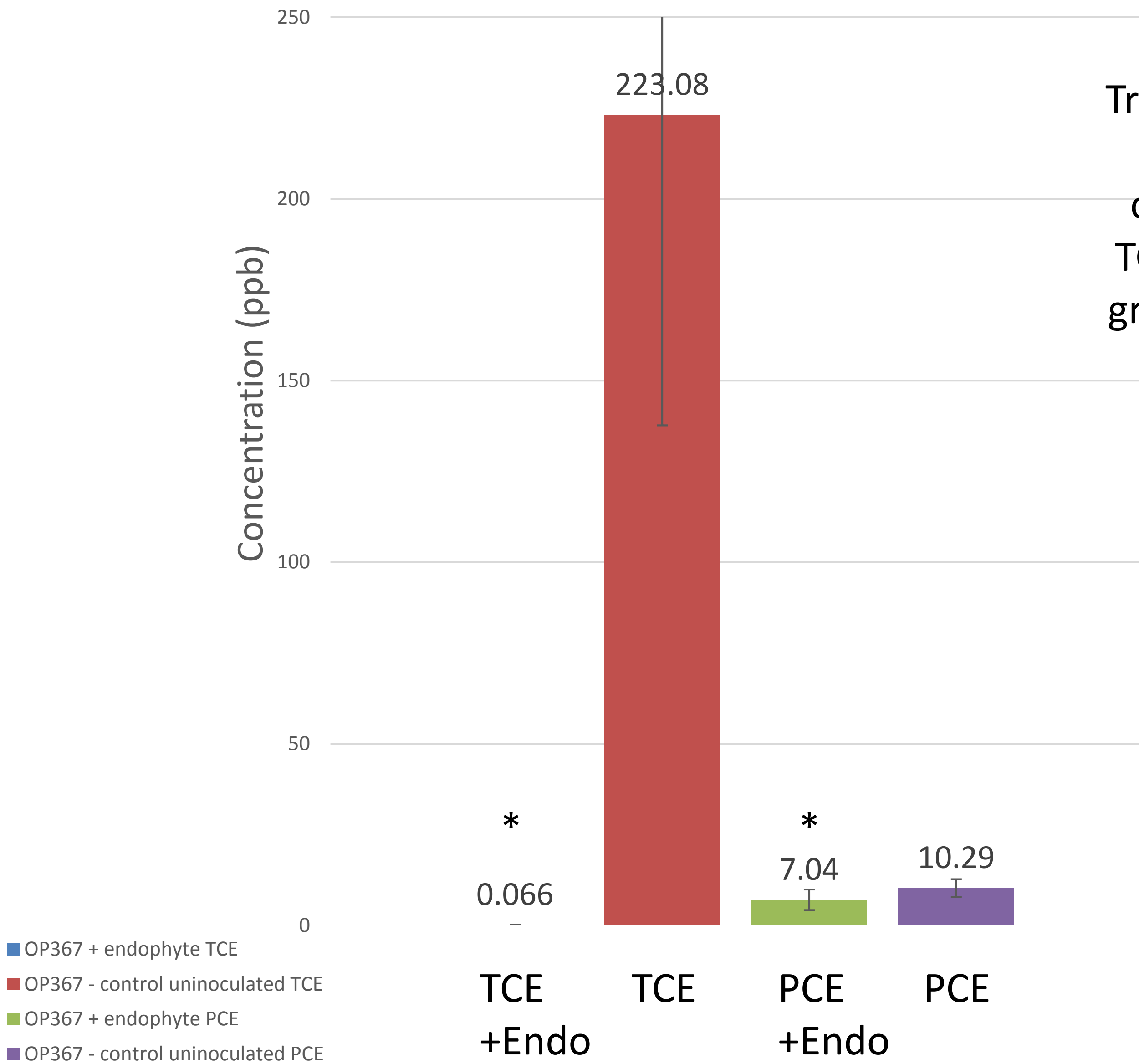


HS-SPME



Limmer MA, Balouet J-C, Karg F, Vroblesky DA, Burken JG*. 2011. *ES&T*. 45(19):8276-8282

Phyto-detection= Using Solid phase micro-extraction + electron capture detection of TCE and PCE in tree cores we can detect presence of internal contaminants and degradation rates



Trees inoculated* with TCE/PCE degrading endophytes consistently had less internal TCE and PCE and are tolerant / grew better than un-inoculated control

n=10 trees each treatment

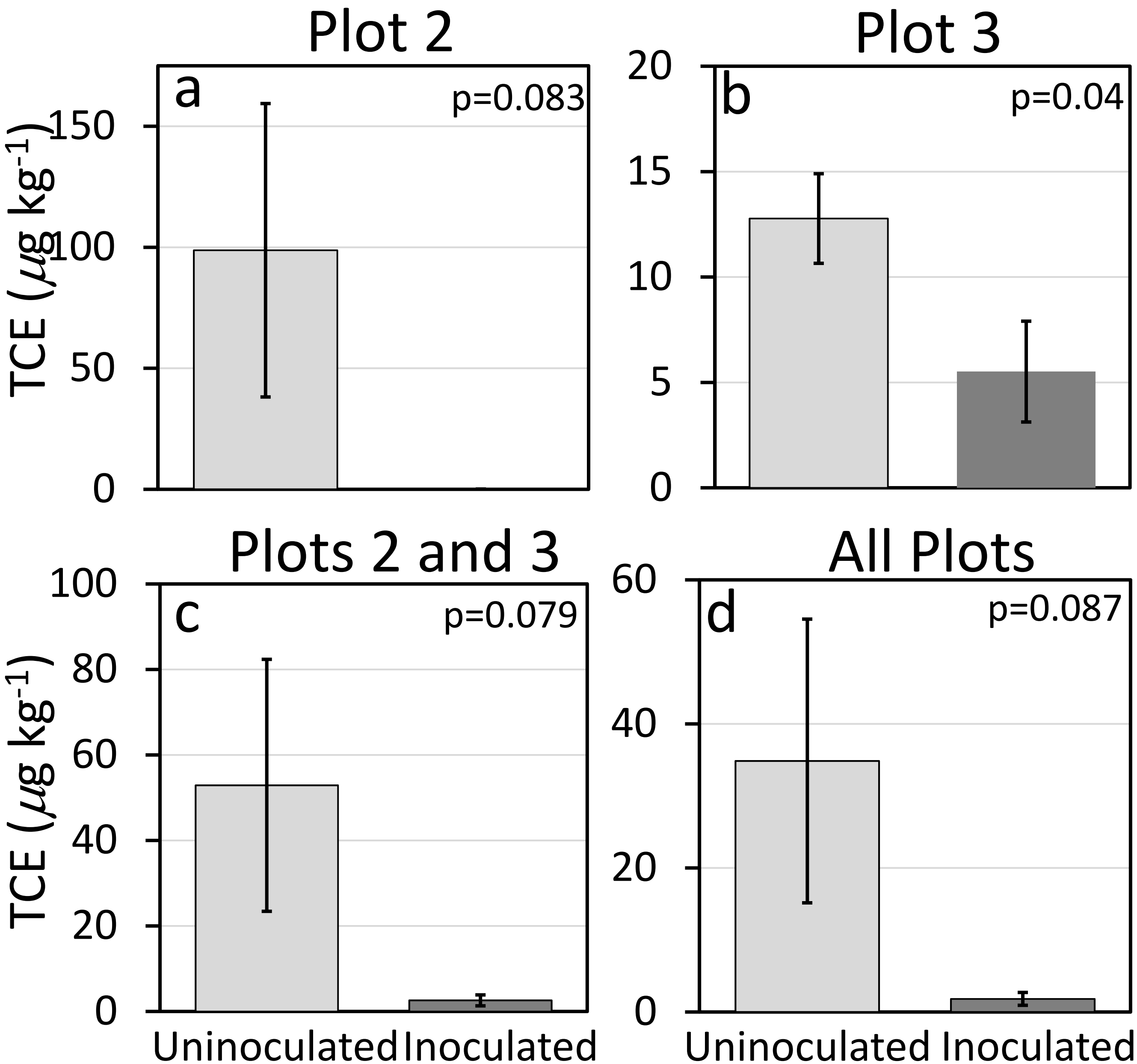
TCE levels in tree core samples (TCE ppb) N=30 trees each treatment

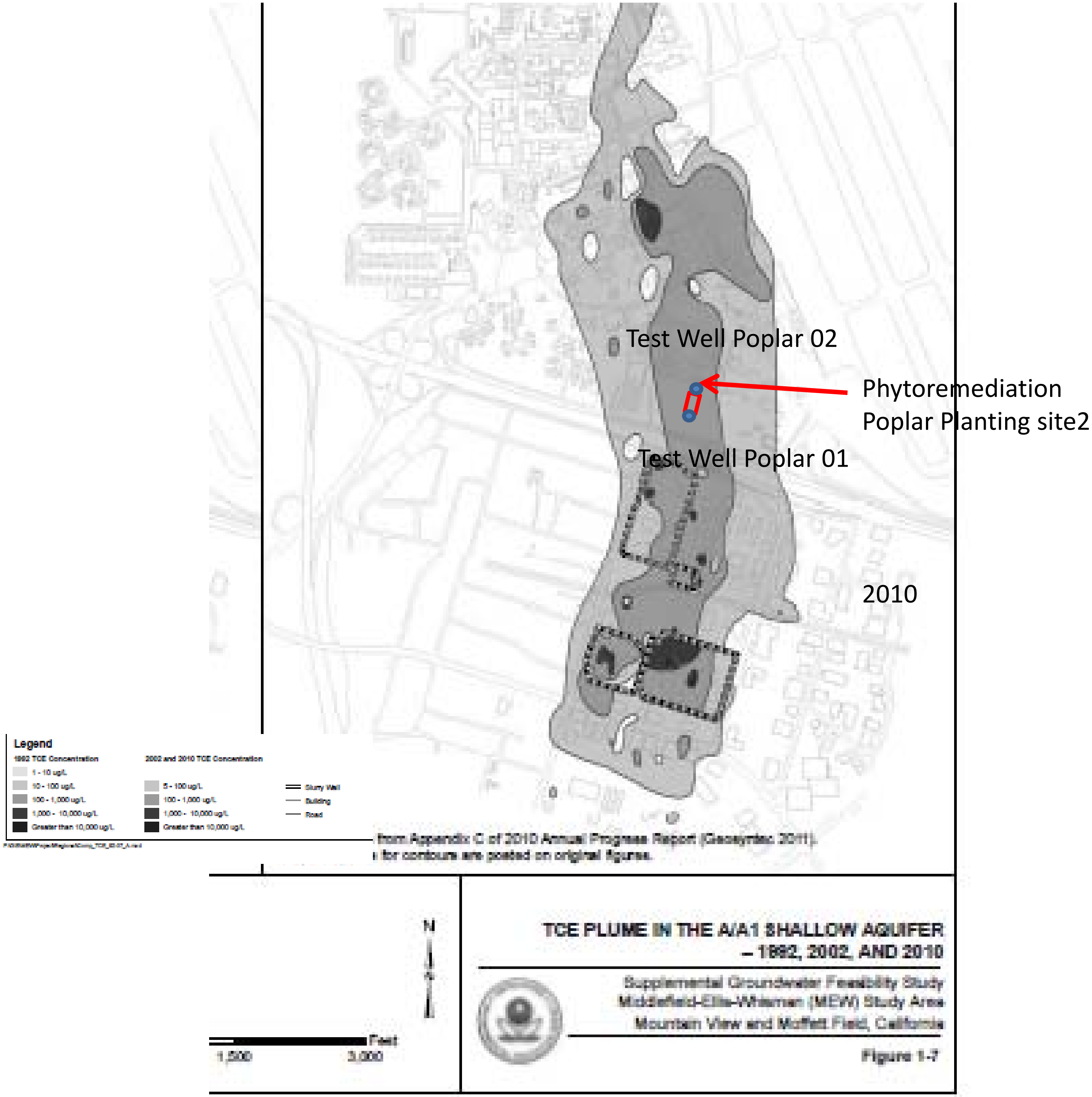
Tree core process allows TCE, PCE, DCE tests to be performed on trees over ground water plumes

can help map concentrations grids or rings of trees around potential hot spots monitor plume movement changes

Endophyte assisted phytoremediation efficacy

Less expensive than test wells




Test Well Poplar 02
Phytoremediation
Poplar Planting site2
Test Well Poplar 01
2010
Legend
1992 TCE Concentration
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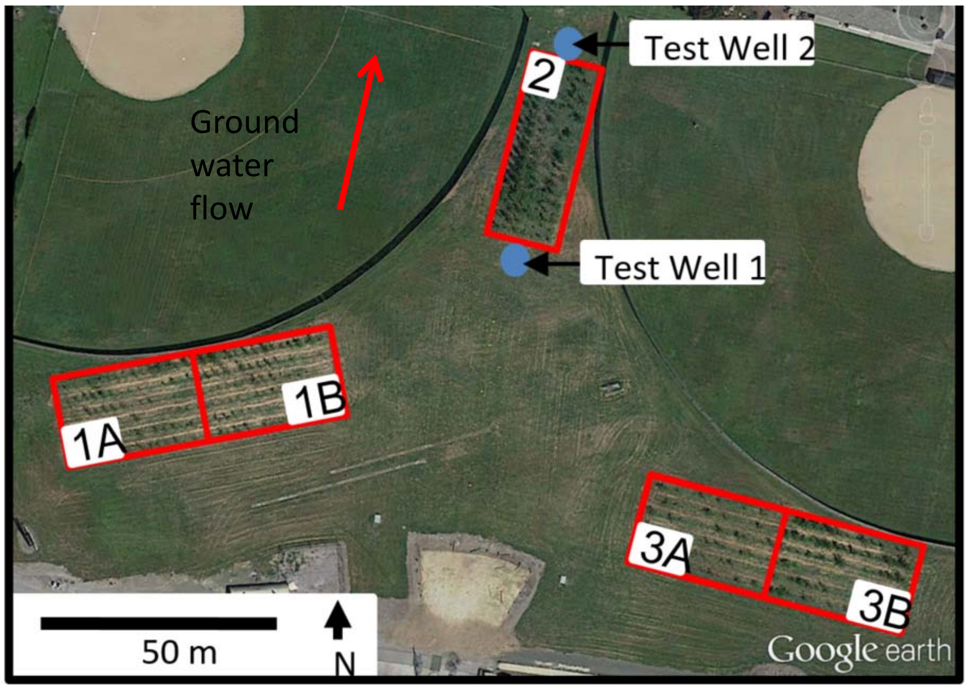

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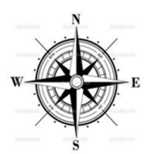

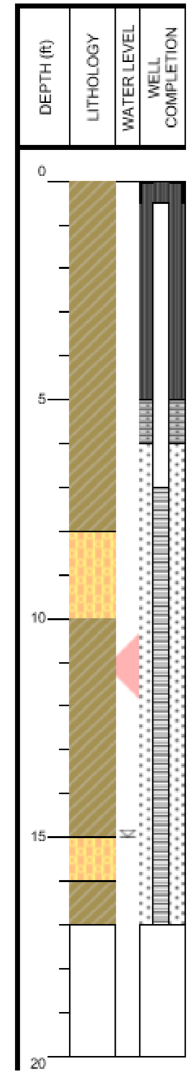

Test Well 1 Upgradient from Trees vs. Test Well 2 Down Gradient from Trees

	August 28, 2016 Test Well		November 21, 2016 Test Well		July 28, 2017 Test Well			
	1	2	1	2	1	2	MDL	Reporting Limit
	(µg/L)							
Trichloroethene	280	ND	300	ND	260	ND	1.1	5
trans-1,2-Dichloroethene	1.7	ND	3.1	ND	2.2	ND	0.13	0.5
cis-1,2-Dichloroethene	140	ND	160	ND	120	ND	1.5	5
1,1-Dichloroethene	7.6	ND	6.8	ND	5.8	ND	0.092	0.5
1,1-Dichloroethane	7.9	0.64	9	0.58	8.1	ND	0.12	0.5
Vinyl chloride	0.54	ND	0.77	ND	ND	ND	0.17	0.5
Tetrachloroethene	0.85	ND	0.88	ND	0.86	ND	0.12	0.5
Chloroform	0.38	ND	ND	ND	ND	ND	0.13	0.5
1,1,2-Trichloro-1,2,2-trifluoroethane (Freon 113)	1.5	ND	1.5	ND	2.2	ND	0.15	0.5

Environmental Science & Technology

Article

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Enhanced Degradation of TCE on a Superfund Site Using Endophyte-Assisted Poplar Tree Phytoremediation

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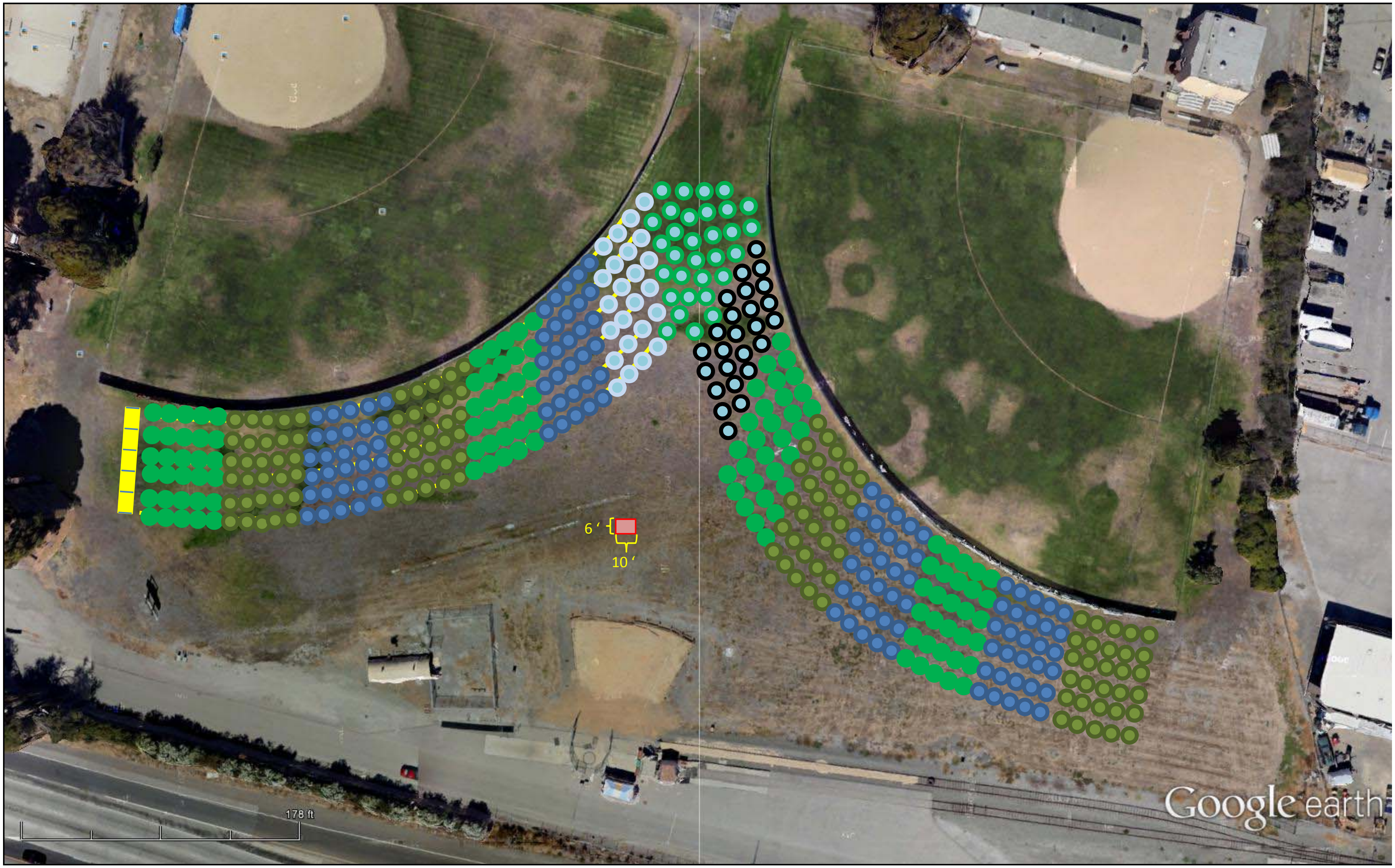
[○]Earth Resources Technology, Inc., Moffett Field, California 94035, United States

Supporting Information

ABSTRACT: Trichloroethylene (TCE) is a widespread environmental pollutant common in groundwater plumes associated with industrial manufacturing areas. We had previously isolated and characterized a natural bacterial endophyte, *Enterobacter* sp. strain PDN3, of poplar trees, that rapidly metabolizes TCE, releasing chloride ion. We now report findings from a successful three-year field trial of endophyte-assisted phytoremediation on the Middlefield-Ellis-Whisman Superfund Study Area TCE plume in the Silicon Valley of California. The inoculated poplar trees exhibited increased growth and reduced TCE phytotoxic effects with a 32% increase in trunk diameter compared to mock-inoculated control poplar trees. The inoculated trees excreted 50% more chloride ion into the rhizosphere, indicative of increased TCE metabolism *in planta*. Data from tree core analysis of the tree tissues provided further supporting evidence of the enhanced rate of degradation of the chlorinated solvents in the inoculated trees. Test well groundwater analyses demonstrated a marked decrease in concentration of TCE and its derivatives from the tree-associated groundwater plume. The concentration of TCE decreased from 300 μg/L upstream of the planted area to less than 5 μg/L downstream of the planted area. TCE derivatives were similarly removed with cis-1,2-dichloroethene decreasing from 160 μg/L to less than 5 μg/L and trans-1,2-dichloroethene decreasing from 3.1 μg/L to less than 0.5 μg/L downstream of the planted trees. 1,1-dichloroethene and vinyl chloride both decreased from 6.8 and 0.77 μg/L, respectively, to below the reporting limit of 0.5 μg/L providing strong evidence of the ability of the endophytic inoculated trees to effectively remove TCE from affected groundwater. The combination of native pollutant-degrading endophytic bacteria and fast-growing poplar tree systems offers a readily deployable, cost-effective approach for the degradation of TCE, and may help mitigate potential transfer up the food chain, volatilization to the atmosphere, as well as direct phytotoxic impacts to plants used in this type of phytoremediation.

Environmental Science & Technology **2017** *51* (17), 10050-10058 DOI: 10.1021/acs.est.7b01504

Now Connected the three test plots, Inoculated all new poplar trees into Phytobarrier
Considering other possible planting sites in Mountain View and elsewhere at NASA Ames



F 2017

- Ramboll Fractured Bedrock Site

Southern California
Arid TCE DCE
Former Military Testing

Salt(s) Boron Tolerant RRR Stock 2016

Salt(s) Boron Tolerant Stock 2017

Soil and Water Thresholds to Consider for RRR Cultivar Use

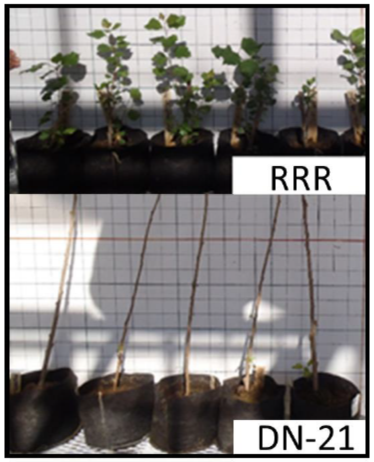
Parameter Tested	Soil	Groundwater	Notes
Sodium (ppm)	≥ 400	≥ 500	Irrigation water threshold is lower than GW values herein, especially if not including leaching fraction, and needs case-by-case consideration
Chloride (ppm)	≥ 1000	≥ 700	
Magnesium	Defer to EC and SAR		Watch for high conc. which increases EC and lowers SAR
Potassium	Defer to EC and SAR		Watch for high conc. which increases EC and increases SAR interpretation
Boron (ppm)	≥ 4	≥ 5 and < 20	
EC (dS/m)	≥ 4	≥ 3	
SAR	≥ 15		Often more important value than Na alone above
pH	≥ 8	≥ 8	Additive impact. Not a standalone, consider with other factors
TDS (mg/L)		≥ 1500	Additive impact. Not a standalone, consider with other factors

challenging situation for any phytoremediation process dealing with mixed wastes such as those found in typical landfill leachates with high levels of salt and boron. At Intrinsyx and PPCU we utilize new proprietary hybrid

poplar tree cultivars that are highly salt and boron tolerant (image to left). These cultivars have undergone continuous cultivation and selection on very high levels of salt and boron found in the west side central valley of California.

At Intrinsyx and PPCU we have propagated, tested, documented and deployed these new salt and boron tolerant hybrid poplar cultivars. They exhibit normal growth, enhanced plant vigor and no signs of salt or boron stress at unusually high concentrations of salts and boron that negatively impact other non-tolerant cultivars.

Salts and boron. This cultivar, known as RRR, was tested and endured groundwater and irrigation water with salinity between 10 dS/m 18 dS/m, and boron of 10 mg/L to 18 mg/L (above picture). Salts found in the soils of the testing sites include Na_2SO_4, NaCl, $CaCl_2$, Na_2SeO_4, $CaSO_4$, $Na_2B_4O_5(OH)_4$, and $CaB_3O_4(OH)_3$) (Na_2SO_4, NaCl, $CaCl_2$, Na_2SeO_4, $CaSO_4$, $Na_2B_4O_5(OH)_4$, and $CaB_3O_4(OH)_3$) and boron; and soil salinity ranges between 4 and 8 dS/m, while soluble boron is found upwards of 8 mg/L. In two tests we compared these salt and boron tolerant cultivars against cultivars (i.e. OP-367, 311-93, DN-21) that have been historically used in phytoremediation applications. This test demonstrated that the RRR cultivar easily tolerated soils with a combination of Na (3500 ppm; 104 meq/L), Cl (92 meq/L), B (4.25 mg/L), salinity of 10.46 dS/m and SAR of 64.31, while the other cultivars did not survive (image to right).

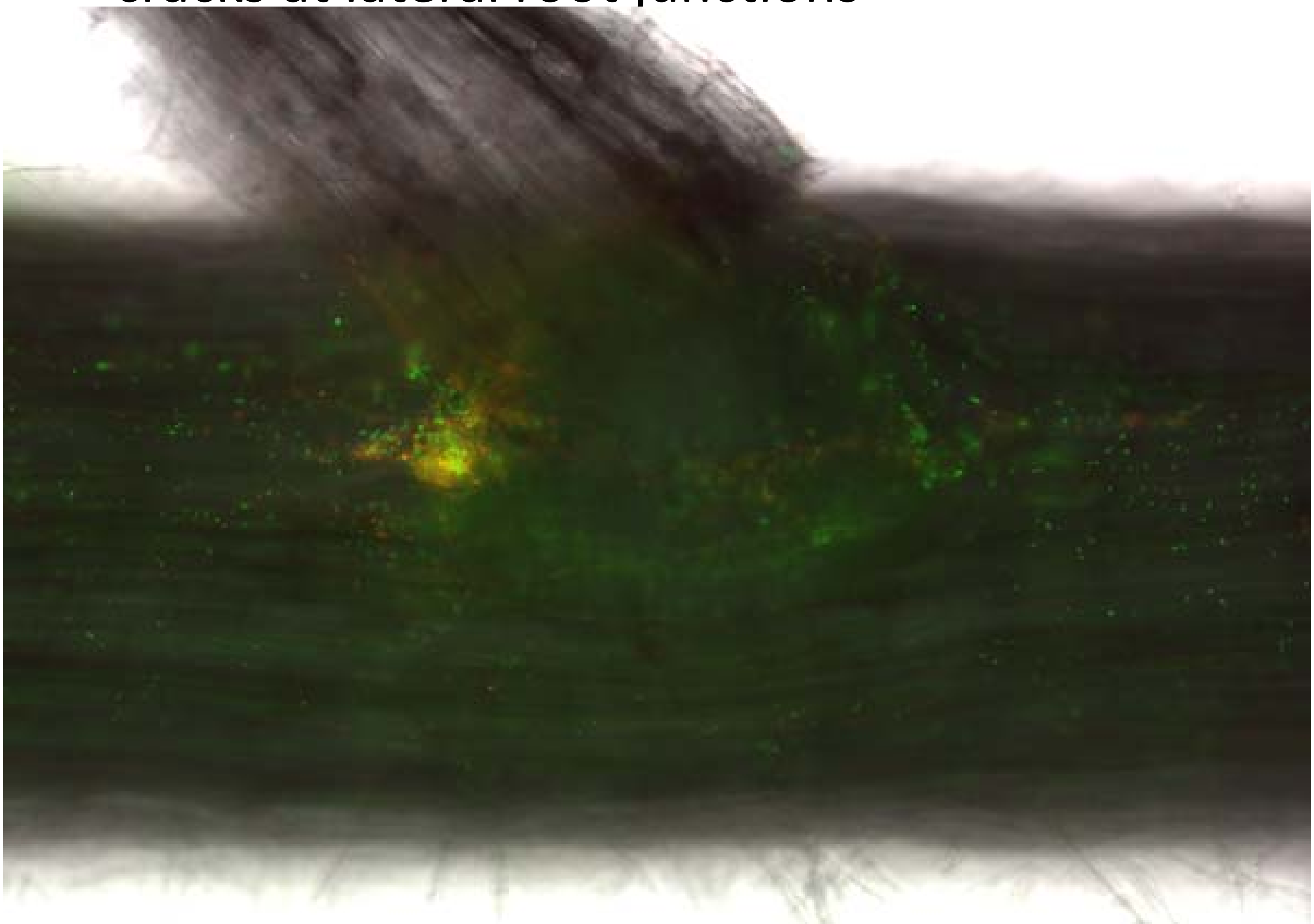


Additional Bacterial Endophyte for Phytoremediation Petroleum Gas

PD1 Willow and Grass Tolerance and Degradation of Phenanthrene

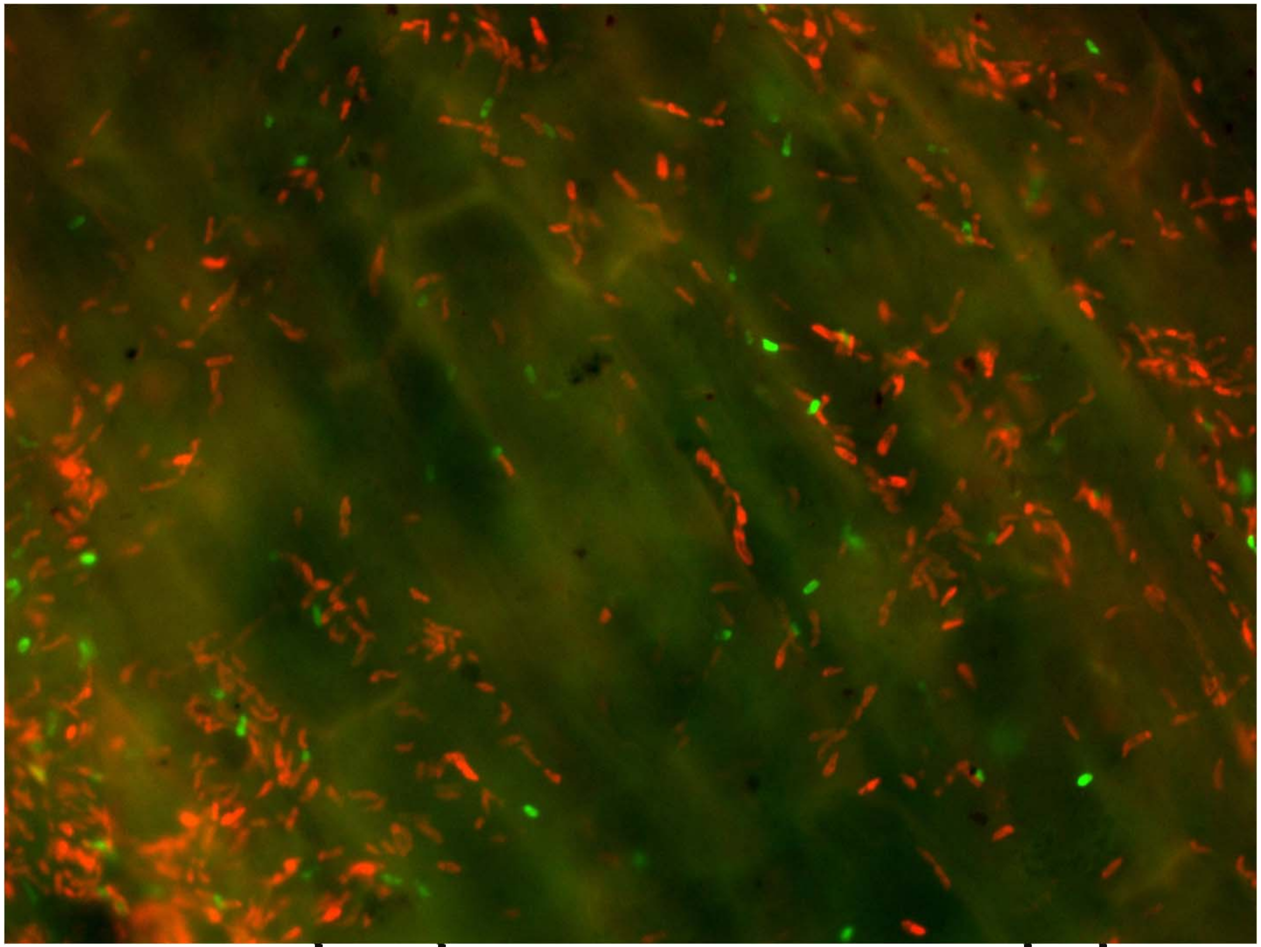
- Selected microbial relationships improve plant growth, tolerance and degradation of soil and water contaminants such as phenanthrene and other PAHs. Endophytes useful for PAHs, TPHs, and BTEX
- Endophyte was found to promote root and shoot growth and protect the plants against the phytotoxic effects of phenanthrene.
- There was an additional 25−40% removal of phenanthrene from soil by the willow and grasses, respectively inoculated with when compared to the un-inoculated controls.

Coinoculated Endophytes enter through cracks at lateral root junctions



PD1 (red) and PDN3 (green) on a poplar seedling root. Andrea Firrincieli, et al.

Endophytes Can be Applied Foliar spray with PD1&PDN3 and result in subsequent root colonization



These two endophytes can travel throughout the plant in the vascular tissue

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Enhanced Degradation of TCE on a Superfund Site Using Endophyte-Assisted Poplar Tree Phytoremediation

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Supporting Information

ABSTRACT: Trichloroethylene (TCE) is a widespread environmental pollutant common in groundwater plumes associated with industrial manufacturing areas. We had previously isolated and characterized a natural bacterial endophyte, *Enterobacter* sp. strain PDN3, of poplar trees, that rapidly metabolizes TCE, releasing chloride ion. We now report findings from a successful three-year field trial of endophyte-assisted phytoremediation on the Middlefield-Ellis-Whisman Superfund Study Area TCE plume in the Silicon Valley of California. The inoculated poplar trees exhibited increased growth and reduced TCE phytotoxic effects with a 32% increase in trunk diameter compared to mock-inoculated control poplar trees. The inoculated trees excreted 50% more chloride ion into the rhizosphere, indicative of increased TCE metabolism *in planta*. Data from tree core analysis of the tree tissues provided further supporting evidence of the enhanced rate of degradation of the chlorinated solvents in the inoculated trees. Test well groundwater analyses demonstrated a marked decrease in concentration of TCE and its derivatives from the tree-associated groundwater plume. The concentration of TCE decreased from 300 μg/L upstream of the planted area to less than 5 μg/L downstream of the planted area. TCE derivatives were similarly removed with cis-1,2-dichloroethene decreasing from 160 μg/L to less than 5 μg/L and trans-1,2-dichloroethene decreasing from 3.1 μg/L to less than 0.5 μg/L downstream of the planted trees. 1,1-dichloroethene and vinyl chloride both decreased from 6.8 and 0.77 μg/L, respectively, to below the reporting limit of 0.5 μg/L providing strong evidence of the ability of the endophytic inoculated trees to effectively remove TCE from affected groundwater. The combination of native pollutant-degrading endophytic bacteria and fast-growing poplar tree systems offers a readily deployable, cost-effective approach for the degradation of TCE, and may help mitigate potential transfer up the food chain, volatilization to the atmosphere, as well as direct phytotoxic impacts to plants used in this type of phytoremediation.

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

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Supporting Information

ABSTRACT: Endophytes have been isolated from a large diversity of plants and have been shown to enhance the remediation efficiency of plants, but little information is available on the influence of endophytic bacteria on phytoremediation of widespread environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs). In this study we selected a naturally occurring endophyte for its combined ability to colonize plant roots and degrade phenanthrene in vitro. Inoculation of two different willow clones and a grass with *Pseudomonas putida* PD1 was found to promote root and shoot growth and protect the plants against the phytotoxic effects of phenanthrene. There was an additional 25−40% removal of phenanthrene from soil by the willow and grasses, respectively inoculated with PD1 when compared to the uninoculated controls. Fluorescent microscopy using fluorescent protein tagging of PD1 confirmed the presence of bacteria inside the root tissue. Inoculation of willows with PD1 consistently improved the growth and health when grown in hydroponic systems with high concentrations of phenanthrene. To our knowledge this is the first time that the inoculation of willow plants has been shown to improve the degradation of PAHs and improve the health of the host plants, demonstrating the potential wide benefit to the field of natural endophyte-assisted phytoremediation.

Based on the Lab and Field Work and because chlorinated solvents and PAH's often coexist in groundwater

Actively Looking for New Ground Water and Soil Impacted Sites!

Thank you

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