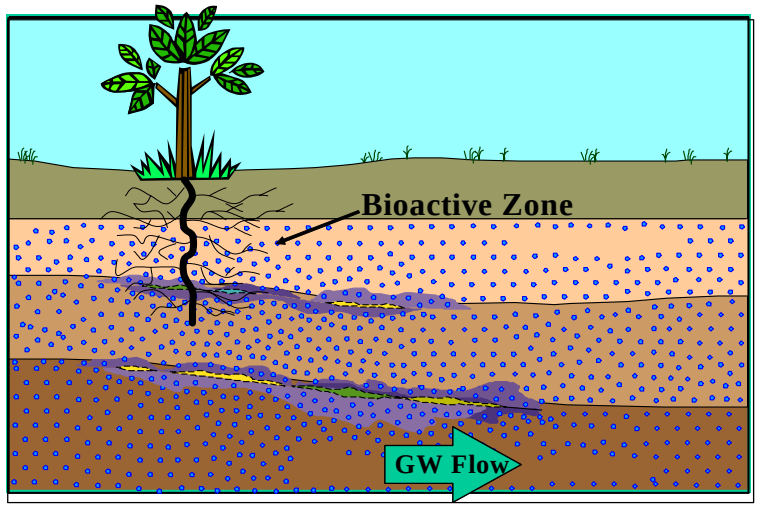
Phytoremediation of TCE at Two Superfund Sites



Assessing Methanotroph Activity in the Rhizosphere

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Personnel Related to This Project

- **Graduate Research Assistant:**
  - Ms. Adriana Pacheco, EES, UF
- **Collaborators:**
  - Dr. Donald Rockwood, Forestry, UF
  - Dr. Jud Isebrands, Environmental Forestry Consultants, New London, WI
  - Dr. Robin Brigmon, Savannah River Laboratory, Aiken, SC
- **UF SBRP Directors:**
  - Dr. Margaret James
  - Dr. Steve Roberts

Web page: www.superfund.ufl.edu

Overview

- Background
  - Phytoremediation
  - Description of Superfund sites in this study
- Broad goals and specific aims
- Initial results
- Current focus

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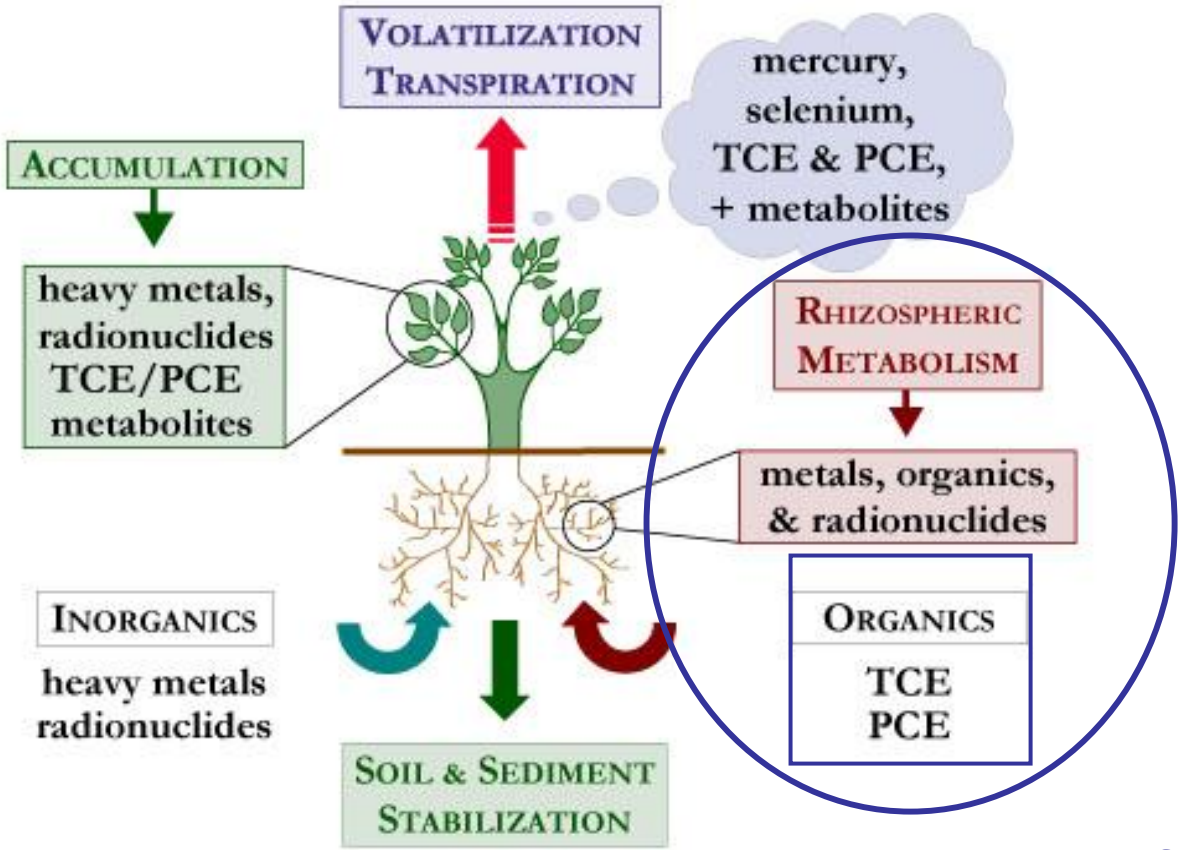
Phytoremediation of Contaminants



Focus of this study

Source:

The University of Georgia
Savannah River Ecology Laboratory

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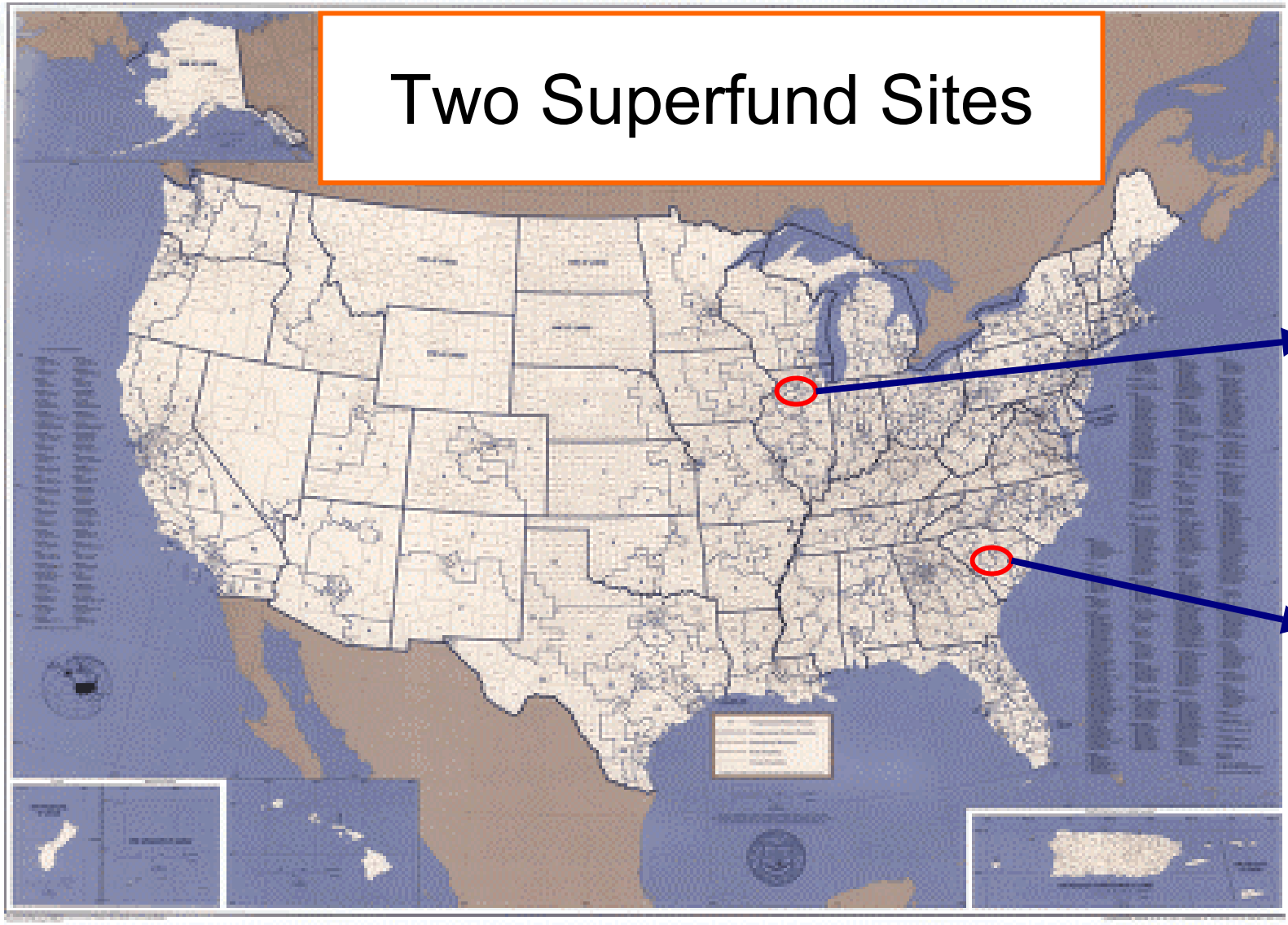
Two Superfund Sites



LaSalle, Illinois (LaSalle Electric Utilities)

Aiken, S.C. (Savannah River Ecology Labs, SRS)

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Description of the Sites

LaSalle

- Former electric utility manufacturing capacitors and in operation between 1943-1982
- Tetrachloroethylene (PCE) and trichloroethylene (TCE) detected in groundwater
- Plant species used in PCE site:
  - PCE site: poplar (*Populus* spp.) in native soil
  - TCE site: juvenile poplar (*Populus* spp.), willow (*Salix* spp.) in PVC pipes
- TCE concentration range: 4-254 ppb
- PCE concentration range: 6-838 ppb

SRS

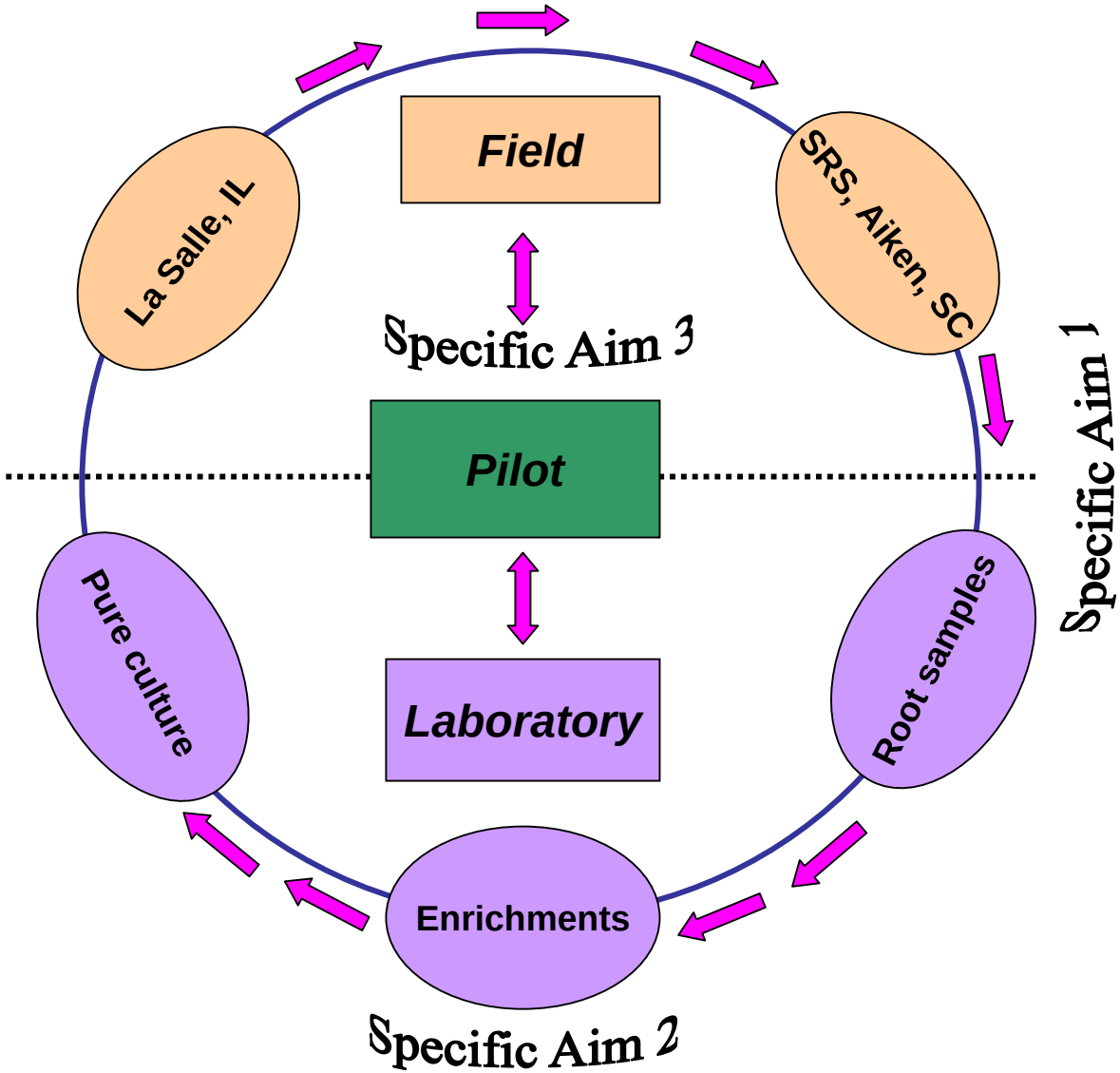
- Constructed in the 1950s to produce basic materials used in nuclear weapons production. A DOE site contracted to Westinghouse Savannah River Company LLC.
- Variety of contaminants, including tritium and plutonium. TCE plume in a seepage successional ecosystem.
- Plant species:
  - Loblolly pine (*Pinus taeda*) in native soil (20 years old)
- TCE concentration range: 6-20 ppb
  - Dechlorination products also detected.

Broad Goals

- Assess the differences in methanotrophic population diversity and activity potentials observed in ***rhizosphere samples*** from a variety of plant species in two U.S. regions

- Verify relationships between plant species and methanotrophic population diversity and activity by simulating ***bench-scale phytoremediation***

Three Levels of Study in the SBRP Phytoremediation Project



Specific Aims:

1. Microbial Community and Activity Analysis of Rhizosphere Samples

2. Laboratory Enrichments and Activity Measurements

3. Bench-scale Studies

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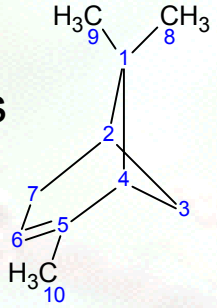
Questions Addressed Here

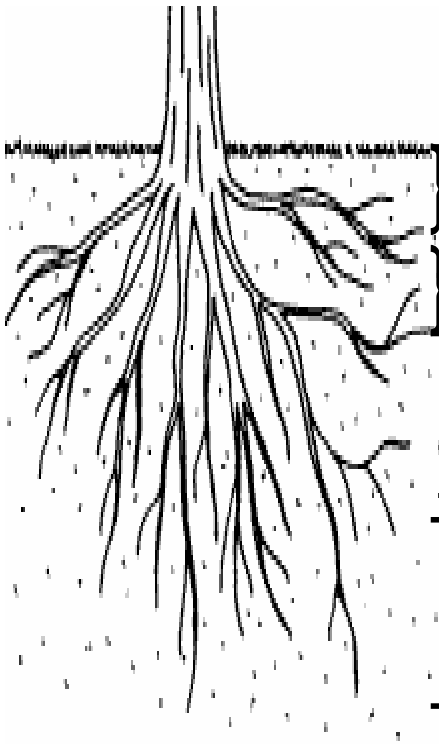
- **Specific Aim 1: Rhizosphere Samples**
  - How do heterotrophic and methanotrophic counts and activity differ
    - By plant type?
    - In roots and soil?
    - With nonvegetated controls?
    - With different moisture levels?

- **Specific Aim 2: Enrichments and Pure Cultures**
  - What effect do terpenes have on TCE biodegradation processes involving methanotrophs?
  - What are the dominant methanotroph species?



***alpha*-pinene**

Sampling Protocol



4 composite soil depth samples X 3 species: Loblolly pine (SRS) Poplar (LaSalle) Willow (LaSalle) X 2 seasons: Mid Summer Mid Winter =

24 samples (Treatment #1)
+
24 samples (Treatment #2)
+
16 samples (Treatment #3)

Overall sample load: 64 samples

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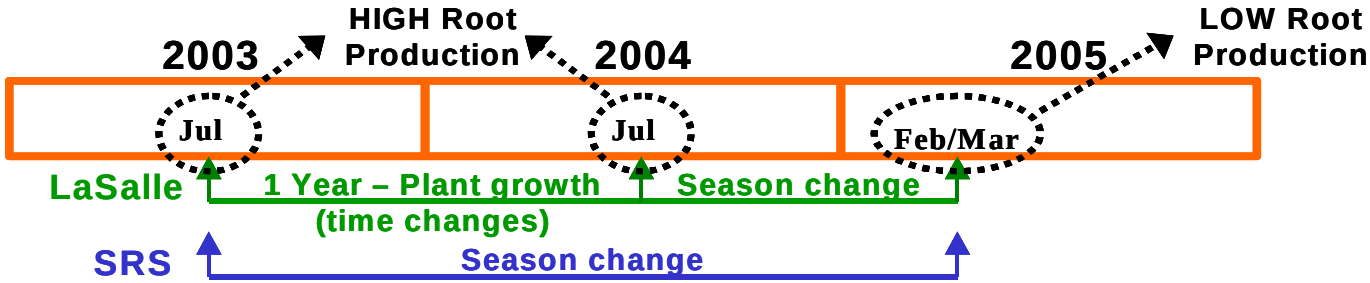
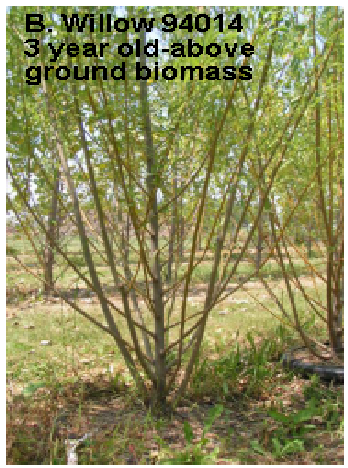
Sampling Schedule





July 2003 ***July 2004***

Next sampling at both sites: February/March 2005

Benefits of Sampling Protocol:

Allows for determining effects of *stage of plant growth, season, and plant maintenance techniques* on populations and activities

Comparison of both sites will provide insight into effects of *climate, plant age, site characteristics,* and *plant type*.

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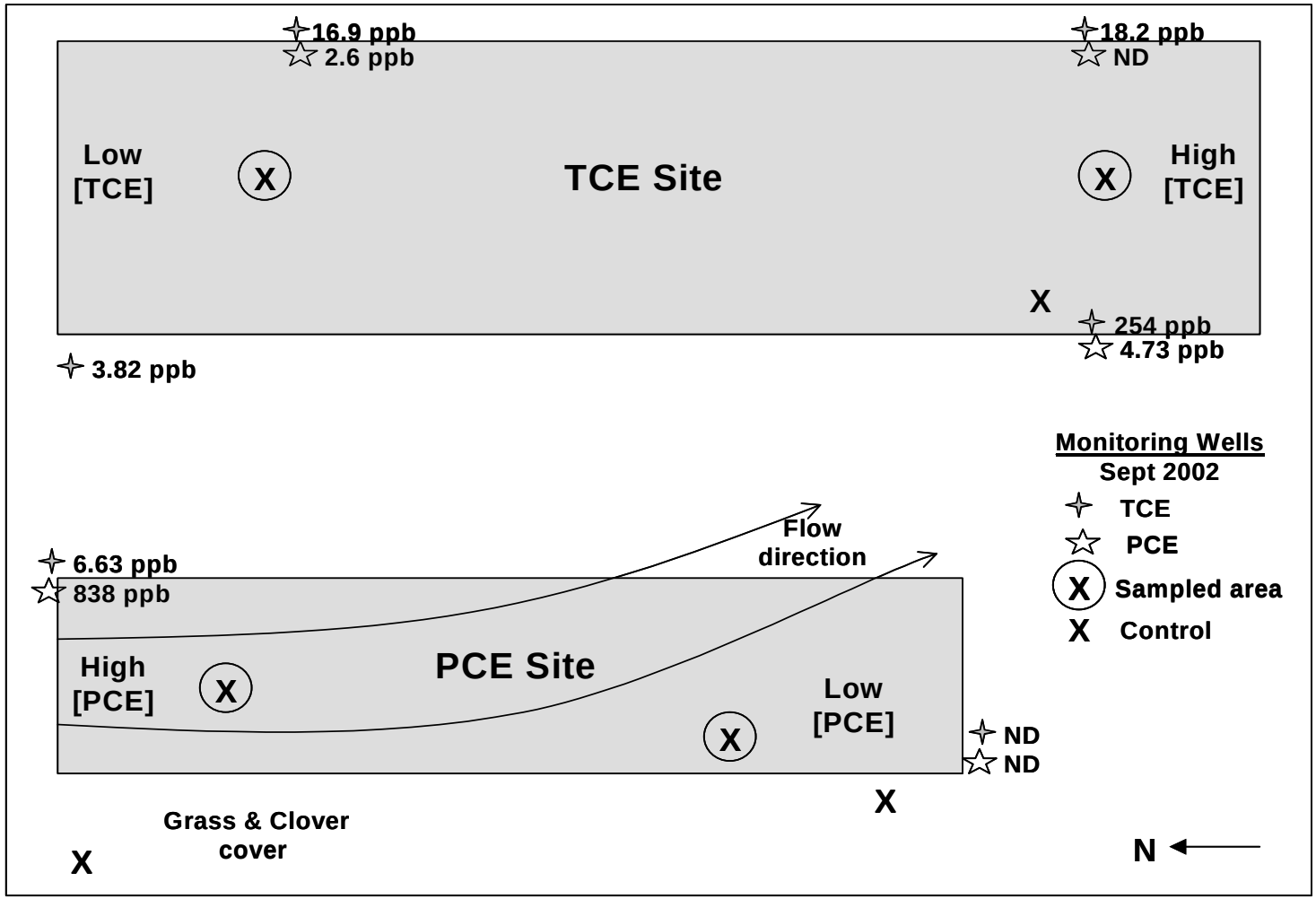
La Salle Site Sampling



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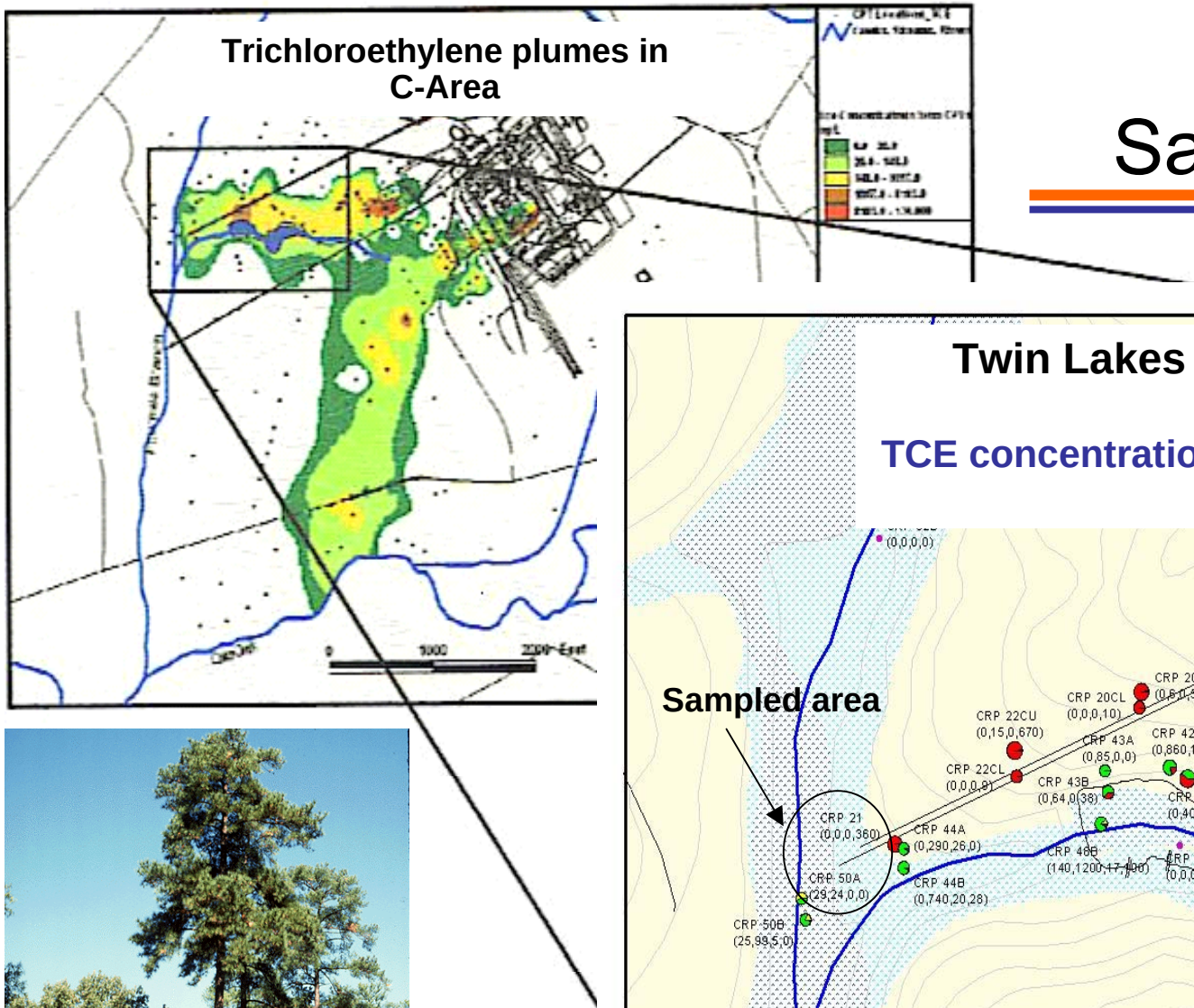
SRS Sampling



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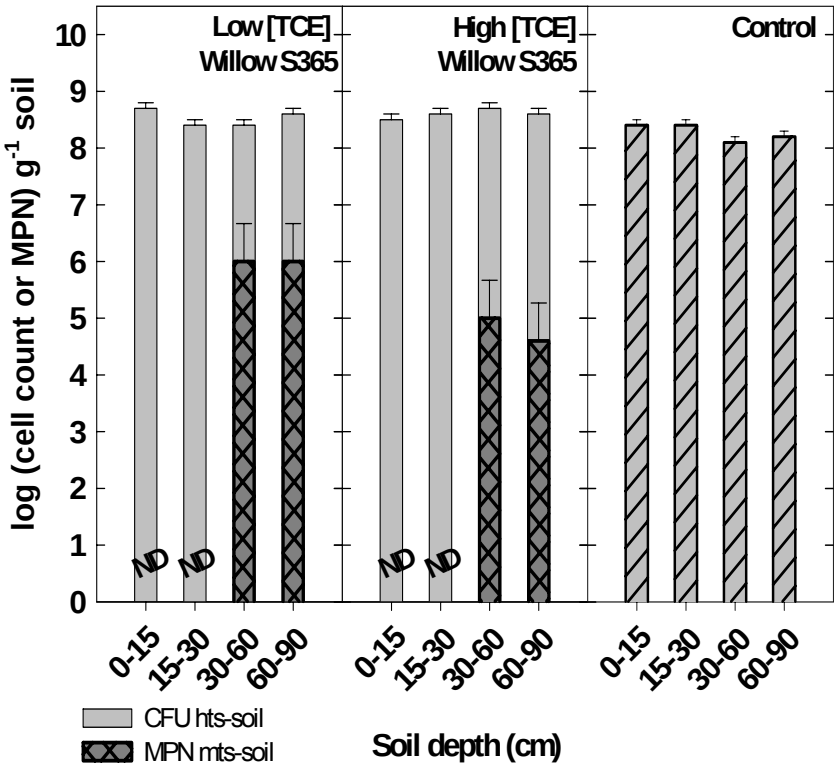
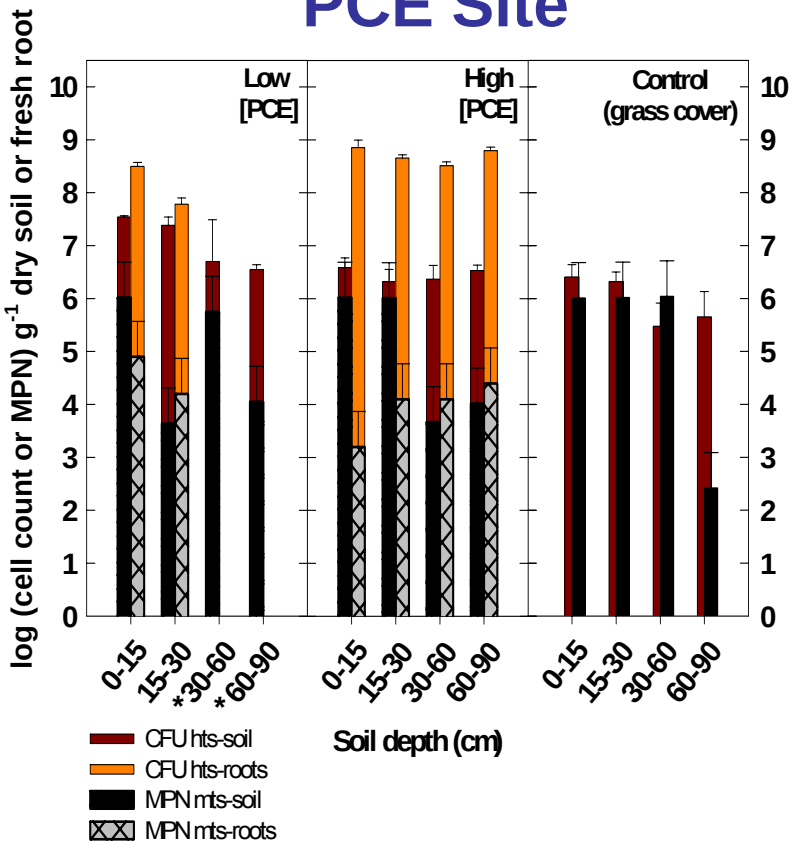
Microbial Counts in 2003 La Salle Samples

TCE Site



PCE Site



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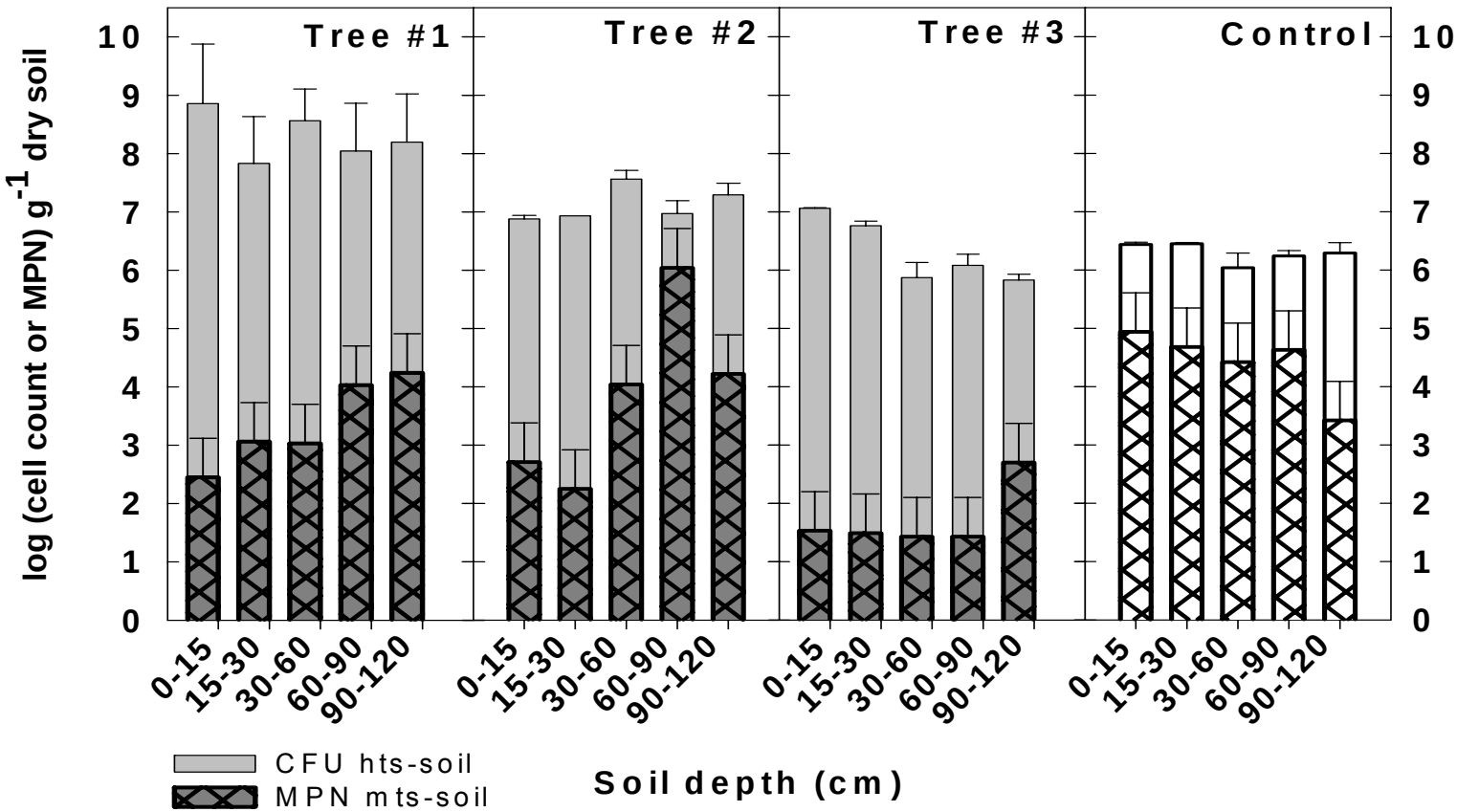
Microbial Counts in 2003 SRS Samples



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Summary of Microbial Counts

Site	Contaminant	Microbial populations counts				Soil characteristics		[TCE] or [PCE] (ppb)
		Heterotrophs (CFU)		Methanotrophs (MPN)		Water content (%)	pH	
		Soil (g^{-1} dry soil)	Root (g^{-1} fresh root)	Soil (g^{-1} dry soil)	Root (g^{-1} fresh root)			
LaSalle	*TCE*	10^8 - 10^9 (10^8)	10^9 - 10^{10}	10^5 - 10^6 (10^5 - 10^6)	10^4 - 10^7	2 - 20 (11 - 21)	7 - 8 (7 - 8)	4 - 254
	PCE	10^6 - 10^8 (10^5 - 10^6)	10^8 - 10^9	10^4 - 10^6 (10^2 - 10^6)	10^3 - 10^5	9 - 33 (9 - 17)	6 - 8 (6 - 7)	ND - 838
SRS	*TCE*	10^6 - 10^9 (10^6)	NA	10^1 - 10^4 (10^3 - 10^5)	NA	12 – 100 (12-17)	4 - 5 (5)	6 - 20

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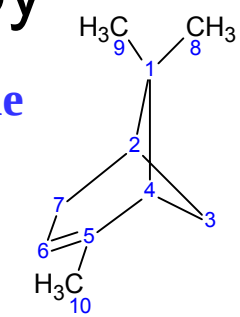
Specific Aim 2

- Impacts of *alpha*-pinene on TCE oxidation by pure methanotrophs:



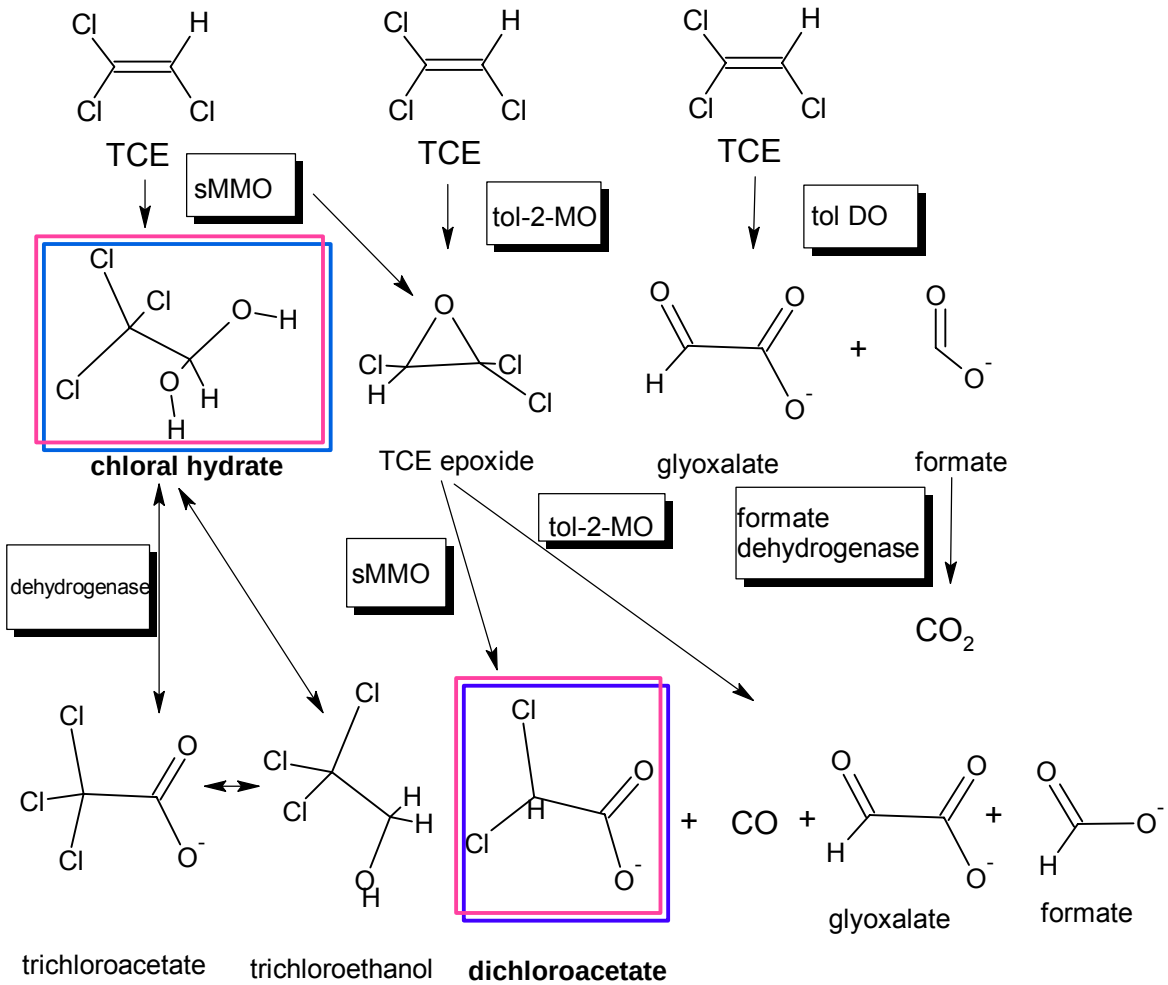
***alpha*-pinene**

  - **Type I: *Methylobacter album* BG8**
  - **Type II: *Methylosinus trichosporium* OB3b**
  - **Type X: *Methylococcus capsulatus* (Bath)**

- Activity and initial phylogenetic characterization of methanotrophic enrichments from field samples

Known Environmental Pathways of TCE: Aerobic Conditions

- Most methanotrophs can express particulate methane monooxygenase (pMMO).
- Some methanotrophs can also express soluble methane monooxygenase (sMMO).
- pMMO and sMMO can oxidize TCE.



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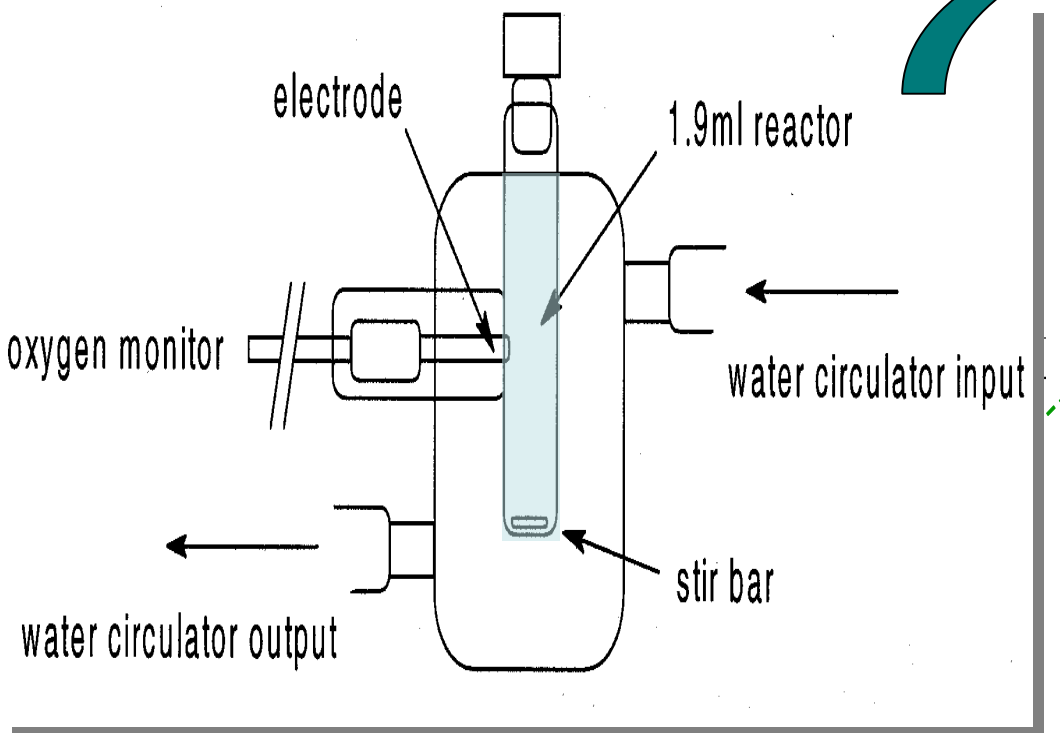
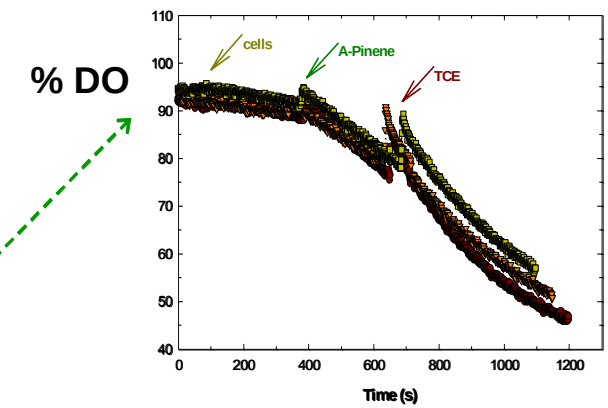
Pure Culture Studies: Oxygen Uptake Analysis to Measure the Effect of α-Pinene on TCE Oxidation

Methanotrophs +

1. **TCE alone**
2. **α-Pinene alone**
3. **TCE + α-Pinene**





Rates are normalized to methane to produce a dimensionless measure of oxygen uptake.

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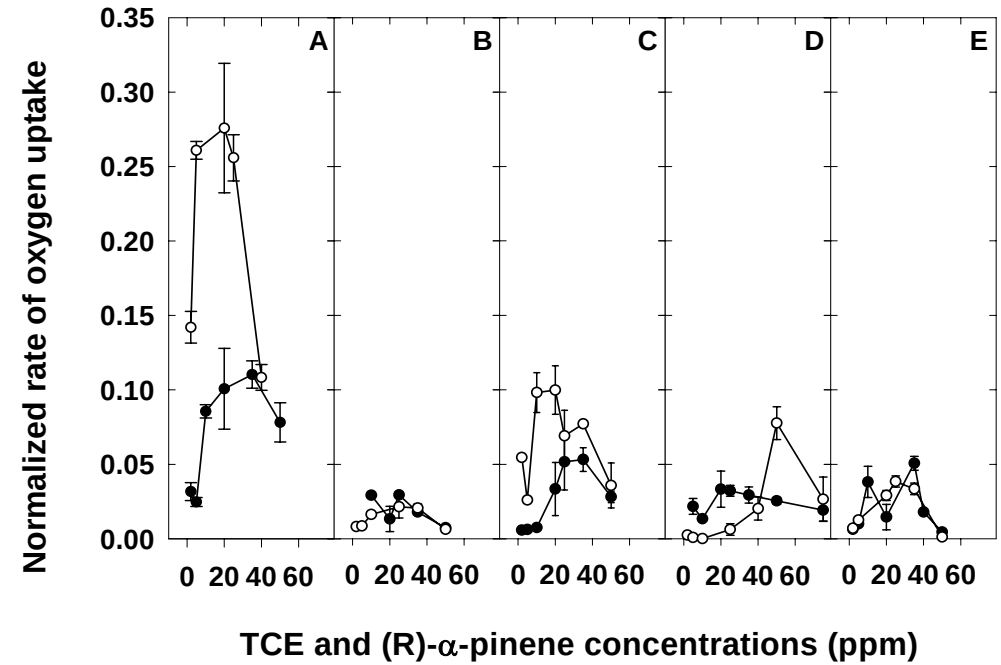
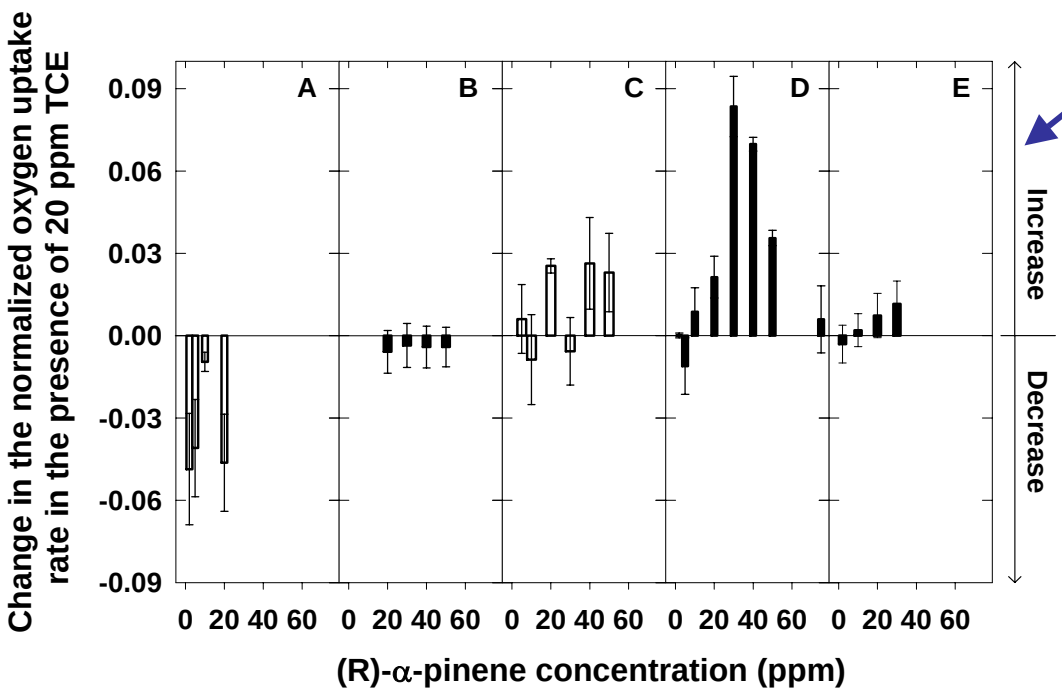
Pure Culture Studies: Effect of *alpha*-Pinene on TCE Oxidation



Pure methanotrophs with *alpha*-pinene and TCE separately



Effect of *alpha*-pinene on oxygen uptake rates at 20 ppm TCE

KEY:
A, **B**: *M. trichosporium* OB3b cultured without and with copper, respectively.
C, D: *M. capsulatus* [Bath] cultured without and with copper, respectively.
E: *M. album* BG8 cultured with copper.
● TCE
○ R-α-pinene

Pacheco and Lindner. 2004. *BECT.* In press.

Description of Enriched Mixed Cultures

- Stable mixed methanotrophic-heterotrophic cultures were isolated from the rhizosphere of poplar (LaSalle), willow (LaSalle), and loblolly pine (SRS) trees.

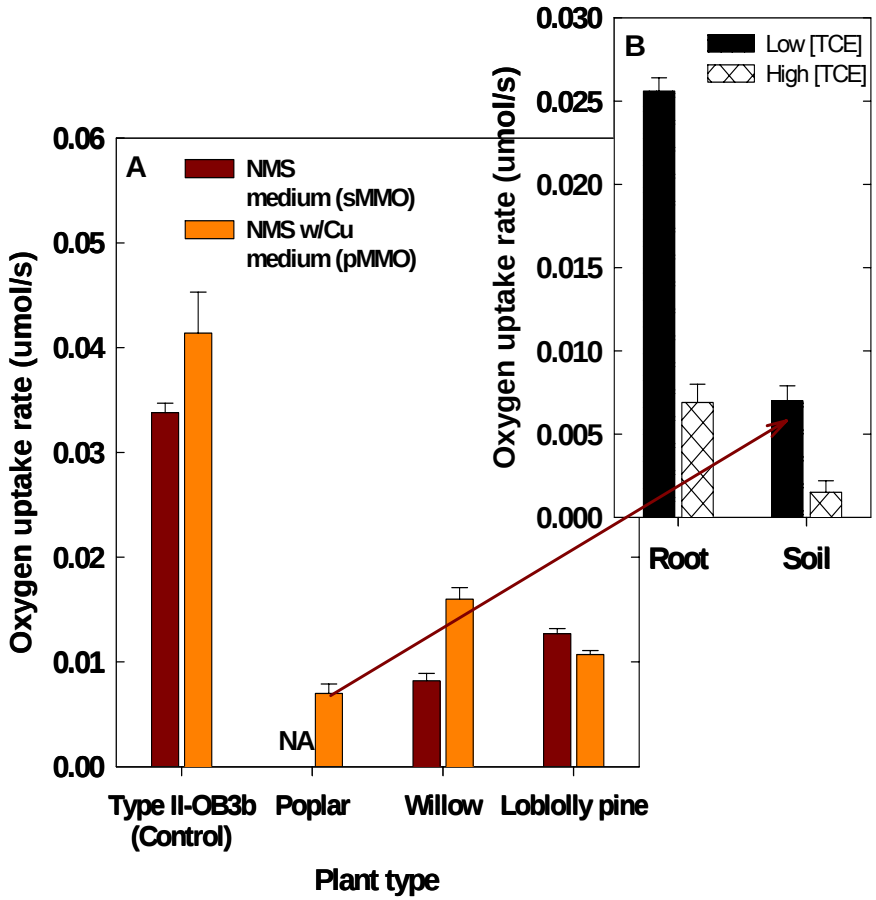
- All mixed cultures can use methane as the only carbon source and exhibit sMMO activity when cultured without copper.

- The LaSalle cultures were shown to be capable of oxidizing TCE and *alpha*-pinene.

- All three cultures exhibited methane oxidation activity…..

Activity of LaSalle Enrichments in the Presence of CH_4

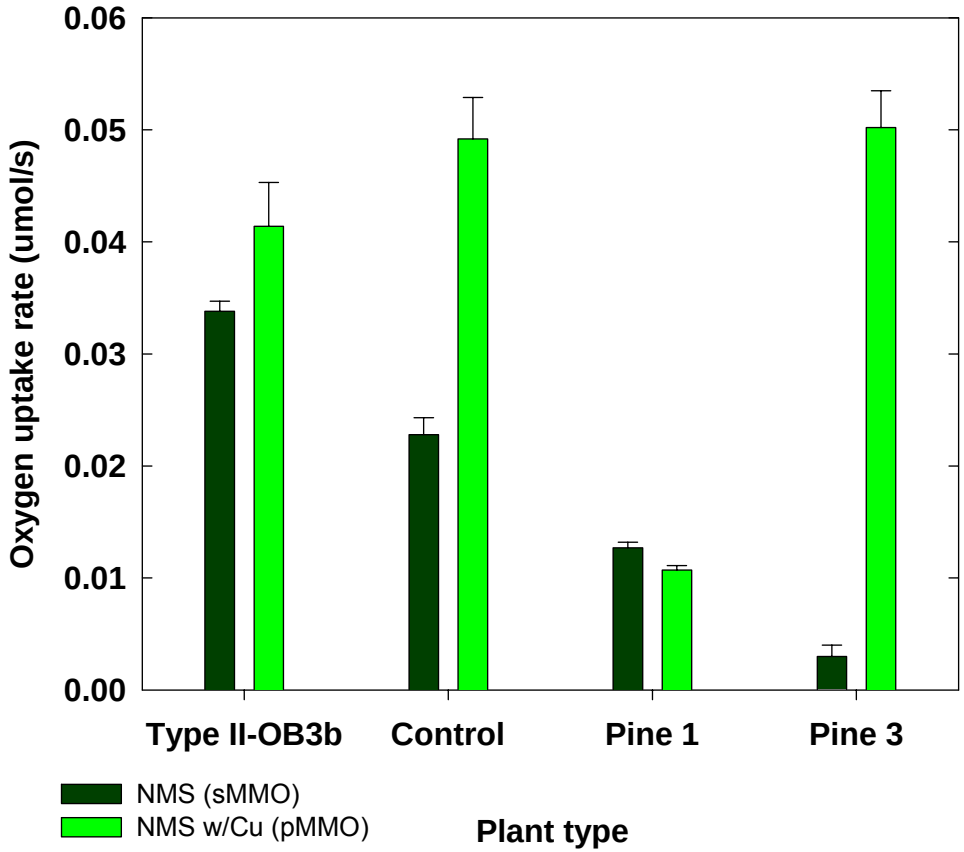
Cultures isolated from poplar soil and root samples



TCE Zone:
Cultures isolated from rhizosphere soil samples of all tree types

PCE Zone:
Culture isolated from rhizosphere soil samples of poplar trees

Activity of SRS Enrichments in the Presence of CH_4



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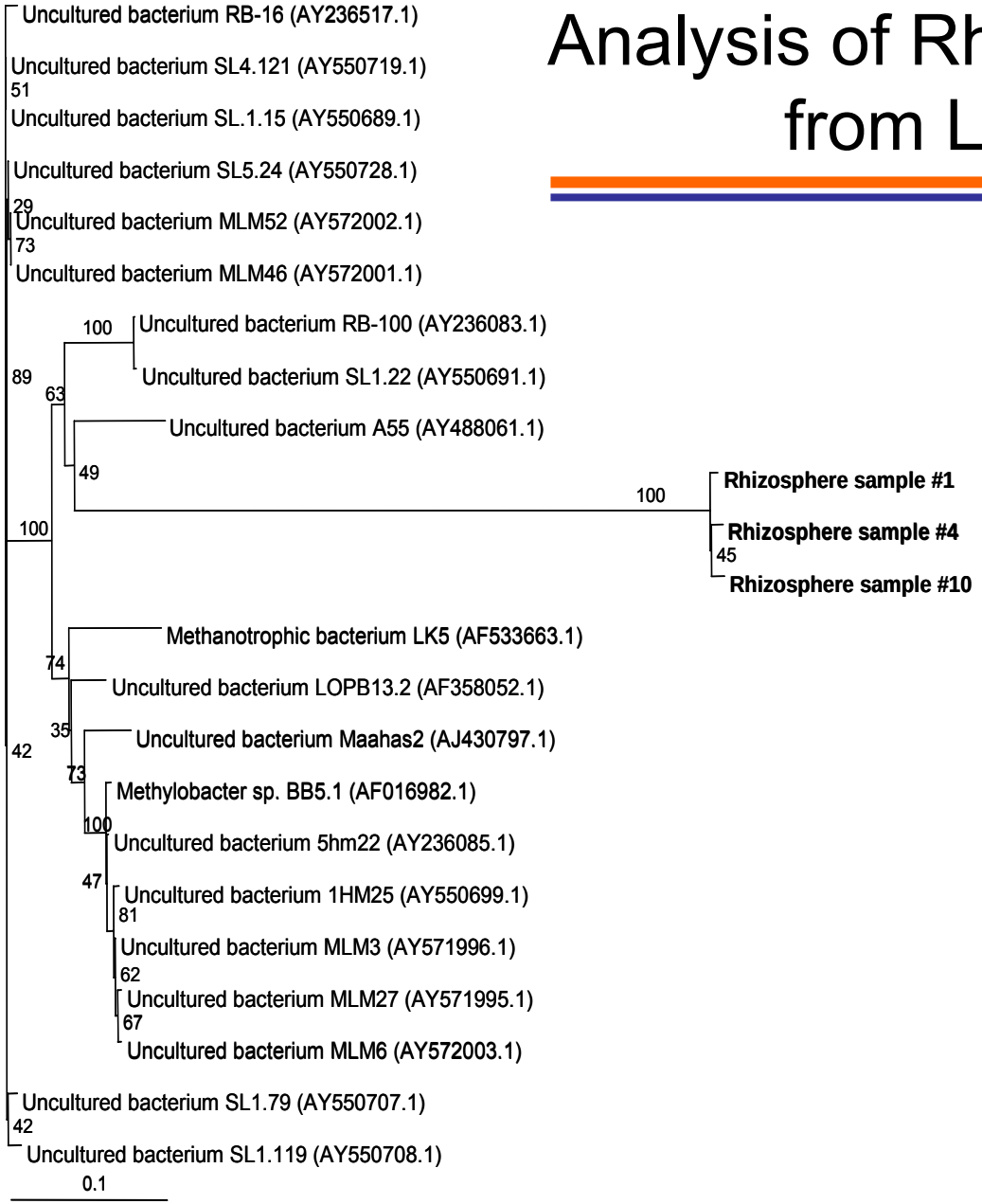
Development of Phylogenetic Analysis of Rhizosphere Enrichments from LaSalle/TCE Site



- Based on the nucleotide sequence of the **pmoA gene** obtained from a rhizosphere root sample
- Length of branches is proportional to % dissimilarity (0.1 base changes per nucleotide sequence position)
- Rhizosphere samples placed in a separated branch (high % dissimilarity)
- Closest branch to rhizosphere samples dominated by uncultured bacteria from peat soils and soda lake sediments

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Conclusions to Date

- **Specific Aim 1**
  - Results from the field have shown that willows, loblolly pine, and certain clones of poplars have been shown to uptake PCE and TCE at both sites.
  - Heterotroph and methanotroph counts in the 2003 root and soil samples seem to be impacted most by soil moisture content, soil/root location, and possibly tree type.
    - Effects of age and tree growth at both sites will be determined by comparing 2004 counts to the 2003 counts.

- **Specific Aim 2**
  - *alpha*-Pinene impacts TCE oxidation; however, with different effects depending on the methanotroph type.
  - Mixed cultures isolated from the roots and soil of each tree type are capable of oxidizing methane, TCE, and *alpha*-pinene. Differences in oxidation potential exist depending on rhizosphere origin (soil or root), tree type, and MMO expression.

- These initial results emphasize the importance of characterizing each phytoremediation site for type of methanotrophs present, bioavailable copper concentrations, and contaminant oxidation potentials.

Current Focus

- **Specific Aim 1:**
  - Counts in one-year samples and next round of sampling
  - Soil testing for determining bioavailable nutrients
  - Protocol development
    - phylogenetic analysis for methanotrophic diversity in soil and root samples
    - stable-isotope probing ($^{14}CH_4$) of samples for methanotrophic activity
- **Specific Aim 2:**
  - Protocol development for analysis for products of *alpha*-pinene and TCE oxidation by pure methanotrophs